Journal of Oil Palm Research
DOI: https://doi.org/10.21894/jopr.2024.0035

MOLECULAR CHARACTERISATION OF OIL PALM (*Elaeis guineensis* Jacq.) HYBRIDS

ROJA RAMANI, G[1]*; KALPANA, M[1]; VENKATSWAMI, D[1] and KALYANABABU, B[2]

ABSTRACT

For genetic mapping research in a crop like oil palm, polymorphic SSR (simple sequence repeat) markers are crucial. Thirty four SSR loci were used for screening in the current work to test a total of 10 hybrids at molecular level. There were between two and four alleles in these, with 22 of them being polymorphic and 12 being monomorphic. The primer SMG00026 *had the greatest Polymorphism Information Content (PIC) value (0.60), while* mEgCIR0408 *had the lowest (0.14), with a mean PIC value of 0.22. Genetic variation scores varied between 0.15* (mEgCIR0408) *and 0.66* (SMG00026)*, with an average of 0.286. Seven highly polymorphic markers,* SMG00026, mEgCIR0074, mEgCIR0353, mEgCIR3350, mEgCIR0555, mEgCIR3886, *and* mEgCIR0905*, were identified based on PIC and other genetic criteria. The oil palm crop development programme can effectively utilise the found polymorphic SSR loci in genetic diversity investigations and mapping. The 10 hybrids were divided into two primary clusters by a total of 22 polymorphic SSRs, and the observed clustering was based on geographic origin. These polymorphic primers can be used effectively because they make it easier to choose promising varieties at nursery stage, which helps both researchers and farmers to modernise the plant breeding programme for oil palm.*

Keywords: allele, polymorphism, primer.

Received: 31 August 2023; **Accepted:** 7 April 2024; **Published online:** 11 June 2024.

INTRODUCTION

The oil palm produces universal and distinctive oil that is most widely manufactured and commercialised of all vegetable oil crops. *Elaeis guineensis* Jacq. an oil palm species is a member of the Arecaceae family (tribe: Cocoineae) and is a monocot that is allogamous and arborescent native to West Africa (Hartley, 1988). Its genetic code, which has 16 homologous chromosome pairs and is diploid (2n = 32), is thought to be 1.800 gigabases (Gb) in size. The reference genome assembly for *E. guineensis* (AVROS, *pisifera* fruit form), which has a total size of 1.535 Gb, was made available to the general public in 2013 (Singh *et al*., 2013). Flow cytometry determined its physical size as 3.79 pg/2C (Rival *et al*., 1997). It can meet market demand in conjunction with a lower planting area than other oil crops due to its better productivity and extremely low production costs, which is especially beneficial for the food industry (Cadena *et al*., 2013; Mozzon *et al*., 2013). This golden palm is one of the greatest possibilities for meeting the nation's needs for edible oil because of its great oil production (4-6 t/ha) capability, which is five times greater than that of other annual oil-yielding crops (Basiron, 2000).

Malaysia and Indonesia are the two countries that produce the most palm oil (Yarra *et al*., 2019), although India is still in the early stages of its expansion in this area. Genetic research is aimed at increasing the effectiveness of oil palm farming because oil palm is naturally highly heterozygous. Genetic diversity assessment and the characterisation of germplasm of oil palm play a significant part in terms of genetic development of the oil palm (Zhou *et al*., 2015). To quantify

[1] Dr. Y.S.R. Horticultural University, Venkatramannagudem, Andhra Pradesh, 534101, India.

[2] ICAR-Indian Institute of Oil Palm Research, Pedavegi, Andhra Pradesh, 534450, India.

* *Corresponding author e-mail: rojaramanigorle60@gmail.com*

the genetic variability of germplasm (Zhao *et al*., 2019) morphological, biochemical and molecular approaches can be used (Mohammadi and Prasanna, 2003).

Oil palm molecular research has advanced somewhat as a result of the development of molecular marker innovation (Lixia *et al*., 2020). Mayes *et al.* (1997) pioneered the use of genetic markers in the oil palm industry by performing Restriction Fragment Length Polymorphism (RFLP)-based genetic mapping. Genetic diversity studies (Bakoume *et al*., 2014), linkage mapping studies (Singh *et al*., 2009), association mapping studies (Pootakham *et al*., 2015), and linkage map construction studies in oil palm all used DNA markers like Rapid Amplified Polymorphic DNA (RAPD) (Sathish and Mohankumar, 2007), Amplified Fragment Length Polymorphism (AFLP) (Rance *et al*., 2001), and Simple Sequence Repeats (SSR) (Billotte *et al*., 2005).

Genomic DNA typification-based molecular assays have been acknowledged as effective techniques for assessing genetic diversity and creating molecular marker-based genetic mapping investigations (Liu *et al*., 2018). Amplification of the Simple Sequence Repeat (SSR) is a common molecular method because of its benefits including hypervariability, broad genomic distribution, co-dominant inheritance, polymorphism, and chromosome-specific locations (Osorio-Guarín *et al*., 2020). In addition to being more practical to use in comparison to other DNA typification assays, PCR-based SSRs are regarded as being the most promising markers to comprehend population genetics (Asadi *et al*., 2019) and identifying probable parental genotypes in the oil palm. Using molecular markers, only some attempts have been done in India to increase the genetic diversity of local germplasm (Satish and Mohankumar, 2007).

Additionally, the data present enough proof to distinguish each variation between the three fruit forms namely, *dura, pisifera* and *tenera* individually (Kalyanababu *et al*., 2017), besides the parent *dura* and *pisifera* jointly. DNA-based polymorphism assays were conducted; this is the initial instance to assess the degree of variation in oil palm types (Satish and Mohankumar, 2007). There have not been any findings, though, on how genetically diverse the indigenous oil palm germplasm. In order to use SSR markers in marker-assisted selection, genetic diversity, and mapping it is essential to discover more polymorphic SSRs (Xiao *et al*., 2014).

Because of this, we used high yield related 34 SSR markers in the current work to identify polymorphic SSRs for genetic diversity investigations in certain genotypes. As a result, the use of SSR markers in oil palm breeding could be beneficial in differentiating high yielding hybrids at early stage, so that best performing hybrids could be multiplied and supplied to farmers in our country by reducing their time and expenses before going for commercial planting at field level and may increase their income.

The objectives of the current study were to identify polymorphic SSR markers of oil palm germplasm and use those markers to analyse the genetic diversity of the chosen germplasm.

MATERIALS AND METHODS

DNA Extraction from Plant Materials

Indian Institute of Oil Palm Research, Palode, Kerala made 10 cross combinations which were collected and planted at Horticultural Research Station, Vijayrai, Eluru with three replications (6 palms per plot) in total were employed and *Table 1* contains information about each hybrid. Genomic DNA was extracted from an unopened oil palm spear leaflet stored in a field gene bank (Gawel and Jarret, 1991) using liquid nitrogen by following standard protocol of CTAB method with few modifications (Babu *et al*., 2019).

TABLE 1. LIST OF TEN HYBRIDS APPLIED IN THE STUDY AND THEIR CROSS COMBINATION

No.	Hybrids	Cross combination
1	NRCOP-1	78 x 435
2	NRCOP-2	90 x 577
3	NRCOP 3	158 x 116
4	NRCOP-4	131 x 435
5	NRCOP-5	5 x 577
6	NRCOP-6	173 x 435
7	NRCOP-7	183 x 577
8	NRCOP-8	70 x 577
9	NRCOP-9	28 x 435
10	NRCOP-10	345 x 577

SSR Amplification using PCR

A total of 34 SSR markers were used to amplify DNA of 10 oil palm hybrids (*Table 2*). The primers forward and reverse sequences came from Billotte *et al*. (2005). A reaction mixture (20 µL) of 10 X buffer (Hi media), 2 µL L of 15 mm $MgCl_2$, 0.2 mM forward and reverse primers, 2 µL of 2 mM dNTPs, 0.2 µL of 1 U Taq DNA polymerase (Invitrogen, USA), and 25-50 ng template DNA was prepared to conduct the thermal reaction. Thermocycler (Biorad, USA) with a programmed initial denaturation of 3 min at 95°C, 35 cycles of 30 s at 95°C, 30 s at 50°C

temperature of annealing, extension of 1 min at 72°C, final extension of 10 min at 72°C, followed by a hold at 4°C was used to carry out the PCR amplifications. On an agarose gel with a 3% super fine resolution (SFR), the PCR results were fractionated. The electrophoresis was conducted at ambient temperature for 3 hr at 100 volts. Agarose gel was manually scored depending on the size of the 100 bp ladder after being viewed using the Bioimaging System (Bio Rad) and stained with ethidium bromide. Power Marker 3.0 was used for the UPGMA analysis and statistical analysis of polymorphism for the generation of dendrograms. The Power Marker V3.0 software was utilised to compute PIC, heterozygosity, gene diversity, allele frequency, and inbreeding co-efficient (Liu and Muse, 2005).

RESULTS AND DISCUSSION

In order to investigate variability in our set of evaluated samples, highly polymorphic SSRs reported by Billotte *et al.* (2005) were sufficient. Using 34 SSR markers, the oil palm's hybrids genetic DNA (*Table 3*) was amplified and produced bands that could be scored. The 34 SSRs were uniformly distributed across the oil palm chromosomes. Out of the 34 primers, 22 loci (64.7% of the total) were discovered to be polymorphic, detecting 58 alleles on average with for each locus, while 12 SSR loci (35.2%) were monomorphic.

With polymorphic primers, the oil palm hybrids produced between 2 and 4 alleles. This figure is less than, the 13.1 alleles per locus discovered by Bakoume *et al.* (2015). Evidently, the quantity of polymorphic alleles per locus depends on the number of investigated samples and sample origin. The modest amount of materials employed in this study *i.e.*, a few number of parents in a breeding programme and a small number of descendants for their intercrosses likely contributed to the low number of alleles. Allele variability tends to decline and the population is impacted by this hybridisation and selection. The SSR loci *mEgCIR0408*, *mEgCIR3808*, *mEgCIR3382*, *mEgCIR3705*, *mEgCIR2347*, and *SMG00227* with 2 alleles were determined to have the most alleles. The SSR locus *SMG00026* registered 4 alleles. *Figure 1* and *2* showed the band pattern suggesting SSR loci polymorphism of *mEgCIR0350* and *mEgCIR0074*, respectively.

The results observed are good , as the markers employed have a 95% efficiency in differentialy unique palms. The capacity of the polymorphic primers to distinguish between different hybrids was demonstrated by the PIC values, which varied between 0.14 to 0.60 for all 10 oil palm hybrids, with an average value of 0.22. It is comparable to PIC values from previous oil palm studies that used markers from comparable sources and were tested on six unique crossings (Budiman *et al*., 2019), also on plants with similar ancestors (Arias *et al*., 2014). The marker's level of informativeness increases with its PIC.

Out of 34 primers, primer *SMG00026* had the highest PIC value in our samples, which was 0.60, is considered to be moderately informative, best in screening oil palm genotypes loci, capable of discriminating between genotypes which can be used for genetic fingerprinting in breeding programmes and the *mEgCIR3808* primer exhibited the lowest PIC value (0.14) (Zaki *et al*., 2012).

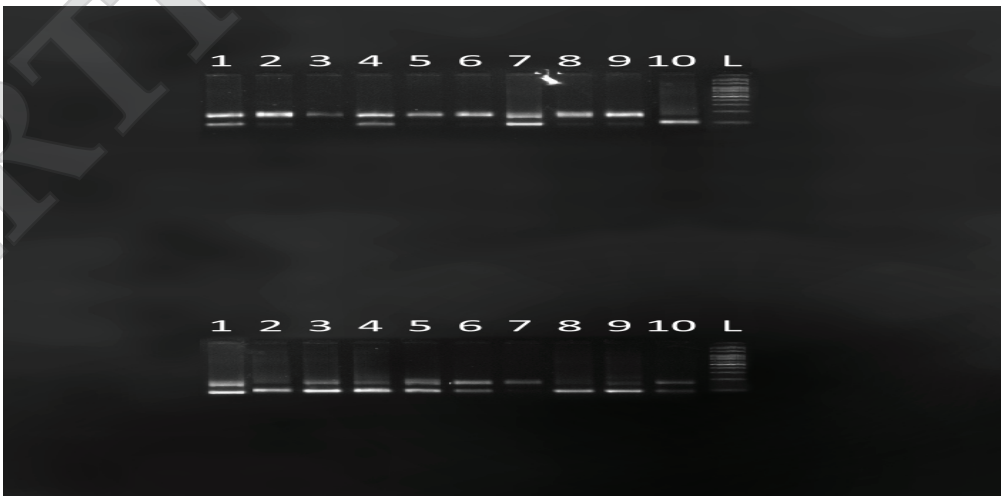

Figure 1. The mEgCIR3350 *loci's SSR banding profile among the 10 oil palm hybrids, where L = 100 bp DNA ladder lane (1-10) oil palm hybrids (please refer to* Table 1 *for label).*

TABLE 2. SSR MARKERS USED TO DETERMINE GENETIC DIVERSITY IN TEN OIL PALM HYBRIDS

No.	Locus	Repeat motif	Primer sequence (5′-3′)		Annealing temperature (°C)	Link age group location[b]
			Forward	Reverse		
1	*SMG00217*	NA	GGTGGAATTAGTTGCTCAGAAG	CGCAGATGTTTCATAATCGAG	52	NA
2	*SPSC00185*	NA	AAGGAGAACTACCACGCGAA	AATTATGTGCGGTTGTTGAGC	52	NA
3	*SMG00227*	NA	TCTATTTCATCCAAATCTGCAC	TTTTCAGTTAGCGCATAGCAT	52	NA
4	*SEG00125*	NA	TACCCTTTTCCCTCCCTCCATA	CATCATCTCCGTTGCCAGTATT	58	NA
5	*SMG00026*	NA	CCCCTACCTTTCTTTCTTACC	ATGAGCAGGAGTTGGAATAG	52	NA
6	*mEgCIR0059*	(GA)15	TGCAGGGGATGCTTTTATT	CCCTTAATTCCTGCCTTATT	52	4
7	*mEgCIR0074*	(GT)7ga	AAGAGAGTTTCACGGTCATA	GACCTCTGCTTGTGTTTCTA	52	4
8	*mEgCIR0195*	(GA)21	CCCACCACCCCTAGCTTCTC	ACCCCGGTCCAAATAAAATC	58	6
9	*mEgCIR0246*	(GA)19	GGTAAGAGATGAGATGGGTTGTC	AGGAATTAAGGGTTGTAGGTGAA	52	8
10	*mEgCIR0353*	(GT)11 (GA)15	AGAGAGAGAGAGTGCGTATG	GTCCCTGTGGCTGCTGTTTC	52	16
11	*mEgCIR0874*	(CA)11 (GA)18	TCCAGTTGTCGAGTTGTAGT	ATTATGGGGTTATGCTTTCA	52	1
12	*mEgCIR0894*	(GA)18	TGCTTCTTGTCCTTGATACA	CCACGTCTACGAAATGATAA	52	7
13	*mEgCIR0555*	(GA)18	TACCATCACTGACCAATAAC	GTCTTTCTTGCTAACTACAC	52	8
14	*mEgCIR0878*	(GA)22	CAAAGCAACAAAGCTAGTTAGTA	CAAGCAACCTCCATTTAGAT	52	11
15	*mEgCIR0773*	NA	GCAAAATTCAAAGAAAACTTA	CTGACAGTGCAGAAAATGTTATAGT	52	NA
16	*mEgCIR2347*	(GA)15	ATTTTGCATGTGTTGAGAGC	CAACCAATTGCACCCTAAAG	52	8
17	*mEgCIR2291*	(GA)11	ATGCCCGGATCTTTGTGTAG	TTGTTCGTGTTAATCAAGTGTATG	52	7
18	*mEgCIR3300*	(GA)19	CATGCACGTAAAGAAAGTGT	CCAAATGCACCCTAAGA	52	7

TABLE 2. SSR MARKERS USED TO DETERMINE GENETIC DIVERSITY IN TEN OIL PALM HYBRIDS (continued)

No.	Locus	Repeat motif	Primer sequence (5′-3′)		Annealing temperature (°C)	Link age group location[b]
			Forward	Reverse		
19	*mEgCIR3382*	(GA)24	TGTAGGTGGTGGTTAGG	TGTCAGACCCACCATTA	52	11
20	*mEgCIR3350*	(GA)16	GGAATAAAGCTTCCAACAAC	CCTGGTCGTTTGGTAGAGA	52	14
21	*mEgCIR3683*	(GA)15	GTAGCTTGAACCTGAAA	AGAACCACCGGAGTTAC	52	2
22	*mEgCIR3555*	(GA)18	CATCAGAGCCTTCAAACTAC	AGCCTGAATTGCCTCTC	52	13
23	*mEgCIR3546*	(GA)15	GCCTATCCCCTGAACTATCT	TGCACATACCAGCAACAGAG	52	14
24	*mEgCIR3569*	(GA)25	AAGGCTTGGAGTTGAGGTAT	CACCATTGCATCATTATTCC	52	14
25	*mEgCIR3705*	(GA)16	CCACCATGCAGAATAGTAAG	GTGTGCCCTCAAAAAGTATG	52	4
26	*mEgCIR3775*	(GA)18	TCTTGATATTAAAAGGTCAGGAGAA	CGTTCCCTTTTTCCATAGAT	52	4
27	*mEgCIR3711*	(GA)17	GTCTCATGTGGCTACCTCTC	AGGCTCCCTGCTTTTAAGT	52	8
28	*mEgCIR3732*	(GA)19	ATTTTATTTGGCTTGGTATA	ACTTTTCTATCTAATTCTTGAAGAT	52	8
29	*mEgCIR3808*	(GA)22	CCGCTAACTTGGTATAC	ATTTCCAGCAGCTAATC	52	8
30	*mEgCIR3557*	NA	CATTGCCATTCCCTTCAAGT	TCCCCTCTGTTCACTCAAGC	52	NA
31	*mEgCIR3260*	NA	GGGCAAGTCATGTTTCCTACA	TAAGGGCGAGGTATTTCTGC	52	NA
32	*mEgCIR0408*	NA	AGCGCAGTTGCTCGGTATAA	CCCTGCAGTGTCCCTCTTTA	52	NA
33	*mEgCIR3886*	NA	TTCTAGGGTCTATCAAAGTCATAAG	AGCCACCACCACCATCTACT	52	NA
34	*mEgCIR0905*	NA	CACCACATGAAGCAAGCAGT	CCTACCACAACCCCAGTCTC	52	NA

Figure 2. The SSR banding profile of the 10 oil palm hybrids of mEgCIR0074 *loci, where L = 100 bp DNA ladder lane (1-10) oil palm hybrids (please refer to* Table 1 *for label).*

Expected heterozygosity (H_e), often known as gene diversity, the values ranged from 0.15 (*mEgCIR0408*) to 0.66 (*SMG00026*), with a mean of 0.286. The observed heterozygosity (H_o), also known as the heterozygosity range (*mEgCIR0074*, *mEgCIR0353*, *mEgCIR0555*, *mEgCIR3886*, *mEgCIR0905*), was seen with an average of 0.10 (*SEG00125*) to 1.00 (*mEgCIR0074*, *mEgCIR3886*, *mEgCIR0905*). But the H_o determined in our investigation, was higher than the projected H_e, demonstrating greater diversity and no heterozygosity deficiency and could be justified by the advanced breeding population (Upit *et al*., 2021) and the stated H_o value is comparable to prior publication (Arias *et al*., 2015).

The major allele at the locus with the highest frequency was *mEgCIR0408* (140 bp allele), which was followed by *mEgCIR3808* (190 bp allele), which was at 88%, *SMG00227* (240 bp allele), which was at 83%, and *mEgCIR3382* (140 bp allele), which was at 83%. This study aids in the identification of small population based on the amount of acquired alleles (Romeo *et al*., 2003). Different populations had different allele frequencies at each locus, and some alleles were exclusive to one or a few populations. The most frequent factors influencing allele frequencies are genetic drift and reproductive isolation which is common in out crossing plants like oil palm, where random mating is expected (Bakoume *et al*., 2009).

Given that genetic diversity functions in various strategies to determine population relationships, the relatively low average gene diversity of all the hybrids (28%), which indicates that the genotypes had low degrees of polymorphism and the lack of correlation between variations assessed by molecular markers should not be regarded as a constraint.

UPGMA analysis generated dendrogram divided the 10 oil palm hybrids into two major categories. NRCOP 5 established a unique cluster that was relatively characteristic of their heterotic group and two further sub clusters were created from cluster II which includes NRCOP-2, NRCOP-1, NRCOP-4, NRCOP-3 and NRCOP-6 in one sub cluster whereas NRCOP-7, NRCOP-8, NRCOP-9 and NRCOP-10 in another sub cluster (*Figure 3*). Both clusters include parents chosen for their high combining ability and production potential.

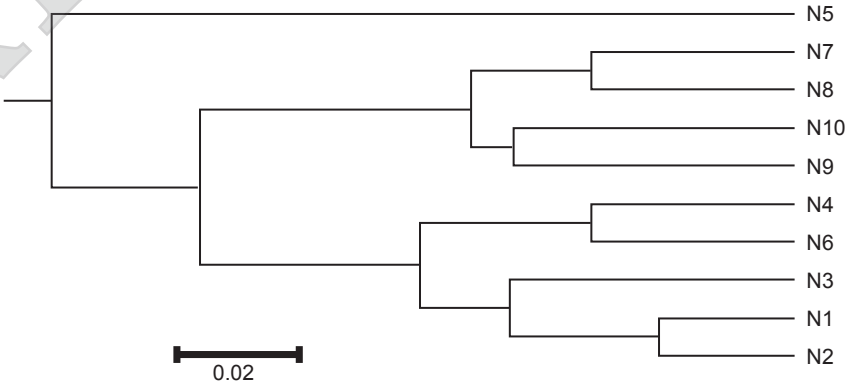

Figure 3. The rectangular dendrogram of 10 oil palm hybrids as determined by UPGMA analysis using the software POWER MARKER. (N1-NRCOP-1, N2-NRCOP-2, N3-NRCOP-3, N4-NRCOP-4, N5-NRCOP-5, N6-NRCOP-6, N7-NRCOP-7, N8-NRCOP-8, N9-NRCOP-9, and N10-NRCOP-10)

TABLE 3. PARAMETERS FOR 34 SSR LOCI GENETIC ANALYSIS OVER THE TEN OIL PALM HYBRIDS

No.	Locus	Major allele frequency	Allele No.	Gene diversity	Heterozygosity	PIC
1	*SMG00217*	0.5714	2.0000	0.4898	0.8571	0.3698
2	*SMG00026*	0.4286	4.0000	0.6633	0.8571	0.6003
3	*mEgCIR0074*	0.5000	2.0000	0.5000	1.0000	0.3750
4	*mEgCIR0195*	0.5556	2.0000	0.4938	0.6667	0.3719
5	*mEgCIR0246*	0.5556	2.0000	0.4938	0.8889	0.3719
6	*SPSC00185*	1.0000	1.0000	0.0000	0.0000	0.0000
7	*SMG00227*	0.8333	2.0000	0.2778	0.3333	0.2392
8	*SEG00125*	0.6500	2.0000	0.4550	0.1000	0.3515
9	*mEgCIR0059*	1.0000	1.0000	0.0000	0.0000	0.0000
10	*mEgCIR0353*	0.5000	2.0000	0.5000	1.0000	0.3750
11	*mEgCIR0874*	1.0000	1.0000	0.0000	0.0000	0.0000
12	*mEgCIR0894*	0.5833	2.0000	0.4861	0.1667	0.3680
13	*mEgCIR0555*	0.5000	2.0000	0.5000	1.0000	0.3750
14	*mEgCIR0878*	0.5556	2.0000	0.4938	0.8889	0.3719
15	*mEgCIR0773*	0.5556	2.0000	0.4938	0.4444	0.3719
16	*mEgCIR2347*	0.7500	2.0000	0.3750	0.0000	0.3047
17	*mEgCIR2291*	1.0000	1.0000	0.0000	0.0000	0.0000
18	*mEgCIR3300*	1.0000	1.0000	0.0000	0.0000	0.0000
19	*mEgCIR3382*	0.8333	2.0000	0.2778	0.3333	0.2392
20	*mEgCIR3350*	0.5000	2.0000	0.5000	0.1111	0.3750
21	*mEgCIR3683*	1.0000	1.0000	0.0000	0.0000	0.0000
22	*mEgCIR3555*	1.0000	1.0000	0.0000	0.0000	0.0000
23	*mEgCIR3546*	0.6000	2.0000	0.4800	0.8000	0.3648
24	*mEgCIR3569*	1.0000	1.0000	0.0000	0.0000	0.0000
25	*mEgCIR3705*	0.7000	2.0000	0.4200	0.2000	0.3318
26	*mEgCIR3775*	1.0000	1.0000	0.0000	0.0000	0.0000
27	*mEgCIR3711*	1.0000	1.0000	0.0000	0.0000	0.0000
28	*mEgCIR3732*	1.0000	1.0000	0.0000	0.0000	0.0000
29	*mEgCIR3808*	0.8889	2.0000	0.1975	0.0000	0.1780
30	*mEgCIR3557*	1.0000	1.0000	0.0000	0.0000	0.0000
31	*mEgCIR3260*	0.6000	2.0000	0.4800	0.8000	0.3648
32	*mEgCIR0408*	0.9167	2.0000	0.1528	0.1667	0.1411
33	*mEgCIR3886*	0.5000	2.0000	0.5000	1.0000	0.3750
34	*mEgCIR0905*	0.5000	2.0000	0.5000	1.0000	0.3750
Mean	-	0.7523	1.7059	0.2862	0.3710	0.2233

According to UPGMA results, genetic similarity is typically not correlated with geographic distance but rather is consistent with geographic dispersal. Combining morphological and agronomic data with similarity groups can be used to select and cross superior plants, maximising the expression of "interpopulation heterosis" and enabling more effective utilisation of the genetic diversity already present.

Jaccard's similarity co-efficient between genotypes ranged from 3.85 to 32% (*Table 4*) with an average of 18%, suggesting a moderate genetic relationship within oil palm hybrids. The highest similarity coefficient value of 32% was observed between hybrids of NRCOP-5 and NRCOP-9 and the least similarity of 3.85% was noticed between hybrids of NRCOP-1 and NRCOP-2. In nine D-P cross combinations, Diana *et al*. (2013) showed similarity coefficients ranging from 0.015% to 0.039% (Zulkifli *et al*., 2012), with a mean genetic distance of 0.251 among oil palm germplasm originating from eleven different African states. Our findings, however, indicated that high similarity values were also seen among oil palm hybrids, indicating that there was either no inter-crossing between them or there was significant inbreeding.

The anticipated heterozygosity of 0.280 was comparable to 0.200, which Diana *et al*. (2013) reported as a high value, and 0.390, which Okoye *et al*. (2016a) recorded for African oil palm. The findings of the genetic diversity analysis conducted to study H_e and H_o published among the utilising genotypes of oil palm using microsatellites are very similar. H_e and H_o measurements collected from Nigeria and Malaysia ranged from 0.167 to 0.778 and 0.153 to 0.643, respectively. With NIFOR oil palm germplasm, H_e of 0.700 and H_o of 0.690 were reported by Okoye *et al*. (2016b). Twelve of the 34 SSRs were monomorphic, and 22 were polymorphic. The gel image depicts the polymorphic SSRs banding patterns, which are seen in *Figure 1* and *2*.

There were 18 microsatellites according to the PIC value that were more numerous, higher than usual due to their comparatively high levels of polymorphism: SSR loci *SMG00026*, *mEgCIR0074*, *mEgCIR0353*, *mEgCIR3350*, *mEgCIR0555*, *mEgCIR3886*, and *mEgCIR0905* stood out among the others. Eighteen SSR loci in all fell into the 0.3-0.6 PIC range, with an average rate of 0.3, whereas 4 loci fell into the 0.14-0.23 PIC range, with 0.18 as the mean. A marker is considered more informative if its PIC is higher.

Out of 22 primers, *SMG00026* has the highest PIC value of 0.6, followed by *mEgCIR0074*, *mEgCIR0195*, *mEgCIR0246*, *mEgCIR0353*, *mEgCIR0555*, *mEgCIR0878*, *mEgCIR0773*, *mEgCIR3350*, *mEgCIR3886* and *mEgCIR0905*. The highest PIC value in commercial oil palm material

TABLE 4. JACCARD'S SIMILARITY COEFFICIENT VALUES BASED ON 34 SSR PRIMERS SEQUENCE DATA OF OIL PALM HYBRIDS

Hybrids	NRCOP-1	NRCOP-10	NRCOP-2	NRCOP-3	NRCOP-4	NRCOP-5	NRCOP-6	NRCOP-7	NRCOP-8	NRCOP-9
NRCOP-1	0.0000	0.1522	0.0385	0.0652	0.1250	0.2391	0.1250	0.2000	0.1731	0.1923
NRCOP-10		0.0000	0.1200	0.1591	0.1458	0.2083	0.1800	0.0625	0.1042	0.0800
NRCOP-2			0.0000	0.0962	0.0556	0.1731	0.0769	0.1800	0.1731	0.1852
NRCOP-3				0.0000	0.1087	0.1739	0.1458	0.1522	0.1667	0.2000
NRCOP-4					0.0000	0.1923	0.0577	0.1667	0.1538	0.1731
NRCOP-5						0.0000	0.1875	0.1667	0.2400	0.3200
NRCOP-6							0.0000	0.1600	0.1400	0.2115
NRCOP-7								0.0000	0.0577	0.0926
NRCOP-8									0.0000	0.1071
NRCOP-9										0.0000

was stated by Arias *et al.* (2010) at 0.822 and Okoye *et al.* (2016a) attained an incredibly elevated mean proportion of polymorphism (85.09%).

The oil palm breeding programme may have involved crossing ten hybrids into two major groups, and the dendrogram suggests that these geographical regions may have sprung from a single genetic background or from genetic origins that are comparable. Similar findings were made in a study by Bakoume *et al.* (2009) using SSR markers on a natural population of oil palms. Since the female lines, namely *dura*, originated from four palms and that oil palm is very heterozygous, it is possible that the same genes contributed to the clustering pattern.

Nevertheless, we offer our SSR results that can be combined with additional markers or used independently for further validation by other authors in search of the most effective identification formula. SSRs are more effective as a quality check to establish the accuracy of the pedigree and authenticity. However, screening more SSRs could make this method better, and more accessions should be developed to create new strains for confirming the findings.

CONCLUSION

Vegetable oil from the golden palm is valuable, and polymorphic markers must be found to identify significant QTLs in the germplasm for use in a breeding program to increase oil yield. Seven highly polymorphic SSR markers (*SMG00026*, *mEgCIR0074*, *mEgCIR0353*, *mEgCIR3350*, *mEgCIR0555*, *mEgCIR3886*, and *mEgCIR0905*) were discovered in this study determined by the following criteria: Polymorphic alleles of ≥2, gene diversity of ≥48, and PIC value of > = 37.

In conclusion, the novel information presented here shows that the developed hybrids are a valuable resource to support additional research to create countrywide breeding plans and conservation initiatives for this vital crop in India. These polymorphic primers and QTL mapping studies can be used effectively because they make it easier to choose promising varieties at an early stage, which helps to shorten the oil palm's lengthy breeding cycle. Additionally, because they showed extremely high polymorphism over other loci, they may also help to modernise the plant breeding programme for oil palm.

ACKNOWLEDGEMENT

The first author is grateful to Dr. Y.S.R. Horticultural University in Venkatramannagudem and the Directors of the ICAR-IIOPR in Pedavegi, Andhra Pradesh for providing the tools needed for me to conduct my study as part of my M.Sc. thesis.

REFERENCES

Arias, D; Gonzalez, M; Prada, F; Ayala-Diaz, I; Montoya, C and Daza, E (2015). Genetic and phenotypic diversity of natural American oil palm (*Elaeis oleifera* (H.B.K.) Cortes) accessions. *Tree Genet. Genomes*, *11(6)*: 122.

Arias, D M; Montoya, C and Romero, H M (2010). Preliminary results on the molecular characterization of oil palm using microsatellites markers. *Palmas, 31(3)*: 35-45.

Arias, D; Ochoa, I; Castro F and Romero, H (2014.) Molecular characterization of oil palm *Elaeis guineensis* Jacq. of different origins for their utilization in breeding programmes. *Plant Genet. Resour., 12*: 341-348.

Asadi, A; Ebrahimi, A; Rashidi-Monfared, S and Basiri, M (2019). Comprehensive functional analysis and mapping of SSR markers in the chickpea genome (*Cicer arietinum* L.). *Comput. Biol. Chem., 84*: 107169.

Babu, BK; Mathur, R K; Naveen, P K; Ramajayam, D; Ravichandran, G; Venu, M V B and Sparjan, S B (2017). Development, identification and validation of CAPS marker for SHELL trait which governs *dura*, *pisifera* and *tenera* fruit forms in oil palm (*Elaeis guineensis* Jacq.). *PLoS ONE., 12(2).*

Bakoume, C; Wickneswari, R; Rajanaidu, N; Kushairi, A and Billotte, N (2009). Screening natural oil palm (*Elaeis guineensis* Jacq.) populations using SSR markers. *International Society for Oil Palm Breeders Seminar*. Kuala Lumpur, Malaysia. p. 1-10.

Bakoume, C; Wickneswari, R; Siju, S; Rajanaidu, N; Kushairi, A and Billotte, N (2015). Genetic diversity of the world's largest oil palm (*Elaeis guineensis* Jacq.) field gene bank accessions using microsatellite markers. *Genet. Resour. Crop. Evol*., *62*: 349-360.

Basiron, Y (2000). Techno-economic aspects of research and development in the Malaysian oil palm industry. *Advances in Oil Palm Research* (Basiron, Y; Jalani, B S and Chan, K W eds.). MPOB, Bangi. p. 1-18.

Billotte, N; Marseillac, N; Risterucci, A M; Adon, B; Brottier, P; Baurens, F C and Singh, R (2005). Microsatellite based high density linkage map in oil palm (*Elaeis guineensis* Jacq.). *Theor. Appl. Genet., 110(4)*: 754-765.

Budiman, L F; Apriyanto, A; Pancoro, A and Sudarsono, S (2019). Genetic diversity analysis of *Tenera* × *Tenera* and *Tenera* × *Pisifera* crosses and D self of oil palm (*Elaeis guineensis*) parental populations originating from Cameroon. *Biodiversitas, 20*: 937-949.

Cadena, T; Prada, F; Perea, A and Romero, H M (2013). Lipase activity, mesocarp oil content and iodine values in oil palm fruits of *Elaeis guineensis, Elaeis oleifera* and the interspecific hybrid O × G (*E. oleifera* × *E. guineensis*). *J. Sci. Food Agric*., *93(3)*: 674-680.

Diana, A; Maria, G; Fausto, P; Edwin, R and Hernan, R (2013). Morpho-agronomic and molecular characterisation of oil palm (*Elaeis guineensis* Jacq.) material from Angola. *Tree Genet., 9*: 1283-1294.

Gawel, N and Jarret, R (1991). A modified CTAB DNA extraction protocol for *Musa* and *Ipomea*. *Plant Mol. Biol., 9*: 262-266.

Hartley, C W S (1988). *The Oil Palm*. 2nd edition. Longman, London. 958 pp.

Kalyanababu, B; Mary Rani, K L; Sarika Sahu; Mathur, R K; Naveen Kumar, P; Ravichandran, G; Anitha, P and Bhagya, H P (2019). Development and validation of whole genome-wide and genic microsatellite markers in oil palm (*Elaeis guineensis* Jacq.): First microsatellite database (OpSatdb). *Sci. Rep*., *9*: 1899.

Liu, K and Muse, S V (2005). Powermarker: Integrated analysis environment for genetic marker data. *Bioinform*., *21(9)*: 2128-2129.

Liu, Z; Shao, W; Shen, Y; Ji, M; Chen, W; Ye, Y and Shen, Y (2018). Characterization of new microsatellite markers based on the transcriptome sequencing of *Clematis finetiana*. *Hereditas., 155*: 23.

Lixia, Z; Rajesh, Y; Zhihao, Z; Longfei, J and Hongxing, C (2020). Development of SSR markers based on transcriptome data and association mapping analysis for fruit shell thickness associated traits in oil palm (*Elaeis guineensis* Jacq.). *Biotech*., *10(6)*: 280.

Mayes, S; Jack, P L; Marshall, D F and Corley, R H V (1997). Construction of a RFLP genetic linkage map for oil palm (*Elaeis guineensis* Jacq.). *Genome, 40(1)*: 116-122.

Mohammadi, S A and Prasanna, B M (2003). Analysis of genetic diversity in crop plant salient statistical tools and considerations: Review and interpretation. *Crop Sci*., *43*: 1235-1248.

Mozzon, M; Pacetti, D; Lucci, P; Balzano, M and Frega, N G (2013). Crude palm oil from interspecific hybrid *Elaeis oleifera* × *Elaeis guineensis*: Fatty acid region distribution and molecular species of glycerides. *Food Chem*., *141(1)*: 245-252.

Okoye, N M; Bakoume, C; Uguru, I M; Singh, R and Okwuagwu, O K (2016a). Genetic relationships between elite oil palms from Nigeria and selected breeding and germplasm materials from Malaysia via simple sequence repeat (SSR) markers. *J. Agric. Sci*., *8(2)*: 159.

Okoye, N M; Michael, U I; Claude, B; Rajinder, S and Christy, O O (2016b). Assessment of genetic diversity of NIFOR oil palm main breeding parent genotypes using microsatellite markers. *Am. J. Plant Sci., 7:* 218-237.

Osorio-Guarín, J A; Berdugo-Cely, J A and Coronado-Silva, R A (2020). Genome-wide association study reveals novel candidate genes associated with productivity and disease resistance to *Moniliophthora* spp. in Cacao (*Theobroma cacao* L.) *G3 (Bethesda) 2020 May 4, 10(5)*: 1713-1725.

Pootakham, W; Jomchai, N; Ruangareerate, P; Shearman, J R; Sonthirod, C; Sangsrakru, D; Tragoonrung, S and Tangphatsornruang, S (2015). Genome-wide SNP discovery and identification of QTL associated with agronomic traits in oil palm using genotyping-by-sequencing (GBS). *Genomics*, *105*: 288-295.

Rance, K A; Mayes, S; Price, Z; Jack, P L and Corley, R H V (2001). Quantitative trait loci for yield components in oil palm (*Elaeis guineensis* Jacq). *Theor. Appl. Genet*., *103*:1302-1310.

Rival, A; Beule, T; Barre, P; Hamon, S; Duval, Y and Noirot, M (1997). Comparative flow cytometric estimation of nuclear DNA content in oil palm (*Elaeis guineensis*) tissue-culture and seedling derived plants. *Plant Cell Rep., 16*: 884-887.

Romero, C; Pedryc, A; Munoz, V; Llacer, G and Badenes, M L (2003). Genetic diversity of different apricot geographical groups determined by SSR markers. *Genome, 46(2)*: 244-252.

Sathish, D K and Mohankumar, C (2007). RAPD markers for identifying oil palm (*Elaeis guineensis* Jacq.) parental varieties (*dura* and *pisifera*) and the hybrid *tenera*. *Indian J. Biotechnol., 6*: 354-358.

Singh, R; Noorhariza, M Z; Ngoot, C T and Rozana, R (2009). Mapping quantitative trait loci (QTLs) for fatty acid composition in an interspecific cross of oil palm. *BMC Plant Biol*., *9*: 114.

Singh, R; Ong-Abdullah, M; Low, E T; Manaf, M A; Rosli, R and Nookiah, R (2013). Oil palm genome sequence reveals divergence of interfertile species in Old and New worlds. *Nature, 500*: 335-339.

Upit, S; Javier, H; Pratiwi, E; Nurcahyono, I; Fahmi, W; Baitha, S; Enrique, R; Zulhermana, S and Dwi, A (2021). Analysis of genetic diversity and discrimination of oil palm DxP populations based on the origins of *pisifera* elite parents. *Breed. Sci., 71*: 134-143.

Xiao, Y; Zhou, L X; Xia, W and Mason, A S (2014). Exploiting transcriptome data for the development and characterization of gene-based SSR markers related to cold tolerance in oil palm (*Elaeis guineensis*).*BMC Plant Biol., 14*: 384.

Yarra, R; Jin, L; Zhao, Z and Cao, H (2019). Progress in tissue and genetic transformation of oil palm: An overview. *Int. J. Mol. Sci., 20*: 5353.

Zaki, N M; Singh, R; Rosli, R and Ismail, I (2012). *Elaeis oleifera* genomic-SSR markers: Exploitation in oil palm germplasm diversity and cross-amplification in Arecaceae. *Int. J. Mol. Sci., 13*: 4069-4088.

Zhao, Y; Zhang, J; Zhang, Z and Xie, W (2019). *Elymus nutans* genes for seed shattering and candidate gene-derived EST-SSR markers for germplasm evaluation. *BMC Plant Biol., 19(1)*: 102.

Zhou, L X; Xiao, Y; Xia, W and Yang, Y D (2015). Analysis of genetic diversity and population structure of oil palm (*Elaeis guineensis*) from China and Malaysia based on species specific simple sequence repeat markers. *Genet. Mol. Res., 14(4)*: 16247-16254.

Zulkifli, Y; Maizura, I and Rajinder, S (2012). Evaluation of oil palm germplasm (*Elaeis guineensi*s Jacq.) populations using EST-SSR. *J. Oil Palm Res., 24*: 1368-1377.