

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

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ABSTRACT

For genetic mapping research in a crop like oil palm, polymorphic simple sequence repeat (SSR) 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.

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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: Coccoineae) 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

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of the oil palm (Zhou *et al.*, 2015). To quantify the genetic variability of germplasm (Zhao *et al.*, 2019) morphological, biochemical and molecular approaches can be used (Mohammadi & Prasanna, 2003).

Oil palm molecular research has advanced somewhat as a result of the development of molecular marker innovation (Zhou *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 & 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 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 (Sathish & Mohankumar, 2007).

Additionally, the data present enough proof to distinguish each variation between the three fruit forms namely, *dura*, *pisifera* and *tenera* individually (Babu *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 (Sathish & Mohankumar, 2007). There have not been any findings, though, on how genetically diverse the indigenous oil palm germplasm is. 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 (six 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 & Jarret, 1991) using liquid nitrogen by following standard protocol of CTAB method with few modifications (Babu *et al.*, 2019).

TABLE 1. LIST OF 10 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 μ L) of 10 X buffer (Hi media), 2 μ L of 15 mM MgCl₂, 0.2 mM forward and reverse primers, 2 μ L of 2 mM dNTPs, 0.2 μ L 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 & 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 differentially unique palms. The capacity of the polymorphic primers to distinguish between different hybrids was demonstrated by the PIC values, which varied between 0.14-0.60 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).

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

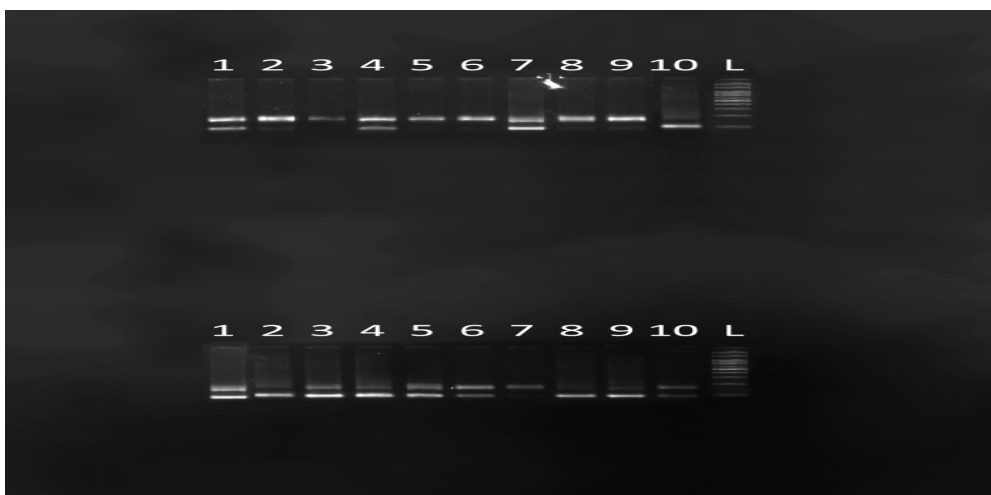


Figure 1. The *mEgCIR3350* loci's SSR banding profile among the 10 oil palm hybrids, where L = 100 bp DNA ladder lane (1-10) oil palm hybrids (please refer to Table 1 for label).

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	GGTGGAAATGATGTGCTCAGAAG	CGCAGATGTTTCATAATCGAG	52	NA
2	SPSC00185	NA	AAGGAGAACTACACCGGAA	AATTATGTGCGGTGTGTGAGC	52	NA
3	SMG00227	NA	TCTATTTTCATCCAAATCTGCAC	TTTTTCAGTTAGCGCATAGCAT	52	NA
4	SEG00125	NA	TACCCCTTTCCCTCCCTCCATA	CATCATCTCCGTTGCCAGTATT	58	NA
5	SMG00026	NA	CCCCTACCTTCTTCTTACC	ATGAGCAGGAGTTGGAATAG	52	NA
6	mE ₈ CIR0059	(GA)15	TGCAGGGATGCTTTTATT	CCCTTAATTCCTGCCITATT	52	4
7	mE ₈ CIR0074	(GT)7ga	AAGAGAGTTTCACGGTCATA	GACCTCTGCTTGTGTTCTA	52	4
8	mE ₈ CIR0195	(GA)21	CCCACCACCCCTAGCTTCTC	ACCCCGGTCCAAATAAAATC	58	6
9	mE ₈ CIR0246	(GA)19	GGTAAGAGATGAGATGGGTTGTC	AGGAATTAAGGGTTGTAGGTGAA	52	8
10	mE ₈ CIR0353	(GT)11 (GA)15	AGAGAGAGAGAGTGGGTATG	GTCCCTGTGGCTGCTGTTTC	52	16
11	mE ₈ CIR0874	(CA)11 (GA)18	TCCAGTTTGTGAGTTGTAGT	AITATGGGGTTAIGCTTTC	52	1
12	mE ₈ CIR0894	(GA)18	TGCTTCTTGTCTTGTGATACA	CCACGCTACGAAATGATAA	52	7
13	mE ₈ CIR0555	(GA)18	TACCATCACTGACCAATAAC	GCTTTTCTTGTAACTACAC	52	8
14	mE ₈ CIR0878	(GA)22	CAAAGCAACAAGCTAGTTAGTA	CAAGCAACCTCCATTTAGAT	52	11
15	mE ₈ CIR0773	NA	GCAAAATTCAAAGAAAACCTA	CTGACAGTGCAGAAAATGTTATAGT	52	NA
16	mE ₈ CIR2347	(GA)15	AITTTGCATGTGTGAGAGC	CAACCAATTCACCCCTAAAG	52	8
17	mE ₈ CIR2291	(GA)11	ATGCCCGGATCTTTGTGTAG	TTGTTCTGTGTTAATCAAGTGTATG	52	7
18	mE ₈ CIR3300	(GA)19	CATGCACGTAAGAAGAAGTGT	CCAAATGCACCCCTAAGA	52	7
19	mE ₈ CIR3382	(GA)24	TGTAGGTGGTGGTTAGG	TGTCAGACCCACCATA	52	11
20	mE ₈ CIR3350	(GA)16	GGATAAAGCTTCCAACAAC	CCTGGTCTGTTGGTAGAGA	52	14
21	mE ₈ CIR3683	(GA)15	GTAGCTTGAACCTGAAA	AGAACCACCGGAGTTAC	52	2
22	mE ₈ CIR3555	(GA)18	CATCAGAGCCTTCAAACCTAC	AGCCTGAATTCGCCTCTC	52	13
23	mE ₈ CIR3546	(GA)15	GCCTATCCCCTGAACCTACT	TGCACATAACCAGCAACAGAG	52	14
24	mE ₈ CIR3569	(GA)25	AAGGCTTGGAGTTGAGGTAT	CACCAITGGATCAITATTC	52	14
25	mE ₈ CIR3705	(GA)16	CCACCAITGCAGAATAGTAAG	GTGTGCCCTCAA AAAAGTATG	52	4
26	mE ₈ CIR3775	(GA)18	TCTTGATATTAAGAAGTCAAGGAAA	CGTTCCTTTTTCATATAGAT	52	4
27	mE ₈ CIR3711	(GA)17	GTCCTCATGTGGCTACCCTCTC	AGGCTCCCTGCTTTTAAAGT	52	8
28	mE ₈ CIR3732	(GA)19	AITTTAATTTGGCTTGGTATA	ACTTTTCTAICTAATTCITGAAGAT	52	8
29	mE ₈ CIR3808	(GA)22	CCGCTAACCTTGGTATAC	ATTTCAGCAGCTAATC	52	8
30	mE ₈ CIR3557	NA	CAITGGCAATCCCTTCAAAGT	TCCCTCTGTTCACTCAAGC	52	NA
31	mE ₈ CIR3260	NA	GGGCAAGTCATGTTTCCCTACA	TAAAGGGCGAGGTATTTCTGC	52	NA
32	mE ₈ CIR0408	NA	AGCCAGTGTCTCGGTATAA	CCCTGCAGTGTCCCTCTTTA	52	NA
33	mE ₈ CIR3886	NA	TTCTAGGGTCTATCAAAGTCATAAG	AGCCACCAACCACATCTACT	52	NA
34	mE ₈ CIR0905	NA	CACCACATGAAGCAAGCAGT	CCTACCACAACCCAGTCTC	52	NA

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_e 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 (Sarimana *et al.*, 2021) and the stated H_e 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.

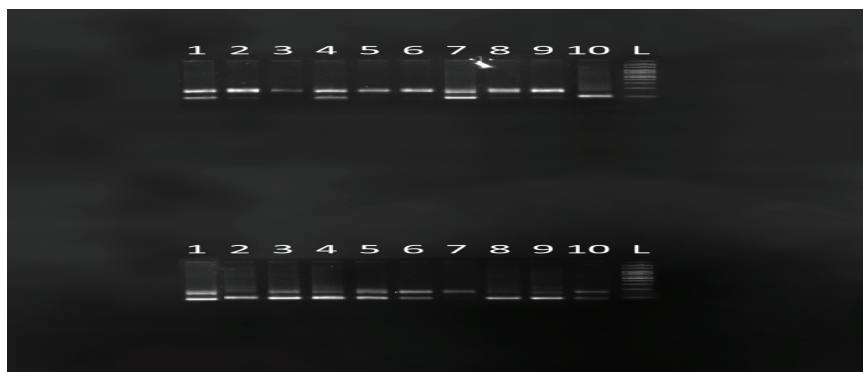
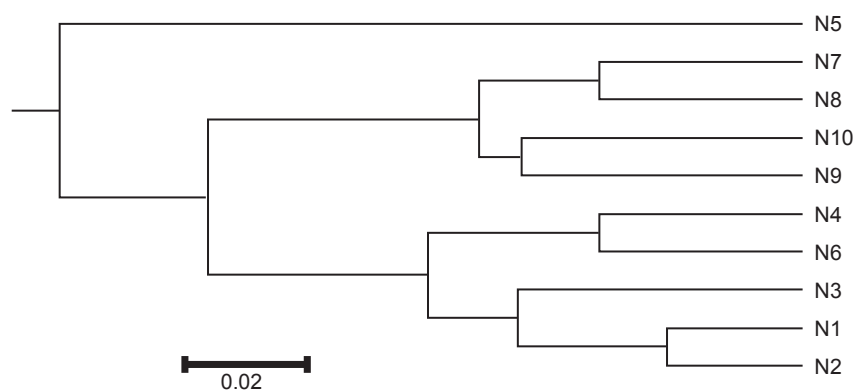


Figure 2. The SSR banding profile of the 10 oil palm hybrids of *mEgCIR0074* loci, where L = 100 bp DNA ladder lane (1-10) oil palm hybrids (please refer to Table 1 for label).



Note: 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

Figure 3. The rectangular dendrogram of 10 oil palm hybrids as determined by UPGMA analysis using the software POWER MARKER.

TABLE 3. PARAMETERS FOR 34 SSR LOCI GENETIC ANALYSIS OVER THE 10 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 3.850%-32.000% (Table 4) with an average of 18.000%, suggesting a moderate genetic relationship within oil palm hybrids. The

highest similarity coefficient value of 32.000% was observed between hybrids of NRCOP-5 and NRCOP-9 and the least similarity of 3.850% was noticed between hybrids of NRCOP-1 and NRCOP-2. In nine D-P cross combinations, Arias *et al.* (2013) showed similarity coefficients ranging from 0.015%-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 Arias *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 0.167-0.778 and 0.153-0.643, 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 four 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 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 10 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.

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

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.

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