RESEARCH ARTICLE

Identification of polymorphic microsatellite markers for genomic studies in oil palm (*Elaeis guineensis* Jacq.)

Ch. Sowmya[1,2*], M. V. B Venu, B. Kalyana babu

[1]ICAR-Indian Institute of Oil Palm Research, Pedavegi-534 450, West Godavari (Dt), Andhra Pradesh
[2]Adikavi Nannaya University, Rajahmahendra varam, Andhra Pradesh
* Corresponding author

E-mail: chavasowmya1994@gmail.com

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ABSTRACT

Oil palm with chromosome number 2n=32 and belongs to the family Arecaceae and having a major share of vegetable oil in India. To meet the edible oil requirement in the country, oil palm is the best options due to its high oil-yield (4-6 t/ha) potential compared to other annual oil-yielding crops. Polymorphic microsatellite markers play an important role in genetic diversity and mapping studies in a crop like oil palm. 30 SSR oil palm markers are selected for assessment of polymorphism and genetic diversity analysis among 24 Oil palm genotypes. The 30 Oilpalm SSR markers yielded 66 scorable alleles, all the loci are found to be polymorphic. The number of alleles ranged from 2 to 4 at an average of 2.2 alleles per locus. The SSR locus mEgCIR0195 was found to have maximum number of allele (4) followed by loci mEgCIR0874, mEgCIR087, mEgCIR0779, mEgCIR3328, (3 allele each). The dendrogram generated through UPGMA analysis grouped all the 24 oil palm genotypes into 2 major groups A and B.

Key words: oil palm, SSR marker, dendrogram, polymorphism

INTRODUCTION

Oil palm (*Elaeis guineensis*, also known as dende oil, from Portuguese) is an edible vegetable oil derived from the mesocarp (reddish pulp) of the fruit of the oil palm. Palm oil is naturally reddish in colour because of high beta-carotene content. It comprises two species of the Arecaceae, or palm family. They are used in commercial agriculture in the production of palm oil. Palm oil formed the basis of soap products, such as Lever Brothers (now Unilever)"sunlight" soap and the American Palmolive Brand. By around 1870, palm oil constituted the primary export of some West African countries, such as Ghana and Nigeria, although this was overtaken by cocoa in the 1880.

Variation in growth and yield results from difference in the genetic makeup (genes) and its interaction with the environment (GxE Interaction). The markers are useful for confirmation of pedigree or legitimacy of parentage, for assessing genetic diversity or in selection of individual or parents for breeding (marker assisted selection, MAS) Selection based genes (DNA) is expected to be reliable, efficient and precise compared to conventional phenotypic selection which is based on morphological traits influenced. The variability in the population is the most important requirement for any breeding programme. Thottappilly et al. (2000), refer to molecular markers as naturally occurring polymorphism which include proteins and nucleic acids that are detectably different.

Initially, several genetic diversity works were based on using RAPD, RFLP and AFLP molecular markers. However, due to certain drawbacks these markers were replaced by SSR and SNP markers. Use of RAPD for genetic diversity study of oil palm was reported for the first time by Shah (1994). Oil palm germplasm accessions collected from Africa (Cameroon, Tanzania, Nigeria and Zaire) were studied using 20 primes and recorded high levels of genetic variation among the accessions. Rival et al. (1998)

studied the suitability of RAPD markers for detection of soma clonal variants in oil palm. The results from the 387 arbitrary primers showed no intra clonal variability and no difference between mother and regenerated palms. The authors opined that RAPD approach is not suitable for the detection of the mantled variant phenotype. Later Mayes et al. (2000) used RFLP markers (40 probes covering 60% oil palm genome) to assess genetic diversity within 54 palms of a specific oil palm breeding program. Molecular marker technologies helps the breeding programmes to a larger extent which reduces time (Babu et al. 2017; Babu and Mathur 2016; Kumar et al. 2018).

Conventional breeding approaches takes more time in identification of genetically diverse oil palm genotypes, since breeding cycle is more. Hence, molecular markers technology plays important role in genetic diversity and mapping studies. The objectives of the present study are, 1) Identification of polymorphic SSR Markers, 2) molecular and genetic diversity analysis of the selected oil palm genotypes.

MATERIALS AND METHODS

Fresh tender spear leaves samples from each of 24 Oil palm genotypes were collected for extraction of DNA. Mid rib of each leaflet was removed and middle portion of the leaflet, which has fewer veins, without pigment was taken for DNA extraction. The DNA was extracted using modified protocol of Babu et al (2017). SSR amplification using PCR

A set of 30 SSR markers were used for amplification in the 24 selected genotypes of oil palm. The forward and reverse sequences of the primers were obtained from Billote et al. (2005). Thermal reaction were carried out in a reaction mixture (20 ìl) consisting of 10 X buffer (Himedia), 2 ìl having 15 mm MgCl2, 0.2 mM of each forward and reverse primer, 2 ìl of 2 mMdNTPs, 0.2 ìl of 1 U of Taq DNA polymerase (Invitrogen, USA) and about 25-50 ng of template DNA. The PCR amplifications were performed in a Thermocycler (Biorad, USA) programmed for an initial denaturation of 3 min at 95^0Cfollowed by 35 cycles of 30s at 95^0C, 30s of 50^0C annealing temperature, extension of 1 min at 72^0C, with a final extension of 10 min at 72^0C, and hold at 4^0C. The PCR products were fractioned on 3 % Agarose gel. The statistical analysis of polymorphism and UPGMA analysis for generating dendrogram was done by using power marker v 3.0 (Liu and Muse, 2005).

RESULTS AND DISCUSSION

In the present study, 30 SSR oil palm markers are selected for assessment of polymorphism and genetic diversity analysis among 24 Oil palm genotypes. DNA was extracted from 24 Oil Palm varieties by using CTAB Extraction Method. Then, quantity and quality of DNA was assessed by agarose gel electrophoresis (0.8%) respectively using ë DNA as marker to ensure the good quality of DNA for SSR assay (Figure 1).

In this present study, we used 30 Oil palm markers. The genomic DNA of the 24 Oil Palm accessions were amplified using 30 Oil palm SSR markers and yielded 66 scorable alleles, all the loci are found to be polymorphic. The SSR locus mEgCIR0195 was found to have maximum number of allele (4) followed by loci mEgCIR0874, mEgCIR087, mEgCIR0779, mEgCIR3328, (3 allele each). The number of alleles ranged from 2 to 4 at an average of 2.2 alleles per locus. Similar results were also obtained by Okoyo *et al.* (2016) where they found an extremely high mean percentage polymorphism (85.09 %) and Arias *et al.* (2012) reported maximum PIC value with 0.822 in commercial oil palm material. The details of the markers along with allele number and polymorphism percentage are given in table 1. The gel banding pattern of mEgCIR0163 is given in figure 2.

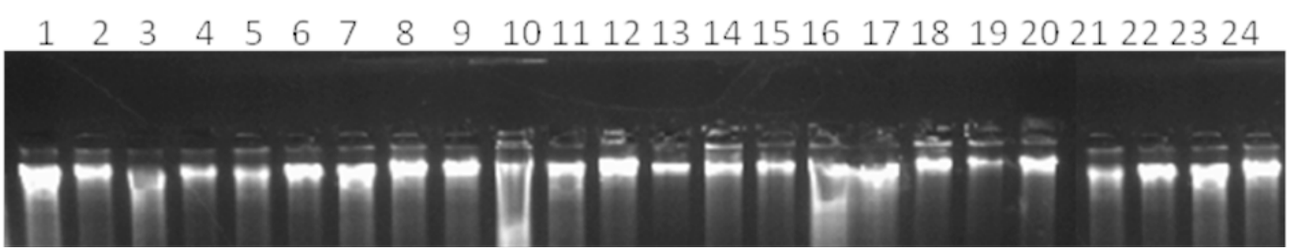

Fig. 1: The banding pattern of genomic DNA of oil palm

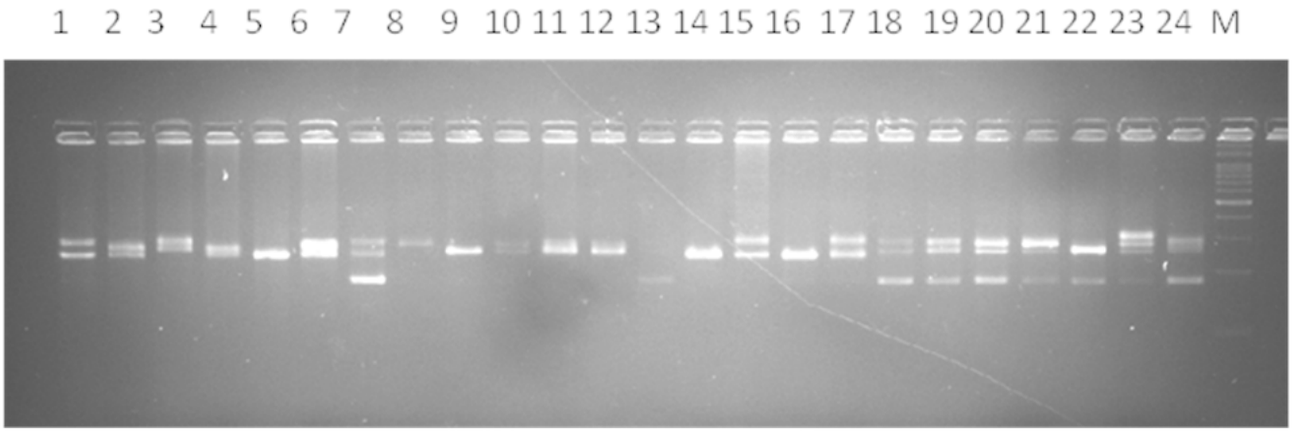

Fig. 2 The gel banding pattern of 24 oil palm genotypes using SSR marker mEgCIR0163

Table 1: The details of the markers along with allele number and polymorphism percentage

S. No.	Markers	Allele	Percentage Of polymorphism
1.	mEgCIR0195	2	100
2.	mEgCIR0163	4	100
3.	mEgCIR0243	2	100
4.	mEgCIR0037	2	100
5.	mEgCIR0177	2	100
6.	mEgCIR0802	2	100
7.	mEgCIR0874	3	100
8.	mEgCIR0793	2	100
9.	mEgCIR0800	2	100
10.	mEgCIR0894	2	100
11.	mEgCIR0555	2	100
12.	mEgCIR0774	2	100
13.	mEgCIR0775	2	100
14.	mEgCIR0825	2	100
15.	mEgCIR0878	3	100
16.	mEgCIR0779	3	100
17.	mEgCIR0773	2	100
18.	mEgCIR0782	2	100
19.	mEgCIR2575	2	100
20.	mEgCIR2518	2	100
21.	mEgCIR2595	2	100
22.	mEgCIR2813	2	100
23.	mEgCIR2387	2	100
24.	mEgCIR2291	2	100
25.	mEgCIR1773	2	100
26.	mEgCIR3282	2	100
27.	mEgCIR3232	2	100
28.	mEgCIR3286	2	100
29.	mEgCIR3310	2	100
30.	mEgCIR3328	3	100
	Average	2.2	

Genetic distances were estimated for pairs of varieties with their hybrids according to Jaccard's coefficient (Jaccard, 1908). High amount of similarity existed between P537 and P536 genotypes, followed 85% similarity between P45 and P43 genotypes. The list of highly similar genotypes given in table 2. Likewise highly dissimilar genotypes were also calculated using jaccard's similarity coefficient. High amount of dissimilarity existed between P42 and P143 genotypes followed by 30% dissimilarity between P143 and P72 genotypes. The list of highly dissimilar genotypes is given in table 3.

Table 2: The list of highly similar genotypes

S. No.	Genotype name	Genotype name	% of Similarity
1.	P537	P536	86%
2.	P45	P43	85%
3.	P143	P142	83%
4.	P539	P537	82%

Table 3: The list of highly dissimilar genotypes

S. No.	Genotype name	Genotype name	% of Dissimilarity
1.	P42	P143	28%
2.	P143	P72	30%
3.	P539	P143	32%
4.	P60	P143	33%
5.	P535	P173	35%

Genetic diversity

The dendrogram generated through UPGMA analysis grouped all the 24 Oil palm genotypes into 2 major groups A and B. The clustering of the Oil Palm genotypes was largely based on their geographical

origin. The following dendogram contains two clusters A&B with different genotypes. Cluster A contains two clusters. The sub clusters A1 and A2 consisted of 19genotypes. However, cluster B contains two sub clusters. The B cluster composed of 5 genotypes viz., P46, P142, P143, P173, and P174. The B cluster composed of all the tall genotypes and they all from Guinea Bissau origin. The genotypes under cluster A were belongs to different geographical origins like Cameroon, Zambia, and Tanzania.

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