RESEARCH ARTICLE

Molecular characterization of oil palm (*Elaeis guineensis*) germplasm using microsatellite markers

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ABSTRACT

The oil palm *(Elaeis guineensis)* belongs to the family Arecaceae which is commonly known as African oil palm. In the present study we aimed at molecular characterization of selected dura and pisifera oil palm genotypes using microsatellite markers. DNA is extracted from 44 oil palm germplasm of selected dura and pisifera plants by using CTAB Extraction Method. Twenty SSR oil palm markers are selected for assessment of polymorphism and genetic diversity analysis among 44 Oil palm genotypes. The 20 oil palm SSR markers yielded 35 scorable alleles, five loci are found to be monomorphic and 11 SSRs were polymorphic. The number of alleles ranged from 1 to 4 at an average of 2.1 alleles per locus. The SSR locus SMG00016 is found to have maximum number of allele (4) followed by loci SPC00065, SMG00217, and SPSC00163 (3 allele each). High amount of similarity existed between D71 and D108 genotypes followed by similarity between D7 and D30, D7 and D37 genotypes. High amount of dissimilarity existed between D4 and D30 genotypes with 44% dissimilarity followed by D5 and D14, D5 and D7 genotypes. The dendrogram generated through UPGMA analysis grouped all the 44 oil palm genotypes into 2 major groups A and B. The clustering of the Oil Palm genotypes is largely based on their dissimilarities. Highly dissimilar dura (D78) and pisifera (P77) (42%) genotypes can be used in high yielding breeding programmes.

Key words: oil palm, SSR markers, genetic diversity, power marker

INTRODUCTION

The oil palm genotypes are divided in to dura, pisifera and tenera forms based on the shell thickness which is a monogenic and co-dominantly inherited trait. With the discovery of the shell gene (Sh gene) by the oil palm researchers in the congo during 1940's led to more focus on increasing the oil palm production. The dura (D) genotypes consists of thick shell (Sh/Sh, dominant homozygote) whereas pisifera (P) genotype has a shell less with recessive homozygous sh/sh allele. The tenera (T) genotype has a shell less which has 30% more mesocarp and oil production than dura and pisifera, which is generally produced as hybrid from the cross between dura and pisifera. The tenera hybrid yields more oil and also is the basis for commercial palm oil production in all the oil palm growing parts of the world. Genetic diversity analysis is carried out by the help of morphological and biochemical markers which may be affected by environmental factors and do not have the resolving power for differentiating between closely related genotypes by the growing environment. Molecular markers are identifiable DNA sequence, found at specific locations of the genome and associated with the inheritance of a trait or linked gene. 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. Rivall 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. Arias et al. (2015) studied genetic and phenotypic diversity of natural American oil palm germplasm. The results from SSR markers and agro-morphological traits showed that analyses of variance for yield and bunch components demonstrated statistically significant differences among countries and geographical regions for several of the traits evaluated. SSR marker analyses revealed high genetic diversity. Recently, few reports on genetic relationship between elite oil palms (Prasanna et al. 2017; Sowmya et al. 2017; Babu et al. 2017). The SSRs are widely used in plants because of their abundance, hyper-variability, and suitability for high throughput analysis. They are randomly tandem repeats of short nucleotide motifs (2-6 bp/nucleotides long). Di-, tri- and tetra-nucleotide repeats, e.g. (GT)n, (AAT)n and (GATA)n, are widely distributed throughout the genomes of plants and animals. The objectives of the present study are 1) Molecular characterization of selected Dura & Pisifera germplasm using microsatellite markers and 2) genetic diversity analysis among dura and pisifera germplasm using SSR markers.

MATERIALS AND METHODS

Fresh tender spear leaf samples from each of 44 selected dura, pisifera oil palm genotypes are collected for extraction of DNA. Mid rib of each leaflet is removed and middle portion of the leaflet, which has fewer veins, without pigment is taken for DNA extraction. The list of the genotypes used in the study is given in table 1.

Table 1: The list of the oil palm genotypes used in the study

SI. No	Cross id	Fruit form	Palm number
1	240D X 281D	Dura	3
2	240D X 281D	Dura	4
3	240D X 281D	Dura	5
4	240D X 281D	Dura	7
5	240D X 281D	Dura	9
6	240D X 281D	Dura	13
7	240D X 281D	Dura	14
8	240D X 281D	Dura	17
9	240D X 281D	Dura	19
10	240D X 281D	Dura	30
11	240D X 281D	Dura	32
12	240D X 281D	Dura	33
13	240D X 281D	Dura	34
14	240D X 281D	Dura	37
15	240D X 281D	Dura	38
16	240D X 281D	Dura	39
17	240D X 281D	Dura	40
18	240D X 281D	Dura	40
19	240D X 281D	Dura	4.
20	240D X 281D	Dura	45
21	240D X 281D	Dura	54
22	240D X 281D	Dura	56
23	240D X 281D	Dura	59
24	240D X 281D	Dura	622
25	240D X 281D	Dura	63
26	240D X 281D	Dura	68
27	240D X 281D	Dura	71
28	240D X 281D	Dura	73
29	240D X 281D	Dura	78
30	240D X 281D	Dura	81
31	240D X 281D	Dura	82
32	240D X 281D	Dura	84
33	240D X 281D	Dura	85
34	240D X 281D	Dura	89
35	240D X 281D	Dura	92
36	240D X 281D	Dura	93
37	240D X 281D	Dura	97
38	240D X 281D	Dura	99
39	240D X 281D	Dura	108
40	-	Pisifera	75
41	-	Pisifera	76
42	-	Pisifera	77
43	-	Pisifera	78
44	-	Pisifera	110

SSR AMPLIFICATION USING PCR AND DATA ANALYSIS

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 950Cfollowed by 35 cycles of 30s at 950C, 30s of 500C annealing temperature, extension of 1 min at 720C, with a final extension of 10 min at 720C, and hold at 40C. 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

SSR polymorphic analysis

In the present study 20 SSR markers are used. In this, 11 markers showed polymorphism (figure 4.15-4.18), 5 markers showed monomorphism and 3 markers did not amplify. Among all the 20 markers primer SPSC00185, SMG00056, SPSC00193, SEG00166 have the major allele frequency with 1.000 and all the primers have mean allele frequency of 0.7738. The banding pattern of SSR primer SEG00156 is given in fig. 1. The major allele number shown by the primer is 4 by SMG00016 and the mean allele number shown by the primers is 2.1. Gene diversity is shown major by primer SMG00016 is 0.67 and the mean gene diversity shown by the primers is 0.29 (Table 2). Heterozygosity is shown maximum by the primer SMG00223 is 1.00 and the mean heterozygosity shown by the primers is 0.37. The PIC (polymorphism information content) is shown maximum by the primer SMG00016 is 0.61 and the mean average shown by the primers is 0.24.

Identification of diversity between dura and pisifera palms

The high amount of diversity observed among dura samples is between dura4 and dura30 (44%) (Table 3), among the dura and pisifera samples high diversity is between dura78 and pisifera 77(42%). (Table 4).

Fig. 1: The banding pattern of the SSR primer SMG00156 across 44 oil palm germplasm.

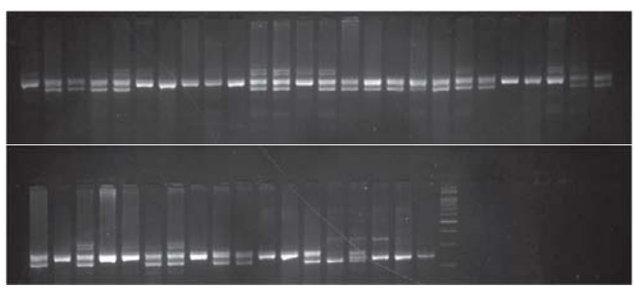

Table 2: The Summary statistics of primers along with allele frequency, number, gene diversity, heterozygosity, PIC.

Marker	Major Allele Frequency	Allele Number	Gene Diversity	Heterozygosity	PIC
SMG00217	0.7558	3.0000	0.3794	0.4419	0.3230
SEG00108	0.8947	2.0000	0.1884	0.0526	0.1706
SPSC00065	0.7436	3.0000	0.3932	0.4615	0.3335
SMG00210	0.5417	2.0000	0.4965	0.9167	0.3733
SPSC00185	1.0000	1.0000	0.0000	0.0000	0.0000
SMG00156	0.7273	2.0000	0.3967	0.5455	0.3180
SEG00094	0.7907	2.0000	0.3310	0.4186	0.2762
SPSC00163	0.6905	3.0000	0.4410	0.5714	0.3626
SPSC00093	1.0000	1.0000	0.0000	0.0000	0.0000
SMG00155	0.9286	2.0000	0.1327	0.0476	0.1239
SMG00227	0.6047	2.0000	0.4781	0.5116	0.3638
SMG00016	0.4186	4.0000	0.6758	0.7209	0.6165
SPSC00063	0.6163	2.0000	0.4730	0.7674	0.3611
SMG00056-	1.0000	1.0000	0.0000	0.0000	0.0000
SPSC00193	1.0000	1.0000	0.0000	0.0000	0.0000
SEG00166	1.0000	1.0000	0.0000	0.0000	0.0000
SMG00223	0.4419	4.0000	0.6028	1.0000	0.5212
Mean	0.7738	2.1176	0.2934	0.3798	0.2437
min	0.4186	1.0000	0.1327	0.0476	0.1239
max	1.0000	4.0000	0.6758	1.0000	0.6165

Table 3: Diversity of dissimilarity among dura oil palm genotypes.

S.NO	Genotype name	Genotype name	% of Dissimilarity
1.	DURA4	DURA30	44%
2.	DURA5	DURA14	43%
3.	DURA5	DURA7	43%
4.	DURA5	DURA82	43%
5.	DURA5	DURA99	43%

Table 4: Diversity of dissimilarity between dura and pisifera oil palm genotypes.

S.NO	Genotype name	Genotype name	% of Dissimilarity
1.	DURA78	PISIFERA77	42%
2.	DURA4	PISIFERA77	42%
3.	DURA30	PISIFERA78	40%

Genetic diversity

The dendrogram generated through UPGMA analysis grouped all the 44 Oil palm genotypes into 2 major groups A and B. The following dendogram contains two clusters A&B with different genotypes. Cluster A contains two clusters (Fig. 2). The sub clusters A1 and A2 consists of 11 genotypes viz., D5, P78, P75, P76, D13, D30, D33, D34, D14, D17, D19 oil palm genotypes. Dura 5 genotype did not cluster with any other genotype. However, cluster B contains two sub clusters. The B cluster consists of 33 genotypes viz., D78, D97, D73, D89, D54, D4, D43, D93, D45, D63, D39, D41, D38, D9, D85, D92, D108, D71, D40, D68, D37, D32, D7, D84, D62, D82, P77, D99, D3, D59, P110, D56 and D81 oil palm genotypes. Highly dissimilar dura (D78) and pisifera (P77) (42%) genotypes can be used in high yielding breeding programmes.

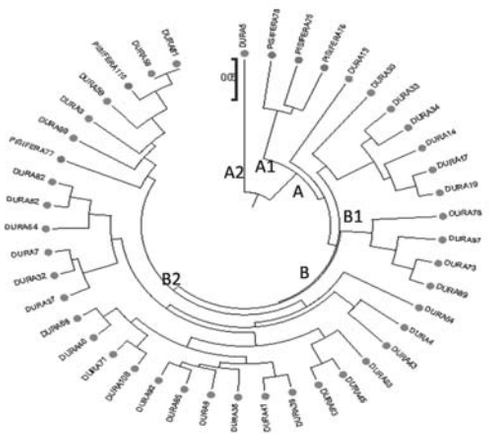

Fig. 2: The dendrogram obtained from 11 polymorphic SSR markers using UPGMA method of power marker software

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