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Genetic diversity in oil palm (*Elaeis guineensis* and *Elaeis oleifera*) germplasm as revealed by microsatellite (SSR) markers

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ABSTRACT

Genetic variability and relationship of 29 germplasm accessions of two species, viz. *Elaeis guineensis* Jacq (25 accessions) and *Elaeis oleifera* (4 accessions) along with two Palode *teneras* were evaluated at ICAR-Indian Institute of Oil Palm Research, Regional Station, Palode, India during 2014-2016 with an objective to assess the germplasm diversity.Nine oil palms specific SSR primer pairs were used to assess the genetic diversity. All the nine primer pairs produced reproducible unambiguous markers. The total number of alleles per primer pair varied from six (sMo00129, sMo00128, mEgCIR3890, sMo00130, mEgCIR0268, mEgCIR0905, sMo00020, sMo00154,) to seventeen (mEgCIR3399). It produced a total of 107 alleles from the selected two oil palm species and all the makers were polymorphic. Cluster analysis, based on UPGMA was performed in order to realize the extent of similarity/ dissimilarity among the germplasm accessions. The dendrogram showed two major clusters at 0.09 similarity coefficient one with *E. oleifera* and other with *E. guineensis*. Among *E. oleifera*, Chithara (*Oleifera* palms identified in the commercial plantation owned by OPIL, Kerala) accessions were grouped separately indicating confirmation of distinctness. Among *E. guineensis* accessions, G1(Nigerian dwarf *tenera*) and G55 (Tanzanian *dura*) showed maximum diversity. Evaluation data obtained in this study on the 'extent of genetic distance' among accessions can be explored carefully for planning hybridization programme so as to accelerate palm oil yield by maximizing the hybrid vigour. The significant genetic diversity observed among the germplasm accessions indicated the scope of introgression into the current breeding programme.

Key words: Diversity, Germplasm, Oil Palm**,** Palode**,** SSR

The narrow genetic base of existing commercial oil palm cultivars has prompted the oil palm breeders to give paramount importance to germplasm (Glaszmann *et al*. 2010) and achieve sustainable development of the crop (Arias *et al.* 2013). In this direction, Pillai *et al*. (2000) collected valuable germplasm material from African sources of primary and secondary centers of origin, viz. Guinea Bissau, Tanzania, Cameroon and Zambia; besides several other sources including American oil palm which were planted at Palode, Kerala, India. Murugesan *et al.* (2015) evaluated phenotypic variations and diversity of African oil palm germplasm in India and reported that Tanzania accessions had highest diversity followed by Zambia. Precise estimation of genetic diversity of germplasm accessions is prerequisite for the effective utilization for oil palm breeding (Bakoumé *et al*. 2014) which is achieved through molecular markers.Isozyme was first used in oil palm to study the genetic diversity in African and Brazilian populations (Ghesquiere, 1985, Ghesquiere *et al.* 1987, Moretszohn, 1995 and Moretzsohn *et al*. 2002). Restriction fragment length polymorphism (RFLP) has also been designed and used in oil palm (Mayes *et al.* 1997, Maizura *et al.* 2006). Barcelos *et al.* (2002) used amplified fragment length polymorphism (AFLP) and RFLP markers to assess genetic relatedness between different accessions of the American oil palm population. Random amplified polymorphic DNA (RAPD) has been employed for the analysis of the genetic variation among African germplasm accessions (Shah *et al.* 1994). SSR was first used on oil palm in a study on the genetic diversity structure of the genus *Elaeis* (Billotte *et al.* 2001). Ferreira *et al*. (2012) reported the effectiveness of SSR markers to detect polymorphism and quantification of genetic diversity within and among progenies of oil palm using Deli *dura* population. Molecular diversity study has not been undertaken for the germplasm available (including *E. oleifera*) at ICAR-IIOPR, Research Centre, Palode (National Active Germplasm Site (NAGs) for Oil Palm) which is prerequisite for their effective utilization. Hence, evaluation was taken up to infer genetic variability, discrimination and relationship of different sources of germplasm using SSR markers.

MATERIALS AND METHODS

Thirty-one genotypes (including 29 germplasm

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accessions and Palode *tenera*) of two species, viz. *Elaeis guineensis* Jacq (25 Samples) and *Elaeis oleifera* (4 Samples) (Table 1) were evaluated during 2014-16 at ICAR-Indian Institute of Oil Palm Research, Research Centre, Palode, Kerala, India.

Genomic DNA was extracted from spindle leaves of palms using the DNeasy mini kit (QIAGEN) following the manufacturer's protocol. The DNA was quantified spectrophotometrically and the bands were checked on 0.8% agarose gel electrophoresis. A total of nine SSR primer pairs specific to oil palm were used (Table 2). The PCR reactions were carried out in 20 µL volume with standardized components: 20 ng genomic DNA, 0.2 µM each of forward and reverse primers, 10 µM dNTPs (M/s Bangalore Genei Pvt Ltd), 10x buffer (10 mM Tris- Hcl (*p*H 8.3), 50 mM KCl, 1.5 mM $MgCl_2$), and 3 units of *Taq* DNA polymerase (M/s Bangalore Genei Pvt Ltd). PCR products were analyzed using the DVA 500 kit of the Shimadzu MCE-202 MultiNA. Samples were run with the reagents from the DNA kit: separation buffer, DNA marker reagent and 25 bp DNA ladder (Invitrogen). Samples were placed into the MultiNA instrument close to the reagents. The statistical analysis of polymorphism and UPGMA analysis for generating dendrogram was done and the samples and reagents mixed automatically on chip and ran using MultiNA control and MultiNA viewer software.

Table 1 List of experimental materials of oil palm germplasm and their important traits

Acc. No	Name of germplasm	Description and fruit forms
DOPRG-1	Nigerian dwarf *tenera*	Dwarf *tenera*
DOPR G-22	Palode-*Oleifera* (Surinam)	Dwarf and deep red at fruit ripe
DOPR G-23	Palode- *E. Oleifera* (Malaysia)	High bunch weight
DOPR G-24	Palode- *E. Oleifera* (Costa Rica)	Large bunch and large kernel
DOPR G-25	Chithara *Oleifera* I	Large kernel
DOPR G-26	Chithara *Oleifera* II	Small fruits
DOPR G-27	*Dura* III	Large fruit and *dura*
DOPRG-28	*Dura* III	Large fruit and *dura*
DOPRG-35	Guinea Bissau 23/312	Long stalk and *dura*
DOPRG-44	Guinea Bissau27/316	Long stalk and *dura*
DOPRG-53	Tanzania - 2	*Virescence* and *dura*
DOPRG-54	Tanzania - 10	*Virescence* and sterile *pisifera*
DOPRG-56	Tanzania - 11	Large bunch and *dura*
DOPRG-59	Zambia - 8	Large bunch, high sex ratio and *dura*
DOPRG-30	Guinea Bissau 27/316	Large kernel, high sex ratio and *dura*
DOPRG-36	Guinea Bissau 10/306	High sex ratio and *dura*
DOPR G-38	Guinea Bissau28/317	Long stalk and *dura*
DOPRG-40	Guinea Bissau12/308	*Dura*
DOPRG-42	Guinea Bissau8/305	Dura and high sex ratio
DOPRG-43	Guinea Bissau 32/321	*Dura*
DOPR G-45	Guinea Bissau81/309	*Dura*
DOPR G-46	Guinea Bissau 3/299	*Dura*
DOPR G-47	Guinea Bissau 8/304	*Dura*
DOPR G-50	Guinea Bissau 30/319	*Dura*
DOPR G-51	Guinea Bissau 21/310	*Dura*
DOPR G-52	Cameroon - 4	*Dura*
DOPRG-55	Tanzania-8	*Tenera*
DOPR G-57	Zambia - 4	*Dura*
DOPR G-58	Zambia - 7	Large kernel and *dura*
D240	Palode - 1	*Tenera*
D80	Palode - 2	Tenera

RESULTS AND DISCUSSION

Nine oil palms specific SSR primer pairs were

Table 2 Details of oil palm specific SSR primers used in the molecular analysis

Primer name	Sequence	Annealing temperature (°C)
mEgCIR3890 F	GTGCAGATGCAGATTATATG	60
mEgCIR3890 R	CCTTTAGAATTGCCGTATC	
mEgCIR0268 F	GCAACACCATTAGAGAGA	60
mEgCIR0268 R	TCCATGCATCCAAACAG	
mEgCIR0905 F	CACCACATGAAGCAAGCAGT	60
mEgCIR0905 R	CCTACCACAACCCCAGTCTC	
mEgCIR3399 F	AGCCAATGAAGGATAAAGG	59
mEgCIR3399 R	CAAGCTAAAACCCCTAATC	
sMo00020 F	CCTTTCTCTCCCTCTCCTTTTG	59
sMo00020 R	CCTCCCTCCCTCTCACCATA	
sMo00128 F	TAGCTCCAACAGCTTGCCT-TAT	57
sMo00128 R	GGTCCCGTCCTATGATTTAT-TCT	
sMo00129 F	TTAGTATTGGGTGTGCATA-AGTGG	59
sMo00129 R	GCTTCCAGCTCCTCTTTC-TACC	
sMo00130 F	TAAGCAAAAGATCAGGG-CACTC	60
sMo00130 R	GGCTGGTGAAAATAGGTTTA-CAAAG	
sMo00154 F	CAAAAGGGTTGTTTGTATAC-GTG	59
sMo00154 R	TGCATGAATATCCTCT-CAAAGTTAC	

utilized to assess the genetic diversity of 29 accessions of germplasm along with two Palode *teneras*. All the nine primer pairs produced reproducible unambiguous markers. The total number of alleles per primer pair varied from six (sMo00129, sMo00128, mEgCIR3890, sMo00130, mEgCIR0268, mEgCIR0905, sMo00020, sMo00154,) to seventeen (mEgCIR3399). It produced a total of 107 alleles from the two oil palm species, *Elaeis oleifera* and *Elaeis guineensis* and all the makers were polymorphic. The present evaluation has given details on the extent of genetic diversity of Palode germplasm and genetic relatedness among the different sources. Cluster diagram based on Dice (1945) similarity matrix by UPGMA analysis was calculated from alleles derived from 31 oil palm accessions from SSR data (Fig 1). The dendrogram showed two major clusters at 0.09 similarity coefficient. Maximum similarity was seen between G27 and G28 (0.96) and minimum similarity is 0.07 for most other accessions (Fig 2). Bakoume *et al*. (2009) evaluated oil palms in natural grooves utilizing SSR markers. Based on the dendrogram, the germplasm accessions could be divided into two main groups. Cluster I consists of four genotypes belonging to *E. oleifera* namely G23, G24, G25 and G26. The Chithara accessions, G25 and G26 were grouped in one cluster and the other two, G23 and G24 in next cluster indicating the probable origins of Chithara (*Oleifera* palms located in plantation owned by OPIL, Kerala) accessions from a single source. The study indicates that *E. oleifera* derived SSR markers were more efficient in revealing the genetic diversity of *E. oleifera* when compared to *E. guineensis* EST-SSR markers. Cluster II included the remaining accessions and was divided into six sub clusters. Accession G55 of Tanzania and G58 of Zambia-7 populations belongs to different sub clusters implying the unique characteristics of these genotypes. The palms of first subcluster were medium tall, where the palms of the second sub cluster were dwarf and with low annual height increment. The sub cluster II consisted of eleven accession, viz. G35, G-43, G-46, G-47, G-44, G-38, G-51, G-52, G-40 and G-50 which are mainly form Guinea Bissau and Cameroon population. The sub cluster IV consisted of accessions G53, G-54, G-56 and G-57. Sub cluster V consisted of the reference accessions, viz. Pld 1, Pld 2 as well as G-42, G-36, G-30, G-59, G-28 and G-27. G27 and G28 are the accessions belonging to Dura-III population and their close resemblance could be justified by their common ancestry. The accessions G-1 and G-22, both having dwarf palms belongs to sub cluster VI (Fig 1). From the 3-D plots, it was clear that there was variation in genetic makeup among most of the germplasm sources. The sources, viz. G-38, G-50 and G- 40 were in different group as per PCA analysis, showing variation among accessions of Guinea Bissau origin (Fig 2). Ajambang *et al.* (2012) studied the diversity of Cameroon's wild oil palm population with the use of microsatellite marker and they found the genetic diversity between *Elaeis guineensis* and *Elaeis oleifera.* Similarly, G27 and G-28 were found

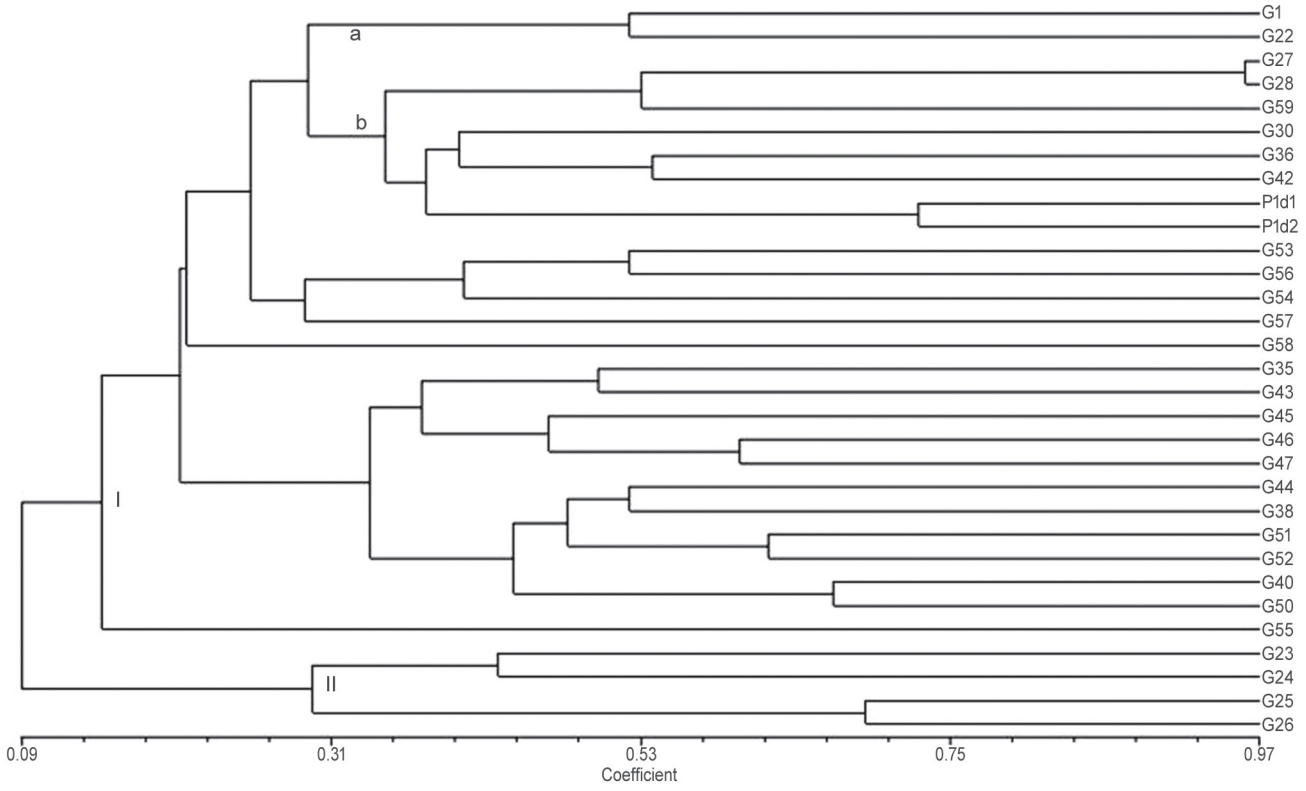

Fig 1 Cluster diagram based on Dice (1945) similarity matrix by UPGMA analysis calculated from alleles derived from 31 oil palm accessions of germplasm from SSR data.

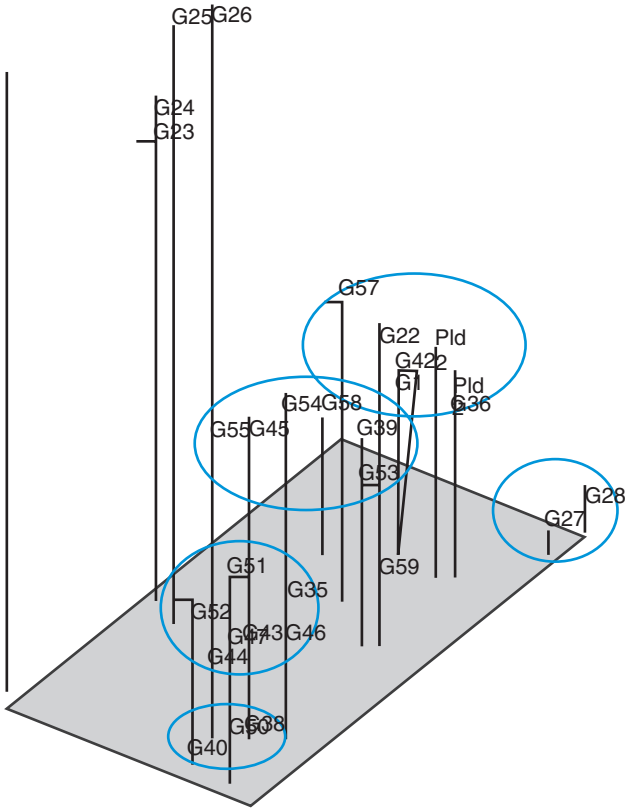

Fig 2 3D Principal Coordinate Analysis (PCA) of genetic identity among 31 oil palm accessions of Palode Research Centre.

in separate group, though they were clustered in the same group of Pld-1 and 2 using UPGMA clustering. This could be due to variation resulting from introgression of alleles from other genotypes while regeneration process. The variability in the size of alleles and sequences reflected the possible mutational processes that had taken place at both the repeat and neighboring regions (Zaki *et al.* 2012). In the nutshell, it is reported that gene diversity existing among all the accessions were high indicating existence of high levels of polymorphisms.Main highlights of the findings are: Nigerian dwarf *tenera* (G1) and Tanzanian dura (G55) showed maximum diversity and *E.oleifera* of chithara I &II (palms identified in commercial planation owned by OPIL, Kerala) accessions (G25 and G26) were grouped separatelyconfirming their separate origin with rest of the *oleifera* accessions (G22, G23 and G24) planted at Palode. However, G23, G24, G25 and G26 grouped together and forms Cluster II, whereas G22 Palode *oleifera* (Suriname) fallen apart and clustered with G1(dwarf Nigerian *tenera*). This Nigerian *tenera* was identified from Nigerian block with the progenitor of 26.3999 D × 25.380P which were introduced to India through National Bureau of Plant Genetic Resources (NBPGR) vide code number E 130756 during 1979 which were field planted at Palode during 1981 and dwarf characters of this Nigerian palm and Surinam *oleifera* were reported by Murugesan *et al*. (2009). So, selected palms (G1 and G22) can be straightway recommended for crossing as they were proven for dwarfness and diversity.

Another interesting palm which was characterized as sterile-*virescence- pisifera* from Tanzania source was G54. It is reported that *virescence* fruit forms are extensively used for developing varieties with homozygous *virescence tenera* by ASD Costa Rica (Http://www.asd-cr.com/paginas/english/molecular_biology.html) (Murugesan and Goutam Mandal, 2010) in view of ease of harvest of matured oil palm bunches. In this respect, *virescence pisifera* palm G-54 has practical utility which could be used as male parent along with potential other *virescence duras*. The study is also revealed existing of high diversity in Nigerian dwarf *tenera* (G1) and reconfirms that Nigeria is one of the primary centres of origin for *E. guineensis*. Evaluation data obtained in this study on the 'extent of genetic distance' among accessions can be explored carefully for planning hybridization so as to accelerate palm oil yield by maximizing the hybrid vigour. Accessions from Guinea Bissau source, viz. G30, G36 and G42 exhibits potential utility as they also have high sex ratio coupled with wide distance from other potential *duras* of other accessions. Guinea Bissau is one of the primary centres of origin and Tanzania and Zambia are the secondary diversity hotspots in Africa. According to Pillai *et al*. (2000), the samples of Guinea Bissau were collected from an area at the edge of natural distribution under FAO programme and planted at Palode centre. Similarly,germplasm accessions from Tanzania source G55 also recorded high diversity. High diversity for the Tanzanian indicates that this germplasm has also has good scope for oil palm improvement. Maizura *et al*. (2006) recorded high mean number of alleles per locus (1.7) and percentage of polymorphic loci (62.1%) in Tanzanian germplasm through RFLP marker study. This study indicates somewhat low to medium distances among Tanzania (G53, G54, G55 and G56) and Zambia accessions (G57, G58 and G59); which may be attributed to dispersal of *E. guineensis* (Zeven 1964) in the African continent from West to centre and East Africa through human interventionand natural selection (Bakoume *et al*. 2007). In the present study, a particular accession (G55) of Tanzanian source had high diversity and morphologically confirmed as *tenera* fruit form. Bakoume *et al.* (2014) reported that Tanzanian germplasm (maintained in MPOB) had highest observed heterozygosity when compared to 49 other population/sources. The diversity observed in Guinea Bissau accessions for medium sized bunches could be used in development of hybrids with medium sized bunches which is desired by the processing industry. *Dura* of Zambian source is characterized by lower shell thickness which can be exploited for development of *tenera* with minimum shell and maximum mesocarp portions, thereby increasing oil yield.

A set of *E. oleifera-guineensis* SSR markers developed were used for understanding the diversity of *E. oleifera* and *E. guineensis* genetic resources. The sequence data showed their ability to amplify DNA, thus analyzing the SSRs to amplify across species and genera in the Arecaceae family. Nigerian dwarf *tenera* (G1) and Tanzanian dura (G55) showed maximum diversity and *E.oleifera* of Chithara

(palms identified in commercial planation owned by OPIL, Kerala) accessions (G25 and G26) were grouped separately confirming their separate origin against rest of the oleifera accessions (G22, G23 and G24) planted at Palode. The significant genetic diversity observed among the accessions indicated that these materials are good sources of new genes for introgression into the current breeding materials for oil palm improvement as well as widening the genetic base.

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