

INTRA- AND INTER-POPULATION GENETIC DIVERSITY OF OIL PALM (*Elaeis guineensis* Jacq.) PISIFERA CLONES ORIGINATED FROM NIGERIA BASED ON SSR MARKERS ANALYSIS¹

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ABSTRACT: The objectives of this experiment were to determine intra- and inter-population genetic diversity of tissue culture derived ramets of pisifera palm collections originated from Nigeria (pisifera Nigeria). A total of 87 ramets of pisifera Nigeria derived from six orteds were used for analysis. In this experiment, the genetic diversity was assessed using 12 loci of oil palm's specific SSR markers. The results of the experiment indicated out of 12 SSR marker loci evaluated, one locus was monomorphic in all pisifera palms evaluated while 11 were polymorphic. The average number of aleles per locus in the analyzed populations was 3.3. Out of six different populations of pisifera Nigeria analyzed, ramets derived from orted # 22, # 24 and # 32 showed uniform alele profiles in all of SSR marker loci tested, indicating the clonal nature of the ramets. On the other hand, at least one ramet derived from orted # 14, # 23 and # 33 exhibited different alele profiles than that of the rest, indicating possibilities of either somaclonal variants or mislabelled materials. These results demonstrated SSR marker can be used to evaluate genetic relatedness among ramets derived from different orteds, uniformity of ramets derived from tissue culture of single orted, and detecting either somaclonal variants or mislabelled ramets.

Key words: Tissue culture, clonal propagation, ramet uniformity, mislabeled materials, somaclonal variants

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1. INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is one of the most productive oil bearing crops because it has, by far, the highest oil yield per unit area. It is an allogamous aborescent monocot of the Arecaceae family (tribe: Cocoinae) [10]. Diploid genome of oil palm consists of 16 pairs of homologous chromosomes ($2n=32$), and it was estimated by flow cytometry to be as much as 3.79 pg/2C [18].

In nature, oil palm is classified into three varietal types based on the presence or absence of fruit shell, a phenotype governed by a major Sh (shell) gene [4]. The three varieties types are (1) dura type (homozygous Sh^+/Sh^+) - produces fruits with a thick shell; (2) pisifera type (homozygous Sh^-/Sh^-) - rarely produces fruits and they are without the shell; and (3) tenera type (heterozygous Sh^+/Sh^-) - produces fruits with an intermediate shell and it is a hybrid between dura and pisifera.

Historically, four dura type oil palms were planted at the Bogor Botanical Garden, Bogor in 1848. Seeds derived from these palms were established in Deli district of Sumatra in 1881 [10] and the famous Deli dura palm were originated from these plantations. The Deli *duras* have provided the foundation for developing commercial oil palm planting materials used by oil palm industry in Indonesia and in other oil palm growing countries.

Since *pisifera* palms are predominantly produce male flowers, they are exploited for pollen sources and for crossing with the *dura* palm to produce the *tenera* (DxP) hybrid. Especially after Beirnaert's discovery in 1939 that shell thickness was governed by single gene in Zaire, a region previously known as Belgian Congo [10]. This discovery was the cornerstone of the oil palm industry and it paved the way for

breeding, selection and production of high yielding DxP oil palm planting materials.

Characterization and quantification of genetic diversity have long been a major goal in oil palm breeding program. Availability of the genetic diversity information among oil palm accessions is essential for a rational use of genetic resources. Furthermore, analysis of genetic variation both within and among elite breeding materials is of fundamental interest to plant breeders since it contributes to germplasm monitoring and is useful for predicting potential genetic gains [1]. Diversity based on phenological and morphological characters usually biased by environmental variations and takes such a long time for evaluating some of these traits. Recently, the rapid development of biotechnology, especially molecular (DNA) markers, allows easy analysis of a large number of loci distributed throughout the genome of plants [13, 7].

Molecular (DNA) markers are powerful tools for assessing genetic variation and elucidating genetic relationships within and among plant species. Molecular (DNA) markers, such as RFLP, RAPD and AFLP have also been used in oil palm genetic analysis [3, 17, 21, 2, and 11]. Among the wide reservoir of available molecular (DNA) markers, microsatellites, also known as simple sequence repeats or SSRs, are a small array of tandemly arranged bases (two to six bases) spread throughout the genomes. Microsatellites markers are more advantageous than other DNA markers because they are highly polymorphic and abundant throughout the genome, codominantly inherited, analytically simple and readily transferable. Microsatellites are more variable than RFLPs, RAPDs or AFLP, and have been widely utilized in genomic studies. It has been demonstrated that among the four marker systems tested:

restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR), SSR marker has the highest information content (ability to distinguish genotypes) [16]. Furthermore, SSR marker has also been used in oil palm genetic analysis [20, 24, 5, 6, 22].

The advantages of microsatellite over other markers are more important and more obvious if it is used to track desirable traits in large-scale breeding programs and to serve as anchor points for map-based gene cloning strategies. SSR markers are also preferred for high throughput mapping, genetic analyses and marker assisted plant improvement programmes [12].

The objectives of this experiment were to determine intra- and interpopulation genetic diversity of tissue culture derived ramets of *pisifera* palm collections originated from Nigeria (*pisifera* Nigeria). A total of 87 ramets of *pisifera* Nigeria derived from six orteds were used for analysis. The evaluated *pisifera* populations have been used as pollen sources for producing commercial oil palm's DxP semi clone planting materials by Sampoerna Agro.

2. MATERIALS AND METHODS

A total of 87 *pisifera* Nigeria ramets used were derived from six *pisifera* orteds. Total nucleic acids were extracted from young leaf using modified [8, 25] CTAB method [15]. Twelve microsatellite loci (Billotte *et al.*, 2005) were used to genotype all of the evaluated ramets. PCR amplifications were performed according to Billotte *et al.* (2001) and the amplicons were separated using 6% denaturing, polyacrylamide gel electrophoresis. The allelic profiles were visualized by silver

staining. Subsequently, the SSR data were inputted and analysed using NTSYSpc version 2.02 software [19]. The similarity based on Dice [9] and Nei and Li [14] coefficients were calculated. Cluster analysis was conducted using unweighted pair-group with arithmetic average (UPGMA) as described by Sneath and Sokal [23] and the appropriate dendrogram was constructed.

3. RESULTS AND DISCUSSION

3.1. Amplification of SSR markers

Twelve primer pairs were used to amplify DNA from 87 individual ramets. They were derived from six orteds of *pisifera* Nigeria. Each primer pair produced a unique banding pattern that can be used to determine identity of the genotypes. Out of 12 primer pairs tested, one was monomorphic to all *pisifera* Nigeria and 11 were polymorphic. The average alele numbers in the tested *pisifera* Nigeria populations were 3.3 aleles per locus.

3.2. Intrapopulation genetic diversity of ramets derived from certain orteds

The results showed that out of six different orteds of *pisifera* Nigeria analyzed, ramet population # 22, # 24 and # 32 showed uniform alele profiles for all SSR marker loci tested indicating the clonal nature of the ramets. However, at least one ramet within ramet population # 14, # 23 and # 33 showed different alele profiles than that of the rest, indicating the possibilities of either existence of somaclonal variants or mislabelled materials.

The dendrogram constructed based on the polymorphic SSR loci (Figure 1) showed that most ramets derived from certain *pisifera* orteds were clearly grouped in one cluster while those derived from different orteds were separated into different

clusters. Results of cluster analysis further showed that ramets within population # 22, # 24 and # 32 were uniform, while that within population # 14, # 23 and # 33 were not. In the later populations, at least one ramet was deviated from the rest of the population members. The number of variant loci showed different allele profile in the variant individual was shown in Table 1.

3.3. Interpopulation genetic diversity of different orteds

Results of interpopulation analysis among orteds showed that all of pisiferas Nigeria orteds were clustered together at 0,65 level of similarity coefficient. Based on 12 SSR loci data, ramets of pisifera Nigeria population # 22 were closely related to population # 24 (0.85 level of similarity coefficient). Moreover, ramets of pisifera Nigeria population # 33 were closely related to that of population # 14 (0.95 level of similarity coefficient).

4. CONCLUSION

The ability of SSR marker to identify ramets showing different alele profiles demonstrated that SSR marker can be used to evaluate genetic relatedness among ramets derived from different orteds, uniformity of ramets derived from tissue culture of single orted, and detecting either somaclonal variants or mislabelled ramets. The generated genetic diversity data from ramet collections of pisifera Nigeria may also be usefull as selection tools for maintaining genetic variability and for assisting future breeding activity.

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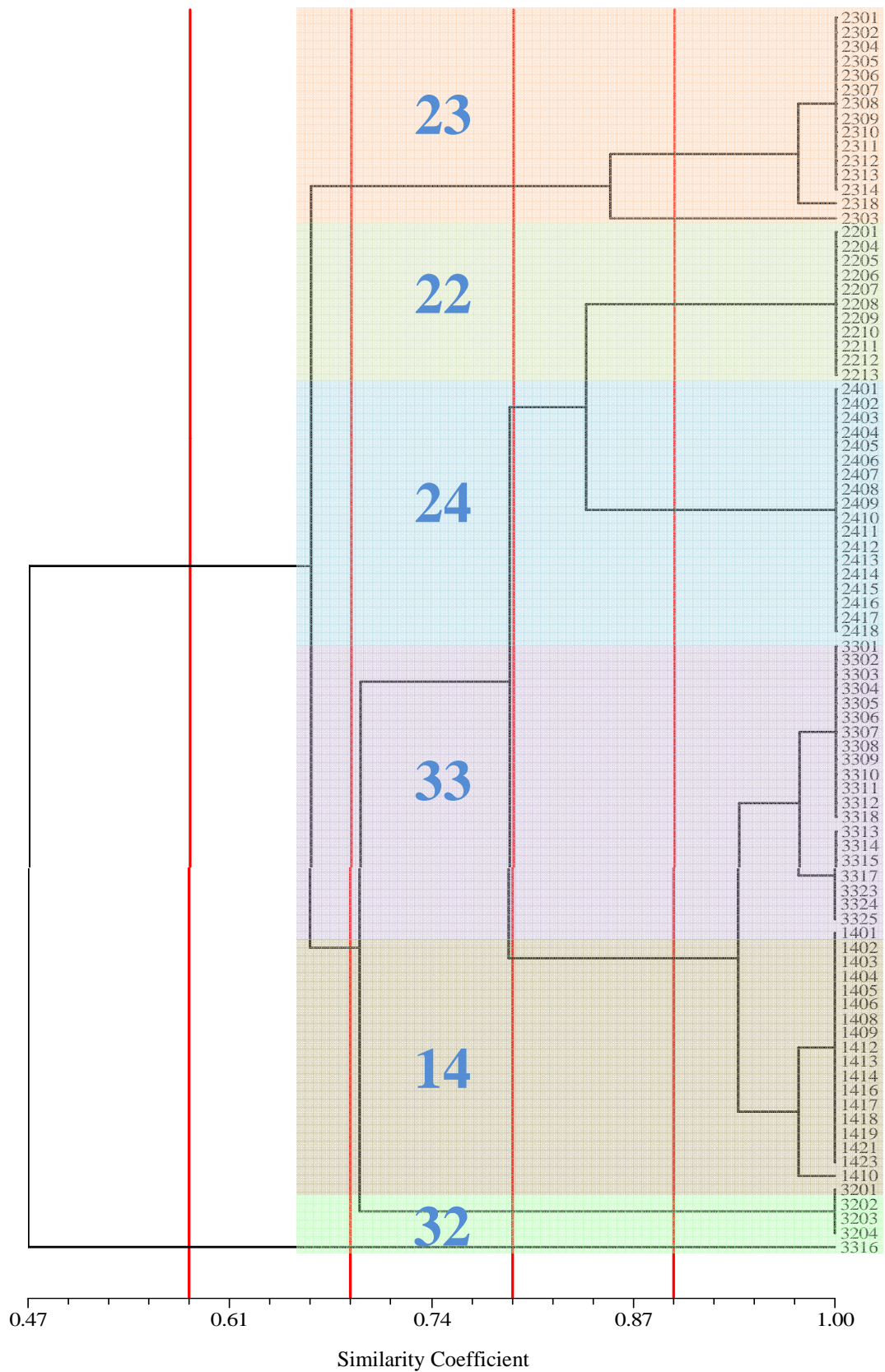


Figure 1. A dendrogram based on the UPGMA clustering of the Nigeria pisifera clones using the similarity coefficient of Dice [9] and Nei and Li [14].

Table 1. Number of variant ramets showing different marker profiles and number of loci exhibiting variant alleles among different population of *Pisifera* Nigeria based on 12 tested SSR loci.

Population	Cluster	No of individual	No of loci exhibiting variant alleles	Coefficient similarity
# 14	14-a	17	0 (uniform)	1.00
	14-b (1410)	1	1	0.97
# 23	23-a	13	0 (uniform)	1.00
	23-b (2303)	1	5	0.86
	23-c (2318)	1	1	0.97
# 33	33-a	13	0 (uniform)	1.00
	33-b	7	1	0.97
	33-c (3316)	1	10	0.47