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GENETIC STRUCTURE OF NIGERIAN AND ANGOLAN OIL PALM (*ELAEIS GUINEENSIS JAQC.*) POPULATION BASED ON FRUIT COLOR PIGMENTATION

F. WENDRA[1,3*], R.A. SUWIGNYO[2], E.S. HALIMI[2], U. SARIMANA[3], P. ERIKA[3], Y. PUJIASTUTI[3], J. HERRERO[4], G. B. SANTIKA[3], E. RITTER[4], Z. SEMBIRING[3], and D. ASMONO[3]

[1]Department of Crop Sciences, Sriwijaya University, South Sumatera, Indonesia
[2]Department of Agronomy, Faculty of Agriculture, Sriwijaya University, South Sumatera, Indonesia
[3]Department of Research and Development, PT Sampoerna Agro Tbk, Palembang, Indonesia
[4]NEIKER - Basque Research and Technology Alliance, Arkaute, Spain
*Corresponding author's email: fahmi.wendra@sampoernaagro.com
Email addresses of co-authors: rujito@unsri.ac.id, esh@unsri.ac.id, upit.sarimana@sampoernaagro.com, pratiwi.erika@sampoernaagro.com, yunita.astuti@sampoernaagro.com, baitha.santika@sampoernaagro.com, jherrero@neiker.eus, zulhermana.sembiring@sampoernaagro.com, dwi.asmono@sampoernaagro.com

SUMMARY

The oil palm (*Elaeis guineensis*. Jacq) is classifiable into two types based on its fruit color pigmentation—virescens, and nigrescens. Virescent fruits are green at the early stage, and then turn bright orange after ripening. Meanwhile, the nigrescent fruit color is dark purple during fruit formation and soon becomes red and purple after ripening. The heredity of virescent traits is favorable in detecting the ripeness of oil palm fruit bunches. The presented study determined the genetic structure characteristics of oil palms in the Nigerian and Angolan populations based on the Virescens (Vir) gene sequence. The 202 palm landraces used included 172 from Angola and 30 from Nigeria (1X and 5X). The young tissues of each palm served for DNA extraction. DNA analysis used a single fragment of a *Vir* gene, totaling 180 bp of DNA sequence. The results showed that a specific haplotype was prominent in the Nigerian and Angolan populations. The average genetic distance of the population was 0.0031 ± 0.0010. However, the highest genetic distance resulted in the Nigerian population 1X (0.0058 ± 0.0022). The lowest one was in the Angolan population (0.0024 ± 0.0012). The landraces were similar in several haplotypes; however, the Angolan population had more haplotypes than the Nigerian population. The Neighbor-Joining phylogenetic tree showed that landraces clustered based on their haplotypes, but this classification did not match the population.

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Keywords: Nigerian and Angolan oil palm (*E. guineensis*), diversity, fruit color, genetic distance, *Vir* gene, DNA sequencing

Key findings: The results indicated that fruit color-specific single nucleotide polymorphisms (SNP) characterized the oil palm (*E. guineensis*) diversity; however, it did not form a cluster in the population.

INTRODUCTION

The palm oil industry is a prime contributor to the global demand for edible vegetable oil. In Indonesia, oil palm (*Elaeis guineensis*) plantation plays an economic and chief role in various industries (Sujadi *et al.*, 2017), especially in the domestic economy, besides providing crucial substances for food, clothing, cosmetics, and edible oil manufacturing (Putri *et al.,* 2009). Intensive efforts are prerequisites to enhance the productivity of this vital national industry, including optimizing seed quality and exploring the germplasm with the highest genetic diversity (Sujadi *et al.,* 2019).

Oil palm is a monoecious species, and its pistillate and staminate flowers appear on the same plant although located on a separate receptacle (Corley and Tinker*,* 2017). These flowers may occur in alternating cycles, producing unisexual male and female inflorescences. The typical fruit considerably varies in external appearance, specifically during the ripening process. In addition, the fruit exocarp appears more pigmented on the outer side than on the inner fruit of the bunch.

Fruit color pigmentation divides the oil palm into Nigrescens or Virescens (Vir). Nigrescens have a color from deep violet to black at the apex and pale to greenish yellow at the base before ripening (Low *et al.,* 2017) (Figure 1). This type of fruit is commonly visible in oil palm, which shows a minimal color variation at the apex during maturation, with at least five loose fruits emerging at harvest (Ying *et al.,* 2007). Contrastingly, Virescens (Vir) fruits are easily identifiable during the ripening season compared with the Nigrescens, showing a distinct color difference during the early and maturity stages. Vir fruit is initially green and turns to orange upon maturity. Therefore, the fruit color is an essential trait that determines the optimal time for harvesting (Singh *et al*., 2013). In oil palm fruit, the causal gene for exocarp color has reached distinction, and the markers are now available for use in breeding for precious selection.

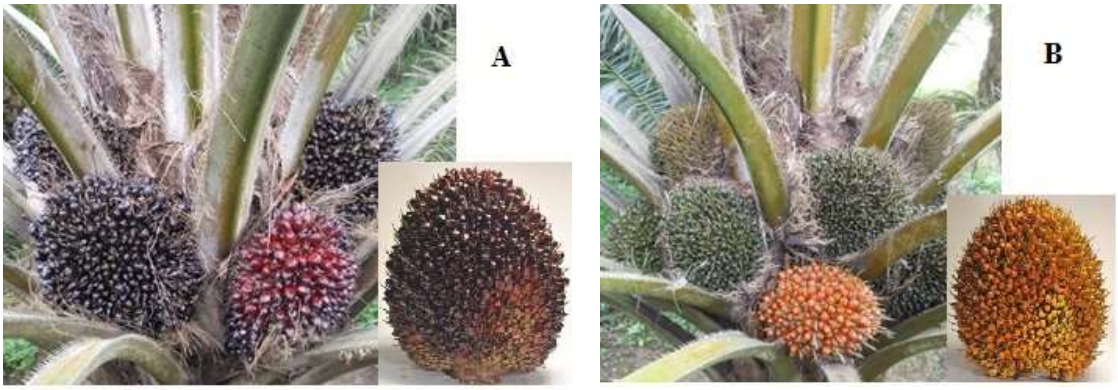

Figure 1. Oil palm fruit color characteristics, A. Nigrescens type (Nig) and B. Virescens type (Vir).

A previous study on the assessment and morphological characterization of oil palm germplasm belonging to the Angolan population found the highest genetic diversity (Maskromo *et al.,* 2017). Genotypes with specific characteristics applicable for breeders to assemble new varieties were available. Among these characteristics, fruit color proved an essential trait for determining the optimal time for harvest. The virescent character is influential in defining the right harvest time, minimizing yield losses during harvest, and maintaining oil quality and other secondary components, such as β-carotene (Singh, 2014).

This research will produce a strategic study of the use of specific characters, especially the virescens, in terms of their contribution, constraints, and potential in oil palm breeding programs. Likewise, it will produce specific virescent primers in Nigerian oil palms. This unique primer can benefit the future selection of male parents for D × P crosses for progeny testing. The breeding strategy to obtain progeny with 100% virescent fruit color characteristics will cross female and male parents, one of which has dominant-homozygous virescent fruit color characteristics (Singh, 2014). The selection to obtain one parent, male or female, with the homozygous dominant virescent fruit color trait is necessary at the next stage of the breeding program.

MATERIALS AND METHODS

Genetic material and genome extraction

The presented study on oil palm transpired in 2021–2022 at the Molecular Laboratory PT Sampoerna Agro Tbk, Palembang, Indonesia. The observations on 202 individual palms comprised Angolan (172) and Nigerian-origin (30) landraces. The Nigerian landraces split into two crossings—5X (Nig5) and 1X (Nig1), with 15 landraces each. The distribution of landraces was according to the existing palm population, considering fruit color characteristics (Table 1). The genomic DNA extraction came from the spear leaves of the individual palms, using a commercial kit (Wizard @ Genomic DNA Purification Kit, Promega Cat. No. A1120) with modifications CTAB (Orozco-Castillo *et al.,* 1994).

DNA amplification

The DNA segments amplification employed the primers, viz., F1-AGC AGC CGC AAG AAA AGT T-3' and R1-5' CAA AGC AAG TCA TCC CAT CC-3', F2-5' TGG TCA GAA GAT CAG CAA TCA-3' and R2-5'ACT TGC ATG GAA ATT TCA GG-3' (Singh *et al.,* 2014). These specially designed primers were for DNA associated with fruit color characteristics through laboratory optimization. The template used in designing Primer-3 software followed the methodology of Singh *et al.* (2013). Polymerase Chain Reaction (PCR) helped amplify approximately 229 bp fragments.

The procedure comprised 25 µl reaction mixture containing 12.5 µL of Go Tag Green Master mix (Promega, Cat. No. 7122 USA), 0.5 µL each primer pair 20 mM, 1 µL DNA template, and 10.5 µL deionized water (ddH2O). The thermocycler conditions contained several processes, such as initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 30 s, annealing at 54 °C for 60 s, extension at 72 °C for 60 s, and final extension at 72 °C for 7 min, with 35x cycles (Ritter *et al.,* 2015). Separating PCR products ensued on a 1% agarose gel earlier stained with Gelred

Table 1. Summary of the oil palm landraces identified based on phenotype, genotype, and the accuracy.

Landraces	Phenotype		Total	Genotype		Accuracy of genotype/ phenotype
	Nig	Vir		Nig	Vir	
Angola	94	78	172	92	78	98.8%
Nigeria	10	20	30	10	17	100%
Total	104	98	202	102	98	99.0%

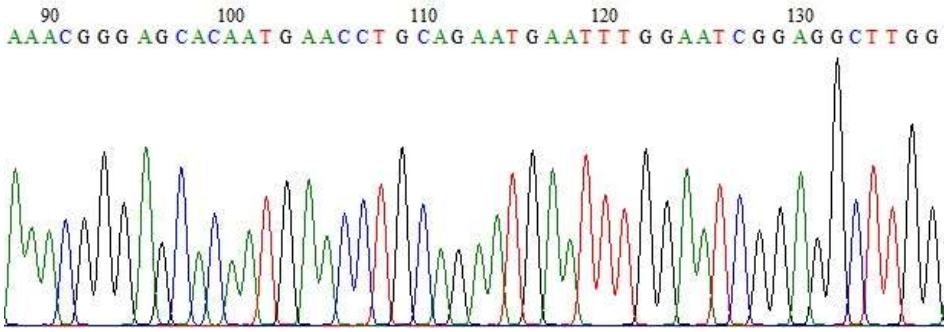

Figure 2. The sample of single nucleotide sequence analyses by using Sanger sequencing.

(Biotium). Afterward, visualization under UV Trans-illuminator helped document the products. Sequencing continued at First Base, Malaysia, using automated DNA sequencing with ABI 3730 XL (Applied Biosystems, USA).

Data analysis

DNA sequence editing and analysis used the MEGA X program (Kumar, 2018) while employing Clustal W for sequence arrangement and nucleotide variations (Tamura *et al.,* 2011). The number of polymorphic sites, number of haplotypes (Hap), haplotype diversity (Hd), and nucleotide diversity (Pi) reached measuring through the DnaSP-6. The data based on the analysis of genetic diversity followed the method of Sarimana *et al.* (2021). Furthermore, the phylogeny tree construction continued with the Kimura 2-parameter evolution model (Kimura, 1980), and 1000x bootstrap replication used the Neighbor-Joining and Maximum Likelihood (ML) analysis methods (Tamura *et al.,* 2007). Based on the findings of Singh *et al*. (2014), there are five different *Vir* gene events, using four in this study as an out-group while constructing the phylogenetic tree.

RESULTS AND DISCUSSION

Haplotype identification and composition

The *Vir* gene fragment succeeded in amplification at 229 bp; however, its analysis continued only at 180 bp of sequence. The DNA sequence comprised the following nucleotide composition: Thymine (T/U) = 22.23%, Cytosine (C) = 11.67%, Guanine (G) = 34.90%, and Adenine (A) = 31.20%. The Guanine (G) comparatively had the highest composition, with the lowest owned by Cytosine (C). The performance of the sample nucleotide sequence is available in Figure 2.

Recognizing 202 landraces came from two individual oil palm types. They showed four alleles and six haplotypes (Table 2) distributed across various populations (Table 3). Haplotype-1 (Hpa-1) appeared in both origins with 38 individuals (Nig: 17 and Ago: 21), while Hpa-2 to Hpa-4 resulted in the Angolan origin. The only oil palm with Hpa-2 was A038_14, Hpa-3 with six members, and Hpa-4 composed of 50 members. Furthermore, three individual oil palms occurred with Hpa-5 and were specific to a Nigerian origin (Nig-1 and Nig-5). Most oil palm individuals (104) had a *Vir* wild-type gene in Hpa-6. In each population, the composition of haplotype frequencies emerged in Table 3. One specific haplotype surfaced on Nigerian origin (Hpa-5) with frequencies of 0.13 on Nig-1 and 0.07 on Nig-5. However, the frequencies of Hpa-2, Hpa-3, and Hpa-4 were specific to Angolan origin, with values of 0.01, 0.04, and 0.29, respectively.

In the presented analysis, all individuals having the nucleotide sequence of the *Vir* gene DNA bore comparison. Variations were visible in four alleles, starting from the 15th base and ending at the 128th base site. The separately conducted analysis showed distinct patterns of variation in each

Table 2. Haplotypes identification summary of 202 landraces of oil palm using SSR markers.

Number of Haplotypes	Alleles (bp)			
	15	23	98	128
Hpa-1	0	1	0	0
Hpa-2	1	1	0	0
Hpa-3	1	0	0	0
Hpa-4	0	0	1	0
Hpa-5	0	0	0	1
Hpa-6	0	0	0	0

Table 3. Frequency of allele composition based on haplotypes of two oil palm populations.

Number of Haplotypes	1	2	3
Hpa-1	0.13	0.67	0.47
Hpa-2	0.01	-	-
Hpa-3	0.04	-	-
Hpa-4	0.29	-	-
Hpa-5	-	0.13	0.07
Hpa-6	0.55	0.20	0.47

Note: Population from Angola (1), Crossing Nigeria 1X (2), Crossing Nigeria 5X (3), and number of haplotypes (Hpa-1 to Hpa-6).

Table 4. Genetic diversity based on haplotypes of two population landraces.

Origin	Genetic variabilities								
	Number of population	n	h	S	A	ɳ	Hd	He	P (%)
Angola	1	172	5	4	176	0.00305	0.489	0,600	86,12
Nigeria	2	30	3	2	178	0.00307	0,528	0,529	15,01

Note: n: number of individuals per population, h: haplotype number, S: number of variation sites, A: number of non-variation sites, ɳ: nucleotide diversity, Hd: haplotype diversity, He: main of pairwise, P: percentage of haplotype polymorphism.

population, including five sites (Angola) and three sites (Nig-1 and Nig-5). The Angolan population had a specific site in the 15th base: A; however, the other population had G in site 98th: T.

The examined base pairs totaled 180. The Angolan origin had the highest number of variations but had the fewest non-variation sites (176) than other populations. Nigeria, on the other hand, only had two variations and 178 non-variation sites. The nucleotide (ɳ) and haplotype diversities (Hd) were high in the Nigerian population. The opposite case was evident in the Angolan population (Table 4). The average number of nucleotide diversity (k) in these two populations was 0.628.

Genetic diversity

The genetic diversity ranged from 0.00392 to 0.00581 in 202 observed landraces from two individual oil palms. The genetic distance between two landraces indicated sequence similarity. The estimation of evolutionary divergence over sequence pairs between the groups appears in Table 5. The average number of base substitutions per site across all the sequence pairs occurred for each group. A bootstrap procedure gave the standard error estimate(s) above the diagonal (1000 replicates). The Kimura 2-parameter model served the analysis (Kimura, 1980). Employing a gamma distribution (shape parameter = 1)

Table 5. Average genetic distance within oil palm populations based on DNA sequence of the *Vir* gene.

Population	Genetic Distance
E. guineensis (population Nig1)	0.0038 ± 0.0027
E. guineensis (population Nig5)	0.0037 ± 0.0029
E. guineensis (population Angola)	0.0031 ± 0.0020

Table 6. Analysis of molecular variance (AMOVA) for the observed five alleles and six haplotypes.

Source of variation (SV)	d.f.	Sum of squares	Variance components	Percentage of variation (%)	Fixation Index
Among origin	2	18,851	0,154	20,525	F_{IT} = 0.99671
Among population within origin	199	237,463	0,595	79,146	F_{IS} = 0.99586
Within population	202	0,500	0,002	0,329	F_{ST} = 0.20524
Total	403	256,814	0,752		

Note: AMOVA calculation performed by 1000 permutation. Degree of freedom (df), fixation index of sample among origin (F_{IT}), fixation index among population within origin, fixation index within population levels (F_{ST}).

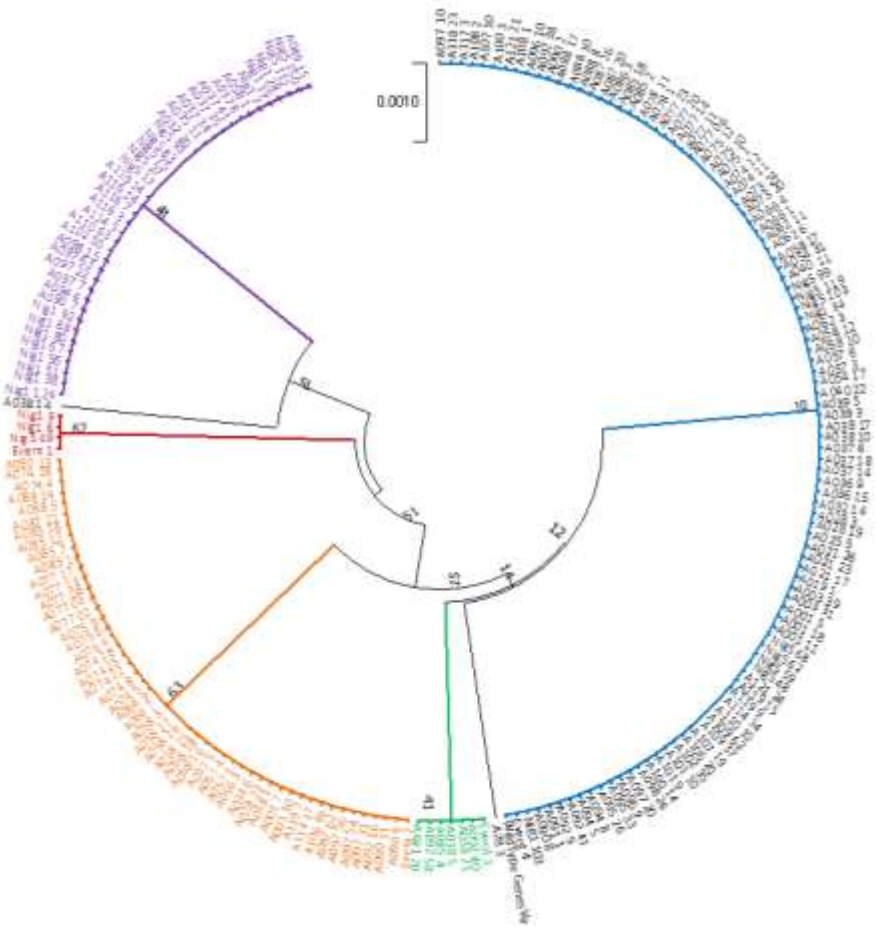

Figure 3. Topology Neighbor-Joining tree based on 180 bp gene Vir sequence data using Kimura-2-parameter substitution model with 1000 bootstrap from 202 oil palm landraces. Event 1: red (Hpa-5), event 2: (Hpa-4) orange, event 3: (Hpa-3) green, wild type gene *Vir* (Hpa-6) blue, and new allele (Hpa-1) purple.

modeled the rate variation across the sites. The AMOVA analysis showed significant variations among the origins, population within origin, and population. However, the highest variation percentage level was notable among the population within the origin (79.145% and Fst = 0.04610) (Table 6).

Based on the Neighbor-Joining (NJ) phylogenetic tree, the landraces used in the latest study did not segregate by origin despite solid support with a 99% bootstrap value. These results gained further authentication by the genetic distance analysis (Figure 3). The blue and green groups proved closely related. They appeared on the same branch with bootstrap values of 12% (NJ). Meanwhile, the blue and red groups were the most distant, occupying different clades on the same large branch with bootstrap values of 62% (NJ). Three huge clades, generated with a 1000 bootstrap value, showed a close kinship between the individuals. The large group comprised most individuals (104 palms) with no nucleotide change, and only two palms were apart from the said group (A078_3 and A038_14).

DISCUSSION

Fruit color is a considerably significant trait in certain plants, such as apples, grapes, and oil palms, with numerous reports from many studies (Lijavetzky *et al.,* 2006; Vimolmangkang *et al.,* 2013). The presented research showed that four different alleles occurred in oil palm. Three of these alleles, namely, allele 1 (event 3), 3 (event 2), and 4 (event 1), proved a match with previous studies as reported by Singh *et al.* (2013). Allele 2 was new and existed between exons three and one. Among six haplotypes, five belonged to the Angolan population. The other was specific to the Nigerian population. Singh *et al.* (2013) also reported that Hpa-5 was only notable in the Nigerian palm population. However, this study also detected the Hpa-1 in the Nigerians. Possibly, the reason was the Nigerian material tested came from Congo. According to Singh *et al.* (2013), Hpa-1 is specific to the oil palm from Congo.

The highest number of haplotypes indicated huge variations. This evidence is visible in the Angolan population. Although specific haplotypes showed diversity sources, the presented study could not conclusively describe the state of diversity, as the populations also shared a haplotype (Hpa-6). These findings were consistent with the previous report of Singh *et al.* (2014), who described that African oil palms have the maximum diversity for morphological characteristics and the broadest wild stands. The study also found that none of the populations had a distinct haplotype from others, meaning that shared haplotypes existed among different oil palm populations. The results further indicated that the haplotype diversity was also minimal, meaning that the composition of each base was not diverse.

The nucleotide diversity value in each population was also low. It could be due to the limited variations in the nucleotide at the DNA sequence sites. This evidence manifests from the seven numbers of sites, as supported by the cladogram. These results implied that diversity based on the *Vir* gene is unfavorable when identifying the palm population. The genetic distance found in this study was relatively smaller than an oil palm population in a previous study (Arias *et al.,* 2015; Prasetyo *et al*., 2000; Fitmawati *et al*., 2022). In another study, the genetic distance between individuals was 0.315 (*E. guineensis*), using 14 SSR markers (Ithnin *et al.,* 2017), which might suggest a close relationship among the oil palm individuals. The small genetic distance extended to both within and among the populations.

In the promising study, the genetic diversity values were much lower than in previous studies. For better understanding, the observed genetic diversity's comparison used several molecular markers, including isozyme (mean of Fst = 0.301) (Hayati *et al.,* 2004), a random amplified microsatellite method (0.15) (Cardona *et al.,* 2018), and a genetic marker method (Bakoume *et al.,* 2015; Okoye *et al.,* 2016). In the entire palm population of the present study, the genetic diversity was only 0.24312. The possible reason was the markers used were specific only to the *Vir* genes.

The phylogenetic tree illustrated the haplotype DNA sequence of *E. guineensis* shared by different population individuals. The closest relationship was evident between populations Nig-1 and Nig-5, which shared the same haplotype. Some individuals also shared the same haplotype with the Angolan population. This close relationship was unstable. It was probably due to a relatively small bootstrap value.

CONCLUSIONS

The study's use of the *Vir* gene helped access the genetic diversity in oil palm (*E. guineensis*). Six haplotypes and four alleles emerged in 202 oil palm landraces belonging to two individuals of *E. guineensis*, Angolans, and Nigerians. Only one specific haplotype resulted in Angolan and Nigerian origins. The genetic hierarchies and differentiations estimated by AMOVA showed significant differences among individual palms in the population.

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