DOI: http://dx.doi.org/10.1590/1678-4499.2017385

Genetic diversity in oil palm (*Elaeis guineensis* Jacq) using RAM (Random Amplified Microsatellites)

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ABSTRACT: The oil palm (*Elaeis guineensis* Jacq) plays an important role in the economy of some tropical countries; Colombia ranks first for production in Latin America and fifth in the world. The aim of this research was to characterize the genetic variability of 51 oil palm genotypes from the Congo with seven randomly amplified microsatellite markers RAM. As a result, 241 alleles were generated and the number of polymorphic loci ranged from 46 to 14 for the ACA and CGA primers, respectively. High genetic diversity was found, with a total heterozygosity of 0.64, along with a high percentage of polymorphic loci, 89%. The coefficient of genetic differentiation (F*st*) was 0.15, indicating that there was moderate genetic differentiation. The genetic diversity study differentiated the 51 genotypes into four groups with a similarity of 0.52. The RAM technique detected the genetic variability of the palm genotypes and showed a high degree of polymorphism and sensitivity for discrimination. These results provide information that can be used to develop conservation strategies for palm germplasm and breeding programs to obtain more productive palm genotypes with superior quality and tolerance to major diseases.

Key words: breeding, genetic markers, germplasm, genetic structure.

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Received: Nov. 4, 2017 – Accepted: Jan. 2, 2018

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is native from Africa and specifically endemic to the southeastern states of Nigeria (Maizura et al. 2006; Bakoumé et al. 2015).

In the year 2015 in the South America, the largest cultivated areas are in Colombia, with more than 400.000 ha (4th largest producer in the world and 1st in America) (RSPO 2016), followed by Ecuador with 240 ha and Brazil with 130 ha (Fedepalma 2016).

The study of oil palm is of enormous economic importance in Asia, Africa and South America, with a wide market of products that include cooking oil, palm kernel, animal feed, soaps and detergents, among many others (Rance et al. 2001).

Because of the perennial characteristic and long cycle of oil palm cultivation, conventional breeding might take several years. This greatly hampers rapid and effective progress on the selection of individuals. Several techniques of molecular biology are currently available to detect genetic variability and similarity between individuals and can be used in various applications of a breeding program (Arias et al. 2012).

Molecular marker methodologies for DNA have broadened the field of crop breeding. Crops genome analyses based on detection of polymorphisms allows the development of gene linkage maps, fingerprints identification, marker-assisted selection and analysis of genetic relationships (Rocha 2003).

The use of molecular markers for oil palm in Colombia started with the preliminary study on tolerant and susceptible materials to bud rot (PC) by Ochoa et al. (1997), who used 303 RAPD primers for looking associations with some PC tolerant materials in populations at the nursery stage. Arias and Rocha (2004) evaluated *E. guineensis* and *E. oleifera* genotypes from Cenipalma's germplasm bank with RAPD, SSR and AFLP markers for selecting resistant or tolerant materials to this disease, and Villegas et al. (2000) evaluated the genetic diversity of 126 genotypes from the Promociones Agropecuarias Monterrey Plantation with 12 RAPD.

Some molecular markers and protein markers have also been used to investigate genetic diversity in oil palm germplasm such as: Restriction Fragment Length Polymorphism (RFLP) of ribosomal DNA (Rajanaidu et al. 1989; Jack et al. 1995; Shah et al. 1993); random amplified polymorphic DNA (RAPD) (Shah et al. 1994; Sathish and Mohankumar 2007; Ochoa et al. 1997; Villegas et al. 2000); microsatellite markers or SSR (Chavarro et al. 2016; Arias et al. 2014; Zhou et al. 2015); RFLP using cDNA probes (Singh et al. 2008); amplified fragment length polymorphism (AFLP) (Galeano 2005); and isoenzyme markers (Purba et al. 2000; Choong et al. 1996).

In general, the genetic diversity of *E. guineensis* Jacq. has been evaluated using molecular markers in wild populations of Africa (Hayati et al. 2004; Barcelos et al. 2002; Mayes et al. 1997; Shah et al. 1994), South America (Billote et al. 2001; Barcelos et al. 2002) and commercial plantations (Villegas et al. 2000). In addition, the genetic diversity of *E. oleifera* species, a native palm from the Americas, has also been evaluated with RAPD markers by Moretzsohn et al. (2002). These studies have provided data that give an idea of the intra- and inter-specific variability of populations, possible dispersion ways and the appropriate materials for breeding, taking into account morphological characteristics and genetic distances.

The Randomized Amplified Microsatellite (RAM) technique is based on Polymerase Chain Reaction (PCR). This methodology is highly reproducible and detects the polymorphism on intra and interspecific DNA. These markers obtained by RAM could be used for population studies and it does not require previous knowledge of sequences or the use of radioactive isotopes (Hantula et al. 1996). The DNA amplified fragments during the reaction are composed by two microsatellites that are close enough for amplifying the area between them by PCR (Zietkiewicz et al. 1994). The source of variability in the fragments obtained may be due to an insertion or deletion between the amplified fragment that could produce a wide polymorphism or the absence of a product, or even variability into microsatellite replication numbers could determine the level of polymorphism (Hantula et al. 1996). The genetic basis of these markers is the same as RAPD because it uses a single primer of arbitrary sequence, therefore the target sequence is also unknown. However, RAM's markers differ from RAPD because the latter have a smaller primer size than RAM. Like RAPD, RAM detects only one allele per locus (Hantula et al. 1996).

The objective of this study was to characterize the genetic diversity of oil palm from the Congo collection of the Unipalma Germplasm Bank using RAM molecular markers, generating fundamental information for the orientation of conservation strategies and use of genetic resources.

MATERIALS AND METHODS

Plant Material and DNA Isolation

The 51 genotypes are part of the Unipalma's oil palm (*Elaeis guineensis* Jacq) germplasm collection originally →

collected from the Republic of Congo (formerly Zaire) and they are located near to the Veracruz Village, municipality of Cumaral (Department of Meta), 241 masl, 4°13'33" N and 73°14'50" W. The DNA isolation was carried out in the Plant Biotechnology Lab and Animal Genetics and Reproductive Lab of the Universidad de Los Llanos, municipality of Villavicencio (Meta), 467 masl, 4°04'30" N and 73°35'07" W.

Total genomic DNA was obtained from young leaves. Tissue was ground by means of liquid nitrogen and the DNA was isolated using the modified protocol of Doyle and Doyle (1990). The quality of DNA was tested by electrophoresis on a 0.8% agarose (w/v) gel. DNA concentration was measured by using a NanoDrop ND-1000 spectrograph. Samples were diluted in sterilized water at a concentration of 10 ng/µl.

RAM Molecular Markers

Eight RAM markers were evaluated with the following amplification conditions: 1X buffer solution, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.625 U Taq Polymerase, 2 µM primer, 0.4 mg/ml BSA (Bovine Serum Albumin) and 10 ng genomic DNA in a final volume of 25 µl. In each group of samples, a negative control was included to detect possible contamination (Hantula et al. 1996).

The amplification conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 37 cycles of a denaturation at 95 °C for 30 sec, a hybridization step at 50 °C – 58 °C for 45 sec (depending on the primer used, Table 1) and extension at 72 °C for 2 min, with a final extension of 72 °C for 7 min. The amplified products were visualized with 7% polyacrylamide gels (37:1 acrylamide-bisacrylamide) at 160 volts for 1 h and stained with silver salts, as described in the standard protocols (Sambrook et al. 1989).

Table 1. RAM markers used to determine the genetic diversity in the oil palm.

Marker	Sequence (5' a 3')	Hybridation T°
CCA	DDB(CCA)5	55
CGA	DHB(CGA)5	58
GT	VHV(GT)5G	58
AG	HBH(AG)7A	50
CT	DYD(CT)7C	55
TG	HVH(TG)7T	55
CA	DBDA(CA)7	50
ACA	BDB(ACA)5	50

The following designations were used for the degenerated sites: H (A or T or C); B (G or T or C); V (G or A or C); and D (G or A or T).

Statistic Analysis

A binary matrix of presence (1) and absence (0) was obtained. For the selection of polymorphic bands, a frequency of the most common allele less than 95% was considered as polymorphic locus. Genetic similarity between individuals was calculated using the similarity coefficient of Nei and Li (1979). From the similarity matrix a dendrogram was performed using an agglomerative hierarchical clustering method known as UPGMA by means of the NTSYS-pc SAHN program (version 2.02g, 1998). The cophenetic correlation coefficient, which is a measure between the similarity values of the dendrogram and those of the original matrix of similarity, was calculated using the COPH and MXCOMP options of the NTSYS-pc package program. In order to estimate the mean heterozygous (H) parameters and the percentage of polymorphic loci, the unbiased formula of Nei (1978) from the statistical package TFPGA (Tools For Population Genetic Analyzes, version 1.3, 1997) was used. The unbiased f statistic was determined with a 95% confidence interval. The genetic structure of the germplasm collection was examined using a Bayesian statistical methods from software Structure version 2.3.4 (Pritchard et al. 2000; Falush et al. 2003). Ten runs were performed by setting the number of clusters (K) from 1 to 12. Each run consisted of a burn-in of 50,000 followed by 100,000 Markov Chain Monte Carlo replications, assuming the admixture model and correlated allelic frequencies. The ΔK value was computed to detect the most probable number of clusters (Evanno et al. 2005). The run with the highest Ln Pr $(X|K)$ value of the 10 was chosen and presented as bar plots per genotype. Individuals with membership probabilities ≥ 0.70 were assigned to corresponding clusters, and individuals with membership probabilities < 0.70 were assigned to a mixed group.

A multiple correspondence analysis (MCA) was performed to associate columns and rows of the binary matrix by determining the association level or determining proximity (Joseph et al. 1992).

RESULTS AND DISCUSSION

The protocol of Doyle and Doyle (1990) yielded a DNA of good quality and quantity for all tissue samples from the evaluated wild palm materials. The genomic DNA concentration ranged from 6.6 to 964 ng/µl.

Seven out of 8 RAM primers that were used to evaluate the oil palm genetic diversity yielded amplified products. Only the GT primer did not amplify any of the evaluated samples. Seven primers generated 241 alleles, with a minimum of 17 alleles on the CGA primer and a maximum of 47 alleles for the ACA primer, with molecular weights between 100 and 1200 bp (Table 2). The number of alleles obtained was adequate to estimate genetic diversity into the *Elaeis guineensis* germplasm and was reliable for statistical analysis (Nybom and Bartish 2000).

The average of observed heterozygosity (Ho) was 0.4135, indicating that the genetic diversity in the oil palm species was relatively high. The expected mean heterozygosity (He) for the total population was 0.64, with a range between 0.35 for the AG primer and 0.98 for the ACA primer, indicating that there was high genetic diversity. This value is similar to that reported by Khierallah et al. (2011), who evaluated the genetic diversity of 30 oil palm cultivars with 22 microsatellite markers, finding a high level of polymorphism and an expected heterozygosity of 0.503 for all cultivars. Okoye et al. (2016a) reported high heterozygosity and genetic diversity values of 0.69 and 0.70, respectively, when studying genetic diversity among NIFOR (Nigerian Institute For Oil palm Research) palm parentals with 10 microsatellite markers. Okoye et al. (2016b) found average values of observed and expected heterozygosity of 0.63 and 0.76, respectively, when studying the genetic relationships between 26 oil palm materials from Nigeria and Malaysia with nine microsatellite markers. Bakoumé et al. (2015) reported Ho and He values of 0.46 and 0.64, respectively, using 16 microsatellite markers. Arias et al. (2015) studied the genetic variation of 788 oil palm accessions using 29 microsatellite markers, and the results revealed important genetic diversity (H_T = 0.759) between oil palm accessions from Angola and Cameroon.

One interesting thing was that, despite of evaluating only seven RAMs over 51 palm genotypes, the He value between 0.35 and 0.98 was higher than those reported by Ting et al. (2010) using 15 SSR markers (Ho and He values of 0.40 and 0.44, respectively), or those reported by Zhou et al. (2015) with 21 polymorphic microsatellite loci (Ho values of 0.37 and 0.40, with an average of 0.39). Also, Rocha-Salavarrieta et al. (2007) found a He value of 0.515 when assessing the genetic diversity of 72 oil palm genotypes from Angola with 16 microsatellite loci. These data indicate that RAM markers could be used to identify polymorphic loci to calculate genetic variation between oil palm genotypes. In addition, the genetic variability found in Congo germplasm can be very useful for a genetic improvement program into the oil palm breeding program because it will allow to identify genotypes of interest that contribute with new allelic variants and therefore being used as parental for the generation of new crosses with better characteristics of agronomic importance for this crop.

The progress of genetic improvement processes relies upon the efficient and systematic use of the genetic resources. This strategy will allow the development of agronomically improved materials through the inclusion of wild and/or landraces germplasm or even from related species containing part of the genetic diversity as a result of both selection and mutational processes in response to adaptation to biotic and abiotic factors (Rey et al. 2007).

Table 2. Estimated Heterozygosity (He), number of loci, number and percentage of polymorphic loci for the seven evaluated RAM primers in 51 *Elaeis* materials.

Marker	No. of Loci	No. of polymorphic Loci	Estimated He (He)	% of polymorphic Loci (95%)
CGA	17	14	0.49	84.4
CT	34	30	0.66	86.9
CA	43	39	0.64	90.2
AG	26	21	0.35	79.8
TG	45	42	0.78	93.7
CCA	29	26	0.53	88.6
ACA	47	46	0.98	96.9
Total	241		0.64	88.6

The highest percentage of polymorphic loci was found for the ACA primer (96.9%) and the lowest for the AG primer (79.8%), with a mean of 88.6 (Table 2). Probably, the high polymorphism found with the primer ACA and its high He (0.98) was due to a higher frequency between the hybridization sites and the complementary DNA strands of that sequence. Because the TG primer exhibited also both high percentages of polymorphic loci and heterozygosity (93.7 and 0.78, respectively), is an indication that these two primers had the highest contribution to the genetic diversity and are suitable for using them for evaluating genetic diversity and population structure of any oil palm germplasm collections.

The Fst (Genetic differentiation Coefficient) mean value was 0.15 (s = 0.05; 1000 replications) indicating that there was moderate genetic differentiation (Wright 1978) on this germplasm collection (Table 3). The ACA primer was the one that made the greatest contribution to the variation with a Fst value of 0.37, which means that it is a good candidate for assessing genetic differentiation between oil palm germplasm.

These results indicate that there may have been some degree of gene flow between individuals on the original site where this oil palm germplasm was collected, therefore, there would be no barriers or marked differentiation between them. Genetic differentiation is primarily a function of gene flow between populations through pollen and seed dispersal (Loveless and Hamrick 1984). Species with discrete or isolated populations experience less gene flow than species with larger or contiguous populations; therefore, they have a relatively lower level of variation within populations and a greater variation between populations. High levels of population differentiation are supported by low levels of gene migration between populations.

Table 3. Population differentiation, F_{st} statistics for the 51 *E. guineensis* materials evaluated with seven RAM markers.

Marker	F_{st}	s
CGA	0.12	0.03
CT	0.16	0.05
CA	0.10	0.04
AG	0.12	0.14
TG	0.13	0.07
CCA	0.08	0.08
ACA	0.37	0.05
Total	**0.15**	**0.05**

Maizura et al. (2006), using RFLP to analyze 359 oil palm accessions from 11 African countries, found that the populations included in the study may have experienced gene flow between populations, resulting in the high level of heterozygosity (He), attributed to the dispersion of seeds through rivers.

Slatkin (1994) and Neigel (1997) show that gene flow could affect the spatial distribution of genes and also processes such as selection, recombination and mutation. Particularly in the oil palm, gene flow could be directly related to the level of pollen migration (mainly from aerial dispersers) and an active dispersion of seeds, avoiding the structuring of the population and favoring the genetic diversity of the species (Ennos 1994; Corley and Tinker 2003).

Diaz et al. (2014) found a low genetic differentiation value (Fst = 0.030, p > 0.001) when evaluating the structure and genetic diversity of 311 oil palm samples from the Republic of Cameroon using 10 microsatellite markers. These authors reported that the Fst value could be associated with the high number of migrants between populations (Nm = 8,093), showing that there is genetic recombination between the evaluated populations, surpassing the effects of drift and impeding local differentiation (Slatkin 1994).

Montoya et al. (2005) reported a Fst value of 0.084 among 48 genotypes from Angola when evaluating with 20 microsatellites. Hayati et al. (2004) reported a Fst value of 0.301 among West Coast and Central African populations. Meanwhile, for the zone comprising the Democratic Republic of Congo, Tanzania and Angola, a Fst value of 0.073 was reported (moderate genetic differentiation).

Barcelos et al. (2002), who studied 38 accessions of *E. guineensis* using RFLP and 22 oil palm accessions with AFLP, reported a low level of genetic structuring within all of the evaluated origins, which was attributed to the dispersion of material without geographical barriers in the African continent.

The result of Bayesian clustering analysis largely agreed with the distance-based cluster analysis. Based on the value of ΔK (Evanno et al. 2005), the 51 genotypes of *Elaeis guineensis* could be grouped into three genetic clusters (Fig. 1). It can be seen that most of the genotypes were grouped in cluster 1 (blue color) and 2 (green color), with a total of 20 and 15 individuals; respectively, eight individuals were grouped in cluster 3 (red color). The remaining individuals were classified into a mixed subgroup. The membership probability was < 0.70 in any given subgroup. The three clusters, on average →

had a membership coefficient (Q value) of 0.89. It can be seen that there are genotypes that mix with other clusters, evidencing that there was a moderate genetic differentiation in the evaluated palm germplasm, which is consistent with the Fst value of 0.15. These results indicate that there may have been some degree of gene flow between individuals, indicating that a majority of the genetic diversity is preserved within populations.

Analysis using the Nei-Li coefficient, at a similarity level of 0.52, differentiated the palm genotypes into four groups (Fig. 2). A high co-phenetic correlation was detected (r = 0.97), which indicates that the dendrogram faithfully represents the values of the similarity matrix (Crisci and López 1983). At a similarity index of 0.70, there was a high concentration of genotypes, suggesting a high similarity between them. This probably happened as a result of several causes as some sort of gene flow between them, due to the natural dispersion of pollen by the entomophilic and anemophilic effect, seeds exchange between producers or even seed movement by animals and water. The latter has a significant impact on the process of seed migration, attributed to part of the process of oil palm dispersion through African rivers and springs (Hayati et al. 2004; Corley and Tinker 2003).

The analysis of the dendrogram revealed the formation of 4 groups. Group A showed the highest number of evaluated genotypes, with a total of 38. Group B had a total of 7 genotypes, while groups C and D were the ones with the lowest number of genotypes (5 and 1 respectively). Group D had the lowest similarity value of (0.37), as compared to the other 50 evaluated genotypes.

In the Multiple Correspondence Analysis (Fig. 3), clusters showed in the dendrogram, we can observe the closeness between groups A and B, moving away slightly from the C group and even more from Group D, in which the genotype identified as 3317 was found. This result suggests that there was genetic differentiation between the evaluated individuals. Typically, allogamous species, which is the case of *Elaeis guineensis*, exhibit high intra-population genetic variation with the consequent detriment of its inter-population genetic variation, being that the divergence within populations is inversely proportional to the amount of genetic flow, so the greater the flow,

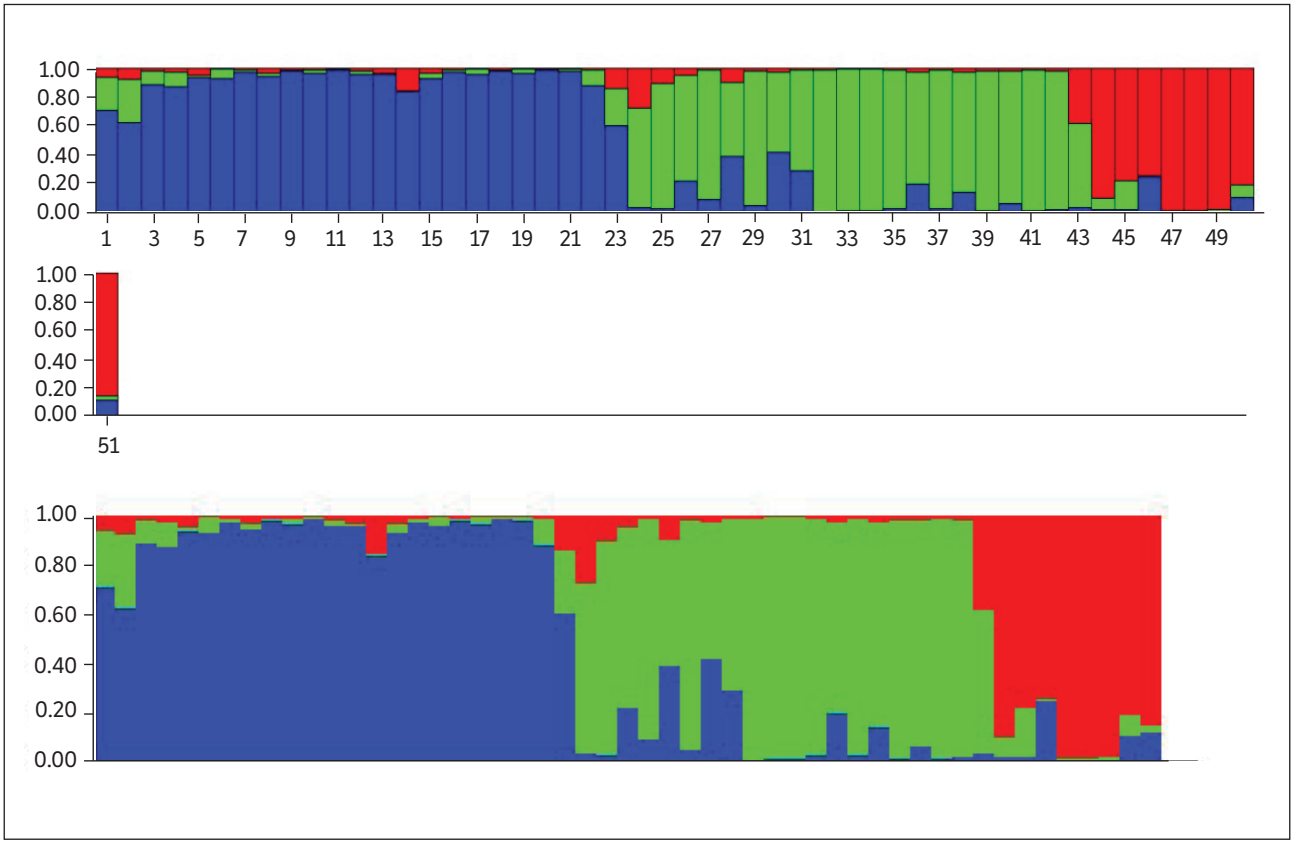

Figure 1. Population structure analysis of 51 genotypes of oil palm using Structure version 2.3.4 (Pritchard et al. 2010). Each vertical line represents one individual genotype. Individuals with multiple colors have admixed genotypes from multiple clusters.

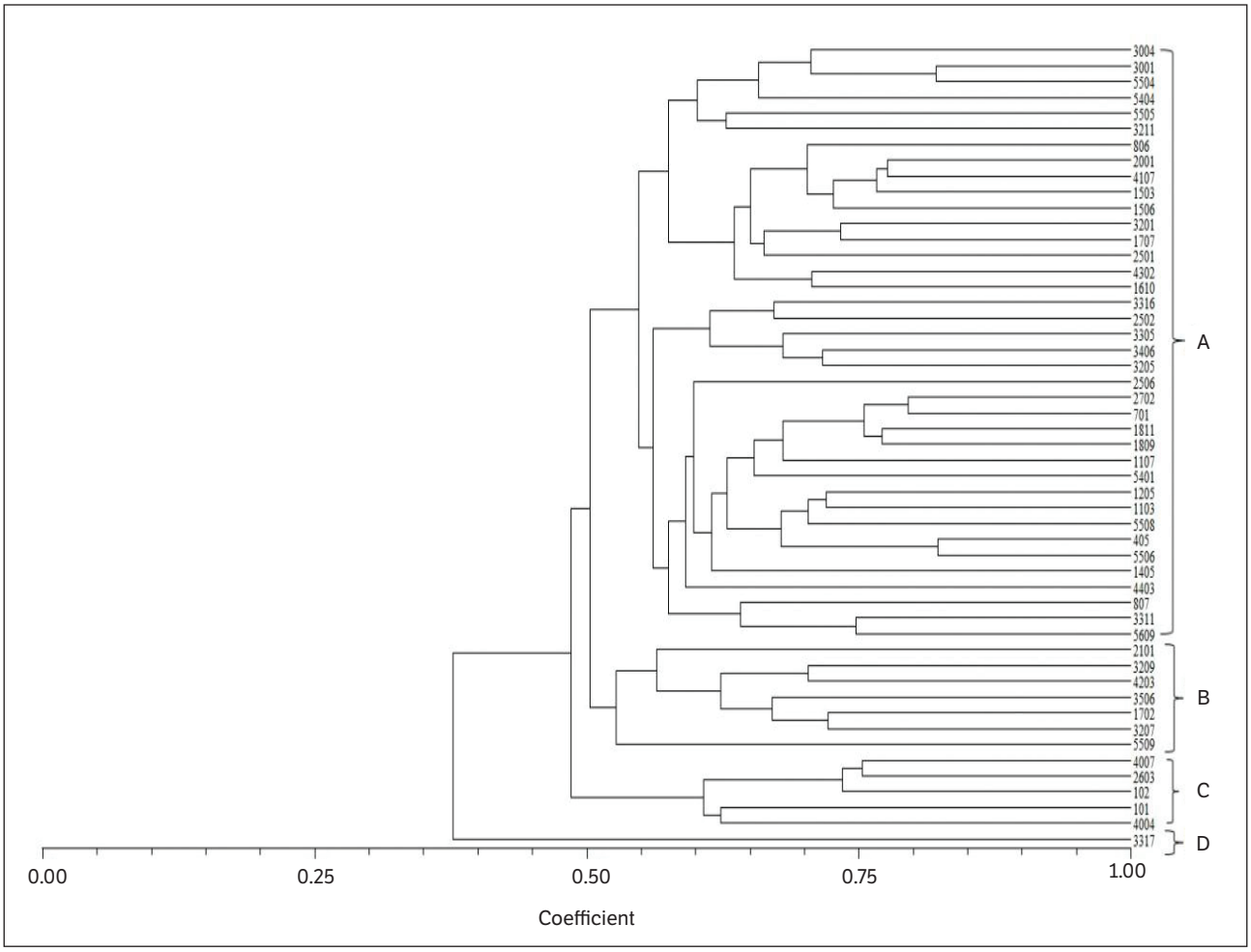

Figure 2. Dendrogram for the 51 *Elaeis guineensis* Jacq genotypes based on the Dice Nei-Li (1978) similarity coefficient and calculated with seven RAM markers with the UPGMA, SAHN and TREE of NTSYs-pc (version 2.02g, 1998) classification methods.

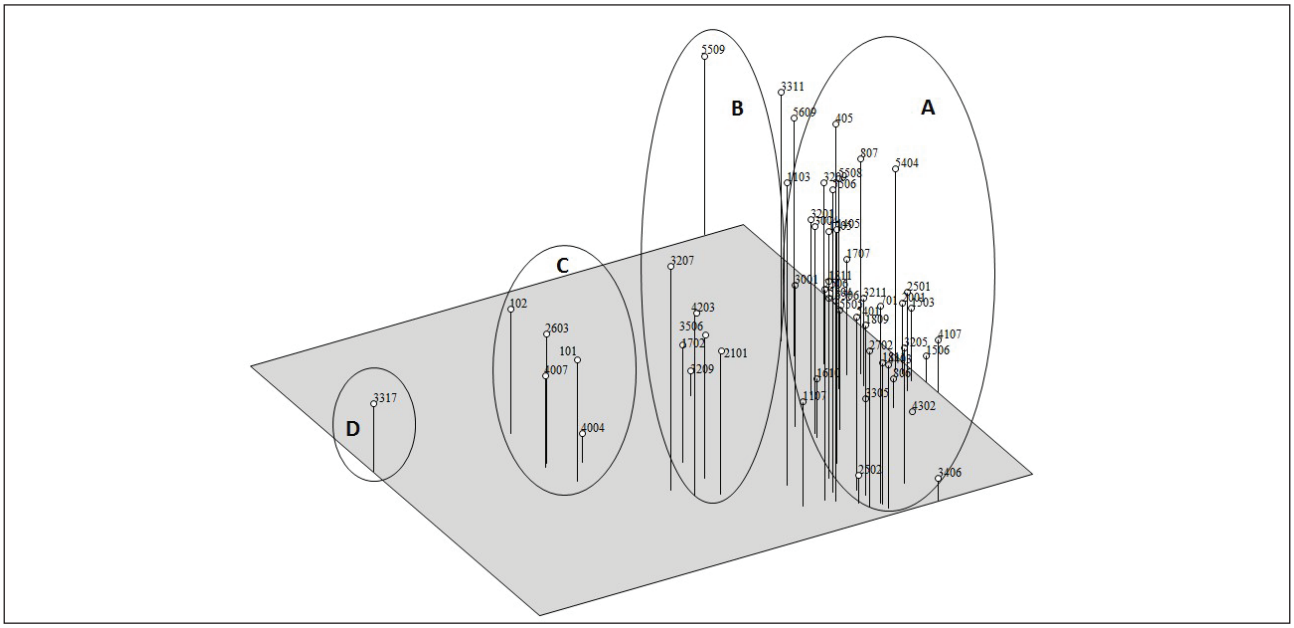

Figure 3. Spatial representation of the genetic structure of 51 *E. guineensis* Jacq individuals using RAM microsatellites.

the lower the intra-population divergence (Hamrick and Loveless 1986).

In germplasm collections, mainly on allogamous plants with strong pollinators activity, as the case of the genus *Elaeis*, high variability is expected within the seeds collected within the same bunch. Molecular markers are an important tool for quantifying variability and its distribution between and within populations (Robinson 1998).

CONCLUSION

The RAM technique was useful in detecting the genetic variability of oil palm genotypes and showed a high degree of polymorphism and sensitivity for their discrimination. This molecular technique is a valuable tool for discriminating oil palm genotypes and assessing their genetic constitution. The high genetic diversity observed in natural oil palm populations indicates that these materials could be used as a good source of new genes for introgression in breeding programs. Therefore, through recombination processes, it is possible to obtain elite genotypes that exhibit characteristics of agronomic importance like high oil yield, better oil quality, and also broad adaptability to biotic and abiotic stresses that limit the crop productivity.

The inclusion of wild genotypes into any oil palm breeding program is of vital importance in order to broaden the genetic basis of the crop and, thereby, ensure the conservation of a wide range of genetic resources of the oil palm.

ACKNOWLEDGMENTS

The authors thank the Empresa Unipalma S.A. and the laboratories of Molecular Biology and Veterinary Medicine and Zootechnics (MVZ) of the University of the Llanos.

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REFERENCES

Arias, D. M. and Rocha, P. J. (2004). Análisis de diversidad genética en materiales tolerantes y susceptibles a la Pudrición del Cogollo en palma de aceite mediante marcadores moleculares. Revista Palmas, 25, 11-27.

Arias, D., González, M. and Romero, H. (2015). Genetic diversity and establishment of a core collection of oil palm (*Elaeis guineensis* Jacq.) based on molecular data. Plant Genetic Resources, 13, 256-265. https://doi.org/10.1017/S1479262114001026

Arias, D., Montoya, C., Rey, L. and Romero, H. (2012). Genetic similarity among commercial oil palm materials based on microsatellite markers. Agronomía Colombiana, 30, 188-195.

Arias, D., Ochoa, I., Castro, F. and Romero, H. (2014). Molecular characterization of oil palm *Elaeis guineensis* Jacq. of different origins for their utilization in breeding programmes. Plant Genetic Resources, 12, 341-348. https://doi.org/10.1017/S1479262114000148

Bakoumé, C. Wickneswari, R., Siju, S., Rajanaidu, N., Kushairi, A. and Billotte, N. (2015). Genetic diversity of the world's largest toil palm (*Elaeis guineensis* Jacq.) field gene bank accessions using microsatellite markers. Genetic Resource Crop Evolution, 62, 349-360. https://doi.org/10.1007/s10722-014-0156-8

Barcelos, E., Amblard, P., Berthaud, J. and Seguin, M. (2002). Genetic diversity and relationship in American and African oil palm as revealed by RFLP and AFLP molecular markers. Pesquisa Agropecuária Brasileira, 37, 1105-1114. http://dx.doi.org/10.1590/S0100-204X2002000800008

Billote, N., Rusterucci, A. M., Barcelos, E., Noyer, J. L., Amblard, P. and Baurens, F. C. (2001). Development, characterization and across taxa utility of oil palm (*Elaeis guineensis* Jacq) microsatellite markers. Genome, 44, 413-425. https://doi.org/10.1139/g01-017

Chavarro, C. F. G., Lozano, M. E. L., Coronado, A. C. M., Ochoa, E. I. and Coronado, Y. M. (2016). Caracterización molecular de

palma de aceite *Elaeis guineensis* Jacq., procedente de diferentes orígenes (Zaire y Camerún) usando marcadores microsatélites. Acta Agronómica, 65, 276-283. http://dx.doi.org/10.15446/acag.v65n3.48413

Choong, C. Y, Shah, F. H, Rajanaidu, N. and Zakri, A. H. (1996). Isoenzyme variation of Zairean oil palm (*Elaeis guineensis* Jacq.) germplasm collection. Elaeis, 8, 45-53.

Corley, R. and Tinker, P. (2003). La palma de aceite. 4th ed. Oxford: Editorial Blackwell Publishing Ltd.

Crisci, J. V. and Armengol, M. F. L. (1983). Introducción a la teoría y práctica de la taxonomía numérica. Washington: CDC, OEA.

Diaz, J. E. P., Moreno, D. M. A., Perez, Z. Z. O. and Angulo, H. M. R. (2014) Diversidad y estructura genética de accesiones de palma de aceite (*Elaeis guineesis* Jacq.) provenientes de Camerún. Revista Colombiana de Biotecnología, 16, 57-67.

Doyle, J. J. and Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. Focus, 12, 13-15.

Ennos, R. (1994). Estimating the relative rates of pollen and seed migration among plant populations. Heredity, 72, 250-259. https://doi.org/10.1038/hdy.1994.35

Evanno, G., Regnaut, S. and Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology, 14, 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Falush, D., Stephens, M. and Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics. 164, 1567-1587.

Fedepalma [Federación Nacional de Cultivadores de Palma de Aceite] (2016). Anuario estadístico 2016. La agroindustria de la palma de aceite en Colombia y en el mundo 2011-2015. Bogotá: Fedepalma.

Galeano, C. H. (2005). Estandarización de la técnica molecular de AFLP en palma de aceite tipo Dura (*Elaeis guineensis* Jacq.) y estudio preliminar de caracterización. Agronomía Colombiana, 23, 42-49.

Hamrick, J. and Loveless, M. (1986). Isozyme variation in tropical trees procedures and preliminary results. Biotropica, 18, 201-207.

Hantula, J. Dusabenyagasanl, M. and Hamelin, R. C. (1996). Random Amplified Microsatellites (RAMs) a novel method for characterizing genetic variation within fungi. European Journal of Forest Pathology, 26, 159-166. https://doi.org/10.1111/j.1439-0329.1996.tb00720.x

Hayati A., Wickneswari R., Maizura I. and Rajanaidu N. (2004). Genetic diversity of oil palm (*Elaeis guineensis* Jacq.) germplasm collections from Africa: implications for improvement and conservation of genetic resources. Theoretical and Applied Genetics, 108, 1274-1284. https://doi.org/10.1007/s00122-003-1545-0

Jack, P. L., Dimitrijevic, T. A. F. and Mayes, S. (1995). Assessment of nuclear, mitochondrial and chloroplast RFLP markers in oil palm (*Elaeis guineensis* Jacq.). Theoretical and Applied Genetics, 90, 643-649. https://doi.org/10.1007/BF00222128

Joseph, H., Anderson, R., Tatham, R. and Black, W. (1992). Multivariate data Analysis with Readings. 3. ed. New York: Riverside.

Khierallah, H. S., Bader, S. M., Baum, M. and Hamwieh, A. (2011). Genetic diversity of Iraqi date palms revealed by microsatellite polymorphism. Journal of the American Society for Horticultural Science, 136, 282-287.

Loveless, M. D. and Hamrick, J. L. (1984). Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics, 15, 65-95. https://doi.org/10.1146/annurev.es.15.110184.000433

Maizura, I., Rajanaidu, N., Zakri, A. H. and Cheah, S. C. (2006). Assessment of Genetic diversity in oil palm (*Elaeis guineensis* Jacq) using restriction fragment length polymorphism (RFLP). Genetic Resources and Crop Evolution, 53, 187-195. https://doi.org/10.1007/s10722-004-4004-0

Mayes, S., Jack, P. L., Corley, R. H. V. and Marshall, D. F. (1997). The construction of a RFLP linkage map for oil palm. Genome, 40, 116-122. https://doi.org/10.1139/g97-016

Montoya, C., Arias, D., Rey, L. and Rocha, P. (2005). Caracterización molecular de materiales de *E. guineensis* Jacq. procedentes de Angola. Fitotecnia Colombiana. 5, 1-10.

Moretzsohn, M. C., Ferreira, M. A., Amaral, Z. P. S., Coelho, P. J. A., Grattapaglia, D. and Ferreira, M. E. (2002). Genetic diversity of Brazilian oil palm (*Elaeis oleifera* H.B.K.) germplasm collected in the Amazon Forest. Euphytica, 124, 35-45. https://doi.org/10.1023/A:1015606304653

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89, 583-590.

Nei, M. and Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restricción endonucleasa. Proceedings of the National Academy of Sciences, 76, 5269-5273. https://doi.org/10.1073/pnas.76.10.5269

Neigel, J. (1997). A comparison of alternative strategies for estimating gene flow from genetic markers. Annual Review of Ecology and Systematics, 28, 105-128. https://doi.org/10.1146/annurev.ecolsys.28.1.105

Nybom, M. and Bartish, I. (2000). Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspectives in Plant Ecology, Evolution and Systematic, 3, 93-114. https://doi.org/10.1078/1433-8319-00006

Ochoa, I. E., Villegas, V. E. and Beebe, S. E. (1997). Identificación de marcadores moleculares RAPD asociados con la resistencia al Complejo de Pudrición de Cogollo en palma de aceite (*Elaeis guineensis* Jacq.). Palmas, 18, 33-38.

Okoye, M. N., Uguru, M. I., Bakoumé, C., Singh, R. and Okwuagwu, C. O. (2016a). Assessment of genetic diversity of NIFOR Oil Palm main breeding parent genotypes using microsatellite markers. American Journal of Plant Sciences, 7, 218-237. http://doi.org/10.4236/ajps.2016.71022

Okoye, M. N., Bakoumé, C., Uguru, M. I., Singh, R. and Okwuagwu, C. O. (2016b). Genetic relationships between elite oil palms from Nigeria and selected breeding and germplasm materials from Malaysia via Simple Sequence Repeat (SSR) Markers. Journal of Agricultural Science, 8, 159-178.

Pritchard, J. K., Stephens, M. and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. Genetics, 155, 945-959.

Purba, A. R., Noyer, J. L., Baudouin, L., Perrier, X., Hamon, S. and Lagoda, P. J. L. (2000). A new aspect of genetic diversity if Indonesian oil palm (*Elaeis guineensis* Jacq) revealed by isozymes and AFLP markers and its consequences for breeding. Theoretical and Applied Genetics, 101, 956-961. https://doi.org/10.1007/s001220051567

Rajanaidu, N., Rao, V., Hassan, A. H. and Ong, A. S. H. (1989). Genetic resources-new development in oil palm breeding. Elaeis, 1, 1-10.

Rance, K. A., Mayes, S., Price, Z., Jack, P. L. and Corley, R. H. V. (2001). Quantitative trait loci for yield component in oil palm (*Elaeis guineensis* Jacq.). Theoretical and Applied Genetics, 103, 1302-1310. https://doi.org/10.1007/s122-001-8204-z

Rey, L., Gómez, P. L., Ayala, I. M., Rocha, P. J. and Prada, F. (2007). La variabilidad del germoplasma y su relación con el éxito de un programa de mejoramiento en palma de aceite. Palmas, 28, 166-175.

Robinson, I. (1998). Aloenzimas na genética de populações de plantas. In A. C. Alfenas (Ed.), Eletroforese de Isoenzimas e Proteínas Afins: Fundamentos e Aplicações em Plantas e Microorganismos (329-380). Viçosa: UFV.

Rocha, P. (2003). Marcadores moleculares, una herramienta de útil para la selección genética de palma de aceite. Palmas, 24, 11-25.

Rocha-Salavarrieta, P. J., Rocío-Meléndez, E. and Rey-Bolívar, L. (2007). Ampliación del análisis de diversidad genética de palma de aceite proveniente de Angola. Revista Respuestas 12, 20-28. http://dx.doi.org/10.22463/0122820X.577

RSPO [Roundtable for Sustainable Palm Oil] (2016). Roundtable for Sustainable Palm Oil; [accessed 2016 November 3]. www.rspo.org/about

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). Gel electrophoresis of DNA. In J. Sambrook, E. F. Fritsch and T. Maniatis (Eds.), Molecular cloning: a laboratory manual (p. 3-60). 2nd ed. New York: Cold Spring Harbor.

Sathish, D. K. and Mohankumar, C. (2007). RAPD markers for identifying oil palm (*Elaeis guineensis* Jacq.) parental varieties (Dura & Pisifera) and the hybrid Tenera. Indian Journal of Biotechnology, 6, 354-358.

Shah, F. H, Omar, R. and Selamat, Z. (1993). Polymorphism of rDNA locus of oil palm: a preliminary investigation. Malaysian Biochemical Society, 17, 81-84.

Shah, F. H., Rashid, O., Simons, A. J. and Dundson, A. (1994). The utility of RAPD markers for determination of genetic variation in oil palm (*Elaeis guineensis*). Theoretical and Applied Genetics, 89, 713-718. https://doi.org/10.1007/BF00223710

Singh, R., Soon-Guan, T., Panandam, J., Rahman, R. A. and Suan-Choo, C. (2008). Identification of cDNA-RFLP Markers and their use for molecular mapping in Oil Palm (*Elaeis guineensis*). Asia Pacific Journal of Molecular Biology and Biotechnology, 16, 53-63.

Slatkin, M. (1994). Gene flow and population structure. In L. Real (Ed.), Ecological Genetics (p. 3-17). Princeton: Princeton University Press.

Ting, N.-C., Zaki, N. M., Rosli, R., Low, E. T. L., Ithnin, M., Cheah, S.-C., Tan, S. G. and Singh, R. (2010). SSR mining in oil palm EST database: application in oil palm germplasm diversity studies. Journal of Genetics, 89, 135-145. https://doi.org/10.1007/s12041-010-0053-7

Villegas, V., Duran, C. and Beebe, S. (2000). Caracterización molecular de materiales Dura. Palmas, 21, 35-40.

Wright, S. (1978). Evolution and the genetics of populations, variability within and among natural populations. Vol. 4. Chicago: University of Chicago.

Zhou, L. X., Xiao, Y., Xia, W. and Yang, Y. D. (2015). Analysis of genetic diversity and population structure of oil palm (*Elaeis guineensis*) from China and Malaysia based on species-specific simple sequence repeat markers. Genetics and Molecular Research, 14, 16247-16254.

Zietkiewicz, E., Rafalsfl, A. and Laubuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics, 20, 176-183. https://doi.org/10.1006/geno.1994.1151