



Genetic similarity among commercial oil palm materials based on microsatellite markers

Similaridad genética entre materiales comerciales de palma de aceite basado en marcadores microsatélites

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ABSTRACT

Microsatellite markers are used to determine genetic similarities among individuals and might be used in various applications in breeding programs. For example, knowing the genetic similarity relationships of commercial planting materials helps to better understand their responses to environmental, agronomic and plant health factors. This study assessed 17 microsatellite markers in 9 crosses (D x P) of *Elaeis guineensis* Jacq. from various commercial companies in Malaysia, France, Costa Rica and Colombia, in order to find possible genetic differences and/or similarities. Seventy-seven alleles were obtained, with an average of 4.5 alleles per primer and a range of 2-8 amplified alleles. The results show a significant reduction of alleles, compared to the number of alleles reported for wild oil palm populations. The obtained dendrogram shows the formation of two groups based on their genetic similarity. Group A, with ~76% similarity, contains the commercial material of 3 codes of Deli x La Mé crosses produced in France and Colombia, and group B, with ~66% genetic similarity, includes all the materials produced by commercial companies in Malaysia, France, Costa Rica and Colombia.

Key words: *Elaeis guineensis* Jacq, breeding programs, alleles, genetic differences.

RESUMEN

Los marcadores moleculares son utilizados para determinar la similaridad genética entre individuos y podrían ser utilizados en diversas aplicaciones en los programas de mejoramiento. El conocer las relaciones de similitud genética de los materiales comerciales ayuda a entender mejor sus respuestas a los factores ambientales, bióticos y abióticos. Este estudio evaluó 17 marcadores de microsatélites en 9 cruces (D x P) de *Elaeis guineensis* Jacq. de varias empresas comerciales en Malasia, Francia, Costa Rica y Colombia, con el fin de encontrar posibles diferencias genéticas y/o similitudes. Se obtuvieron 77 alelos, con un promedio de 4,5 alelos por cebador y una gama de 2-8 alelos amplificados. Los resultados muestran una reducción significativa de los alelos, en comparación con el número de alelos reportados para las poblaciones silvestres de palma de aceite. El dendrograma obtenido muestra la formación de dos grupos con base a su similitud genética. El grupo A con similitud ~76% contiene material comercial de 3 códigos de cruzamientos Deli x La Mé producido en Francia y Colombia, y el grupo B con ~66% de similitud genética que incluye todos los materiales producidos por empresas comerciales en Malasia, Francia, Costa Rica y Colombia.

Palabras clave: *Elaeis guineensis* Jacq, programa de mejoramiento, alelos, diferencias genéticas.

Introduction

The African oil palm (*Elaeis guineensis* Jacq.) is a species native to Africa. It is very important because of the oil extracted from the endosperm of its fruits, which is refined to obtain products of great commercial value, which have multiple uses in the food industry and for biofuels. The oil palm has the highest yields per hectare of all the oil crops. The expansion of oil palm cultivation began to develop in Africa and South-East Asia around the colonial period because of its multiple industrial and food uses (Corley and Tinker, 2003). According to population growth estimates, global consumption of palm oil is expected to reach 37.9 million t by 2020. This represents an average annual growth

rate of 3.3% (Fedepalma, 2008). To meet these targets, it is necessary to produce improved, high-yielding genetic material with better oil quality, slower growth and high tolerance to pests and diseases; characteristics that are crucial to commercial plantations for ensuring adequate returns (Bakoumé *et al.*, 2007).

The oil palm is normally monoecious, with male or female flowers, but sometimes both; with inflorescences developing in the axils of the leaves. It is an allogamous species and its diploid genome is comprised of 16 pairs of homologous chromosomes (Singh *et al.*, 2008). In oil palm, there are three types that produce different fruit, depending on the thickness of the shell and its ability to produce oil. The Dura type has fruits with thick shells (2 to 8 mm), occasionally

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less; accounting for 25-55% of the fruit weight, with 35-70% pulp and 1-20% kernel. The Pisifera type has shell less fruit and the plants generally exhibit female sterility, the total fruit pulp accounts for 82-99% of the total weight and the kernels 1-18%. The Tenera type is a product of the intra-specific cross between the Dura and pisifera types. Its fruit has a thin shell, 0.5 to 4.0 mm, representing 5-30% of the fruit weight with 3-15 and 60-91% kernel pulp; and constitutes the material grown at the commercial level (Corley and Tinker, 2003).

Oil palm breeding programs are characterized by using the reciprocal recurrent selection scheme which uses two Dura and Pisifera-type starting populations to make the crossings and progeny testing of which the best parents are chosen; and Tenera-type seeds are produced. Subsequently, new starting populations are generated and the cycle is repeated (Corley and Tinker, 2003). History indicates that the oil palm material grown commercially in the world comes from four plants planted in the Bogor Botanical Garden in Java (Indonesia) in 1884. This very narrow genetic base has led to uniformity in the production of the material that is distributed worldwide. Therefore, planted materials share the same female parental stock, Deli *dura*, with some introgressions, and are combined with three male parental stocks (Pisiferas), the Avros, La Mé and Yagambi (Bakoumé, 2007; Cochard, 2009). This narrow genetic base has driven oil palm breeders to place greater importance on genetic resources of the species to increase the genetic variability in breeding programs.

Due to the perennial nature and long generative cycles of oil palm, conventional breeding can take several years, which greatly hampers rapid and efficient progress in the selection of individuals. Various molecular biology techniques are available today for detection of genetic variability and for establishing genetic similarity relationships among individuals and can be used in various applications in breeding programs. For example, microsatellite markers or SSR (Simple Sequence Repeats) have been developed in oil palm since 1999 to carry out studies on genetic diversity (Billotte *et al.*, 2001; Billotte *et al.*, 2005; Bakoumé *et al.*, 2007; Singh *et al.*, 2008), varietal identification (Norziha *et al.*, 2008), pedigree analysis, genome mapping and QTL detection for molecular marker-assisted selection (Billotte *et al.*, 2010). SSRs have great advantages over other markers such as isozymes (Hayati *et al.*, 2004), RAPD (Shah *et al.*, 1994), restriction fragment length polymorphism (RFLP; Maizura *et al.*, 2006) and amplified fragment length polymorphism (AFLP; Kularatne, 2001). SSRs have the highest Polymorphism

Information Content (PIC) and high distribution of loci within the genome (Ferreira and Grattapaglia, 1998). Additionally, Mohammadi and Prasanna (2003) underpin the importance of using microsatellite markers for the following reasons: i) each locus is defined as codominant, so they are ideal for marker-assisted breeding, genetic mapping and diversity measurements; ii) they are highly variable and can distinguish between closely related cultivars and iii) they are highly reproducible through PCR.

Additionally, it is a tool that would allow us to keep track of parents and their offspring. The aim of this study is the molecular characterization of 9 crosses (D x P) of *Elaeis guineensis* Jacq. from various commercial companies in Malaysia, France, Costa Rica and Colombia; planted in the Palmar de la Vizcaina Research Center (Barrancabermeja, Colombia), to find possible genetic differences and/or similarities among these commercial materials.

Materials and methods

Plant material and DNA isolation

Plants of 189 commercial materials of 9 crosses of oil palm produced by commercial companies in Malaysia, France, Costa Rica and Colombia were used. The origins of the materials used are: Deli Dumpy x Avros; Deli x La Mé; Deli x Avros; Deli x Ghana; Deli x Nigeria; Deli x Yagambi; Deli (Avros x Yagambi); Compact x Ekona and Compact x Ghana. The materials were coded from 1 to 10 within the same cross and a letter of the alphabet was assigned to differentiate between commercial companies. The label of the materials is given by the name of their parents. Thus, the individual Deli x Yagambi A10 represents the individual numbered ten from a Deli x Yagambi cross of commercial company A. These materials are planted in a Cenipalma agronomic trial at the Palmar de la Vizcaina Research Center, Barrancabermeja, Colombia.

Total genomic DNA was obtained from young leaflets. Each leaf sample was macerated with liquid nitrogen and the DNA was isolated using a Qiagen Extraction Kit (Ref: 69106, Gentech, Medellín, Colombia), following the manufacturer's instructions. The DNA was quantified using agarose gel and a spectrophotometer, and finally the samples were diluted to a concentration of 5 ng μL^{-1} .

PCR amplification

The 189 DNA samples were evaluated using Polymerase Chain Reaction (PCR) with 17 microsatellite markers (Tab. 1) according to the amplification conditions reported by Billotte *et al.* (2001) and Singh *et al.* (2008). To visualize the

TABLE. 1. List of the 17 microsatellite markers evaluated in oil palm.

SSR Locus	Motive	Forward primer (5'-3')	Reverse primer (5'-3')
mEgCIR0008	(GA)18	CGGAAAGAGGGAAGATG	ACCTTGATGATTGATGTGA
mEgCIR0009	(GA)20	CAGTCTTTAAGTACGGCTATGAT	GAATTTTATGTTCAACCAGGTAGA
mEgCIR0018	(GA)18	CCTTATTTTCTTTGCTTACC	TTCTATTTTATTTTCTTCCT
mEgCIR0046	(GA)19	AGCCTTAGTATTTTGTGAT	CCTCTGATTTGTCTTTTGG
mEgCIR006	(GA)17	TACACAACCCATGCACAT	AAAAACATCCAGAAATAAAA
mEgCIR0219	(GA)17	TTTGCTCGGCGGATACAT	CTCACTGGCCTCTTCTT
mEgCIR0221	(GA)11	TGCCATGTTCAGAGAGC	TTCAGATTTTCCGACTTC
mEgCIR0230	(TA)6GAG(GA)19	CCCTGGCCCCGTTTTTC	AGCGCTATATGTGATTCTAA
mEgCIR0254	(GA)18	CCTTTTGTGCTTTCTTC	GCTGTGCACTAGGTTTC
mEgCIR1772	(GT)22	ACCTTGATTAGTTTGCCA	CTTCCATTGTCTCATTATTCTCTTA
mEgCIR0437	(CCG)6	CCAACCCAACCCAACATAAA	GGTCCCGATCCCGTCTACT
mEgCIR0465	(CCG)6	TCCCCACGACCCATTC	GGCAGGAGAGGCAGCATTC
sEg00067	(TGTA)6	GTCAGCCCGTAGAAGATTGC	CTTTCGGATAGCCAAAACGA
sEg00090	(AT)9	TATGCGGGTGATCAAGTGAA	CCACCATGGTTCTCAGGAAA
sEg00125	(GCG)6	TACCCTTTTCCCTCCCTCCATA	CATCATCTCCGTGCCAGTATT
sEg00126	(CGC)7	CCGTCTCAAAGCCCTAAAC	TTGTTGTCCCACTCCCTCTT
sEg00127	(TTC)9	CTAAATTCCTCATCGTCTC	CTCGAAGCTCATCGTCTCTC

product of the amplification, a denaturing polyacrylamide gel stained with silver nitrate was used.

Statistical analysis

The number of amplified alleles was determined for each locus using the amplification patterns reported by Billotte *et al.* (2001) and Singh *et al.* (2008) as a reference. The total number of alleles and average polymorphic information content (PIC) were calculated for each locus from the algorithms included in the FSTAT software (Goudet, 2002).

The data were analyzed using the NTSYS pc 2.11L software (Rohlf, 2000) to generate a similarity matrix according to Nei and Li's (1979) definition as follows:

$$S_{ij} = 2a / (2a + b + c) \quad (1)$$

Where S_{ij} is the similarity between individuals i and j ; a is the number of bands present in both i and j ; b is the number of bands present in i but absent in j ; and c is the number of bands present in j but absent in i . From the similarity matrix, a dendrogram was generated using the Unweighted Pair-Group Mean Arithmetic Average method (UPGMA). Additionally, a principal coordinate analysis was performed, based on which a diagram was generated so that the geometric distances among individuals might reflect genetic distances with minimal distortion. The aggregation patterns in the diagram reveal genetic similarity among individuals (Mohammadi and Prasanna, 2003). To this end, the statistical analysis program NTSYS pc 2.11L (Rohlf, 2000) was used.

To determine allele frequencies, number of alleles per locus and the list of single alleles per cross, the GenAlex ver. 6.0 program (*Genetic Analysis in Excel*®) was used (Peakall and Smouse, 2006).

Results and discussion

Polymorphism

The amplification patterns obtained from the 17 microsatellite markers showed that some markers had more polymorphism within and between genetic origins (Fig. 1a), while other primers were polymorphic, but had fewer differences within and between genetic origins (Fig. 1b).

The 17 amplified microsatellite markers were polymorphic, the percentage of missing data was 0.9. A total of 77 alleles were obtained for the 189 assessed samples of oil palm *E. guineensis*, with an average of 4.5 alleles per primer and a range of 2-8 amplified alleles. The PIC showed a maximum value of 0.822 for locus mEgCIR1772, which suggests that this locus is highly informative and locus mEgCIR0437 was the least informative (PIC = 0.141) (Tab. 2). However, only nine markers had a PIC value = 0.5, which means they could be selected for studies on breeding oil palm material.

Results show a significant reduction of alleles as compared to the number of alleles reported by Billotte *et al.* (2001) in 18 accessions of *E. guineensis* from germplasm collections of different origins and the number of alleles reported by Singh *et al.* (2008) in 66 wild *E. guineensis* from seven

countries (Tab. 2). The low number of alleles found could be explained by the fact that the materials used as breeding material and the crosses are generally derived from a few individuals, which affects the population and decreases allele variability.

TABLE 2. Comparison between the number of amplified alleles and the number of reported alleles.

SSR locus	PIC	Number of amplified alleles	Number of reported alleles by author ¹	% Missing data
mEgCIR0008 ^a	0.572	4	6	0.5
mEgCIR0009 ^a	0.679	5	4	1.0
mEgCIR0018 ^a	0.298	4	11	0.5
mEgCIR0046 ^a	0.378	3	5	2.1
mEgCIR0067 ^a	0.765	8	8	2.6
mEgCIR0219 ^a	0.714	7	7	1.0
mEgCIR0221 ^a	0.492	4	7	1.0
mEgCIR0230 ^a	0.682	5	6	0
mEgCIR0254 ^a	0.785	8	10	2.6
mEgCIR1772 ^a	0.822	7	12	4.7
mEgCIR0437 ^a	0.129	2	3	0
mEgCIR0465 ^a	0.539	5	4	0
sEg00067 ^b	0.500	2	4	0
sEg00090 ^b	0.390	4	8	0
sEg00125 ^b	0.340	3	4	0
sEg00126 ^b	0.451	2	2	0.5
sEg00127 ^b	0.141	4	6	0
Total		77	107	
Average		4.5	6.3	0.9

PIC: polymorphic information content. 1: ^a (Billotte *et al.*, 2001), ^b (Singh *et al.*, 2008).

Indeed, different studies show a general tendency of loss of diversity after several cycles of selection. Bakoumè *et al.* (2007) determined the allelic diversity of 3 breeding materials, using 16 microsatellite markers and found that alleles that were rare in wild oil palm populations showed a reduction due to many years of selection in these materials. Condón *et al.* (2008) studied the genetic diversity in improved barley varieties with 70 microsatellite markers

and found that allelic diversity indices decreased from an average of 5.89 alleles per locus in the most ancestral group to 2.43 alleles per locus after four decades of breeding. Fu *et al.* (2006) analyzed wheat cultivars released from 1845 to 2004 using 37 EST-derived SSRs and found a significant reduction in the number of alleles (16 alleles in 14 loci found in pre-cultivars in 1910 were missing in cultivars released after 1990) and a reduction in variation in four ancestral families from 14.7 to 12.8% after six periods of breeding. Therefore, the results of this study show a general trend of loss of genetic diversity caused by the genetic improvement of the oil palm.

Dendrogram and principal coordinates analysis

The dendrogram (Fig. 2) shows the formation of two groups based on their genetic similarity. Group A, with ~76% similarity, contains commercial materials Deli x La Mé (3 different codes) produced by commercial companies in France and Colombia. These materials have a high genetic similarity between themselves but low similarity with other commercial materials (~50%), reflecting a genetic gain in terms of heterozygosity. It is important to note that there were 7 private alleles in the cross: a single 225-bp allele at locus mEgCIR0008 two single 175-pb and 168-pb alleles at locus mEgCIR0067; a single 200-pb allele at locus mEgCIR0221; a single 137-pb allele at locus mEgCIR0465; a single 152-pb allele at locus sEg00090 and a single 167-pb allele at locus sEg00127. Single alleles are important because they are the raw material of the evolutionary process and are likely to allow a population to respond to a change in the environment. Group B, with ~ 66% of genetic similarity, is the largest one and comprises materials produced by commercial companies in Malaysia, France, Costa Rica and Colombia. This reflects the narrow genetic base of Deli and Avros populations used for these crosses. The high value of genetic similarity between all of the palms analyzed could be explained by different factors such as: a) the fact that they have the same female Deli parent (Deli

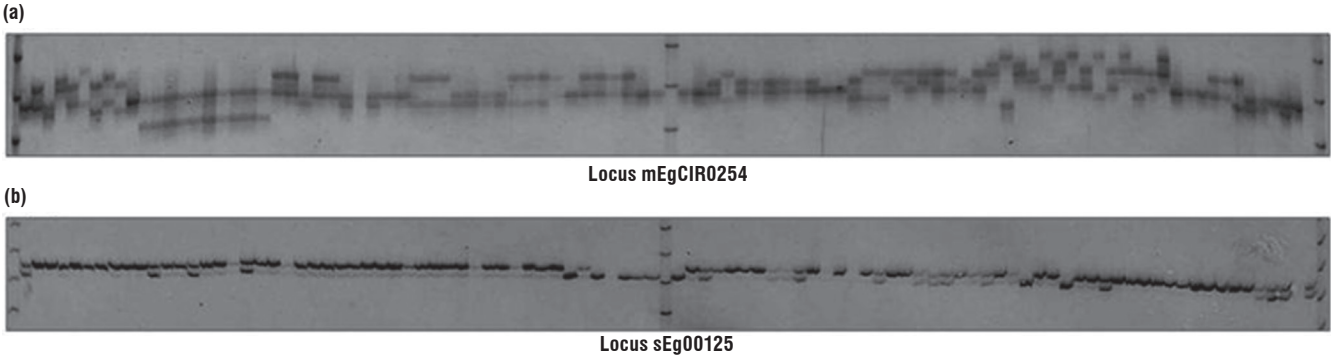


FIGURE 1. Amplification pattern obtained from two microsatellite markers in the various origins of commercial oil palm materials. (a) Amplification pattern of marker mEgCIR0254. (b) Amplification pattern of marker sEg00126.

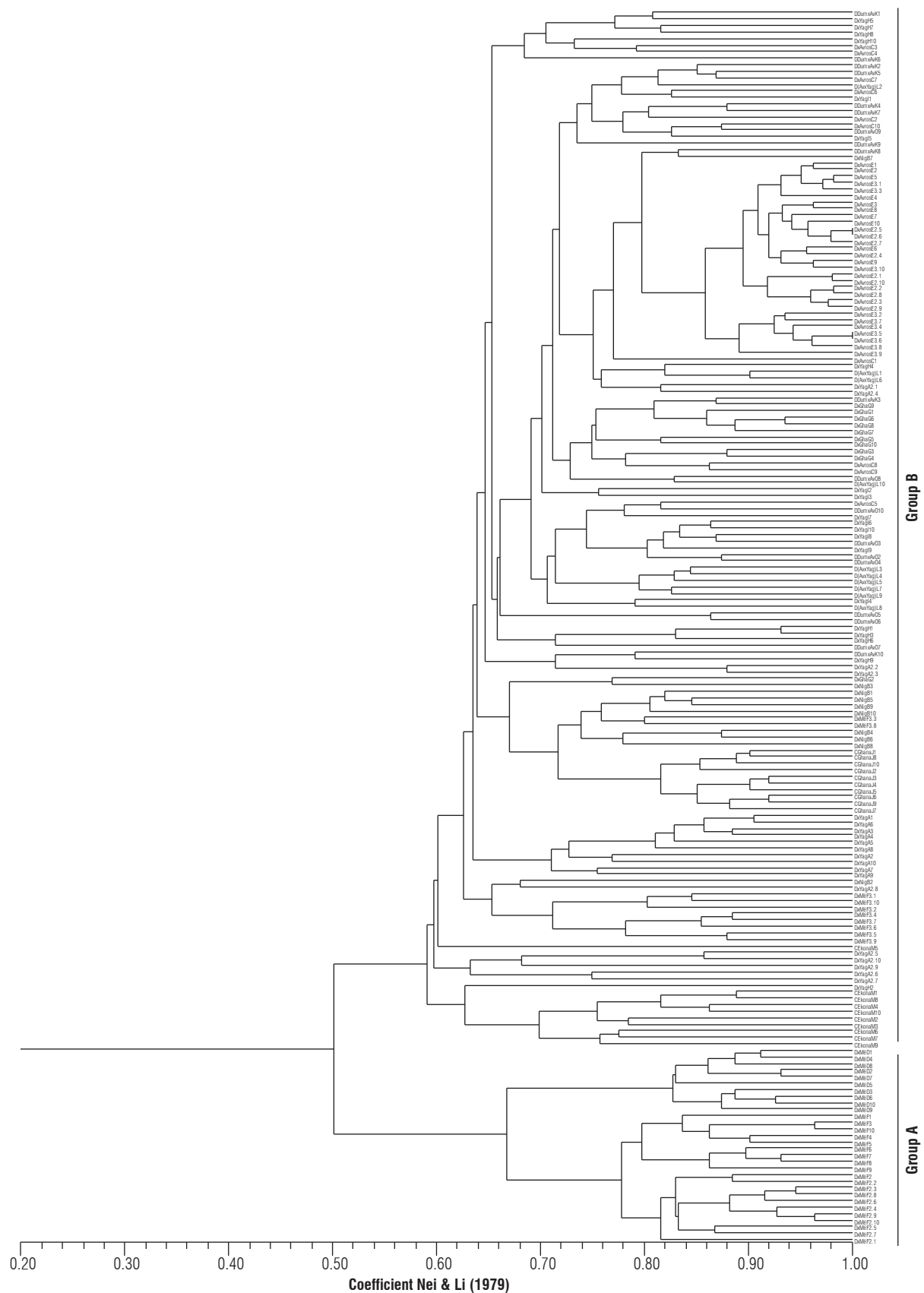


FIGURE 2. Dendrogram for commercial oil palm *E. guineensis* individuals; obtained using Nei and Li's genetic similarity coefficient and the UPGMA clustering method.

dura Deli Dumpy and Compact), and different male parents (AVROS, Yagamby, La Mé, Ghana, Nigeria and Ekona), b) the global exchange of plant resources that breeding programs of the various commercial companies have done for many years (Richardson, 1995; Sterling *et al.*, 2002) and c) the limited origin of these populations, which has been described by several authors (Bakoumé, 2007; Dumortier, 2003; Cochard, 2009). The analysis of principal coordinates (Fig. 3) also shows the composition of these two groups (A and B). Dimension 1 shows the separation of the genotypes Deli × La Mé (Group A) from the other commercial genotypes: Deli Dumpy × Avros; Deli × Avros; Deli × Ghana; Deli × Nigeria; Deli × Yagamby; Deli (Avros × Yagamby); Compacta × Ekona and Compact × Ghana (Group B). Dimension 2 shows the distribution of each individual within each group. The high degree of observed genetic similarity is consistent with the results reported by different genetic diversity studies of wild *E. guineensis*, which include Deli *dura*-type materials as a reference; and it could be concluded that genetically improved populations have less genetic diversity (expressed as heterozygosity values and the percentage of polymorphic loci). According to an analysis using RFLP (Restriction Fragment Length Polymorphism) markers, improved materials are grouped with 80% of genetic similarity (Mayes *et al.*, 2000) and 17% of polymorphic loci, versus 65% in wild populations from Cameroon (Maizura *et al.*, 2001).

The study found a loss of genetic variability in terms of average number of alleles per locus and proportion of polymorphic alleles, but concludes that gene frequency differences among the populations is one of the main factors contributing to the expression of heterozygosity and increased genetic distance among the varieties. The differences between the two groups could be based on the origin and type of progenies. Due to the selection process, different types of alleles have been fixed according to the objectives of each breeding program. For group A, the majority of seed production is based on the selection of *Dura*-type genotypes of the Deli base as almost their sole *dura* source, and selection of *Pisifera* has been performed mainly from La Mè and Yangambi (Corley and Tinker, 2003). This selection is done through the subsequent evaluation of progenies for high oil yield, following the Reciprocal Recurrent Selection (RRS) method (Gascon and Berchoux, 1964). For group B, the selection of *Dura*-type genotypes has been based on the original Bogor palms, but after four generations of breeding, the best progeny was established, the so called “Ulu Remis” populations from which many breeding programs in the world take the Deli *dura* breeding population (Corley and Tinker, 2003). The method adopted for seed production was the Family and Individual Selection (FIS) method using phenotypic selection (Hardon, 1970).

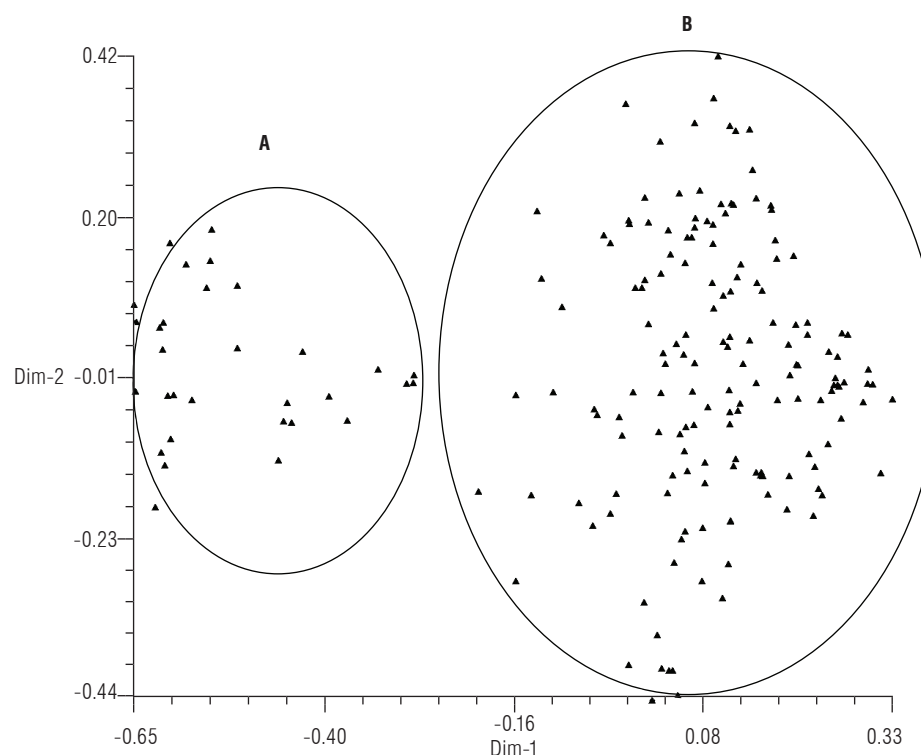


FIGURE 3. Principal Coordinate analysis of commercial oil palm (*E. guineensis*) materials. Letters A and B represent the resulting groups.

In the seed production of palm oil, the choice of parental lines is fundamental, representing a greater opportunity to recombine genes so that they can maintain and/or maximize the heterozygosity of the new materials, to develop in each cycle. This ensures a way of genetic variability and therefore new allelic combinations within a species. However, several generations of selection and selfing can reduce the number of alleles and increase the coefficient of inbreeding. This circumstance has prompted oil palm breeders to place greater importance on the genetic resources of the species, in order to increase the genetic variability of the base populations through crosses between populations with a wide range of origins, which allows for a rapid selection progress, exploiting the additive genetic variance to the fullest extent and therefore, significant genetic progress in their productive potential. Wild populations of oil palm are found in central and western tropical Africa and on the island of Madagascar or kept in ex situ conditions in various germplasm banks (Corley and Tinker, 2003). Two of the world's most important ex situ collections are found in the MPOB (Malaysian Palm Oil Board) and the NIFOR (Nigeria Institute for Oil Palm Research) with natural populations of oil palm from more than 12 African countries, including Tanzania and Madagascar (Rajanaidu, 1986; Corley and Tinker, 2003).

Conclusion

The results obtained in this research show the conformation of two groups, which reflects the fixation of alleles depending on selection and breeding methods and the base breeding populations used.

While it is true that the breeding programs carried out by commercial seed companies have introduced *E. guineensis* germplasm with excellent results, there is no doubt that this process must continue, because failure to do so could have negative effects on the required rapid development of new varieties with low vulnerability to adverse environmental factors. A successful breeding program depends on the available genetic variability and on how it is used during the selection process to find high quality palms. The importance of genetic diversity in breeding programs and the knowledge of plant genetic resources are of great social and scientific value for the economic development and food security of humankind. Therefore, it is imperative to provide the greatest genetic diversity possible to breeders and farmers to obtain new cultivars that respond to different soil and weather conditions with enhanced resistance to biotic and abiotic stresses.

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