Genetic variability of wild palms *Euterpe precatoria*, *Euterpe oleracea* and *Mauritia flexuosa* with molecular markers ISSR

Variabilidad genética de palmas silvestres *Euterpe precatoria*, *Euterpe oleracea* y *Mauritia flexuosa* con marcadores moleculares ISSR

Yacenia Morillo-Coronado[1]; Salvador Rojas-González[2]; Ana Cruz Morillo-Coronado[3]; Christian Camilo Castañeda-Cardona[4]; Karem Julieth Mendoza-Romero[5]; Jaime Marín-Colorado[6]

Authors Data

1. Professor, Ph.D., Universidad Nacional de Colombia, Palmira, Colombia, ymorilloc@unal.edu.co, https://orcid.org/0000-0003-1974-3464

2. Researcher, Ph.D., AGROSAVIA - Corporación Colombiana de Investigación, Villavicencio, Colombia, srojas@agrosavia.co, https://orcid.org/0000-0002-0765-3587

3. Professor, Ph.D., Universidad Pedagógica y Tecnológica de Colombia. Tunja, Colombia, ana.morillo@uptc.edu.co, https://orcid.org/0000-0003-3125-0697

4. Researcher, M.Sc., Universidad de los Llanos, Villavicencio, Colombia, christian.castaneda@unillanos.edu.co, https://orcid.org/0000-0001-5429-9739

5. Biologist, Universidad de los Llanos. Villavicencio, Colombia, karem.mendoza@unillanos.edu.co, https://orcid.org/0000-0003-0269-9719

6. Researcher, Ph.D., AGROSAVIA - Corporación Colombiana de Investigación, Nataima, Colombia, jamarin@agrosavia.co, https://orcid.org/0000-0001-5628-4637

ABSTRACT

Wild palms constitute a group of plants of particular economic importance in the tropics, being a valuable source for obtaining construction materials, fabrics, fuel, food, ornamental and medicinal plants. Despite their economic, social, and environmental importance, the genetic variability existing in wild palms, mainly of the genus Mauritia and Euterpe, is unknown, which limits their potential use and the identification of promising genotypes to be included in recombination programs. The genetic variability of three wild palm species (*Euterpe oleracea*, *Euterpe precatoria,* and *Mauritia flexuosa*) was evaluated using Inter-Simple Sequence Repeat (ISSR) markers. A total of 51 genotypes were analyzed with seven ISSR primers, which generated a total of 153 bands, with a percentage of polymorphic loci of 90.24%. The cluster analysis revealed the formation of six groups at a similarity level of 37%, grouping the palm genotypes by species and geographical origin. Molecular variance analysis revealed that within group variations contributed more to genetic diversity (89%) than between group variations (11%). The average value of F_{st} was 0.20, demonstrating moderate genetic differentiation. The high genetic variability found in this study indicates the valuable genetic potential present in palm germplasm, which could be used for future improvement programs of the species.

Key words: Arecaceae; genetic diversity; gene flow; germplasm; plant breeding; polymorphism.

RESUMEN

Las palmas silvestres constituyen un grupo de plantas de particular importancia económica en los trópicos, siendo una fuente valiosa para la obtención de materiales de construcción, tejidos, combustibles, alimentos, plantas ornamentales y medicinas. A pesar de su importancia económica, social y ambiental se desconoce la variabilidad genética existente en las palmas silvestres principalmente del género Mauritia y Euterpe, lo cual limita su uso potencial y la identificación de genotipos promisorios para ser incluidos en programas de recombinación. Se evalúo la variabilidad genética de tres especies de palmas silvestres (*Euterpe oleracea*, *Euterpe precatoria* and *Mauritia flexuosa*) mediante marcadores de Secuencia Inter-Simple Repetida (ISSR). Un total de 51 genotipos fueron analizados con siete cebadores ISSR, los cuales generaron un total de 153 bandas, con un porcentaje de loci polimórficos de 90.24%. El análisis cluster reveló la formación de seis grupos a un nivel de similitud del 37%, agrupando los genotipos de palma por especies y por origen geográfico. El análisis de la varianza molecular reveló que, dentro de los grupos, las variaciones contribuyeron más a la diversidad genética (89%), que las variaciones entre grupos (11%). El valor promedio de F_{st} fue de 0.20, lo que demuestra una moderada diferenciación genética. La alta variabilidad genética encontrada en este estudio indica el valioso potencial genético presente en el germoplasma de palma evaluado, el cual podría ser utilizado para futuros programas de mejoramiento genético de la especie.

Palabras clave: Arecaceae; diversidad genética; flujo genético; germoplasma; mejoramiento vegetal; polimorfismo.

INTRODUCTION

The Arecaceae family is one of the most numerous in the plant kingdom, highlighting its diversity in the tropical zones of the world (Galeano & Bernal, 2010). This family is made up of 190 genera and 2,360 species distributed in tropical and subtropical areas around the world, with Asia and America being the tropics' richest in species (Galeano & Bernal, 2010). Colombia is the second-richest country in palm trees in America and the third in the world, after Malaysia and Brazil (Galeano *et al*., 2015; MADR - Ministerio de Agricultura y Desarrollo Rural, 2021). Since it has approximately 250 species belonging to 45 genera (Galeano & Bernal, 2005) geographically distributed in the Amazon, the Caribbean, the Pacific, and the Andes of the country (Galeano & Bernal, 2010).

Wild palms are one of the most useful groups of plants in the tropics, and their ethnobotanical importance is undeniable (Bernal *et al.,* 2011). In addition to their ecological importance and contribution to biodiversity, palms constitute a resource of socio-cultural and economic importance and have been traditionally used as raw materials for various purposes (Valois-Cuesta *et al.,* 2013).

Mauritia flexuosa is known by the names of moriche in the Eastern Plains of Colombia, canangucha, cananguche or canangucho in the departments of Caquetá and Putumayo, burití and mirití in Brazil, moriche in Venezuela, aguaje in Peru, morete in Ecuador, and palm real in Bolivia (Trujillo-González *et al.,* 2011). It is abundant in the Orinoco savannahs and the Amazon Rainforest and is considered an important socioeconomic resource for indigenous communities due to its multiple uses and edible fruits (Gilmore *et al.,* 2013; Virapongse, 2017a). Its fruit is widely used in the production of sweets

and ice cream, while the oil is used for cooking and serves as a raw material for the cosmetics industry (Freire *et al.,* 2016; Milanez *et al.,* 2016; Speranza *et al.,* 2016). Previous studies have highlighted the importance of this species for its nutritional value due to the presence of antioxidants and antimicrobial capacity (Koole *et al.,* 2013). Due to its high anthocyanin content, the fruit decreases free radical levels, lipid peroxidation, and proinflammatory state (Quiroga *et al.,* 2017). This species is a source of fibers for the elaboration of handicrafts for indigenous Sikuani communities (Mesa-Castellanos *et al.,* 2017).

Other palms that stand out for their importance in the communities that inhabit the Amazon region belong to the *Euterpe* genus, such as *edulis*, *oleraceae,* and *precatoria*. The latter (*E. precatoria* Mart.) is known in countries like Brazil and Colombia as heart of palm. Its fruits are the traditional food of the Amazonian inhabitants; they are harvested from natural populations, employing management practices that have repercussions on the harvested populations and their productivity (Aranguren *et al.,* 2014). They have a high economic potential because all parts of the plant are used for food, such as the fruit in smoothies, ice creams and juices; for the construction of houses using the leaves; and for pharmaceutical use to treat muscle pain and snake bites where the roots and stem are used (Aranguren *et al.,* 2014).

E. oleracea, better known as asaí or açaí in Portuguese, is used by the native populations of the Amazon basin of Brazil, Colombia, Venezuela, Ecuador, and Suriname as food in drinks and desserts (Galate *et al.,* 2014). Regarding its economic importance, the açaí pulp obtained from fruits shows a remarkable richness in bioactive compounds, dietary fibers, vitamins, and minerals (Clarke *et al.,* 2011; Yamaguchi *et al.,* 2015).

Despite the economic, social and environmental importance, the genetic variability existing in wild palms, mainly of the genus Mauritia and Euterpe, is unknown, which is a limitation to having a better understanding of the genetic structure, dispersal, phylogenetic relationships, and population dynamics of the species that make up these genera. In addition to the above, the lack of knowledge of genetic diversity limits the identification of elite genotypes, as well as their possible use as a source of potentially useful genetic variation.

The concern for the loss of variability and the extinction of valuable species with great economic potential has aroused interest in carrying out genetic diversity studies in order to obtain information that contributes to their conservation and sustainable use. Molecular markers are a valuable tool that has been frequently used in studies like these (Ramos *et al.,* 2021).

Inter-Simple Sequence Repeat (ISSR) molecular markers are DNA segments located between repetitive nucleotide sequences, which generally occur in non-coding regions of the genome. They are dominant markers and have the advantages of being simple, fast, highly reproducible, and highly polymorphic, as well as requiring small DNA amounts, which makes them highly informative (Gupta *et al.,* 2021; Ghanbari *et al.,* 2022).

The ISSR technique has been used successfully for the assessment of genetic diversity and phylogenetic studies in plants (Ma *et al.,* 2021; Yancheva & Mavromatis, 2021; Mir *et al.,* 2021; Hocaoglu-Ozyigit *et al.,* 2022). Molecular characterization studies in wild palms have been carried out with ISSR markers (Rossi *et al.,* 2014; Chagas *et al.,* 2015; Pinheiro *et al.,* 2017; Madar *et al.,* 2019). The objective of this study was to evaluate the genetic diversity of wild palms *Mauritia flexuosa*, *Euterpe precatoria,* and *Euterpe oleracea* with ISSR molecular markers.

MATERIALS AND METHODS

Plant material. Fifty-one genotypes of wild palms from the departments of Meta, Nariño, Guaviare, Caquetá, Guainía, Putumayo, Vaupés, and Amazonas were evaluated. The species *M. flexuosa*, *E. oleracea,* and *E. precatoria* were included, which consisted of 11, 18, and 22 genotypes, respectively (Table 1). Three to four leaflets were taken from each accession, in good phytosanitary condition, stored in silica gel and transported to the laboratory, where they were stored at a temperature of -20°C.

Table 1. Genotypes of wild palms used for molecular characterization with ISSR markers.

ID	Species	Department	Municipality	Latitude	Length
6	*E. oleracea*	Meta	Villavicencio	04°03′32.98″N	073°27′55.29″O
8	*E. oleracea*	Vaupés	Mitú	01°15′58.12″N	070°12′16.61″O
17	*E. oleracea*	Guainía	Inírida	03°54′4.25″N	067°54′00.69″O
18	*E. oleracea*	Vaupés	Mitú	01°15′58.12″N	070°12′16.61″O
20	*E. oleracea*	Nariño	Tumaco	01°47′18.16″N	078°46′44.94″O
21	*E. oleracea*	Nariño	Tumaco	01°47′18.16″N	078°46′44.94″O
22	*E. oleracea*	Nariño	El rosario	01°44′32.93″N	077°20′09.85″O
23	*E. oleracea*	Nariño	Tumaco	01°44′32.97″N	077°20′10.73″O
25	*E. oleracea*	Nariño	Tumaco	01°47′18.16″N	078°46′44.94″O
26	*E. oleracea*	Nariño	Tumaco	01°47′18.16″N	078°46′44.94″O
27	*E. oleracea*	Nariño	S. Domingo	01°36′20.06″N	078°50′40.92″O
28	*E. oleracea*	Nariño	S. Domingo	01°36′20.06″N	078°50′40.92″O
33	*E. oleracea*	Guaviare	San José	02°31′36.05″N	072°38′47.52″O
34	*E. oleracea*	Guaviare	San José	02°31′36.05″N	072°38′47.52″O
46	*E. oleracea*	Amazonas	Leticia	04°08′38,4″ S	069°57′22,0″ W
47	*E. oleracea*	Amazonas	Leticia	04°08′42,0″ S	069°57′22,2″ W
50	*E. oleracea*	Amazonas	Leticia	03°45′50,2″ S	070°23′30,2″ W
51	*E. oleracea*	Amazonas	Leticia	03°45′50,7″ S	070°23′25,1″ W
1	*E. precatoria*	Meta	Villavicencio	04°03′32.98″N	073°27′55.29″O

ID	Species	Department	Municipality	Latitude	Length
2	*E. precatoria*	Guaviare	San José	02°31'49.83''N	072°40'06.76''O
3	*E. precatoria*	Putumayo	Mocoa	0°22'32.26''N	075°05'42.89''O
4	*E. precatoria*	Putumayo	Mocoa	0°22'32.26''N	075°05'42.89''O
5	*E. precatoria*	Guaviare	San José	02°33'54.76''N	072°35'49.72''O
7	*E. precatoria*	Caquetá	Florencia	01°36'48.74''N	075°39'17.33''O
9	*E. precatoria*	Guainía	Inírida	03°54'4.25''N	067°54'00.69''O
10	*E. precatoria*	Guaviare	San José	02°31'36.05''N	072°38'47.52''O
11	*E. precatoria*	Guainía	Inírida	03°54'4.25''N	067°54'00.69''O
12	*E. precatoria*	Guaviare	San José	02°31'49.83''N	072°40'06.76''O
13	*E. precatoria*	Guaviare	San José	02°31'49.83''N	072°40'06.76''O
14	*E. precatoria*	Amazonas	Leticia	03°48'37.54''S	070°17'53.03''O
15	*E. precatoria*	Guaviare	San José	02°31'36.05''N	072°38'47.52''O
19	*E. precatoria*	Guainía	Inírida	03°54'4.25''N	067°54'00.69''O
24	*E. precatoria*	Putumayo	Mocoa	0°22'32.26''N	075°05'42.89''O
29	*E. precatoria*	Meta	Villavicencio	04°03'32.98''N	073°27'55.29''O
30	*E. precatoria*	Guaviare	San José	02°36'4.43''N	072°37'10.28''O
31	*E. precatoria*	Guaviare	San José	02°36'4.43''N	072°37'10.28''O
32	*E. precatoria*	Guaviare	San José	02°36'4.43''N	072°37'10.28''O
48	*E. precatoria*	Amazonas	Leticia	04°07'54,1'' S	069°57'14,4'' W
49	*E. precatoria*	Amazonas	Leticia	04°07'54,5'' S	069°57'15,1'' W
52	*E. precatoria*	Amazonas	Leticia	04°08'41,1'' S	069°57'18,3'' W
35	*M. flexuosa*	Meta	Puerto Gaitán	04°18'30.53''N	072° 5'21.81''O
36	*M. flexuosa*	Meta	Puerto Gaitán	04°18'30.53''N	072° 5'21.81''O
37	*M. flexuosa*	Meta	Puerto Gaitán	04°18'30.53''N	072° 5'21.81''O
38	*M. flexuosa*	Meta	Puerto Gaitán	04°18'31.42''N	072° 5'22.73''O
39	*M. flexuosa*	Meta	Puerto Gaitán	04°18'31.42''N	072° 5'22.73''O
40	*M. flexuosa*	Meta	Villavicencio	04° 2'49.50''N	073°32'47.50''O
41	*M. flexuosa*	Meta	Villavicencio	04° 5'37.56''N	073°36'49.88''O
42	*M. flexuosa*	Meta	Villavicencio	04° 5'37.64''N	073°36'49.97''O
43	*M. flexuosa*	Meta	Villavicencio	04° 5'37.35''N	073°36'50.39''O
44	*M. flexuosa*	Meta	Villavicencio	04° 5'37.35''N	073°36'50.80''O
45	*M. flexuosa*	Meta	Villavicencio	04° 5'37.54''N	073°36'50.83''O

DNA extraction and PCR conditions. DNA extraction was carried out in the Plant Biotechnology, Animal Reproduction, and Genetics laboratories at the Universidad de los Llanos (Villavicencio, Meta). Genomic DNA was extracted using the DNA Extraction Kit (PowerSoil®) and DNA Isolation Kit - MO BIO Laboratories. DNA quality evaluation was performed using 0.8% agarose gels run in 0.5X TBE Buffer (0.045M Tris-Borate;

0.001M EDTA) at 80 volts for 45 min, using a Maxicell Primo EC-340 Electrophoresis Gel System chamber. To determine the amount and concentration of extracted DNA, the NanoDrop ND-1000 spectrophotometer was used. The quantified DNA was diluted in distilled water with a volume of 100μl to 10ng/μl. Seven ISSR-type primers (Technologies Inc.), whose sequence is shown in Table 2, were used.

Table 2. ISSR primers used to determine genetic diversity in wild palms.

Primer	Sequence (5' - 3')
CCA	$DDB(CCA)_5$
CGA	$DHB(CGA)_5$
TG	$HVH(TG)_7T$
AG	$HBH(AG)_7A$
CT	$DYD(CT)_7C$
CA	$DBDA(CA)_7$
ACA	$BDB(ACA)_5$

Degenerate sites: H (A or T or C); B (G or T or C); V (G or A or C); D (G or A or T); Y (C or T).

The amplification reaction was prepared in a final volume of 25 μl. The reaction mixture included 1X Buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1U Taq Polymerase, 2 μM primer, and 10ng genomic DNA. Amplification was carried out in a LabNet Multigene Optimax thermal cycler. Initial denaturation was at 95°C for 5 minutes; denaturation at 95°C for 30 seconds; annealing at 50°C (AG, CA and ACA primers); 55°C (CCA, CT and TG primers) and 58°C (CGA primer) for 45 seconds; an extension at 72°C for 2 minutes; 37 cycles from denaturation to extension; and finally, an extension at 72°C for 7 minutes. The amplified products were separated by electrophoresis on 1.2% agarose gels stained with GelRed. The samples were run at 150 volts for 1 hour, and visualization was performed in a Benchtop UV transilluminator M-15.

Statistic analysis. A binary matrix of absence (zero) and presence (one) was constructed. The similarity estimate was calculated using the method of Nei & Li (1979). The cluster analysis was performed using the UPGMA method, and a dendrogram was generated using the statistical package NTSYS-pc version 2.0. The cophenetic correlation coefficient, which is a measure between the similarity values of the dendrogram and those of the original similarity matrix, was calculated using the COPH and MXCOMP programs of the NTSYS-pc statistical package version 2.0.

An analysis of molecular variance (AMOVA) was performed using GenAlEx software version 6.2 (Peakall & Smouse, 2012) with 999 permutations in order to determine the differences between and within the groups formed. To estimate the average heterozygosity parameters (H_E) and the percentage of polymorphic loci, the unbiased

formula of Nei & Li (1979) was used, which is defined as $S_{ij} = 2N_{ij}/(N_i + N_j)$, where N_{ij} is the number of bands present in cultivars i and j, and N_i and N_j represent the number of bands present in cultivars i and j, respectively. The unbiased F statistic was determined with a 95% confidence interval. These analyzes were performed using the TFPGA statistical package (Tools for Population Genetic Analyzes, version 1.3) (Miller, 1997).

RESULTS AND DISCUSSION

Genetic diversity with ISSR. The seven primers used for the characterization of 51 palm genotypes generated a total of 153 amplified bands with molecular weights between 200 and 1000 bp, which ranged from 15 for the CT primer to 34 for the CGA primer (Table 3).

Table 3. Genetic diversity statistics (Nei, 1973) estimated from ISSR data evaluated in palm genotypes.

Primer	Bands	Polymorphic loci	H_E	Polymorphic loci (%)	F*st*
ACA	30	29	0.87	95.49	0.26
AG	18	16	0.66	87.00	0.08
CA	18	17	0.54	93.62	0.16
CCA	20	16	0.47	79.81	0.18
TG	18	16	0.78	91.20	0.27
CT	15	13	0.64	87.64	0.18
CGA	34	33	0.97	96.90	0.29
Mean	**153**	**140**	**0.70**	**90.24**	**0.20**

H_E: expected average heterozygosity. F*st*: coefficient of genetic differentiation.

In a study of three populations of the moriche palm *M. flexuosa* using nine ISSR markers, 97 polymorphic bands were reported in 51 individuals evaluated (Rossi *et al.,* 2014). Cardona *et al.* (2018) obtained 241 estimated alleles with a minimum of 17 alleles on the CGA primer and a maximum of 47 alleles for the ACA primer by evaluating seven ISSR primers in African palm (*E. guineensis* Jacq.). When comparing these studies carried out in different plant species, the number of estimated alleles obtained (153) in this research can be considered adequate to estimate genetic parameters.

The genetic diversity of Nei or diversity or expected heterozygosity (H_E) was 0.70, which reveals a high genetic diversity in the palm genotypes evaluated. The highest H_E values were found in the CGA primers with 0.97, ACA with 0.87, and TG with 0.78, while the lowest value corresponded to the CCA primer with 0.47 (Table 3). The high H_E value (0.70) indicates that the results could be influenced by the allogamous nature of the species evaluated, which tends to favor the conservation of a high percentage of

heterozygotes. According to Gomes *et al.* (2011), the high genetic diversity observed in populations of *M. flexuosa* is due to the fact that the species is allogamous. Regarding the species *E. oleracea* and *E. precatoria*, despite being monoecious plants, the fertilization process is generally crossed due to the high degree of self-incompatibility, as well as the non-synchronization in the maturation of the reproductive organs, which favors allogamy (Calzavara, 1972).

The results found here agree with those reported by Ramos *et al.* (2021), who found high genetic diversity (H_E= 0.66) when evaluating the genetic structure in populations of *Euterpe precatoria* Mart. in the Brazilian Amazon with microsatellite markers. Carvalho *et al.* (2020) found high heterozygosity values (He= 0.68) in their study of genetic diversity of *E. edulis* with microsatellite markers. In studies of genetic diversity in the species *M. flexuosa* using microsatellite markers, are reported the investigations of Sander *et al.* (2018), who found high genetic variation (H_E = 0.63–0.74), Melo *et al.* (2018) obtained H_E values between 0.52–0.88 and Montúfar *et al.* (2020) average H_E values of 0.70.

Similar results were found in other palm species, such as the date palm *Phoenix dactylifera* (Yusuf *et al.,* 2015), the oil palm *Elaeis guineensis* Jacq. (Gan *et al.,* 2021), and *Oenocarpus bataua* (Escobar *et al.,* 2018), which could indicate a behavior common to plants of the Arecaceae family.

The average polymorphism percentage obtained was 90.24%, where the ACA and CGA primers showed the highest values, corresponding to 95.49 and 96.90%, respectively (Table 3). These primers were the ones that contributed the most to diversity and may be appropriate for use in future research on the evaluation of genetic diversity and population structure of wild palms. It should also be noted that the primers AG, CA, CCA, TG, and CT showed important values of polymorphism; however, the CCA primer was the one with the lowest value (79.81), for which reason it was considered the primer with the least contribution to genetic diversity. Similar results were reported by Castro *et al.* (2022), who found that the level of polymorphism detected in the evaluated populations of *M. flexuosa* using microsatellite markers was high, above 90%.

In the characterization of 10 date palm cultivars, *Phoenix dactylifera,* using ISSR and AFLP molecular techniques, it was found that the level of polymorphism between cultivars for ISSR ranged from 20 to 100% with an average of 85%, while the levels of polymorphism for AFLP varied from 63 to 84% with an average of 76% (Sabir *et al.,* 2014). Gomes *et al.* (2011), when evaluating the genetic diversity in four populations of *M. flexuosa* with AFLPs markers, observed an average percentage of polymorphism comprised between 82% and 99.1%.

The fixation index (F_{st}) yielded a value of 0.20. According to Wright (1978), this value indicates a moderate genetic differentiation. This is possibly due to the existing gene flow between the palm tree genotypes evaluated, showing that there are no crossability

barriers or marked differences between the species. The TG and CGA primers presented a high level of polymorphism, as well as exhibiting high F_{st} values of 0.27 and 0.29, respectively, concerning the other primers evaluated (Table 3). Therefore, they could be useful to achieve greater differentiation between the genotypes of the genera *Euterpe* and *Mauritia* evaluated. The AG primer was the one that yielded a F_{st} of only 0.08, thus being the lowest of the primers used.

The moderate differentiation found in the present study can be explained by the cross-pollination typical of these palm species, the transport of plant material carried out by man, or by migration events of strains or seeds by water currents, birds and other animals. Works on palms have reported that the fruits of *E. precatoria* and *E. oleracea* are part of the diet of several animals that are seed dispersers in the forest, being the birds of the Psittacideae, Ramphastidae, and Cracidae families, the main wild animals that help in seed dispersal (Rocha & Viana, 2004). According to Virapongse *et al.* (2017b), the swamps where populations of *Mauritia flexuosa* develop host a high density and diversity of animals (primates, ungulates, rodents, frugivorous birds, and even reptiles and fish) that consume their lipid-rich fruits. Consequently, it has been assumed that this faunal diversity could favor an important seed-mediated gene flow (Montúfar *et al.,* 2020).

Ramos *et al.* (2018), in their study of the natural populations of *E. precatoria*, found low genetic differentiation and high pollen flow. The authors reported that these results could be influenced by animals as the main pollen dispersal vector, which pollinate different plant species in tropical forests. Similar results have been reported using ISSR (Sugai *et al.,* 2016) and SSR (Carvalho *et al.,* 2020) markers.

For their part, Coelho *et al.* (2020) found a moderate to high F_{st} value among the *E. precatoria* populations analyzed (from 0.10 to 0.37), indicating limited gene flow among the populations analyzed. The authors affirm that the differences found among the populations of this palm should not be related to habitat fragmentation but to evolutionary events that occur naturally, such as local adaptation (Brancalion *et al.,* 2018). Likewise, they affirm that the pattern of genetic differentiation found among the populations is probably influenced mainly by morphological differences.

In contrast, Santos-Cochev-da-Cruz *et al.* (2022), in their work on genetic diversity and population structure in populations of *E. precatoria*, found high genetic differentiation (F_{st}=0.77), low heterozygosity, and high inbreeding rate in all loci evaluated. According to the authors, the results could be influenced by the low flow of pollinators and by the reduction in the number of *E. precatoria* individuals in the fragments.

Cluster Analysis

The UPGMA average genetic clustering analysis based on the Nei & Li (1979) similarity coefficient clustered the palm genotypes into six groups at a similarity level of 37%

(Figure 1). According to the cophenetic correlation coefficient, a high correlation was detected (r=0.83), which indicates that the dendrogram faithfully represents the values of the similarity matrix.
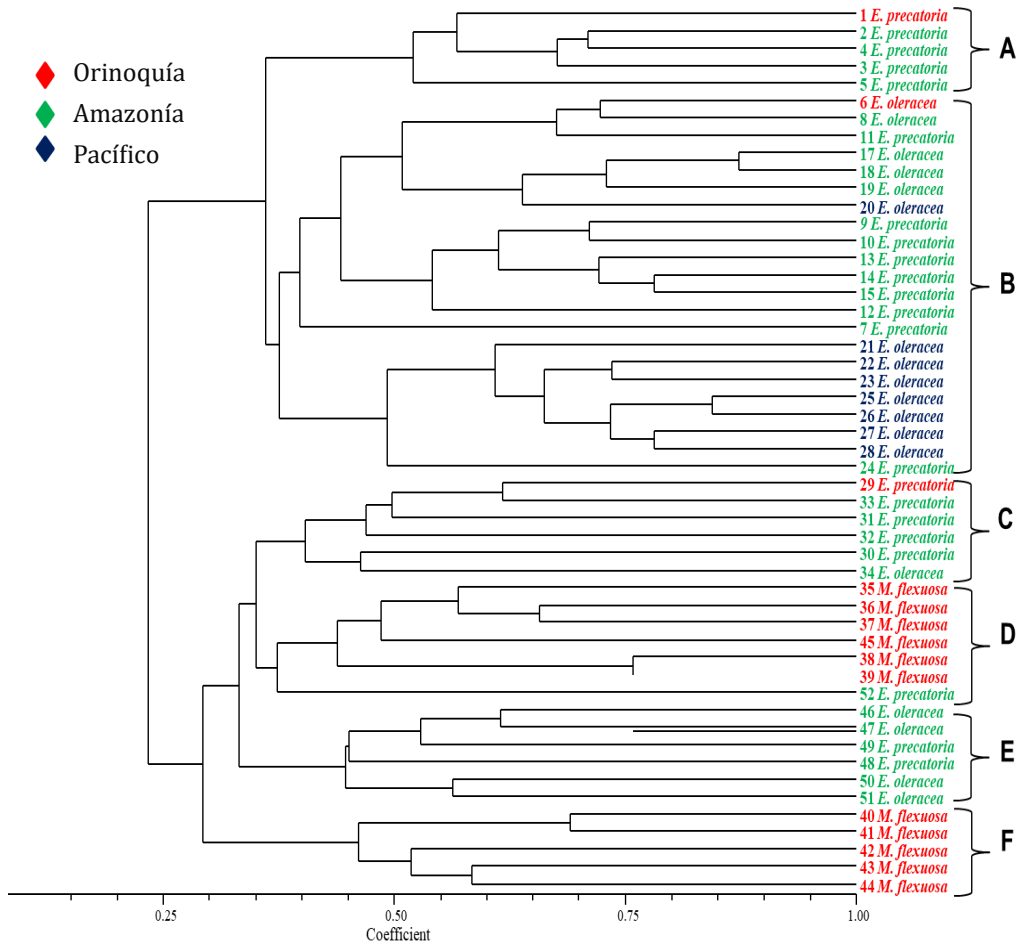
Figure 1. Dendrogram for the 51 wild palm genotypes, based on the Nei-Li (1979) similarity coefficient and estimated with seven ISSR markers.

It can be seen that group A was formed by five genotypes, while group B was the one that gathered the highest number of genotypes (22) corresponding to *E. oleracea* species with 13 genotypes and *E. precatoria* with nine from the Amazon and Pacific regions. Within this group, a subgroup with a similarity level of 40% was formed mainly by genotypes of the *E. oleracea* species collected in the department of Nariño.

Group C consisted of two *E. oleracea* genotypes and four *E. precatoria*, for a total of six genotypes. Similarly, group E consisted of six genotypes, of which half corresponded to the species *E. oleracea* and the rest to *E. precatoria*. The tendency of grouping genotypes of these two species in groups A, B, C, and E is possibly due to the constant flow of genes or the presence of common alleles that share the genotypes because they are species of the same genus.

Groups D and F were made up of individuals of the species *M. flexuosa*, which were collected in the department of Meta, which is part of the Orinoquía region. It is worth mentioning that group F was the one that presented the lowest degree of similarity, corresponding to 29%. Possibly, this is due to the morphological characteristics of the species as well as the presence of unique alleles, which make the individuals separate from the rest of the formed groups. The characterization with morphological descriptors could be useful to complement the molecular characterization and thus identify promising genotypes that can be included as parents in recombination programs.

The ISSR technique allowed for the discrimination of genotypes not only by the species to which they belong, i.e., *E. oleracea*, *E. precatoria,* and *M. flexuosa*, but also by their place of origin. Therefore, this technique can be considered a useful tool for assessing genetic diversity in populations.

The AMOVA revealed that 89% of the variation observed in this study was attributed to genetic differentiation within the groups formed, and the remaining 11% was due to the genetic variance component between groups (Table 4). Our results agree with those reported by Rossi *et al.* (2014) when evaluating three populations of *M. flexuosa* with ISSR markers, where AMOVA showed that 84.1% of the total genetic variation was within populations, while 15.9% was between populations. Similar results have been reported by Ithnin *et al.* (2017) and Montúfar *et al.* (2020).

The high variation found within the groups formed suggests that it is necessary to complement this information with other types of analysis, such as genetic structure, phylogenetic studies, as well as morpho agronomic characterization of characteristics of economic importance, among others. This will allow a better understanding of the genetic structure and population dynamics of the species evaluated in this study.

Table 4. Molecular Analysis of Variance (AMOVA) for the groups formed with the seven ISSR markers.

Source of variation	Degrees of freedom	Sum of squares	Middle Square	Total variation
Between groups	2	127.48	63.74	11%
Within groups	48	999.22	20.82	89%
Total	50	1,126.71		100%

CONCLUSIONS

The seven ISSR markers used for characterizing the fifty-one (51) palm genotypes generated a total of 153 amplified bands, showcasing a polymorphism range from 87.00 to 96.90, with an average of 90.24.

The expected heterozygosity (H_E) has a range of 0.47 to 0.97, with a mean of 0.70, which reveals a high genetic diversity in the evaluated palm genotypes. The high H_E value (0.70) indicates that the results could be influenced by the allogamous nature of the species evaluated, which tends to favor the conservation of a high percentage of heterozygotes.

The fixation index F_{st} has a value between 0.08 and 0.29, with an average of 0.20, showing a moderate genetic differentiation. The moderate differentiation found in the present study can be explained by the cross-pollination typical of these palm species, the transport of plant material carried out by man, or by migration events of strains or seeds by water currents, birds, and other animals.

The UPGMA average genetic clustering analysis based on the Nei & Li (1979) similarity coefficient clustered the palm genotypes into six groups at a similarity level of 37%, indicating a high correlation (r=0.83).

Conflict of interest: The authors declare that there is no conflict of interest.

BIBLIOGRAPHIC REFERENCES

Aranguren, C.I.; Galeano, G.; Bernal, R. (2014). Manejo actual del Asaí (*Euterpe precatoria* Mart.) para la producción de frutos en el Sur de la Amazonia Colombiana. *Colombia Forestal*. 17(1): 77-99.

Bernal, R.; Torres, C.; García, N.; Isaza, C.; Navarro, J.; Vallejo, M. (2011). Palm Management in South America. *The Botanical Review*. 77(4): 607-646. https://doi.org/10.1007/s12229-011-9088-6

Brancalion, P.H.; Oliveira, G.C.X.; Zucchi, M.I.; Novello, M.; van Melis, J.; Zocchi, S.S.; Chazdon, R.L.; Rodrigues, R.R. (2018). Phenotypic plasticity and local adaptation favor range expansion of a Neotropical palm. *Ecology and Evolution*. 8(43): 7462-7475. https://doi.org/10.1002/ece3.4248

Calzavara, B. (1972). As possibilidades do Açaizeiro no Estuário Amazônico. *Boletim da Faculdade de Ciências Agrárias do Pará*. (5): 1-103.

Cardona, C.C.; Morillo, C.Y.; Morillo, C.A.; Ochoa, I. (2018). Genetic diversity in oil palm (*Elaeis guineensis* Jacq.) using RAM (Random Amplified Microsatellites). *Bragantia*. 77(4): 546-556. https://doi.org/10.1590/1678-4499.2017385

Carvalho, M.S.; Ferreira, M.F.; Oliveira, W.B.; Marçal, T.; Guilhen, J.H.; Mengarda, L.; Ferreira, A. (2020). Genetic diversity and population structure of *Euterpe edulis* by REML/BLUP analysis of fruit morphology and microsatellite markers. *Crop Breeding and Applied Biotechnology*. 20(4): e31662048. https://doi.org/10.1590/1984-70332020v20n4a61

Castro, D.A.; Costa, T.S.; Cardoso, A.S.; Ramos, H.C.; López, J.A.; Diniz, L.E. (2022). Genetic structure analysis of *Mauritia flexuosa* natural population from the Lençóis Maranhenses region using microsatellite markers. *Scientia Agricola*. 79(1): e20200112. https://doi.org/10.1590/1678-992X-2020-0112

Chagas, K.P.; Sousa, R.F.; Fajardo, C.G.; Vieira, F.A. (2015). Seleção de marcadores ISSR e diversidade genética em uma população de *Elaeis guineensis*. *Revista Brasileira de Ciências Agrárias*. 10(1): 147-152. https://doi.org/10.5039/agraria.v10i1a5133

Clarke, C.; Schreckenberg, K.; Haq, N.N. (2011). *Fruit products for profit*. 16° edition. Rome: Food and Agriculture Organization of the United Nations. 16-80p.

Coelho, G.M.; Santos A.S.; de Menezes, I.P.; Tarazi, R.; Souza F.M.; Silva, M.D.; Gaiotto, F.A. (2020). Genetic structure among morphotypes of the endangered Brazilian palm *Euterpe edulis* Mart (Arecaceae). *Ecology and Evolution.* 10(12): 6039-6048. https://doi.org/10.1002/ece3.6348

Escobar, S.; Pintaud, J.C.; Balslev, H.; Bernal, R.; Moraes Ramírez, M.; Millán, B.; Montúfar, R. (2018). Genetic structuring in a Neotropical palm analyzed through an Andean orogenesis-scenario. *Ecology and Evolution*. 8(16): 8030-8042. https://doi.org/10.1002/ece3.4216

Freire, J.A.P.; Barros, K.B.; Lima, L.K.; Martins, J.M.; Araújo, Y.C.; Oliveira, G.L.; Aquino, J.A.; Ferreira, P.M. (2016). Phytochemistry profile, nutritional properties and pharmacological activities of *Mauritia flexuosa*. *Journal of Food Science*. 81: 611-622. https://doi.org/10.1111/1750-3841.13529

Galate, R. dos, S.; Mota, M.G.; da, C.; Gaia, J.M.D.; Costa, M. do, S.S. (2014). Distância fenotípica entre matrizes de açaizeiro (*Euterpe oleracea* Mart.) procedentes do nordeste do Pará. *Semina: Ciências Agrárias*. 35(4): 1667-1681. https://doi.org/10.5433/1679-0359.2014v35n4p1667

Galeano, G.; Bernal, R. (2005). Palmas. En: Calderón, E.; Galeano, G.; García, N. *Libro Rojo de Plantas en Colombia*. pp. 59-224. Volumen 2. Bogotá, Colombia: Instituto Alexander von Humboldt Instituto de Ciencias Naturales de la Universidad Nacional de Colombia.

Galeano, G.; Bernal, R. (2010). *Palmas de Colombia. Guía de Campo*. Colombia, Bogotá: Editorial Universidad Nacional de Colombia. 688p.

Galeano, G.; Bernal, R.; Figueroa, C.Y. (2015). *Plan de conservación, manejo y uso sostenible de las palmas de Colombia*. Primera Edición. Bogotá: Editorial Universidad Nacional de Colombia, Bogotá. 134 pp.

Gan, S.T.; Teo, C.J.; Manirasa, S.; Wong, W.C.; Wong, C.K. (2021). Assessment of genetic diversity and population structure of oil palm (*Elaeis guineensis* Jacq.) field genebank: A step towards molecular-assisted germplasm conservation. *PLoS ONE.* 16(7): e0255418. https://doi.org/10.1371/journal.pone.0255418

Ghanbari, M.A.; Salehi, H.; Moghadam, A. (2022). Genetic diversity assessment of Iranian Kentucky Bluegrass accessions: I. ISSR markers and their association with habitat suitability within and between different ecoregions. *Molecular Biotechnology*. 64(2): 1244-1258. https://doi.org/10.1007/s12033-022-00502-3

Gilmore, M.P.; Endress, B.A.; Horn, C.M. (2013). The socio-cultural importance of *Mauritia flexuosa* palm swamps (aguajales) and implications for multiuse management in two Maijuna communities of the Peruvian Amazon. *Journal of Ethnobiology and Ethnomedicine*. 9(1): 29. https://doi.org/10.1186/1746-4269-9-29

Gomes, L.R.; Lopes, M.T.; Bentes, J.L.; Barros, W.S.; Neto, P.Q.; Contim, L.A. (2011). Genetic diversity in natural populations of Buriti (*Mauritia flexuosa* L. f.). *Crop Breeding and Applied Biotechnology*. 11(11): 216-223. https://doi.org/10.1590/S1984-70332011000300003

Gupta, P.; Mishra, A.; Lal, R.K.; Dhawan, S.S. (2021). DNA fingerprinting and genetic relationships similarities among the accessions/species of *Ocimum* using SCoT and ISSR markers system. *Molecular Biotechnology*. 63(2): 446-457. https://doi.org/10.1007/s12033-021-00316-9

Hocaoglu-Ozyigit, A.; Ucar, B.; Altay, V.; Ozyigit, I.I. (2022). Genetic diversity and phylogenetic analyses of Turkish cotton (*Gossypium hirsutum* L.) lines using ISSR markers and chloroplast trnL-Fregions. *Journal of Natural Fibers.* 19(5): 1837-1850. https://doi.org/10.1080/15440478.2020.1788493

Ithnin, M.; Teh, C.K.; Ratnam, W. (2017). Genetic diversity of *Elaeis oleifera* (HBK) Cortes populations using cross species SSRs: implication's for germplasm utilization and conservation. *BMC Genetics.* 18(1): 37. https://doi.org/10.1186/s12863-017-0505-7

Koole, H.F.; Da silva, M.A.; Gozzo, F.; De Souza, A.Q.; De Souza, A.D. (2013). Antioxidant, antimicrobial activities and characterization of phenolic compounds from buriti (*Mauritia flexuosa* L. f.). *Food Research International*. 51(2): 467-473. https://doi.org/10.1016/j.foodres.2013.01.039

Ma, S.; Khayatnezhad, M.; Minaeifar, A. (2021). Genetic diversity and relationships among *Hypericum* L. species by ISSR markers: a high value medicinal plant from Northern of Iran. *Caryologia.* 74(1): 97-107. https://doi.org/10.36253/caryologia-968

MADR - Ministerio de Agricultura y Desarrollo Rural. (2021). Cadena de Palma de Aceite. https://sioc.minagricultura.gov.co/Palma/Documentos/2021-06-30%20Cifras%20Sectoriales.pdf

Madar, U.; Thangadurai, D.; Kulkarni, S.S.; Gai, P.; Saliyavar, B. (2019). Genetic variation in *Arenga wightii* Griff. (Arecaceae) populations using Inter Simple Sequence Repeat (ISSR) markers. *Plant Archives*. 19(1): 1646-1652.

Melo, W.A.; Freitas, C.G.; Bacon, C.D.; Collevatti, R.G. (2018). The road to evolutionary success: Insights from the demographic history of an Amazonian palm. *Heredity*. 121(2): 183–195. https://doi.org/10.1038/s41437-018-0074-1

Mesa-Castellanos, L.; Toro, B.A.; Isaza, A.C. (2017). Manejo de *Mauritia flexuosa* L.f. para la producción de artesanías en la altillanura colombiana. *Colombia Forestal*. 20(1): 85-101. https://doi.org/10.14483/udistrital.jour.colomb.for.2017.1.a07

Milanez, J.T.; Neves, L.C.; Silva, P.M.; Bastos, V.J.; Shahab, M.; Colombo, R.C.; Roberto, S.R. (2016). Pre–harvest studies of buriti (*Mauritia flexuosa* L. f.), a Brazilian native fruit, for the characterization of ideal harvest point and ripening stages. *Scientia Horticulturae*. 202: 77-82. https://doi.org/10.1016/j.scienta.2016.02.026

Miller, M.P. (1997). Tools for population genetic analysis (TFPGA), 1.3: A windows program for the analysis of allozyme and molecular population genetic data. https://www.scienceopen.com/document?vid=91146e4d-17b1-42e6-b5e0-7717b8d6600d

Mir, M.A.; Mansoor, S.; Sugapriya, M.; Alyemeni, M.N.; Wijaya, L.; Ah-mad, P. (2021). Deciphering genetic diversity analysis of saffron (*Crocus sativus* L.) using RAPD and ISSR markers. *Saudi Journal of Biological Sciences.* 28(2): 1308-1317. https://doi.org/10.1016/j.sjbs.2020.11.063

Montúfar, R.; Recalde, A.; Couvreur, T.L.P. (2020). High genetic diversity with low connectivity among *Mauritia flexuosa* (Arecaceae) stands from Ecuadorean Amazonia. *Biotropica.* 53(1): 152-161. https://doi.org/10.1111/btp.12855

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America*. 70(12): 3321-3323. http://dx.doi.org/10.1073/pnas.70.12.3321

Nei, M.; Li, W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*. 76(10): 5269-5273. https://doi.org/10.1073/pnas.76.10.5269

Peakall, R.; Smouse, P. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research- an update. *Bioinformatics*. 28(19): 2537-2539. https://doi.org/10.1093/bioinformatics/bts460

Pinheiro, L.G.; Chagas, K.P.; Freire, A.S.M.; Ferreira, M.C.; Fajardo, C.G.; Vieira, F.A. (2017). Anthropization as a determinant factor in the genetic structure of *Copernicia prunifera* (Arecaceae). *Genetics and Molecular Research*. 16(3). https://doi.org/10.4238/gmr16039768

Quiroga, Y.; Gómez, M.S.; Lares, M. (2017). Componentes Bioactivos del Asai (*Euterpe oleracea* Mart. y *Euterpe precatoria* Mart.) y su efecto sobre la salud. *Archivos Venezolanos de Farmacología y Terapéutica*. 36(3): 58-66.

Ramos, S.L.; Dequigiovanni, G.; Sebbenn, A.M.; Lopes, M.T.; de Macêdo, J.L.; Veasey, E.A.; Alves-Pereira, A.; da Silva, P.P.; Garcia, J.N.; Kageyama, P.Y. (2018). Paternity analysis, pollen flow, and spatial genetic structure of a natural population of *Euterpe precatoria* in the Brazilian Amazon. *Ecology and Evolution.* 8(22): 11143-11157. https://doi.org/10.1002/ece3.4582

Ramos, S.L.F.; Dequigiovanni, G.; Lopes, M.T.G.; Aguiar, A.V.; Lopes, R.; Veasey, E.A.; Macêdo, A.L.V.; Alves-Pereira, A.; Fraxe, T.J.P.; Wrege, M.S.; Garcia, J.N. (2021). Genetic structure in populations of *Euterpe precatoria* Mart. In the Brazilian Amazon. *Frontiers in Ecology and Evolution*. 8:603448. https://doi.org/10.3389/fevo.2020.603448

Rocha, E.; Viana, V.M. (2004). Manejo de *Euterpe precatoria* Mart. (Açaí) no seringal Caquetá, Acre, Brasil. *Scentia Forestalis*. 65: 59-69.

Rossi, F.S.; Rossi, A.; Dardengo, J.D.; Brauwers, L.R.; da Silva, M.L.; Sebbenn, A.M. (2014). Diversidade genética em populações naturais de *Mauritia flexuosa* L.f. (Arecaceae) com uso de marcadores ISSR. *Scientia Forestalis*. 42(104): 631-639.

Sabir, J.S.; Abo-Aba, S.; Bafeel, S.; Zari, T.A.; Edris, S.; Shokry, A.; El-Domyati, F.M. (2014). Characterization of ten date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia using AFLP and ISSR markers. *Comptes Rendus Biologies*. 337(1): 6-18. https://doi.org/10.1016/j.crvi.2013.11.003

Sander, N.L.; Pérez-Zavala, F.; Da Silva, C.J.; Arruda, J.C.; Pulido, M.T.; Barelli, M.; Rossi, A.B.; Viana, A.P.; Boechat, M.S.B.; Bacon, C.D.; Cibrián-Jaramillo, A. (2018). Rivers shape population genetic structure in *Mauritia flexuosa* (Arecaceae). *Ecology and Evolution*. 8(13): 6589-6598. https://doi.org/10.1002/ece3.4142

Santos-Cochev-da-Cruz, J.; de-Freitas-Encinas-Dardengo, J.; Souza-Rodrigues, A.; Vicente-Tiago, A.; Moreno-de-Pedri, E.; Müller-Zortéa, K.; Gonçalves-Garcia, M.; Alves-da-Silva-Neves, S.; Bandini-Rossi, A. (2022). Diversity and genetic structure of natural populations of the palm tree *Euterpe precatoria* (Arecaceae). *Revista de Biología Tropical*. 70(1): 213-221. https://doi.org/10.15517/rev.biol.trop.v70i1.42942

Speranza, P.; Falcão, A.O.; Macedo, J.A.; Silva, L.H.; Rodrigues, A.M.; Macedo, G.A. (2016). Amazonian Buriti oil: chemical characterization and antioxidant potential. *Grasas Aceites*. 67(2): 1-9. https://doi.org/10.3989/gya.0622152

Sugai, K.; Watanabe, S.; Kuishi, T.; Imura, S.; Ishigaki, K.; Yokota, M.; Suyama, Y. (2016). Extremely low genetic diversity of the northern limit populations of *Nypa fruticans* (Arecaceae) on Iriomote Island, Japan. *Conservation genetics*. 17(1): 221-228. https://doi.org/10.1007/s10592-015-0773-6

Trujillo-González, J.M.; Torres, M.A.; Santana-Castañeda, E. (2011). La palma de Moriche (*Mauritia flexuosa* L.f) un ecosistema estratégico. *Orinoquia*. 15(1): 62-70. https://doi.org/10.22579/20112629.43

Valois-Cuesta, H.; Martínez-Ruiz, C.; Rentería Cuesta, Y.Y.; Panesso Hinestroza, S.M. (2013). Diversidad, patrones de uso y conservación de palmas Arecaceae en bosques pluviales del Chocó, Colombia. *Revista de Biología Tropical*. 61(4): 1869-1889. https://doi.org/10.15517/rbt.v61i4.12858

Virapongse, A. (2017a). Social mechanisms and mobility: Buriti Palm (*Mauritia flexuosa)* extractivism in Brazil. *Human Ecology*. 45(1): 119-129. https://doi.org/10.1007/s10745-016-9887-9

Virapongse, A.; Endress, B.A.; Gilmore, M.P.; Horn, C.; Romulo, C. (2017b). Ecology, livelihoods, and management of the *Mauritia flexuosa* palm in South America. *Global Ecology and Conservation*. 10: 70-92. https://doi.org/10.1016/j.gecco.2016.12.005

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

Yamaguchi, K.K.; Pereira, L.F.; Lamarão, C.V.; Lima, E.S.; da Veiga-Junior, V.F. (2015). Amazon acai: chemistry and biological activities: a review. *Food Chemistry*. 179: 137–151. https://doi.org/10.1016/j.foodchem.2015.01.055

Yancheva, S.; Mavromatis, P. (2021). Phylogenetic analysis of selected olive genotypes by ISSR markers. *Journal of Central European Agriculture*. 22(1): 127-132. https://doi.org/10.5513/JCEA01/22.1.2960

Yusuf, A.O.; Culham, A.; Aljuhani, W.; Ataga, C.D.; Hamza, A.M.; Odewale, J.O.; Enaberue, L.O. (2015). Genetic diversity of Nigerian date palm (*Phoenix dactylifera*) germplasm based on microsatellite markers. *International Journal of Bio-Science and Bio-Technology*. 7(1): 121-132. https://doi.org/10.14257/ijbsbt.2015.7.1.12