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Genomic Characterization of SNPs for Genetic Differentiation and Selection in Populations from the American Oil Palm [*Elaeis oleifera* (Kunth) Cortés] Germplasm Bank from Brazil

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Abstract: In this study, we used SNP markers to access the genetic components occurrence of genetic differentiation resulting from the selection processes applied to collect and maintain the germplasm bank of *Elaeis oleifera* (Kunth) Cortés from the Brazilian Amazon rainforest. A set of 1667 higher quality SNPs—derived from a previous GBS study—was used for genomic characterization and calculation of genetic parameters. There is differentiation in the distribution of alleles between populations for 78.52% of the tested loci. Genotypic diversity test results indicated strong evidence of genotypic differentiation between populations. Sixteen out of the nineteen tested deviated significantly from the expected allele frequencies in HWE, reinforcing the hypothesis that there was maybe a selection in the evaluated populations. A group of 568 loci with a higher probability of being under selection effects were selected, both directional and stabilizing. In total, 1546 and 1274 SNPs aligned in the genomes of *E. oleifera* and *E. guineensis* Jacq., respectively. These markers showed a wide distribution throughout the genome of the two species. In conclusion, the *E. oleifera* GB from the Brazilian Amazon rainforest has specific genetic structures and good genetic variability within populations.

Keywords: caiaué; SNP markers; diversity; selection; conservation strategy

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1. Introduction

Elaeis guineensis Jacq. and *Elaeis oleifera* (Kunth) Cortés comprise the two only species of this genus. The first one is the African oil palm, a crop of great commercial importance and source of the largest share of vegetable oil consumed in the world [1,2]. The second one is the American oil palm, native to and widely distributed in the Central and Northern regions of South America [3]. Caiaué is the common name given to the American oil palm in Brazil.

These two species are monoecious (male and female reproductive organs on different parts of the same plant), producing male and female flowers during distinct sexual cycles [4,5]. There are no differences in the development of inflorescence structures between the two species [6]. Entomophilous pollination is the primary method of pollination in this genus, although manually assisted pollination is a common agronomic practice in some countries; however, there are some differences in the flowers of *E. oleifera* and the interspecific hybrids when compared with *E. guineensis*, which reduces the effectiveness of insect pollination [7].

Although not a commercially attractive species due to its low productivity—an oil to bunch ratio of 5%, compared to 25% of oil palm—caiaué has pronounced importance to oil palm breeding programs in Brazil and elsewhere for the development of superior

interspecific hybrids by crossing with the African oil palm. Among the characteristics that proved to be superior to African oil palm and, therefore, subject to introgression via breeding programs, the following stand out: (i) resistance to fatal yellowing, a disease of unknown etiology severely affecting oil palm plantations in Brazil [8]; (ii) smaller plant size and slower growth, which facilitates cultivation and prolongs the time of commercial exploitation; (iii) the quality of the oil, which has a higher content of carotenoids and more unsaturated oil than palm oil; (iv) lower lipase activity in the fruits' mesocarp, allowing more time between harvesting and processing; and (v) wide genetic variability, allowing genetic gain through the production of hybrids.

To have better access to the genetic variability, providing consequently higher genetic gains in developing economically sustainable interspecific hybrids, proper maintenance of the germplasm bank (GB) followed by genetic and morphological characterization is a must. The great commercial importance of *E. guineensis* has encouraged the publication of many studies reporting the genetic variability of different populations and their genetic differentiation [9–11]. On the other hand, the information available for *E. oleifera* is scarcer, mainly those involving the use of molecular markers in large numbers. In the last 20 years, only a few studies have applied DNA markers (RFLP, AFLP, RAPD, microsatellites, and SNPs) to characterize genetic and phenotypic diversity, population structure, or even design a core collection of the American oil palm [3,12–17]. The *E. oleifera* collection at Embrapa has a moderate degree of genetic diversity and a high interpopulation genetic differentiation [17].

The current study adds to a previous one carried out by our group on identification, selection, and use of SNP markers to characterize the genetic diversity and population structure and to design a core collection of the *E. oleifera* Germplasm Bank (GB) maintained in Brazil by Embrapa [17]. Using a set of 1667 SNPs allowed the identification and characterization of possible markers under the effect of selection; these markers were characterized at the genomic level in *E. oleifera* and *E. guineensis*, seeking to understand their distribution and organization. Our approach allows us to select those with a high probability of transferability between species, aiming, above all, to underpin decisions related to the conservation of the species as well as to breeding programs of the African oil palm through the conscious and efficient use of the American oil palm germplasm available in Brazil.

2. Materials and Methods

2.1. Plant Materials

Plants used in this study belong to the Brazilian *E. oleifera* Germplasm Bank (GB) and are maintained in vivo at the Rio Urubu Experimental Station—Embrapa Western Amazon, located 140 km from Manaus, in the municipality of Rio Preto da Eva, Amazonas, Brazil, latitude 2°35′ S, longitude 59°28′ W, and altitude 200 m. This GB was established based on a series of expeditions in the Brazilian Amazon rainforest organized by Embrapa and CIRAD in the early 1980s [18]. These plants are from 206 different half-sibling families (subsamples) out of the 246 subsamples that make up the entire GB. Each half-sibling family is a group of 10 plants originated from seeds collected from a plant. The number of subsamples per locality varied considerably (Table 1). There are 19 populations in the GB; each one is composed of all subsamples from that locality. These distinct localities spread throughout six geographic regions in the States of Amazonas and Roraima (Table 1). For this study, we collected and used leaves from three plants per subsample.

Table 1. Origin and number of 553 plants, representing 206 subsamples (half-sibling families) collected from the *Elaeis oleifera* Germplasm Bank at Embrapa Western Amazon (CPAA). Plants from 19 different populations (localities) originally collected at six distinct geographic regions in the Brazilian Amazon rainforest (Manaus, Rio Amazonas, Rio Solimões, Rio Negro, Caracaraíe Rio Madeira).

Population	Geographic Region	Locality	Number of Subsamples	Number of Plants
1	Manaus	Caldeirão	7	18
2		Careiro	25	68
3		Manacapuru	1	3
4		Iranduba	2	6
	Subtotal		35	95
5	Rio Amazonas	Amatari	11	31
6		Autazes	11	28
7		Maués	11	32
	Subtotal		33	91
8	Rio Solimões	Anori	3	9
9		B. Constant	1	3
10		Coari	19	54
11		Tefé	5	14
12		Tonantins	4	12
	Subtotal		32	92
13	Rio Negro	Acajatuba	10	29
14		Barcelos	2	2
15		Moura	11	32
	Subtotal		23	63
16	Caracaraí	BR174	12	35
17		Vila Moderna	06	18
	Subtotal		18	53
18	Rio Madeira	Manicoré	58	140
19		Novo Aripuanã	7	19
	Subtotal		65	159
	TOTAL		206	553

2.2. Genotyping by Sequencing and SNP Selection

Leaves from individual trees, collected fresh in the field, were stored at −80 °C until DNA extraction. Total DNA was extracted according to a modified CTAB protocol [19] and sent to DArT Pty® to perform genotyping by sequencing (GBS) using the DArTseq Technology. At DArT Pty®, due to quality problems in some DNA samples, 65 out of the 618 initial plants were discarded and the GBS performed using the remaining 553. After removing the barcodes, the resulting sequences underwent trimming at 69 bp (5 bp restriction site plus 64 bases with a minimum Q score of 10), and virtually identical reads (i.e., less than three polymorphisms) were combined so that one or more SNPs in the read did not confuse the analysis. A low coverage consensus sequence was generated and used as a reference in the discovery of SNPs by aligning the 69 bp reads using the Bowtie v0.12 program [20].

The DArT Pty® pipeline generated 7461 SNP markers, divided into 5365 higher, 146 high, and 1950 lower quality SNPs. After filtering the higher quality SNPs based on a Call Rate higher than 0.90 and Minor Allele Frequency (MAF) higher than 0.05, a set of 1667 SNP markers was generated and used to run the genetic analysis.

2.3. Genetic Analysis

The Genepop software version 4.2, a population genetics software package, was used to analyze the following genetic parameters: the number of migrants (Nm), percentage of polymorphic loci (criterion 0.95), expected and observed heterozygosity (He and Ho), and Wright's F statistics (Fis and Fst—[21]). Chi-square tests were performed for each locus for deviation of genotypes concerning the Hardy–Weinberg equilibrium.

Genotypic differentiation was tested by evaluating the distribution of genotypes across the population, using an unbiased estimator of the *p*-value of an exact test (G test). The tested nullity hypothesis is that the genotypic distribution is identical across all tested populations. The estimates of the exact *p*-values for the tests of conformity with the expectations of the Hardy–Weinberg equilibrium were calculated using the Monte Carlo randomization method via Markov chains (MCMC) [22] and the expected number of heterozygotes was computed using Levene's test [23].

To identify adaptive SNP (putative loci under selection) and the neutral loci, we used a coalescent simulation processed by the LOSITAN software, a workbench to detect molecular adaptation based on a Fst-outlier method [24]. A simulation was first performed with the gross degree of genetic differentiation values calculated for each one of the 1667 SNPs, aiming to obtain the neutral degree of genetic differentiation for the data set not biased by extreme values. The LOSITAN software was also used to separate SNP loci possibly under the selection from neutral loci. For this, a coalescent simulation was initially performed through obtaining a neutral Fst value, based on all 1667 analyzed SNP loci. Then, this average neutral Fst was used to perform a new simulation to identify loci outliers, which are possibly under selection effect.

2.4. Alignment of SNPs Sequences to Elaeis guineensis and E. oleifera Genomes

SNP sequences were aligned to: (a) the African oil palm reference genome [25]—files downloaded from the National Center for Biotechnology Information (BioProject PRJNA192219; BioSample SAMN02981535) on April 2021; and (b) a local preliminary assembly (version 1.0) of the genome of *E oleifera* access from the Amazon rainforest, Manicoré, belonging to the *E. oleifera* Germplasm Bank of Embrapa [26].

2.5. Genomic Characterization and Functional Annotation

The 1667 SNPs were mapped against the reference genome of *E. guineensis* and *E. oleifera* by means of a Blastn (blastn-task blastn-short-max_target_seqs 3), the alignments were filtered based on e-value (less than e-10) and alignment coverage (greater than 90). Based on the GFF (General Feature Format) genome file, intragenic and intergenic SNPs were identified in the analyzed set (only those aligned with 97–100% identity to the reference). The distribution of SNPs in the chromosomes of *E. guineensis* and the synteny analysis were visualized using the chromoMap R package. Functional annotations of genes containing SNPs (intragenic) were performed using the Blast2go software implemented in the OmicsBox package [27].

3. Results

3.1. Genetics Analysis—Genotypic Differentiation in E. oleifera

The genetic parameters were calculated based on the 1667 selected SNP markers. By calculating the average frequency of private alleles [28,29] in the populations evaluated ($p = 0.05$) and the average sample size (N = 21.54), the estimated number of migrants (Nm)—the gene flow in the Brazilian GB of *E. oleifera*—was 2.65. This Nm value estimated indicates that the frequency of private alleles is inversely proportional to the migration rate in the GB used in this study.

To investigate whether the alleles in various genotypes are generated from the same distribution for all populations, we applied the test for allelic differentiation. A total of 1309 (78.52%) SNP loci were significant at 5% by the Fisher method when tested for allelic differentiation, indicating that there is differentiation in the distribution of alleles among

populations for the vast majority of the loci tested. Genotypic diversity test, which analyzes the distribution of diploid genotypes in various populations, was also applied to test the differentiation of populations. A total of 1613 (96.76%) SNP loci were significant, indicating strong evidence of genotypic differentiation between individuals in the populations tested. The results from allelic differentiation and genotypic diversity tests indicated the occurrence of population structure, even though the number of migrants was estimated at 2.65. Therefore, there is evidence of the occurrence of selection in the populations evaluated.

Another complementary strategy to analyze population differentiation consists of investigating whether the allele frequencies within populations and the total population match the frequencies expected in the Hardy–Weinberg equilibrium. Deviations from this equilibrium indicate the occurrence of inbreeding, selection, migration, or even a combination of these factors. When considering the 19 populations as a single population, the results indicated a complete deviation from the Hardy–Weinberg equilibrium towards the excess of heterozygotes (p-value = 0). On the other hand, tests within populations resulted in three populations in Hardy–Weinberg equilibrium (4, 9, and 14), while the other 16 (1, 2, 3, 5, 6, 7, 8, 10, 11, 12, 13, 15, 16, 17, 18, and 19) deviated significantly ($p = 0.01$) from the expected allele frequencies in equilibrium (Table 1). These results reinforce the hypothesis that maybe there was a selection in the evaluated populations, which would justify the occurrence of a population structure even with Nm = 2.65.

The analysis carried out using the Genepop software to calculate the genetic variance between and within the sub-populations returned significantly high and negative inbreeding coefficient (Fis) values on all populations (from −0.62 to −0.82), indicating a high rate of outbreeding among individuals within a population and an excessive number of heterozygotes (Table 2). These data may seem contradictory at first, but the high occurrence of inbreeding and the number of heterozygotes in excess can be explained by a recent genetic drift so that the total population has not yet had time to re-balance itself.

Table 2. Genetic variances (inter- and intra-population) and fixation index (Fis) per population from the Brazilian *E. oleifera* Germplasm Bank for the set of 1667 SNP loci. 1-Qintra: intrapopulation allelic diversity. 1-Qinter: interpopulation allele diversity. Fis: diversity measure.

Population	1-Qintra	1-Qinter	Fis
1	0.617	0.353	−0.745
2	0.629	0.378	−0.665
3	0.629	0.382	−0.645
4	0.629	0.380	−0.655
5	0.634	0.384	−0.650
6	0.611	0.377	−0.619
7	0.594	0.361	−0.644
8	0.623	0.376	−0.656
9	0.613	0.369	−0.661
10	0.629	0.378	−0.663
11	0.600	0.365	−0.647
12	0.632	0.367	−0.720
13	0.649	0.357	−0.820
14	0.647	0.372	−0.741
15	0.667	0.378	−0.765
16	0.651	0.365	−0.785
17	0.612	0.357	−0.713
18	0.591	0.366	−0.617
19	0.636	0.375	−0.727

Another parameter associated with Fis that can help clarify the genetic relationships between the populations studied is the fixation index (Fst). It measures the influence of the relationship between drift and gene flow in the population structure. Genomic loci or regions with high Fst values and highly variable between populations are potentially associated with selection processes. Since selection—whether natural or artificial—tends

to increase genetic differentiation, the higher the Fst value, the stronger the evidence of differentiation is [30]. The gross results of the Fst values assigned to each SNP ranged from −0.097 to 0.343, with an average of 0.031. The average neutral Fst was then used to perform a new simulation to identify loci outliers, possibly under selection effect (Figure 1). A list with the top 50 loci under positive selection and the 50 loci under negative selection is presented in the Table S1.

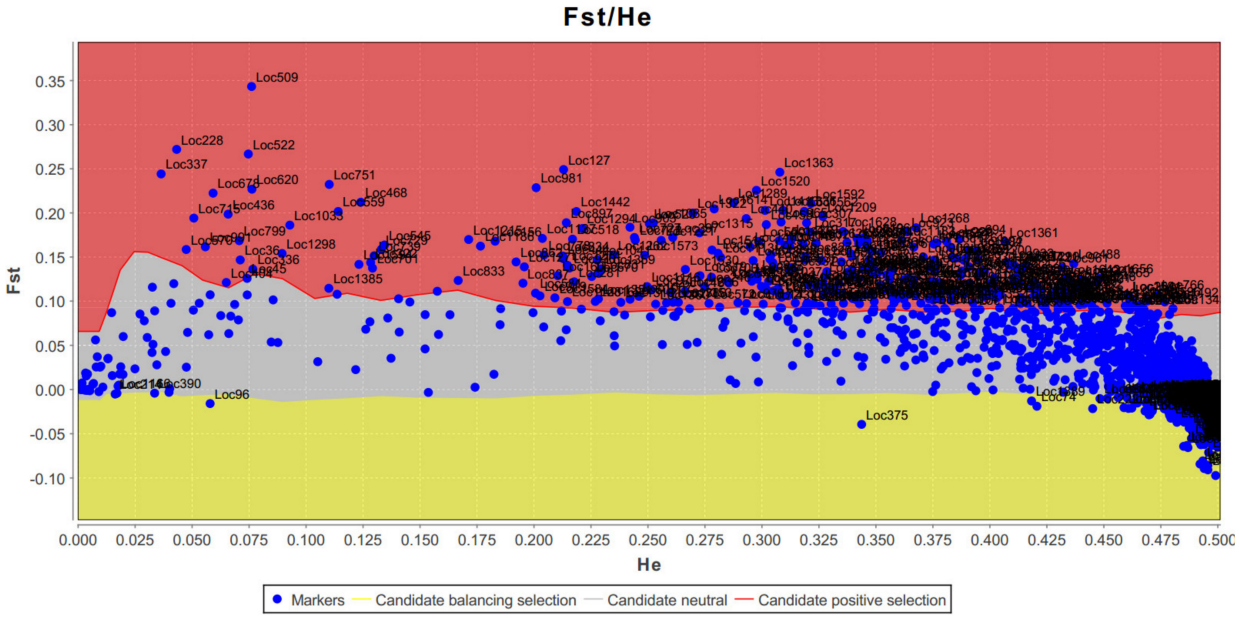


Figure 1. Identification of outlier loci for neutral Fst, performed in the LOSITAN software using the set of 1667 SNP loci analyzed in 553 *E. oleifera* plants from 206 half-sibling families, which make up for a group of 19 populations sampled in six major geographic regions of the Brazilian Amazon rainforest.

Obtaining a neutral Fst value from a coalescent simulation performed in the LOSITAN software allowed the identification and segregation of potential loci under selection. Based on the distance from their Fst value to the neutral Fst calculated in the coalescent simulation, we identified SNP loci with different potentials of selection intensity. The outlier loci were then identified one by one, with information on their distribution in the different genotypes. In this way, 568 loci with a higher probability of being under selection effect were selected, both directional and stabilizing, and characterized regarding the location in the genome (Table S2).

3.2. Genomic Characterization of SNPs in E. oleifera and E. guineensis

From a total of 1667 SNPs, it was possible to align 1546 and 1274 SNPs loci in the genomes of *E. oleifera* and *E. guineensis*, respectively. When searching for intragenic SNPs, 261 and 222 SNPs were identified in *E. oleifera* and *E. guineensis*, respectively. SNPs are distributed homogeneously along all 16 chromosomes of *E. guineensis* (Figure 2); however, for the *E. oleifera* draft, SNPs were found over 1208 scaffolds (Table S3).

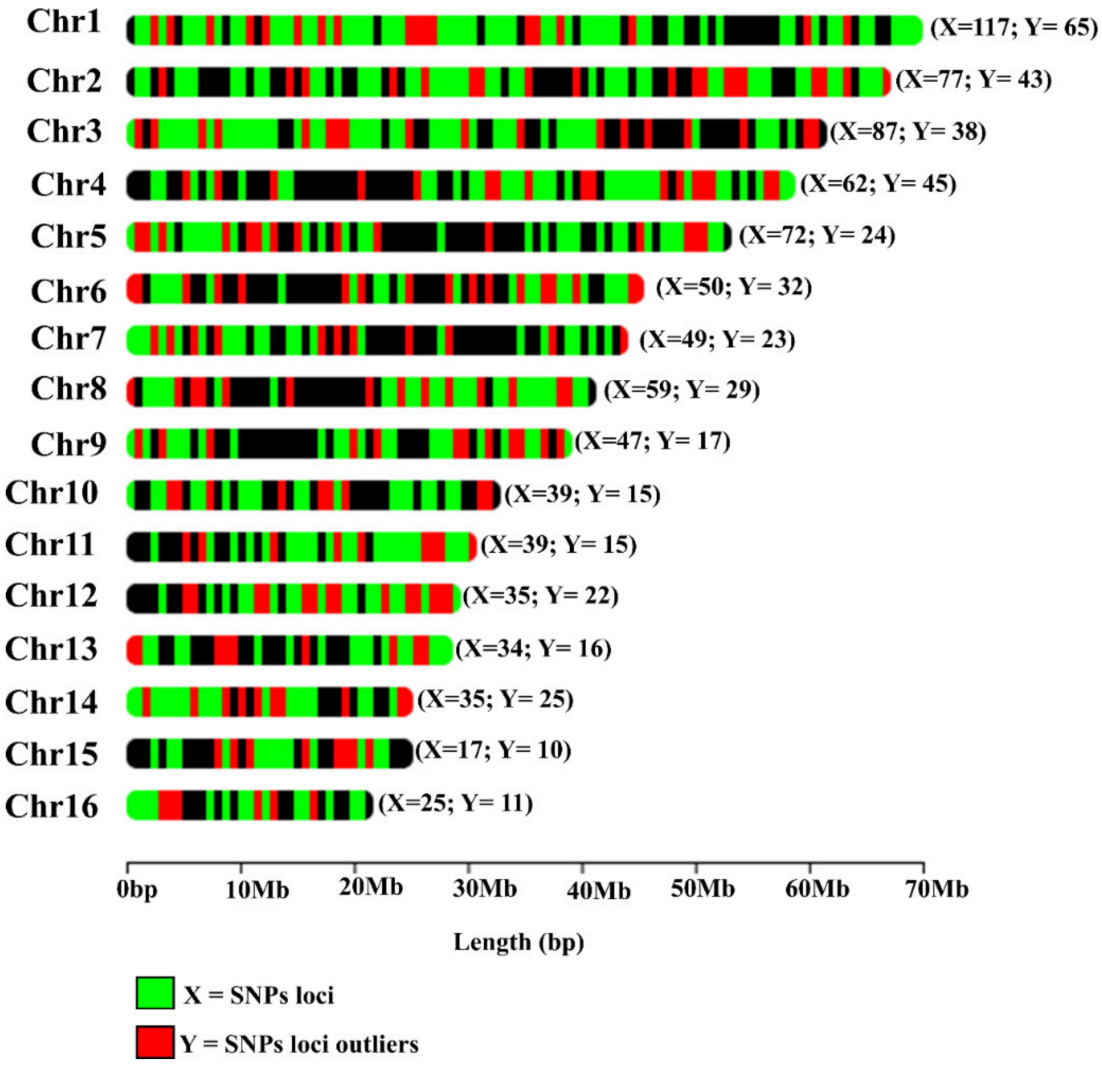
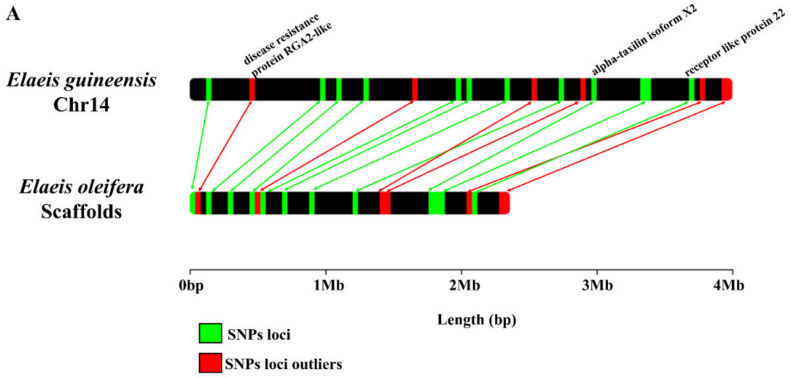


Figure 2. Distribution of 1274 SNPs loci aligned against *E. guineensis* chromosomes (PRJNA192219), visualized through the R package chromoMap. X = SNPs loci; Y = SNPs loci outliers.

To investigate the SNP collinearity between these two species, we analyzed a region of Eg chromosome 14 (4 Mb) containing 20 SNPs which is corresponding to 15 *E. oleifera* scaffolds (2.1 Mb) (Figure 3A). The *E. oleifera* scaffolds have a variation of 1 to 2 SNPs, where the smallest one had a size of 7448 bp and the largest one, 304,670 bp (Figure 3B). Based on this analysis, it is possible to observe collinearity between the SNPs of the two species and a probable transferability between these markers.

Genes containing intragenic SNPs from both species were functionally annotated according to the three main ontologies (cellular component—CC; molecular function—MF; and biological process—BP). For *E. oleifera*, terms such as intracellular component (CC), intrinsic component (CC), catalytic activity (MF), carbohydrate-binding (MF), and response to stress (BP) were identified (Figure 4A). For *E. guineensis*, terms such as hydrolase activity (MF) and catalytic activity (MF) were found (Figure 4B). When comparing the two species, it was possible to identify 69 genes containing intragenic SNPs unique to *E. oleifera* (Figure 4C and Table S4).



B

Scaffold	Number of SNPs	Length (bp)
1-Scaffold_12263	1	25049
2-Scaffold_15208	1	34159
3-Scaffold_108	2	348774
4-Scaffold_4796	2	57504
5-Scaffold_30087	1	7448
6-Scaffold_3137	1	108806
7-Scaffold_2402	1	91872
8-Scaffold_121	1	337750
9-Scaffold_204	2	294816
10-Scaffold_6363	1	50372
11-Scaffold_145	1	304670
12-Scaffold_670	2	186548
13-Scaffold_370	2	230603
14-Scaffold_4711	1	59031
15-Scaffold_14727	1	24024
Total	**20**	**2161426**

Figure 3. Synteny and collinearity of 20 SNPs loci between *E. guineensis* and *E. oleifera* (**A**). Table with the sizes of scaffolds of *E. oleifera* used for the comparative analysis (**B**). Visualized through the R package chromoMap.

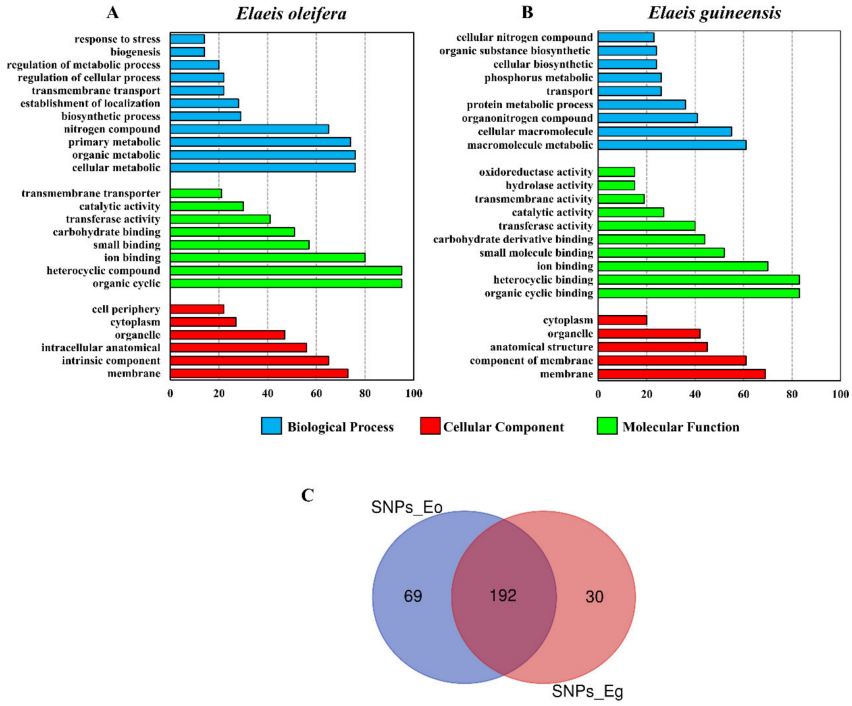


Figure 4. Functional annotation of genes containing SNPs in *E. oleifera* (**A**) and *E. guineensis* (**B**). Venn diagram comparing genes containing intragenic SNPs in *E. oleifera* and *E. guineensis* (**C**). Functional annotation was performed on the OmicsBox package.

4. Discussion

The GBS technique has been successfully used in SNP discovery, mapping genome-wide markers, genomic diversity study, genetic linkage analysis, and genomic selection in a wide range of crop species [31], such as rice [32], soybean [33], maize [34,35], and wheat [36,37]. In this study, a GBS approach was applied to select a set of 1667 SNP markers and use it to access the genetic components of the *E. oleifera* Germplasm Bank (GB) maintained in Brazil by Embrapa, as well as to investigate the genomic distribution of these markers and occurrence of genetic differentiation resulting from the selection processes used to establish this GB.

According to Slatkin and Barton [38], gene flow values determine whether genetic drift is the only cause of high genetic variability between different sites. Nm values greater than 1.0 indicate that the gene flow is acting against the forces of genetic drift and, as a consequence, the populations that are exchanging individuals will be homogenized. Values below 1.0 indicate the occurrence of genetic drift and consequent population differentiation. However, the occurrence of selection can lead to population differentiation even with Nm values greater than 1.0. For this reason, other tests for population differentiation were carried out to investigate the possibility of occurrence of selection and population structure even with values of migration rate greater than 1.0.

The dispersion of alleles between different populations is called gene flow and can be caused by the dispersion of pollen and seeds, among other types of dispersion mechanisms. A high level of gene flow causes a reduction in genetic differentiation between the populations involved [39]. Thus, gene flow throughout evolutionary history was responsible for the greater homogenization of populations, minimizing the effects of selection and genetic drift [40]. As an economically important crop, cultivated in many countries, oil palm does not have genetic differentiation concerning the place of origin due to the continued expansion and commercialization of its seeds. Conversely, the dispersion of *E. oleifera* seeds is more restricted, due to its low level of commercialization [14,41]. When a small portion of a population separates from the parental population, the gene frequencies of the new population can be quite different from the one that gave rise to it [42]. Some authors support the hypothesis that these populations have experienced drift effects and recent bottleneck events [3,43].

The few previous genetic studies using molecular markers in *E. oleifera* available observed lower genetic diversity of *E. oleifera* [12,15,44,45], compared to *E. guineensis* [46], with greater genetic differentiation between populations. Arias, González, Prada, Ayala-Diaz, Montoya, Daza and Romero [42] analyzed the genetic diversity of natural populations of caiaué from four countries (Brazil, Colombia, Ecuador, and Peru), and all samples were grouped according to their country of origin. The grouping associated with the geographic origin and location-specific alleles is consistent with the results obtained by Barcelos, Amblard, Berthaud and Seguin [3], who discriminated groups between populations of *E. oleifera* from French Guiana, Suriname, and Peru, through specific alleles. Moretzsohn, Ferreira, Amaral, Coelho, Grattapaglia and Ferreira [12] also pointed out that the distribution of genotypes along the Amazon River is more determinant in the grouping of caiaué plants than their geographical distances, indicating that Rio acts as a seed disperser. In a previous work, the set of SNPs used in this study was applied in the analysis of genetic diversity and defined a core collection model for the germplasm bank of caiaué, the genetic diversity found was moderate, with greater interpopulation differentiation [17].

Among the advantages of the GBS technique, the sampling of markers along the entire genome is highlighted, thus it is possible to use these markers for studies of linkage disequilibrium and genomic selection [47–49]. Based on our results, it was possible to observe a wide distribution of markers along the genomes of *E. oleifera* and *E. guineensis*, thus, together with phenotypic data, these markers can be used for genomic association studies. *E. oleifera* and *E. guineensis* are phylogenetically closely related species [25]. Based on our analysis, it was possible to observe a wide synteny and collinearity of SNPs markers in the genomes of the two species and a high similarity in the annotation of genes containing

SNPs (intragenic SNPs), these results indicate a potential transferability of these markers that can also be applied in breeding genetic of *E. guineensis*. The intergenic and intragenic SNPs identified were used for the analysis of genetic diversity and population structure of these genotypes [17] and in-depth analysis through genomic re-sequencing can better clarify the genetic and genomic differences between these genotypes.

The analysis of synteny and collinearity between the SNPs highlights the difficulty of comparing complete genomes (*E. guineensis*) and drafts (*E. oleifera*). This small number of SNPs was used due to the difficulty of comparing in the correct order between the *E. oleifera* draft assembled in 85612 scaffolds [26] and the *E. guineensis* genome assembled in 16 scaffolds (chromosomes) [25]. This approach can be computationally optimized and automated in order to correctly order small scaffolds and improve the assembly of genome drafts, especially complex plant genomes.

Understanding genetic diversity and distribution are essential for the conservation and use of *E. oleifera*. These results demonstrate that this species has a specific genetic structure and good genetic variability within the 19 populations that make up for the Brazilian GB of caiaué. In this way, the ex situ conservation strategy to be applied must prioritize a larger number of individuals rather than a large number of populations. The identification of genes associated with SNP loci under strong selection will subsidize decisions for conservation or use in breeding programs.

5. Conclusions

The *E. oleifera* populations from the Brazilian Amazon rainforest evaluated in this study have specific genetic structures and good genetic variability within and between them. There is evidence of selection occurring in these populations. We have selected 568 loci most likely to be under selection effect (both directional and stabilizing), these SNPs have a wide distribution along the genomes of *E. oleifera* and *E. guineensis*, and many of them are intragenic; thus, they are markers that can be selected for further studies of linkage disequilibrium, transferability, and genomic selection.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14040270/s1, Supplementary Table S1: List of the 568 SNP loci outliers identified with the greatest significance, both for stabilizing and directional selection; from a set of 1667 SNP loci previously identified in the Brazilian *E. oleifera* Germplasm Bank via a sequencing genotyping approach. Supplementary Table S2: Mapping of SNP loci in the reference genomes of *Elaeis oleifera* and *Elaeis guineensis*. Supplementary Table S3: (A). Intragenic SNPs present in both species (*E. oleifera* and *E. guienenis*); (B). Intragenic SNPs present exclusively in *E. oleifera*, and (C). Intragenic SNPs present exclusively in *E. guineensis*. Supplementary Table S4: Top 100 outlier loci (50 under positive selection and 50 under negative selection) for neutral Fst, performed in the LOSITAN software using the set of 1667 SNP loci analyzed in E. oleifera plants from half-sibling families, which make up for a group of 19 populations sampled in six major geographic regions of the Brazilian Amazon rainforest.

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Data Availability Statement: The data-sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

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