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Assessment of Genetic Diversity of NIFOR Oil Palm Main Breeding Parent Genotypes Using Microsatellite Markers

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Abstract

The genetic diversity among 15 NIFOR breeding parents was assessed using 10 microsatellite markers. A high genetic diversity was observed with a total of 64 alleles including 23 rare alleles or alleles at frequencies less than 0.05. The NIFOR *tenera* parents recorded the highest number of rare alleles. The average observed heterozygosity and mean gene diversity across all parental groups were 0.6889 and 0.7029, respectively. Higher genetic diversity was detected among the NIFOR *dura* and *tenera* parents compared to that of the Deli *dura* parents in absolute terms. Analysis of molecular variance (AMOVA) showed that 87% of the total variation ($p < 0.001$) observed was due to differences among parents. Rogers' genetic distance ranged from 0.2988 to 0.8000 (mean = 0.5570). The dendrogram constructed on the basis of Rogers' genetic distance clustered the parents in three groups. They generally clustered in heterotic manner rather than by geographic origins. The groupings obtained through PCoA confirmed the results obtained by cluster analysis. The results obtained are strong assets for NIFOR breeding programme.

Keywords

Allelic Diversity, *Elaeis guineensis* Jacq, Genetic Distance, Heterosis, Molecular Characterization, NIFOR, Population Genetics, Selection, SSR Markers

1. Introduction

The Nigerian Institute for Oil Palm Research (NIFOR) oil palm Main Breeding Programme (MBP) is a modification of the Reciprocal Recurrent Selection (RRS) proposed by Comstock *et al*. [1]. One of the most important features of this scheme of selection is the improvement of the hybrid by a gradual assemblage of favourable alleles, together with the maintenance of genetic variability within the base populations of the breeding programme to ensure yield progress from one generation to the other. The success of this breeding method depends on the effectiveness of selecting distinct parents with complementary yield components combined in their offspring whose yields are superior to those of the parents. Accordingly, the value of a breeding population as potential source of new hybrid variety depends on the magnitude of its genetic variability for economic traits. The higher the genetic divergence between parents used in the crossing scheme that produces the population, the higher the genetic variability within the population.

Plant breeders have often postulated that geographical distance is related to genetic distance in selecting parents for high heterosis effect in the hybrids. The NIFOR first selection cycle of the breeding programme had limited genetic diversity because it was based mainly on the early collections from Aba and Calabar natural oil palm groves, in spite of the abundance oil palm germplasm available to the Institute [2]. New oil palm materials were introduced at NIFOR in view of enlarging the genetic base of parents of the second selection cycle. Following the evaluation of the first cycle breeding populations, five Deli *dura*, eight African *dura* and 13 *tenera* oil palms were selected as parents of the second selection cycle of the MBP on the basis of general combining ability [3] [4]. New introductions were selected based on individual and family performance. In selecting the African *dura* and *tenera* parents, full advantage was taken of the vast introductions of oil palm germplasm from various groves in the country especially the Ufuma, Umuabi, Igala, Opobo, and the more recent Aba accessions. Two new Deli *dura* parents were introduced from Ecuador and Malaysia (Ulu Remis x Malaya) to enhance diversity of the NIFOR Deli breeding population and ensure effective selection progress [2] [5]-[7].

Since RRS is a long-term breeding procedure, the maintenance of genetic variability during the cycles of selection is essential to guarantee sufficient magnitudes of selection responses for subsequent cycles. Besides, oil palm improvement programme in NIFOR and elsewhere aims at producing high quality oil yielding *tenera* (D × P) planting materials. Therefore, the maintenance of adequate genetic variability among parent populations used for hybrid seed production is critical for maximum utilization of heterosis and sustained genetic progress in the selection cycle [8].

Studies on the genetic diversity of oil palm breeding populations on the basis of agro-morphological variables have been extensively carried out in NIFOR [2] [9]-[16]. However, genetic diversity evaluation based on agro-morphological information alone is no longer sufficient because of low polymorphism, long juvenile phase, and vulnerability to environmental effects [17]. An additional and objective measure of genetic variation which will permit the monitoring of genetic variability within and between populations, efficient selection of parents to maximize heterosis among populations and sustained genetic progress in the RRS programme is compulsory [18] [19].

The application of DNA marker technology in the NIFOR oil palm breeding programme would not only reduce the breeding duration but ensure greater precision in the production of planting materials [20] [21]. Molecular markers offer great scope for assessing genetic diversity and relationships among natural or breeding populations because they are impervious to environmental conditions and are detectable in all stages of plant growth and development [22]. Among the likely alternatives, isozymes are not satisfactorily variable due to low polymorphism [23] [24]. Random amplified polymorphic DNA (RAPD) has also been examined [25], but poor reproducibility of amplification products limits their generalisation in genetic diversity studies [26]. Other more robust molecular markers such as restriction fragment length polymorphic DNA (RFLP) [27] are complex; requiring relatively large amounts of purified and high molecular weight DNA, time-consuming and laborious. Finally, the amplified fragment length polymorphism (AFLP) [28] is a dominant marker which rarely detects heterozygosity and is scored as a presence/absence polymorphism.

Microsatellites or simple sequence repeats (SSRs) are considered as ideal genetic marker for plant genetic and breeding studies because they are characterized by high polymorphism [29], co-dominant inheritance [30], reproducibility and abundance throughout the genome [31]. Also, they are readily transferable [32], and easily assayed using polymerase chain reaction (PCR) and capillary electrophoresis [33] [34]. Furthermore, multiplexing PCR products on single gels also reduces the workload for studies requiring a large number of samples [35].

SSR markers are useful in oil palm population genetics and breeding studies [36]-[41], varietal identification [42]-[46], pedigree analysis, genome mapping, and QTL detection for molecular marker-assisted selection [47]-[49]. But to date, studies on the diversity of the current NIFOR oil palm breeding populations have not been carried out using molecular markers. Earlier efforts in this direction have utilized the conventional agromorphological analysis. This has created a yawning gap in the establishment of a reliable genetic constitution which can only be resolved with molecular marker analysis. The need to fill this gap has prompted the present study aiming at (1) evaluating the efficiency of sixteen microsatellite markers in detecting genetic variation among NIFOR main breeding parental genotypes, (2) estimating genetic similarities among the parents and (3) classifying them on the basis of genetic relationships.

2. Materials and Methods

2.1. Plant Material

Fifteen parent oil palms (4 Deli *dura* NIFOR, 4 NIFOR *dura*, and 7 NIFOR *tenera*) intercrossed in the second cycle of the reciprocal recurrent selection of the NIFOR oil palm breeding programme were sampled at the NIFOR Main Station, Benin City, Nigeria. Fresh leaf tissues were collected from an unopened spear. Detailed information concerning the parent trees visited is presented in Table 1. Each leaf sample was placed in a separate labeled zip lock polyethylene bag in an airtight container with ice chips and temporarily kept in a cold room at −80˚C at the Bioscience Centre, International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria until DNA extraction.

Table 1. NIFOR parental oil palms sampled.

S/No.	PARENT CODE	PROVENANCE	FRUIT FORM	BASES FOR SELECTION
1	DD1	ex Serdang Avenue (Malaysia)	Deli *dura*	Good combing ability for bunch yield and very good fruit and bunch composition
2	DD2	ex Serdang Ave. x IRHO—Pobe	Deli *dura*	Good combining ability for bunch yield. Excellent fruit and bunch composition (high breeding value for *tenera* progeny)
3	DD3*	Deli Ulu Remis x ex Sabah	Deli *dura*	Family and within family selection for high oil yield and good fruit and bunch composition
4	DD4	Deli Ecuador	Deli *dura*	High yielding. Family selection for very good fruit and bunch composition
5	AD1	Calabar	Nigerian *dura*	High GCA for bunch yield and excellent fruit and bunch composition. Individual selection for high average bunch weight
6	AD3*	Ufuma	Nigerian *dura*	High yielding progeny with heavy bunches and good fruit and bunch composition
7	AD4*	Opobo	Nigerian *dura*	Individual within family selection for consistent high yield of heavy bunches and good bunch composition
8	AD5*	Aba	Nigerian *dura*	Individual and within family selection for high bunch yield and excellent fruit and bunch composition
9	T1	Ufuma ex Aba	Nigerian *tenera*	Excellent GCA for high bunch yield and bunch number, within family selections for high oil to mesocarp ratio
10	T2	Calabar	Nigerian *tenera*	Excellent GCA for fruit and bunch composition, Family selection for high oil to mesocarp ratio
11	T3	Aba ex Calabar	Nigerian *tenera*	GCA for high average bunch weight and average bunch number
12	T5*	Ufuma	Nigerian *tenera*	Family and within family selection for high bunch yield, high bunch number and very good fruit and bunch composition
13	T6*	Umuabi OP	Nigerian *tenera*	Individual and within family selection for high bunch number, high oil to mesocarp ratio and very good fruit and bunch composition
14	T7	Aba	Nigerian *tenera*	High GCA for bunch yield and high fruit to bunch ratio
15	T8	Ufuma ex Angola	Nigerian *tenera*	Individual and within family selection for good fruit composition with high bunch yield and bunch number

*New introductions, GCA: General combining ability.

2.2. DNA Extraction

Total genomic deoxyribonucleic acid (DNA) was extracted from fresh leaf tissues using the CTAB (Cetyl trimethyl ammonium bromide) DNA isolation protocol of Doyle and Doyle [50] with minor modifications. Approximately 1.0 - 2.0 g of each fresh leaf tissue was used. The concentration and purity of isolated DNA was determined using a Multiskan GO spectrophotometer (Thermo Fisher Scientific Inc., Denver). Optical density (OD) readings were obtained at wavelengths 260 and 280 nm. All DNA samples were stored at −20°C until microsatellite analysis at the Genomics Unit of Advanced Biotechnology and Breeding Centre (ABBC), Malaysian Palm Oil Board (MPOB) Selangor, Malaysia. The DNA samples were diluted to an optimum concentration of 25 ng/µl by addition of sterile distilled water or appropriate amount of TE (Tris-EDTA) buffer and stored at 4°C until polymerase chain reaction (PCR) amplification.

2.3. PCR Reaction and Genotyping

A total of 16 microsatellite loci selected for their high polymorphism and reproducibility were tested on 15 NIFOR oil palm main breeding parents. Nine of the sixteen microsatellite markers were developed at the Genomics Unit of ABBC-MPOB [39] [51] and seven at the French Centre de Coopération en Recherche Agronomique pour le Développement (CIRAD; **Table 2**) [52]. Every forward microsatellite marker was M13-tailed for labeling with one of the four florescent dyes *i.e.* NED, 6-FAM. VIC, and PET at the five-prime end to permit multiplexing of four marker loci during scoring of banding patterns. Differences in dyes' colours allowed distinguishing loci and corresponding alleles in the data output whose size ranges overlapped one another.

Each polymerase chain reaction contained 2 µl of 25 ng genomic DNA, 6.625 µl MilliQ water, $1 \times$ PCR standard buffer (NEB, USA), 0.2 µl of 10 mM deoxynucleotide triphosphates (dNTPs) (NEB, USA), 0.025 µl of each of the M13-tailed forward primer and untailed reverse primer for every primer pair, 0.025 µl dye, and 0.1µl of Taq DNA polymerase (5 U/µl) (NEB, USA) for a total reaction volume of 10 µl. PCR was performed using Perkin Elmer 9700 thermo-cycler (Life Technologies, Thermo-Fisher Scientific, USA). The PCR programme consisted of an initial 3 min denaturing at 95°C, followed by 35 cycles of denaturation at 95°C for 30 sec, primer annealing for 30 sec at 50°C - 58°C depending on the primer annealing temperature and an extension temperature of 72°C for 30 sec, terminated by a final extension at 72°C for 2 min. The amplification products were resolved on 0.9% of SFR agarose gel run in $1 \times$ TAE buffer. Band sizes were determined by reference to the 100 bp ladder (Thermo-Scientific) run in a horizontal electrophoresis system (Horizon model 20 - 25; **Figure 1**). The PCR products were stored at 4°C until scoring of banding patterns.
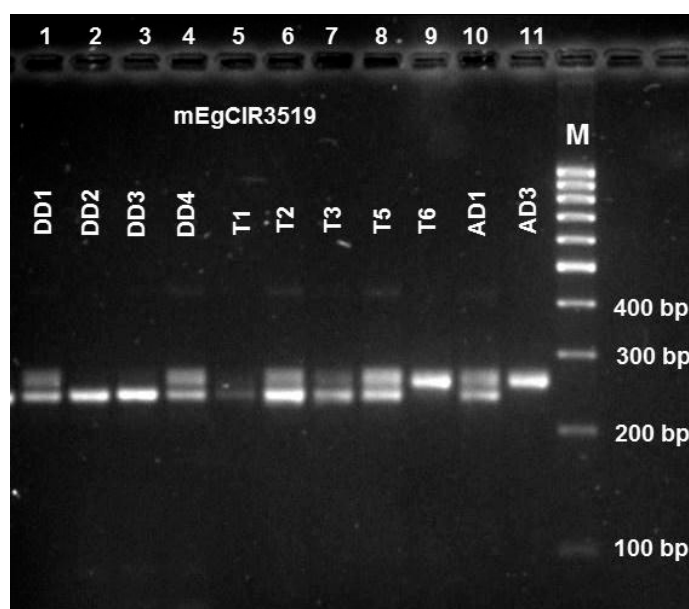
Figure 1. Banding pattern of SSR marker mEgCIR3519 used for genotyping NIFOR oil palm parents (M = 100 base pair ladder).

Table 2. Microsatellite primer pairs used for population genetic analysis of oil palm.

| S/N	SSR LOCI	LINKAGE GROUP	TA (°C)	PRIMER SEQUENCE	SSR REPEAT MOTIF	EXPECTED FRAGMENT SIZE (bp)	FLUORESCENT LABEL	ACCESSION NUMBER
1	mEg CIR3813	1	52	F-CATACCCTGCTTATCTTTC R-GTAGATACCCGTTAGTTGAC	(GA)19	167	FAM	AJ578734
2	mEg CIR0793	2	56	F-GTACTTCGCAACTATTCCTTTTCTT R-AGTTGATCGTGGTGCCTGAC	(GA)15	149	FAM	AJ578545
3	mEg CIR0425	3	58	F-AGCAAGAGCAAGAGCAGAACT R-CTTGGGGGCTTCGCTATC	(CCG)9	232	NED	AJ578521
4	sMg 00156	4	50	F-GGTGTCATAACTTCGTTGTTGCT R-ATGCTCAAAAGTGGGTTTCTCTC	(CT)15	237	NED	Pr010615888[*]
5	mEg CIR3828	5	50	F-AGCCAGATGGAAATACAC R-GTGCGATAAAGAGGAGAGT	(GA)23	282	VIC	AJ578738
6	sEg 00154	6	57	F-TCCCCCAATACTCATCATGC R-TGATCGACGGTTGTCACATT	(CAG)5	238	FAM	EY410356[**]
7	sMo 00102	7	53	F-ATGAGATGGGACAAATCAAAC R-ACCATACCAACTAGAGAACTAAACA	(AG)11	235	FAM	Pr010615939[*]
8	sMg 00228	8	54	F-CACGTATATGAGCAGGATTTGA R-CTCCAAACCAACTAGAGCTGA	(AT)25	205	PET	Pr010615913[*]
9	sMg 00016	9	52	F-GCGATTCCGGTTATCTTTAG R-GAGTTTTTGCTGTGATGATTAG	(GA)13	274	VIC	Pr010615861[*]
10	mEg CIR3519	10	52	F-CCACTGCTTCAAATTTACTAG R-GCGTCCAAAACATAAATCAC	(GA)15(GT)8	236	NED	AJ578672
11	sMg 00120	11	54	F-GATCAATGCGAGAAATCAGG R-GATCATGCTTATCCTTTCCAAGT	(AT)11	152	NED	Pr010615881[*]
12	mEg CIR0790	12	52	F-TTGGTGGTCCTTTTGAATATC R-ACAAACCCAGCACTTAAAATAAC	(GA)19	215	VIC	AJ578544
13	sEg 00151	13	57	F-ATCACAACAGCAGCAGCATC R-CGCATCAAGAAACATGGAGA	(CAG)8	219	PET	EY411661[**]
14	sMg 00179	14	54	F-AACCCCTTTTTCATGCTCTAA R-CTGATTTTGGAATCAGAGGTG	(AAAAG)6	214	VIC	Pr010615893[*]
15	sMg 00087	15	58	F-CACCTAAAAACGGCAAGGAAC R-GGAGGAGAGAAATGGAAGACG	(AG)19AA(AG)	212	PET	Pr010615880[*]
16	mEg CIR3745	16	52	F-GGAAGTCTTGATGTTGAAAG R-ATCAAGCAGTCGCATAATAC	(GA)18	215	PET	AJ578718

TA = Annealing temperature, [*]Probe Unique Identifiers (PUIDs) of NCBI Probe Database, [**]Accession numbers of NCBI GenBank.

Four PCR products labeled with different fluorescent dyes were pooled in the ratio of 2:1:1:1. 2 µl of the pooled PCR products was combined with 7.84 µl of formamide (Applied Biosystems, Foster City, CA) and 0.16 µl of the GeneScan-500 LIZ size standard (Applied Biosystems, Foster City, CA). The samples were heated at 95°C for 3 min in a 96-well PCR microplate and placed at 4°C before loading on ABI 3730 DNA Genetic Analyzer (Applied Biosystems, USA) for an automated capillary electrophoresis.

The allele sizes for each SSR locus were identified using Genemapper 4.1 software (Applied Biosystems, USA). Electropherogram profiles (sample plots) were generated and allele sizes for the set of SSR markers were exported as data table for genotyping (**Figure 2**).

3. Data Analysis

The oil palm parent samples were subdivided into three groups depending of the fruit form and the provenance of the parental material *i.e.* one group comprising the NIFOR *dura* parents, one for NIFOR *tenera* parents, and one group of Deli *dura* NIFOR parents. Estimates of genetic diversity were analyzed for each locus as well as each group of oil palm parents. The genetic diversity parameters estimated were allelic frequencies (F_x), number of genotypes, mean number of alleles per locus (A_o), and effective number of alleles per locus (A_e) [53]. The relative allele frequencies (F_x) were calculated according to the method of Marshal and Brown [54] for a better
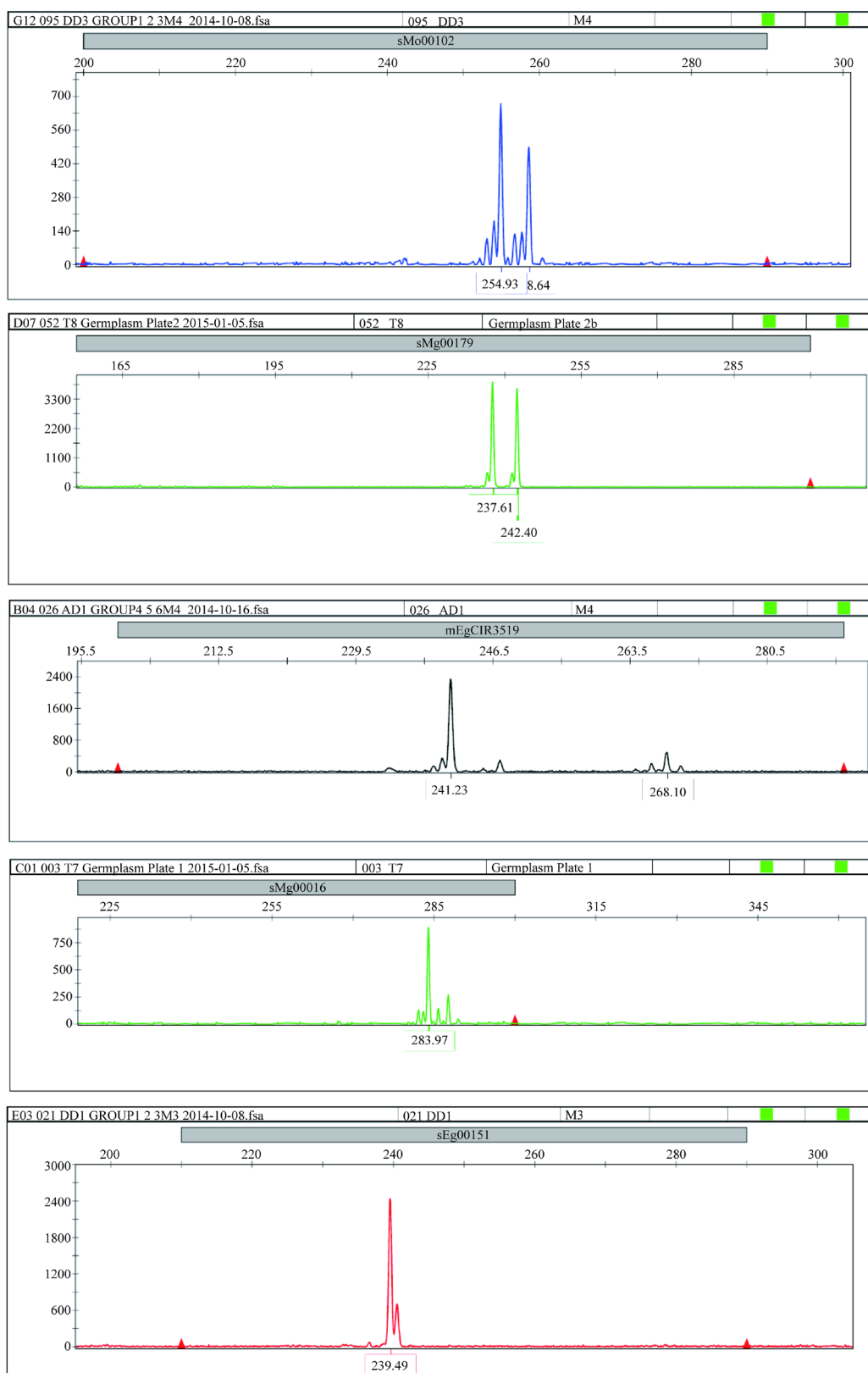
Figure 2. Electropherogram profiles from ABI 3730 DNA Genetic Analyzer showing allele peaks and sizes of some SSR loci (sMo00102, sMg00179, mEgCIR3519, sMg00016, and sEg00151).

comparison of the distribution of common alleles (alleles at frequency $p \geq 0.05$) and rare alleles (alleles at frequency $p < 0.05$).

The polymorphism information content (PIC) value, that provides an estimate of the discriminatory power of a molecular locus by taking into account the observed number of alleles per locus (A_o) and their relative frequencies (F_x) in the studied population, was calculated for each marker as described by Botstein *et al*. [55] and Anderson *et al*. [56];

$$\text{PIC} = 1 - \sum_{i=1}^{n} f_i^2 - \sum_{i-1}^{n-1} \sum_{j=i+1}^{n} 2 f_i^2 f_j^2$$

where n is the number of alleles; and f_i and f_j are the frequencies of the ith and j^{th} alleles, respectively.

Observed or direct heterozygosity (H_o) was estimated by dividing the number of heterozygous individuals by the total number of individuals sampled. Expected heterozygosity (H_e) is often referred to as gene diversity, and defined as the probability that two randomly chosen alleles from the population are different [57]. It was calculated for each SSR locus according to the formula:

$$H_e = 1 - \sum (pi)^2$$

where p is the frequency of the ith allele for the population and $\sum (pi)^2$ is the sum of squared population allele frequencies. All of the calculations were performed using PowerMarker software version 3.25 [58]. Wright's Fixation Index (F) which is the overall inbreeding coefficient within the entire population was calculated per locus;

$$F = \frac{H_e - H_o}{H_e}$$

where H_o is the observed heterozygous per locus and H_e is the expected heterozygous per locus. In order to assess the genetic relationships among parents, Rogers' [59] genetic distance was computed;

$$D_R = \frac{1}{m} \sum_{j}^{m} \sqrt{\frac{1}{2} \sum_{i}^{a_j} (p_{ij} - q_{ij})^2}$$

where p_{ij} and q_{ij} are the frequencies of ith allele at the jth alleles in populations X and Y, respectively, a_j the number of alleles at the jth locus, and m the number of loci examined. The distance matrix was subjected to cluster analysis to produce a hierarchical representation of the relationships among parents using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) as described by Sneath and Sokal [60]. All genetic distance calculations and construction of dendrograms were performed using PowerMarker software version 3.25 [58] and MEGA software version 4.0 [61] respectively. To further assess the genetic relationships between the parents, principal coordinate analysis (PCoA) was performed based on dissimilarity matrix using DARwin version 6 [62]. To quantify the extent of population differentiation and distribution of genetic variation in the sampled groups of parents, analysis of molecular variance (AMOVA) was computed using GenAlEx version 6.5 software [63] [64].

4. Result

4.1. Genetic Diversity in the Oil Palm Parents of the NIFOR Main Breeding Programme

Ten of the 16 SSR loci which generated PCR products were used for the study and the rest dismissed (**Table 3**). A total of 64 alleles were detected among the 15 NIFOR oil palm parent genotypes. The number of alleles per SSR locus varied from 4 at sMg00016 to 9 at mEgCIR0790 with an average of 6.4 alleles (**Table 3**). The frequency of the major allele (highest proportion in all alleles) for the oil palm parents varied from 0.23 at sMg00087 to 0.57 at mEgCIR3519 with a mean of 0.44 at each locus. Allele frequencies were low, particularly for loci with higher number of alleles. Rare alleles or alleles at frequency ($F_x < 0.05$) were observed at all but one of the microsatellite loci (sMg00179) with a total of 23 (35.94%) rare alleles across all loci (**Table 4**). The highest number of rare alleles (5) was recorded at the SSR loci mEgCIR0790 followed by sMg00156 (4). Rare

Table 3. Microsatellite primer pairs used for population genetic analysis in the NIFOR oil palm main breeding parents.

S/N	MARKER	LINKAGE GROUP	TA (°C)	REPEAT MOTIF	EXPECTED FRAGMENT SIZE (bp)	A_o	A_e	MAF	G	PIC	H_o	H_e	F
1	mEgCIR0793	2	56	(GA)15	149	7	3.285	0.53	7	0.64	0.7333	0.6667	−0.0999
2	sMg00156	4	50	(CT)15	237	7	6.368	0.42	9	0.68	0.6154	0.7249	0.1510
3	sEg00154	6	57	(CAG)5	238	5	2.557	0.50	5	0.63	0.7333	0.6711	−0.0927
4	sMo00102	7	53	(AG)11	235	7	3.634	0.27	8	0.77	0.7692	0.7988	0.0370
5	sMg00016	9	52	(GA)13	274	4	3.000	0.54	5	0.47	0.7692	0.5592	−0.3757
6	mEgCIR3519	10	52	(GA)15(GT)8	236	5	3.041	0.57	7	0.56	0.4667	0.6089	0.2336
7	mEgCIR0790	12	52	(GA)19	215	9	3.061	0.53	11	0.65	0.7333	0.6733	−0.0891
8	sEg00151	13	57	(CAG)8	219	6	4.723	0.47	9	0.65	0.6667	0.6956	0.0415
9	sMg00179	14	54	(AAAAG)6	214	6	4.971	0.32	8	0.76	0.8571	0.7883	−0.0874
10	sMg00087	15	58	(AG)19AA(AG)	212	8	2.268	0.23	10	0.82	0.5455	0.8430	0.3529
	Mean					**64**	**3.691**	**0.44**	**7.9**	**0.66**	**0.6889**	**0.7029**	**0.0071**

TA = Annealing temperature, A_o = Number of allele, A_e = Effective number of alleles, **MAF** = Major allele frequency, H_o = Observed heterozygosity, H_e = Expected heterozygosity/gene diversity, **G** = Genotype number, **PIC** = Polymorphism information content, **F** = Fixation index(inbreeding-like effects within the entire population). Position of markers in linkage groups of an oil palm genetic map is indicated in column 3.

Table 4. Allele frequencies in the 15 NIFOR oil palm main breeding parents using ten microsatellite markers.

Al	mEgCIR0793	Al	sMg00156	Al	sEg00154	Al	sMo00102	Al	sMg00016	Al	mEgCIR3519	Al	mEgCIR0790	Al	sEg00151	Al	sMg00179	Al	sMg00087
A	0.5333	A	0.0385	A	0.5	A	0.2692	A	0.5385	A	0.5667	A	0.5333	A	0.2	A	0.1786	A	0.1818
B	0.1333	B	0.0385	B	0.1	B	0.2308	B	0.3846	B	0.2	B	0.1667	B	0.4667	B	0.3214	B	0.2273
C	0.0333	C	0.2308	C	0.1667	C	0.2308	C	0.0385	C	0.0333	C	0.0333	C	0.0667	C	0.2143	C	0.0909
D	0.1	D	0.4231	D	0.0333	D	0.0385	D	0.0385	D	0.1667	D	0.0333	D	0.2	D	0.0714	D	0.0909
E	0.0333	E	0.1923	E	0.2	E	0.1154			E	0.0333	E	0.0333	E	0.0333	E	0.1429	E	0.0455
F	0.333	F	0.0385	F	0.333	F	0.0769					F	0.0667	F	0.0333	F	0.0714	F	0.1364
G	0.1333	G	0.0385			G	0.0385					G	0.0667					G	0.0455
												H	0.0333					H	0.1818
												I	0.0333						

Al = Allele letter.

alleles occurred in all the NIFOR oil palm breeding parents with the exception of Deli *dura* NIFOR parents (DD2 and DD3). The highest number of rare alleles (14) was detected in the NIFOR *tenera* parents with a minimum of 1 rare allele (T7) and maximum of 3 rare alleles (T3 and T6). SSR loci mEgCIR0790 exhibited 1 unique allele (unique allele, *i.e.* present in one parent but not in any other) in one of the NIFOR *dura* parents (AD4). All the 10 microsatellite loci scored in this study were polymorphic, displaying high values of PIC from 0.4708 to 0.8237 (mean = 0.66) (**Table 3**).

The number of genotypes obtained per loci ranged from 5 at sEg00154 and sMg00016 to 11 at mEgCIR0790. Observed heterozygosity (H_o) values (per marker) ranged from 0.4667 at mEgCIR3519 to 0.8571 at sMg00179 (mean = 0.6890). H_e values ranging from 0.5592 at sMg00016 to 0.8430 at sMg00087 (mean = 0.7030).

4.2. Genetic Diversity among Fruit Forms and Provenances of the NIFOR Main Breeding Programme

In order to better understand the contribution of the various parental genotypes to the total genetic diversity of

the NIFOR oil palm parents, the 15 oil palm parent trees were divided into three groups' on the basis of fruit form and provenance of the parental oil palm viz., Deli *dura* NIFOR (DDN), NIFOR *dura* (ND) and NIFOR *tenera* (NT). The average numbers of alleles in DDN, ND, and NT were 3.4, 3.7, and 5.0, respectively (**Table 5**). The effective number of alleles per group (A_e) varied from 2.739 in Deli *dura* NIFOR to 3.403 in NIFOR *tenera* with an overall group average of 3.091. Major allele frequency varied from 0.4829 in the NIFOR *tenera* parent group to 0.5042 in the NIFOR *dura* group. **Table 6** shows the distribution of common and private alleles among the three NIFOR oil palm parent groups screened with 10 SSR loci. Results showed that the private alleles accounted for the highest proportion in the NIFOR *tenera* group. Eight private alleles were specific to groups of NIFOR *dura* parents and two alleles for the Deli *dura* NIFOR group respectively.

The observed heterozygosity per group ranged from 0.650 (NIFOR Deli *dura*) to 0.725 (NIFOR *dura*) with an average value of 0.683 (**Table 5**). The observed heterozygosity of Deli *dura* NIFOR and NIFOR *dura* groups are quite comparable. The NIFOR Deli *dura* group had the lowest values among all the populations for all the allelic variability parameters in absolute terms. Contrary to this observation, NIFOR *dura* recorded the least expected heterozygosity value (0.602) among the different groups of parents.

Table 5. Estimates of genetic diversity in the three groups of parents detected by 10 microsatellite markers.

GROUP	N	MAF	A_o	A_e	H_o	H_e	PIC
DELI *DURA* NIFOR (DDN)	4	0.4875	3.400	2.739	0.650	0.622	0.5560
NIFOR *DURA* (ND)	4	0.5042	3.700	3.132	0.725	0.602	0.5489
NIFOR *TENERA*(NT)	7	0.4829	5.000	3.403	0.674	0.650	0.6088
GRAND MEAN			**4.033**	**3.091**	**0.683**	**0.625**	

N = Number of samples, **MAF** = Major allele frequency, A_o = Number of allele, A_e = Effective number of alleles, H_o = Observed heterozygosity, H_e = Expected heterozygosity/gene diversity, **PIC** = Polymorphism information content.

Table 6. Distribution of common and private alleles among the 3 groups of NIFOR oil palm parent populations for 10 microsatellite loci.

SSR LOCI	NUMBER OF ALLELES	COMMON ALLELES/ LOCUS (>5%)	TOTAL PRIVATE ALLELES/ LOCUS	PRIVATE ALLELES/ GROUP DELI *DURA* NIFOR (DDN)[†]	PRIVATE ALLELES/ GROUP NIFOR *DURA* (ND)[†]	PRIVATE ALLELES/ GROUPNIFOR *TENERA* (NT)[†]
mEgCIR0790	9	3	6	1 (DD4)	2 (AD3, AD4)	3 (T1, T5, T6, T7)
mEgCIR3519	5	3	2	1 (DD1)	1 (AD5)	-
mEgCIR0793	7	4	3	-	-	3 (T3, T6)
sEg00154	5	4	1	-	1 (AD1)	-
sEg00151	6	3	3	-	3 (AD1, AD3, AD4, AD5)	-
sMg00179	6	6	-	-	-	-
sMg00016	4	1	3	-	-	2 (T2, T3)
sMg00087	8	6	2	-	-	3 (T1, T2, T5, T6)
sMg00156	7	3	4	-	1 (AD3)	3 (T2, T6, T8)
sMo00102	7	4	3	-	-	3 (T1, T7, T8)
TOTAL	**64**	**37**	**27**	**2**	**8**	**17**

[†]Code in parenthesis indicates individual parent(s) with private allele.

4.3. Analysis of Molecular Variance (AMOVA)

The examination of the hierarchical partitioning of genetic variation by AMOVA demonstrated that genetic differentiation was significant at $p < 0.001$ using the co-dominant allelic distance matrix for calculation of F_{ST} [63]-[65] (**Table 7**). There was a clear genetic differentiation both among and within the groups of parent populations using the significance tests based on 999 permutations calculated according to Wright [65] and Excoffier *et al*. [66]. The genetic variation was higher within groups with a variance component of 0.418 than among groups (with a variance component of 0.063). Of the total diversity, 13% was attributed to group differences while 87% was attributed to differences within groups. Both the F_{ST} index ((0.131) and G_{ST} value of 0.136 ($p < 0.002$) were low.

4.4. Genetic Distance Analysis

Based on the genotypic data obtained at the ten microsatellite loci, Rogers' genetic distance coefficients were estimated for all pair-wise comparisons of the 15 parent trees. The average distance between parents was moderate (0.5570) and ranged from 0.2988 between AD4 and T8 to 0.8000 between DD4 and AD5 (**Table 8**). The highest genetic distance among the *tenera* parents (0.6500) was found between T3 and T5 and the lowest (0.4086) between T2 and T6. The minimum genetic distance within the Deli *dura* NIFOR parents is 0.4293 between DD1 and DD4 and the maximum distance of 0.6086 between DD2 and DD4, and DD3 and DD4. AD3 and AD4 recorded the highest genetic distance (0.7988) among the NIFOR *dura* parents.

**Table 7.** Analysis of molecular variance (AMOVA) among the NIFOR oil palm parents.

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	ESTIMATED VARIANCE	PERCENTAGE OF VARIATION (%)	*P*-value
Among groups	2	2.048	1.024	0.063	13%	<0.001
Within groups	27	11.286	0.418	0.418	87%	<0.001
Total	29	13.333		0.481	100%	

$F_{ST} = 0.131$ ($p < 0.001$)
$G_{ST} = 0.136$ ($p < 0.002$)

Table 8. Rogers' [59] genetic distance matrix between 15 NIFOR main breeding oil palm parents generated by microsatellite markers.

	AD1	AD3	AD4	AD5	DD1	DD2	DD3	DD4	T1	T2	T3	T5	T6	T7
AD3	0.5672													
AD4	0.7155	**0.7988**												
AD5	0.5586	0.7586	0.3000											
DD1	0.3464	0.5586	0.7988	0.6172										
DD2	0.6000	0.7086	0.5143	0.4172	0.5379									
DD3	0.5379	0.3257	0.4310	0.3172	0.5086	0.4964								
DD4	0.4379	0.6293	0.7500	**0.8000**	**0.4293**	**0.6086**	0.6086							
T1	0.6086	0.7586	0.5488	0.5586	0.5086	0.4172	0.5672	0.5586						
T2	0.5586	0.4964	0.5976	0.5172	0.6586	0.5672	0.4379	0.5586	0.4879					
T3	0.5879	0.6086	0.7155	0.6586	0.6293	0.7293	0.4379	0.5293	0.6172	0.5086				
T5	0.4293	0.6879	0.5488	0.3586	0.4672	0.4586	0.4879	0.5793	0.5379	0.4379	**0.6500**			
T6	0.6293	0.7293	0.5976	0.5172	0.6172	0.5379	0.4757	0.5086	0.5379	**0.4086**	0.6086	0.4586		
T7	0.5095	0.6762	0.3964	0.4172	0.5746	0.6206	0.4961	0.6302	0.5095	0.5191	0.6072	0.5191	0.5881	
T8	0.6992	0.7873	**0.2988**	0.5000	0.5881	0.6667	0.5651	0.5651	0.4310	0.6532	0.5651	0.6437	0.5976	0.4865

4.5. Cluster Analysis

The 15 oil palm parents were grouped using Unweighted Pair Group Method with Arithmetic mean (UPGMA) dendogram [60] (**Figure 3**). Accordingly, the parents were grouped into three major clusters designated I, II and III. The number of parents for each of the three groups varied from 4 to 6. Cluster I contained, in part, NIFOR *dura* and *tenera* parents, Cluster II is predominantly made up of Deli *dura* NIFOR parents (DD1, DD4, and DD2) and two *tenera* parents (T5 and T1). Cluster III included three *tenera* parents (T3, T2, and T6) and one Ufuma (NIFOR) *dura* (AD3) and one Ulu Remis ex Sabah *dura* (DD3). The two *tenera* parents (T2 and T6) from Calabar and Umuabi were grouped in the same sub-cluster. In general, the groupings of the parents of NIFOR Main Breeding Programme within the different clusters were not consistent with their assumed genealogies.
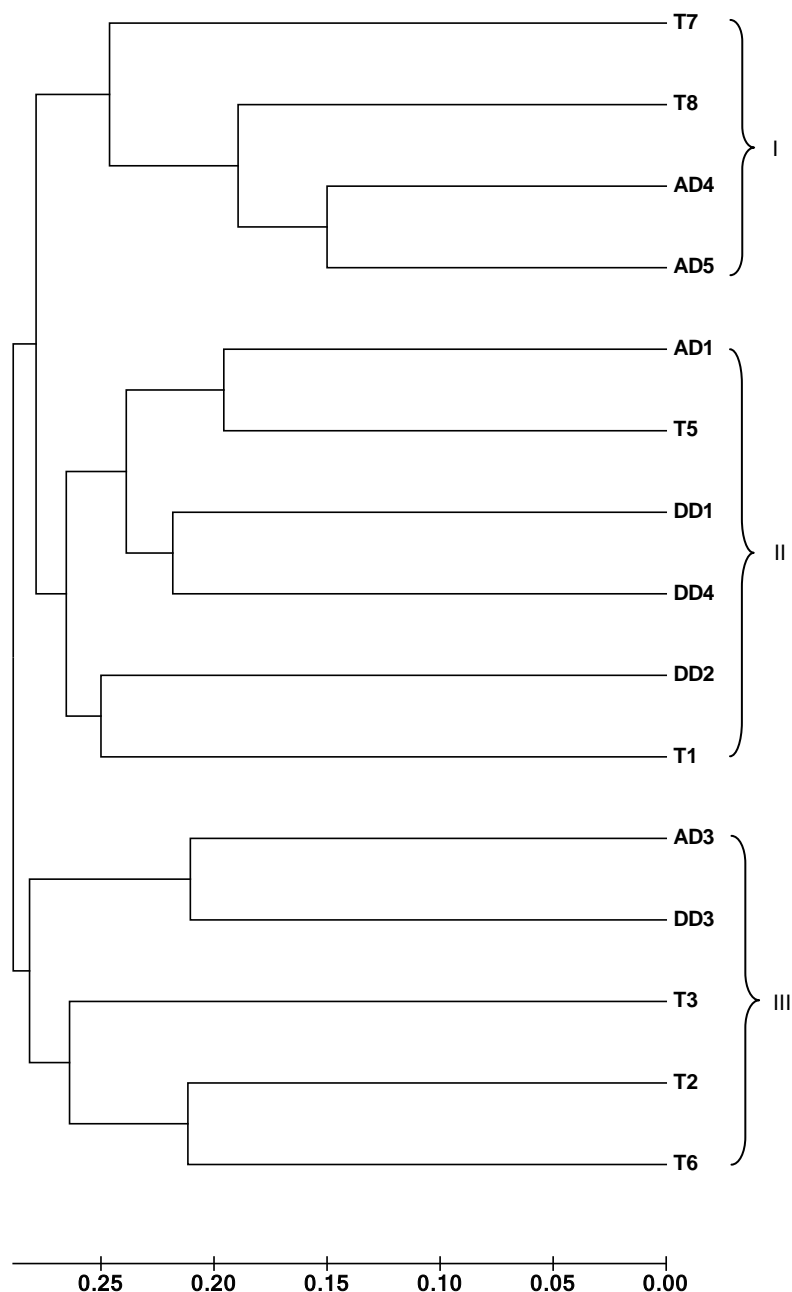
Figure 3. Dendogram revealed by the UPGMA cluster analysis of parents of the NIFOR oil palm Main Breeding Programme based on Rogers' [59] genetic distance.

4.6. Principal Co-Ordinate Analysis

A multidimensional scatter plot (MDS) was used to further understand the genetic relationships among parents of the NIFOR oil palm breeding programme. Principal co-ordinate analysis (PCoA) clustered the 15 parents into three groups (**Figure 4**). The first three coordinates explained 64.43% of the total variation, with 38.51% explained by the first coordinate and 14.61% by the second coordinate. Parents generally clustered in heterotic manner rather than by geographic origins. The grouping obtained through PCoA confirmed the results obtained by UPGMA cluster analysis.

5. Discussion

5.1. Allelic Frequencies

Allelic frequencies varied at each locus at the individual parent's level as well as among the three distinct parental groups. The unequal distribution of allele frequencies among the parents could be due to drift and selection. However, more than 40% of the individual parents and their groups shared a common major allele (alleles shared between many parents) at any given locus. Rare alleles ($p < 0.05$) were found in NIFOR *dura* and NIFOR *tenera* but not in Deli *dura* NIFOR (ex Serdang Ave. NIFOR x IRHO—Pobe and Ulu Remis Deli x ex Sabah). The absence of rare alleles in the Deli *dura* could be explained by several generations of breeding resulting to fixation of some alleles. Meanwhile, it can be presumed that NIFOR *dura* and NIFOR *tenera* parents which have barely undergone two cycles of selection are not too genetically different from their wild relatives. They have experienced little loss and negligible fixation of alleles. It is also possible that the provenance specific alleles were derived from a mutation event since SSRs loci are known to have a high rate of mutation per locus per generation of 25×10^{-5} to 1×10^{-2} [67]. The prevalence of private alleles in the NIFOR *dura* and *tenera* parents may be due to the low number of samples used in this study. In general, only elite palms which are usually in very small number are introduced in a breeding programme. It is the case of the 5 oil palms including Ufuma *dura* (AD3) and *tenera* (T5), Aba *dura* (AD5), Opobo *dura* (AD4), and Umuabi *tenera* (T6) recently introduced in the NIFOR Main Breeding Programme. However, the speculation of adaptive genetic variants as
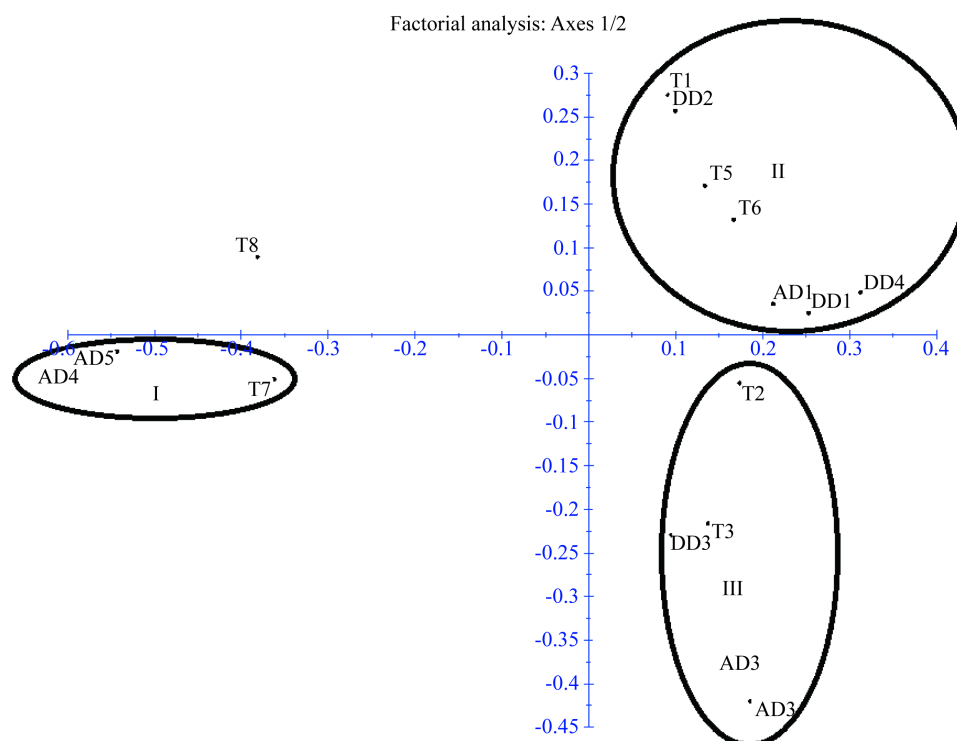
Figure 4. Principal coordinates analysis (PCoA) in the NIFOR main breeding parents based on the dissimilarity matrix using 10 SSR markers.

proposed by Zeng *et al.* [68] cannot be precluded because the geographical origin of the palms, ecology, agro-climatic conditions, pedology, and ethnical behaviour of local farmers could justify the presence of private alleles. All the parents are derived from palms originally selected on account of their high yields or good fruit composition from small oil palm groves at Aba, Calabar, Ufuma, Umuabi, and Opobo, which form part of a very large contiguous population constituting the oil palm belt of Nigeria. The highest number of private alleles occurred in the *tenera* parents from Umuabi. This location is derived savannah ecology and generally regarded as marginal for oil palm production with rainfall of about <2000 mm per annum. The oil palm occurs in isolated but dense groves in valleys and conical hills and ridges which cover the entire region. The private alleles found in such a marginal environment may have an adaptive value. The inhabitants of this area, as in all other areas of the Nigerian oil palm belt, live in homesteads within the groves. The palms are characterized by slow stem increment, high bunch yield, and palm wine (alcoholic beverage from oil palm sap) production.

Two parents (AD3 and T5) were selected from Ufuma, representing the heart of the eastern oil palm belt of Nigeria with annual rainfall of about 2000 to 2500 mm. This area is characterized by an unusually high proportion of *tenera* (thin-shelled) palms even before the inheritance of the fruit forms was fully understood. The immediate cause of this could not be ascertained but ethnical behaviour of the farmers/grove owners as regards method of seed selection/exchange with preference to high mesocarp to fruit and oil to bunch ratio may possibly explain this occurrence (personal interview of local farmers, 2015). The high palm oil production from this region seems to support this claim. The Aba *dura* parent (AD5) selection was carried out on an impoverished soil which is typical of the main oil palm belt of Nigeria. Selection of high bunch yielding palms in such condition held out hopes of breeding palms capable of high productivity under such conditions. Similar instances were revealed using isozymes [69] and microsatellite markers [38], which were attributed to adaptive genetic variants.

5.2. Polymorphism Information Content

In recognition of the relative superiority of SSRs in detecting DNA polymorphism [70], the discriminative power of each SSR marker was assessed by calculating polymorphic information content (PIC). A PIC value of greater than 0.7 is considered to be highly informative, whereas a value of 0.44 is considered to be moderately informative. In the present study the result showed a high average PIC value (mean = 0.66) in all the tested loci. The result further revealed that with the exception of locus sMg00016 (PIC = 0.47); sMg00087 (PIC = 0.82) would be best in screening NIFOR oil palm genotypes followed by sMo00102 (PIC = 0.77), and sMg00179 (PIC = 0.76). Accordingly, the PIC value indicates that about nine of these loci were informative and capable of discriminating between genotypes. Very similar results were obtained in a study that involved the analysis of 8 microsatellite loci in a population of 48 parent trees of oil palm (*E. guineensis*) from the breeding plantation of Univanich Palm Oil Public Company Ltd. where PIC ranged from 0.580 to 0.821 [71], confirming that oil palm microsatellites are very informative. Arias *et al.* [40] also reported a maximum PIC value of 0.822 in a comparative study of 189 oil palm materials produced by different commercial companies using 17 SSR markers. The result of the present study is in agreement with earlier reports from Billotte *et al.* [52] and Singh *et al.* [39] on the high polymorphism of CIRAD and MPOB oil palm microsatellite markers used in this study. These highly polymorphic set of microsatellite marker pairs has been used for genetic fingerprinting in breeding programmes.

5.3. Mean Number and Effective Number of Alleles per Locus

Microsatellite markers are multi-allelic and co-dominant, hence their relative superiority in detecting DNA polymorphism. A comparison of the results obtained in the present study (4 - 9 alleles per locus with a mean of 6.4 alleles/locus) with those published earlier indicates that the average number of alleles per locus was relatively higher than those earlier reported for two parents (LM2T and LM10T) of BRT10 first selection cycle oil palm population with an estimated average of 1.75 alleles/locus [72]. It was also higher than that reported for improved Nigerian germplasm samples of NIFOR origin and some survey materials from Ayangba and Bida (5.4 and 5.3, respectively) maintained at the Centre National de la Recherche Agronomique (CNRA) in Côte d'Ivoire [8] and 9 D x P oil palm crosses from different Colombian commercial companies (4.5 alleles per locus) using 16 SSR loci [40]. However, the average alleles per locus in the present study is lower compared to the 8 alleles per locus reported by Thongthawee *et al.* [71] among 132 parent trees from the Thai oil palm breeding programme carried out at Univanich Palm Oil Public Company Ltd., using 8 microsatellite loci.

Some of this variability could be explained by differences in the numbers of genotypes involved, coupled with

the number of SSR markers used. The number of alleles per locus is affected by the number of markers and sample size analyzed. Singh *et al*. [39] previously reported lower number of alleles per locus (2.2 - 3.2) in *E. guineensis* germplasm using a smaller set of SSRs and the number of alleles increased (2.8 - 3.9) when Ting *et al*. [51] employed a larger set of SSRs to evaluate a wider pool of oil palm germplasm. In fact, Augustina *et al*. [73] reported a total of 163 alleles (mean = 8.2) among the 85 *pisifera* accessions from germplasm collections of different origins at Sampoerna Agro Tbk (SA) in Indonesia. Bakoumé *et al*. [37] detected a total of 209 alleles, with a mean number of 13.1 alleles per locus in a sample of 494 oil palm derived from 10 African countries. Cochard *et al*. [8] obtained a total of 202 alleles, with an average of 14.5 alleles per locus within 318 oil palm samples from eight countries. The low number of alleles in the present study suggests the small size of materials used, *i.e*., few parents of a breeding programme and limited number of descendants for their inter-crosses. This hybridization and selection tend to affect the population and decrease allele variability. Several studies have shown a general tendency of loss of diversity after several cycles of selection. In a related study, Bakoumé *et al*. [37] investigated the allelic diversity of 3 breeding materials, using 16 microsatellite markers and observed that rare alleles were common alleles in wild oil palm populations showing a reduction due to many years of selection in the materials.

5.4. Observed Heterozygosity and Expected Heterozygosity in the Parents of the NIFOR Oil Palm Main Breeding Programme

The genetic diversity of NIFOR oil palm main breeding parents studied was relatively high (H_e = 0.703). This result is consistent with studies of genetic variation in oil palm using RAPD [74], AFLP [28], isozyme, [69], and RFLP [27]. However, the extent of the gene diversity of the studied NIFOR oil palm parents (H_e = 0.703) was lower than those reported by Bakoumé *et al*. [38] for five natural populations of oil palm from Nigeria (Umuabi = 0.736; Ibono = 0.712; Vabiti = 0.803; Ogbalato = 0.751; and Ologbo = 0.739), respectively. On the other hand, the present result is slightly higher than the average H_e value detected in the Nigerian improved germplasm (NIFOR = 0.696; Ayangba = 0.697; and Ahoada = 0.704) materials reported by Cochard *et al*. [8] using 14 SSRs. Nonetheless, comparisons were not on the same basis as the origin and number of samples was different coupled with the number of SSRs assayed. Similarly, the observed heterozygosity (H_o = 0.683) was comparable to what was reported by Cochard *et al*. [8] for oil palm accessions from Ahoada (H_o = 0.685) and a bit higher than in Ayangba (H_o = 0.673), but much higher than the results (0.516 to 0.551) of Bakoumé *et al*. [38] for the five natural oil palm populations from Nigeria. Although there were few significant departures from HWE, the observed heterozygosity was lower than the expected heterozygosity. In congruence with this study, Nybom [75] compiled 79 microsatellite based studies and found that grand means for H_o was lower than H_e in 64 of those studies. Similarly, most of the genetic diversity studies in oil palm using SSRs supported this finding [8] [38] [51] [73] [76] [77]. Fixation index values close to zero are expected under random mating and according to Bruford *et al*. [78], substantial positive values as recorded in this study may be due to presence of undetected null alleles or perhaps allele dropout which show a high deficit of heterozygosity at loci sMo00102.

5.5. Observed Heterozygosity and Expected Heterozygosity in the Three Groups of NIFOR Oil Palm Main Breeding Parents

The high genetic diversity observed among the groups of parents suggests the broad genetic base of the parents which was expanded beyond the early Calabar and Aba groves selections of the NIFOR 1st selection cycle parents (H_e = 0.602 for NIFOR *dura* and H_e = 0.650 for NIFOR *tenera*). The *dura* and *tenera* parents available at NIFOR are mainly from the early Aba and Calabar groves. However, full advantage was taken of the vast introductions of oil palm germplasm from various groves in the country especially the Ufuma and more recent Aba introductions, the coastal (Opobo) and hinterland (Umuabi) introductions, including the very valuable Angola introductions to ensure a wide genetic variability and genetic gain expected from breeding projects. In contrast to the NIFOR *dura* and *tenera* parents, the introgression among Deli from different origins and introduction of new Deli parents from Ecuador and Ulu Remis ex Sabah may have enhanced the diversity of the Deli *dura* NIFOR breeding population. In the present study, the expected heterozygosity value (H_e = 0.622) in the Deli *dura* NIFOR was high and might have been due to the out-crossing behaviour of oil palm. High genetic diversity implies a high amount of additive genetic variance on which breeding can still capitalize although Deli *dura* NIFOR has gone several selection cycles. Different sources of Deli *dura* corresponds to different populations of

Deli *dura*. Every oil palm research station in the world developed Deli *dura* using its own selection preferences leading to favouring different alleles and different allele combinations. The acquisition of Deli *dura* from different sources has enriched the Deli *dura* NIFOR materials with different alleles and different allele combinations. When compared to the previous report of Cochard *et al.* (2009), lower values of expected heterozygosity ($H_e = 0.373$) for Deli NIFOR and ($H_e = 0.510$) for 6 Deli *dura* populations from different origins using 14 SSR markers. Also, Ting *et al.* [51] revealed even lower values ($H_e = 0.340$) for Deli *dura* via 15 EST-SSRs. Recently, Bakoume *et al.* [38] reported H_o values (0.310 and 0.211) and H_e values (0.549 and 0.559) for Deli *dura* MPOB (Malaysia) and Deli *dura* Dabou (Côte d'Ivoire) with 16 microsatellite markers. These figures are lower than the reported result in this study. This low genetic diversity of the Deli breeding population reinforces the very narrow genetic base of the Deli materials having been selected from four palms introduced in Bogor (Indonesia) in 1848.

5.6. Fixation Index

In the present study, fixation index (F) was estimated using the F-statistics of Wright [65]. It is a measure of the excess or deficit of heterozygotes in the entire parental materials due to non random mating. In general, F value for the entire set of parental genotypes was low but positive (F = 0.0071) indicating a deficit of heterozygotes. Selfing and intra-groups crosses performed during the breeding programme could have ineluctably led to increased homozygosity in both the *dura* group and *tenera/pisifera* group (Bakoumé 2015, personal communication).

5.7. Genetic Differentiation among the Three Groups of Parental Genotypes

The analysis of molecular variance (AMOVA) shows that most of the variation in the NIFOR oil palm main breeding parents lies within populations, a result compatible with those from AFLP, Isozymes, and SSR studies involving oil palm germplasm collections and out-breeding plant species [8] [28] [69] [79]. Consequent upon partitioning of genetic diversity, there is a considerable genetic diversity within populations that may be exploited in population improvement programme. The F_{ST} value for the entire parent population was 0.131. According to Wright [80] and Hartl and Clark [81], a genetic differentiation ($F_{ST} = 0.05 − 0.15$) is moderate. Therefore, we consider that the overall F_{ST} value found in this study ($F_{ST} = 0.131$, P = 0.001), indicates significant differences among groups of parents.

5.8. Genetic Relatedness among Parents

SSR genetic distance refers to the genetic divergence among populations, which was measured with Rogers' dissimilarity coefficient. It is a function of the coefficient of co-ancestry and therefore, suitable for the uncovering of pedigree relationships among operational taxonomic units such as the detection of essentially derived varieties in plant breeding or the identification of duplicates and collection gaps in seed banks [82]. The mean genetic distances among NIFOR oil palm main breeding parents was moderate ($D_R = 0.5570$) indicating a considerable degree of relatedness which underpins low genetic diversity within the populations. Lower genetic distance values (0.050 to 0.573) were reported for 19 MPOB oil palm parent trees using 9 SSR markers [43]. Information on the genetic distance among individuals in a breeding population is an important tool for breeders aiming at exploiting heterosis effect on which optimum oil palm growth and fresh fruit bunch yields depend.

   The purpose of clustering analysis is to group together individuals that are similar and provide a picture of the overall relationship among the individuals sampled. Microsatellite markers have been reported to be useful in assigning inbred lines into known heterotic groups, especially for those accessions without clear and accurate pedigree records [83] [84]. In this study, parents of NIFOR oil palm Main Breeding Programme are genetically close to each other. Grouping of the parents revealed by the present analysis did not agree with the pedigree of parents, such as the grouping of Serdang Avenue ex Pobe *dura* (DD2) and Ufuma x Aba *tenera* (T1) parents, and Ufuma *dura* (AD3) and Ulu Remis *dura* (DD3) parents in clusters II and III respectively. Both clusters comprise parents selected on the basis of good combining ability for bunch yield and, good fruit and bunch composition respectively. Clusters were rather representative of the parent's heterotic groups. The UPGMA dendogram grouping of the parents from different origin/pedigree in the same cluster indicate lack of correlation between origin/pedigree and genetic distance among the parents. Similar result was also observed in maize

[84]-[86], which revealed that grouping of inbreds based on molecular data do not always concur with the available pedigree information. This may be due to several possible factors [84] in addition to the fact that DNA markers may be affected by selection, drift and mutation. The clustering process and the method selected may also result in incongruities [83]. This was demonstrated where in some clusters, an inbred line that is related to two other inbred lines may fall in one of two separate clusters [85].

Information about the relationship among breeding materials and the inherent genetic variation is important in making choices of parents in breeding programmes. This is significant in hybrid breeding where detection and utilization of heterotic patterns between different sources are important for genetic success. The principal co-ordinate analysis (PCoA) based on genetic distance estimates determined by SSR data for the 15 oil palm parents, provided a distinct separation of parents from different origin into heterotic groups. The *tenera* parent, T8 (Ufuma x Angola) was separated from the rest of the parents. Similar results were recorded in the earlier mentioned studies.

6. Conclusion

Results of this first evaluation of the genetic diversity of the current NIFOR oil palm main breeding parents demonstrated the presence of high genetic variation within and between the NIFOR main breeding groups of oil palm parents. NIFOR *tenera* parent genotypes were more diverse than the NIFOR *dura* and Deli *dura* NIFOR. The ten microsatellite makers independently distinguished an average of 8 genotypes out of the 15 genotypes evaluated in this study. Hence, further analysis using large numbers of microsatellite markers and samples is proposed. Moreover, involving more SSR markers for genotyping is expected to group the parental genotypes in a better manner. The high PIC value indicated that the microsatellite markers were preferentially valuable for genetic diversity analysis. Loci mEgCIR0790, mEgCIR0793, sMg00087, sMg00154 and sMo00102 were the most efficient microsatellite markers in detecting genetic variation among parent genotypes. The lowest pair-wise genetic dissimilarity coefficient was recorded between parent genotypes AD4 and T8. As a result, these parent genotypes could be used to study the correlation between genetic distance and heterosis in the NIFOR oil palm Main Breeding Programme. Generally, results of this study would be helpful to design crosses among these parents for future breeding and selection programme.

Acknowledgements

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