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Allelic variability of oil palm inter-population progenies based on simple sequence repeats markers

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The genetic structure and variability was described among 52 oil palm inter-population crosses after two selection cycles using simple sequence repeats markers (SRR). The 10 sets of SSR markers covering 62.5% of the oil palm genome showed a high level of polymorphism (average number of alleles per locus = 6.7; unbiased expected heterozygosity = 0.655; G"st = 0.346) across the oil palm samples. On the basis of allelic variability indices, three genetically diverse progenies (P1, P2 and P5) with estimates higher than the overall mean values were identified. Analysis of molecular variance revealed that partitioning of variance was higher (82%) among palms within each progeny than among the progenies (18%). High pairwise fixation index ($F_{ST} > 0.150$) among progenies was evident, particularly between progeny P4 and progenies P1, P2 and P3. Crossing of selected palms from highly differentiated progenies could generate offsprings with more genetic variation. The mean Nei's standard genetic distance across progenies was 0.364. The lowest genetic distance was observed between progeny P2 and P5 (0.090) and the highest was found between progeny P1 and P4 (0.653). Based on the results, there is ample variation among the inter-population progenies for maximum exploitation of heterosis and further gains in future breeding programme.

Key words: *Elaeis guineensis* Jacq., microsatellite markers, NIFOR, reciprocal recurrent selection.

INTRODUCTION

Indigenous to West Africa but with widest occurrence in the South-Eastern States of Nigeria, oil palm (*Elaeis guineensis* Jacq.) is cultivated for palm oil and palm kernel oil extracted from the mesocarp and kernel, respectively (Ataga and Van der Vossen, 2007). Palm oil is the most preferred natural oil in the diets of Nigerians

both as crude red palm oil and as refined oil (olein). Modest but steady progress has been made in the genetic improvement of the crop in the past eight decades, culminating in an increased average yield from 2.5 to 5.0 metric tonnes of fresh fruit bunch ha⁻¹year⁻¹ and 0.5 to 1.0 tonnes oil ha⁻¹year⁻¹ between 1930s and 1950s

to 20-25 metric tonnes of fresh fruit bunch ha^{-1} year^{-1} and 3.5 to 4.0 tonnes oil ha^{-1}year^{-1} to date (Okwuagwu et al., 2005). Even with this progress, there is still a huge discriminating and competitive demand on the yield and quality of palm oil for edible and non-edible industries. With an estimated 3 million hectares of land under cultivation, Nigeria's share of the global palm oil production is less than 2% with about 1.01 million metric tonnes of palm oil per annum (USDA, 2019). Although this level of production is below the domestic consumption of 1.34 million metric tonnes, the deficit in supply is complemented by an annual importation of about 325,000 metric tonnes. Consequently, continuous and systematic improvement for these traits of economic importance with a proportionate expansion of area under cultivation using improved genetic stock is highly desirable to meet industry demand and ensure a country-wide sufficiency of oils and fats.

The Nigerian Institute for Oil Palm Research (NIFOR) has continued to make advances in the improvement of bunch yield and oil quality of oil palm by the implementation of modified reciprocal recurrent selection (RRS) scheme. It was reported that the RRS scheme had increased oil yield by almost 18% per cycle compared to the base population (Okwuagwu et al., 2005; Durand-Gasselin et al., 2009). During each cycle of selection, the *dura* (D) and *tenera/pisifera* (T/P) base populations are kept separate and outstanding *dura* and *tenera* palms are identified through the performance of D × T and D × P progenies. These inter-population crosses of the base population are essential in the comparative trials of the breeding programme because it represents the evaluation units to identify the best crosses for prospective inter-population hybrid production. Additionally, it is one of the main sources of parents for the development of new populations exploitable in subsequent recombination cycles of the breeding programme (Falconer, 1989). However, frequent hybridization and selection among few parents in the RRS scheme tend to affect the effective population size, allele variability and genetic structure of the populations which risks long-term genetic progress in the scheme (Cao, 1995). For rapid and continued progress in oil palm breeding, the potential variability of the populations must be considered because genetic variability is required to achieve genetic gains.

Genetic variability can be estimated through the application of different molecular techniques and to date a series of different genetic markers have been explored and developed (Powell et al., 1996). Molecular genetic markers, especially Simple Sequence Repeats (SSRs) provide an important method of assessing genetic variability and have been widely employed in a variety of plant species including oil palm (Powell et al., 1996; Bakoumé, 2016). The preference for SSR markers is associated with their high information content, co-dominance, abundance in the genome, reproducibility and PCR based detection (Powell et al., 1996).

This study is a subset of a larger programme designed to describe the genetic structure and variability among NIFOR oil palm breeding populations using molecular markers. The present paper aims at estimating the available genetic variability and population structure within and between five inter-population progenies of the NIFOR oil palm main breeding programme with SSR markers. Results obtained from this study would be exploited in the selection of new parent palms for the breeding programme as well as for the production of commercial planting material.

MATERIALS AND METHODS

Fifty-two individual palms from five divergent *dura* x *tenera* inter-population crosses in the comparative trials of NIFOR oil palm main breeding programme, referred to as P1 to P5 were evaluated. The *dura* (female parent) population is characterized by consistent high yield of heavy bunches and good bunch composition traits while the *tenera* (male parent) population has a high bunch number with excellent oil-to-mesocarp ratio. The genetic backgrounds of the parent palms were *dura* from Ecuador, Calabar, and Ufuma, with the *tenera* mainly from Umuabi open pollinated genetic collections (Okwuagwu, 1985). The experimental site is located at Benin City, Nigeria; 06°312 N latitude and 05°402 E longitude and at an altitude of 149.4 m.a.s.l. The annual rainfall range from 1595 to 1958 mm and a mean annual temperature of 31.8 to 32.0°C. The number of evaluated palms in each cross ranged from 8 to 13 individuals after hybrid validation of the progenies.

Total genomic DNA was extracted from lyophilized leaf tissues from an unopened spear leaf of individual palms following the protocol described by Doyle and Doyle (1990) with minor modifications. This protocol was originally designed for relatively large-scale DNA isolation but was scaled down to suit the present study. The amount of DNA needed for the SSR reaction is very small, only about 25 ng for a single reaction of a total volume of 10 μl. The PCR conditions using M13-tailed primers described by Ting et al. (2013) was used for the amplification of 10 SSR loci/primer pairs (mEgCIR0793, sMg00156, sEg00154, sMo00102, sMg00016, mEgCIR3519, mEgCIR0790, sEg00151, sMg00179, and sMg00087) developed for oil palm (Billotte et al., 2005; Singh et al., 2008). The detection of amplification products was carried out with an automated capillary DNA genetic analyzer (ABI 3739, Applied Biosystems, USA) at the Genomics unit of Advanced Biotechnology and Breeding Centre (ABBC), Malaysian Palm Oil Board (MPOB) Malaysia. Allele calls and sizing was performed using the software GeneMapper® version 4.1 (Applied Biosystems, USA).

Marker informativeness was evaluated based on the standardized genetic differentiation measure (G"st; Hedrick 2005) after correction for small population size as described by Peakall and Smouse (2006; 2012). In addition, estimates of population genetic variation such as average number of alleles (Ao), effective number of alleles (Ae), number of private alleles (PA) - number of alleles unique to a particular population, Shannon information index (I), observed heterozygosity (Ho), unbiased expected heterozygosity (uHe), and fixation index (F_{ST}) were computed for each progeny across the 10 SSR loci. The proportion of genetic variability components within and between the inter-population progenies was determined by analysis of molecular variance (AMOVA). Pairwise (Wright, 1978) and genetic distance values among progenies based on unbiased Nei's standard genetic distance (Nei 1978) were estimated to provide information of the relatedness among the progenies. All calculations were facilitated

Table 1. Characteristics and allelic diversity in SSR loci evaluated in 52 oil palm inter-population crosses.

SSR loci	Linkage group	Ta(°C)	SSR repeat	Expected fragment size (bp)	Accession number	Ao	Ho	uHe	G"st
mEgCIR0793	2	56	(GA)15	149	AJ578545	6	0.481	0.685	0.635
sMg00156	4	50	(CT)15	237	Pr010615888*	7	0.808	0.720	0.187
sEg00154	6	57	(CAG)5	238	EY410356**	5	0.596	0.696	0.381
sMo00102	7	53	(AG)11	235	Pr010615939*	6	0.731	0.702	0.366
sMg00016	9	52	(GA)13	274	Pr010615861*	5	0.381	0.533	0.528
mEgCIR3519	10	52	(GA)15(GT)8	236	AJ578672	9	0.372	0.482	0.081
mEgCIR0790	12	52	(GA)19	215	AJ578544	7	0.404	0.504	0.197
sEg00151	13	57	(CAG)8	219	EY411661**	6	0.667	0.725	0.329
sMg00179	14	54	(AAAAG)6	214	Pr010615893*	7	0.731	0.681	0.180
sMg00087	15	58	(AG)19AA(AG)	212	Pr010615880*	9	0.683	0.825	0.541
Mean						**6.7**	**0.585**	**0.655**	**0.346**

Ta = annealing temperature; Ao = number of different alleles; Ho = observed heterozygosity; uHe = unbiased expected heterozygosity; G"st = Hedrick's standardized Gst, corrected when population size is small; *Probe Unique Identifiers (PUIDs) of NCBI Probe Database; **Accession numbers of NCBI GenBank

using GenAlEx version 6.5 software (Peakall and Smouse, 2006; 2012).

RESULTS AND DISCUSSION

Genetic variation is fundamental for populations to be able to face the present environmental changes and to ensure long term response to selection for traits of economic interest. In this study, we have examined levels of allelic variability and population structure within and among five inter-population progenies of oil palm, using SSR markers. Table 1 gives the synopsis of the 10 SSR markers including the basic genetic diversity parameters estimated. The marker informativeness (G"st) value for all the scored markers in this study ranged from 0.081 to 0.635. Six markers namely, mEgCIR0793, sEg00154, sMo00102, sMg00016, sEg00151 and sMg00087 revealed high G"st values (>0.250) which provided

sufficient discrimination to assess genetic variation within and between the progenies. The number of different alleles across the 10 SSR markers varied from 5 (sEg00154 and sMg00016) to 9 (mEgCIR3519 and sMg00087) with a mean value of 6.7 alleles per locus (Table 1).

The observed heterozygosity (Ho) values ranged from 0.372 (mEgCIR3519) to 0.808 (sMg00156) with an overall average of 0.585. The unbiased expected heterozygosity (uHe) showed slightly higher values than Ho with values ranging from 0.482 (mEgCIR3519) to 0.825 (sMg00087) and a mean value of 0.655. The high mean uHe value suggests a high expected heterosis effect upon which oil palm yield depends. The high allelic variation and genetic diversity across the 10 SSRs were relatively higher than those reported by Putri et al. (2017; Ao = 3.0; He = 0.543) for MTG oil palm commercial variety from Socfindo, Indonesia. However, the extent of SSR diversity in the current study (He = 0.655) was lower than that

reported by Augustina et al. (2013) among 85 oil palm *pisifera* accessions from SA Indonesia (Ao = 8.2; He = 0.70). This variability could be explained by differences in the number, origin of genotypes and the number of SSR markers used.

Results of the allelic patterns across the progenies revealed the presence of 19 different alleles varying from 3.2 (Progeny P4) to 4.4 (Progeny P2) with a mean value of 3.8 alleles per locus (Figure 1).

The highest number of effective alleles (Ae = 2.733) was exhibited by Progeny P2 while Progeny P3 showed the lowest value (Ae = 2.341) with an average of 2.509. The average Ao (3.8) and Ae (2.509) revealed per locus was lower than 5.0 and 3.3 alleles from a previous study on natural oil palm collections from 10 African countries maintained at MPOB (Malaysia) and three breeding materials (Bakoumé et al., 2015), but higher than 2.0 and 1.627 reported on 30 individual clones of BTC A-group collection of PT
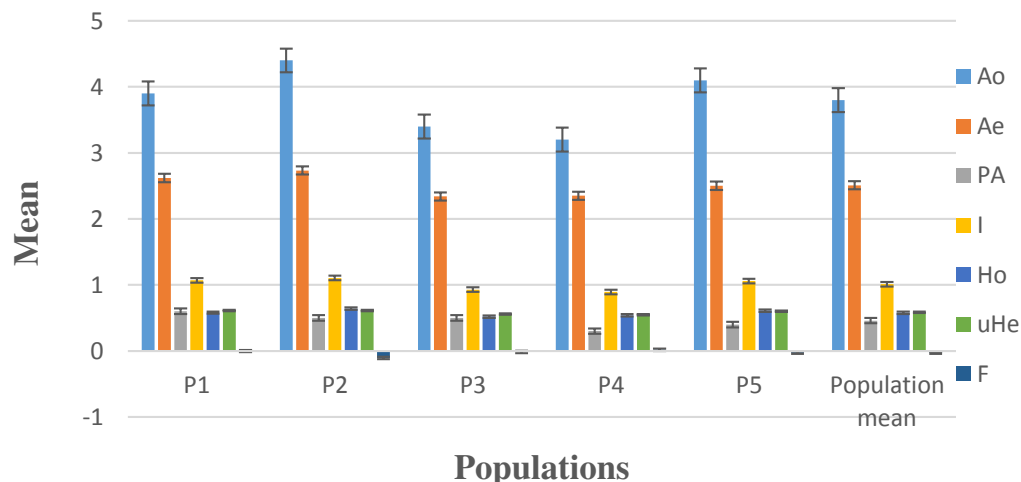
Figure 1. Mean allelic patterns across inter-population progenies; Ao = number of different alleles; Ae = number of effective alleles; PA = private alleles; I = Shannon's Information Index; Ho = observed heterozygosity; He = unbiased expected heterozygosity; F = inbreeding coefficient.
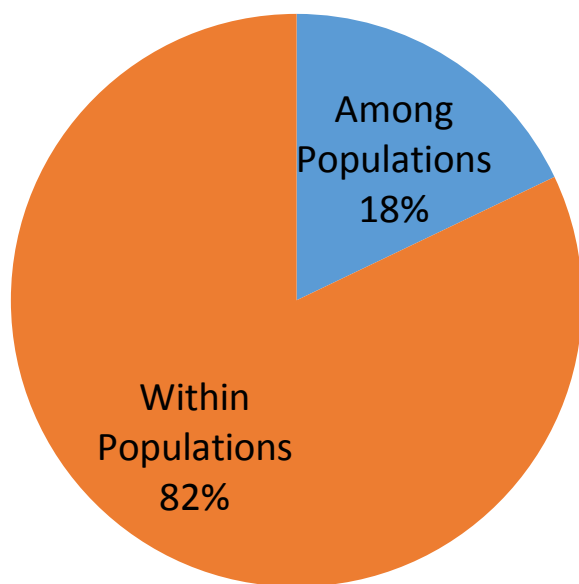

Figure 2. Pie chart of molecular variance estimated by AMOVA.

Socfindo (Putri et al., 2018). It is possible that the lower number of alleles observed among the progenies is a consequence of selection pressure on the parents of the progenies. Several studies have shown a general tendency of allele loss or reduction in the number of alleles after several cycles of selection (Cochard et al., 2009). Private alleles (PA) were identified among the progenies evaluated (Figure 1). The progenies with the most private alleles were P1 (0.6), P2 (0.5), and P3 (0.5) respectively. Progenies with private alleles can be used as sources of parents in the breeding programme to

broaden the genetic base of the breeding population as well as development of more adapted variety. Progeny P4 recorded the least Shannon's index (I = 0.894) whereas P2 revealed the highest (I = 1.105). The mean observed (Ho) and unbiased expected (uHe) heterozygosities across the progenies were 0.578 and 0.590 respectively, implying that the progenies are highly heterozygous regardless of the two generations of selection by the parents. The uHe values ranged from 0.55 (P4) to 0.61 (P1 and P2). Meanwhile, Ho was lowest for P3 (0.52) while the highest was for P2 (0.64). The uHe values obtained in these progenies were high compared to those obtained from Ulu Remis Deli (He = 0.493; Bakoumé et al., 2007) and *E. oleifera* (He = 0.221; Maizura et al., 2017). However, the values were lower than those reported for oil palm germplasm from Owerri (He = 0.745; Bakoumé et al., 2007). Mean F-value (inbreeding coefficient) was low and negative (-0.027), implying excess of heterozygosity due to heterotic selection in the inter-population crosses. An excess of heterozygosity suggests the impact of selection, which favours heterozygous individuals. The negative F-values for most of the populations showed the prevalence of outcrossing, whereas the positive value in P4 indicated some level of inbreeding. Considering the high Ho and uHe values in most of the populations (P1, P2 and P5), the presence of private alleles unique to a specific population may explain the excess of heterozygosity. Figure 2 represents the components of genetic variance of the oil palm inter-population progenies estimated by AMOVA.

The analysis of molecular variance showed that 82% of the total genetic variations were due to differences within populations, while 18% were due to genetic variation among populations. The maximum percentage of variation (82%) present was among palms within

Table 2. Pairwise F_{ST} (upper diagonal) and unbiased Nei's standard genetic distance (below diagonal) between the 5 populations.

Population	P1	P2	P3	P4	P5
P1	0.000	0.063	0.121	0.167	0.090
P2	0.200	0.000	0.053	0.155	0.029
P3	0.430	0.147	0.000	0.175	0.056
P4	0.653	0.563	0.620	0.000	0.123
P5	0.321	0.090	0.155	0.402	0.000

populations, while the remaining 18% of the total variation was partitioned among populations. Permutation tests suggest that the total genetic differentiation among populations (*PhiPT*) was significant (0.179; p = 0.001) which indicates that differences among progenies are significant. This high level of intra-population diversity and low but significant genetic differentiation among oil palm progenies is in agreement with previous reports on oil palm (Bakoumé et al., 2007; Allou et al., 2008; Maizura et al., 2017), and consistent with outbreeding perennial species maintaining most of their variation within populations (Hamrick et al., 1992).

Genetic differentiation estimated by pairwise F_{ST} values among progenies were in the range of 0.029 to 0.179 (Table 2; upper diagonal). Progeny P4 was highly differentiated from P1, P2 and P3 with F_{ST} values above 0.150. According to Wright (1978), F_{ST} values between 0.15 and 0.25 indicate a high level of inter-population divergence. This high genetic relationship between individual palms of different progenies will enhance selection of parents for the establishment of crossing programmes to maximize the probability of finding transgressive hybrids in any of the following crosses; P4 x P1, P4 x P2 and P4 x P3. The unbiased Nei's standard genetic distance values ranged from 0.090 (P2 vs P5) to 0.653 (P1 vs P4) with an average distance of 0.364 (Table 2; lower diagonal). Considering the wide range of distance between the progenies and the assumption that progenies with the greatest distance is associated with inter-population heterotic potential, it may be reasonable to infer that crossing of the most distant progenies (P1 vs P4) with the other progenies will lead to the development of superior hybrids.

Conclusion

The results suggest that the inter-population progenies showed considerably high levels of allelic variation that will ensure continued progress in subsequent selection cycles of the breeding programme. Since high degree of genetic variation was observed among palms within the progenies, more palms should be sampled for a better insight on the genetic structure within and between the inter-population progenies. Within the framework of genetic differentiation and genetic distance of the inter-

population hybridization programme, crossing of the genotype P4 with P1, P2 and P3 could offer a possible alternative for the maximum exploitation of heterosis and maintenance of genetic variation requisite for further gains in future breeding programme. The crossing between P1 and P3 may be acceptable for some gain in heterosis, although this gain will likely be lower than the crossing between P4 and P3.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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