Allelic Diversity of 22 Sampoerna Agro's Oil Palm Pisifera Based on Microsatellite Markers [1)]

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ABSTRACT

Knowledge on genetic distances and relationships among breeding materials are important in plant breeding and have significant impact on crop improvement. Sampoerna Agro (SA) has established oil palm germplasm collections consisted of 22 pisifera populations originated from various regions. Microsatellites markers are highly reliable; inherited in co-dominant fashion; therefore, they are able to distinguish heterozygous to homozygous individual; easy to score and rapidly produced using PCR. In this experiment, allelic diversity among SA's pisifera collection (85 palms from the total populations) were investigated using SSR marker (20 SSR loci). Results of the investigations indicated the presence of at least 163 alleles in the SA's pisifera collection evaluated. Mean number of alleles per locus was 8.2 while mean of *polymorphic information content* (PIC) of the SSR marker analyzed was 67 %. Observed mean of heterozygosity was 0.4 while expected mean of heterozygosity was 0,7. Results of genetic dissimilarity coefficient calculation and dendogram construction using DARwin 5.05 indicated that the SA's pisifera populations were clustered into four groups. Some accessions of Yangambi origin formed group I; some other Yangambi and Ekona origin clustered into group II; Ghana, LaMé, Dami, and the rest of Nigeria origin clustered into group III, and a number of Avros origin were in Group IV, respectively. Implication of the observed allelic diversity to the SA's oil palm breeding program will be discussed in detail.

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is currently one of the strategic plantation commodities in Indonesia. Therefore, activities to improve oil palm characters are needed to develop better planting materials. Such objectives may be achieved through oil palm breeding program. Effective oil palm breeding activities depend on the availability of oil palm germplasm collections and their genetic variability.

A number of microsatellite markers evaluations have been done for oil palm germplasm collections of Sampoerna Agro. The objectives of those studies were genetic diversity analysis and genetic uniformity evaluation for oil palm clonal materials (ramets). The main objectives of this study were to utilize microsatellite markers for analyzing Sampoerna Agro's (Sumatra, Indonesia) 22 pisifera populations originated from various regions and to characterize their genetic diversity. The generated data will be used to draw preliminary conclusion on this germplasm for breeding purposes.

MATERIALS AND METHODS

Plant material and DNA extraction.

The evaluated oil palm populations consisted of 22 combinations of tenera by pisifera crosses (TxP families) of PT Sampoerna Agro Tbk (SA) in Indonesia. Each *TxP family* was represented by 3-4 *pisifera* descents. Total genomic DNA was extracted from fresh leaf samples of each individual palm using a conventional CTAB method. The genomic DNA concentration was estimated with a fluorimeter (Fluoroskan Ascent®, Thermo Fisher Scientific®, USA) and the DNA quality was checked using agarose minigel electrophoresis.

E. guineensis microsatellite primers pairs and genotyping.

Twenty independent microsatellite loci (Table 1) were chosen from the oil palm reference map published by Billotte *et al.* (2005), based on the following: (i) high polymorphism within the *E. guineensis* species and (ii) good pan-genomic coverage (generally one locus per homologous chromosomes pair). SSRs were genotyped as described by Roy *et al.* (1996) using an automated infrared fluorescence technology of a Li-Cor IR2 sequencer (Lincoln, Neb.). PCR amplification of genomic DNAs using the SSR primers and separation of the amplification products by electrophoresis were carried out as described by Billotte *et al*. (2005). The amplified SSR allelic patterns were analysed with the SAGAGT® software (LI-COR, Lincoln, USA) and alleles were identified according to their base pair size.

Data analysis.

The genetic diversity was estimated under PowerMarker v3.0 (Liu and Muse, 2005) by determining allelic diversity (total number of alleles, allele frequency per group), observed and expected heterozygosities (H_o and H_e) (Nei, 1987), and polymorphism information content (PIC) according to Botstein *et al.* (1980). Two types of descriptive analysis on genetic diversity were performed under DARwin5 (Perrier and Jacquemoud-Collet, 2009), such as: (i) a Principal Coordinates Analysis (PCoA), the factor analysis type to investigate the main origin groups and (ii) a Neighbour-Joining tree according to Saitou and Nei (1987) to gain a clearer picture of relations among individuals. The Neighbour-Joining analysis was un-weighted and un-rooted. These descriptive analyses were carried out based on a pair-wise dissimilarity matrix calculated by Simple Matching in accordance with the formula:

$$d_{ij} = 1 - \frac{1}{L}\sum_{l=1}^{L}\frac{m_l}{\pi}$$

by which dij is the dissimilarity between i and j, L the number of loci, π the ploidy and m_l the number of common alleles between i and j for locus l.

RESULTS AND DISCUSSIONS

Allelic variability

Results of the analysis revealed the presence of a total of 163 SSR alleles among the 85 pisifera accessions tested (Table 1). The number of alleles per locus varied widely from 3 in mCnCIR 0067 locus to 13 in mEgCIR 3346 locus. The mean of alleles per locus was 8.2. Eight loci (mEgCIR0802, mEgCIR0894, mEgCIR2414, mEgCIR3346, mEgCIR3362, mEgCIR3546, mEgCIR3785, and mEgCIR3886) gave very good results, with number of polymorphic alleles ranged from 10-13.

The pisifera originated from Nigeria showed the highest value forPIC and mean number of alleles per locus (58.7% and 4.5, respectively). Meanwhile, the La Mé origin was the lowest for the same parameters (16.2% and 1.55, respectively). Data for percentages of polymorphic amplification for each pisifera origin was presented in Table 2. In general for all observed genetic parameters, five pisifera origins (Nigeria, Ekona, Yangambi, Dami and Ghana) showed higher polymorphism than the other two (Avros and L Me). Moreover, pisifera of Avros origin showed lower polymorphism than that of La Mé. High diversity of pisifera of Nigeria origin that has been used as parent for production of comercial seeds (Bina Sawit Makmur, 2004), showed high level of heterozygosities. Such data may indicate that this pisifera origins may have previously been exposed to only little selection pressure. Germplasm collection that had high polymorphism level could be recommended for breeding purposes and further germplasm exploitation.

This slightly lower numbers of alleles, with a higher major allele frequency and a lower H_o mean value (0.4) of the SA pisifera accessions, were in accordance to the effects of past hybridization and selections previously conducted. The past hybridization and selections tended to decrease the average number of alleles per locus and the proportion of heterozygous loci. Expected heterozygosities (H_e) in the SA's pisifera samples indicated a high average polymorphism rate (0.7) for the loci. The polymorphism information content (PIC) values of the loci were generally high with average of 67 % for SA pisifera populations evaluated, indicating that SA pisifera germplasm is still characterised by a high degree of genetic diversity.

Genetic relationship among pisifera *accessions*

Analysis using factorial methods was conducted since the purpose of analysis was mainly to give an overall representation of diversity and not the individual effects. The Principal Coordinates Analysis (PCoA) identified several independent axes or eigenvectors that are linear combinations of the characters studied (SSRs in this case), which account for the largest part of the variation (SSR length polymorphism). The PCoA was performed on the 163 alleles, revealed by the 20 SSR loci over the 85 SA pisifera accessions. Distinct groups were discriminated, with axes 1 and 2 explaining 37.9% of the total molecular variation (*Figure 1*). One group was formed by Yangambi accessions. A second group was made of Avros along with one Nigeria accession (PN-17_3). A third group on the axes 1 and 3 was formed by Ekona along with Nigeria accession (PN-12_1) and Yangambi accession (PY-24_2). A fourth group was formed by Dami, La Mé, Ghana and Nigeria accessions. The two axes of our PCoA identified a genetic structure of distinct groups of accessions, consistent with the four main origins. Three individuals (PN-17_3, PN-12_1, and PY-24_2) were separated from their main group. These materials may have been mislabelled materials. Such materials should be discarded and should not be used as pollen sources and in breeding program.

As described by Perrier and Jacquemoud-Collet (2006), tree method was another approach for presenting diversity structure. This method indicated individual relationship that might be less accurate than factorial analyses on the overall structure. Our results of unrooted Neighbour-Joining tree gave a quite nice picture about the relations among SA pisifera accessions (*Figure 2*). Four major clusters were formed: Yangambi, Avros, Ekona and a fourth broader cluster made of Dami, La Mé, Ghana and Nigeria accessions. A selection of African breeding materials formed broader grouping. These results also indicated that Avros material had the least intra-population variability.

All of these demonstrated that results of SSR analysis were usefull for genetic diversity of pisifera oil palms germplasm. Moreover, SSR-genotyping should allow more efficient selection of pisifera parents for oil palm breeding programs. Selection of more genetically divergent parents would theoretically maximize heterosis and thus increase hybrid vigour of oil palm hybrids.

CONCLUSIONS

We have demonstrated that SSR polymorphism provides a valuable tool for the analysis of pisifera oil palms germplasm. The patterns of variation detected confirm previously reported theories on the genetic diversity of oil palm. In addition, these results have significant implications for pisifera conservation purposes, since they could be used to identify a core genepool for ex situ pisifera conservation and for future SA's oil palm breeding programs.

Based on generated SSR markers, mean number of allele per locus, expected heterozygosities and PIC value, the selected pisifera originated from Nigeria, Ekona and Yangambi had high level of allelic diversity. The geographic and genetic structures of the pisifera oil palm diversity suggested the possibility of new breeding approaches. Within the frame of an inter-population hybridization program using Nigeria, Ekona and Yangambi origins of pisifera, more favorable heterosis effects on the palm oil production might be obtained.

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Table 1. Synopsis of 20 Microsatellite Loci Used in the SSR Analysis of Sampoerna Agro's Pisifera Oil Palm Genetic Diversity.

SSR Locus	Linkage Group	Accession Number	Allele Size (bp)	Annealing Temperature (^{0}C)
mCnCIR 0038	13	AJ865151	104	52
mCnCIR 0067	1	AJ865174	146	52
mEgCIR0173	3	AJ578503	132	52
mEgCIR0353	16	AJ271935	102	52
mEgCIR0802	1	AJ578549	217	52
mEgCIR0886	8	AJ578561	157	52
mEgCIR0894	7	AJ578562	186	52
mEgCIR2414	12	AJ578595	195	52
mEgCIR2422	12	AJ578597	248	52
mEgCIR2813	5	AJ578618	210	52
mEgCIR3282	2	AJ578632	245	52
mEgCIR3346	15	AJ578649	237	52
mEgCIR3362	11	AJ578652	151	52
mEgCIR3392	1	AJ578660	260	52
mEgCIR3533	4	AJ578674	139	52
mEgCIR3543	6	AJ578678	232	52
mEgCIR3546	14	AJ578680	286	52
mEgCIR3745	16	AJ578718	273	52
mEgCIR3785	10	AJ578726	284	52
mEgCIR3886	9	AJ578743	187	52

Table 2. Mean number of Alleles Per Locus, Polymorphic Information Content (PIC), Observed (Ho) and Expected (He) Heterozygosities of 20 Microsatellite loci.

Microstellite Loci	Number of Alleles/locus	PIC (%)	Ho	He
mCnCIR0038	8	65.9	0.235	0.695
mCnCIR0067	3	43.8	0.153	0.547
mEgCIR0173	6	48.9	0.282	0.518
mEgCIR0353	7	74.3	0.412	0.778
mEgCIR0802	12	76.9	0.435	0.79
mEgCIR0886	6	61.5	0.424	0.672
mEgCIR0894	10	84.7	0.435	0.867
mEgCIR2414	10	81.1	0.435	0.836
mEgCIR2422	6	40.2	0.271	0.425
mEgCIR2813	5	54.7	0.459	0.614
mEgCIR3282	7	70.6	0.447	0.751
mEgCIR3346	13	80.1	0.506	0.823
mEgCIR3362	11	77.3	0.435	0.800
mEgCIR3392	7	54.3	0.047	0.571
mEgCIR3533	6	52.7	0.412	0.605
mEgCIR3543	6	62.5	0.376	0.686
mEgCIR3546	10	82.2	0.647	0.845
mEgCIR3745	8	70.4	0.388	0.745
mEgCIR3785	11	82.7	0.412	0.85
mEgCIR3886	11	70.6	0.518	0.737
Mean	8.2	67	0.4	0.7

Tabel 3. Mean Number of Alleles Per Locus, Polymorphic Information Content (PIC), Observed (Ho) and Expected (He) Heterozygosities of 7 Sampoerna Agro's pisifera Oil Palm Based on Their Origin

Origin	Number of population	Mean number of alleles per locus	PIC (%)	Ho	He
Avros	8	3.05	34.8	0.279	0.401
Nigeria	3	4.50	58.7	0.482	0.663
Ekona	3	3.85	56.2	0.604	0.645
Ghana	3	2.85	39.6	0.373	0.471
Yangambi	2	3.50	48.0	0.356	0.567
Dami	2	3.00	42.3	0.463	0.520
La Me	1	1.55	16.2	0.250	0.225

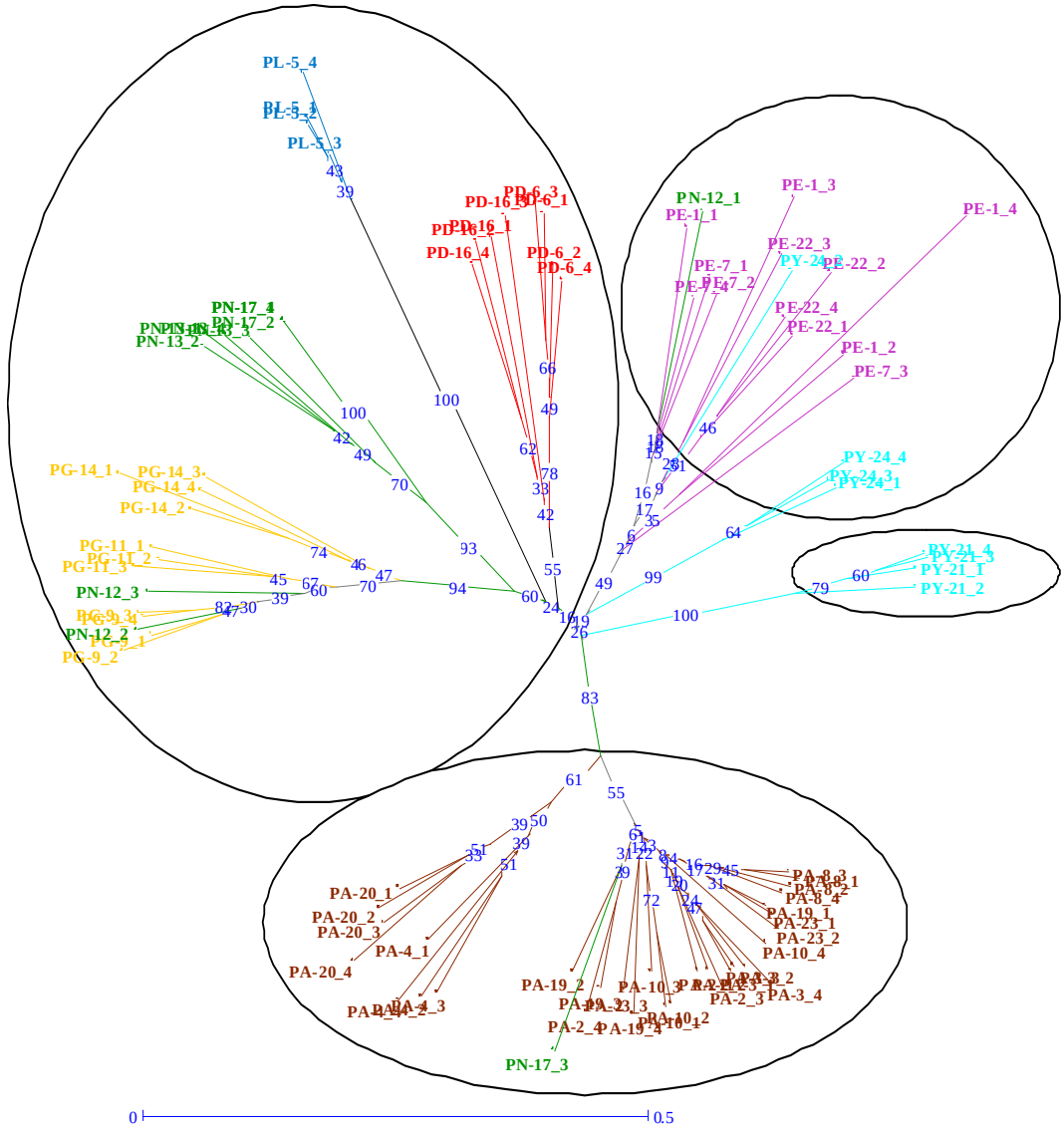

Figure 1. Clustering of 85 individues of Sampoerna Agro's pisifera oil palm germplasm collections using Neighbor-Joining trees based on *Simple Matching Dissimilarity Matrix* analysis

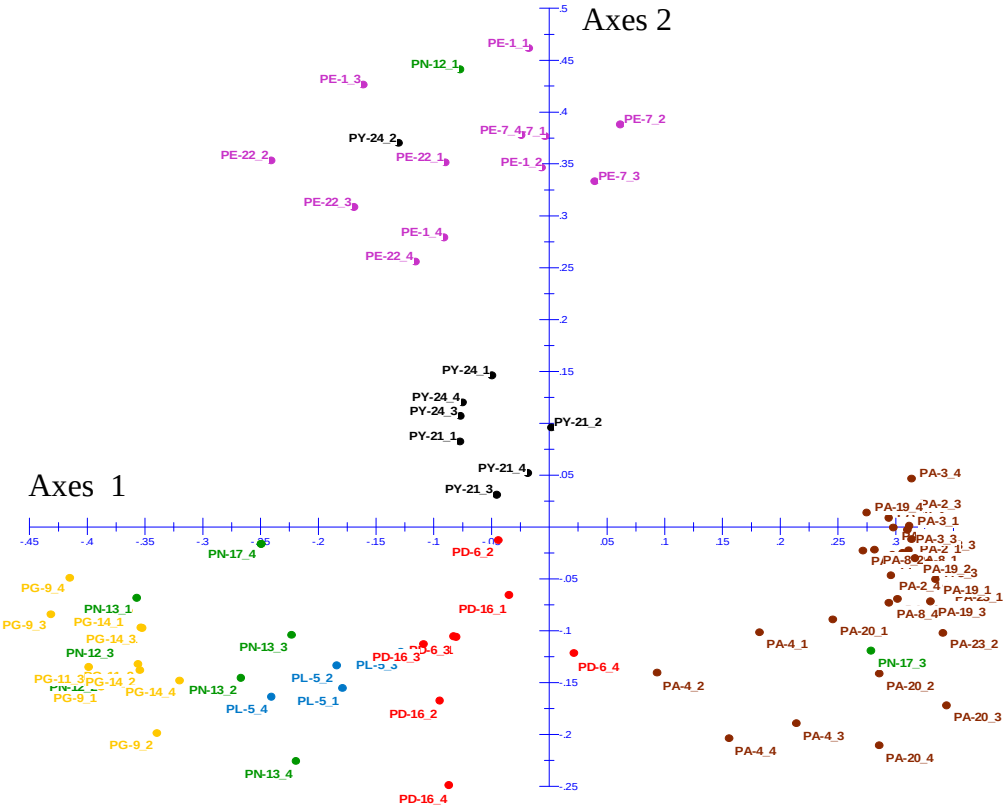

Figure 2. Multivariate factorial analysis on axis 1 and axis 2 of 85 individues of Sampoerna Agro's pisifera oil palm germplasm collections based on 20 microsatellite locus