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Bio Diversity

Elixir Bio Diver. 81 (2015) 31773-31775

ISSN: 2229-712X

Genetic Diversity among some Oil Palm Accessions at the Oil Palm Research Institute-Ghana using Microsatellites Markers

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ARTICLE INFO

Article history:
Received: 12 February 2015;
Received in revised form:
15 March 2015;
Accepted: 9 April 2015;

Keywords
Oil Palm,
Microsatellites,
Diversity,
Breeding.

ABSTRACT

The study was carried out to assess genetic diversity among nineteen oil palm accessions collected for conservation, evaluation and future incorporation into Oil Palm Research Institute (OPRI) breeding programmes. Genetic diversity and relationship among the nineteen accessions were evaluated using 10 microsatellites. The number of alleles revealed by each of the polymorphic loci ranged from 2 to 3 with an average of 2.5. Cluster analysis resolved the nineteen accessions oil palm accessions into two clusters. The percentage similarity between the oil palm accessions ranged from about 63% to 100%. This study suggest that there was not much genetic diversity among the nineteen accessions used in the study compared to other similar genetic diversity studies in oil palm using microsatellites.

Introduction

Oil palm (*Elaeis guineensis*) is one of major economic crops in Ghana after Cocoa. Oil palm is a crop that provides multiple outputs and it is the only plant whose fruit produces two types of oil, palm oil and palm kennel oil. Oil palm is a monocot and diploid (2n = 32 chromosomes) perennial tree crop.The conservation of oil palm genetic resources provides a reservoir of genes for the development of novel traits, a prerequisite for the improvement of profitability of the crop. The major limitations for the in situ conservation of oil palm are the large area needed and the high maintenance cost. These drive the need to conserve only those collections which are allelically diverse (Claude *et al.*, 2007) The prerequisite for the efficient utilization of germplasm for crop improvement programmes is that, it must be properly evaluated, characterized and documented so that any group of entries carrying desired characteristics can be easily retrieved and used in breeding programs (Reddy *et al.*, 2006). Information about genetic diversity permits the classification of germplasm into heterotic groups, which is particularly important to hybrid breeding as crosses between unrelated and consequently genetically distant parents, show greater hybrid vigour than crosses between closely related parents (Stuber, 1994; Hallauer, 1999).Microsatellite markers show co-dominant inheritance, multiallelic in nature, abundant, allow extensive genome coverage and can be detected using PCR. Microsatellites also appear to be the most promising molecular marker system for understanding the population genetic structure and gene flow (Singh et al. 2008).This study, therefore, seeks to conduct studies to some characterize the natural oil palms collections at OPRI and ascertain the genetic relatedness amongst them using microsatellites. This will provide important information to define future collection expeditions for germplasm conservation and their use in breeding programmes.

Materials and Methods

Genetic materials

Nineteen accessions of local oil palm genotypes were collected from the gene bank of the Oil Palm Research institute of the Council of Scientific and Industrial Research.

DNA Extraction

Genomic DNA extraction was using the CTAB method described by Murray and Thompson (1980) with some modification. Extraction buffer was composed of 2% CTAB, 50Mm Tris-HCI pH 8.0, 10Mm EDTA, 0.7M NaCl,0.1% SDS, 0.1mg/ml Proteinase K, 2% insoluble PVP and 2% 2-mercaptoethanol. Chloroform extraction was to remove cellular debris and proteins by using chloroform-isoamyl alcohol (24:1 v/v), DNA was precipitated by addition of 2-isopropanol and the precipitate was washed twice in 70% ethanol. The final precipitate was dissolved in 50 ul of 1/10 TAE solution containing RNase A, incubated at 200C overnight, and stored at 40C. The quality of the DNA was verified on a 1% agarose gel and diluted to a working concentration of 5 ng/ul.

Selection of the Microsatellites

Ten Microsatellites were selected from Oil palm reference map published by Billotte *et al.* (2005)

DNA Amplification and Gel Electrophoresis

Polymerase chain reaction amplifications(PCR) conditions were: 3 min denaturation at 94 °C followed by 35 cycles of denaturation at 94 °C for 30 s, 60 s at the appropriate primer annealing temperature, and 60 s extension at 72 °C and a final 10 min extension at 72 °C. Amplified products were resolved by electrophoresis on 2% agarose gels run in tris-acetate buffer, pH 8 for 2 hours at 80 volt. The gel was stained with Ethidium bromide (10mg/ml) and scanned with the gel documentation system.

Band Scoring and Microsatellites Data analysis

Bands generated were sized and then binary coded as 1 or 0 for the presence or absence an allele in each genotype, and scored in a binary data matrix. To visualize the relationship between the accessions a dendogram was constructed by clustering of the accessions based on similarity matrix using unweighted pair group method with UPGMA arithmetic algorithm in Sequential Agglomerative Hierarchical Nested method (SAHN) Module using NTYSC Software. Mean number of alleles were also computed.

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Table 1.0 list of the plant materials and their locations

Code	Location
GHA01	Abesre-Eastern Region(E/R)
GHA10	Adum-dominase-Western Region(W/R)
GHA506/06	Dorma-Ahenkro-Brong Ahafo Region(BA/R)
GHA15	Nankese-E/R
GHA29	Bonakrom-E/R
GHA53	Jasikan –Volta Region(V/R)
GHA21	Sekyi-Nkawanta-W/R
GHA57	Techiman-BA/R
GHA25	Kwamo-A/R
GHA44	Kwamo-A/R
GHA37	Fomena-A/R
GHA505/01	Sefwi-wiaso-W/R
GHA2011/103	Bawku-Upper East Region(UE/R)
GHA2011/101	Nandowli-Upper West Region(UW/R)
GHA2011/104	Mamprusi-Nothern Region(N/R)
GHA2011/204	Assin Praso-Central Region (C/R)
GHA2011/207	Mfoum-C/R
GHA2011/201	Dunkwa-Offin-C/R
GHA502/O1	NyinahinW/R

Table 2.0 List of the name of the markers and their sequences

Microsatellite loci	Motif	Primer sequence (5'–3')
mEgCIR0173	$(GA)_{18}$	TGAACAAGAAGGCGGAAAGAGA TGCGGGCGAGGAAAGGT
mEgCIR0353	$(GT)_{11}(GA)_{15}$	ATTTCGTAAGGTGGGTGT CCTCCAAACTCCTCTGT
mEgCIR0802	$GA)_{12}$	CTCCTTTGGCGTATCCTTTA TACGTGCAGTGGGTTCTTTC
mEgCIR0832	$(GA)_{19}$	CTCCGATGGTCAAGTCAGA AAATGGGGAAGGCAATAGTG
mEgCIR1753	$(GA)_{21}$	GCAGGGATTAAGTTTGATAT TTTGATGTTGCTTCTTTGAT
mEgCIR1730	$(CT)_{17}(GT)_5$	AATTTCAAATACAGCATAGC CATAGTAAGTTTTGGATGATTATTA
mEgCIR3543	$(GA)_{15}$	GTTCCCTGACCATCTTTGAG GTCGGCGATTGATTAGATTC
mEgCIR3546	$(GA)_{15}$	GCCTATCCCCTGAACTATCT TGCACATACCAGCAACAGAG
mEgCIR3300	$(GA)_{19}$	CATGCACGTAAAGAAAGTGT CCAAATGCACCCTAAGA
mEgCIR3292	$(GA)_{20}$	AGCCATGAGTGAATCATATC ACCACGATGTCAATCTCTAT

Results and Discussion

Polymorphism

The amplification pattern obtained from the ten microsatellites showed that two out of the ten microsatellites are polymorphic. Two of the primers, mEgCIR3543 and mEgCIR3292 were polymorphic (fig1).The rest were monomorphic therefore could not reveal any differences among the nineteen oil palm accessions. The number of alleles revealed by each of the polymorphic loci ranged from 2 to 3 with an average of 2.5. The study suggest that there was not much genetic diversity among the nineteen accessions used in the study compared to other similar genetic diversity studies in oil palm using microsatellites.

Fig 1. Sample of DNA profile generated by mEgCIR3543 M-100bp ladder; lanes 1-19

Corresponding to the code of the 19 Oil palm accessions in Table 1.Ajambang et al (2012) recorded an average number of alleles of 4.71 on 39 Oil palm accessions using 16 microsatellites. The low level of genetic diversity among the 19 Oil palm accessions might be due to the origin and the background of the accessions, the accessions are landraces which are scattered around the locations where they were collected. The human activities of Palm wine tapping resulted in a considerable loss of the palm in the wild, also the establishment of Cocoa plantations also caused wiping off of wild palms thus resulting in the loss of genetic diversity. Another factor that may influence the reported genetic diversity in the study is the few number of primers used in the study, is only two polymorphic primers that were included in the genetic diversity analysis the use of more polymorphic primers may reveal more genetic diversity among the accessions. Agarose gel was used in separating the PCR products, this may also influence the reported genetic diversity, and (Agrama and Tuinstra 2003) reported in their study that acryl amide gels have greater resolving power than Agarose gels.

Cluster analysis

Cluster analysis resolved the nineteen accessions oil palm accessions into two clusters (Fig2.0).The percentage similarity between the oil palm accessions ranged from about 63% to 100%.Cluster A consist of four accessions which separated from the other 15 accessions at 63% percent similarity level. Cluster B consist of 15 accessions, 14 of the accessions with 100% similarity. Accession GHA 2011/207 separated from the other accessions in cluster B. Some of the accessions collected from the same geographical regions tend to cluster together; GHA 2011/103/01, GHA 2011/101/103 and GHA 2011/104/01 were collected from the northern regions of Ghana. From the dendogram, it could be realize that there is a high level of similarity between the accessions; this could be from the genetic background of the accessions

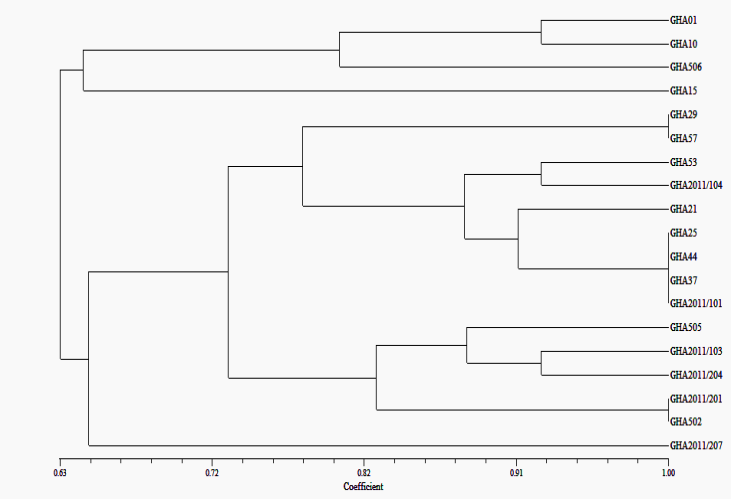

Fig 2. Dendogram generated by UPGMA cluster analysis showing relationship among 19 Oil Palm Accessions based on Nei and Li similarity

Conclusion

Very low level Genetic diversity was observed among the nineteen accessions used in the study. Cluster analysis also revealed a high level of similarity among the Oil palm accessions. The study should be repeated with more microsatellites markers.PCR products should be resolved on a polyacrylamide gel instead of Agarose gel. The molecular data should be compared with Agro morphological data after the evaluation of the accessions on the field.

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