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Diversity Assessment of Selected Coconut (*Cocos nucifera L.*) Accessions using Microsatellite Markers

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Abstract

Coconut (*Cocos nucifera* L.) is a multipurpose plant that has a very high level of cultivar diversity Almost all parts of the coconut plant, (*Cocos nucifera* L.) can be utilized and provide benefits for human life, In the fruit, from the skin to the coconut water, each has different functions and uses. This study aimed to determine the genetic diversity of selected coconut varieties. A total of 12 microsatellite markers were used to assess genetic diversity, 293 coconut individuals consisting of 19 coconut varieties, A total of 63 alleles were detected with an average of 5.25 alleles per locus. Polymorphic information content, (PIC) ranged from 0.1518 (C22) to 0.6778 (C2) with an average of 0.3546 per locus. Pairwise genetic distances ranged from 0.0361 (Malayan Red Dwarf-Malayan Green Dwarf) to 0.6585 (Malayan Yellow Dwarf-Rotuman). This value indicates a high similarity between the varieties Malayan Red Dwarf and MGD. The neighbor dendogram shows that the tall varieties Laguna, Rotuman, Markham, and Kar Kar are clustered together. Meanwhile, all dwarf varieties (MRD, MGD, MYD, Nias, Catigan, and CRD) are clustered together with the dwarf x dwarf hybrids Careca and Careni. These results provide important insights for future breeding activities and conservation management programs

Keywords: *Cocos nucifera*, DNA fingerprinting, Genetic variability, Germplasm Characterization, SSR markers

1. Introduction

Coconut is a multipurpose plant with a very high level of cultivar diversity. Almost all parts of the coconut plant (*Cocos nucifera* L.) can be utilized and provide benefits to human life. The fruit, from the skin to the coconut water, each has its own function and use (Riono, 2013). This species is believed to have spread through human migration and coconuts carried by ocean currents and floating in the ocean. There are two main types of coconut, namely tall coconut and dwarf coconut, which have many different characteristics. Tall coconut has strong vertical growth, high productivity, precocity less productive, more precocious, and generally self-pollinating. The study of genetic diversity of coconut accessions or germplasm is very important for breeding, especially in the context of creating hybrid varieties. Indeed, this should allow the arrangement of ecotypes into genetic groups, thereby identifying redundant accessions in the collection and rationalizing the choice of crosses to be tested. In addition to the Tall-Dwarf duality, there is considerable morphological variability among ecotypes, including fruit characteristics and vegetative organs. Shrub breeders use a wider variety of parents in improving varieties, because without genetic variability there will be no improvement in plant characteristics. Genetic diversity can be evaluated at several different biological levels. To date, three methods have been recognized for evaluating genetic diversity, namely through morphological-agronomic characters, biochemical characters and DNA markers (Astuti et al., n.d.). Germplasm collections, which contain significant genetic diversity within and among coconut populations, are crucial for effective crop improvement programs. Therefore, assessing genetic diversity within coconut populations is becoming increasingly important for germplasm conservation and utilization in breeding programs. Diversity analysis in oil palm has been carried out based on morphological traits, biochemical, and molecular markers (Rajesh et al., 2008). Morphological and

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biochemical markers are less attractive in coconut genetic syudies due to the long juvenile phase, the high cost of investigation, the need for long-term field evaluation, the strong influence of environmental factors on phenotype, and the limited number of informative phenotypic descriptors (Manimekalai et al., 2006). In contrast, molecular markers detect variation directly at the DNA level and can be applied at any developmental stage. Because DNA markers provide genome-wide coverage and are largely independent of environmental effects, they overcome many limitations associated with morphological and biochemical approaches (Rognon, 2000; Upadhyay et al., 2004; Manimekalai et al., 2006). Among available marker systems, SSRs are particularly useful due to their multi-allelic and co-dominant nature, high polymorphism, and reproducibility, making them well suited for cultivar identification and diversity assessment. Compared with previous coconut diversity studies that relied primarily on morphological descriptors, the present work adopts a standardized SSR genotyping approach coupled with quantitative marker characterization (e.g., allele number, PIC, gene diversity, and heterozygosity) and distance-based clustering. In addition, genetic relationships were inferred using shared-allele genetic distance and visualized through a neighbor-joining dendrogram, enabling clearer resolution of tall, dwarf, and hybrid groupings within the MARDI germplasm collection. This provides a more objective and reproducible framework for germplasm curation, identification of potential duplicates, and strategic parent selection for future breeding programme.

2. Material and Methods

2.1. Plant Material

Young fresh leave of 239 coconut individuals which represent 19 coconut accessions were collected to estimated their diversity level. The samples were collected from MARDI Bagan Datok, Perak (3º53’25”N 100º51’37”E, 10m). Each leave was dried using silica gel prior to DNA extraction. The details about the plant materials were summarized in Table 1.

Table 1. Sample information on selected coconut accessions

No	Accession	Type	No of Samples	No	Accession	Type	No of Samples
1	MYD	Dwarf	12	**11**	Careca	Hybrid	15
2	MRD	Dwarf	12	**12**	Careni	Hybrid	13
3	MGD	Dwarf	15	**13**	Myleca	Hybrid	15
4	CRD	Dwarf	13	**14**	Marleka	Hybrid	9
5	NIAS	Dwarf	10	**15**	Mylag	Hybrid	14
6	Catigan	Dwarf	12	**16**	Marena	Hybrid	15
7	Rotuman	Tall	12	**17**	MRDxMarkham	Hybrid	6
8	Karkar	Tall	19	**18**	MRDxKarkar	Hybrid	10
9	Markham	Tall	15	**19**	MRDxRotuman	Hybrid	10
10	Laguna	Tall	12				

2.2. Genomic DNA extraction

The total genomic DNA was extracted using high throughput CMDV In-house DNA extraction protocol. Approximately 1 gram of each individual was stuffed into 96 well plate containing stainless steel beads for grinding purpose. The samples were ground using TissueLyser (Qiagen, Netherlands). The ground samples were incubated with DNA extraction buffer at 65°C for 1 hour. Then, the DNA then was precipitated with an equal volume of cold isopropanol before washed twice with 70% ethanol. The DNA pellet was left dried before resuspended with 50 µl TE-RNase solution. The DNA integrity was measured using 1.0% agarose gel and the DNA concentration was measured using Thermo Labsystems Fluoroskan Ascent ™ (Thermo Scientific, USA).

2.3. SSR marker genotyping

A total of twelve SSR markers from previously published studies were selected to resolve the diversity in this study. Each of the forward primer of the SSR markers was concatenated with M13 sequence (Schuelke, 2000). The details regarding the SSR markers were summarized in Table 2. The PCR reaction was performed using Peltier Thermal Cycler, DNA Engine Tetrad 2 (Biorad, USA) with a total volume of 10 µl containing 2.0 µM $MgCl_2$, 10X PCR buffer, 2mM dNTP, 10 µM forward and reverse primer, 5 mM M13 fluorescent labelled (Fam/Pet/Ned/Vic), 0.1 µl BSA (Bovine serum albumin) as enhancer and 1 unit of Taq Polymerase (Invitrogen, USA). The PCR reaction was performed with the initial pre-denaturation at 94^0 C for 2 Minutes, followed by 33 cycles of 94^0 C for 30 seconds, 44.8^0 C – 61.1^0 C (Table 2) for 40 seconds and 75^0 C for 40 seconds. The reaction was completed with a post-extension process at 75^0 C for 5 minutes. The amplified products were multiplex up to four pair of primers with combination of different dye before genotyped using ABI 3730xl DNA Analyzer (Applied Biosystem, USA). Standard GS500LIZ was used as a Standard Ladder.

2.4. Data Scoring and Genetic diversity analysis

Fragment analysis files generated by ABI 3730xl DNA Analyze were analyzed and scored using GeneMapper version 5.0 (Applied Biosystems). The allele sizes then were imported into Microsoft Excel File. The number of allele, Polymorphic Information Content (PIC) value, major allele frequency, gene diversity and heterozygosity of each SSR marker was calculated using Power Marker 3.5. In addition, Pairwise genetic distance based on shared allele also was calculated using Power Marker version 3.5 (Liu & Muse, 2005). MEGA version 7.0 (Kumar et al., 2016) was used to generate dendogram using Neighbour Joining approach based on genetic distance matrices produced by Power Marker version 3.5 software. Figure 1 summarized the flow of the research for this study.

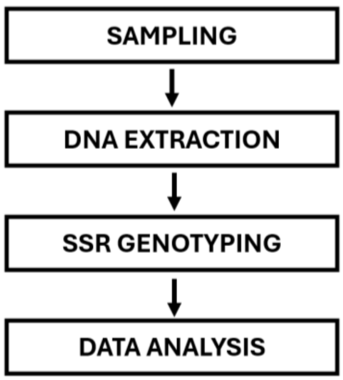

Figure 1. Research flow diagram

3. Results and Discussion

3.1. SSR markers characterization

SSRs remain the most trusted markers for genotyping plants for more than the past 20 years owing to their characteristics of being informative, highly polymorphic, multi-allele, co-dominant, experimentally reproducible, and transferable across the species (Vieira et al., 2015). Moreover, the microsatellite is greatly useful in diversity assessment measured in terms of genetic distance, cultivar identification through DNA fingerprinting, linkage and QTL analysis, and even in evolutionary studies (Kalia et al., 2011). In this study a total of 12 SSR markers have been selected in order to evaluate the genetic diversity of 19 coconut accession. The study also reveal the number of alleles ranged from three (C5, C13, C20, C22) to nine (C2) with an average of 5.25. The polymorphism information content (PIC) and major allele frequency values of each SSR marker range from 0.1518 and 0.4170 to 0.6778 and 0.9139 with and average of 0.3546 and 0.7196, respectively. The details characterizations of these 12 SSR markers were summarized in Table 2.

Table 2. Characterization of twelve polymorphic SSR markers based on this study

Marker	Major Allele Frequency	Allele No	Gene Diversity	Heterozygosity	PIC
CAC03	0.4170	9.0000	0.7182	0.5605	0.6778
CAC08	0.5085	3.0000	0.5121	0.2222	0.3935
CAC20	0.7768	8.0000	0.3835	0.1545	0.3678
CAC50	0.6578	8.0000	0.5233	0.4178	0.4847
CAC52	0.8814	3.0000	0.2122	0.1483	0.1951
CAC56	0.7957	7.0000	0.3561	0.2681	0.3426
CAC68	0.7911	5.0000	0.3560	0.3207	0.3332
CAC71	0.7227	4.0000	0.4031	0.2479	0.3252
CAC84	0.8848	3.0000	0.2105	0.1870	0.2000
CN1C6	0.6709	5.0000	0.4598	0.4093	0.3786
CN1G4	0.9139	3.0000	0.1600	0.1429	0.1518
CN2A5	0.6142	5.0000	0.4987	0.4095	0.4050
Mean	0.7196	5.2500	0.3995	0.2907	0.3546

The major allele frequency is the proportion of a specific, most common variant (allele) of a marker within a population. Allele No refers to number of alleles detected. Gene diversity refer to the probability that two randomly chosen alleles from the population are different. Heterozygosity refers to the proportion of heterozygous individuals in the population, PIC is abbreviation for polymorphism information content which measures the informativeness and variability of genetic markers.

3.2. Diversity of 19 coconut accessions

The Neighbour Joining dendogram generated from genetic distance matrices showed the present of 2 major groups namely Group A and Group B (Figure). Group A consisted of dwarf and hybrid varieties whereas Group B consisted of tall varieties. The dendogram also revel the hybrid varieties namely Careca and Careni were flanked by dwarf variety in Group A. This might be caused by both hybrid varieties is dwarf X dwarf variety. The dendogram also reveal each variety able to be differentiating from each other suggested the markers could be used to develop the DNA profile for each variety. The pairwise genetic distance (Table 3) reveals the lowest value of genetic distance for MGD and MRD with the value of 0.036 which indicate high similarity between both variety owing to both varieties are Malayan Dwarf type variety. The table also showed highest pairwise genetic distance between MYD and Rotuman with the value of 0.658 which suggested highest dissimilarity between both varieties.

3.3. Molecular characterization and genetic relationships among selected coconut breeds

The results of the hierarchical cluster analysis, produced in the form of branching patterns in the dendrogram, indicate the grouping of coconut varieties based on the level of similarity of the analyzed characters. The dendrogram divides all varieties into two main groups, namely Group 1 and Group 2, which reflect differences in the level of kinship between coconut varieties. Group 1 is the largest group, dominated by dwarf and hybrid varieties, indicating a relatively high level of similarity in characters among the varieties. The MYD, CRD, and CARECA varieties are on adjacent branches, indicating a very close kinship relationship and possibly having similar agronomic traits, while the MRD, MGD, and NIAS varieties also form a subgroup. tersendiri, yang mengindikasikan kesamaan karakter pertumbuhan dan morfologi buah. Varietas hibrida seperti MYLAG, MARLECA, dan MYLECA are in the same cluster, According to The further the genetic distance between parents, the greater the opportunity to produce new cultivars with wide genetic variability will be. Conversely, crossing between closely related parents will result in narrow genetic variability. One of the limitations of success in crossing is the genetic relationship between parents (Tenda & Tulalo, 2009). Group 2 consists of deep coconut varieties (high variety) such as Laguna, Markham, Karkar, and Rotuman, which are quite far apart from Group 1, showing significant differences in character. According to (Maskromo et al., 2015) The use of specific primers in analysis using SSR markers produces amplification products from certain target loci thereby increasing the accuracy of detecting differences or similarities between individuals, Due to the high level of accuracy, researchers can use SSR markers in different laboratories with relatively similar results.

The results of the genetic similarity analysis shown in the table show a variation in similarity values between genotypes, ranging from 0.036 to 0.658. This range of values indicates that the tested genotype collection has quite broad genetic diversity. This broad genetic diversity reflects that each genotype has a different allele composition and genetic variation, which has the potential to be utilized in plant breeding programs. According to (Damayanti et al., 2018), differences or similarities in the appearance of the external morphology of a plant species can be used to determine the closeness of kinship relationships. Genotypes with a high level of similarity need further evaluation because they are likely varieties that share the same origin or genetic background. According to (Tulalo et al., 2019), one of the priorities for coconut plant breeding for the future is to obtain coconuts with shorter stems. The desired stem growth characteristics in coconut plants are slow growth, denser leaf growth on the stem, making it easier to harvest. In general, the pattern of variation in genetic similarity values obtained can also be used as a basis for phylogenetic analysis and genetic clustering, which will help in understanding the kinship relationships between genotypes, determining breeding strategies, for example in selecting parents to be mated to obtain superior gene combinations. The phenotypic information of these varieties was described and discussed by Maizura et al (2025).

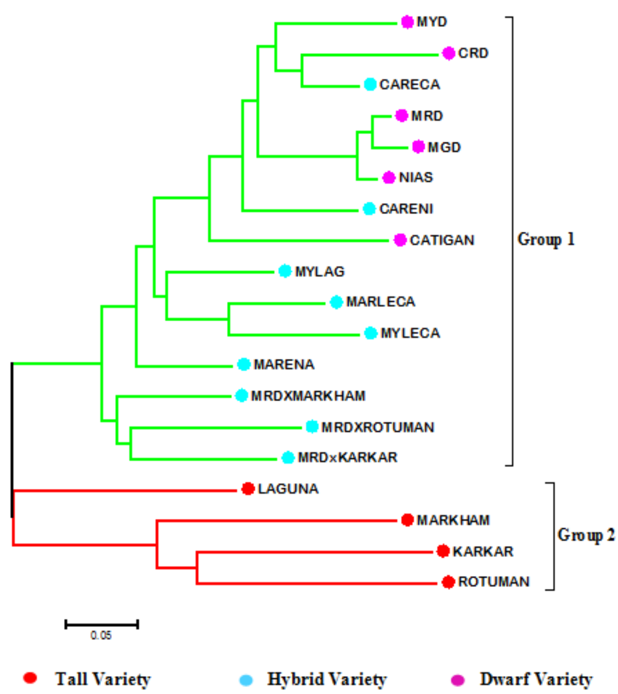

Figure 2. Dendogram analysis based on nineteen polymorphic microsatellite markers.

Table 3. Pairwise genetic distances of the studied offspring based on nineteen microsatellite markers.

OTU	CARECA	CARENI	CATIGAN	CRD	KARKAR	LAGUNA	MARENA	MARKHAM	MARLECA	MGD	MRD	MRDx KARKAR	MRDX MARKHAM	MRDX ROTUMAN	MYD	MYLAG	MYLECA	NIAS	ROTUMAN
CARECA	0.000	0.162	0.148	0.133	0.569	0.388	0.219	0.525	0.255	0.211	0.175	0.340	0.274	0.294	0.169	0.216	0.301	0.188	0.552
CARENI	0.162	0.000	0.266	0.162	0.550	0.402	0.205	0.500	0.294	0.177	0.162	0.288	0.274	0.309	0.245	0.190	0.291	0.159	0.528
CATIGAN	0.148	0.266	0.000	0.282	0.537	0.334	0.212	0.551	0.321	0.233	0.217	0.368	0.309	0.305	0.210	0.256	0.311	0.205	0.570
CRD	0.133	0.162	0.282	0.000	0.632	0.469	0.311	0.535	0.338	0.203	0.167	0.398	0.354	0.368	0.167	0.261	0.340	0.182	0.636
KARKAR	0.569	0.550	0.537	0.632	0.000	0.373	0.476	0.344	0.484	0.615	0.629	0.339	0.402	0.479	0.650	0.468	0.508	0.596	0.329
LAGUNA	0.388	0.402	0.334	0.469	0.373	0.000	0.266	0.445	0.428	0.455	0.434	0.357	0.302	0.405	0.448	0.296	0.416	0.417	0.472
MARENA	0.219	0.205	0.212	0.311	0.476	0.266	0.000	0.525	0.218	0.297	0.290	0.217	0.183	0.207	0.212	0.156	0.266	0.276	0.501
MARKHAM	0.525	0.500	0.551	0.535	0.344	0.445	0.525	0.000	0.473	0.489	0.514	0.465	0.421	0.503	0.644	0.386	0.440	0.482	0.373
MARLECA	0.255	0.294	0.321	0.338	0.484	0.428	0.218	0.473	0.000	0.267	0.275	0.251	0.211	0.227	0.271	0.215	0.158	0.269	0.471
MGD	0.211	0.177	0.233	0.203	0.615	0.455	0.297	0.489	0.267	0.000	0.036	0.394	0.354	0.350	0.203	0.198	0.220	0.054	0.584
MRD	0.175	0.162	0.217	0.167	0.629	0.434	0.290	0.514	0.275	0.036	0.000	0.403	0.354	0.347	0.167	0.182	0.226	0.038	0.610
MRDxKARKAR	0.340	0.288	0.368	0.398	0.339	0.357	0.217	0.465	0.251	0.394	0.403	0.000	0.191	0.220	0.348	0.265	0.322	0.370	0.396
MRDXMARKHAM	0.274	0.274	0.309	0.354	0.402	0.302	0.183	0.421	0.211	0.354	0.354	0.191	0.000	0.205	0.271	0.224	0.305	0.331	0.441
MRDXROTUMAN	0.294	0.309	0.305	0.368	0.479	0.405	0.207	0.503	0.227	0.350	0.347	0.220	0.205	0.000	0.261	0.276	0.301	0.323	0.423
MYD	0.169	0.245	0.210	0.167	0.650	0.448	0.212	0.644	0.271	0.203	0.167	0.348	0.271	0.261	0.000	0.284	0.337	0.191	0.658
MYLAG	0.216	0.190	0.256	0.261	0.468	0.296	0.156	0.386	0.215	0.198	0.182	0.265	0.224	0.276	0.284	0.000	0.179	0.163	0.449
MYLECA	0.301	0.291	0.311	0.340	0.508	0.416	0.266	0.440	0.158	0.220	0.226	0.322	0.305	0.301	0.337	0.179	0.000	0.211	0.474
NIAS	0.188	0.159	0.205	0.182	0.596	0.417	0.276	0.482	0.269	0.054	0.038	0.370	0.331	0.323	0.191	0.163	0.211	0.000	0.577
ROTUMAN	0.552	0.528	0.570	0.636	0.329	0.472	0.501	0.373	0.471	0.584	0.610	0.396	0.441	0.423	0.658	0.449	0.474	0.577	0.000

4. Conclusion

SSR markers proved to be an effective and reproducible tool for characterising genetic diversity and relationships among the selected coconut accessions. Using 12 polymorphic SSR loci across 19 accessions, this study generated a robust molecular dataset with informative marker performance and clear genetic differentiation among tall, dwarf, and hybrid groups. The resulting clustering patterns provide an objective basis for identifying closely related accessions as well as highly divergent materials that may serve as promising parents in breeding programmes.

Based on these findings, SSR fingerprints and shared-allele genetic distance estimates can be applied to verify accession identity, detect potential duplicates, and prioritise genetically unique materials for conservation and regeneration. In addition, the observed divergence between tall and dwarf groups can guide more strategic parent selection to maximise the likelihood of heterosis, while avoiding crosses among highly similar genotypes when broader variability is required. Future work should expand the marker panel and include additional accessions to further refine germplasm curation and strengthen breeding decisions.

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