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Molecular Characterization of Pineapple (*Ananas comosus L, Merr.*) using Microsatellite Markers

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Abstract

The molecular characterization of pineapple germplasm (*Ananas comosus* L. Merr.) is crucial for evaluating genetic variation and supporting conservation and breeding initiatives. A total of 25 pineapple accessions were used in this study. The accessions included cultivated types designated with the code (MP) and ornamental types designated with the code (OC). Pineapple accessions were characterized using six simple sequence repeat (SSR) markers. These SSR markers revealed moderate levels of polymorphism, with the number of alleles per locus ranging from 2 to 6, an average gene diversity of 0.63, and polymorphic information content (PIC) values ranging from 0.37 to 0.65. Pairwise genetic distances ranged from 0.000 to 0.917, reflecting a broad spectrum of divergence among the accessions. The UPGMA dendrogram grouped all accessions into two main clusters, with one ornamental accession (OC11) forming a distinct cluster, while the other ornamental and cultivated types were intermixed, indicating partial genetic separation rather than complete divergence. The moderate number of markers and the limited number of loci constrained the clustering resolution. The strength of this study lies in the availability of genetic distance and clustering information, which can be used to identify the most divergent accessions as candidate parents for crossing and to recognize highly related accessions to avoid collection redundancy. It is recommended that accessions originating from different clusters and exhibiting high genetic distances be prioritized as sources of genetic diversity in breeding programs. Additionally, increasing the number of polymorphic SSR markers is advised to improve identification accuracy and clustering resolution.

Keywords: Fingerprint DNA Finger, Genetic Divergence, Genetic Diversity, Germplasm, Relationship Kinship

1. Introduction

Pineapple (*Ananas comosus* L. Merr) is a member of the Bromeliaceae family, native to tropical regions. It is highly sought after both domestically and internationally for its sweet, refreshing, slightly sour taste. Pineapple contains various nutrients and vitamins, including calories, protein, fat, carbohydrates, calcium, vitamin A, vitamin C, and small amounts of vitamin B. As an agricultural product, pineapple holds high economic value and significant export potential (Nuraini et al., 2022). Besides being consumed fresh, pineapple is also used as a raw material in industrial processing and in traditional medicine (Akrinisa et al., 2019). Pineapple plants grown in Indonesia exhibit considerable diversity, serving as a valuable germplasm resource for pineapple breeding programs (Prasetyo et al., 2023). However, genetic information from pineapple germplasm collections remains limited, despite its critical importance for plant breeding initiatives.

A fundamental obstacle to pineapple commodity development is the limited database of verified genetic identity and diversity for both local and commercial accessions. This situation leads to erroneous variety identification, overlapping local names (synonyms), and the potential duplication of accessions in germplasm collections. Consequently, the selection of crossbreeding parents is often not based on clear kinship information, resulting in limited genetic diversity used in breeding, slowing the rate of variety improvement, and inefficient conservation efforts. Furthermore, unclear genetic identity also complicates the standardization of planting material, the consistent development of superior varieties, and the strengthening of competitiveness for markets and exports.

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Most people, especially pineapple farmers, only know pineapples in limited morphological terms. For example, farmers can only distinguish pineapples by their fruit shape and colour (Tamsar et al., 2022). However, characterizing only by morphology is not appropriate in the breeding process, as environmental factors easily influence plant morphology. Character morphology does not always reflect genetic differences between accessions (Erliani et al., 2010; Van Den Ende *et al.*, 2023). Consequently, there are difficulties in managing germplasm collections, detecting duplications, and determining kinship relationships between accessions. Therefore, a more accurate, stable, and environmentally unaffected molecular marker-based approach is needed.

Microsatellites, or Simple Sequence Repeats (SSRs), are very useful for various genetic analyses, including understanding genome evolution, analyzing phylogenetic relationships between taxa, genetic mapping, DNA fingerprinting, and physical mapping. Currently, SSRs have been used in genetic diversity studies across many plant species, including sugar palm (Zebua & Anwar, 2025), chili (Rahayu, 2025), and citrus (Oktaviana et al., 2024). The advantages of SSRs compared to other molecular markers are that they produce high polymorphism, are codominant, and require only a relatively small amount of DNA sample for analysis (Hidayatun et al., 2011). SSR can detect the number of alleles and high levels of heterozygosity in a population (Vieira et al., 2016). Markers. This can also be used for selecting for genome introgression, gene markers, and selection-strain breeding in Marker Assisted Selection (MAS) (Brown & Kresovich, 1996; Sahin et al., 2018). However, the SSR application for characterizing pineapple germplasm remains relatively limited, especially for germplasm collection in Southeast Asia.

Previous research on pineapple has generally focused on characterizing specific commercial cultivars, collections originating from limited regions, or using other molecular markers primarily for general grouping. Furthermore, some studies have not yet emphasised the development of standardised DNA fingerprint profiles for variety identification and detection of collection duplication. The novelty of this research lies in the use of selected SSR markers to map genetic diversity while simultaneously constructing DNA fingerprinting profiles in pineapple germplasm accessions encompassing both cultivated and ornamental varieties within a single panel. This approach yields information on genetic distances and grouping patterns that can be readily integrated for collection management, accession identity verification, and more targeted selection of crossbreeding parents.

This study aims to characterize and fingerprint several pineapple germplasm accessions using selected SSR markers. The results are expected to provide valuable genetic information for the effective management of germplasm collections, variety identification, and to support pineapple breeding programs aimed at developing superior new varieties. Additionally, the DNA fingerprinting results can serve as a reference for developing a national genetic database and for implementing DNA fingerprinting technology to verify pineapple varieties in the future.

2. Material and Methods

2.1. Genomic DNA isolation and material plant

Two tens five pineapple accessions, each repeated 5 times, selected for characterization, using marker microsatellites. Five samples from every accession collected. Pineapple varieties that will isolate DNA originating from Institute Study and Development Malaysian Agriculture (MARDI), Pontian, Johor, Malaysia (1º30'23.40"N 103º26'54.63"E, 4m), place special pineapple cultivation. Ingredients: plants that will be isolated; DNA is from leaf, fresh, young , collected, and saved in a plastic *zip lock* containing silica gel. *Deepwell* 96- *well plate* containing steel balls Stainless steel plates (2.3 mm in diameter) were prepared. Small pieces of young pineapple leaves were placed in *deepwell plates* and frozen at -80°C for 12 hours. The frozen leaf tissue was ground using *a Tissue Lyser* (Qiagen, Germany) and added to the extraction buffer (2% CTAB, pH 8, 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, 0.05% β-mercaptoethanol). Total pineapple leaf DNA was extracted following the protocol described by Mace et al. (2003). DNA quality was measured using a 0.8% agarose gel, and DNA quantity was measured using a Fluoraskan Ascent (Thermo Fisher Scientific, United States).

2.2. SSR genotyping

The six SSR markers used in this study were obtained from Shoda et al. (2012). These are listed in Table 1. PCR was performed as described by Schuelke (2000). First, a non-fluorescently labelled M13 sequence (TGTAA AACGACGGCCAGT) and a fluorescently labelled M13 adapter (FAM, PET, NED, or VIC) were used. This dye helps detect the PCR results. These two sequences are used to attach the primers (forward and reverse) to the DNA to be amplified. The PCR reaction mixture is made with a total volume of 10 µL. It contains: 1× buffer (Invitrogen, USA), 10 µM forward and reverse primers, 5 µM fluorescently labelled M13 adapter, 2 µM dNTP, 1 unit of Taq polymerase, and 0.1 µL BSA (Bovine Serum Albumin), which helps stabilize the PCR process. Amplification was performed using the GeneAmp® PCR System 9700 (Applied Biosystems, USA). Stages his reaction: Denaturation at 94°C for 2 minutes to separate the DNA strands. 35 cycles reactions consisting of denaturation at 94°C for 30 seconds, primer attachment (annealing) at a temperature of 41–65°C for 45 seconds, DNA elongation (extension) at 72°C for 45 seconds, and ended with

extension end on temperature of 72°C for 5 minutes. After PCR is complete, the amplification products from several primers with different fluorescent colours are combined. The mixture was then added to Hi-Di formamide and GeneScan 500 LIZ, namely standard heavy molecules used to aid in the reading process. Finally, the PCR results were analyzed using an ABI 3130xl Genetic Analyzer (Applied Biosystems, USA). This tool performs size-based separation and detection of DNA fragments, where every SSR marker has been identified with clarity

2.3. Analysis statistics

GeneMapper Version 5 (Thermo Fisher Scientific, United States) is used to evaluate allele sizes. The method described by Arif et al. (2010) was used as a basis for the evaluation and analysis of the electropherogram. The number of alleles, allele frequency, main genetic diversity, level of heterozygosity, and Content Information Polymorphism (PIC) for each microsatellite marker were calculated using PowerMarker. From the matrix distance calculated from allele-sharing microsatellites, the Method *Unsaturated Polyester Glass Mat* (UPGMA) was created. Next, UPGMA is used in the MEGA7 software (Kumar et al., 2016) to produce a dendrogram for this study.

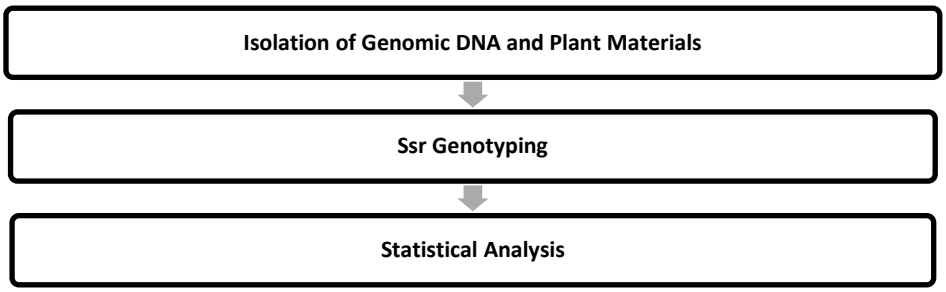

Figure 1. Diagram flow implementation study

3. Results and Discussion

3.1. Microsatellite Characterization of Certain Pineapple Germplasm

A total of six SSR markers (AC008, AC013, AC030, AC039, AC041, and ACOM828) were analyzed in 25 pineapple accessions representing cultivated (MP) and ornamental (OC) groups. The number of alleles per locus ranged from 2 to 6, with an average of 4.67, indicating a moderate level of allelic richness across the tested germplasm. Major allele frequencies ranged between 0.37 and 0.59, indicating that no single allele dominates at the locus. Genetic diversity ranged from 0.48 (AC041) to 0.70 (AC030, ACOM828), with an average of 0.63, reflecting considerable polymorphism among loci. The observed heterozygosity (Ho) averaged 0.59, while PIC values ranged from 0.37 to 0.65, with an overall mean of 0.56, indicating that most loci were highly informative (PIC > 0.5). Similar PIC ranges have been reported in other pineapple SSR studies, confirming the informativeness of these loci (Ismail *et al*, 2020).

These indices collectively indicate that the SSR loci used can detect moderate to high levels of genetic variation among pineapple accessions. The high PIC values of AC030 and ACOM828 highlight their potential utility for future studies of germplasm diversity and discrimination. As this is a preliminary study, these results provide a valuable molecular basis for further evaluation of genetic diversity and relationships between cultivated and ornamental *Ananas comosus* (L) Merr. (Makaranga *et al.*, 2018) .

Table 1. Characterization SSR markers

Marker	Freq major allele	Amount allele (Na)	Genetic Diversity	Heterozygosity	PIC
AC008	0.3704	5.00	0.6824	0.6296	0.621
AC013	0.4074	6.00	0.6578	0.8148	0.5958
AC030	0.4444	6.00	0.6962	0.4074	0.6507
AC039	0.5926	3.00	0.5487	0.5185	0.4762
AC041	0.5926	2.00	0.4829	0.5185	0.3663
ACOM828	0.3889	6.00	0.7023	0.6296	0.6522
Mean	0.466	4.6667	0.6284	0.5864	0.5604

Note: Freq major allele: Frequency of the most common allele (major allele) in a sample. Amount allele (Na): Number of detected alleles on the locus. Gene diversity: The probability that two randomly selected alleles in a population are different.Heterozygosity: The proportion of individuals heterozygous at a locus in a population for a given locus. PIC (Polymorphic Information Content): Indexes the informativeness of markers to differentiate genotypes; the taller the value, the more markers are discriminant/informative.

3.2. Genetic Diversity of Selected Pineapple Germplasm

The genetic relationships among pineapple accessions were evaluated using the *Unweighted Pair Group Method with Arithmetic Mean (UPGMA)* based on genetic *distance from shared alleles (Sokal & Michener, 1958)*. Pairwise genetic distance values ranged from 0.000 to 0.917,

indicating a wide spectrum of genetic divergence. This range is higher than that reported in other studies (0.001–0.495 in Malaysia; 0.000–0.78 in Taiwan), indicating a possible greater divergence between our ornamental and cultivated accessions (Lin *et al*., 2015; Ismail *et al*, 2020).

The UPGMA dendrogram (Figure 2) groups the 25 pineapple accessions into two main clusters. Cluster 1 (red) contains only one ornamental accession (OC11), while Cluster 2 (blue) contains the remaining accessions, where the cultivated (MP) and ornamental (OC) types are clustered together. This pattern indicates limited genetic differentiation between the two groups, with OC11 showing the greatest divergence from the others.

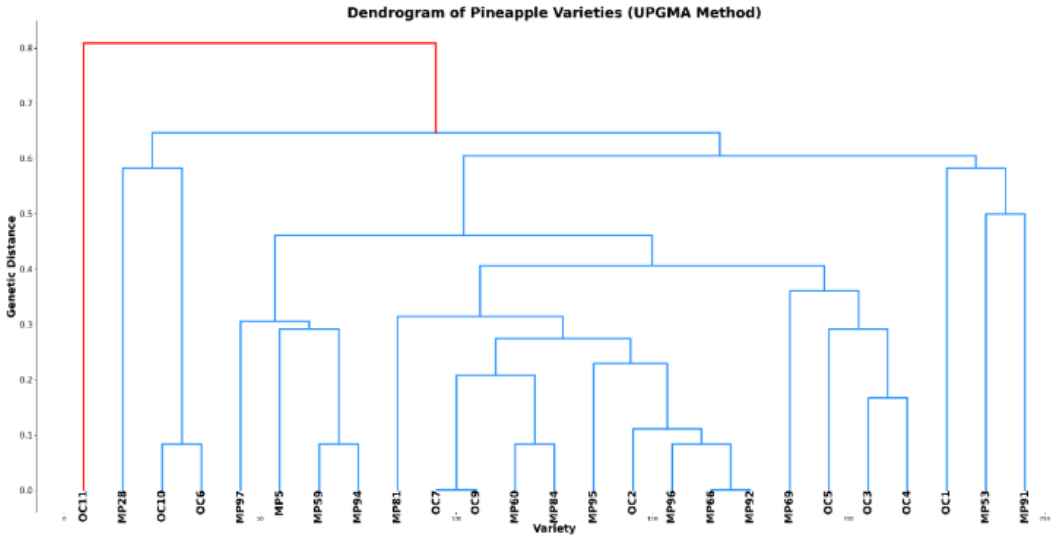

Figure 2. UPGMA dendrogram for 25 pineapple accessions using 6 SSR markers

Pairwise genetic distances (Figure 3) support the clustering pattern observed in the dendrogram. Deeper colors (red-orange) indicate higher genetic distances, while lighter colors (blue-green) indicate closer relationships. Overall, the distribution of genetic distance values shows partial separation, if not complete divergence, between ornamental and cultivated pineapples, suggesting that some ornamental accessions may share a similar genetic background or experience gene flow with cultivated types. Figure 4 shows morphological traits of several pineapple accessions. For further morphological characterization, please refer to Rozlaily et al. (2020).

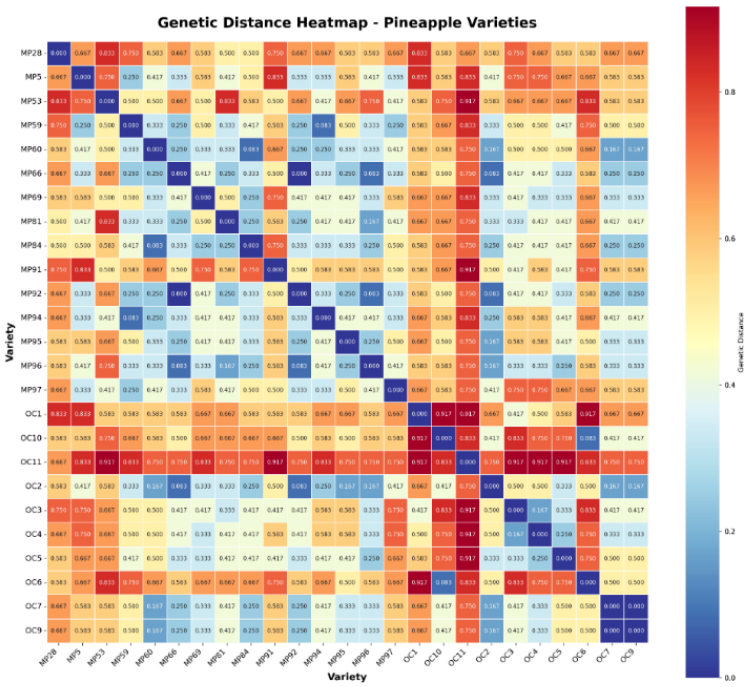

Figure 3. Genetic distance of 25 pineapple accessions based on 6 SSR markers

However, the limited number of SSR markers (six loci) used in this study reduced the resolution of the dendrogram and the ability to clearly distinguish subgroups within the germplasm. To improve the robustness of clustering and relationship inference, future studies should integrate morphological and agronomic character data with molecular information, and expand the marker set to include additional polymorphic SSRs or high-throughput single-nucleotide polymorphism (SNP) markers. This combined approach is expected to provide a more comprehensive understanding of the genetic structure and diversity among *Ananas comosus* accessions.

Nevertheless, SSR-based UPGMA analysis provides an informative framework for understanding genetic divergence and for guiding future conservation and breeding strategies of *Ananas comosus* germplasm.

Figure 4. Research morphology for accession of MP5 and MP28.

4. Conclusion

This study demonstrates that effective SSR markers can be used to evaluate the genetic variation of pineapple germplasm (*Ananas comosus* L. Merr.) and provide valuable information on kinship for collection management. Analysis of 25 accessions, including both cultivated types (MP) and ornamental types (OC), revealed partial genetic differentiation, with the OC11 accession being the most distinct. Several ornamental and cultivated accessions were found to be closely related. The main advantage of this study is the availability of a genetic distance database and grouping information that can be used to verify accession identity, detect potential duplicates, and select parental lines from different clusters to expand genetic diversity. It is recommended that accessions from different clusters with high genetic distance be prioritized as parental sources in breeding programs. Additionally, incorporating more polymorphic SSR markers or using SNP markers is advised to increase identification accuracy and improve grouping resolution.

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