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# Characterization of Mangoes In Cirebon Using The Single Nucleotide Polymorphism Method (Snp)

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**ABSTRACT**

Mango (Mangifera indica L.) is a leading horticultural commodity in Cirebon Regency, boasting a diverse array of local varieties with distinct fruit characteristics. Varietal identification has traditionally been based on morphological characteristics, but this method is often inaccurate due to environmental factors. Therefore, a more stable and objective molecular-based approach is needed, one of which is Single Nucleotide Polymorphism (SNP) analysis. This study aims to characterize the genetic diversity of mangoes in Cirebon using SNP markers as a basis for kinship information and potential variety development. The research methods included leaf sampling from several local Cirebon mango varieties, genomic DNA isolation, DNA amplification using the Polymerase Chain Reaction (PCR) method, and sequencing analysis to identify SNP variations. The SNP data obtained were analyzed to assess genetic diversity and kinship among mango varieties. The results showed genetic differences between local mango varieties that could be clearly distinguished using SNP markers. In conclusion, SNP-based characterization of Cirebon mango is effective for revealing genetic diversity and can serve as a basis for variety identification, preservation of local germplasm, and support for mango breeding and development programs in the Cirebon region.

**Keywords**: mango, genetic characterization, Single Nucleotide Polymorphism (SNP), PCR, Cirebon.

## 1. INTRODUCTION

Mango (*Mangifera indica* L.) originates from the area around Bombay and the area around the foothills of the Himalayas, then from that area spreads outside the region, including some reaching Latin America, especially Brazil, some to the African continent, and also countries in the Southeast Asian region, such as Vietnam, the Philippines, and Indonesia. The genus Mangifera has 62 species, but only 16 produce delicious fruit, and the most commonly consumed mango is Mangifera indica. The world's major producers of mangoes are India, Mexico, Brazil, Pakistan, Thailand, China, Indonesia, the Philippines, and Bangladesh, with India as the main producer, accounting for around 40% of global production. (Al., 2005).

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Analysis of genetic diversity using molecular markers has become a standard method with accurate results, although each marker has its own advantages and disadvantages. A molecular marker is a fragment of DNA located at a specific location in the genome that is linked to a trait. The molecular marker used depends on factors such as the suitability of the study and the desired information content. (Hafizah et al., 2018).

Mango is suggested to have a partial allopolyploid genome based on cytogenetics. However, genetic markers for mango have been reported to be inherited in a disomic fashion by several authors (Duval et al., 2005; Schnell et al., 2005, 2006; Viruel et al., 2005) suggesting that mango maybe treated as diploid. Mango has a total of 40 chromosomes, which suggests a haploid number of chromosomes as 20 and similarly 20 linkage groups. The haploid genome size is estimated at ~439 Mb. (Kuhn et al., 2017)

Genetic variation within a single mango species can produce new phenotypes or simply produce genetic variation at the DNA level without altering the phenotype of the fruit. Morphological characters have been routinely used to identify mango phenotypes, but morphological identification is difficult in mangoes with a high potential for genetic variation, especially in open-pollinated varieties. DNA analysis technology can be considered an additional identification tool for mango plants, including for cultivar identification and genetic purity determination. In Indonesia, mangoes are the third-largest fruit crop, contributing approximately 10.07% to national fruit production after bananas and oranges, and in the 2003–2005 period ranked fifth among the world's mango-producing countries (Rebin & Karsinah, 2010). Indonesia's leading export varieties are Arumanis 43 and Gedong Gincu, but each still has limitations in skin color and fruit size. (Prasetiyono, 2015).

Mangoes are a leading product in Cirebon Regency due to their distinctive characteristics: a sharp aroma, bright red lipstick-like color, and high fiber content. Their distinctive flavor, with a slightly sweet and sour aftertaste, distinguishes Cirebon mangoes from other regions. As a popular horticultural commodity, mangoes also have the potential to improve the welfare of mango farming families due to their high economic value. (Di & Cirebon, 2019)

Cirebon mangoes are widely known in the domestic market as a superior variety due to their sweet taste, distinctive texture, and good adaptation to the agroecological conditions of the north coast region of West Java. However, compared with national mango varieties, information on the genetic diversity of Cirebon mangoes is still limited. The use of SNP methods to characterize Cirebon mangoes can reveal hidden genetic potential that cannot be seen through morphological

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observations and is important for ensuring variety authenticity, preventing genetic erosion, and strengthening the position of Cirebon mangoes in conservation and breeding programs. The genetic diversity of local Cirebon mangoes, as assessed by SNP analysis, shows that cultivars grown in this region exhibit a fairly high level of genetic variation, despite often appearing morphologically uniform. Understanding genetic diversity in mangoes is crucial for germplasm conservation and improving breeding programs, as well as for preventing genetic erosion driven by the dominance of certain commercial varieties. (Liang et al., 2024)

Therefore, the application of the SNP method for the characterization of local Cirebon mangoes is very important to support the preservation, breeding, and protection of local superior varieties through scientific research, given the limited genetic research on Cirebon mangoes using the SNP approach.

## 2. RESEARCH METHOD

*Types of research*

This research is an experimental laboratory study that aims to obtain pure DNA and analyze the genetic diversity of mango using a molecular approach based on Single Nucleotide Polymorphism (SNP).

*Time and place*

The research was conducted from November to December 2025. Sampling was conducted in the Cirebon area, while laboratory analysis was performed at the Faculty of Medicine Laboratory, Universitas Swadaya Gunung Jati, Cirebon.

*Sample*

This study used two mango varieties from Cirebon: Arumanis from Lemahabang District and Gedong Gincu from Dukupuntang District. Samples were healthy, undamaged young mango leaves with high chlorophyll content (Fig. 1), which facilitated molecular analysis. After picking, the mango leaf samples were placed in labeled plastic sample tubes and transported from the field to the laboratory in a cooler bag containing ice packs. The use of the cooler bag aims to maintain a low temperature throughout the journey, keeping the sample fresh and preventing degradation. Upon arrival at the laboratory, the samples were immediately isolated overnight and stored at -80℃.

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Figure 1. Leaf from Gedong Gincu (left) and Arumanis (right)

*Tools and materials*

The tools used in this study included a micropipette, a centrifuge, a vortex, a PCR machine, and an electrophoresis apparatus. The materials used in this study were mango leaves, AD Buffer, VB Buffer, Isopropanol, RNase-free water, primers, agarose, and TAE buffer.

*Research procedure*

All samples were stored and isolated at −80°C to prevent DNA degradation. DNA extraction was carried out from 0.400 grams of crushed leaves using 350 µL AD Buffer and 350 µL VB Buffer until homogeneous, then vortexed and centrifuged to obtain 0.1 µL of pure DNA, which was then washed with 70% alcohol for 15 minutes and re-dissolved in NFW buffer for further analysis.

The next stage is PCR, which begins with pre-denaturation for 2–5 minutes, followed by denaturation for 1 minute, and annealing at 50°C for 1 minute to allow the primers to attach to the target DNA.

Primer (Fig. 2) based on:

https://www.ncbi.nlm/nih.gov/nuccore/NC_058151.1?from=10730344&to=10731853&report=genbank&strand=true

Add 200 bp on the downstream (10.731.853 + 200 = 10.732.053)

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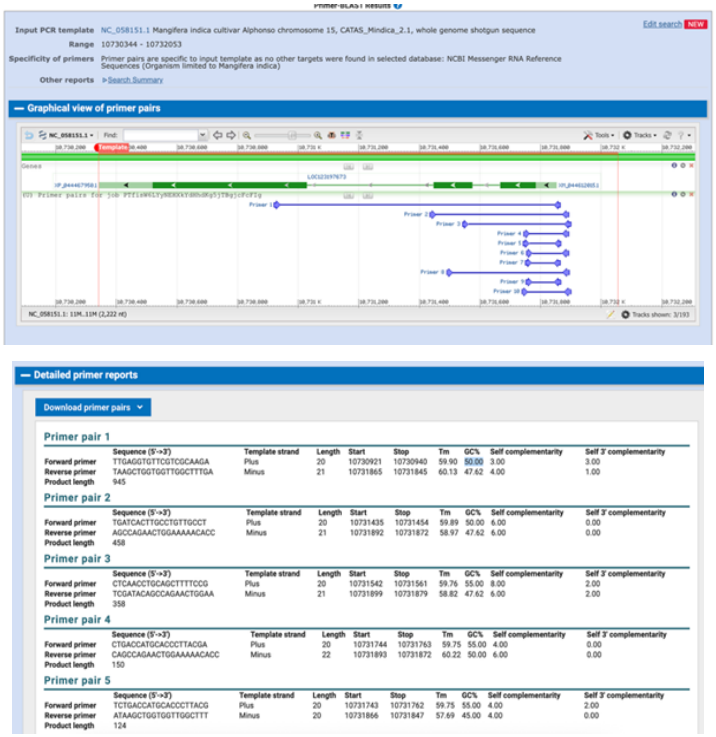


Figure 2. Primary design

The first design in this study was conducted using the genome of the Mangifera indica cultivar Alphonso as a template, with reference to the whole genome sequence (NC_058151.1) obtained from the NCBI database. The amplification target is on chromosome 15, so the designed primers are specific to the mango (Mangifera indica) genome and do not cross-react with other organisms.

Based on the primer design above, several primer pairs were obtained that bind to different locations within the target region. Visualization of primer positions shows that each primer has a forward and a reverse orientation, allowing PCR to amplify DNA fragments. This design ensures that the resulting fragments originate from specific and controlled genomic locations.

The next stage, the extension stage (Fig. 3), lasts 1–2 minutes, during which Taq polymerase extends the new DNA strand, followed by a final extension of 1 hour and 45 minutes after the PCR cycle is complete. PCR results are visualized by 2% agarose gel electrophoresis using 1 g agarose in 50 mL TAE buffer, run for 45 minutes to observe the DNA banding pattern as a basis for genetic diversity analysis.

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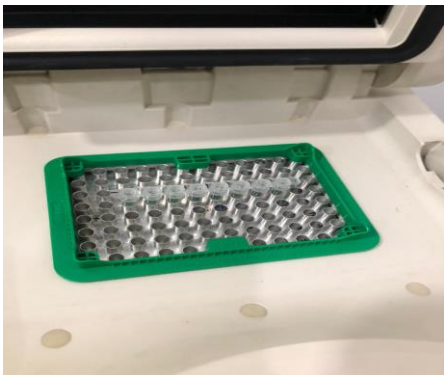

Figure 3. Extension process

## 3. RESULTS AND DISCUSSION

Characteristically, the primers used range in length from 20 to 22 bases, with GC content of ±50–60%. These GC content values indicate that the primers have fairly good stability during annealing. Furthermore, the primers' melting temperatures (Tm) are relatively uniform, supporting efficient and consistent PCR amplification.

The analysis results also showed that the self-complementarity and self-3'-complementarity values of the primers were relatively low. This indicates that the primers have a minimal risk of forming secondary structures such as hairpins or primer dimers, thereby increasing the specificity and success of amplification. The size of the PCR products generated from each primer pair varied, ranging from approximately 350 bp to 470 bp. This variation in product size leads to distinct DNA banding patterns when visualized by agarose gel electrophoresis. These differences in banding patterns were then used to distinguish the Arumanis and Gedong Gincu mango varieties.

Overall, the primer designs used in this study met the criteria for good primers: specificity to the target genome, optimal length and GC content, and minimal secondary structure formation. Therefore, these primers are considered suitable and effective for molecular identification of mango varieties based on differences in banding patterns observed in PCR amplification results.

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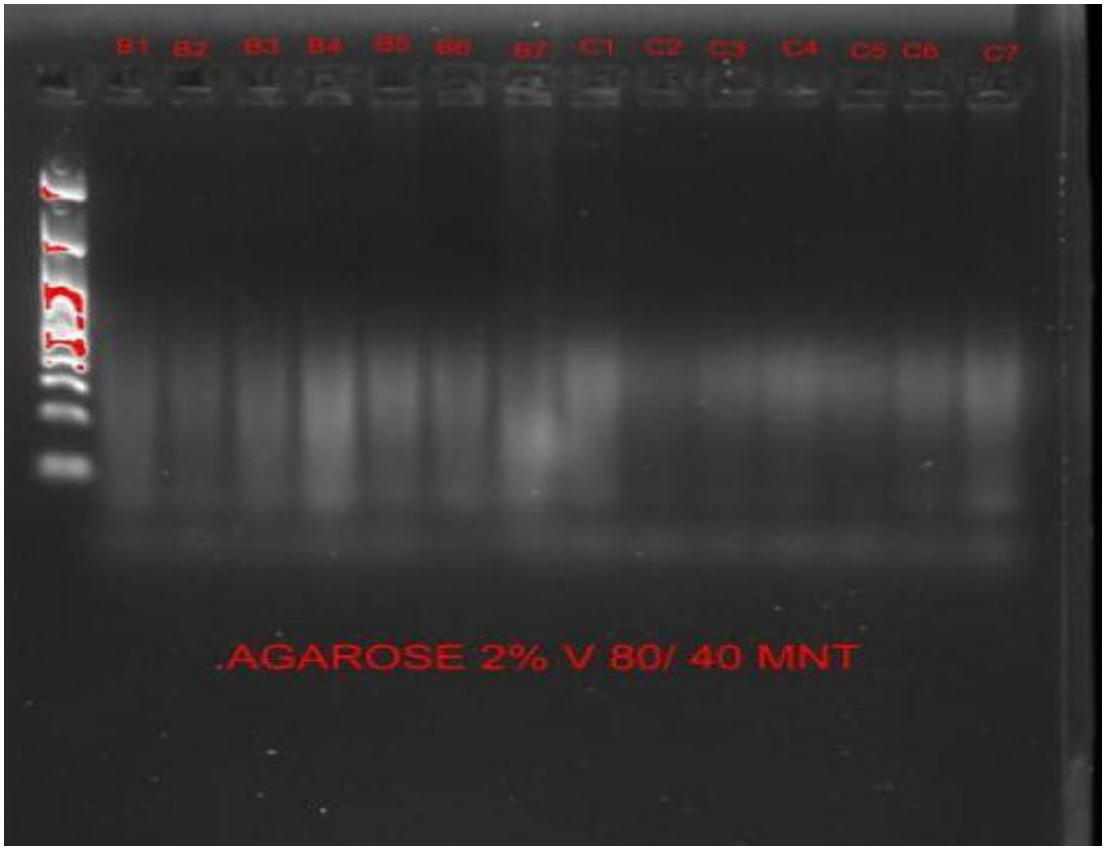


Figure 4. DNA of Arumanis and Gedong Gincu

Based on the PCR results, the primers designed in this study can specifically amplify the target DNA region in two common mango varieties in Indonesia, namely Arumanis and Gedong Gincu, which are widely cultivated in the Cirebon region.

PCR amplification results revealed clear differences in DNA banding patterns between the two groups of mango samples analyzed. Based on the electrophoresis results, the samples were divided into two groups with distinct banding patterns. This difference in banding patterns serves as a key indicator in distinguishing the two mango varieties, Arumanis and Gedong Gincu, from Cirebon.

The DNA banding patterns generated by the amplification process indicate that each variety possesses distinct molecular characteristics. Samples from one group showed similar banding patterns, whereas those from other groups showed different patterns. This indicates genetic variation between mango varieties, which can be detected through the primer designs used in this study.

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The results show that samples 1-7 are Arumanis mangoes, while samples 8-14 are Gedong Gincu. The differences in DNA banding patterns in gel electrophoresis, both in terms of size and presence, allow each variety to be clearly distinguished. This difference indicates that the primers used successfully recognized genetic variation between the mango varieties. Thus, the PCR process not only successfully amplified DNA but also served as an effective tool for identifying and differentiating the Arumanis and Gedong Gincu varieties based on their genetic characteristics.

PCR products were analyzed by electrophoresis on a 1% agarose gel at 100 volts for 25 minutes, visualized under ultraviolet light using a UV transilluminator, and observed for bands based on molecular weight. The amplified mango genomic DNA, previously confirmed by PCR, was then subjected to direct sequencing using degenerate primers in a bidirectional approach (forward and reverse). The entire sequencing process was carried out by Macrogen Inc. (Singapore). The sequencing results were analyzed using the BioEdit program version 7.2.5 (Hall, 1999) to obtain the base sequence of the sequenced fragments. The base sequence was then analyzed using the BLAST program available on the NCBI website (www.ncbi.nlm.nih.gov). Multiple sequence alignment analysis was performed using the ClustalW program. The results of the Nanodrop spectrophotometer showed that the modified method of Uddin et al. (2014) can be recommended for mango peel DNA isolation, as it is more consistent than other methods, with an average purity of 2.12 and an average DNA concentration of 641.06 ng across five mango peel samples. This is because, in the first stage of the DNA isolation process, extraction uses 0.4 M glucose to improve DNA quality and prevent contamination; PVP is used to bind phenolic compounds; and β-mercaptoethanol is used as an antioxidant. (Ashgi, 2021).

The washing or cleaning process with chloroform-isoamyl alcohol is repeated three times. Furthermore, isopropanol is added to the DNA precipitation stage in the aqueous phase, allowing the DNA to clump together to form a fiber structure and form a pellet after centrifugation. Isopropanol can also be used to remove chloroform residues from the extraction stage. (Lade et al., 2014).

SNP discovery in mango and other non-model systems usually relies on next generation sequencing and de novo transcriptome analysis of a few accessions to develop markers for genotyping collections. A‘Tommy Atkins’ mango transcriptome from multiple plant tissues identified 30,000 transcripts and mapped RNA sequences from 24 genetically diverse mango accessions to identify ˜400,000 SNPs that led to 640 high-quality, well isolated SNPs in protein-coding genes. Another study developed 239 SNPs from mango cultivars ‘Keitt’ and ‘Tommy

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Atkins' and was used to genotype 74 Israeli accessions. Recently, 1,054 single nucleotide polymorphism (SNP) markers were used from the three sources described to create a genetic map for mango. (Kuhn et al., 2019).

The markers from Kuhn et al were used to genotype seven mapping populations of mango totaling 807 individuals. A consensus genetic map was produced that defined 20 linkage groups for mango as expected. In the present study, 384 SNP markers were used to analyze diversity. The majority of these markers are evenly distributed across the 20 linkage groups to capture diversity across the genome.

In mangoes, the red coloration of the fruit is caused by the anthocyanin pigment content. Anthocyanin biosynthesis in mangoes is controlled by the MYB transcription factor gene. According to Colanero et al. (2020), the MYB transcription factor gene plays a crucial role in anthocyanin pigmentation. In Irwin mangoes, the MYB transcription factor gene is a key regulator of anthocyanin biosynthesis in mango peel. MYB gene expression in Irwin mangoes increases with light intensity and is directly proportional to the accumulation of anthocyanin biosynthesis, which produces the red color in the fruit peel. Single Nucleotide Polymorphism is one of the markers used to determine phenotypic variations in mango peel color by observing differences in DNA sequences of each mango variety. SNPs in the exon (coding region) are categorized as the most important because they can affect the function of the MYB transcription factor gene. (Ashgi, 2021).

Molecular markers have been widely used to determine kinship relationships within and between species. Allele-frequency data or dissimilarity matrices are used to determine kinship relationships among individuals. In the second method, the choice of the similarity coefficient is crucial for the clustering results. (Rijzaani et al., 2015).

This research aligns with several studies that reported that PCR techniques with specific primers can be used to differentiate mango varieties based on their genetic variation. Viruel et al. (2005) stated that DNA analysis using molecular markers, including PCR, is effective for identifying genetic differences among Mangifera indica varieties due to nucleotide-level variation. In addition, research by Schnell et al. (2005) showed that PCR-based molecular markers in mangoes are useful for both identification and germplasm conversion. Therefore, the results of this study support previous findings and confirm that the PCR method, using appropriately designed primers, is a reliable approach for genetically distinguishing Arumanis and Gedong Gincu mango varieties.

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## 4. CONCLUSIONS

Based on the research results, it can be concluded that the primers designed and used were able to amplify polymorphic DNA fragments, thereby enabling differentiation between the Arumanis and Gedong Gincu mango varieties originating from the Cirebon region. Therefore, the primer design is considered effective and has the potential to serve as a molecular identification tool for mango varieties based on differences in DNA banding patterns observed in PCR results.

The differences in banding patterns observed across varieties confirm that the PCR method, with appropriate primer design, can be used as a molecular approach for identifying and characterizing plant varieties, particularly local mangoes. These findings are expected to support the development of more accurate and efficient molecular-level techniques for mango variety identification.

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