Review Article

Genetic Diversity and Development of Molecular Marker Toolkit for Early Detection of Seedlings Based on Early Maturity of Sugar Palm (*Arenga pinnata* Merr.) in Indonesia: A Review

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ABSTRACT

Sugar palm (*Arenga pinnata* Merr.) is a promising estate crop with significant potential as a raw material for palm sugar, starch, fiber, and bioethanol production. However, its utilization and cultivation in Indonesia have not been fully optimized. This plant is found throughout Indonesia's tropical rainforests, from Sumatra to Papua. While several genetic diversity studies of sugar palm in Indonesia have been conducted using morphological traits, isozymes, and molecular markers, these efforts are less extensive than those focused on other members of the Arecaceae family. To date, the genetic diversity studies of sugar palm in Indonesia are still limited in terms of the number of markers, population coverage, and the use of modern genomic approaches. This paper aims to review the current status of genetic diversity research on sugar palm in Indonesia and the development of a molecular marker toolkit for the early identification of early maturing sugar palm seedlings. The introduction of this toolkit is expected to accelerate sugar palm breeding programs in Indonesia, particularly in developing improved varieties that can benefit farmers. Further dissemination and broader availability of this kit are necessary to increase recognition of its benefits and facilitate its widespread adoption within the community.

Keywords: detection toolkit, genetic diversity, molecular marker, plant breeding, estate crop

Introduction

Indonesia is a megadiverse country located near the equator, home to extensive tropical rainforests and a wide array of plant and animal species. However, many of these plants have yet to be thoroughly researched, and their potential uses and economic value are largely unknown. One such plant, sugar palm (*Arenga pinnata* Merr.), locally known as *aren*, is among many plant species in Indonesia's rainforests and holds considerable potential, though it is still underutilized.

Sugar palm is a member of the

Arecaceae family (Rambey et al., 2020). Compared to its relatives, such as coconut (*Cocos nucifera* L.), date palm (*Phoenix dactylifera* L.), or oil palm (*Elaeis guineensis* Jacq.), sugar palm is still relatively unknown, despite its multifunctionality. Almost all parts of sugar palm can be utilized and have high economic value, including as a raw material for making palm sugar, starch, alcohol, and fiber (Muda & Awal, 2017).

One of the primary products of the sugar palm is its sap, known locally as *nira*, which serves as the raw material for producing palm sugar. Analysis of the sugar palm sap reveals a sucrose content ranging from 8–17% and a sugar yield of 12–18% (Saleh, 2004). Palm sugar offers several advantages, including a significantly higher market price compared to other types of sugar, a more fragrant aroma, and superior nutritional value compared to cane and beet sugar (Baharuddin et al., 2007; Lempang, 2012). Additionally, the production of palm sugar is relatively simple and requires minimal equipment, making it well-suited for micro, small, and medium enterprises (UMKM) (Agustina et al., 2022).

A study conducted by Pontoh (2012) indicated that the primary carbohydrate content of palm sugar is sucrose, with a concentration of 80–85%, followed by reducing sugars, glucose, and fructose. Furthermore, palm sugar also contains 1.7–2.4% protein. Meanwhile, a previous study done by Assah et al. (2021) showed that the granulated sugar contain higher levels of sucrose, reducing sugars, and fructose than those in molded and liquid forms. The high sucrose content in palm sugar has great potential to be developed as an alternative to cane sugar, especially to fulfill the national sugar demand which is increasing from year to year.

In addition to being used for sugar production, sugar palm sap can also be utilized in the creation of *nata pinnata*, alcohol, and acetic acid. *Nata pinnata* is a refreshing food or dessert similar to *nata de coco*, known for its high water and fiber content (Lempang, 2017). Another product of sugar palm sap fermentation is an alcoholic beverage called *tuak* or *captikus*, which is popular in North Sulawesi (Wenur & Waromi, 2018). Besides its use as a beverage, the alcohol derived from the fermentation of palm sap holds potential as bioethanol fuel. Bioethanol is a biofuel produced by fermenting sugar into ethanol, sourced from high-carbohydrate plants like sugarcane, beetroot, potato, cassava, sugar palm, corn, sorghum, wheat, and barley (Arlianti, 2018). The development of bioethanol is expected to help reduce reliance on nonrenewable fuel oil.

The endosperm of the sugar palm fruit, locally known as *kolang kaling*, is also useful as a food source of fiber in making foods and drinks, such as *kolak*, sweets, and *es campur*. Other functional parts of the sugar palm plant are its leaves, which can be used to make roofs for houses or huts; its roots, which can be used to make flower vases or fruit baskets; its fibers, which can be used to make brooms, brushes, and ropes (Wulantika, 2015). When the sugar palm plant is no longer able to produce sap, the stem pith contains starch can be used to make sugar palm flour, which is also useful as an ingredient in cakes (Lempang, 2012).

The availability of new improved varieties is a crucial focus for the future development of the sugar palm farming system. Several sugar palm local varieties have been registered by the local government in Indonesia, including Genjah Kutim from Kutai Timur (East Borneo), Dalam Toumuung from Tomohon (North Sulawesi), Smulen ST-1 from Bengkulu, and Parasi from Banten (Wijaya, 2024). However, farmers have largely utilized local sugar palm plants that grow wild in the forests surrounding their homes. In recent years, many forest areas have been converted into land for other agricultural crops or residential development, highlighting the need for more intensive sugar palm cultivation and the availability

Table 1. Genetic diversity studies of sugar palm in Indonesia.

No.	Number of genotypes	Genotype origins	Marker type	Number of markers	Reference
1	40	Lima Puluh Kota (West Sumatra)	Morphology traits	13	Wulantika (2015)
2	80	South Tapanuli (North Sumatra)	Morphology traits	53	Harahap et al. (2018)
3	83	Temanggung (Central Java); Banjar (South Kalimantan); Rejang Lebong (Bengkulu); Tomohon (North Sulawesi)	Isozymes	4	Haryjanto et al. (2011)
4	20	North Sumatra and Southeast Sulawesi	RAPD	2	Putri et al. (2016)
5	24	South Tapanuli (North Sumatra)	RAPD	10	Harahap (2017)
6	40	Cianjur (West Java); Cibaliung and Rangkasbitung (Banten)	SSR	7	Terryana et al. (2019)
7	141	Bangka Island; Lampung; Lebak (Banten); Bogor and Tasikmalaya (West Java); Brebes (Central Java); Gowa, Bombana, and Muna (South Sulawesi)	SSR	9	Rinawati et al. (2021)

of high-quality seedlings from high-yielding varieties (Tenda et al., 2010).

The development of new sugar palm varieties through conventional breeding faces challenges, such as the relatively long juvenile period, which lasts over 5 years. Tenda et al. (2010) identified two types of sugar palm based on their juvenile stages: early maturity dan late maturity. The sap from early maturing sugar palm can be harvested at 5–6 years of age, while sap from late maturing sugar palm is harvested at 10–12 years. Genjah Kutim is an example of a early maturing variety, while Dalam Toumuung represents a late maturing variety. Additionally, there is a medium maturing variety, which can be harvested at 6–8 years, such as Smulen ST-1 and Parasi (Wijaya, 2024).

To accelerate the sugar palm breeding program, the use of a biotechnology approach, specifically molecular markers, is essential. A molecular marker toolkit for detecting sugar palm seedlings based on their early maturity character presents a valuable tool for breeders in selecting parental lines and progenies. Additionally, this toolkit can be used to verify the authenticity of sugar palm seedlings in the market, identifying whether they are early, medium or late maturity types. This article reviews the latest research on the genetic diversity of sugar palm in Indonesia, utilizing morphological, biochemical, and molecular markers. It also discusses the efforts to develop a molecular marker toolkit for detecting early maturity traits in sugar palm seedlings, along with future development prospects.

Geographic Distribution and Habitat of the Sugar Palm in Indonesia

According to Mogea et al. (1991), sugar palm originates from the Indo-Malayan Peninsula, with its distribution center located in Indonesia. Sugar palm can also be found in other South and Southeast Asian regions, such as India, Sri Lanka, Malaysia, Philippines, Myanmar, Thailand, Vietnam, Papua New Guinea, Guam Island, and Ryukyu Islands (Elberson & Oyen, 2010). In Indonesia, sugar palms are distributed across 15 provinces, such as Aceh, North Sumatra, West Sumatra, Bengkulu, Banten, West Java, Central Java, East Kalimantan, South Kalimantan, North

Sulawesi, South Sulawesi, Southeast Sulawesi, Maluku, North Maluku, and Papua (Fiani, 2015; Mogea et al., 1991).

Sugar palms generally grow in primary and secondary forests near residential areas and are distributed not only in areas with fertile soils rich in organic matter, but also in areas with heavy clay, loamy sand, and laterite soil (Elberson & Oyen, 2010). This plant can adapt from lowlands along the coast to highlands at an altitude of 1,400 m above sea level (Mogea et al., 1991). According to Effendi (2010), sugar palm exhibits high adaptability to various land and agroclimatic conditions and grows rapidly due to its extensive root system and dense crown. As a result, sugar palm has significant potential to be developed on marginal lands, which are abundant across Indonesia. There are several sugar palm local varieties that have been registered in Indonesia, such as Genjah Kutim, Dalam Toumuung, Smulen ST-1, and Parasi (Tenda et al., 2010; Wijaya, 2024).

Current Status of Genetic Diversity Studies of Sugar Palm in Indonesia

The genetic diversity studies of sugar palm in Indonesia, both using morphological, isozyme, and molecular markers, have been conducted as presented in Table 1. These studies are valuable for assessing the level of diversity within a specific sugar palm population in a given region. The genetic diversity analysis offers important insights into the genetic relationships both within populations and between populations from different regions (Haryjanto et al., 2011). Additionally, the broad geographical distribution of sugar palms can serve as the basis for the development of their genetic diversity.

The availability of sugar palm germplasms with a high level of genetic diversity is essential for developing new improved varieties of sugar palm. According to Harahap (2017), genetic diversity is crucial for a species' adaptability, particularly in response to changes in environmental conditions, as it enables the species to better survive with these changes. In practice, farmers typically use local sugar palm plants that are grown in their yards or sourced from nearby forests.

The genetic diversity analysis based on morphological marker have been previously reported in several studies, i.e. Wulantika (2015) and Harahap et al. (2018). The genetic diversity of sugar palm from Lima Puluh Kota (West Sumatra), based on morphological characters which was conducted by Wulantika (2015), showed the clustering of 40 sugar palm genotypes into two main clusters with a genetic similarity coefficient of 40–88%. Based on the analysis results, from the total of 35 morphological characters observed, as many as 16 characters showed similarities, while a total of 19 characters showed variation.

According to Wulantika (2015), the similarities of morphological characters might arise because there is a possibility that the genotypes which grew in similar locations, originated from a similar parent tree. The sugar palm seeds produced from one parent tree can be spread due to the role of Asian palm civets (*Paradoxurus hermaphroditus*) which eat sugar palm fruit then the undigested seeds are excreted through their feces, then the seeds germinate and grow in a place quite far from the parent tree. Meanwhile, the existence of genetic differences among the genotypes is likely caused by cross-pollination that naturally occurs in sugar palm mediated by insects.

A study by Harahap et al. (2018) using 80 sugar palm genotypes originating from South Tapanuli (North Sumatra) revealed the clustering into four main groups at a genetic similarity coefficient of 75%. The first cluster consisted of sugar palm genotypes whose phenotypic characters are suitable for producing fruit from female inflorescences, the second cluster consisted of sugar palm genotypes which did not possess female inflorescences, the third cluster consisted of sugar palm genotypes whose characters are suitable for producing sap from male inflorescences, and the fourth cluster consisted of sugar palm genotypes which did not possess male inflorescences. The results of this analysis are useful for identifying sugar palm accessions that can be developed for fruit production and those suitable for sap production.

The use of biochemical markers such as isozymes to analyze the genetic diversity of sugar palm in Indonesia has been reported by Haryjanto et al. (2011). In this study, a total of four populations of sugar palm from Temanggung (Central Java), Banjar (South Kalimantan), Rejang Lebong (Bengkulu), and Tomohon (North Sulawesi) were analyzed using isozyme markers based on four types of enzymes, such as esterase (EST), glutamate oxaloacetate transamirase (GOT), diaphorase (DIA), and 6-phosphogluconate dehydrogenase (6Pg). Each population consisted of 20–22 randomly selected plants. The results showed that as many as nine alleles were successfully detected and spread across four polymorphic loci. The average number of alleles per locus obtained was 2.2500 and the average number of effective alleles per locus was 1.8377.

The Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) analysis revealed the separation of the sugar palm population into two main clusters. The first cluster included populations from Central Java and South Kalimantan, while the second cluster comprised populations from Bengkulu and North Sulawesi. Interestingly, the population grouping in this study did not follow the geographic origins of the populations, suggesting limited genetic flow between them, likely due to geographic isolation by the sea.

The utilization of random amplified polymorphic DNA (RAPD) has been reported in several studies, such as Putri et al. (2016) and Harahap (2018). A study by Putri et al. (2016) reported the use of two RAPD primers (OPD-20 and OPH-06) to analyze 20 sugar palm genotypes. The results showed the separation of the sugar palm genotypes into three clusters: the first cluster consisted of sugar palm genotypes originating entirely from North Sumatra, the second cluster consisted of a mixture of sugar palm genotypes from North Sumatra and Southeast Sulawesi, while the third cluster consisted of sugar palm genotypes originating entirely from Southeast Sulawesi. This clustering pattern suggested that the sugar palm genotypes from North Sumatra and Southeast Sulawesi might share a common ancestor, indicating a potential shared origin of sugar palm in Indonesia. However, since the study only used two RAPD primers, this limited the accuracy of the genetic diversity analysis due to the small number of markers.

Meanwhile, a previous study from Harahap (2017), revealed the separation of 24 sugar palm genotypes into five main clusters, with the pattern of genotypes originating from Sipirok being spread across almost all clusters, indicating that these genotypes have a close genetic relationship with the genotypes from other regions, while there is one accession from South Angkola which separated into a distinct cluster because of its unique character.

The use of microsatellite or simple sequence repeat (SSR) markers for sugar palm diversity analysis has been demonstrated in the studies by Terryana et al. (2020) and Rinawati et al. (2021). In the study by Terryana et al. (2020), a total of three sugar palm populations from Cianjur (West Java), also Cibaliung and Rangkasbitung (Banten) were analyzed using seven SSR markers. The UPGMA analysis showed the populations were grouped into two main clusters. The Cianjur population existed in a separate cluster from the Cibaliung and Rangkasbitung populations, which were grouped in a similar cluster. On the other hand, in Rinawati et al. (2021) study, a total of 141 Indonesia's sugar palm genotypes originating from several regions, such as Bangka Island, Lampung, Lebak, Bogor, Tasikmalaya, Brebes, Gowa, Bombana, and Muna, were analyzed using nine SSR markers. The results showed the grouping of the 141 genotypes into three main clusters, with a clustering pattern that was not based on geographic origin. The sugar palm genotypes from Lampung and Muna showed the farthest genetic distance, while the genotypes from Bangka Island and Lebak showed the closest genetic distance.

Morphological, biochemical, and molecular markers are all useful for analyzing genetic diversity, but each type has its own strengths and weaknesses. For instance, morphological markers are simple to use and cost-effective, making them ideal for studies with limited budgets. However, using morphological markers for genetic diversity analysis has some disadvantages, such as the influence of environmental factors, especially on quantitative traits, challenges in distinguishing between closely related genotypes, and in some cases, the long wait required for floral and fruit production (Chesnokov et al., 2020; Dida, 2022).

Most isozymes have been obtained from enzymes involved in intermediary metabolism, such as those in the glycolytic pathway, even though it is possible that isozyme developed from any type of enzyme (Jaiswal, 2024). The use of isozymes as markers has several limitations, such as enzyme expression being influenced by the plant growth stage, a limited number of enzymes available that cannot cover the entire plant genome, and some enzymes only expressing certain traits in specific plant tissues (Azrai, 2005; Porth & El-Kassaby, 2014). To address these issues, molecular markers offer significant advantages. They are not affected by the environmental factors, less time-consuming, provide higher accuracy and reproducibility, and can be directly designed for the desired target gene (Amom & Nongdam, 2017). As a result, molecular markers have great potential to be utilized as a selection toolkit in plant breeding programs. However, the combination of all these markers will produce more robust data in the study of genetic diversity of sugar palm.

Development of Molecular Marker Toolkit for Sugar Palm Seedling Detection Based on Early Maturity Traits and Its Commercialization Prospects

Sugar palm is a perennial crop that starts bearing fruit after five years. In Indonesia, the breeding program aimed at developing new improved sugar palm varieties with high sap production and early maturity characters, is challenging due to the long juvenile period. As a result, selecting progenies from hybridization is time-consuming, as it requires waiting for fruit production. To accelerate the sugar palm breeding program in Indonesia, a study is being conducted to develop a molecular marker toolkit for detecting early maturity traits in sugar palm seedlings.

The molecular marker toolkit is the result of a collaboration among Balai Penelitian Tanaman Palma/Balitpalma (currently called as BRMP Palma), Balai Besar Penelitian dan Pengembangan Bioteknologi dan Sumber Daya Genetik Pertanian/BB Biogen (currently called as BRMP Biogen), and IPB University by a collaboration project initiated from 2015 to 2018 through Kerjasama Kemitraan Penelitian dan Pengembangan Pertanian Strategis (KKP3S) program which was continued through Kerjasama Penelitian, Pengkajian, dan Pengembangan Pertanian Strategis (KP4S) program which was funded by the Indonesian Agency for Agricultural Research and Development/IAARD (currently called as BRMP) at that time.

The development of the molecular toolkit began with the *de novo* assembly of the sugar palm draft genome, which had not been widely published at that time. The result of this draft genome assembly has been published by Rijzaani et al. (2017).

From *de novo* assembly results, a number of tandem repeat sequences (SSRs) were successfully identified both in the contig region (as many as 359 SSRs) and scaffold (approximately 1,000 SSRs). The identified repeat sequences were then used to design SSR primers that can be used to differentiate between the early and late maturing sugar palm genotypes. A total of 140 pairs of SSR primers were successfully designed and used to amplify ten samples of early maturing sugar palm variety, namely Genjah Kutim, and tall sugar palm variety, namely Dalam Toumuung, respectively. Of the total 140 primers analyzed, as many as ten SSR primers were obtained having a high level of polymorphism and were able to distinguish between the early and latye maturing sugar palm samples in different clusters.

The molecular marker toolkit was granted a patent by the Directorate General of Intellectual Property (DGIP), Ministry of Law and Human Rights of the Republic of Indonesia in 2019 (Patent No. IDP000058280). The analysis can be conducted at the seedling stage, eliminating the need to wait until the plants bear fruit, and it requires only standard molecular laboratory equipment (Lestari et al., 2016). To date, the toolkit has been validated across multiple sugar palm populations from different regions, such as Rejang Lebong (Bengkulu); Lebak and Pandeglang (Banten); Cianjur, Sumedang, and Leuwiliang (West Java); Kendal, Ungaran, and Rembang (Central Java); as well as Kulonprogo (Yogyakarta) and Malang (East Java) (Lestari et al., 2018).

This molecular marker toolkit is designed for a range of users, including researchers and breeders working with sugar palm, growers as well as companies involved in sugar palm plantations. For plant breeders, the toolkit helps in selecting parentals and progenies; while for the companies, it assists in verifying the authenticity of sugar palm seeds or seedlings, determining whether they are early or late maturing genotypes. Although the toolkit has advanced to the patent registration stage and has been showcased at several exhibitions. Based on the current policy on research in Indonesia, especially with the existence of the National Research and Innovation Agency (BRIN), further development of this molecular marker toolkit shows higher opportunities which could be beneficial for sugar palm farmers in Indonesia.

Conclusion

The sugar palm presents considerable opportunities for future development. Studies have revealed a wide range of genetic diversity among sugar palms in Indonesia. Therefore, creating new improved sugar palm varieties with high production and early maturing character is essential. To date, the genetic diversity studies of sugar palm in Indonesia are still limited in terms of the number of markers, population coverage, and application of modern genomic approaches. The development of a molecular toolkit capable of identifying the early maturing trait will significantly accelerate the breeding process, allowing for rapid selection of parental lines and progenies. It is important to continue promoting and making this kit available so that its advantages are more broadly recognized and adopted by the community.

Acknowledgements

This research received no specific funding from public, commercial, or not-for-profit agencies.

Conflict of Interest

The authors declare that they have no conflict of interest.

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