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Research Article

Biosystematics of Sweet Starfruit Cultivars (*Averrhoa carambola* L.) Based on Morphology and DNA Barcoding in Tarub Village, Grobogan Regency, Central Java

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ABSTRACT
The sweet starfruit (*Averrhoa carambola* L.) has high economic value, but research on the biosystematics of its cultivars in Indonesia is still limited. The morphological similarities among starfruit cultivars cause difficulties in identifying the cultivars. This leads to misidentifications and overlapping names in society. The Karangsari and Bangkok Merah cultivars are often considered the same, despite differences in habitus and fruit. This study aims to identify and to examine the genetic relationships among starfruit cultivars using morphological and DNA barcoding approaches. The research samples are five cultivars of sweet starfruit found in the starfruit plantations in Tarub Village, Grobogan Regency, Central Java. The analysis was conducted based on morphological traits and the Internal Transcribed Spacer (ITS) molecular marker to identify and to determine their genetic relationships. The identification results found five cultivars of *A. carambola*, namely Dewa, Dewi, Madu, Bangkok Merah, and Karangsari. Based on phenetic and phylogenetic analysis, it was shown that the Bangkok Merah and Karangsari cultivars have a very close genetic relationship, as do the Dewa and Dewi cultivars. Meanwhile, the Madu cultivars is distinct from the others due to significant morphological and genetic differences. Haplotype analysis showed a high haplotype diversity value, with a haplotype diversity (hd) value of 0.98, indicating a wide genetic diversity within the studied starfruit population.

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INTRODUCTION

Local tropical fruits in Indonesia exhibit a wide range of cultivars with significant potential for development. One type of horticultural commodity with high economic value is sweet starfruit (*Averrhoa carambola* L.). Sweet starfruit (*Averrhoa carambola* L.) exhibits a wide diversity of cultivars across various countries, including Malaysia, India, Thailand, Taiwan, China, and many others. Starfruit cultivars in Indonesia include Dewi, Dewa, Demak Kunir, Demak Jingga, Demak Kapur, Welahan, Bangkok Merah, Filipina, Penang, Rawasari, Karangsari, Madu, Wulan, Malaya, Sembiring, and Paris (Muthu et al. 2016).

Morphological similarities between cultivars make it difficult to distinguish each cultivar. In addition, there is still overlapping in the naming of cultivars, which is still not quite right in the community (Meilina et al. 2024). Taxonomic issues of the *A. carambola* include the fact that some people consider the Karangsari and Bangkok Merah cultivars to be the same, while others consider them to be distinct due to differences in their habitus and fruit characteristics. Identification of cultivars based on morphological characters contributes to plant breeding programs to obtain good-quality plants so that superior cultivar candidates are obtained in plants (Pavlinov 2020). An important initial step in plant breeding is to analyse the kinship relationships between these cultivars (Xin et al. 2024). The kinship relationships between individuals are analysed based on the similarities in the number of characters they have. Therefore, further research is needed on the biosystematics of various starfruit cultivars through various approaches, such as morphological or molecular approaches, to answer these problems.

Morphological characterisation activities play a role in exploring the diversity of a plant-based on phenotype. Morphological information can be used in plant breeding, potential exploration, knowing kinship relationships, conservation, and developing classification systems. In starfruit plants, morphological characterisation helps distinguish cultivars and provides important information about genetic potential that can be utilized in breeding programs (Tuo et al. 2024). The biodiversity identification process initially only used morphological identification, but now it has developed using a molecular taxonomy approach. Molecular identification is the process of grouping and characterisation based on the similarity of nucleotide sequences in the organisms studied (Salgotra & Chauhan 2023). Molecular taxonomy using DNA barcoding has several advantages, including identifying species accurately, precisely, and quickly (Mahima et al. 2022; Chika et al. 2024).

Studies on the diversity and kinship relationships between starfruit cultivars in Indonesia are still minimal. Research on the diversity and kinship between starfruit plant cultivars has been conducted morphologically in the Cibinong Plant and Animal Germplasm Garden collection. Characterisation with a molecular approach must be carried out to support and strengthen the morphological identification process in a species. This is because molecular identification is more stable and unaffected by environmental factors (Zhang et al. 2015; Mishra et al. 2016). ITS DNA barcode was chosen because it has a high level of variation compared to other regions (Takano & Okada 2002). Comparative analysis of ITS sequences can be carried out on several closely related species. In addition, the ITS DNA barcode has successfully identified five cultivars of *Syzygium samarangense* from Dong Thap Province in Vietnam (Nguyen et al. 2020). ITS DNA barcode has also been used for species identification in the *Averrhoa* genus, namely in two types of wild starfruit (*Averrhoa dolichocarpa* and *Averrhoa leucopetala*) (Senjaya et al. 2021). ITS DNA barcode is estimated to be suitable for identifying and analyzing the genetic variation of starfruit cultivars. Based on this, further research is needed to integrate morphological and molecular analysis in the biosystematics of starfruit culti-

vars.

Grobogan Regency, especially in Tarub Village, is one of the centres of starfruit cultivation with several superior cultivars, namely Dewa, Dewi, Madu, Bangkok Merah, and Karangsari. Research on identifying sweet starfruit cultivars in Indonesia, especially in Grobogan Regency, is still minimal and has not been widely carried out. This study aims to identify and to determine the kinship relationships between starfruit cultivars based on morphological and molecular approaches. The results of this study are expected to help developing knowledge about identification, providing scientific information that supports plant breeding, and serving as a reference for selecting effective DNA barcodes in plants at the cultivars level.

MATERIALS AND METHODS

The sampling location in three starfruit cultivation areas in Grobogan Regency, namely in Tarub Village, Tawangharjo District, is divided into Station 1, station 2, and Station 3 (Table 1 and Figure 1). The sampling was conducted on October 2024. The identification of cultivars was based on the Annex of the Minister of Agriculture Decree No. 717/Kpts/TP.240/8/98, Annex of the Minister of Agriculture Decree No. 718/Kpts/TP.240/8/98 regarding the description of sweet starfruit (*A. carambola*). A total number of 30 samples were collected for morphological analysis and 5 samples from each cultivar was collected for molecular analysis.

Table 1. List of Sampling Locations and Cultivars of *A. carambola* from Tarub Village.

No.	Sampling Locations	*A. carambola* Cultivars
1.	Amelia Starfruit Garden (Station 1)	Dewa, Dewi, Madu, Bangkok Merah, Karangsari
2.	Maulana Starfruit Garden (Station 2)	Dewa, Madu, Bangkok Merah
3.	Sami Starfruit Garden (Station 3)	Dewa, Karangsari

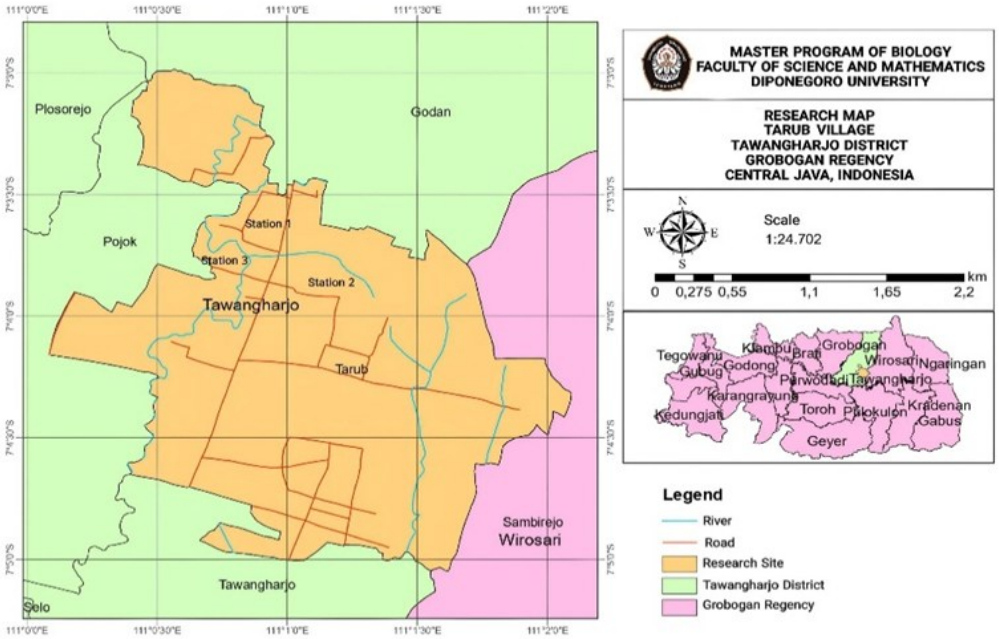


Figure 1. Sampling point, Tawangharjo District, Grobogan Regency, Central Java.

Morphological Characterization

Morphological characterisation was carried out on each cultivar. Each plantation was repeated three times, so the total number of samples was 30 *A. carambola* and three *A. bilimbi*. Furthermore, all samples were measured, and the

morphological characteristics of the stems, leaves, flowers, fruits, and seeds were recorded. The stem diameter was measured on stems with a height of about 10 cm from the ground surface. The selected leaves were of uniform age, healthy, and had no wounds. The colour was determined using the RGB (Red-Green-Blue index) colour index. Flower characterisation was observed using a light microscope connected to the Optilab application, and images were saved for each observation. The list of characters used in this study is presented in Table 2.

Table 2. List of Morphological Characters of *A. carambola* from Tarub Village.

Plant parts	Morphological character
Stem	Habitus, crown shape, stem type, stem position, branching type, stem growth direction, stem shape, stem diameter, stem surface texture, stem colour, nodules on the surface of branches.
Leaf	Leaf type, lateral leaflet structure, tip leaflet structure, base leaflet structure, leaf arrangement, leaflet arrangement, leaf margin, lateral leaf tip angle, tip leaf angle, base leaf angle, leaf texture, upper surface colour of young leaves, lower surface colour of young leaves, upper surface colour of mature leaves, lower surface colour of mature leaves, lateral leaf length, lateral leaf width, tip leaf length, tip leaf width, base leaf length, base leaf width, upper surface texture of leaves, lower surface texture of leaves, number of leaflets, leaflet spacing.
Flower	Flower location, flower sex, complete/incomplete flower, number of petals, corolla arrangement, flower diameter, flower base colour, corolla pattern, flower corolla structure, flower corolla length, flower corolla width, flower stalk colour, flower petal structure, calyx colour, flower calyx length, flower calyx width, flower bud structure, flower bud colour, flower bud length, flower bud width, stamen shape, stamen colour, stamen length, stamen shape, pistil colour, pistil length, corolla pattern, flower stalk characteristics.
Fruit	Fruit type, young fruit skin colour, mature fruit skin colour, fruit skin surface, fruit tip angle, fruit length, fruit diameter, fruit weight, fruit rib/rib edge colour, mature fruit rib/rib depth, fruit stalk characteristics, mature fruit flesh colour, fruit flesh texture, seed shape, number of seeds, seed length, seed diameter, seed texture, seed colour, fruit storage capacity after harvest, young fruit taste, mature fruit taste, productivity/tree/year, harvest age since flowering.

Molecular Analysis

DNA isolation followed the manufacturer's protocol of the Quick-DNA Magbead Plus Kit (ZymoResearch, D4082). ITS Barcode DNA amplification was performed by polymerase chain reaction (PCR) technique using (GGAAGKARAAGTCGTAACAAGG) primer pair ITS-u1F and ITS-u4R (RGTTTCTTTTCCTCCGCTTTA) (Cheng et al. 2016). The primers were synthesized by Integrated DNA Technologies, Singapore. PCR was performed in a volume of 50 µL with the composition of MyTaq HS Red Mix 25 µL, ITS forward primer 2 µL, ITS reverse primer 2 µL, DNA template 8 µL, sterile ddH2O 13 µL. The amplification stages in the PCR program include pre-denaturation at 95 °C for 2 minutes, denaturation at 95 °C for 30 seconds, annealing at 52 °C for 30 seconds, extension at 72 °C for 30 seconds, and final extension at 72 °C for 5 minutes. The denaturation, annealing, and extension stages were repeated 35 times. The samples successfully isolated and amplified were then subjected to electrophoresis using a 1 % agarose solution. The amplicon results were then sent for sequencing. The sequencing process was carried out at 1st BASE (Malaysia) using the Sanger sequencing method.

Data Analysis

The morphological data of starfruit cultivars were arranged in a table and characterised, followed by scoring and standardisation. Based on binary data, the similarity index between starfruit cultivars was calculated to reconstruct the phenogram using MVSP Version 3.1 software (Kovach 2007; Wulandari et al. 2024). The Simple Matching Coefficient calculated the similarity index (Sokal & Michener 1958). The analysis results were in the form of a phenogram using the UPGMA method (Sneath & Sokal 1973).

Molecular data analysis was carried out using the MEGA 11 and BioEdit applications (Hall et al. 2011; Tamura et al. 2021). The contig sequences that had been obtained were then BLAST (Basic Local Aligment Sequence) https://blast.ncbi.nlm.nih.gov/Blast.cgi analysis on the NCBI (National Centre for Biotechnology Information) https://www.ncbi.nlm.nih.gov/ page. Phylogenetic tree reconstruction used the MEGA 11 application with the Maximum Likelihood method and the Jukes-Cantor model with a bootstrap value of 1000 times (Jukes & Cantor 1969; Tamura et al. 2021). Genetic variation analysis was carried out using the DnaSP6 application to determine haplotypes in the *A. carambola* sequence.

RESULTS AND DISCUSSION

Morphological Variation of Sweet Starfruit Cultivars (*A. carambola* L.) in Tarub Village, Grobogan Regency

The characterization results show variations in morphological characters in sweet starfruit cultivars Dewa, Dewi, Madu, Bangkok Merah, and Karangsari in Tarub Village, Grobogan Regency. Each cultivar has different morphological character variations as a differentiator from other cultivars. Morphological variations among starfruit cultivars include crown shape, stem diameter, leaflet arrangement, leaf apex, flower corolla pattern, stamen shape, pistil shape, fruit colour, and fruit taste.

The crown shape of the five *A. carambola* cultivars has three variations: rounded, picturesque, and spreading. The rounded crown shape is found in the Dewa and Dewi cultivars. The picturesque crown shape is only found in the Madu cultivars. The spreading crown shape is found in the Bangkok Merah and Karangsari cultivars. The variations in the crown shape of *A. carambola* are presented in Figure 2.

The rounded tree crown is shaped like a ball and tends to be symmetrical, with regular and even branches. The picturesque tree crown is asymmetrical, with branches that grow irregularly. Meanwhile, the spreading crown shape has branches that grow horizontally (Booth 1983). The shape of the crown affects the process of absorbing sunlight for photosynthesis and transpiration. A more open crown shape will facilitate the process of absorbing sunlight optimally; conversely, if the crown is too dense, it will inhibit the absorption of sunlight, and the efficiency of photosynthesis will be reduced (Niinemets 2023).

The variation in stem diameter size has three variations, namely small (stem diameter 18-29 cm), medium (stem diameter 30-39 cm) and large (stem diameter 40-50 cm). Large stem diameter is found in the Dewi cultivars. Medium stem diameter is found in the Dewa cultivars and Madu cultivars. Small stem diameter is found in the Bangkok Merah cultivars and the Karangsari cultivars. Stem diameter variation affects plants' ability to support structures, to transport water and nutrients, store energy, and to adapt to environmental conditions. Stems with larger diameters indicate that the vascular tissue, namely the xylem and phloem, is also increasing. This allows storing more water and nutrients to support plant growth (Rahmawati et al. 2025). The variation in stem diameter of *A. carambola* is presented in Figure 3.

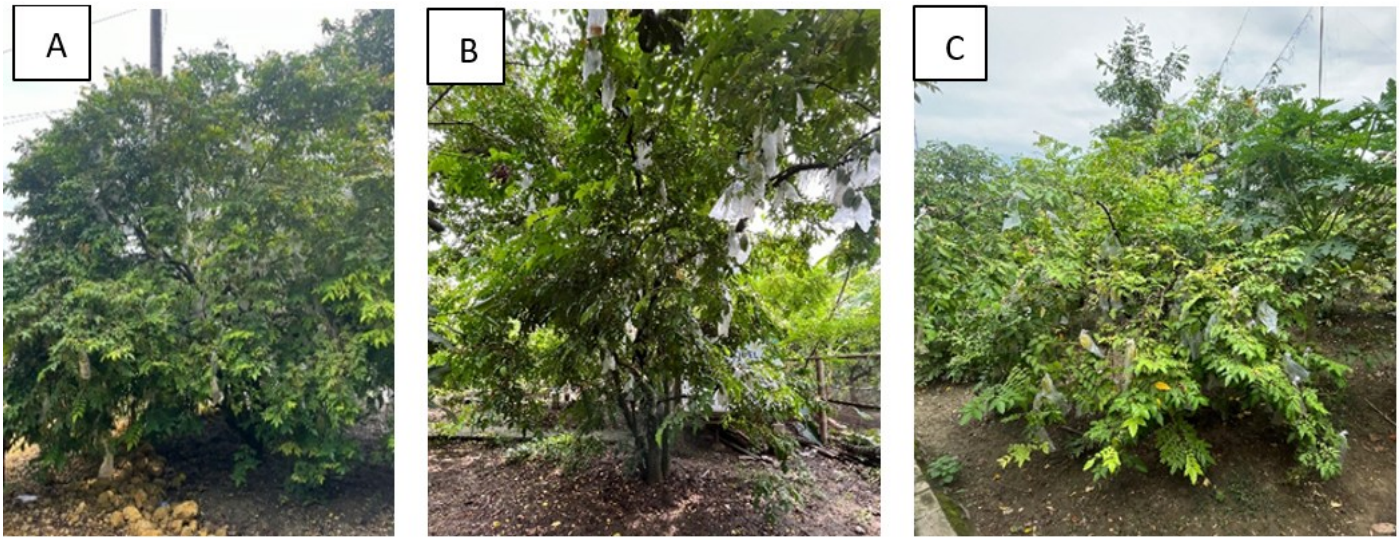


Figure 2. Variations in the shape of the crown *A. carambola*, (A) rounded, (B) picturesque, dan (C) spreading.



Figure 3. Variation in stem diameter of *A. carambola*, (A) small, (B) medium, and (C) large.

The sweet starfruit leaves of the five cultivars in Tarub Village, Grobogan Regency, exhibit several trait variations, including leaf arrangement and apex folii. There are three variations in leaflet arrangement in *A. carambola* cultivars: alternate, subopposite, and opposite. Alternate leaflet arrangements are found in Dewi, Bangkok Merah, and Karangsari cultivars. The subopposite leaflet arrangement is found in the Madu cultivars, and the opposite is found in the Dewa cultivars. Alternate arrangement, where the leaflets are arranged alternately along the leaf stalk, with each leaflet growing on opposite sides. Subopposite arrangement, where the leaflets are arranged almost opposite and slightly shifted from a symmetrical position. Meanwhile, opposite arrangement, where the leaflets are arranged symmetrically opposite on the same side of the leaf stalk. Variations in leaflet arrangement play a role in increasing the efficiency of sunlight absorption to support the photosynthesis process in plants (Ellis et al. 2009). Variations in the leaflet arrangement of the *A. carambola* are presented in Figure 4.

There are two variations of the apex folii, namely acuminatus and acutus. Apex folii acutus is found only in the Madu cultivars. Meanwhile, apex folii acuminatus is found in the Dewa, Dewi, Bangkok Merah, and Karangsari cultivars. The main difference between apex folii acuminatus and acutus is that "acuminatus" refers to a leaf tip that is gradually pointed and narrow, while "acutus" refers to a leaf tip that is abruptly sharp and pointed. Apex

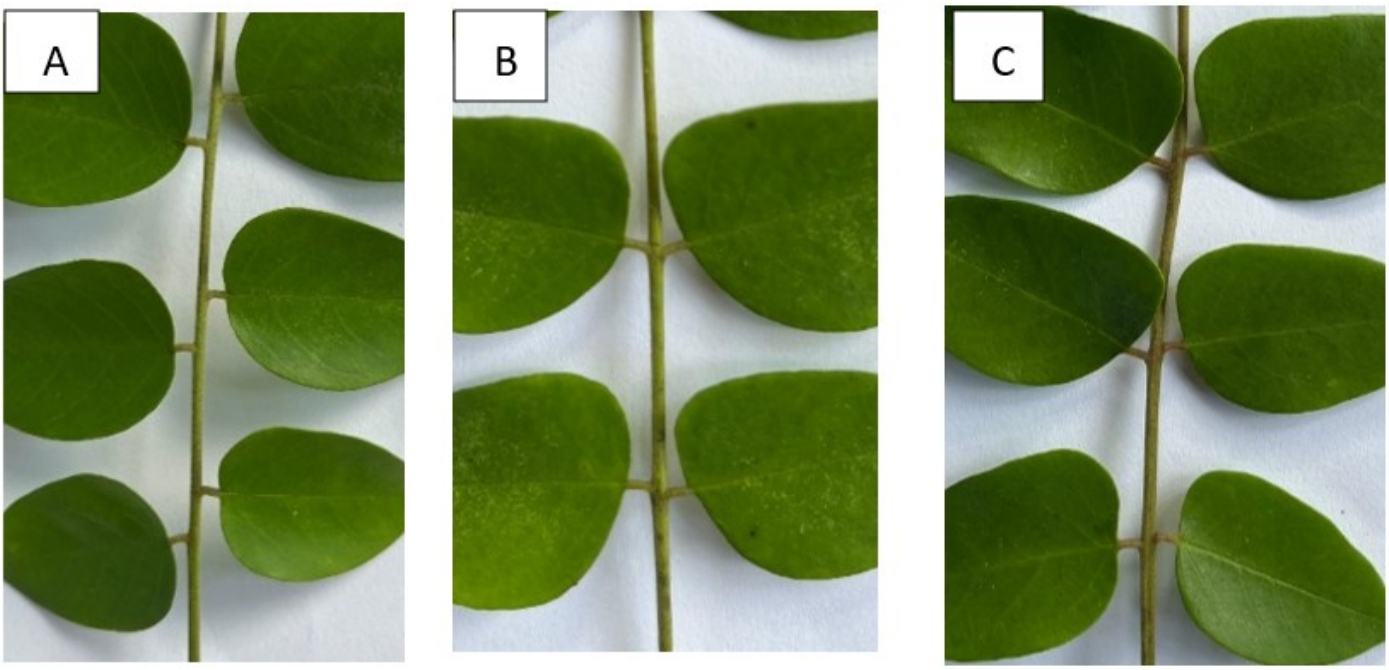


Figure 4. Variations in leaflet layout, (A) alternate, (B) opposite, (C) subopposite.

folii affects the orientation of the leaf to sunlight. The apex folii can optimise the amount of light received by the leaf for the photosynthesis process (Wang et al. 2022). Variations in the lateral leaf tip angle are presented in Figure 5.

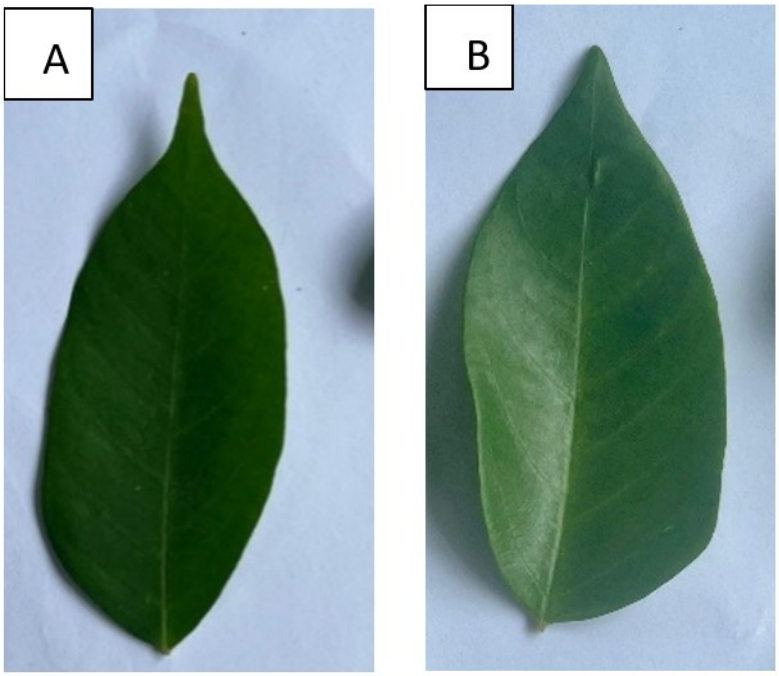


Figure 5. Variations in the apex folii, (A) acutus, (B) acuminatus.

The flowers of the sweet starfruit cultivars in Tarub Village, Grobogan Regency, exhibit variations in petal pattern, stamen shape, and pistil shape. The flower corolla pattern has three variations: magenta-plum, medium orchid-white, and purple-white. The magenta-plum flower corolla pattern colour is found in the Dewa and Karangsari cultivars. The medium orchid-white flower corolla pattern colour is found in the Dewi and Bangkok Merah cultivars. The purple-white flower corolla pattern colour is found in the Madu cultivars. The colour variations of the flower corolla pattern are presented in Figure 6.

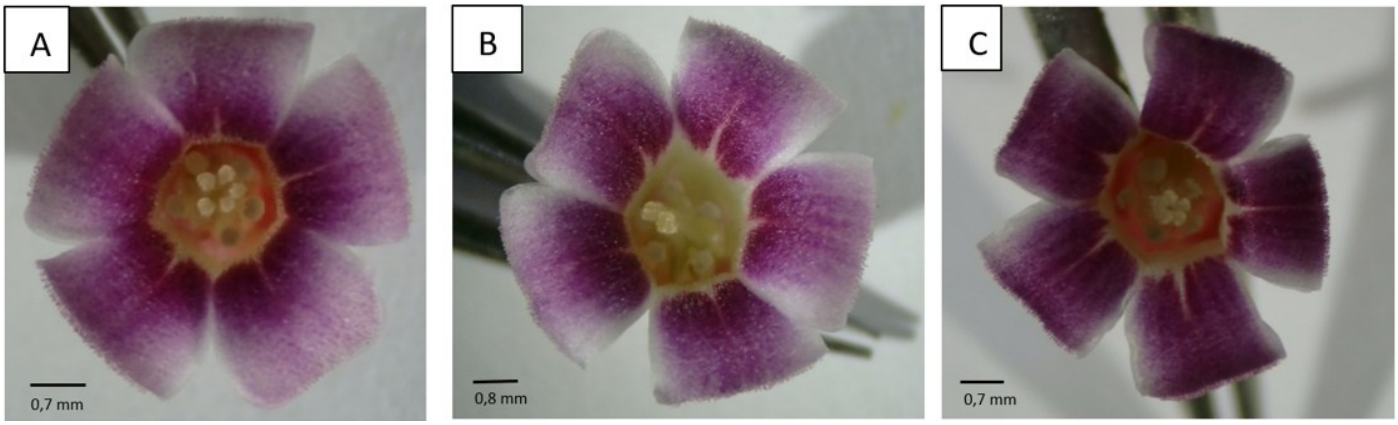


Figure 6. Colour variations of flower corolla patterns: (A) magenta-plum, (B) medium orchid-white, (C) purple-white.

The flower corolla's colour variations are designed to attract the attention of pollinating insects, thereby increasing the chances of pollination and reproduction. In addition, differences in flower corolla colour can indicate species diversity, which is essential for plant identification (Trunschke et al. 2021).

The stamen shape of the five *A. carambola* cultivars has three variations, namely straight, straight hooked, and curved. The straight stamen shape is only found in the Dewi cultivars, and the straight hooked stamen shape is only found in the Madu cultivars. Meanwhile, the curved stamen shape is found in the Dewa, Bangkok Merah, and Karangsari cultivars. The variation in stamen shape is presented in Figure 7.

The variation in stamen shape supports the reproduction process and plant survival. The variation in pistil shape adapts to various pollination methods to ensure successful plant reproduction (Shivanna 2020).

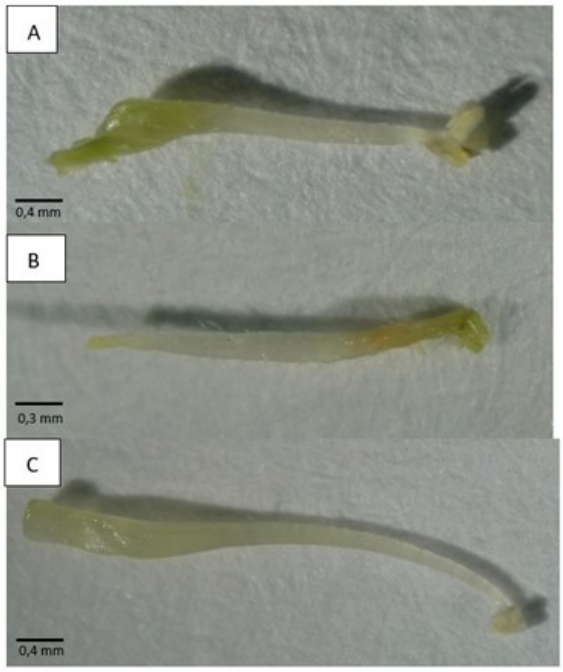


Figure 7. Stamen shape variation, (A) Straight, (B) Straight hooked, (C) Curved.

The pistil shape of the five *A. carambola* cultivars has two variations: straight and wavy. The straight pistil shape is only found in the Dewa cultivars. Meanwhile, the wavy pistil shape is found in the Dewi cultivars, Madu cultivars, Bangkok Merah cultivars, and Karangsari cultivars. The variation of pistil shape is presented in Figure 8.

Pistils in plants have variations in shape that play an essential role in the reproduction process and survival of plants. The primary function of the pistil is to receive pollen from the stamens, which then carry out the pollination process and allow fertilisation to occur. This variation in pistil shape supports pollination efficiency (Shivanna 2020).

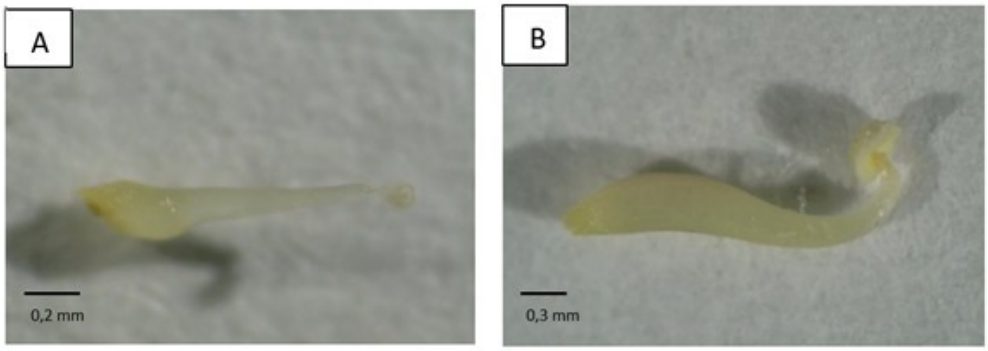


Figure 8. Variation of pistil shape, (A) Straight, (B) Wavy.

The mature fruit of the five *A. carambola* cultivars has four colour variations: orange, goldenrod, gold, and yellow. The Dewa cultivars have a goldenrod colour, the Dewi cultivars is gold, the Madu cultivars is orange, the Bangkok Merah cultivars is goldenrod, and the Karangsari cultivars is yellow. The taste of mature *A. carambola* fruit has three variations: very sweet, sweet, and sweet with a slightly sour taste. The Madu cultivars has a very sweet flavour. The Bangkok Merah cultivars have a sweet taste. Meanwhile, the cultivars that have a sweet with a slightly sour taste are the Dewa cultivars, the Dewi cultivars, and the Karangsari cultivars. Various genetic and environmental factors physiologically and morphologically influence the colour and taste variations of star fruit. Physiologically, the taste of star fruit also varies depending on the fruit's sugar content (such as glucose and fructose) (Ali et al. 2021). The morphology of mature *A. carambola* fruit is presented in Table 3 and Figure 9.

Table 3. Colour and Taste Variations of Fruit.

No.	*A. carambola* Cultivars	Fruit Colour	Fruit Taste
1.	Dewa	Goldenrod	Sweet with a slightly sour taste
2.	Dewi	Gold	Sweet with a slightly sour taste
3.	Madu	Orange	Very sweet
4.	Bangkok Merah	Goldenrod	Sweet
5.	Karangsari	Yellow	Sweet with a slightly sour taste

Phenetics Analysis

Based on the characteristics of the five cultivars of *A. carambola* from Tarub Village, Grobogan Regency, a phenetic analysis was carried out using the UPGMA method (Sneath & Sokal 1973). The similarity index was calculated using the Simple Matching Coefficient (Sokal & Michener 1958). The phenogram of *A. carambola* cultivars is presented in Figure 10.

The phenogram of 30 samples of *A. carambola* cultivars and three (3) outgroup samples of *A. bilimbi* had a similarity coefficient between 0.25-0.98. Based on the 80 % phenon line, four *A. carambola* clusters and one outgroup cluster were obtained. The analysis showed that cluster 1 consisted of nine samples of *A. carambola* cultivars Dewa with a similarity coefficient of 0.97. Cluster 2 consisted of the Dewi cultivars with a similarity coefficient of 0.95. Cluster 1 and cluster 2 are grouped with a similarity coefficient 0.76,



Figure 9. Fruit Cultivars *A. carambola*, (A) Dewa, (B) Dewi, (C) Madu, (D) Bangkok Merah, (E) Karangsari.

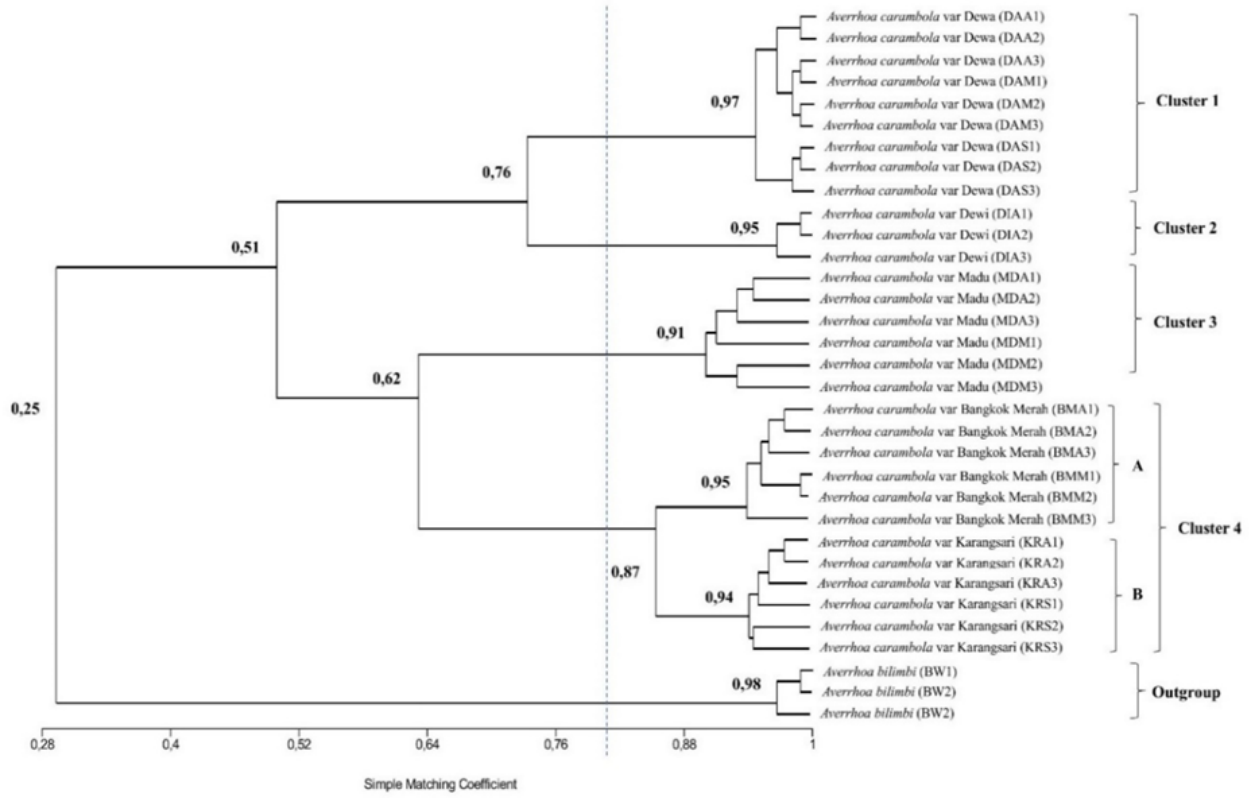


Figure 10. Phenogram of *A. carambola* cultivars based on the Simple Matching Coefficient similarity index.

indicating that the Dewa cultivars is closely related to the Dewi cultivars compared to other cultivars. Similarities in character between cluster 1 and cluster 2 include crown shape, leaf tip angle (lateral leaves), the upper surface colour of mature leaves, flower corolla pattern, flower petal colour, stamen colour, pistil colour, and mature fruit flavour.

Cluster 3 consists of the Madu cultivars with a similarity coefficient of 0.91. Cluster 4 consists of two sub-clusters, including sub-cluster A, namely the Bangkok Merah cultivars with a similarity coefficient of 0.95 and sub-cluster B, consisting of the Karangsari cultivars with a coefficient of 0.94. Sub-clusters A and B are grouped with a similarity coefficient of 0.87, indicating that the Bangkok Merah and Karangsari cultivars are closely related. Meanwhile, the similarity coefficient between the Madu cultivars (cluster 3), the Bangkok Merah cultivars and the Karangsari cultivars (cluster

4) is 0.62. The similarity of characters between cluster 3 and cluster 4 is the leaf tip angle (base leaf), number of leaflets, stamen colour, pistil shape, pistil colour, and fruit flesh texture.

The outgroup in this study, *A. bilimbi*, is located in the lowest cluster with a similarity coefficient 0.98. The similarity coefficient between *A. carambola* and *A. bilimbi* is 0.25. The outgroup functions as a comparison in plant systematics to form a clear and accurate phenogram in classifying a species' kinship (Wilberg 2015). A total of 71 out of 88 morphological characters were found to differ between *Averrhoa carambola* and its outgroup *A. bilimbi*. These include variations in canopy shape, stem diameter, stem surface texture and color, presence of lenticels, leaflet shape and arrangement, leaf apex and base morphology, leaf texture and colour (both young and mature), floral structures (such as corolla arrangement, flower diameter, petal and calyx shape, size, and colour), stamen and pistil form, petal patterns, bud morphology, and inflorescence position. Significant differences also occur in fruit characters including skin colour (immature and mature), surface texture, fruit angle and ridges, fruit size and weight, flesh texture and colour, seed morphology, flavor (young and ripe), postharvest shelf life, productivity, and time to harvest after flowering. *A. bilimbi* was chosen as an outgroup because it is still in the same *Averrhoa* genus. In general, the samples are grouped based on the similarity of cultivars. The Bangkok Merah and Karangsari cultivars are closely related, with a similarity coefficient of 0.87. The Dewa cultivars is closely related to the Dewi cultivars. Meanwhile, the Madu cultivars is in cluster 3. The similarity coefficient value describes the ratio between characters similar to the number of characters. The more similarity of characters, the greater the similarity coefficient, which indicates that the kinship relationship is also closer. Kinship relationships are indicated by many character similarities (Sneath & Sokal 1973).

Molecular Analysis

A. carambola samples of Dewa, Dewi, Madu, Bangkok Merah, and Karangsari cultivars were successfully amplified using Internal Transcribed Spacer (ITS) DNA barcodes. The visualized DNA was by the target of the ITS sequence, which was around 600-700 bp. The five contig sequences of the *A. carambola* samples were BLAST analysis on the NCBI GenBank page. The BLAST results (Table 4) show a list of possible genes, and the sequence with the highest per cent identity value (90-100 %) was selected, with the maximum value being better. The higher the percent identity value, the more significant the sample's homology (similarity) level with the comparison sequence. The query cover value with the highest percentage (90-100 %) shows better and more accurate results. The selected query cover data should have a sequence that is not too long or short (Pearson 2013). The query cover value must also include specific target genes in the identification process (Pal et al. 2017).

A total of five (5) *A. carambola* sample sequences that have been contig, 12 BLAST comparison sequences from NCBI Genbank, and one sequence from the *Oxalis* genus as an outgroup were then aligned using the ClustalW method in the MEGA 11 application. The alignment results of all trimmed sequences have an average length of 645 bp. This is by the Internal Transcribed Spacer (ITS) region, which is 600-700 bp in size (Deb & Kamba 2022).

The reconstruction of the *Averrhoa* phylogenetic tree is presented in Figure 11. The phylogenetic tree was reconstructed using a combination of the Maximum Likelihood (ML) method with the Jukes-Cantor model and Neighbor Joining (NJ) with the p-distance model and a bootstrap value of 1000x (Jukes & Cantor 1969; Tamura et al. 2021).

Maximum Likelihood is a statistical method that uses sequence

Table 4. List of BLAST result sequences used in the phylogenetic tree.

No.	Sample	BLAST Result	Query Cover	Percent Identity	Accession
1.	*A. carambola* Dewa	*Averrhoa carambola* (Bogor)	100 %	99.86 %	MZ014947.1
		Averrhoa carambola (Bogor)	99 %	100 %	EU436862.1
		Averrhoa carambola (Bogor)	98 %	99.71 %	MW507602.1
		Averrhoa carambola (India)	93 %	99.55 %	MN511172.1
		Averrhoa carambola (China)	100 %	98.02 %	KU569491.1
		Averrhoa carambola (South Africa)	100 %	95.49 %	EU436863.1
		Averrhoa bilimbi	99 %	95.31 %	MZ014946.1
		Averrhoa dolichocarpa	99 %	95.01 %	MZ014942.1
		Averrhoa dolichocarpa	99 %	94.87 %	MW507601.1
		Averrhoa carambola (India)	87 %	95.00 %	KR905603
		Averrhoa leucopetala	100 %	93.69 %	MZ014945.1
		Averrhoa leucopetala	99 %	93.90 %	MW507598.1
2.	*A. Carambola* var. Dewi, Madu, Bangkok Merah, and Karangsari are identical to the BLAST results of *A. carambola* var. Dewa				

comparison in the character-based alignment process by considering the possible values in each tree (Yang & Rannala 2012; Chika 2024). Neighbor-Joining reconstructs a phylogenetic tree based on the genetic distance (distance) owned by the sequences analysed (Fernández et al. 2023). The reconstruction of the phylogenetic tree is divided into four ingroup clades and one outgroup. *A. carambola* cultivars Bangkok Merah and Karangsari are very closely related. The Dewa cultivars is closely related to the Dewi cultivars. *A. carambola* cultivars Dewa, Dewi, Bangkok Merah, and Karangsari from Grobogan Regency are closely related to *A. carambola* originating from Bogor. Meanwhile, the *A. carambola* cultivars Madu from Grobogan Regency is closely related to the *A. carambola* originating from India with accession number MN511172.1 and the Madu cultivars is separated from the other four cultivars. *A. carambola* originating from China and South Africa is located at the bottom line among the other *A. carambola* sequences.

The results of the genetic distance analysis between starfruit cultivars from Grobogan Regency ranged from 0.003-0.011. The lowest genetic distance (0.003) was between the Dewa, Dewi, Bangkok Merah, and Karangsari cultivars. Meanwhile, the genetic distance between the Madu and Dewa cultivars was 0.008. The Madu cultivars had a distance of 0.011, with the Dewi, Bangkok Merah, and Karangsari cultivars. These results align with the phylogenetic tree, which shows that the Madu cultivars is separated from the other four cultivars.

This grouping occurs because there are nucleotide variations in each sequence. *A. carambola* cultivars Madu from Grobogan Regency is separated from the other four cultivars sequences because it has more varied nucleotides than other samples and indicates the spread and evolution in the South Asian region. *A.carambola* cultivars Dewa, Dewi, Bangkok Merah, and Karangsari from Grobogan Regency have close kinship with *A. carambola* originating from Bogor and India, which can be due to having similar haplotypes and originating from the same ancestor in the Asian region. A haplotype is a collection of alleles in an organism that are inherited together from one parent. The arrangement of gene sequences with similar haplotypes indicates that the sequences originate from the same ancestor (Soltis & Soltis 2021).

Based on the research results, the phylogenetic tree reconstruction based on the Maximum-Likelihood method showed a high bootstrap value (81-100 %) and a low value (43-60 %). Meanwhile, the Neighbor-Joining method showed a high bootstrap value (76-100 %) and a low value (45-61 %). The bootstrap value is a number written on each branch of the phylogenetic tree.



Figure 11. Reconstruction of *Averrhoa* phylogenetic tree using Maximum Likelihood (ML) with Jukes-Cantor model, bootstrap value 1000x and Neighbor Joining method with p-distance model, bootstrap value 1000x (* sign is *A. carambola* sample from Grobogan Regency.

In phylogenetics, the bootstrap value (<50 %) is low, and (>70 %) shows a high value and indicates grouping stability. The bootstrap value indicates the accuracy value at each branch. The greater the bootstrap value, the higher the level of accuracy of the phylogenetic tree formed (Nei & Kumar 2000).

The results of the alignment of the *A. carambola* species in the ITS region showed that the length of the entire sequence was 629 bp, consisting of 585 monomorphic bp and 44 polymorphic bp (34 bp singleton sites and 10 bp parsimony sites), 12 gaps in the form of deletions and insertions (Table 5). A gene is considered monomorphic when the entire sequence has the same

nucleotide sequence at a particular position. In contrast, a gene is considered polymorphic if there is genetic variation in the nucleotide sequence at a specific position (Weinberg 2013).

Table 5. Total genetic variation of *Averrhoa carambola* based on the ITS region.

Variation	Number
Number of haplotypes	10
Variable site	44
Singletone site	34
Parsimony site	10
Gap	12
Nucleotide length (bp)	629
Haplotipe diversity (hd)	0,98

The analysis results showed that the haplotype diversity value of all *A. carambola* sequences was 0.98. According to Nei (1972), a low hd value is 0.1-0.4, a medium hd value is 0.5-0.7, and a high hd value is 0.8-1.0. Based on this grouping, the haplotype diversity in *A. carambola* is of high value. The high and low haplotype diversity in *A. carambola* is caused by transitions, transversions, insertions and deletions in the nucleotide sequence. Haplotype diversity plays a significant role in plant breeding by providing genetic variation to improve agronomic characteristics. Haplotype diversity also influences plant resistance, adaptation to changing environmental conditions, and abiotic and biotic threats. The higher the haplotype diversity value, the higher the plant's ability to adapt, be resistant to disease and support the field of plant breeding (Roychowdhury et al. 2023). Haplotype diversity describes genetic variation in a population that plays a role in adapting to environmental changes. Populations with high genetic diversity will also have a greater chance of being exposed to selection pressures, climate change, and disease (Chung et al. 2023). Genetic variation analysis was carried out using the DnaSP6 application to determine haplotypes in the *A. carambola* sequence. Based on the analysis, it is known that the *A. carambola* sequence has 10 haplotypes. The list of *A. carambola* haplotypes based on the ITS region is presented in Table 6.

Table 6. *Averrhoa carambola* haplotypes based on the ITS region.

Haplotipe	Total sample	Sample
Haplotipe 1	2	MZ014947.1 *A. carambola* (Bogor)
		MZ014948.1 *A. carambola* (Bogor)
Haplotipe 2	1	DWA1 *A. carambola* Dewa (Grobogan)
Haplotipe 3	1	DWI1 *A. carambola* Dewi (Grobogan)
Haplotipe 4	1	MDU1. *A. carambola* Madu (Grobogan)
Haplotipe 5	1	BKM1. *A. carambola* Bangkok Merah (Grobogan)
Haplotipe 6	1	KRS1 *A. carambola* Karangsari (Grobogan)
Haplotipe 7	1	MW507602.1 *A. carambola* (Bogor)
Haplotipe 8	1	KU569491.1 *A. carambola* (China)
Haplotipe 9	1	MN511172.1 *A. carambola* (India)
Haplotipe 10	1	EU436863.1 *A. carambola* (South Africa)

Table 6 shows that Haplotype 1 consists of two sequences, namely *A. carambola* originating from Bogor with accession numbers MZ014947.1 and MZ014948.1. Haplotype 2 consists of one sequence, namely *A. carambola* Dewa Cultivars, originating from Grobogan Regency. Haplotype 3 consists of one sequence, namely *A. carambola* Dewi Cultivars, originating from Grobogan Regency. Haplotype 4 consists of one sequence, *A. carambola* Madu Cultivars, originating from Grobogan Regency. Haplotype 5 consists of one

Table 7. Genetic Variation in *Averrhoa carambola* haplotypes in the ITS region.

Nucleotide Site*	10	20	30	40	47
Nucleotide Number**		1111111122	2222223444	4444455555	5666666
	-133456779	1356788901	1112795001	1134800355	6122222
	1846141896	8039089584	7892147691	3574767917	3234567
Hap_1	ACACAACCTT	ACATCCCGAA	CGAATGCGTT	CAACACCACT	-TGTCAG
Hap_2	.....	.....A..	.....	.....	-.....
Hap_3	.....	.....T...	.....	.....	-.....
Hap_4	.....	.....A..	.....	.....	-.TCAGC
Hap_5	.....	.....	.....	..G.....	-.....
Hap_6	.....G..	.....	.....	.....	-.....
Hap_7	.....	.....	....C-....	.....	-.....
Hap_8	.G..C..G..	.T..TTG.GG	.....T...	..GT....T.	-.....
Hap_9	-.....	.....	.....	.....	C.....C
Hap_10	..GGCGTGCC	C.GGAAG.GT	TCGT...ACC	TG..CGTGTC	-C.....

* read horizontally, ** read vertically

Note : . = same nucleotide as above
Red = transition
Blue = transversion
Green = insertion
Orange = deletion

sequence, namely *A. carambola* Bangkok Merah Cultivars, originating from Grobogan Regency. Haplotype 6 consists of one sequence, namely *A. carambola* Karangsari Cultivars originating from Grobogan Regency. Haplotype 7 consists of one sequence, *A. carambola* originating from Bogor Regency with accession number MW507602.1. Haplotype 8 consists of one sequence, *A. carambola*, which originates from China. Haplotype 9 consists of one sequence, *A. carambola*, which originates from India. Haplotype 10 consists of one sequence, *A. carambola*, which originates from South Africa.

In Table 7, the genetic variation among *Averrhoa carambola* haplotypes is presented based on sequence differences identified in the ITS region. The results of morphological and molecular analysis showed a harmonious relationship in determining the kinship of *A. carambola* cultivars. Bangkok Merah and Karangsari cultivars are very closely related morphologically and molecularly. The differences between the two cultivars morphologically include stem diameter, flower base colour, flower petal colour, flower corolla pattern, mature fruit skin colour, number of seeds, seed colour, and mature fruit taste. These two cultivars have differences in genetic variation. The Bangkok Merah cultivars is haplotype 5, which has one mutation in the form of a transition.

Meanwhile, the Karangsari cultivars is haplotype six, which has one mutation in the form of a transversion. This shows that these two cultivars are different even though they are closely related. The Dewa cultivars is closely related to the Dewi cultivars. Meanwhile, the Madu cultivars is separated from the other cultivars because there are more significant differences morphologically and in genetic variation. The variations in morphological characters possessed by the Madu cultivars include crown shape, stem diameter, leaflet layout, the upper surface colour of mature leaves, flower base colour, flower stalk colour, flower bud colour, stamen shape, corolla pattern, mature fruit skin colour, and mature fruit taste.

In terms of molecular analysis, the Madu cultivars has seven variations in transitions at four nucleotides and transversions at three nucleotides. It has a greater genetic distance than the other four cultivars. Morphological and

molecular analysis was carried out so that the data obtained was more accurate, comprehensive, and valid. This study has a high novelty value because it analyzes morphological variations in *A. carambola* cultivars Dewa, Dewi, Madu, Bangkok Merah, and Karangsari. In addition, this study examines the relationship between the five cultivars of kinship. The results of the morphological analysis are supported by molecular analysis, which explores the kinship relationships among the five cultivars based on genetic data. The sequencing results of these cultivars have been uploaded to NCBI GenBank, where the *A. carambola* species from Indonesia is still underrepresented, with only three sequences available. This effort contributes to genetic conservation and the analysis of haplotypes in the five *A. carambola* cultivars from Grobogan Regency.

CONCLUSIONS

The results showed significant morphological and genetic variations between the Bangkok Merah and Karangsari cultivars, indicating that these two cultivars are different, although closely related. Kinship relationships based on morphological and molecular data were also analyzed, namely that the *A. carambola* cultivars Dewa is closely associated with the Dewi cultivars. Meanwhile, the Madu cultivars is separated from other cultivars because there are more significant differences morphologically and in genetic variation. The analysis results indicate that the haplotype diversity of *A. carambola* is high, with a haplotype diversity (hd) value of 0.98. Further research should add samples of *A. carambola* cultivars in Indonesia from many regions.

AUTHOR CONTRIBUTION

All authors have contributed to this paper. S.C. collecting research data and drafting the article; L.K. and N.K.D.C. revising manuscripts and final revision of the manuscript; S.C., L.K., and N.K.D.C. creating research concepts and revising manuscripts.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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