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Molecular Identification Validates Morphological Identification of Javanese Cardamom from Banyumas in Central Java, Indonesia

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Abstract. Cardamom is one of the prominent plants with significant economic value in the Banyumas Regency, Central Java, Indonesia. Although Javanese cardamom is traditionally categorized under *Amomum compactum*, the morphological variations observed create ambiguity about its exact species status. DNA barcoding using the maturase K (matK) and ribulose-bisphosphate carboxylase (rbcL) genes is proven as a reliable technique to elucidate the taxonomic status of morphological variable plant cultivars. This study aimed to characterize cardamom from Banyumas Regency using morphological and molecular approaches for taxonomic status identification and genetic diversity evaluation. The matK and rbcL genes were selected as genetic markers and sequenced using a bidirectional sequencing technique. Morphological examination showed significant color variations at the cardamom stem base. All samples had high genetic identities to reference species in databases and were supported by high query cover and zero e-values. Therefore, molecular characterization, alongside geographic distribution assessment, established that this plant belongs to a single species, *Amomum compactum*. Additionally, the analysis conducted showed a low level of genetic diversity, as evidenced by haplotype and nucleotide diversity. Low-level genetic diversity provides additional data to convince that cardamon in Banyumas Regency belongs to a single species. These results are essential data in seed selection for further cultivation.

Keywords: *Amomum*, barcoding, genetic diversity, homology, similarity.

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INTRODUCTION

Cardamom (*Amomum*) is a spice plant with high economic value as well as essential applications in cooking and medicine, leading to a consistent increase in market demand. Boer et al. (2018) emphasized the economic significance of various cardamon species, including *Amomum compactum* Sol. ex Maton (Javanese cardamom), *A. subulatum* Roxb. (black cardamom), and *A. verum* Blackw. (cardamom from Cambodia and Thailand). Additionally, it was reported that *Amomum* is a medicinal or traded plant that plays a vital role in the forest ecosystem (Juwitaningsih et al., 2020; Nurcholis et al., 2021). It also contains bioactive (skin-lightening agent) that can be utilized in the cosmetic industry (Batubara et al., 2016).

Amomum is the second largest genus after *Alpinia* Roxb. and consists of 300 species across Southeast Asia and Australia (Alkandahri et al., 2021). Furthermore, in international trade, cardamom is known as Cardomum and is divided into three types, namely green, black, and Madagascar cardamom. Green cardamom is called true cardamom and consists of only one species, *Elettaria cardamomum*. Black cardamon comprises four *Amomum* species, including *A. aromaticum, A. compactum, A. subulatum, and A. testaceum*. Madagascar cardamom has three Aframomum genus species, namely *A. angustifolium, A. corrarima*, and *A. melegueta* (Silalahi, 2017). Meanwhile, Indonesia grows at least 20 cardamom types, one of which is *Amomum compactum* (Javanese cardamom) with high economic value (Juwitaningsih et al., 2020).

Over the past two decades, Cardamom production has declined substantially in several countries due to factors such as climate change, the emergence of very deadly diseases, and the

lack of varietal resistance. Efforts to overcome this situation include conducting genetic improvement in plants. However, Cardamom cultivars are generally characterized based on morphology, necessitating the use of molecular markers to support the identification process (Subba et al., 2021).

Jain et al. (2017) and Aftab et al. (2020) reported that identifying plant accessions based on morphological characteristics proves challenging due to the complexity of classification. The limitation of morphological identification is the inability to obtain detailed descriptions for closely related species. Additionally, traditional identification methods face obstacles because plants are easily influenced by the ecological environment and development stage.

Ho et al. (2021) and Wattoo et al. (2016) stated that DNA barcoding showed superior performance when compared with traditional identification through taxonomic and morphological characteristics. Additionally, Aftab et al. (2020) reported traditional methods to be highly problematic during cryptic species examination. The chloroplast genes ribulose-bisphosphate carboxylase (rbcL) and maturase K (matK) have recently been recognized as plant DNA barcode regions by the Consortium for the Barcoding of Life (CBOL) (Gong et al., 2022; Gong et al., 2021). Previous studies reported the reliability of matK and rbcL genes as barcode markers (Wattoo et al., 2016; Ho et al., 2021; Singh et al., 2021). According to Subba et al. (2021), matK locus and rbcL were identified as promising candidates for barcoding large cardamom cultivars.

Genetic diversity is an important attribute of plants in the nature. Plants' genetic diversity can be assessed using molecular markers (Bandari et al., 2017). The matK and rbcL genes were also common markers in genetic diversity and population genetic study of plants (Bieniek et al., 2015; Khan et al., 2022). Bieniek et al. (2015) proved that matK and rbcL genes showed high sequence variation in Triticeae tribe (Poaceae). Similarly, Khan et al. (2022) reported high genetic diversity in rbcL and matK genes of *Ulmus villosa*. Therefore, it assumed that matK and rbcL genes are also reliable markers for the population genetic study of cardamom in Banyumas Regency.

The currently available information about cardamom cultivars in the Banyumas Regency is limited. The only study on *A. compactum* was about genetic diversity but based on isozyme diversity (Setyawan et al., 2014). No study is available on Java cardomen based on DNA markers, especially barcoding and genetic diversity analysis. Therefore, this study aimed to identify the cultivars based on morphological and molecular characteristics. Molecular characterization was carried out by DNA barcoding of both matK and rbcL genes. The results are expected to contribute valuable insights to the database of cardamom cultivars in Indonesia and serve as a foundation for effective varietal resistance management.

METHODS

Study area

Amomum accessions for this study were collected from five sampling locations in Banyumas Regency, Central Java, Indonesia. These locations included Sunyalangu, Dawuhan Kulon and Baseh, Karangtengah, as well as Banjarsari Villages in Districts of Karanglwas, Kedungbanteng, Baturraden, and Sumbang, respectively (Figure 1). The geographic positions of each sampling location are presented in Table 1.

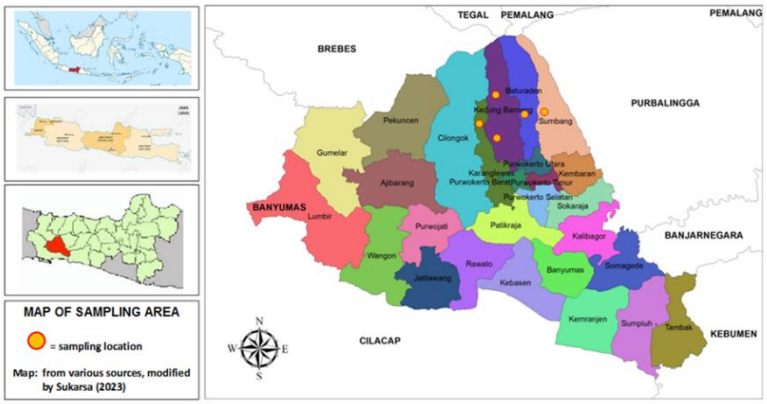

Figure 1. Map of Banyumas Regency showing sampling locations (orange circles).

Table 1. The geographic position of each sampling location

No	Location	Geographic position
1	Sunyalangu	7°21’48.9”S 109°10’25.4”E
2	Dawuhan Kulon	7°22’53.7”S 109°11’31.8”E
3	Baseh	7°22’08.1”S 109°11’09.4”E
4	Karangtengah	7°21'07.0"S 109°13'32.8"E
5	Banjarsari	7°21'33.7"S 109°15'18.1"E

Table 2. Code of sample and its code from each location

No	Location	Sample code	Sample name
1	Banjarsari	2540-1 and 2632-6	Kap-1 and Kap-6
2	Karangtengah	2540-2 and 2632-7	Kap-2 and Kap-7
3	Dawuhan Kulon	Kap and 2632-8	Kap and Kap-8
4	Baseh	2540-3 and 2632-9	Kap-3 and Kap-9
5	Sunyalangu	2540-4 and 2632-10	Kap-4 and Kap-10

Plants material

A total of ten *Amomum* accessions were examined, with two specimens being identified from each location as presented in Table 2.

Morphological characterization

Morphological characterization included observing the features of plant stems, leaves, flowers, and fruits as explained by Droop & Newman 2014; de Boer et al. (2018) and Karunarathne et al. (2021). The observation results were presented in images and described following the method of Iroka et al. (2015).

Molecular characterization

Molecular characterization was performed using both MatK and rbcL as the genetic markers. The extraction of total genomic DNA and amplification of the selected genetic marker was conducted at the Genetic Laboratory of PT. Genetika Science Indonesia by the molecular procedures outlined by the company. Subsequently, the used marker was subjected to bi-directional sequencing at 1st BASE laboratory in Kuala Lumpur, Malaysia using the Sanger method.

Data analysis

Morphological data were analyzed descriptively by comparing the observed features of the sample with reference *Amomum* characteristics (Droop & Newman 2014; Iroka et al., 2015; de Boer et al., 2018; Karunarathne et al. 2021). Additionally, the sequences of MatK-rbcL genes were manually edited and trimmed in Bioedit 7.0 (Hall 2011). The final results were translated into functional sequences through the online software ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder) with specified search parameters consisting of minimal ORF length (300 nucleotides), standard genetic code, any sense start codon, and ignore nested ORF. Online verification against available sequences in GenBank was conducted to ensure correctness using a basic local alignment search tool (BLAST). Furthermore, the taxonomic status of the cardamom accessions was determined based on genetic identity and similarity to reference species, with a threshold of 99% genetic identity being considered as a species border. In cases where gene identity showed similar percentages to more than one conspecific reference, taxonomic status was determined based on the geographic range of the species. Statistical calculations of the number of nucleotide bases, as well as total genetic diversity based on polymorphism, nucleotide, and haplotype diversity, were performed using Arlequin 3.5 software (Excoffier & Lischer 2010).

RESULTS AND DISCUSSION

Morphological characterization

The results of morphological observations of cardamom plants originating from five villages in the Banyumas Regency, Central Java, Indonesia showed variations in stem base color, number of flower heads, size of leaves, and flower color. In general, the plants grew in clusters with false stems, which were round and covered by midribs.

The stem base of cardamom plants from the sampling locations contained reddish-green and greenish-white midribs. Samples from the villages of Dawuhan Kulon, Banjarsari, and Karangtengah had reddish-green midribs at the stem base. In contrast, those from the Baseh and Sunyalangu villages had greenish-white and reddish-green midribs (Figure 2). Plants with greenish-white

stem bases were found in Kap-9 samples from Baseh and Kap-10 from Sunyalangu. The stem height varied, ranging from 1-2 m for cardamom with reddish-green branches and 1.5-3.5 m for those with greenish-white stem bases.

According to Figure 3, the leaves in all samples contained relatively the same shape and color. Each of these appeared single, green, lanceolate, with slightly tapered tips and bases. Furthermore, the leaves were arranged in a spread-out pattern, with flat and slightly wavy edges, as well as green to yellowish-green color. Samples with reddish-green stem bases had shorter and narrower leaves compared to those with greenish-white stem bases.

The morphology of flowers and fruits in *Amomum* is quite diverse, and the structure of both parts plays a significant role in identifying the species. Previous studies proved that flower and fruit morphology are among the important characters for plant identification (Ganie et al., 2015; Sunandar & Kurniasih, 2019). The cardamom flowers from the samples observed generally showed similarities, including being attached to the stem base, compound in appearance, and tuber-shaped. The flowers had a very short stalk, tubular corolla, elliptical anthers, and hairless pistil. This tubular shape correlated to the report of Droop & Newman (2014) for the *Amomum Roxb.* flower from Sumatra (Figure 4), with a labellum side also covered by the dorsal corolla lobe.

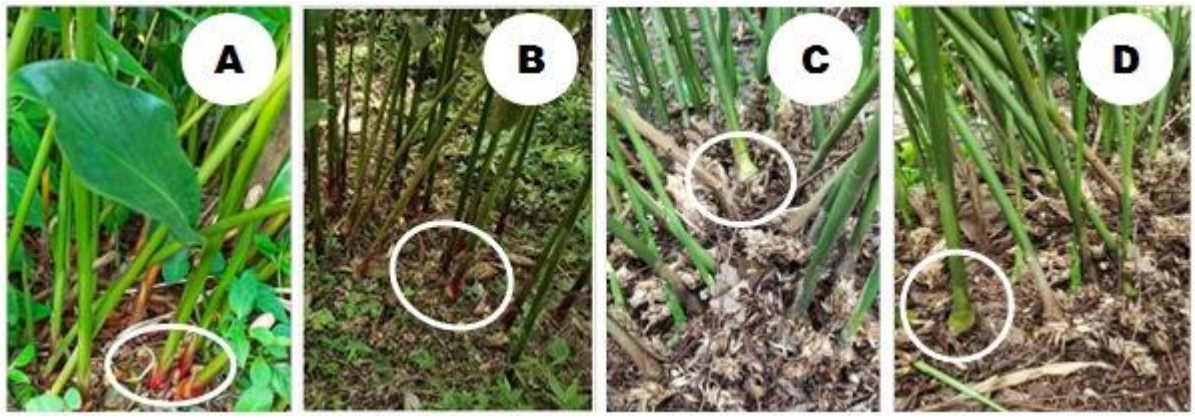

Figure 2. Color stem base of Banyumas Cardamom

Remarks:
Kap (A) and Kap-7(B), reddish-green stem base
Kap-9(C) and Kap-10 (D), greenish-white stem base

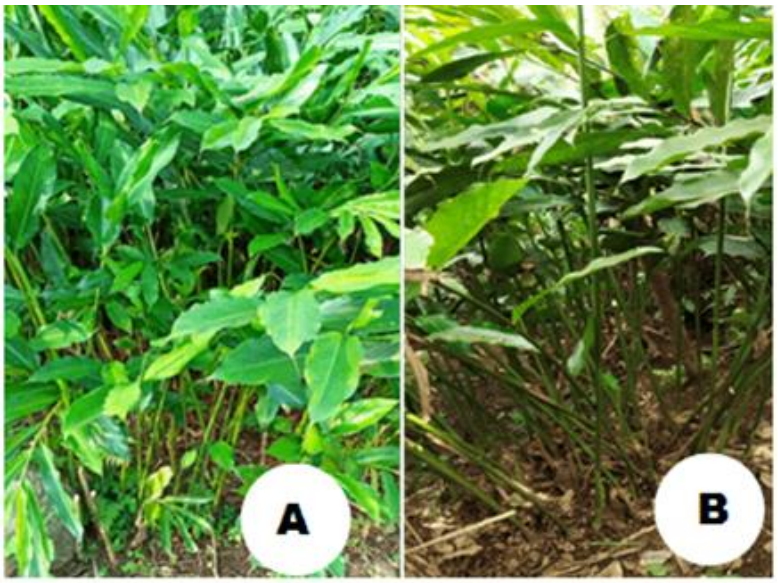

Figure 3. General morphology of leaves of Kap (A) and Kap-9 (B) samples

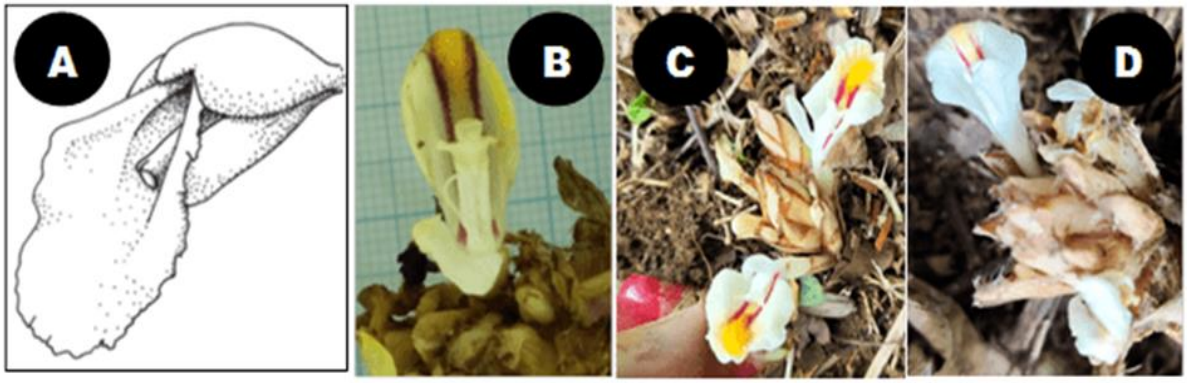

Figure 4. Flower petal shape and color

Remarks:
A. Schematic diagram of the flower crown of *Amomum Roxb.* from Sumatera (Droop & Newman, 2014)
B. Form and color of the flower crown of *Amomum* from Dawuhan Kulon Village (Kap)
C. Form and color of the flower crown of *Amomum* from Karangtenga Village (Kap-7)
D. Shape and color of the flower crown of *Amomum* from Baseh Village (Kap-9)

The observed flower crowns showed color variations, specifically the Kap sample from Dawuhan Kulon Village had a reddish-green stem base and yellowish-white flowers, with two parallel dark red grooves and yellow middle (Figure 4B). Furthermore, the Kap-7 sample from Karangtengah Village was found on a plant that had a reddish-green stem base. These were white flowers comprising two parallel pink lines, a yellowish-white middle, and a slightly wider tip with a solid yellow color (Figure 4C). The Kap-9 sample from Baseh Village had a white flower crown, with two parallel pink lines and a white middle. The ends were not comprehensive and appeared to be faintly yellow (Figure 4D), while the sources were plants with greenish-white stem bases. The history of cardamom classification showed that only minor attention was provided to fruit morphology. Several species have been reported without detailed fruit descriptions (Droop & Newman 2014). However, Ganie et al. (2015) and Sunandar & Kurniasih (2019) established that fruit morphology an important characteristics capable of reflecting the genetic structure of the genus.

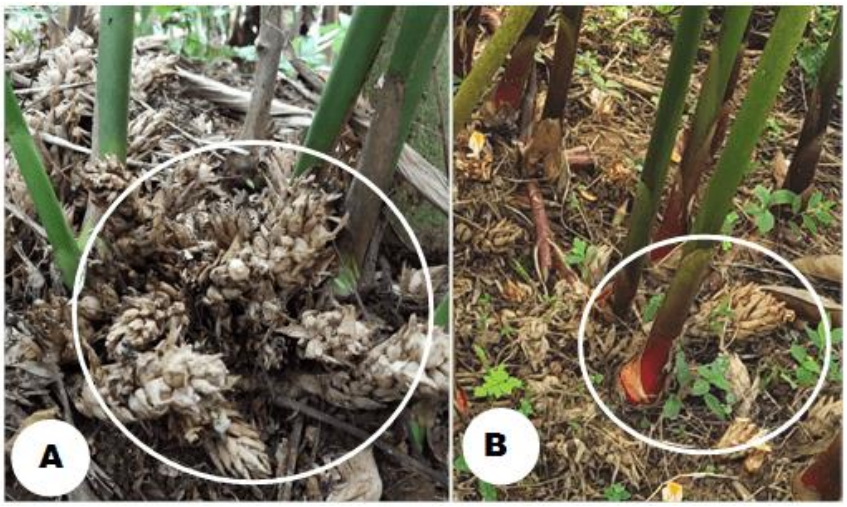

Figure 5. Cardamom fruit bunches at the base of stems, white (A) and red (B).

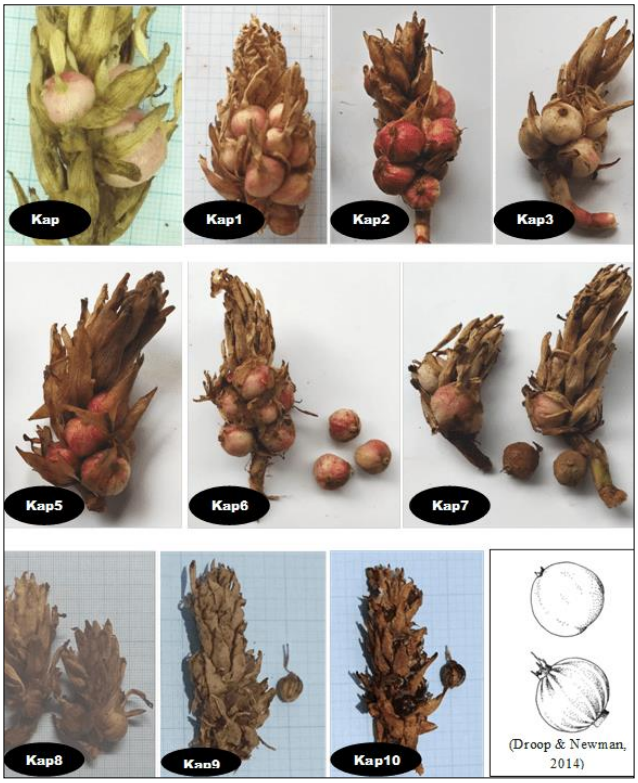

Figure 6. Fruit bunches

The observations result in Figure 5 showed that cardamom fruits were in tightly arranged bunches. On plants with white stem bases, the fruit bunches also appeared white, growing closely together (Figure 5A). Meanwhile, those on plants with red stem bases were reddish-white, relatively sparse, and did not grow near the stem bases (Figure 5B). The fruit content in each bunch varied, ranging from 3 to 17, as evident from the collected samples (Figure 6).

The fruits were round, three in number, and each ranging from sizes of 8-11mm contained 12 - 16 seeds (Figure 6.). In the fresh state, the fruit skin appeared white, reddish-white, and red (Figure 6). During slightly dry or dry conditions, the skin color changed to brownish-white and black. According to Figures 6 and 7, the skin surface was slightly wrinkled, featuring precise grooves. Plants with red stem bases produced reddish-white fruits, while those with white stem bases yielded soft hairy fruits.

Cardamom seeds were found to be tiny and protected in a whitish seed coat, while the fruits were divided into three locules, each containing 2 seeds. As fruit maturity level increased, the color of the seeds also changed, from initially reddish-white (light pink) to brownish or blackish brown (Figure 7).

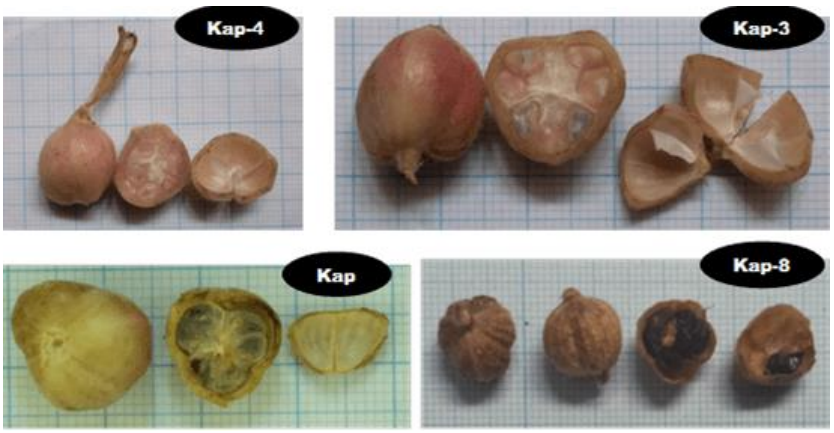

Figure 7. Morphology of fruits and seeds of *Amomum*

Observations of fruits and seeds characteristics in the samples were conducted following guidelines from Droop & Newman (2014) for classification purposes. The cardamom plants identified from the five locations in Banyumas Regency belonged to the *Amomum* genus. Considering that Javanese cardamom was the most cultivated type in Indonesia, the observed samples had a high tendency to be *Amomum compactum*. However, molecular characterization must be performed to confirm the status of the species.

The *Amomum* genus, locally known as 'cardamom,' has a diverse morphological appearance, similar to the variability observed in its cultivars. Therefore, morphological identification is essential to obtain comprehensive data and information about this plant. Zahara (2020) and Hassemer et al. (2020) asserted that morphological characteristics commonly play a fundamental role in describing and identifying plants in the field of taxonomy.

Despite morphological identification being classified as a traditional method in taxonomy, it relies on observable characteristics for group division (Ganie et al. 2015). This method unveils the development, shape, and external structure of plants as well as proves valuable for exploring the similarities and origins (Iroka et al., 2015). According to Iroka et al. (2015), morphological characteristics serve as diagnostic tools or key features in the identification, description, and classification of plants, alongside the resolution of taxonomic problems. These traits can be described and observed qualitatively, namely the shape of the leaf blade, or quantitatively, for example, the length of the leaf blade.

Molecular characterization

Taxonomic status

The genetic species concept was determined based on genetic identity values for reference species available in GenBank (Table 3). Additionally, query cover and e-value were considered during the BLAST process. The geographic distribution of the conspecific references presented in Table 4 was observed when the BLAST parameters showed similarity to several top-hit reference species.

Table 3. Query cover, e-value, genetic identity, and genetic similarity of the samples to reference species in two global databases.

Sample Code	Gene	GenBank			Reference Species
		Query (%)	e-value	Identity (%)	
Kap	matK	100	0.00	99.87	*Wurfbainia testacea* KY620250
		100	0.00	99.87	*Wurfbainia scmidtii* KY510016
		100	0.00	99.87	***Amomum compactum* NC_036992**
		100	0.00	99.87	*Amomum krervanh* NC_036935
	rbcL	100	0.00	100	***Amomum compactum* NC_036992**
		100	0.00	100	*Amomum krervanh* NC_036935
		100	0.00	99.65	*Wurfbainia villosa* MK389642
		100	0.00	99.65	*Wurfbainia longiligularis* NC_044774
Kap-1	matK	100	0.00	100	*Wurfbainia testacea* KY620250
		100	0.00	100	***Amomum compactum* NC_036992**
		100	0.00	100	*Amomum krervanh* NC_036935
		100	0.00	99.87	*Wurfbainia longiligularis* MK889505
	rbcL	100	0.00	99.83	***Amomum compactum* NC_036992**
		100	0.00	99.83	*Amomum krervanh* NC_036935
		100	0.00	99.48	*Wurfbainia villosa* MK389642
		100	0.00	99.48	*Wurfbainia longiligularis* NC_044774
Kap-2	matK	100	0.00	99.39	***Amomum compactum* NC_036992**
		100	0.00	99.39	*Amomum krervanh* NC_036935
		100	0.00	99.27	*Wurfbainia testacea* KY620250
		100	0.00	99.15	*Wurfbainia longiligularis* MK889505
	rbcL	100	0.00	99.49	***Amomum compactum* NC_036992**
		100	0.00	99.49	*Amomum krervanh* NC_036935
		100	0.00	99.15	*Wurfbainia villosa* MK389642
		100	0.00	99.15	*Wurfbainia longiligularis* NC_044774

Sample Code	Gene	GenBank			Reference Species
		Query (%)	e-value	Identity (%)	
Kap-3	matK	100	0.00	100	***Amomum compactum* NC_036992**
		100	0.00	100	*Amomum krervanh* NC_036935
		100	0.00	99.88	*Wurfbainia testacea* KY620250
		100	0.00	99.88	*Wurfbainia scmidtii* KY510016
	rbcL	100	0.00	99.83	***Amomum compactum* NC_036992**
		100	0.00	99.83	*Amomum krervanh* NC_036935
		100	0.00	99.48	*Wurfbainia villosa* MK389642
		100	0.00	99.48	*Wurfbainia longiligularis* NC_044774
Kap-4	matK	100	0.00	100	***Amomum compactum* NC_036992**
		100	0.00	100	*Amomum krervanh* NC_036935
		100	0.00	100	*Wurfbainia testacea* KY620250
		100	0.00	99.87	*Wurfbainia longiligularis* MK889505
	rbcL	100	0.00	99.83	***Amomum compactum* NC_036992**
		100	0.00	99.83	*Amomum krervanh* NC_036935
		100	0.00	99.48	*Wurfbainia villosa* MK389642
		100	0.00	99.48	*Wurfbainia longiligularis* NC_044774
Kap-6	matK	100	0.00	99.77	***Amomum compactum* NC_036992**
		100	0.00	99.77	*Amomum krervanh* NC_036935
		100	0.00	99.65	*Wurfbainia testacea* KY620250
		100	0.00	99.54	*Wurfbainia longiligularis* MK889505
	rbcL	100	0.00	99.66	***Amomum compactum* NC_036992**
		100	0.00	99.66	*Amomum krervanh* NC_036935
		100	0.00	99.32	*Wurfbainia villosa* MK389642
		100	0.00	99.32	*Wurfbainia longiligularis* NC_044774
Kap-7	matK	100	0.00	99.88	***Amomum compactum* NC_036992**
		100	0.00	99.88	*Amomum krervanh* NC_036935
		100	0.00	99.76	*Wurfbainia testacea* KY620250
		100	0.00	99.64	*Wurfbainia longiligularis* MK889505
	rbcL	100	0.00	99.83	***Amomum compactum* NC_036992**
		100	0.00	99.83	*Amomum krervanh* NC_036935
		100	0.00	99.48	*Wurfbainia villosa* MK389642
		100	0.00	99.48	*Wurfbainia longiligularis* NC_044774
Kap-8	matK	100	0.00	99.77	***Amomum compactum* NC_036992**
		100	0.00	99.77	*Amomum krervanh* NC_036935
		100	0.00	99.65	*Wurfbainia testacea* KY620250
		100	0.00	99.54	*Wurfbainia longiligularis* MK889505
	rbcL	100	0.00	99.32	***Amomum compactum* NC_036992**
		100	0.00	99.32	*Amomum krervanh* NC_036935
		100	0.00	98.98	*Wurfbainia villosa* MK389642
		100	0.00	98.98	*Wurfbainia longiligularis* NC_044774
Kap-9	matK	100	0.00	99.77	***Amomum compactum* NC_036992**
		100	0.00	99.77	*Amomum krervanh* NC_036935
		100	0.00	99.65	*Wurfbainia testacea* KY620250
		100	0.00	99.54	*Wurfbainia longiligularis* MK889505
	rbcL	100	0.00	99.16	***Amomum compactum* NC_036992**
		100	0.00	99.16	*Amomum krervanh* NC_036935
		99	0.00	99.15	*Etlingera pauciflora* KX893482
		100	0.00	98.82	*Wurfbainia villosa* MK389642
Kap-10	matK	100	0.00	99.88	***Amomum compactum* NC_036992**
		100	0.00	99.88	*Amomum krervanh* NC_036935
		99	0.00	99.88	*Wurfbainia testacea* KY620250

Sample Code	Gene	GenBank			Reference Species
		Query (%)	e-value	Identity (%)	
	rbcL	100	0.00	99.64	*Wurfbainia longiligularis* MK889505
		100	0.00	99.49	***Amomum compactum* NC_036992**
		100	0.00	99.49	*Amomum krervanh* NC_036935
		100	0.00	99.15	*Wurfbainia villosa* MK389642
		100	0.00	99.15	*Wurfbainia longiligularis* NC_044774

Table 3 shows that amomum samples from Banyumas Regency have a high genetic similarity to four different species listed in GenBank. A related result was obtained based on the similarity test conducted using matK or rbcL genes because the similarity values for all reference species exceeded 97%, namely 99.15% to 100%. Previous studies have reported that that matK is a reliable marker for plant identification (Hartvig et al. 2015; Li et al., 2015; Tallei & Kolondam, 2015; Roslim et al., 2023). Other studies suggested a threshold of 97% genetic similarity for assigning samples to a particular category as reference species. Genetic similarity values below 97% were accepted as a species border based on certain considerations. However, the results obtained were distinct from the observations reported by Subba et al. (2021) during differentiation between *A. villosum* cultivars using matK and rbcL genes. This study encountered challenges in distinguishing among amomum species using the same genetic markers due to the samples being sourced from agricultural areas. Cultivated plants often pass through directional selection for specific desired characteristics (Bhandari et al., 2017), leading to a similar genetic identity among cultivars.

The genetic similarity of all samples to four different reference species exceeding 97% showed that matK and rbcL genes were not suitable barcode markers for *Amomum*. The unsuitability of these genes as barcode markers was also reported by Olson et al. (2022), which proved that closely related pine could not be identified using matK and rbcL genes. Consequently, additional information such as the geographic distribution of the samples (Table 4) is needed for accurate characterization. Based on an in-depth examination, most tests showed that the first top hit was *Amomum compactum,* which was synonymous with *Wurfbainia compacta*. Considering the geographic distribution of reference species and the first top hit, this study identified amomum samples from Banyumas as *Amomum compactum* or *Wurfbainia compacta*. The characterization of Banyumas amomum samples was further supported by the rbcL marker consistently yielding *Amomum compactum* as the highest top hit during the BLAST process.

Genetic diversity

The total haplotype diversity was 0.378 ± 0.181, and nucleotide diversity was 0.061% ±0.054%. Both values showed that the *Amomum compactum* population in Banyumas Regency, Central Java, Indonesia had low genetic diversity. This phenomenon is common in cultivated plants due to directed selection for human-desired traits, such as high yield and resistance to diseases or pests. Previous studies stated that breeding and human-directed selection contributed to substantial allelic loss and crop genetic erosion (Fu, 2015). This phenomenon means that cultivated crops tend to have low genetic diversity due to directed selection. A parallel observation of low allele and heterozygosity has been reported in the widely cultivated cowpea, Vigna unguiculata (Xiong et al., 2016). A previous study reported the genetic diversity of Java Cardamom. However, that study utilized isozymes as a genetic marker (Setyawan et al. 2014). However, no genetic study using DNA-based markers. Therefore, exploration of low genetic diversity among the cardamom population grown in the Banyumas Regency was necessary. This study used matK-rbcL and was not entirely correspondent to Xiong et al. (2016) which applied simple sequence repeat (SSR) markers. However, both markers proved that cultivated crops tended to show low genetic diversity due to being the product of human-directed selection to meet farmer and breeder preferences, as extensively reviewed in several studies (Fu, 2015; Bhandari et al., 2017; Riaz et al., 2018).

This study proved that Java Cardamom from Banyumas Regency was molecularly identified as A. compactum and showed low genetic diversity although showed morphological variation. These data are essential data in seed selection for further cultivations to avoid inbreeding depression.

Table 4. Geographic distribution of each reference species.

No	Species	Native geographic distribution
1	*Amomum compactum* or *Wurfbainia compacta*	Jawa, Sumatera
2	*Amomum krervanh* valid as *Wurfbainia vera*	Cambodia, Sumatera, Thailand, Vietnam
3	*Wurfbainia testacea*	Borneo, China South-Central, China Southeast, Malaya, Thailand, Vietnam
4	*Wurfbainia villosa*	Bangladesh, Cambodia, China South-Central, China Southeast, East Himalaya, Laos, Myanmar, Thailand, Vietnam
5	*Wurfbainia longiligularis*	China Southeast, Hainan, Laos, Thailand, Vietnam
6	*Etlingera pauciflora*	Jawa, Malaya, Thailand

Source: Royal Botanical Garden Kew (Plant of the World Online: https://powo.science.kew.org/taxon)

CONCLUSION

In conclusion, the results showed that cardamom samples from different locations in the Banyumas Regency had morphological variations. Identification based on both geographic distribution and genetic similarity established this cardamom as belonging to *Amomum compactum* but with low genetic diversity. Molecular identification validated morphological identification by placing the Javanese Cardamom into *Amomum compactum*. This study was preliminary and carried out on a small number of samples and narrow areas. Further study working on a large number of samples and wider areas is necessary to obtain a statistically more reliable conclusion.

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