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Research Article

Identification of DNA Barcodes from rbcL Chloroplast DNA in Katokkon Chili (*Capsicum annuum* var. *chinense*) Origin of Tana Toraja, South Sulawesi

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Abstract
Katokkon chili (*Capsicum annuum* var. chinense) is a type of chili which are commonly found in Tana Toraja. It has a distinctive aroma, high spiciness, and a potential economic value but has not been widely identified and explored, thus, it is necessary to carry out molecular identification using DNA barcodes from chloroplast DNA. The aim of this study was to determine genetic variation and the results of constructing a phylogenetic tree from DNA sequences katokkon chili (*C. annuum* var. chinense) using the rbcL marker. This study used 6 samples of katokkon chilies (*C. annuum* var. chinense) and 3 outgroup samples *(C. frutescens*, *C. chinense* and *C. baccatum*). The stages of the research included total DNA isolation, qualitative and quantitative tests, PCR amplification using rbcL primers, and sequencing. Data analysis used is sequence alignment, phylogenetic, genetic distance matrix, haplotypes and phylogeography. The results showed genetic variation with 7 polymorphisms consisting of 4 singleton sites at the nucleotide base sequences of 6th, 525, 715 and 737, and 3 parsimony informative sites at the nucleotide base sequences 370, 616 and 902 and the haplotype distribution is divided into 4 haplotypes namely Hap_1 (A1, A2, A3, B1, B2 and B3), Hap_2 (C1), Hap_3 (C2), and Hap_3 (C3). The phylogenetic tree construction formed two clades, namely clade I consist of six samples of katokkon chilies (*C. annuum* var. chinense) and clade II consisting of three outgroup samples. Mark the highest bootstrap is 96 and the lowest bootstrap value is 29. Genetic distance matrix values are in the range of 0.000–0.005.

Keywords: *Capsicum annuum* var. chinense, DNA barcode, phylogenetic tree, rbcL, genetic variation

1. INTRODUCTION

Indonesia is a tropical country with a high diversity of plants, even though Indonesia's territory is only about 1.3% of the earth's area. Plants in Indonesia are about 25% of the world's plants, with a total of 20,000 species and 40% are endemic plants. Plant diversity has enormous potential to meet food needs, economic needs and renewable energy as well as being the basis for determining superior varieties of a species [1].

South Sulawesi is geographically located in a transitional area between the continents of Asia and Australia, so it has unique plants, one of which is the katokkon chili (*C. annuum* var. chinense) found in Tana Toraja. Katokkon chilies have a very distinctive aroma and a high level of spiciness and have a unique shape resembling bell peppers but are smaller and fatter in size [2]. Katokkon chili powder is also very spicy compared to other chili powders. Hot pepper varieties with Scoville Heat Units 30,000–50,000 SHU. Katokkon chili is about 100–120 cm in length, type of shrub habitus, and has a root system, namely taproot. The shape of the fruit is fat, blunt and short, green when it is young and bright red when it is ripe and has a thick fruit skin [3].

Katokkon chili is one of the chili varieties in Indonesia with high potential economic value but has not been identified and explored much [4]. Identification of a plant can be done based on morphological characteristics in all parts of the plant, and then matched with existing specimen collections. This system has weaknesses, namely the identification process is only for mature plants, requires a long time and is influenced by environmental factors and experts in the field of taxonomy are limited [5]. Thus, we need the process for identifying differences in a plant species using a faster and more accurate method through DNA barcodes. Identification of a species was

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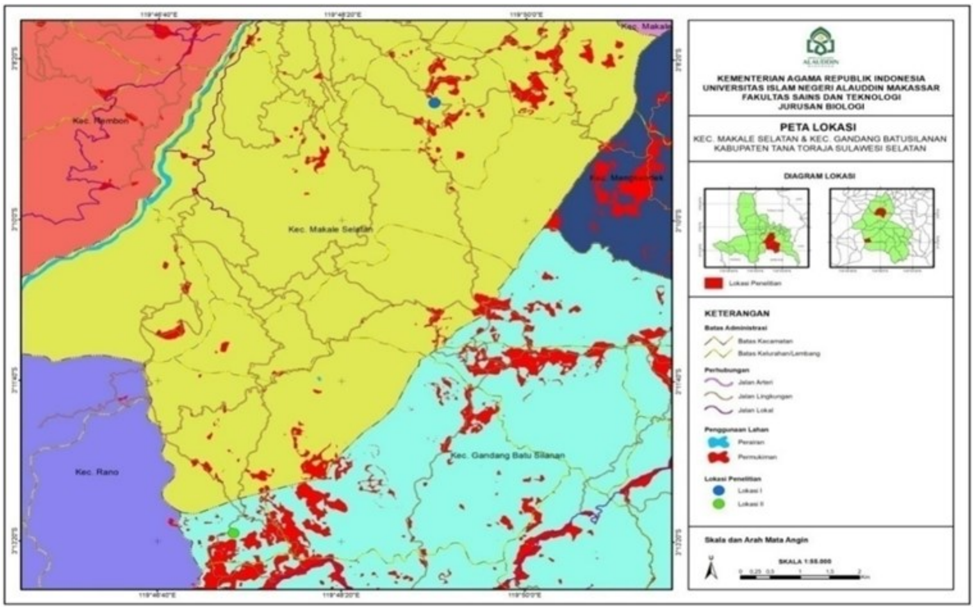

Figure 1. Katokkon chili research location map. Location 1 (Pasa Village, Makale Selatan District) (03008'30.86"S. 119049'05.19"E) and location 2 (Lembang Kaduaja, Gandang Batusillanan sub-district) in Tana Toraja Regency (03013'09.84"S. 119047'43.53"E).

initially only based on morphological characters. However, now it has developed species identification based on molecular characters.

DNA barcodes are short DNA sequences that are used to simplify and speed up the process of identifying a species on a molecular basis and have a very important contribution to diversity and taxonomy research. Barcoding DNA sequences are orthologous and could differentiate between species [6]. DNA barcodes aim to identify a species by molecular technique that has not been previously identified [7]. In addition, DNA barcodes aim to help taxonomists identify species that are difficult to identify [8]. The advantage of DNA barcoding is that it enables fast and accurate species identification using short, standardized genes as internal species markers and helps taxonomists accelerate the rate of species discovery [9]. Another advantage of the DNA barcode identification technique is that it identifies organisms with incomplete and even degraded forms of DNA [10].

Molecular markers that are often used in determining plant barcodes are the maturase-K (matK) andribulose-1,5-bisphosphate carboxylase (rbcL) genes [11]. In this study, the rbcL gene was used as a molecular marker. The rbcL gene has a high success rate during the amplification process and plays a role in encoding the RuBisCO protein which causes the rbcL gene to have a low mutation rate, making it advantageous in intraspecies phylogenetic studies and genetic variation [7]. This study aims to determine the genetic variation of DNA sequences katokkon chili using rbcL markers and to construct a phylogenetic tree from the rbcL sequence of katokkon chili. Result of phylogenetic tree showed analysis of diversity and family relationships can provide information on these Biological genetic relationships between genotypes. This information is important for overall planning. Plant breeders can identify and select local genotypes as parents for breeding based on population relatedness. This study could provide DNA barcode that impact the urgency of the research on katokkon chili and has been highlighted in the context of its potential applications and benefits, especially in the preservation of seed diversity and food security.

2. MATERIALS AND METHODS

2.1. Materials

In total, the collection of 6 living plant specimens of the katokkon chili fruit (*Capsicum annuum* var. chinense) carried out at two locations in Tana Toraja Regency, namely Pango-Pango, Lembang Pasang, Makale Selatan District and Sangbua, Lembang Kaduaja, Gandang batu Sillanan District (Figure 1). In addition, their close relative species were used as outgroup; *Capsicum frutescens* (JX996068.1), *Capsicum chinense* (KF917580.1), and *Capsicum baccatum* (U08610.1).

2.2. Methods

2.2.1. Morphological Characterization

Samples were taken from two districts in Tana Toraja. Morphological characterization is observed fruit such as fruit color before ripening and when ripe, fruit shape, fruit cross-sectional shape, fruit shape at the tip of the bloom and the character of the fruit position. Chili leaves used are young leaves and fruit samples were randomly taken from about 30 fruits directly from the farmer's field. The samples obtained were stored in plastic clips which were given silica gel. Physical parameters of the environment were also observed, such as pH and soil moisture, air temperature and humidity, light intensity, wind speed, and altitude.

2.2.2. Isolation of Total DNA of Plant Tissue from Leaves

DNA isolation was performed using The Wizard® Genomic DNA Purification Kit Promega. The sample was frozen using liquid nitrogen and

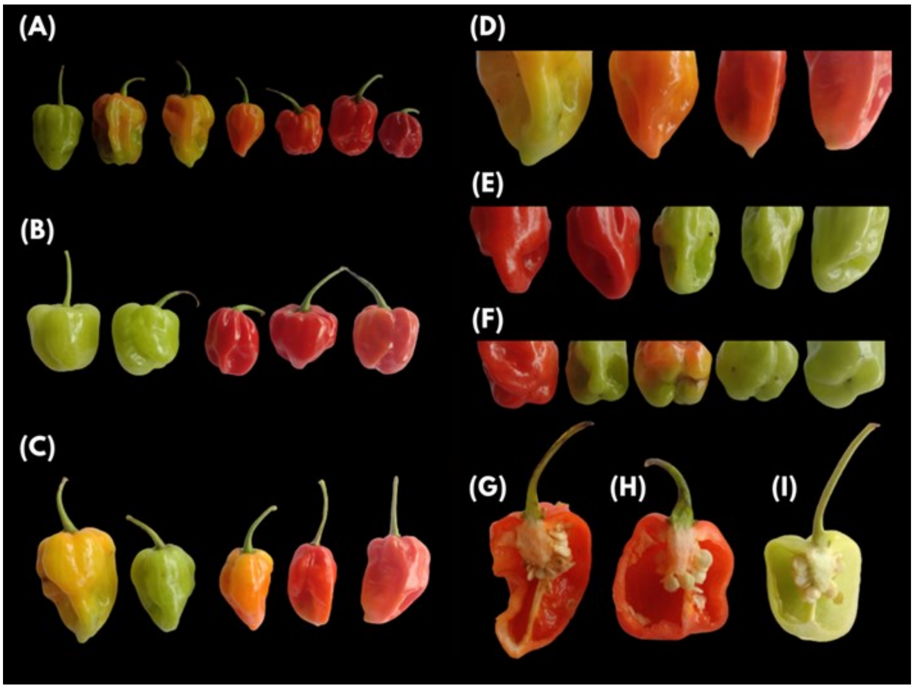

Figure 2. Katokkon chili fruit morphology location I. (A) Color intensity (green, orange color of fruit before ripening and red color after ripening), (B) square shape of fruit, (C) triangular fruit shape, (D) pointed fruit shape at flower tip, (E) blunt fruit shape at flower tip, (F) the shape of the fruit is concave at the end of the flower, (G) the cross-section of the fruit is wavy, (H) slightly wavy, and (I) not wavy.

Table 1. Observation results of physical environmental factors.

No.	Environmental Physical Factors	Research sites	
		1	2
1.	Altitude (masl)	1348	1532
2.	Temperature (°C)	27.5	29.3
3.	Air Humidity (%RH)	68.5	51.2
4.	Light Intensity (Lux)	85.3	120
5.	Wind Speed (m/s)	1.0	1.5
6.	Soil pH	5.5	5.5
7.	Soil Moisture (%RH)	9	9

*Location 1 (Pasa Village, Makale Selatan District) and location 2 (Lembang Kaduaja, Gandang Batusillanan sub-district) Tana Toraja Regency

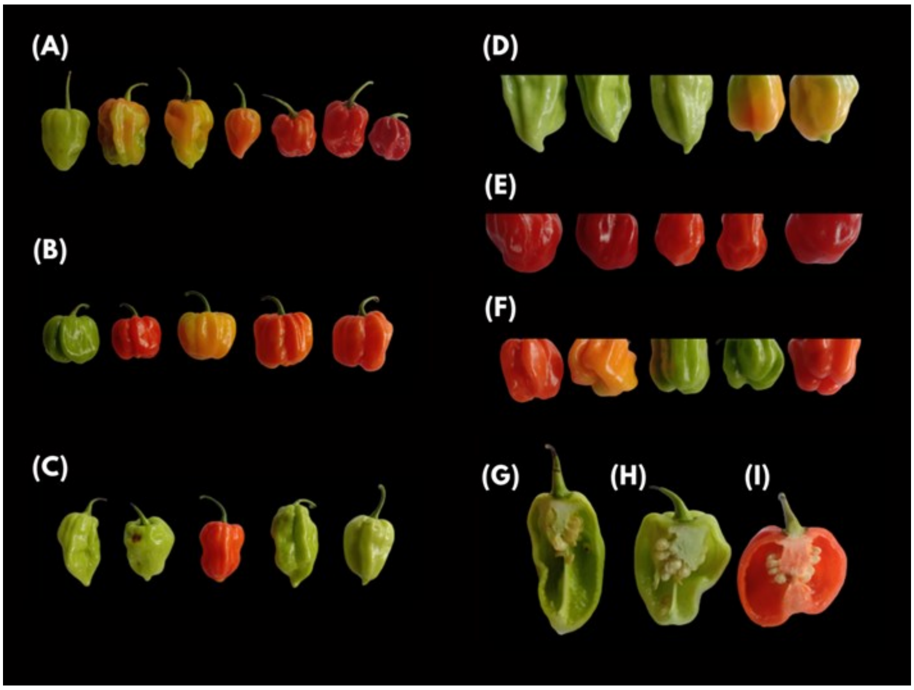

Figure 3. Katokkon chili fruit morphology location II. (A) Color intensity (green, purple spots, orange color before ripening and red after ripening), (B) square fruit shape, (C) triangular fruit shape, (D) fruit shape pointed at the tip of the flower, (E) fruit shape blunt at the end flower, (F) the shape of the fruit is concave at the end of the flower, (G) the cross-section of the fruit is wavy, (H) slightly wavy, and (I) not wavy.

then pulverized using a mortar and pestle to form a powder. The leaf powder was taken as much as 40 mg and put into a 1.5 mL tube and added by 600 mL of nuclei lysis solution and then vortexed for 1–5 seconds. The suspension formed was then incubated for 15 min at 65 °C. Then 3 µL of RNAase solution was added to the suspension, followed by closing the tube and inverting it 2–5 times so that the suspension was evenly mixed. The suspension was incubated again for 15 min at 37 °C, after which the suspension was allowed to stand for 5 min at room temperature.

The next step is protein precipitation in DNA suspension by adding 200 µL of protein precipitation solution in a tube and vortexing for 20 s. Then, the DNA suspension was centrifuged at speeds ranging from 13,000–16,000 rpm for 30 min to separate the molecules based on weight, then the supernatant was taken in the tube and transferred to a new tube measuring 1.5 µL and 600 µL isopropanol DNA was added. The mixture between DNA and isopropanol is homogenized by slowly inverting the tube in a closed state. Once homogeneous, centrifuge again for 1 min at room temperature at 13,000–16,000 rpm.

The next process is DNA purification where the mixture of DNA and isopropanol that has been centrifuged is taken as part of the supernatant and discarded, while the pellet portion is added to 600 µL of 70% ethanol (room temperature). The tube containing the solution is slowly inverted several times to wash the sample DNA. Then the solution was separated again by centrifuging for 1 min at 13,000–16,000 rpm at room temperature.

Then, the supernatant is taken using a micropipette and disposed of carefully because at this stage the DNA pellet easily separates from the tube. The pellets that had been cleaned from the supernatant were air-dried for 15 min after which 100 µL of DNA rehydration solution was added and then homogenized by inverting the tube 1–5 times. The last step of DNA isolation is the DNA solution, which is incubated for 1 h at 65 °C, and then stored in a refrigerator at 4 °C so that it can be used for a long time.

2.2.3. Qualitative and Quantitative Tests

Results DNA isolation was then tested qualitatively using electrophoresis with 1% agarose gel, while the quantitative test was carried out using nanodrops.

2.2.4. PCR Amplification

The isolation was then amplified using a PCR machine in 30 µL of a solution consisting of 15 µL PCR Master Mix Nexpro, 3 µL DNA template sample (100 ng/µL), 6 µL water, 3 µL primer (10 pmol each forward and reverse primer). The primers used in the PCR-amplification were forward rbcL1 (TGTCACCAAAAACAGACT) and reverse rbcL2 (TTCCATACTTCACAAGCAGC) primers. Amplification was carried out with the following temperature settings, pre-denaturation at 95 °C for 2 min, then followed by 35 cycles consisting of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 120 s. Then the post elongation process was carried out at 72 °C for 7 min. The PCR results were then sequenced using the sequencing service of 1st BASE Laboratories Sdn Bhd.

2.2.5. Data Analysis

Identification is carried out using Basic Local Alignment Search Tool (BLAST) analysis at the National Center of Biotechnology Information (NCBI), sequence alignment, phylogenetic tree construction and determination of genetic distance matrix values using MEGA XI, determination haplotypes and phylogeography using DnaSP and haplotype networks.

3. RESULTS AND DISCUSSIONS

3.1. Observation of Environmental Factors and Fruit Morphology of Katokkon Chili

There are seven parameters of physical environmental factors observed in the observations, namely altitude, temperature, air humidity, light intensity, wind speed, soil pH, and soil moisture (Table 1). In observing fruit morphology several fruit morphological characteristics were observed such as color intensity, fruit shape, fruit tip shape, fruit cross section, and fruit genotypic position (Fig 2 and 3). The morphological character of katokkon chili fruit shows that there are several colors of fruit before it ripens such as green, purple spots, orange and the color of the fruit when ripe is red (Fig 2(a) and 3(a)). The observed fruit shape characters were square (Fig 2(b) and 3(b)) and triangular (Fig 2(c) and 3(c)). The shape of the fruit at the end of the flower is blunt (Fig 2(d) and 3(d)), concave (Fig 2 (e) and 3(e)), and pointed (Fig 2(f) and 3(f)). The cross-sectional shape of the fruit is wavy (Fig 2(g) and 3(g)), slightly wavy (Fig 2(h) and 3(h)), and not wavy (Fig 2(i) and 3(i)) influenced by the surface appearance of the fruit. The position of the fruit on the katokkon chili is facing down.

3.2. PCR Amplification and Sequencing

Amplification using the forward rbcL1 and reverse rbcL2 primers has been successfully carried out on katokkon chili. Visualization of the target gene amplified by all samples showed ±1500 bp DNA fragments (Figure 4). Sequencing was successfully carried out from the forward direction with a length of ±1000 bp. The results of the rbcL

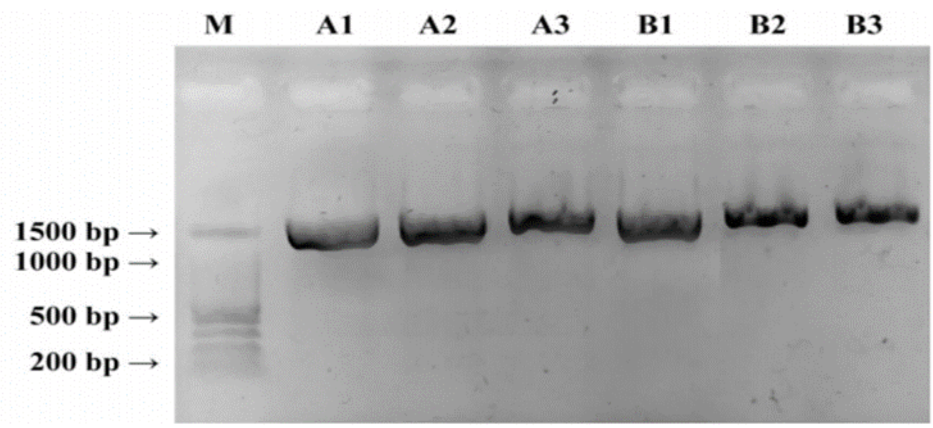

Figure 4. PCR amplification results of katokkon chili.

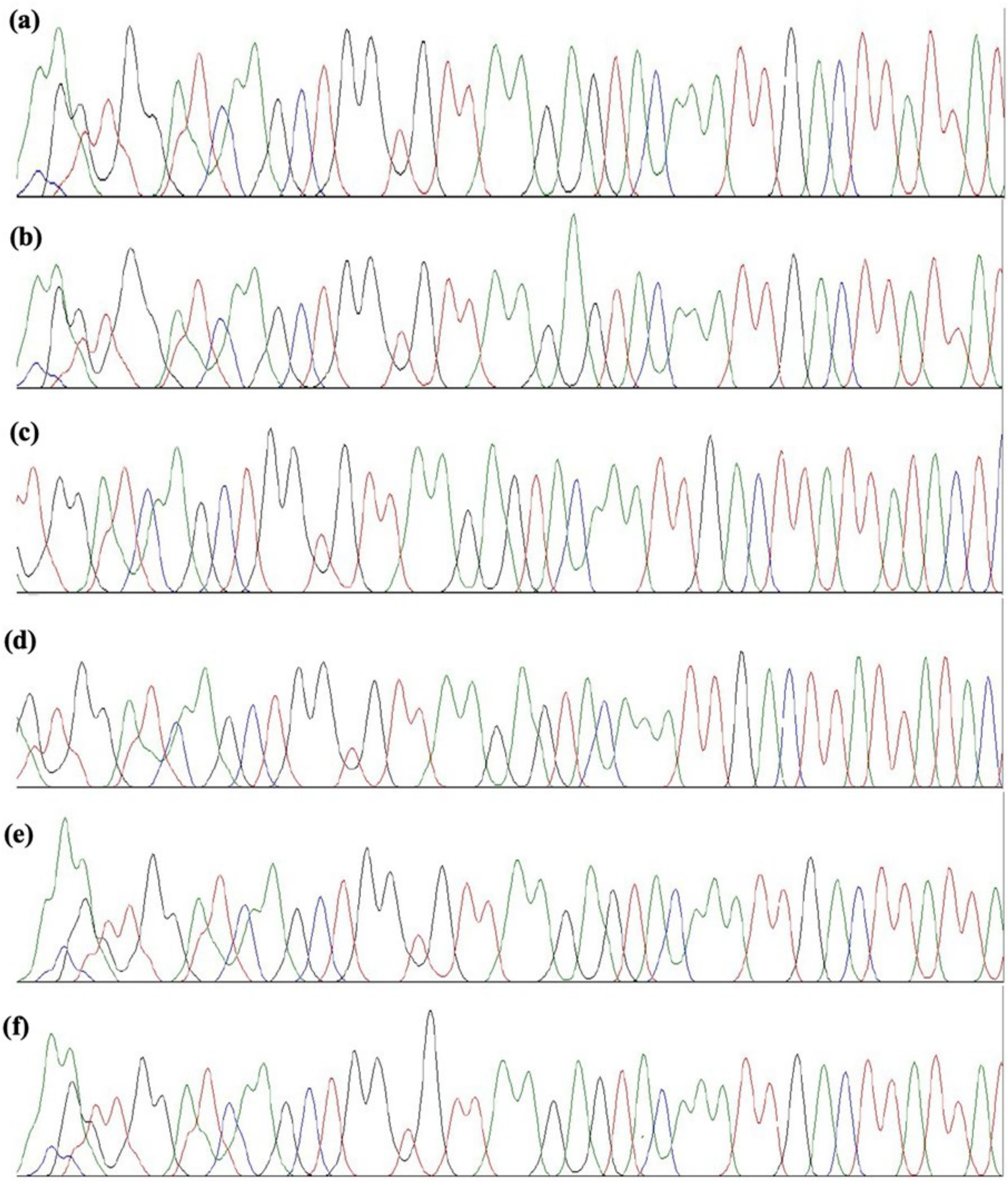

Figure 5. Chromatogram of sample (a) A1, (b) A2, (c) A3, (d) B1, (e) B2, and (f) B3 sequencing results.

gene sequencing showed a high-quality chromatogram, marked by clear and non-overlapping peaks (Figure 5).

Sequencing data of katokkon chili were identified using the Basic Local Alignment Search Tool (BLAST) analysis at the National Center of Biotechnology Information (NCBI). BLAST results show that the six samples katokkon chili *(C. annuum* var. chinense) were identified to have similarities with *C. annuum* (MH559329.1) (Table 2).

3.3. Alignment of The Katokkon Chili DNA Sequences

Alignment of DNA sequences was carried out with the aim of matching the same characters and analyzing the presence of gaps in the same sequence (Figure 6). Sample code C1 = cayenne pepper (*Capsicum frutescens*), C2 = habanero chili

(*Capsicum chinense*) as well as C3 = *Capsicum baccatumas* an outgroup sample. Alignment in the nucleotide base sequences of the nine samples yielded 972 bp as the final basepare amount. Sequence alignment using the rbcL molecular marker on six katokkon chili samples and three outgroup samples showed that the aligned sequence length was 972, and there were 7 polymorphism events consisting of 4 singleton variable sites and 3 parsimony informative sites. On results, phylogeography is divided into 4 haplotype distributions and has a haplotype diversity of 0.5833 (Figure 7).

3.4. Construction of Phylogenetic Trees and Genetic Distance Matrices of Katokkon Chili

The construction of phylogenetic trees from sequences that have been aligned aims to determine the level of kinship in six samples of katokkon chili and three outgroup samples. Results of phylogenetic tree construction on katokkon chili and outgroup samples using the rbcL molecular marker found that two large clades were formed (Figure 8). Samples A1 and A2 are closely related to sample A3 with the highest bootstrap value of 96. Both samples are in the same branching group as sample A3 with the same bootstrap value.

Genetic distance matrix values katokkon chili and the outgroup sample showed the lowest genetic distance matrix value was 0.000 and the highest genetic distance was 0.05 (Table 3). The farthest genetic distance matrix values occur in samples C1 and C3 with samples A1, A2, A3, B1, B2, and B3. The value of the katokkon chili genetic distance matrix and outgroup samples obtained from the construction of phylogenetic trees.

3.5. Discussion

Variation in a plant is also influenced by environmental factors, not only determined by the diversity of genes it has. The traits possessed by each individual are the result of the interaction process between the environment and genes. Individuals with identical gene characters do not necessarily have the same morphological characteristics. There are several environmental factors that affect the morphological characteristics of plants, namely location altitude, humidity, temperature, and climate [12]. Observation of environmental factors at the first location (Pasa Village, South Makale sub-district) and the second location (Lembang Sangbua, Gandangbatu Sillanan District) have differences in environmental factors such as altitude, temperature, light intensity, air humidity, and wind speed. As well as having similarities in soil pH and moisture (Table 1). Cignore katokkon it only grows well in the highlands with altitudes ranging from 1000–1500 meters above sea level and is susceptible to temperatures around 16–24 °C [13]. Rain intensity required for optimal growth is around 1500–3500 mm/year with humidity around 82–86%. Soil pH suitable for growth cignore katokkon is around 3.5–5.0. Based on the results of observations on the morphology of the katokkon chili fruit that there is no difference in color, fruit shape, fruit tip, cross-section and fruit position between the two sites with different heights. It is presumably that both locations are still within the same range, so there are no morphological differences due to the

Table 2. Identification test results based on the NCBI Database.

No.	Sampel	NCBI Identification	NCBI Similarities	NCBI Acc Number
1.	A1	*Capsicum annum*	98.82%	MH559329.1
2.	A2	*Capsicum annum*	98.60%	MH559329.1
3.	A3	*Capsicum annum*	99.15%	MH559329.1
4.	B1	*Capsicum annum*	97.79%	MH559329.1
5.	B2	*Capsicum annum*	99.48%	MH559329.1
6.	B3	*Capsicum annum*	99.24%	MH559329.1

Note: Sample code A1, A2 and A3 (First location), sample code B1, B2, and B3 (Second location)

Figure 6. The results of the alignment of the katokkon chili sequences and outgroup samples.

different biophysical and environmental status of both locations, katokkon chili at the first location and the second location is a local variant (Limbong Sangpolo).

PCR is an enzymatic DNA replication process using a PCR machine with a working principle that involves enzymatic reactions using DNA polymerase [14]. The working procedure of PCR is divided into three stages, namely denaturation, annealing, and extension. There are several factors that affect the success rate of the PCR process such as temperature at the annealing stage, DNA polymerase enzymes, and concentrations of PCR components. Six samples katokkon chili succeeded in amplification characterized by thick and clear DNA bands. PCR visualization results for the six samples yielded ±1500 bp DNA bands (Figure 4). The PCR process goes well if thick and clear bands are obtained as a sign that PCR has reached optimum conditions so that it can proceed to the sequencing process [15].

The peaks of the sequenced chromatogram have different colors for each purine base and pyrimidine base. Adenine is green, guanine is black, thymine is red and cytosine is purple. A chromatogram of the sequencing of the rbcL gene in six chili samples katokkon shows quality results with clear peaks and no overlap between one peak and another. The chromatogram of good sequencing results is indicated by clear peaks, separated from one another, not overlapping, and having high peaks [16]. Meanwhile, the chromatogram of poor sequencing results is shown by short peaks, unclear, overlapping and not separated from one another.

The results of the analysis of the BLAST for the rbcL gene of the six samples katokkon chili (*C. annuum* var. chinense) has a high homology value with *C. annuum* which has an accession number MH559329.1. The similarity level identified varies from 97.79% to 99.82% (Table 2). The higher the percentage of similarity obtained, the higher the level of homology of the two sequences being analyzed [15]. The high homology value of a sequence causes low genetic variation between two individuals.

Alignment of DNA sequences was carried out to determine similarities between aligned sequences by comparing sequence homology and genetic variation [15]. Based on the alignment results of the nine samples, it was found that these samples had polymorphisms or differences in the nucleotide base sequence. The difference occurs due to mutation events in the nucleotide base sequence. Polymorphism or differences in the nucleotide base sequence in an individual can be caused by mutations. Mutations are divided into several types such as deletions, substitutions and insertions. Deletion mutations occur when there is a loss of a nucleotide base and an insertion mutation is when there is an insertion of a nucleotide base. Substitution mutations are further divided into two, namely transversion and transition substitution. Transversion mutations are the replacement of pyrimidine bases with purines or purines with pyrimidines (TA, TG, CA, CG), while transition mutations are the replacement of pyrimidine nucleotides with pyrimidines (TC) and purines with purines (GA) [14].

Based on the results of the analysis of sequence alignment, it can be seen that there are polymorphisms consisting of 4 singleton sites and 3 parsimony informative sites. Polymorphism on the singleton sites contained the 6th, 525th, 715th and 737th nucleotide base sequences. Parsimony informative sites on the 370th, 616th and 902th nucleotide bases. The 6th nucleotide bases, 370, 525, 616, 715 and 737 underwent mutations transition. Meanwhile, the 902nd nucleotide base underwent a transition mutation.

Results phylogeography shows that the haplotype distribution is divided into 4 haplotypes, namely Hap_1 (A1,A2, A3, B1, B2 and B3), Hap_2 (C1), Hap_3 (C2), and Hap_3 (C3). Has a haplotype diversity (hd) value of 0.5833. The hd value indicates that the 9 samples have a low level of haplotype diversity. Muqaddas *et al* (2020), explained that hd values > 0 <0.5 (having low haplotype diversity) and hd values > 0.5 <1 (having high haplotype diversity) [17]. Haplotype values with low diversity indicate that the level of genetic diversity that occurs in a population is low and vice versa.

A total of six samples katokkon chili and three outgroup sample which has been aligned is then used to construct a phylogenetic tree using the Neighbor-Joining method. The Neighbor-Joining method is a method used to construct phylogenetic trees based on evolutionary distances by looking at the differences between the sequences used [18]. Results of phylogenetic tree construction katokkon chili and outgroup sample using the molecular marker rbcL showed that the nine samples formed two large clades. Clad I consists of six samples katokkon chili with codes A1, A2, A3, B1, B2 and B3. Clad II consists of three samples outgroup with codes C1, C2 and C3. Sample outgroup who is in Clade II the most polymorphism occurs. The formation of clades I and clades II is supported by the many mutations that occur in the nucleotide base sequence [19]. This is in accordance with the results obtained that the sample on Clade II polymorphism does not occur, whereas in Clade I there are 7 polymorphisms caused by mutations.

Clade formation is also influenced by bioecological differences between katokkon chili and outgroup samples. This is due to the outgroup sample sequences obtained from NCBI coming from different countries katokkon chili, so

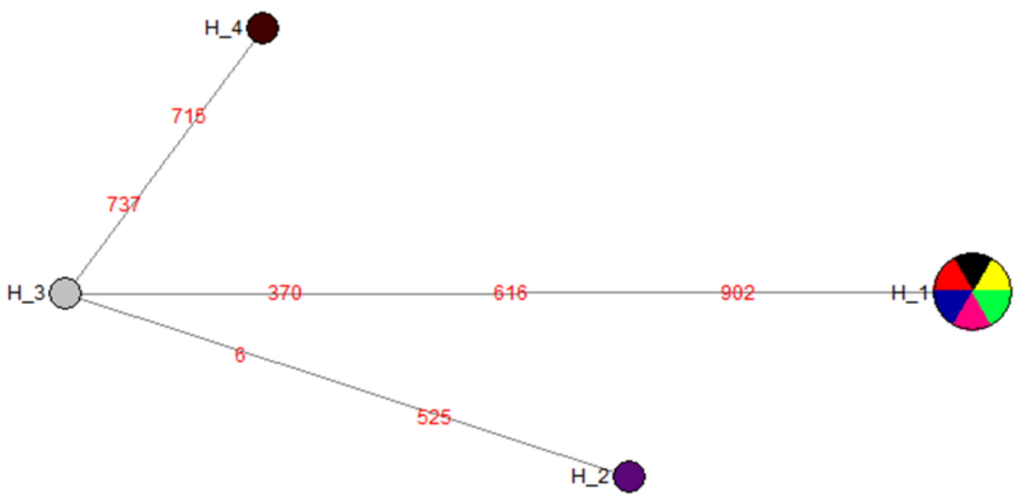

Figure 7. Phylogeography of katokkon chili and outgroup samples.

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Table 3. The value of the katokkon chili genetic matrix and outgroup samples.

Sequence Code	A1	A2	A3	B1	B2	B3	C1	C2	C3
A1									
A2	0.000								
A3	0.000	0.000							
B1	0.000	0.000	0.000						
B2	0.000	0.000	0.000	0.000					
B3	0.000	0.000	0.000	0.000	0.000				
C1	0.005	0.005	0.005	0.005	0.005	0.005			
C2	0.003	0.003	0.003	0.003	0.003	0.003	0.002		
C3	0.005	0.005	0.005	0.005	0.005	0.005	0.004	0.002	

Note: Sample codes A1, A2 and A3 (First location), sample codes B1, B2, and B3 (Second location) and sample codes C1, C2 and C3 (Outgroup Sample).

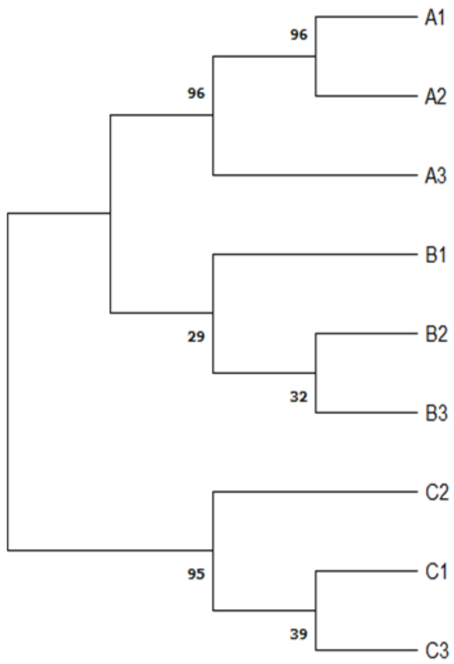

Figure 8. Construction of the katokkon chili phylogenetic tree and outgroup samples.

environmental factors affect the growth of katokkon chilies and samples of different outgroups. Environmental factors such as altitude, temperature, light intensity, soil pH, moisture and availability of nutrients in the soil are indicators that determine physiological and metabolic processes in a plant [20]. Differences in physiological and metabolic processes will affect the genotype and sequence of a plant species.

Phylogenetic tree construction using the Neighbor-Joining method uses 1000 replications for bootstrap values. The bootstrap value used aims to test the validity of the phylogenetic tree topology. Evaluation of phylogenetic tree bootstrap values based on the value of each cladogram [21]. Mark bootstrap katokkon chili and outgroup samples show that samples A1, A2 and A3 have value the highest bootstrap is 96 and the three samples are closely related. Meanwhile, sample B1 has the lowest bootstrap value with a value of 29 and is included in the same group as samples B2 and B3 with a bootstrap value of 32. The nine samples have a bootstrap value range from very weak to high (29 –96%). This is in accordance with the previous work, which states that bootstrap values are categorized starting from <50% (very weak), 50–

69% (weak), 70–85% (moderate), and >85% (high) [22].

Phylogenetic tree construction can also be used to view the genetic distance matrix of six katokkon chili sequence samples (*C. annuum* var. chinense) and three outgroup samples (*C. frutescens, C. chinense, C. baccatum*). Genetic distance is one of the determining factors used to see the level of kinship of the species studied [23]. The lowest genetic distance matrix value will have a very close kinship relationship, obtained in the sequence of samples A1, A2, A3, B1, B2 and B3 with samples A1, A2, A3, B1, B2 and B3 which is 0.000. While the highest genetic distance value will have a distant kinship relationship, obtained in the sequence of samples C1 and C3 with samples A1, A2, A3, B1, B2 and B3, namely 0.005. The low genetic distance between samples is due to the lack of differences between the nucleotide bases. Conversely, if the genetic distance is high, the samples have many differences in the nucleotide base sequence. This is in accordance with the previous opinion that the farther the value of the genetic distance matrix of an individual, the further the kinship relationship [14]. Vice versa, the closer the value of the genetic distance matrix of an individual, the closer the level of kinship will be. Sample C1 (*Capsicum frutescens*) and sample C3 (*C. baccatum*) with samples A and B of katokkon chili (*C. annuum var.* chinense) have a large genetic distance value because the three chilies are different chili species. According to Ferniah (2018), domestication of the Capsicum genus divided chilies into five species, namely, *C. frutescens, C. annuum, C. pubescens, C. chinense* and *C. Bataccum* [24].

Genetic distance matrix values katokkon chili and outgroup samples are in the range of 0.000–0.005 which indicates that the genetic distance of the six samples katokkon chili and the three outgroup samples were included in the very low group. The genetic distance values are grouped into three, namely the genetic distance values between 1.00–2.00 including high, the genetic distance values with susceptibility between 0.10–0.99 including moderate and the genetic distance values with susceptibility between 0.010–0.099 including low [25].

4. CONCLUSIONS

We conclude that the genetic variation of the six samples katokkon chili (*C. annuum* var. chinense) and three outgroup samples that resulted in 7 polymorphisms consisting of 4 singleton sites and 3 parsimony informative sites and 4 haplotype distributions. The phylogenetic tree construction forms two clades and the value of the genetic distance matrix is in the range of 0.000–0.005. Suggestions for further research, namely the method for reconstructing phylogenetic trees can use methods other than the Neighbor-Joining Tree and it is recommended that different islands be sampled so that it will produce polymorphism and distribution of various haplotypes.

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Conflicts of Interest

The authors declare no conflict of interest.

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REFERENCES

[1] C. Kusmana and A. Hikmat. (2015). "The Biodiversity of Flora in Indonesia". *Journal of Natural Resources and Environmental Management.* **5** (2): 187-198. 10.19081/jpsl.5.2.187.

[2] A. S. Iryani and A. D. M. Bali. (2021). "Farmer Group of Cabe Bakul (Lada Katokkon) in Rantepao District, North Toraja Regency". *Mattawang: Jurnal Pengabdian Masyarakat.* **2** (1): 27-35. 10.35877/454RI.mattawang204.

[3] M. Mutmainnah and M. Masluki. (2017). "The Effect of Giving Organic and Inorganic Fertilizer Types on the growth and production of local Toraja varieties of katokkon chilies". *Sustainable Agriculture Journal.* **5** : 21-30.

[4] D. Flowrenzhy and N. Harijati. (2017). "Pertumbuhan dan Produktivitas Tanaman Cabai Katokkon (Capsicum chinense Jacq.) di Ketinggian 600 Meter dan 1.200 Meter di atas Permukaan Laut". *Biotropika.* **5** (2): 44-53. 10.21776/ub.biotropika.2017.005.02.2.

[5] M. Virgilio, K. Jordaens, F. C. Breman, T. Backeljau, and M. De Meyer. (2012). "Identifying insects with incomplete DNA barcode libraries, African fruit flies (Diptera: Tephritidae) as a test case". *PLoS One.* **7** (2): e31581. 10.1371/journal.pone.0031581.

[6] F. Y. Amandita, K. Rembold, B. Vornam, S. Rahayu, I. Z. Siregar, H. Kreft, and R. Finkeldey. (2019). "DNA barcoding of flowering plants in Sumatra, Indonesia". *Ecology and Evolution.* **9** (4): 1858-1868. 10.1002/ece3.4875.

[7] E. Harnelly, Z. Thomy, and N. I. R. Fathiya. (2018). "Phylogenetic analysis of Dipterocarpaceae in Ketambe Research Station, Gunung Leuser National Park (Sumatra, Indonesia) based on rbcL and matK genes". *Biodiversitas Journal of Biological Diversity.* **19** (3): 1074-1080. 10.13057/biodiv/d190340.

[8] W. Sunaryo. 2015. "Aplikasi DNA Barcoding untuk analisis keragaman genetik lai-durian (Durio zibethinus x kutejensis) asal Kalimantan Timur". presented at the Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia. 10.13057/psnmbi/m010602.

[9] A. Gismondi, M. F. Rolfo, D. Leonardi, O. Rickards, and A. Canini. (2012). "Identification of ancient Olea europaea L. and Cornus mas L. seeds by DNA barcoding". *Comptes Rendus Biologies.* **335** (7): 472-9. 10.1016/j.crvi.2012.05.004.

[10] S. Ahmed, M. Ibrahim, C. Nantasenamat, M. F. Nisar, A. A. Malik, R. Waheed, M. Z. Ahmed, S. C. Ojha, and M. K. Alam. (2022). "Pragmatic Applications and Universality of DNA Barcoding for Substantial Organisms at Species Level: A Review to Explore a Way Forward". *BioMed Research International.* **2022** : 1846485. 10.1155/2022/1846485.

[11] J. Yu, X. Wu, C. Liu, S. Newmaster, S. Ragupathy, and W. J. Kress. (2021). "Progress in the use of DNA barcodes in the identification and classification of medicinal plants". *Ecotoxicology and Environmental Safety.* **208** : 111691. 10.1016/j.ecoenv.2020.111691.

[12] S. Shrestha, F. Asch, J. Dusserre, A. Ramanantsoanirina, and H. Brueck. (2012). "Climate effects on yield components as affected by genotypic responses to variable

environmental conditions in upland rice systems at different altitudes". *Field Crops Research.* **134** : 216-228. 10.1016/j.fcr.2012.06.011.

[13] N. Kasim, N. D. P. Panggula, F. Haring, F. Ulfa, A. Dachlan, N. Widiayani, and D. Yulsan. (2020). "Growth and production of Katokkon (Capsicum chinense Jacq) chili plants in lowland applied with gibberellins and liquid organic fertilizer". *IOP Conference Series: Earth and Environmental Science.* **486** (1). 10.1088/1755-1315/486/1/012121.

[14] D. I. Roslim and A. Fitriani. (2021). "Barkoding DNA pada Tumbuhan Durik-Durik (Syzygium sp.) Asal Riau Menggunakan Daerah Gen ndhF". *Jurnal Bios Logos.* **11** (1). 10.35799/jbl.11.1.2021.31191.

[15] D. A. G. Perwitasari, S. Rohimah, T. Ratnasari, B. Sugiharto, and M. Su'udi. (2020). "DNA Barcoding of Medicinal Orchid Dendrobium discolor Lindl. Tanimbar Using rbcL and ITS genes". *Buletin Penelitian Tanaman Rempah dan Obat.* **31** (1). 10.21082/bullittro.v31n1.2020.8-20.

[16] E. S. Wardi, J. Jamsari, I. Irwandi, D. Sartika, and A. R. Ningsih. (2020). "Barkod Dna Pada Tanaman Gambir (Uncaria Gambir (Hunter) Roxb.) Berdasarkan Gen Matk Dan Rbcl". *Jurnal Ilmiah As-Syifaa.* **12** (1): 22-28. 10.33096/jifa.v12i1.587.

[17] H. Muqaddas, N. Mehmood, and M. Arshad. (2020). "Genetic variability and diversity of Echinococcus granulosus sensu lato in human isolates of Pakistan based on cox1 mt-DNA sequences (366bp)". *Acta Tropica.* **207**. 10.1016/j.actatropica.2020.105470.

[18] Y. Hong, M. Guo, and J. Wang. (2021). "ENJ algorithm can construct triple phylogenetic trees". *Molecular Therapy Nucleic Acids.* **23** : 286-293. 10.1016/j.omtn.2020.11.004.

[19] I. Masruroh, N. Triesita, S. Sulistiono, and A. Santoso. 2018. "Bamboo Kinship Based on the Rbcl Gene Based on In Silico Analysis as Evidence of Molecular Evolution". presented at the Proceedings of the VI National Seminar on Biology.

[20] N. Nursanti, A. A. Adriadi, and S. i. Sai'in. (2022). "Komponen Faktor Abiotik Lingkungan Tempat Tumbuh Puspa (Schima Wallichii Dc. Korth) Di Kawasan Hutan Adat Bulian Kabupaten Musirawas". *Jurnal Silva Tropika.* **5** (2): 438-445. 10.22437/jsilvtrop.v5i2.14566.

[21] D. A. Lestari, R. Azrianingsih, and H. Hendrian. (2017). "Taxonomical position of Annonaceae species from East Java, Indonesia: Collections of Purwodadi Botanic Garden based on morphological character". *Biodiversitas Journal of Biological Diversity.* **18** (3): 1067-1076. 10.13057/biodiv/d180326.

[22] W. J. Kress, L. M. Prince, and K. J. Williams. (2002). "The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data". *American Journal of Botany.* **89** (10): 1682-96. 10.3732/ajb.89.10.1682.

[23] R. Elfianis, J. Warino, R. Rosmaina, S. Suherman, and Z. Zulfahmi. (2021). "Analisis Kekerabatan Genetik Tanaman Padi (Oryza Sativa L.) Di Kabupaten Kampar Dengan Menggunakan Penanda Random Amplified Polymorphic Dna (Rapd)". *Jurnal Agroteknologi.* **11** (2). 10.24014/ja.v11i2.10013.

[24] R. Ferniah, S. Pujiyanto, and H. P. Kusumaningrum. (2018). "Indonesian red chilli (Capsicum annuum L.) capsaicin and its correlation with their responses to pathogenic Fusarium oxysporum". *NICHE Journal of Tropical Biology.* **1** (2): 7-12. 10.14710/niche.1.2.7-12.

[25] M. Nei. (1972). "Genetic Distance between Populations". *The American Naturalist.* **106** (949): 283-292. 10.1086/282771.