Genetic Diversity and Relationship of Durian (*Durio* spp.) Germplasm Based on the Internal Transcribed Spacer (ITS) Region: *In Silico* Analysis

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ABSTRACT

Durian (*Durio* spp.) is a germplasm with a relatively high species diversity, with an estimated 27 species worldwide. However, the existence of several species has been threatened. This study aimed to reconstruct the DNA barcode of the durian and its relatives (*Durio* spp.) and analyze the genetic diversity and its relationship based on the internal transcribed spacer (ITS) region. Sixteen sequences of durians ITS were collected from GenBank (NCBI) and analyzed *in silico* using the BLAST, MultAlin, and MEGA-X software, then reconstructed phylogenetically by the UPGMA and Maximum Likelihood methods. The results show that the ITS region of *Durio* spp. has a base length of about 702 bp, where several mutations occur, substitution (transversion and transition) and indel (insertion and deletion). At the nucleotide level, this germplasm shows a relatively high diversity of 0.065. The cluster analyses (UPGMA and Maximum Likelihood) can separate this germplasm into four clusters and five main clades, respectively. In this study, *D. zibethinus*, the most popular species in the *Durio* genus, is closely related to *D. lowianus* and far from *D. griffithii*. This information is beneficial as reference data to support durian conservation and breeding programs, locally and globally, especially in Indonesia.

Keywords: Chloroplast DNA, Breeding, Durian, Genetic diversity, Phylogenetic

ABSTRAK

Durian (Durio spp.) merupakan salah satu plasma nutfah yang memiliki keragaman spesies relatif tinggi, diperkirakan mencapai 27 spesies di seluruh dunia, namun keberadaannya telah terancam. Penelitian ini bertujuan untuk merekonstruksi DNA barcoding durian dan kerabatnya, serta menganalisis keragaman dan kekerabatan genetiknya secara in silico berdasarkan sekuen gen internal transcribed spacer (ITS). Sebanyak 16 sekuen gen ITS Durio spp. telah dikoleksi dari GenBank (NCBI) dan dianalisis secara in silico menggunakan software BLAST, MultAlin dan MEGA-X, serta direkonstruksi secara filogenetik menggunakan metode UPGMA dan Maximum Likelihood (ML). Hasil penyejajaran memperlihatkan bahwa region tersebut memiliki panjang basa sekitar 702 bp, yang didalamnya terdapat beberapa peristiwa mutasi, baik substitusi (transversi dan transisi) dan indel (insersi dan delesi). Hasil analisis lebih lanjut menunjukkan bahwa plasma nutfah ini memiliki keragaman genetik relatif tinggi, sebesar 0,065. Sementara itu, analisis UPGMA dan ML mampu memisahkan plasma nutfah Durio spp., masing-masing kedalam empat kluster dan lima klad utama. Dalam penelitian ini, D. zibethinus yang merupakan spesies paling populer dalam genus Durio memiliki kekerabatan sangat dekat dengan D. lowianus dan berkerabat jauh dengan D. griffithii. Informasi ini diharapkan sangat bermanfaat sebagai data acuan untuk mendukung program pelestarian dan pemuliaan durian (Durio spp.), baik secara lokal dan global, terutama di Indonesia.

Kata kunci: *DNA kloroplas, Pemuliaan, Durian, Keragaman genetic, Filogenetik*

INTRODUCTION

Durian (*Durio* spp.), belonging to the Malvaceae family, is a higher plant with a relatively high diversity of species (Mursyidin et al., 2022). This germplasm is estimated to reach 27 species (Kurniadinata et al., 2019). It spreads widely, especially in the Asian region, including Cambodia, India, Myanmar, Sri Lanka, Thailand, Vietnam, Malaysia, and Indonesia (Sundari et al., 2019). In Indonesia, 20 species of durian are found on several large islands, including Kalimantan (18 species), Sumatra (7 species), Java (1 species), Bali (1 species), Sulawesi (1 species), and Maluku (1 species) (Mursyidin & Daryono, 2016). In Kalimantan, local terms have named these durians, such as *lahong* for *D.*

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dulcis, *kerantungan* for *D. oxleyanus*, and *lai* for *D. kutejensis*. Several others are known as *tupaloh* (*D. acutifolius*), *apun* (*D. excelsus*), *lai kuyu* (*D. griffithii*), and *tekawai* (*D. lowianus*) (Uji, 2004).

In general, most durian species have economically and ecologically essential values (Aziz & Jalil, 2019). For example, nine durian species have edible fruits, namely *D. lowianus*, *D. graveolens*, *D. kutejensis*, *D. oxleyanus*, *D. testudinarium*, *D. grandiflorus*, *D. dulcis*, *Durio excelcus*, and *D. zibethinus* (Aziz & Jalil, 2019). Even *D. zibethinus* is an agricultural commodity with prominent export prospects (Cheon et al., 2017). Indonesia, for example, one of the biggest durian producers in the world, was able to export this fruit to several other countries, including Middle Eastern countries, with a total value of 232,000 USD in 2020 (Rizaty, 2021). In addition, this country produced over 1.19 million metric tons of durian in the same year (Statista Research Department, 2021).

Apart from producing fruit, 14 species of durian also generate wood that can be useful as interior materials. In addition, the bark of several types of durians is also used in medicine, for example, *D. oxleyanus* and *D. griffitii* as malaria drugs because they contain tannin compounds (Feng et al., 2016). However, due to various human activities, such as deforestation and excessive land conversion, especially for plantations, agriculture, settlements, and industry, several durian species have been threatened (Wilcove et al., 2013).

The *International Union for Conservation of Nature* or IUCN (2021) states that *D. acutifolius*, *D. dulcis*, *D. grandiflorus*, *D. kutejensis*, *D. pinangianus*, and *D. testudinarium* are included as vulnerable, whereas *D. lanceolatus is* the near-threatened. Consequently, employing conservation or preservation, including cultivation and breeding efforts, is indispensable. According to Wintle et al. (2019), conservation is an activity directed at saving and preserving the existence of endangered species. Meanwhile, breeding/cultivation activities aim to explore and utilize functional genes for developing new superior cultivars (van Huylenbroeck, 2018). In this case, characterization is also urgent to support both activities. However, this activity is performed using morphological markers, which have several limitations because it is time-consuming and highly influenced by environmental factors (Mursyidin & Khairullah, 2020).

The molecular markers provide speed and high accuracy in germplasm characterization activities. Among the existing molecular ones, ITS is a marker with advantages for characterizing germplasm, including durian (Santoso et al., 2017). According to Prahl et al. (2021) and Soumya & Nair (2017), this gene is located between the structural ribosomal RNA (rRNA) of a similar precursor transcript and a non-functional RNA unit with a rapid evolutionary rate. As a result, it can be used to determine germplasm relationships at the genus, species, and subspecies levels (Qin et al., 2017).

Previously, the genetics of durian germplasm have been studied by various molecular markers, such as RAPD (Mursyidin & Daryono, 2016; Prihatini et al., 2016; Hariyati et al., 2013), SSR, and ISSR (Ho et al., 2020; Santoso et al., 2016). However, these markers are highly subjective. In addition, poor consistency, limited repeatability, and complicated operation limit their effectiveness (Wu et al., 2021). This study aimed to reconstruct a DNA barcoding motif, as well as determine and analyze the genetic diversity and relationship of 16 durian species (*Durio* spp.) *in silico* by utilizing internal transcribed spacer (ITS) gene sequence data provided in GenBank or the National Center for Biotechnology Information (NCBI). According to Sayers et al. (2019), GenBank has a comprehensive database of freely accessible nucleotide sequences or formal gene descriptions. Hence, such a study

MATERIALS AND METHODS

This study was conducted from March-May 2020 *in silico* at the Laboratory of Genetics and Molecular Biology, Faculty of Mathematics and Natural Sciences, University of Lambung Mangkurat. This research used internal transcribed spacer (ITS) gene sequence data from 16 durian species (*Durio* spp.) found in GenBank or NCBI (Table 1). In general, this study includes three main activities: nucleotide sequence search and homology (similarity) analysis, nucleotide (multiple) sequence alignment, and analysis of genetic diversity and relationship of those durians obtained.

Nucleotide Sequence Search and Homology Analysis

The internal transcribed spacer (ITS) gene sequence of *D. zibethinus, which is* available on the GenBank or NCBI website (https://www.ncbi.nlm.nih.gov/) with accession number MF629779.1, was used as a reference in this study (Table 1). The homology (similarity) analysis with other durian species, also available on the GenBank website, was then carried out using the BLAST (Basic Local Alignment Search Tool) software. All of the durians (*Durio* spp.) ITS sequences were then copied into text (notepad) format for further analysis.

Multiple Sequence Alignments

Nucleotide (multiple) sequence alignment of the ITS durian region and its relatives (*Durio* spp.) was carried out online using the MultAlin software (Babar et al., 2014). The software is available online at http://multalin.toulouse.inra.fr/multalin/.

Analysis of Genetic Diversity and Relationship

Analysis of genetic diversity and the relationship of Durio spp. was performed using Molecular Evolutionary Genetics Analysis or MEGA-X software (Kumar et al., 2018). This analysis was started by inputting all ITS durian sequence data (text format) into the MEGA-X software. Before analysis, the sequences were first converted to fasta (.fas) or mega (.meg) format and aligned in the software. After that, genetic diversity analysis was carried out using the nucleotide diversity index (π) method (Nei & Li, 1979). Meanwhile, phylogenetic reconstruction was employed using the UPGMA and Maximum Likelihood methods (Swenson, 2019). The statistical (bootstrap) analysis was then applied to evaluate the internal branches of the phylograms (Kumar et al., 2018).

RESULTS AND DISCUSSION

Multiple Sequence Alignments

Multiple sequence alignment is one of the biological studies which is most frequently used data analysis models (Shi et al., 2021). This modeling is applied to look at the phylogeny of a whole genome, proteins, identification of horizontally transferred genes, and detection of combined sequences (Zielezinski et al., 2017). As data sequencing technology advances, the use of this modeling is increasing (Katoh et al., 2018). The increasing use of multiple sequence alignment modeling has made this area an active research topic, so more than 100 methods have been used. In multiple alignment analysis, all data will be entered from a point in a set of sequences into equivalent classes based on their respective similarities for all members of a common ancestor (Maiolo et al., 2018).

The durian and its relatives (*Durio* spp.) have a total length of ITS gene sequences of around 702 base pairs (Figure 1), in which several mutation events, both substitutions (transitions and transversions) and insertion-deletion (indel), are found. Table 2 provides detailed information about mutation events in the ITS durian sequences. There were 217 loci experiencing mutations in the durian ITS gene sequence (Figure 1 and Table 2).

Transversion was the most common type of mutation (95 loci) compared to others. Compared to other studies, the number of mutations in durian is higher than in other species. Soumya & Nair (2017) reported that in the ITS region of *Averrhoa* (L.), there were only 87 loci mutations out of a total of 615 bases it had. Similar other cases were shown in *Anoectochilus* (Thinh et al., 2020), *Aquilaria* (Lee et al., 2017), *Litsea* (Fijridiyanto & Murakami, 2019), *Uncaria* (Zhu et al., 2020), and *Zanthoxylum* (Zhao et al., 2018).

These study results align with Soumya & Nair (2017), stating that the ITS region is a part of ribosomal RNA with a high evolutionary rate but is universal for different taxa. Moreover, it can be utilized in phylogenetic studies, molecular ecology, detection, and identification of individual pathogens and non-pathogens (Zhang et al., 2021). According to Skuza et al. (2019), the ITS region has proven to be one of the most informative regions for forming genetic relationships between species in the genus.

In this case, however, the deletion was the lowest found in the ITS sequence of durians (13 loci). A similar result was found in fungi (Zhang et al., 2021). According to Houde et al. (2019), such a

Table 1. Species of Durians used in this study, the nucleotide length, and GenBank accession number

Species	Nucleotide length (bp)	GenBank Accession Number
D. acutifolius	684	AF287700.1
D. affinis	692	AF287705.1
D. beccarianus	695	AF287707.1
D. carinatus	689	AF287708.1
D. dulcis	689	AF287713.1
D. grandiflorus	683	AF233320.1
D. graveolens	729	MF629770.1
D. griffithii	684	AF233310.1
D. kutejensis	730	MF629750.1
D. lanceolatus	686	AF287709.1
D. lowianus	688	AF287711.1
D. oblongus	692	AF287703.1
D. oxleyanus	688	AF233306.1
D. singaporensis	696	AF287701.1
D. testudinarium	694	AF287704.1
*D. zibethinus**	747	MF629779.1

*Reference species

Table 2. Mutations on the ITS region of *Durio* spp. germplasm

Mutation type	Number of Mutation
Deletion	13
Insertion	15
Substitution-transition	94
Substitution -transversion	95
Total	217

Table 3. Information of internal transcribed spacer (ITS) of *Durio* spp*

Parameter	Value
Nucleotide length (bp)	684-747
Number of the polymorphic site	217
Bayesian information criteria (BIC)	4538.350
Akaike information criteria (AIC)	4320.399
Maximum likelihood value (lnL)	-2130.112
Transition/tranversion bias value (R)	1.010
GC content (%)	68.710
Nucleotide diversity (π)	0.065

* following Kimura 2-parameter model

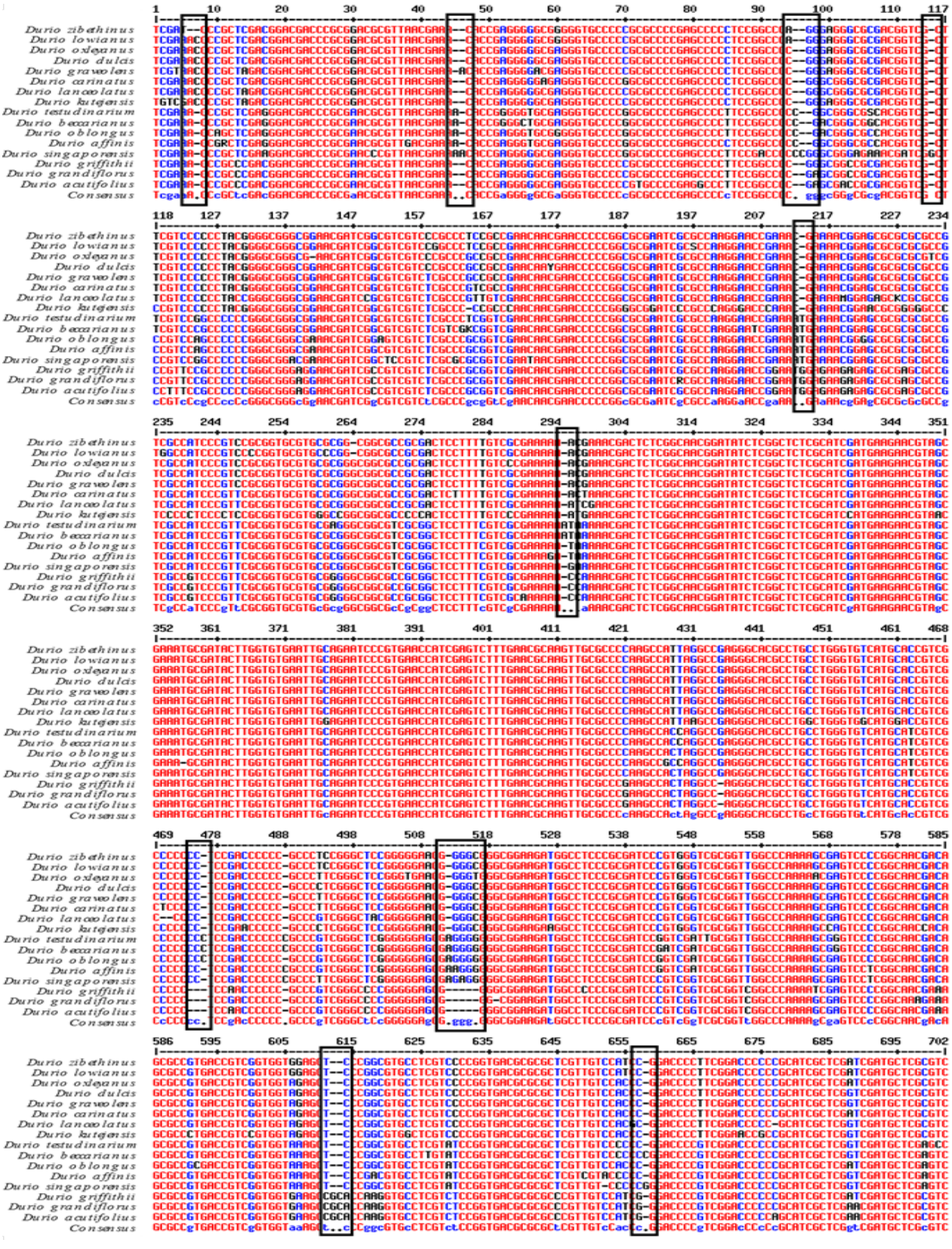

Figure 1. Multiple sequence alignment of the ITS region of *Durio* spp., showing a unique DNA barcoding motif, where mutation, like indels, present therein (close rectangle)

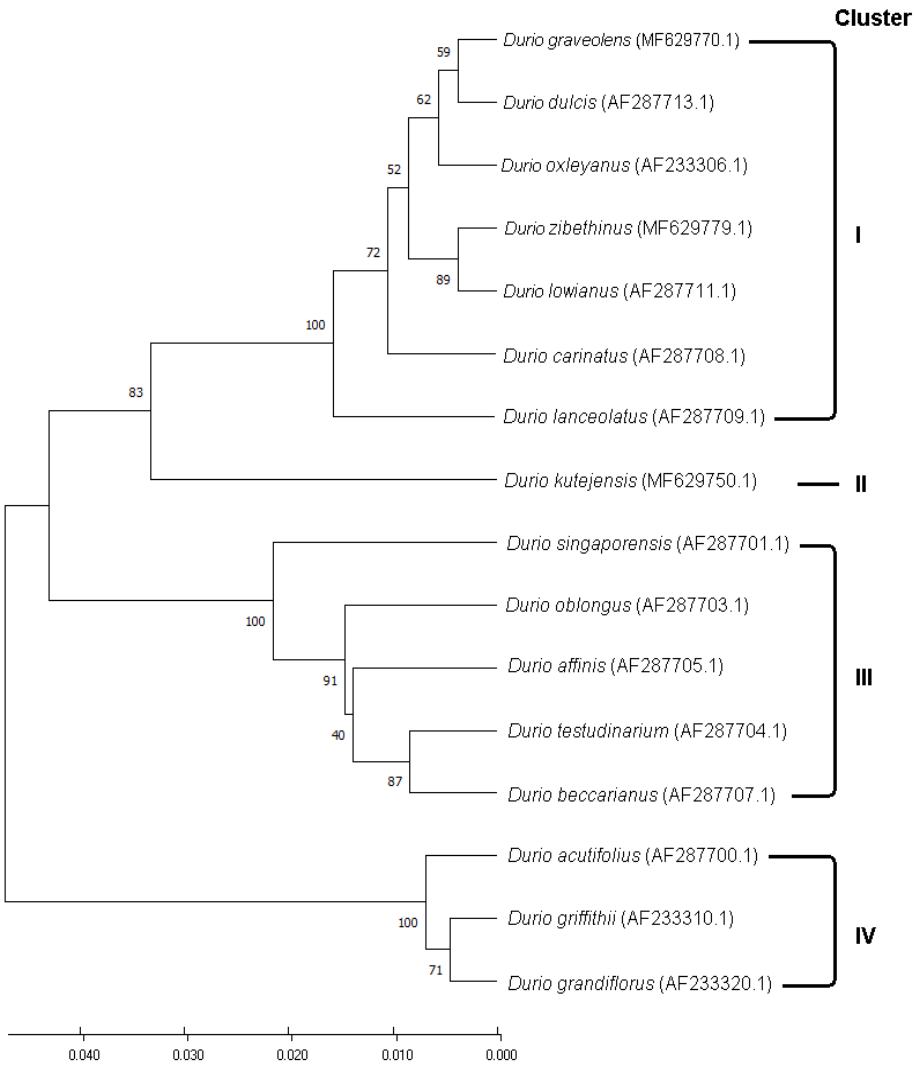

Figure 2. The genetic relationship of *Durio* spp. based on UPGMA analysis. The value on the internal branch shows the results of the bootstrap analysis 1000 replicates

mutation has a significant value in phylogenetic relations or increases the resolution of evolutionary genetic relationships between the studied taxa. Among candidate DNA barcoding regions, ITS is a non-coding region that generally shows high genetic diversity, including indel polymorphism, so it has the potential ability to be applied in species identification (Qin et al., 2017).

Genetic Diversity and Relationship of *Durio* spp.

Durian and its relatives (*Durio* spp.) showed relatively high genetic diversity at the nucleotide level, recorded at 0.065 (Table 3). This genetic diversity is closely related to mutation events in the ITS durian sequences studied. Based on Table 3, the *Durio* spp. has ITS sequence character with relatively high polymorphic sites (217 loci), relatively high GC content (68.71%), and the substitution bias value (transition/transversion) is also high (1.01).

According to van Dorp et al. (2020), mutations are the main factor in the emergence of genetic diversity. In other words, this phenomenon is the primary source that plays a vital role in knowing the differences and evolutionary events between

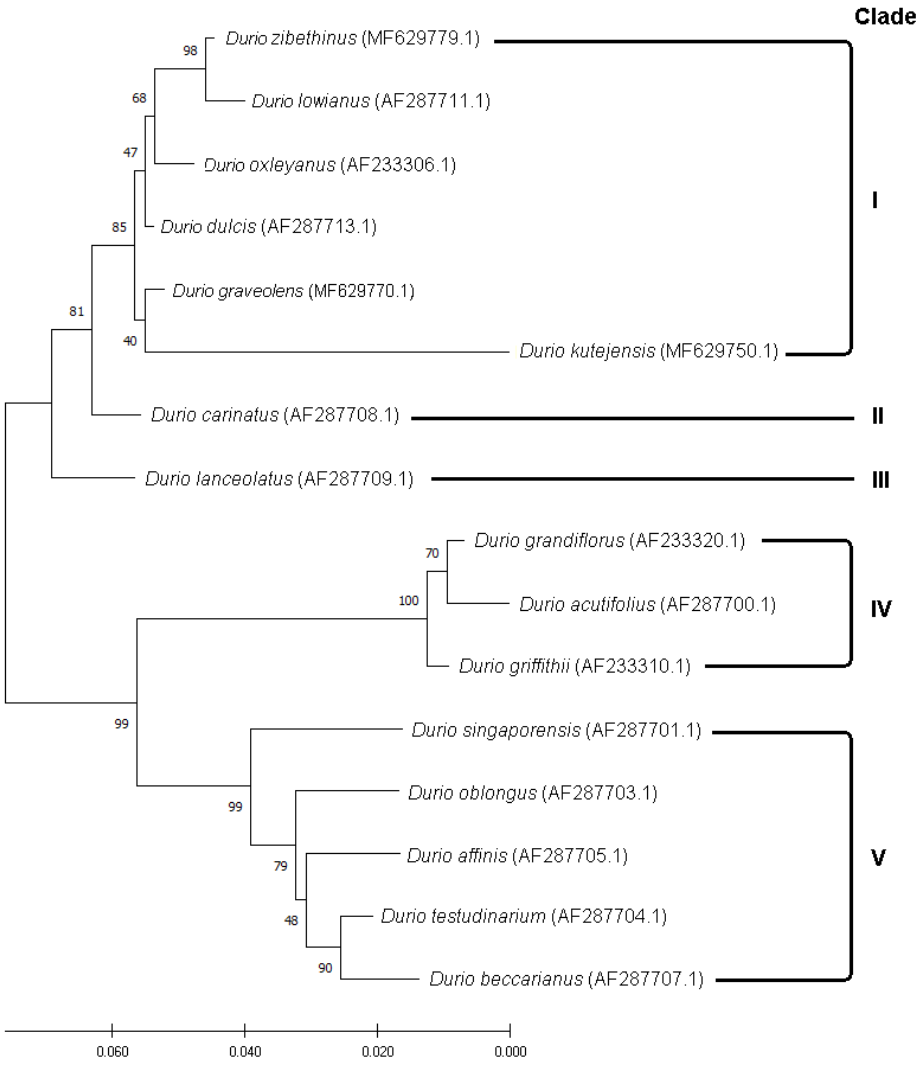

Figure 3. The genetic relationship of *Durio* spp. based on Maximum Likelihood (ML) analysis. The value on the internal branch shows the results of the bootstrap analysis 1000 replicates

species. In short, a mutation is an event of permanent changes in genetic material, genes, genomes, and chromosomes (Maluszynski et al., 2017). At the nucleotide (gene or genome) level, mutations include four types, namely deletions, insertions, transitions, and transversions. Deletion and insertion mutations can cause a shift in the coding of the nucleotide triplet. Meanwhile, transition and transversion mutations can change the amino acid composition formed (Guo et al., 2017). According to Qin et al. (2017), internal transcribed spacer (ITS) sequences generally have mutations that cause variations between species.

Based on the UPGMA method (Figure 2) and Maximum Likelihood or ML (Figure 3), the durian germplasm (*Durio* spp.) was separated into four clusters and five main clades, respectively. The separation is generally relatively consistent for all species that group together, either into the same cluster or clade. It is strongly related to the bootstrap analysis results and is relatively high in the resulting internal branches of the dendrogram (Figure 2) and phylogram (Figure 3).

In this study, *D. zibethinus* closely related to *D.*

lowianus but very far from *D. griffithi* (see Figures 2 and 3). This study's results align with Nyffeler & Baum (2000) using the ITS marker, stating that *D. zibethinus* is closely related to *D. lowianus*. According to Nyffeler & Baum (2000), these two durian species have morphological similarities, such as calyx, filament arrangement, and anther architecture. Based on other studies, mainly using markers *ndh*F and ITS, a close relationship between *D. zibethinus* and *D. oxleyanus* was reported (Nyffeler & Baum, 2001). Meanwhile, based on the ITS nr-DNA marker, the relationship between *D. zibethinus* and *D. kutejensis* was reported (Santoso et al., 2017). Furthermore, following the UPGMA (Figure 2) and ML (Figure 3), *D. carinatus* and *D. lanceolatus* are nearly related. Naufal (2021) reported that these two durians had the same primitive character in the form of a straight pistil stalk and flowering present on the branches.

However, the relationship between these organisms can show the results of molecular evolution during a time course in the presence of genetic differences (Guerrero et al., 2019). In other words, phylogenetic trees obtained from polymorphisms in a genome, such as chloroplasts, can be used to determine the bar code of an organism and identify differences in taxonomic status and evolution, including population genetics (Nguyen et al., 2017; Xu et al., 2020). Furthermore, information on these relationships benefits the conservation of endangered species and increases plant breeding in general (Bi et al., 2018).

CONCLUSION

Durian germplasm (*Durio* spp.) shows a unique DNA barcode motif based on the internal transcribed spacer (ITS) region sequence. The alignment of these sequences shows that the ITS *Durio* spp. has a base length of about 702 bp, in which there are several mutation events, both substitution (transversion and transition) and indel (insertion and deletion). The results also show that this germplasm has a relatively high genetic diversity, amounting to 0.065. Meanwhile, the cluster analysis (UPGMA) and Maximum Likelihood (ML) can separate *Durio* spp. into four clusters and five main clades, respectively. In this study, *D. zibethinus*, the most popular species in the genus *Durio*, is closely related to *D. lowianus* and distantly related to *D. griffithii*. This information is beneficial as reference data to support durian (*Durio* spp.) conservation and breeding programs, both locally and globally, especially in Indonesia.

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