Amplification rate of matK and rbcL genes in three types of durian

Y F Cahyaningsih1, Sukartini1, A Sutanto1, P J Santoso1, M A Arsyad2 and S H Larekeng3

1 Indonesian Tropical Fruits Research Institute, Jl. Raya Solok-Aripan Km 8, Aripan, X Koto Singkarak, Solok, West Sumatera
2 Agrotechnology, Faculty of Agriculture, Halu Oleo University, Kampus Bumi Tridharma, Jl. HEA Mokodompit, Kendari, South East Sulawesi
3 Forestry department, Faculty of Forestry, Hasanuddin University, Jl. Perintis Kemerdekaan Km 10, Makassar, South Sulawesi

Email: sittihalimah@unhas.ac.id

Abstract. Durian is a tropic fruit having numerous variations on its fruits. Its variations are not only in its shape but also in its aril fruit, aril color, flavor, and aril thickness. In addition to its fruit variations, the genus Durio also has many species which quite hard to distinguish morphologically, except during flowering and fruiting times. This study aimed to determine the genetic relationship among Durian, Pelangi Atuturi Variety Durian, Durio graveolent, and Durio zibethinus based on chloroplast genes (RbcL and matK genes). The primers were previously designed for amplifying matK and rbcL genes based on the Durio zibethinus sequence. Both genes were used because of having great competence to describe genetic relationships between plant species. The rbcL primer could amplify all evaluated samples. Meanwhile, matK primer generated a smeared band in Durian Pelangi; thus, we did not obtain any sequence of this plant. Sequence analysis showed no variation of rbcL sequence in these evaluated species. A similar result was also observed on D. zibethinus and D. graveolent. Overall, both genes could not describe the genetic relationship among the evaluated durians, and they were grouped in the same cluster in phylogenetic.

1. Introduction

Durian (Durio zibethinus) is a tropical fruit from the genus Durio. Twenty species of this genus grow in Indonesia, and eighteen species can be found in Borneo Island. Apart from D. zibethinus, this genus also has other edible species, D. graveolens, D. dulcis, D. kutejenensis, D. testudinarum, D. grandifloras, D. oxleyanus, D. lowianus, and D. excelsus [1].

Based on the morphology of the fruit, plants in the genus Durio have highly diverse aril colors, from white to red. D. zibethinus has variations in the aril color ranging from white (like durian susu variety), yellow (like durian matahari variety) to orange. Red aril color belongs to D. graveolent. Color gradations in the aril color were also observed on the Pelangi atururi variety durian. This durian has red-orange and yellow color gradation (Figure 1). Based on this aril color, Pelangi variety durian is presumed closely related to D. zibethinus and D. graveolent.
The analysis of chloroplast gene sequences can be utilized to identify the relationship among these durians. Two chloroplast genes that are commonly used for the analysis are matK and rbcL. According to the CBOL plant working group [2], both genes are the best combination of gene loci for analyzing relationships in the plant species.

The matK gene can describe the plants even in a very close relationship [3]. On the other hand, this gene has a low amplification rate. Whereas the rbcL gene has a high amplification rate, but its capability to describe plants is not better than the matK gene [2].

This study aimed to determine the amplification rate of matK and rbcL genes in three types of durian, namely D. zibethinus, Pelangi atururi variety durian, and D. graveolent, as well as identify the relationship among those durians based on the sequences of matK and rbcL genes. Both genes have good capability to describe genetic relationships in plants, high amplification rate, and generate high quality of sequences.

2. Methodology
This research was conducted at the Molecular Laboratory and Seed Quality Test, Indonesian Tropical Fruits Research Institute, Solok, West Sumatra, Indonesia, from February to June 2021.

2.1. Plant materials
The samples used consisted of three types of durian (D. zibethinus, D. graveolent, and Durian Pelangi Atururi). Samples were collected from the genetic resources collection garden of the Indonesian Tropical Fruits Research Institute.

2.2. DNA extraction
DNA was extracted using the Geneiad Mini Kit Plant Extraction with modifications. The modifications were the incubation stage carried out for 3 hours at 65°C [4]. The quantity of DNA was tested using a spectrophotometer (Eppendorf). DNA quality was tested by electrophoresis using 1% agar dissolved in 1x SB buffer. The electrophoresis process was performed at 100 V for 45 minutes. The DNA concentration for DNA working was 20 ng/mL. The isolated DNA was stored at -20°C.

The matK and rbcL primers were designed for DNA amplification. The primers were designed using matK and rbcL sequences that have been deposited in the Gene Bank with accession number NC_036829.1 [5]. The designed primers were used to amplify the 600-900 bp of matK and rbcL gene regions. The primer sequences were designed using Primer Tree Plus online software.
2.3. DNA amplification

DNA amplification was performed using a PCR thermal cycle (Eppendorf) machine with an initial denaturation stage at 95°C for 5 minutes, followed by 35 cycles consisting of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 52°C, extension for 30 seconds at 72°C. Final extension for 10 minutes at 72°C.

The PCR mix solution for each reaction was 25 µL. The solution consisted of 12.5 µL of PCR mix My Taq™ Red Mix (Bioline), 1µL of 10µM for each primer, 2µL of DNA template, and ddH₂O. The primers used to amplify the samples were matK1 and rbcL1 (Table 1).

The PCR product and the 1Kb ladder were electrophoresed using 1.2% agarose gel in 1× SB buffer at 50 Volts for 90 minutes. The staining process was carried out by soaking the gel in EtBr for 10 minutes and distilled water for 15 minutes. Gel documentation was carried out using a BDA Digital (Biometra) gel doc. The PCR products were then sequenced using a sequencing service (1st BASE Malaysia).

Quality control (QC) of sequences was carried out using Genestudio software. The alignment sequence was using Geneious software. The phylogenetic and genetic distances were constructed using the UPGMA method in MEGA X software [6].

3. Results and discussion

3.1. DNA amplification

The primer used to amplify the sample consisted of two pairs of primers, one matK and one rbcL (Table 1). The rbcL primer produced band ± 650bp. The optimum primer temperature was 54°C, where the primer produced a clear and specific band. All samples were successfully amplified using rbcL primer.

The matK primer produced band ± 850bp. The specific and clear band was produced at 52°C. Not all amplified samples produced specific bands. The primers produced smear bands on the Pelangi variety Durian samples; thus, they could not be sequenced and were not analyzed further (Figure 2).

![Electropherogram of PCR product separation of evaluated durians base on matK and rbcL primers](image)

Figure 2. Electropherogram of PCR product separation of evaluated durians base on matK and rbcL primers

The results of the amplification using the selected primers showed that the amplification level of the gene rbcL was better than the matK gene. The matK primer generated a smeared band in the durian Pelangi sample, and thus the sample could not be sequenced. If this sample was sequenced, the
quality of the sequence would be low. A similar result was reported on plants of the genus Acacia. The amplification rate of the rbcL gene was higher at 7% than the matK gene [7]. Smear bands can be caused by improper annealing temperature. But we eliminate this possibility. Because at the time of optimization of DNA amplification (PCR process), the annealing temperature gradient produces a firm and clear band starting at temperature lowest to highest. Another factor that can cause the primer to produce a smeared band is an unspecified primary sequence. An unspecified primary sequence can be stuck to other places that are not the target of amplification to produce smears bands or multiple bands.

3.2. Nucleotide composition

Sequence analysis of the matK gene showed that there was no difference in the number of nucleotides A, T, C, and G in sequence species *D. zibethinus* and *D. graveolent* (Table 1). This indicates that the sequence of Durio species is highly conserved, even in the gene matK, which has a high mutation rate. MatK sequences have high levels of nucleotide substitution, accumulation of important insertions and deletions (indel), sequence informative, and sequence variation is also greater than [8].

| Species         | Nucleotide A | Nucleotide T | Nucleotide G | Nucleotide C | Sequence length |
|-----------------|--------------|--------------|--------------|--------------|----------------|
| *D. zibethinus* | 232          | 300          | 124          | 140          | 796            |
| *D. graveolent* | 232          | 300          | 124          | 140          | 796            |

Similar results were also obtained for the rbcL gene sequence, with no difference in amount nucleotides from the rbcL gene sequence in *D. zibethinus*, *D. graveolent*, and Durian Pelangi species (Table 2).

| Species         | Nucleotide A | Nucleotide T | Nucleotide G | Nucleotide C | Sequence length |
|-----------------|--------------|--------------|--------------|--------------|----------------|
| *D. zibethinus* | 166          | 176          | 137          | 133          | 612            |
| *D. graveolent* | 166          | 176          | 137          | 133          | 612            |
| Durian Pelangi  | 166          | 176          | 137          | 133          | 612            |

3.3. Phylogenetic Relationship and Genetic Distance

The phylogenetic results based on the matK and rbcL gene sequences showed the three types of durian tested are in the same cluster (Figure 3 and 4). Similar results were obtained from the distance where the genetic distance value of the three types of durian is zero. This indicates that the matK and rbcL gene sequences used have not been able to describe the differences between the three types of durian. The absence of sequence variation in the sample causes the obtained phylogenetic tree to group them on the identical line although different species. Phylogenetics is based on variations in the sequence analyzed using a specific algorithm. Sequence variations generally occur because of insertions, deletions, or substitutions in gene sequences that signal evolutionary processes. Sequence matK has a higher mutation rate than rbcL because that matK is very informative used to make phylogenetic [9]. According to [10], rbcL has a universal sequence that is highly conserved and has a slow evolutionary rate. Several research results also show that the matK and rbcL genes have not been able to discriminate against the plants tested. Research Das et al. (2013) [11] showed the matK gene has not been able to identify the bamboo species from Northeast India. The same result too obtained by [12] where matK and rbcL have not been able to discriminate four species of Styrax L growing in...
North Sumatra. The research results of Kang et al. (2017) [10] showed that the combination of matK and rbcL genes was not suitable for the identification of forest plants tropical at the species level. Further research needs to be done using other types of genes which can describe genetic diversity to the species level in tropical plants.

Figure 3. Phylogenetic tree types of durian base on matK sequence

Figure 4. Phylogenetic tree types of durian base on rbcL sequence

4. Conclusion
The rbcL gene had a higher amplification rate compared to the matK gene in the durians tested. The rbcL gene amplified all tested samples, while the matK gene produced a smeared band in the Pelangi durian sample. MatK and rbcL genes cannot describe the relationship tree type of durian.
Reference
[1] Uji T 2005 Keanekaragaman Jenis dan Sumber Plasma Nutfah Durio (Durio Spp.) di Indonesia Bul. Plasma Nutfah 11 28–33
[2] CBOL 2009 A DNA barcode for land plants are PNAS 106 12794–7
[3] Osman S A and Ramadan W A 2019 DNA barcoding of different Triticum species Bull. Natl. Res. Cent. 43
[4] Santoso P J 2015 Pemetaan Marka Mikrosatelit DNA terpaut Karakter Tahan penyakit Phytiaeae pada Durio sp. (Intitut Teknologi Bandung)
[5] Cheon S ., Jo S, Kim H ., Kim Y ., Sohn J . and Kim K J 2017 he Complete plastome sequence of durian, Durio zibethinus L (Malvaceae) ’itchonrial part B Resour 2 763–4
[6] Tamura K, Stecher G, Peterson D, Filipski A and Kumar S 2013 MEGA6: Molecular evolutionary genetics analysis version 6.0 Mol. Biol. Evol. 30 2725–9
[7] Ismail M, Ahmad A, Nadeem M, Javed M A, Khan S H, Khawaish I, Sthanadar A A, Qari S H, Alghanem S M, Khan K A, Khan M F and Qamer S 2020 Development of DNA barcodes for selected Acacia species by using rbcL and matK DNA markers Saudi J. Biol. Sci. 27
[8] Barthet M M and Hilu K W 2007 Expression of matK: Functional and Evolutionary Implications Am. J. Bot. 94 1402–12
[9] Larekeng S H 2019 Selection of Dominant and Co-dominant Markers for Red Wood (Pterocarpus indicus Willd) Polymorphism from Five Provenances in East Nusa Tenggara ICOST (EUDL)
[10] Kang Y, Deng Z, Zang R and Long W 2017 DNA barcoding analysis and phylogenetic relationships of tree species in tropical cloud forests Sci. Rep. 7 1–9
[11] Das M M, Mahadani P, Singh R, Karmakar K and SK G 2013 MatK sequence based plant DNA Barcoding failed to identify Bambusa (Family: Poaceae) species from Northeast India J Env Sociobiol 10 49–54
[12] Susilowati A, Hendalastuti H, Kholibrina C R and Ramadhani R 2017 Short communication: Weak delineation of Styrax species growing in North Sumatra, Indonesia by matK + rbcL gene Biodiversitas 18 1270–4