Isolation and amplification of mangrove plants using DNA barcode in Percut Sei Tuan, North Sumatra, Indonesia

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Abstract. Mangroves are a collection of several species of trees or shrubs that distribute around the coastline and can survive in high salinity environments. Around 60% of mangrove forests in North Sumatra are reported to have been damaged, the main factors of this damage being the mangrove forests conversion into ponds and the expansion of oil palm plantations. Identification of mangrove species is very important in protecting and applying the biodiversity of mangrove forests. Identification of living things has evolved from morphological characterization to molecular identification. This study aims to explain the DNA isolation and PCR methods to identify mangrove species in North Sumatra. The results suggested that the rbcL primer used can detect mangrove species that were visualized in the form of DNA bands. The length of DNA fragments of mangrove species Acrosticum aureum ranged 632.0-619.6 bp, species Rhizophora apiculata 619.6-585.8 bp, species Nypa fruticans 600-592.9 bp, species Avicennia alba 549.1-533.5 bp, species Hibiscus tiliae was not detected, and mangrove species Acanthus ilicifolius 480.3 bp.

1. Introduction
Mangroves are a collection of several trees or shrubs species that distribute around the coastline and can live in high salinity environments. Mangrove forests provide an important role, as a source of livelihood, because they can produce various products of high economic value including fuel (firewood and charcoal), building materials (beams, roofs, etc.), fisheries, food raw materials, medicines, and agro-tourism [1-4].

Mangrove loss in North Sumatra was found in primary mangrove forests significantly reduced 61.21% between 1990 and 2015, main deforestation was from 1990 to 2000 became secondary mangrove forest and swamp shrub [5]. The main factor in the destruction of mangroves during this period was conversion to ponds. In the next two decades, it is estimated that the expansion of ponds and the expansion of oil palm plantations will still be the main factors for mangrove loss in Indonesia, especially in North Sumatra [5].

Identification of mangrove species is important to conserve and utilize the biodiversity, which seems to be deterred by taxonomic expertise. The method of identification of living species has evolved from morphological identification to molecular identification [6]. The primer used was rbcL primer. The rbcL
gene has a low mutation rate compared to other barcode genes so that this gene provides an advantage for in-depth studies of interspecies genetic and phylogenetic variation [7]. This study aims to report a DNA extraction and PCR amplification prior was used for the identification of mangroves in North Sumatra.

2. Materials and Methods

2.1. Plant material

The plant material used was DNA from mangrove leaves obtained of species *Acrosticum aureum*, *Rhizophora apiculata*, *Nypa fruticans*, *Avicennia alba*, *Hibiscus tiliaceus*, *Acanthus ilicifolius* from Percut Sei Tuan, North Sumatra Province, Indonesia as depicted in Table 1.

Figure 1. Sample of mangrove leaves (A= Acrosticum aureum, B= Rhizophora apiculata, C= Nypa fruticans, D= Avicennia alba, E= Hibiscus tiliaceus, F= Acanthus ilicifolius).

2.2. Method

2.2.1. DNA Extraction. DNA isolation using the Genomic DNA Mini Kit (Plant). Briefly, before the leaves are used, the leaves are first cleaned with 70% ethanol. 50 mg of leaves were cut and froze with liquid nitrogen, then ground the leaves using a mortar. The sample was transferred to a 1.5 ml tube then
added 400µl Buffers GP1 and 5 µl RNase, then vortex. The sample was incubated at 60°C for 10 minutes, alternating samples every 5 minutes. 100µl buffer added GP2 and vortexed and then incubated on ice for 3 minutes. Samples were centrifuged at 13,000 rpm for 6 minutes. The supernatant was transferred into the filter column and then centrifuged at 15,000 rpm for 2 minutes. Next step buffer GP3 is added into the 1.5 ml tube as much as 700µl as previously described [8].

Then the supernatant was transferred into a 1.5 ml tube that has been filled with Buffer GP3 mix supernatant then transfer 700 µl supernatant that has been mixed into the GD Column then centrifuged at 15,000 rpm for 2 minutes. The filter was removed and then transferred the remaining mixture into the GD Column, centrifuged at 15,000 rpm for 2 minutes, and discarded the filter. 400µl buffer W1 was added into the GD Column and then centrifuged at 15,000 rpm for 30 seconds then discarded the filter results. Then 600µl wash buffer was added then centrifuge 15,000 rpm for 30 minutes, discarded the filter, centrifuged 15,000 rpm for 3 minutes to dry the matrix column. The top of the GD Column was transferred to a new 1.5 ml tube. 100µl added the heated elution buffer to the center of the matrix column. Samples were left for 3-5 minutes then centrifuged at 14,000 rpm for 1 minute. Finally, the DNA was stored in the freezer (-20°C).

2.2.2. PCR amplification. Mix PCR was made with 3.6 µl of ddH2O composition, GoTaq 2.5 µl, primer of 0.5 µl, and DNA of 1 µl, along with rbl and trnH-psbA primers. Amplification was carried out with a PCR machine. Amplification program was performed at 94 °C preheat cycle for 3 minutes, denaturation cycle for 94°C at 30 seconds, annealing at 53°C for 1 min, and elongation at 72°C for 1 min, final heating at 72°C for 7 minutes [9].

2.2.3. Visualization of PCR amplification. Visualization of the results of PCR amplification was carried out by electrophoresis of the PCR product produced by making 2% agarose gel by dissolving 0.8 gram of agarose with 40 mL of TAE 1 X, heated in a microwave for 2 minutes, and visualized by Ultraviolet (UPV).

2.2.4. Data analysis. The DNA amplicon was determined with UV-1D software (UV-Tex ver v16.09b) as previously reported [10].

3. Results and Discussion
Table 1 shows the length of amplicon fragments viewed with UV-ID after irradiation with UV light. The length of PCR product for A. aureum mangroves ranged from 619.6-632.0, R. apiculata 619,588-585,786 bp, N. fruticans 600- 592.9 bp, A. alba species 549,135-533,58 bp, H. tiliaceus species were not detected, and mangroves A. ilicifoilus 480.3 bp.
Table 1. DNA amplification of mangrove species in Percut Sei Tuan.

| Line | Mangrove species         | Amplicon (bp) |
|------|--------------------------|---------------|
| 1    | Acrosticum aureum        | 632.0         |
| 2    | A. aureum                | 625.8         |
| 3    | A. aureum                | 619.6         |
| 4    | Rhizopora apiculata      | 585.8         |
| 5    | R. apiculata             | 613.1         |
| 6    | R. apiculata             | 619.6         |
| 7    | Nypa fruticans           | 600.0         |
| 8    | N. fruticans             | 592.9         |
| 9    | N. fruticans             | 578.6         |
| 10   | Avicennia alba           | 533.5         |
| 11   | A. alba                  | 549.1         |
| 12   | A. alba                  | 549.1         |
| 13   | Hibiscus tiliaceus       | Na            |
| 14   | H. tiliaceus             | Na            |
| 15   | H. tiliaceus             | Na            |
| 16   | Acanthus ilicifolius     | 480.2         |

Na= not amplification

PCR and PCR primer used can detect mangrove species that are visualized in the form of DNA bands. The rbcL primer is an effective marker for identifying mangroves, (Harisam et al., 2019). According to the research [11], which tested the primer rbcL and matK to identify mangrove species and they recommended matK to be a worthy candidate primer for identifying mangrove species. However, other studies found that rbcL and trnH-psbA have a high amplification and sequencing success rate, indicating that these two markers are suitable for species identification in mangrove plants. [11, 12].

Figure 2. PCR Results primer rbcL of Mangrove Samples (L = Ladder, lines 1-16 = PCR results) documented with UV-1D software (UV-Tex ver v16.09b).

Figure 2 showed the amplification of mangrove plant samples, from 16 samples, only 3 mangrove samples were not detected. Each sample has a different length of the fragment depending on mangrove species. These samples were further investigated to clarify the correct identification using sequence.
4. Conclusion
The primer rbcL could be used as a DNA barcode in identifying mangrove species in North Sumatra Province.

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