Molecular Identification and Genetic Diversity of *Thalassia hemprichii* Through DNA Barcoding Using Internal Transcribed Spacer gene (ITS) from Awur Bay Jepara, Indonesia

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**Abstract.** *Thalassia hemprichii* is a type of seagrass that dominates in Awur Bay of Jepara. Conventional identification has many obstacles and the environmental pressure causes seagrass stand damage and incomplete. This leads to difficulty in morphological identification. Therefore, it is necessary to do molecular identification. DNA barcode using ITS gene is one method of molecular identification using short sequences that are efficient and have a high level of accuracy. The purposes of this research were to identify *Thalassia hemprichii* using DNA barcode of the Internal Transcribed Spacer (ITS). The research methods includeds DNA extraction using CTAB, PCR amplification of ITS fragment, electrophoresis, DNA sequencing, and analysis with MEGA 6 and BLAST. The result of this research showed that all of the samples studied were *Thalassia hemprichii*. DNA barcode of ITS is a gene marker which capable of identifying seagrass species of *Thalassia hemprichii*.

**Keywords :** *Thalassia hemprichii*, barcode, ITS

1. Introduction

Seagrass is a high-level plant that lives and adapted to submerged in sea water during its life cycle [1]. There are 50 species of seagrass worldwide, 12 of which are found in Indonesian waters [12].

DNA barcode is an identification system that uses one or more short gene sequences taken from the genome standard and used for type identification [8]. This technique is important because there can be some changes in plant morphology so that it looks similar to each other [9]. Plant DNA (DNA barcode) markers are quite a lot, however Consortium for the Barcoding of Life (CBoL) Plant Working Group [13] recommends three genes that can be used for patching DNA, which is *rbcL*, *matK* and ITS. The Internal Transcribed Spacer (ITS) is DNA in the ribosomal nucleus (nrDNA) that is very useful for obtaining the approval and biogeography of a species. ITS is able to identify plant species to species level [6].

Awur Bay is one of the waters in Jepara that has a relatively high diversity of seagrass species. Seagrass conditions in Awur Bay and its surrounding areas have high cover and distribution [10]. However, seagrass beds in that area are classified as vulnerable ecosystems. The nature of seagrasses is vulnerable and as plants are attached to a substrate, the changes that occur in the water will affected seegrass. The environmentl pressure caused damage to the seagrass. *Thalassia hemprichii* is a type of seagrass that has the most damage, because *T. hemprichii* is a type of seagrass that grows dominantly in Awur Bay. The existing environmental pressure can affect the genetic of a biota. On the other hand, the damage caused seagrass shape difficult to recognize morphologically. It is necessary to identify molecularly to find *Thalassia hemprichii* seagrass in Teluk Awur Jepara.
2. Materials and Methods

Sample Collection

Samples have been taken from the Awur Bay, Jepara, West Java, Indonesia. Sampling was done in the front area of the mangrove. The first location is ± 100 meters from the beach and the second location in ± 300 meters (break water area). Seagrass samples were selected base on morphology from the part of the leaf that has no defects. Leaf samples were taken 10 seagrass stands.

DNA Extraction

DNA extraction was done using CTAB buffer according to Dole and Doyle (1990) with modification. The composition of CTAB are 100 mM Tris pH 8, 1.4 M NaCl, 50 mM EDTA pH 8.1%, Polyvinylpyrrolidone (PVP), 3% β-mercaptoetanol. Before the extraction process, the CTAB buffer was heated at 65 °C. As much as 0.5 gram young leaf sample was placed in a mortar and 1 ml of CTAB extracting buffer was added, then crushed. The mixture was incubated at 65°C for 30 minutes and centrifuge for 8,000 rpm for 15 minutes. The supernatant was transferred to the new microtube and chloroform: isoamilalkohol (24:1) and the mixed using vortex for 1 minute followed centrifugation at 8,000 rpm for 5 minutes. Furthermore, the supernatant was transferred to a new microtube, then 500 µL of cold isopropanol was added incubated at -20 °C for a minimum of 2.5 hours. The tubes were centrifuged at a speed of 8,000 rpm 3 minutes and the supernatant was discarded. The pellets were washed using 70% ethanol and centrifuged at 8,000 rpm for 3 minutes. Furthermore, 70% ethanol was removed and the microtube and the pellet was air dried. Sterile aquades of 50 µL were added to dissolve the DNA pellet and to remove RNA, 2 µL of RNase was added and incubated at 37 °C for 30 minutes. The quality DNA was evaluated using 1% agarose gel electrophoresis with atidium bromide staining the quantity of total DNA was determine using comparison with lambda DNA (Thermo Scientific, USA).

DNA Amplification (PCR) and Sequencing

The primer were used is S2F (ATGCGATACTTGGTGTAAT) and S3R (GACGCTTCTCCAGACTACAAT) [3]. The total reaction of PCR was 25 µL consisted of H2O 9.75 µL, PCR buffer 2.5 µL, dNTP 5 µL, MgCl2 3 µL, S2F Primer 1.25 µL, S3R Primer 1.25 µL, and taq polymerase 0.25 µL and template DNA 2 µL. The PCR cycles were pre-denaturation 95°C 5 minutes, denaturation 95°C 1 minute, annealing 62°C 1 minute 30 seconds, extension 72°C 2 minutes, repetition 38 times. PCR products were evaluated using 1% agarose gel, electrophoresis. The PCR products were sent to PT. Genetika Indonesia. A total of 7 of the best samples were sent for sequencing.

Data analysis using MEGA 6 and BLAST

The DNA sequences were aligned using the ClustalW program on Mega 6 software. Dendogram was developed using Neighbor Joining. The DNA sequences were also subjected to BLAST (Basic Local Alignment Search Tool) alalysis to serch or sequence homology.

3. Results and Discussion

DNA and PCR Extraction Results using ITS Primer

DNA extraction aims to collect DNA so that it can be used for bioinformatics, phylogenetic, relationship and diversity analysis[5]. The DNA extraction of T. hemprichii can be said to be successful even though smeared DNA was observeds. Lamda DNA with known concentratien (λ2 50 ng and λ4 100 ng) was used to determine DNA concentration (Figure 1).
Identify using BLAST

The Basic Local Alignment Tool (BLAST) method was used to find the similarity of nucleotide sequences so that samples can be known for their type. BLAST is accessed online at NCBI (http://ncbi.nlm.nih.gov). The results showed that samples were similar to *Thalassia hemprichii* seagrass species (table 1).

| No. | Sample | Max score | Total score | Query cover | E value | Ident. | Name of Species |
|-----|--------|-----------|-------------|-------------|---------|--------|-----------------|
| 1.  | 2 S2F  | 145       | 145         | 18%         | 2e-30   | 97%    | *Thalassia hemprichii* |
| 2.  | 8 S2F  | 593       | 593         | 72%         | 4e-165  | 87%    | *Thalassia hemprichii* |
| 3.  | 9 S2F  | 619       | 619         | 68%         | 3e-173  | 89%    | *Thalassia hemprichii* |
| 4.  | 11 S2F | 847       | 847         | 74%         | 0.0     | 97%    | *Thalassia hemprichii* |
| 5.  | 16 S2F | 916       | 916         | 74%         | 0.0     | 99%    | *Thalassia hemprichii* |
| 6.  | 17 S2F | 972       | 972         | 86%         | 0.0     | 97%    | *Thalassia hemprichii* |
| 7.  | 21 S2F | 529       | 529         | 59%         | 4e-146  | 99%    | *Thalassia hemprichii* |

The results of the identification of 7 ITS gene samples showed mixed results based on BLAST. Samples of 16 S2F and 21 S2F have ident values of 99%. Samples of 2 S2F, 11 S2F and 17 S2F have ident values of 97%, which means species that are compared have identical similarities. While samples of 8 S2F and 9 S2F have ident values of 87% and 89%, indicating insignificant similarities. The ident value of 97 - 100% shows that species are compared identically, 92 - 96% shows sufficient or moderate similarity and ≤91% shows similarity is not significant [2]. All samples show the same species, *Thalassia hemprichii*, with Different ident values indicate the presence of genetic diversity in *Thalassia hemprichii* in Teluk Awur Jepara.

Genetic Diversity

The genetic diversity of *Thalassia hemprichii* was shown as dendrogram in Figure 2. The 7 sample differed genetically based on ITS sequences. The results showed that there were 2 large groups. *Thalassia hemprichii* with code 11 S2F is close to 17 S2F, and still in one large group with 16 S2F and 21 S2F. The next large group containing 8 S2F, 9 S2F the outgroup used is a sequence of seagrass *Enhalus acoroides*. The differences shown by each sample indicate a high diversity in the population, according to Dharmayanti's statement [4] the branching pattern and length of lines determine the genetic diversity of a group.
Identification of seagrass there are ± 33 different bases. The result of genetic distance matrix shows moderate difference values even though it is still in one type of seagrass. It is meaning that seagrass in Awur Bay has a moderate diversity genetic [1].

4. Conclusion

Identification of seagrass Thalassia hemprichii in Awur Bay Jepara shows homology levels between 83% - 99%, this shows that ITS DNA barcodes can be used to identify seagrass Thalassia hemprichii in Awur Bay Jepara, Central Java. The genetic distance matrix shows that seagrass in Awur Bay has a moderate diversity genetic.

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