The GAPDH partial gene of durik-durik (Syzygium sp.) from Riau Province of Indonesia

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Abstract. GAPDH gene encoding glyceraldehyde-3-phosphate dehydrogenase enzyme is one of housekeeping genes which is often used as an internal control in gene expression analysis. This study is aimed to isolate the GAPDH gene from durik-durik (Syzygium sp.). Fresh leaves of Syzygium sp. were collected from Kajuik Lake which is located in Langgam District, Pelalawan Regency, Riau Province, Indonesia. The methods included total DNA isolation using plant DNA isolation kit (Qiagen), polymerase chain reaction, electrophoresis, and sequencing. Data was analyzed using BioEdit7 and MEGA6 softwares, and BLAST program. The DNA partial sequence of GAPDH gene was obtained from Syzygium sp. with the length of 623 bp. The DNA sequence was predicted consisting of 2 exons and 3 introns and encoding 68 amino acids. The DNA sequence of Syzygium sp. GAPDH gene had 74%-89% similarity to one of other plant species, but none was member of Syzygium genus. The deduced amino acid sequence had 100% similarity to one of Tropaeolum minus dan Mentha spicata plants. The GAPDH partial gene obtained in this study is the first one reported from Syzygium genus and this sequence can be a basis for isolating of GAPDH gene from other species in Syzygium genus.

1. Introduction
A floodplain lake is a low land which is flooded by overflow of river in rainy season and then forms a lake. There are many floodplain lakes in Riau Province, for example Kajuik Lake in Kampar River Flow Area (DAS Kampar). Many plants – such as tuntun angin (Elaeocarpus floribundus), durik-durik (Syzygium sp.), pandan (Benstonea sp.), rotan (Calamus sp.), rengas (Gluta renghas), and putat (Planchonia valida) [1, 2, 3, 4, 5] - grow well there and they are often submerged up to approximately 2 meters during the rainy season. Most likely they carry flooding tolerant genes. Those plants play important roles for the survival of riparian ecosystem because they can maintain water quality and quantity in rivers and lakes, resist erosion, be a nutrition source for animals, and be a place for spawning, nursing, and hiding [6,7].

Syzygium sp. has been adapted well to flooded conditions. Most likely, this plant has many flooding tolerant genes so it can survive and reproduce well in Kajuik Lake. To understand the expression and role of these genes, the reference genes are needed as internal controls. The genes are commonly used as internal controls which come from housekeeping genes class. The reasons to use the housekeeping genes as internal controls are their expression tends to be stable and abundant in various tissues and development stage of eukaryotic organisms and also is not influenced by certain
conditions [8]. Some of them belong to the group of housekeeping genes: tubulin (TUB), ubiquitin (UBQ), actin (ACT), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 26S rRNA, and 18S rRNA [9, 10].

The GAPDH genes encode member of glyceraldehyde-3-phosphate dehydrogenase protein family that are key enzymes in glycolysis. There are two groups of GAPDH protein in higher plant, i.e. GAPA and GAPB proteins that are involved in Calvin cycle and the genes that encoded them are located in chloroplast genom; and GAPC protein that are involved in glycolysis pathway and the gene are positioned in nuclear genom [11]. A GAPDH gene has been isolated and characterized in Haloxylon salicicormicum plant [12] and has been validated as reference gene in Brassica juncea [13]. The housekeeping genes have never been isolated from Syzygium sp. plant, including the GAPDH gene. Therefore, this study is aimed to isolate the GAPDH gene from durik-durik (Syzygium sp.).

2. Material and Methods

2.1. Materials
Leaves of Durik-durik (Syzygium sp.) plant were collected from Kajuik Lake which is located in Langgam District, Pelalawan Regency, Riau Province, Indonesia. A primer pair designed by Gantasala et al. [14] was used to amplify the GAPDH gene of Syzygium sp. as follows: gapdh_F 5‘-AAC CGG TGT CTT CAC TGA CAA GGA-3’ and gapdh_R 5‘-GCT TGA CCT GCT GTC ACC AAC AAA -3’.

2.2. Extraction of Total DNA
Extraction of total DNA was conducted using DNeasy plant mini kit (Qiagen). The fresh leaf was cut into small pieces then grinded using mortar and pestle with adding liquid nitrogen. The powder was then poured into 1.5 ml tube for DNA extraction. The success of extraction was checked by electrophoresis on 1.0% agarose gel. Electrophoresis was performed in 1X TBE buffer (Tris-Borate-EDTA pH 8.0) at 65 volts for 30 minutes. Camera Olympus SP-500 UZ was using to record the total DNA band on stained gel.

2.3. GAPDH Gene Amplification
The GAPDH gene of Syzygium sp. was amplified using polymerase chain reaction (PCR) technique. The PCR components are as follows: 1X PCR buffer, 0.2 mM dNTPs, 2.4 μM primer forward, 2.4 μM primer reverse, 2 U Dream Taq DNA polymerase (Thermo Scientific), 15 ng the total DNA, and aquabidestilata. The PCR was performed with the following conditions: 5 minutes at 94 °C for 1 cycle followed by 45 seconds at 94 °C, 45 seconds at 55 °C, and 1 minute at 72 °C for 35 cycles and then ended with 1 cycle of post-PCR for 10 minutes at 72 °C.

2.4. Electrophoresis
The PCR product was checked by electrophoresis technique on 1.0% agarose gel in 1X TBE buffer, at 75 volts for 30 hours using Mupid®-exU. Ethidium bromide solution with concentration about 5 μg/ml was used to stain the band on the gel. UV lamp transilluminator (WiseUv WUV-M20, Daihan Scientific) was then used to visualize the stained-band. After that, Camera Olympus SP-500 UZ was using to record the total DNA band on stained gel

2.5. Sequencing
The tube containing PCR product was sealed and packed then sent to PT Genetika Science Indonesia located in Jakarta, Indonesia. The company was then continued sending it to 1st Base Malaysia for sequencing. Sequencing was conducted using the PCR primer pair.
2.6. Data Analysis
BioEdit version 7.0.0 software was used to get the complete sequence by aligning the forward and reverse sequences [15]. BLAST program (Basic Local Alignment Search Tool) at http://www.ncbi.nlm.nih.gov/BLAST was used to search the similarity between the Syzygium sp. gapdh sequence and the sequences deposited in GenBank database [16]. MEGA version 6.06 software (Build#: 6140226) (Molecular Evolutionary Genetics Analysis) was used to create the phylogenetic tree by using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) statistical method and Kimura 2-parameter model for the DNA sequence and Poisson model for the amino acid sequence with 1000 bootstrap [17]. Some GAPDH genes of some selected plant species and an ubiquitin DNA sequence of Glycine max which was used as a comparison were picked up from GenBank database.

3. Results
The total DNA of Syzygium sp. was obtained and used as template in PCR process. The PCR product obtained was approximately 650 bp in size (Figure 1). After sequenced and analyzed, the length obtained was 623 bp (Figure 2).

![Figure 1](http://example.com/f1.png)  
**Figure 1.** The GAPDH DNA fragment profile of durik-durik (*Syzygium* sp.) migrated on 1.0% agarose gel. (M) 100 bp DNA ladder. (1) The GAPDH DNA fragment.

![Figure 2](http://example.com/f2.png)  
**Figure 2.** The GAPDH partial DNA sequence of durik-durik (*Syzygium* sp.).

The alignment analysis (BLASTn) showed that the DNA sequence obtained in this study was a part of GAPDH gene and had 74%-89% similarity to one of other plant species, but none were member of Syzygium genus (Table 1). Based on the BLASTn analysis, 2 exons and 3 introns were detected in the GAPDH DNA sequence obtained in this study. Both exons were predicted to encode 68 amino acids. The alignment analysis (BLASTp) showed that GAPDH amino acid sequence of *Syzygium* sp. had
93%-100% similarity to one of other plant species (Table 2).

Table 1. The BLASTn analysis on the GAPDH DNA sequence of durik-durik (Syzygium sp.).

| Description            | Max score | Total score | Query cover | E value | Ident | Accession     |
|------------------------|-----------|-------------|-------------|---------|-------|---------------|
| Lonicera maackii       | 219       | 219         | 50%         | 8e-53   | 76%   | KM200570.1    |
| Gazania rigens         | 210       | 210         | 53%         | 4e-50   | 74%   | XX215158.1    |
| Tropaeolum minus       | 205       | 205         | 50%         | 2e-48   | 76%   | MF074114.1    |
| Tradescantia pallida   | 205       | 205         | 53%         | 2e-48   | 74%   | JX183244.1    |
| Oxalis vulcanicola     | 203       | 283         | 35%         | 6e-48   | 89%   | HM988728.1    |
| Mentha spicata         | 197       | 197         | 52%         | 3e-46   | 75%   | KF282613.1    |

Table 2. The alignment analysis using BLASTp on the GAPDH amino acid sequence of durik-durik (Syzygium sp.).

| Description            | Max score | Total score | Query cover | E value | Ident | Accession     |
|------------------------|-----------|-------------|-------------|---------|-------|---------------|
| Lonicera maackii       | 137       | 137         | 100%        | 4e-47   | 97%   | KM200570.1    |
| Gazania rigens         | 132       | 132         | 100%        | 4e-45   | 93%   | XX215158.1    |
| Tropaeolum minus       | 142       | 142         | 100%        | 2e-48   | 99%   | MF074114.1    |
| Tradescantia pallida   | 140       | 140         | 100%        | 7e-45   | 95%   | JX183244.1    |
| Oxalis vulcanicola     | 132       | 132         | 97%         | 9e-49   | 100%  | HM988728.1    |
| Mentha spicata         | 142       | 142         | 100%        | 9e-49   | 100%  | KF282613.1    |

Phylogenetic tree constructed based on the DNA (Figure 3, above) and amino acid (Figure 3, below) sequences of GAPDH gene and ubiquitin gene of Glycine max as a comparison indicated that the DNA and amino acid sequences of Syzygium sp. formed one cluster with the DNA and amino acid sequences of some plant species GAPDH genes and separated from G. max ubiquitin gene. These results supported the BLAST results.

Figure 3. Phylogenetic tree created based on DNA (above) and amino acid (below) sequences of GAPDH gene. ubq = ubiquitin gene; GAPDH = gene encoding glyceraldehyde-3-phosphate dehydrogenase.
The amino acid sequences of GAPDH gene were very different from one of ubiquitin gene. The amino acid sequence analysis of GAPDH gene showed the presence of 6 different amino acids among the comparable species (Figure 4).

| Accessions | No amino acid |
|------------|---------------|
| Syzygium sp clone Riau - gapdh | GKKVVIASDAPKDFVGVVKEKVRGPEPVNSYTNCLAPKLKIVOR | FGIVKLMTTVEIST |
| Lonicera maackii - gapdh | TAKVVIASDAPKDFVGVVKEKVRGPEPVNSYTNCLAPKLKIVOR | FGIVKLMTTVEIST |
| Osulisc vulcanicolle - gapdh | TAKKVIASDAPKDFVGVVKEKVRGPEPVNSYTNCLAPKLKIVOR | FGIVKLMTTVEIST |
| Gossamia rigens - gapdh | TAKKVIASDAPKDFVGVVKEKVRGPEPVNSYTNCLAPKLKIVOR | FGIVKLMTTVEIST |
| Tropaeolum minus - gapdh | TAKKVIASDAPKDFVGVVKEKVRGPEPVNSYTNCLAPKLKIVOR | FGIVKLMTTVEIST |
| Tradescantia palida - gapdh | TAKKVIASDAPKDFVGVVKEKVRGPEPVNSYTNCLAPKLKIVOR | FGIVKLMTTVEIST |
| Mentha spicata - gapdh | TAKKVIASDAPKDFVGVVKEKVRGPEPVNSYTNCLAPKLKIVOR | FGIVKLMTTVEIST |
| Glycine max - ubiquitin | MQC1KWTGLTITLAEVEVSDFFYKAVQKQREGIFDQQILFAGKQLEDGYPAHQKQKSTJHLYKLHGMQFXTT |

**Figure 4.** The alignment of GAPDH amino acid sequence. The numbers arranged vertically show amino acid positions according to Durik-durik (Syzygium sp.). Hypens (-) show deletion on the particular position. Amino acids in the box are conserved regions.

4. Discussion
Gene expression studies are performed to determine gene functions and to understand molecular biological processes. However gene expression studies are sensitive to human error like pipetting error when taking PCR components. Reference genes can be applied to solve the problem. Those genes act as internal control because their expressions are abundant in every kind of tissues and developmental stages of eucaryotic organisms and not influenced by certain conditions and treatments or in other words their expressions are constitutive [9,10,13,14].

GAPDH gene is a key enzyme in glycolysis and gluconeogenesis [11] that is already applied as an internal control. The GAPDH partial gene has been isolated and characterized in Haloxylon salicornicum plant [12]. In this study, amino acids numbers 37 to 43 (Figure 4) are GAPDH family signature [12].

The DNA sequence of GAPDH gene obtained in this study can be used to design GAPDH primer specific for Syzygium sp. The primer is designed in the exon regions and then can be used as an internal control and also as a DNA contamination indication in Syzygium sp. genes expression analysis. The total cDNA which is contaminated by DNA will produce two kinds of amplicon with different size. The small amplicon is a product of cDNA amplification because cDNA contains only exon regions without introns while the large amplicon is a product of DNA amplification because DNA contains both exons and introns. Consequently, the result of target gene expression becomes invalid.

The absence of species from Syzygium genus in the BLAST results showed that the DNA sequence of Syzygium GAPDH gene is never recorded in GenBank database. Therefore, the DNA sequence obtained in this study is the first reported GAPDH gene from Syzygium genus. In addition, validation of the DNA sequence is required so it can be used as an internal control in Syzygium sp.

5. Conclusion
The DNA partial sequence of GAPDH gene obtained from Syzygium sp. with the length of 623 bp. The DNA sequence was predicted consisting of 2 exons and 3 introns and encoding 68 amino acids. The DNA sequence of Syzygium sp. GAPDH gene had 74%-89% similarity to one of other plant species, but none were member of Syzygium genus. The deduced amino acid sequence had 100% similarity to one of Tropaeolum minus and Mentha spicata plants. The GAPDH partial gene obtained in this study is the first gene to be reported from Syzygium genus and this sequence can be a basis for isolating of GAPDH gene from other species in Syzygium genus.

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References
[1] Elvyra R and Yus Y 2012 *Ikan Lais dan Sungai Paparan Banjir di Provinsi Riau* (Pekanbaru: UR Press Pekanbaru)
[2] Roslim DI, Khumairah S and Herman 2016a *Biosaintifika: Journal of Biology & Biology Education* 8 392
[3] Roslim D I, Nurkhairani P, Herman and Elvyra R 2016b *TPGM* 3 11
[4] Roslim D I and Herman 2017 *Biosaintifika: Journal of Biology & Biology Education* 9 209
[5] Roslim D I 2017 *SABRAO Journal of Breeding and Genetics* 49 346
[6] Zeug SC and Winemiller KO *River. Res. Applic.* 24: 90–102 DOI: 10.1002/rra.1061
[7] Pease A A, Davis J J, Edwards M S and Turner T F 2006 *Freshwater Biology* DOI:10.1111/j.1365-2427.2005.01506.x.
[8] Thellin O, Zorzi W, Lakaye B, Borman BD, Coumans B, Hennen G, Grisar T, Igout A and Heinen E 1999 *J. Biotechnol.* 75 291
[9] Jain M, Nijhawan A, Tyagi AK and Khurana JP 2006 *Biochemical and Biophysical Research Communications* 345 646
[10] Caldana C, Scheible WR, Mueller-Roeber B and Ruzicic S 2007 *Plant Methods* 3 7
[11] Cerff R and Chambers SE 1979 *J. Biol. Chem.* 254 6094
[12] Al Mazrooei S S and Ghazala W S 2017 *Journal of King Saud University – Science* http://dx.doi.org/10.1016/j.jksus.2017.07.004 [in press]
[13] Chandna R, Augustine R and Bisht NC 2012 *PLoS ONE* 7(5) e36918 doi:10.1371/journal.pone.0036918
[14] Gantasala N P, Papolu P K, Thakur P K, Kamaraju D, Sreevathsa R and Rao U 2013 *BMC Research Notes* 6 312
[15] Hall T A 1999 *Nucl. Acids Symp. Ser.* 41 95
[16] Altschul S F, Madden T L, Schaffer A A, Zhang J, Zhang Z, Miller W and Lipman D J 1997 *Nucleic Acid Res.* 25 3389
[17] Tamura K, Stecher G, Peterson D, Filipski A and Kumar S 2013 *Molecular Biology and Evolution* 30 2725