Biosaintifika 10 (2) (2018) 237-244

Biosaintifika
Journal of Biology & Biology Education

http://journal.unnes.ac.id/nju/index.php/biosaintifika

Isolation of Housekeeping Genes on Durik-durik (*Syzygium* sp)

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DOI: http://dx.doi.org/10.15294/biosaintifika.v10i2.14234

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History Article

Received 29 April 2018
Approved 23 June 2018
Published 30 August 2018

Keywords

Actin; Beta-tubulin; Durik-durik; EF1α; GAPDH; Syzygium sp; Ubiquitin

Abstract

Housekeeping gene is a gene expressed with a fixed level and in abundant amounts under various conditions. After validation, the housekeeping gene can be used as an internal control to normalize gene expression data. This study reports the isolation of several housekeeping genes in Durik-durik plant (*Syzygium* sp). This plant material in form of fresh leaves from Durik-durik plants are taken from Kajuik Lake, Riau Province. The next stage is total DNA isolation, polymerase chain reaction, electrophoresis, sequencing and data analysis using bioinformatic tools. The isolated housekeeping genes included 18S rRNA, actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Beta-tubulin and Ubiquitin with sizes of 400 bp, 679 bp, 1134 bp, 836 bp, 1167 bp and 2155 bp, respectively. In addition to 18S rRNA, the five housekeeping genes are the first reported from the genus *Syzygium* and referable to isolate housekeeping genes in other species in this genus. The six housekeeping genes can be used as internal controls on Durik-durik plants after validation.

How to Cite

Roslim, D. I., Yusfi, L. A., Hutagalung, D. R., Faizah, N., Andriani, L., Azrial, Herman, & Lestari, W. (2018). Isolation of Housekeeping Genes on Durik-durik (*Syzygium* sp). *Biosaintifika: Journal of Biology & Biology Education*, 10(2), 237-244.

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p-ISSN 2085-191X
e-ISSN 2338-7610

INTRODUCTION

Gene expression studies need some internal controls that commonly come from housekeeping genes group. Housekeeping genes are group of genes expressed constitutively at a constant level under various conditions. This gene group encodes the proteins involved in the various metabolisms that are essential for the survival of a cell (Koonin, 2000). For example, actin and tubulin proteins are involved in cytoskeleton formation because they play a role in maintaining cellular turgidity and structure (Keren *et al.,* 2008; Kueh & Mitchison, 2009). Another example is the ubiquitin protein involved in the recycling of cell components through the ubiquitination process (Callis, 2014; Stone, 2014). Another example is the 1-alpha protein elongation factor which is involved in elongation of the polypeptide chains during translation (Sasikumar *et al.,* 2012). Glyceraldehyde-3-phosphate dehydrogenase and 18S ribosomal RNA are also a member of housekeeping gene group.

Some housekeeping genes have been isolated from various plants, such as the actin from the plant of *Arabidopsis thaliana* (McDowell *et al.,* 1996), *Melastoma malabathricum* (Hannum *et al.,* 2010), *Oryza sativa* (McElroy *et al.,* 1990), *Zea mays* (Staiger *et al.,* 2000), *Jatropha curcas* (Yuniati *et al.,* 2011) and *Elaeocarpus floribundus* (Roslim & Herman, 2017a). The tubulin-coding gene has been isolated from the *Arabidopsis thaliana* plant (Snustad *et al.,* 1992) and *Linum usitatissimum* (Gavazzi *et al.,* 2017). Another example is the ubiquitin-coding gene which has been isolated from the *Mesembryanthemum crystallinum* plant (Azad *et al.,* 2013). The gene encoding of glyceraldehyde-3-phosphate dehydrogenase has been isolated from the *Haloxylon salicornicum* plant (Al Mazrooei & Ghazala, 2017) and the 1-alpha elongation factor from the *Rosa hybrid* plant (Klie & Debener, 2011).

Isolation of housekeeping genes are important because these gene groups are often used as internal controls for normalizing the expression data of certain genes, such as the abiotic stress tolerant gene. However, to function as an internal control, this group of genes must be validated. The validation of housekeeping genes has been done in some plants (Galeano *et al.,* 2014; Sinha *et al.,* 2015; Hou *et al.,* 2017).

Durik-durik (*Syzygium* sp) is one of the many plants that grow in Kajuik Lake, Riau Province. This lake is one of the floodplain lakes in Riau. This lake contains the typical flora and fauna that are spesific to Riau (Roslim *et al.,* 2016a, 2016b; Roslim, 2017; Roslim & Herman, 2017b). One of the peculiarities of the Durik-durik and other plants that grow in Kajuik Lake is their resistance to flooding stress for several months during the rainy season. This resistance is certainly because these plants carry the tolerant genes of flooding stress. To isolate and study these tolerant genes through the study of gene expression requires internal control. However, no single internal control has ever been reported from Durik-durik plants. Therefore, this study reports the isolation of housekeeping genes in Durik-durik plants.

METHODS

Materials

The fresh leaves of Durik-durik (*Syzygium* sp) plants were taken from Kajuik Lake, Riau for total DNA isolation. The primer pairs used for isolation of tubulin-encoding genes were designed based on Einax & Voigt (2003), 18S rRNA based on Gantasala et al. (2013), and a 1-alpha elongation factor based on *Rosa multiflora* mRNA sequence (accession number JN399225). The previous studies have isolated the actin-coding gene from Durik-durik using the degenerate primer designing by McDowell et al. (1996) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) glyceraldehyde gene using primer pairs designing by Gantasala et al. (2013). The sequences of actin and GAPDH obtained were then used to design the Durik-durik specific primers. Primers for the amplification of ubiquitin-coding genes were designed on the basis of conserved regions of the ubiquitin-coding gene in some plants (Table 1).

Extraction of Total DNA

Young and fresh leaves of Durik-durik plants were taken for total DNA isolation using Genomic DNA Mini Kit Plant (Geneaid). A total of 0.1 grams of leaves were cut into small pieces and then inserted into the mortar and crushed using pestel with the help of liquid nitrogen. After the leaves became powder, they were then put into a 1.5 ml tube for continued total DNA isolation by procedure following the manufacturer's instructions from the kit. The total DNA solution obtained was then stored in the refrigerator.

Polymerase Chain Reaction (PCR)

Housekeeping genes were isolated by PCR techniques using primers as listed in Table 1. The PCR components included 1X Supreme NZYTaq 2 X Green Master Mix (NZYTECH), 0.2 μM forward primer, 0.2 μM reverse primer, 1 μl total DNA, and aquabidestilata up volume of

Table 1. Primer pairs for amplification of housekeeping genes on Durik-durik (*Syzygium* sp).

Primer	5'----------3'	Annealing Temperature (°C)	Region
18S_F	CGCGCAAATTACCCAATCCTGACA	55.0	18S ribosomal RNA
18S_R	TCCCGAAGGCCAACGTAAATAGGA		
Syz-act-F2	AGAAGGATGCCTATGTTGG	53.0	actin
Syz-act-R2	GTCGGGCAGCTCATAGTT		
Syz-gapdh-F	TGGATGCCCTTGTGTTCTGT	57.8	glyceraldehyde-3-phosphate dehydrogenase
Syz-gapdh-R	TCCCCTTCAGTTTGCCCTCA		
EF1α_F	GACTCTGGAAAGTCGACCA	54.7	elongation factor 1-alpha
EF1α_R	TGGTGCATCTCCACAGACTT		
P-bTub3-F	TGGGCCAAGGGICACTAYAC	58.4	tubulin
P-bTub4-R	GCCTCRGTGAACTCCATCTCGTCCAT		
Gg-Ubq_F1	TCATCCAATTTCTCGAT	49.6	ubiquitin
Ubiq_R	ACTCCTTCTGGATGTTGTAGTC		

PCR reaction to 50 µl. The PCR process included pre-PCR at 95°C for 5 min, followed by 35 cycles consisting of three stages: denaturation at 95°C for 45 seconds, annealing at the corresponding primer annealing temperatures (Table 1) for 45 seconds and extention at 72°C for 1 minute 30 seconds. The last stage was post-PCR at 72°C for 10 minutes.

Electrophoresis

Total DNA molecules and PCR products were migrated to 1.2% agarose gel containing 5 µg/ml of ethidium bromide to determine their quality and quantity. The electrophoresis buffer used was 1X TBE (Tris Borate EDTA pH 8.0). Electrophoresis was performed at 50 volts for 45 minutes. After that, the gel was placed on top of the UV lamp to visualize the DNA bands. DNA bands were photographed using a digital camera (Olympus SP-500 UZ) for analysis purposes.

Delivery of PCR Products for Cloning and Sequencing

A total of 45 µl of PCR products from housekeeping genes and 30 µl primer per sample were packed and sent to PT Gentika Science Jakarta, Indonesia for gel purification, cloning and sequencing at 1st Base in Malaysia. Gel purification and sequencing were performed on PCR products of the 18S rRNA (Syz-18S), actin (Syz-act), elongation factor 1-alpha (Syz-ef1a) and glyceraldehyde-3-phosphate dehydrogenase (Syz-gapdh) genes, while cloning and sequencing were performed on PCR products of the beta-tubulin (Syz-βtub) and ubiquitin (Syz-ubq) genes. Gel purification using Zymoclean™ Gel DNA Recovery Kit (Zymo Research) and sequencing using forward and reverse PCR primers. Cloning used pTA2 vector with Toyobo Target Clone -Plus- (Toyobo) and tansformed into *E. coli* Zymo 5a with Mix and Go Competent Cells™ (Zymo Research). The bacteria colony was PCR using T3 and T7 promoter primers by using KOD FX Neo (Toyobo). Plasmid was isolated using ZR Plasmid MiniPrep (Zymo Research) and subsequently plasmid was sequenced in two directions using T3 and T7 promoter primers.

Data Analysis

DNA sequence data were analyzed using the BLASTn (Basic Local Alignment Search Tool) program at http://www.ncbi.nlm.nih.gov/BLASTn (Altschul *et al.,* 1997) to determine the similarity of DNA sequences, exon-intron regions and amino acids. Primers spesific for Durik-durik were designed using Primer3 v.0.4.0 program (http://bioinfo.ut.ee/primer3-0.4.0/) (Koressaar & Remm, 2007; Untergasser *et al.,* 2012).

RESULTS AND DISCUSSION

Profile of Total DNA and PCR Products

The total DNA molecule from the Durik-durik plant has been obtained with sufficient band thickness and is feasible for PCR use (Figure 1). The PCR products obtained in encoding genes of 18S rRNA (Syz-18S), actin (Syz-ACT), glyceraldehyde-3-phosphate dehydrogenase (Syz-GAPDH), elongation factor 1-alpha (Syz-EF1α), beta-tubulin Syz-βTUB) and ubiquitin (Syz-

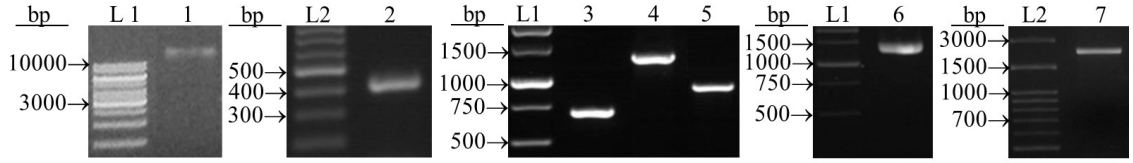

Figure 1. The DNA band profiles of (1) total DNA, (2) Syz-18S rRNA, (3) Syz-ACT, (4) Syz-GAP-DH, (5) Syz-EF1α, (6) Syz-βTUB, and (7) Syz-UBQ. L1 = 1 kb DNA ladder; L2 = 100 bp DNA ladder.

UBQ) were measuring approximately 400 bp, 700 bp, 1200 bp, 900 bp, 1200 bp and 2000 bp, respectively (Figure 1). The DNA bands of the Syz-18S, Syz-ACT, Syz-GAPDH and Syz-EF1α genes were relatively thick and can be directly sequenced. Meanwhile, the DNA bands of the Syz-βTUB and Syz-UBQ gene were relatively thinner and longer in size and should be cloned into the plasmid before sequencing.

Analysis of Housekeeping Gene Sequences

The lengths of the housekeeping gene sequences from Durik-durik obtained in this study after be sequenced were 421 bp for Syz-18S rRNA, 679 bp for Syz-ACT, 1134 bp for Syz-GAPDH, 836 bp for Syz-EF1α, 1167 bp for Syz-βTUB and 2155 bp for Syz-UBQ. The sequences have already been registered to GenBank database (Figure 2).

>MG836255 | *Syzygium* sp 18S ribosomal RNA gene, partial sequence
TCGCGCAAATTACCCAATCCTGACACGGGGAGGTAGTGACAATAAATAACAATACCGGGCT
CTTTGAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAACGAGGATCCATTGGAGG
GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTG
CAGTTAAAAAGCTCGTAGTTGGACCTTGGGTTGGGTCGACCGGTCCGCCTCACGGTGTGCA
CCGATTGGCTCGTCCCTTCTACCGGCGATACGCTCCTGGCCTTAACTGGCCGGGTCGTGCC
TCCGGTGCTGTTACTTTGAAGAAATTAGAGTGCTCAAAGCAAGCCTACGCTCTGTATACAT
TAGCATGGGATAACATCATAGGATTCCGGTCCTATTTACGTTGGCCTTCGGGAAA

>MH177933 | *Syzygium* sp actin gene, partial sequence
TATGTTGGTGATGAAGCCCAATCTAAAAGAGGTATTCTTACCTTGAAATATCCGATTGAGC
ATGGTATTGTCAGCAACTGGGATGACATGGAGAAAATATGGCATCACACCTTCTACAATGA
GCTCCGTGTTGCTCCGGAAGAGCATCCTGTCCTTTTAACTGAAGCGCCTCTTAATCCGAAG
GCAAACAGAGAGAAGATGACCCAAATCATGTTCGAAACTTTCAATGTACCGGCCATGTATG
TTGCCATTCAGGCTGTTCTGTCTCTCTATGCCAGTGGTCGTACAACTGGTGGGTAATTTTC
CTCTTTTCCTCAATTTTGAGCCAGTTAGTCCTGCCTTTTATAGTTTATTTACTGAAGTCAA
GTTTCCCATTTTTAAATGTTATTATTTTTATTGGAACAGGTATTGTGCTGGATTCTGGTGA
TGGTGTGAGTCACACTGTCCCAATCTATGAAGGTTATGCTCTTCCCCATGCCATTCTCCGT
CTTGATCTTGCTGGTCGTGATCTCACTGATTCCTTGATGAAGATTCTCACTGAGAGGGGTT
ACATGTTCACCACCACTGCTGAACGGGAAATTGTTCGTGACATGAAGGAAAAGCTGGCATA
TGTTGCCCTTGACTATGAGCAGGAGCTTGAAACGGCTAAGAGCAGCTCGTCTGTTGAGAAA
AACTATGA

>MH177936 | *Syzygium* sp glyceraldehyde-3-phosphate dehydrogenase gene, partial cds
GTCTGGATTTTTAGACATTATGTATGCTGAGTGGCATGCCCTCCGTCTTCTGGTGGACATA
CCGGTCGATCTAGCGAAGATTCTGGTGATTTGGCATAAGTGACTTGATTCTTGATTACTGG
CGTAGTACTTGCGATGATAGTGGCCCCTAGTGTTTCATCATCTGGGTTAGTGAAATCGCGA
TGTGTCGTGTAAGATTGACGAGGCATTTTATCTGTTTTTAGGGTGGAGCTAAGAAGGTAGT
CATTTCTGCCCCCAGTAAGGATGCGCCCATGTTTGTTGTGGGTGTGAACGAGAAAGAGTAC
AAGCCAGAGCTTGACATTGTATCCAACGCCAGTTGCACTACCAACTGTCTTGCCCCTCTTG
CCAAGGTTCGTAACATGATCAATTGCATTCTTTGAGAGTTCATAATCATTCCTTGGAAATG
TGCAGGTCATTTGTGAACTGACCTTATCCTTGCAAATTTCAGGTTATCAATGACAGGTTTG
GCATTGTTGAGGGACTTATGACCACTGTCCACTCCATCACTGGTGAACCGTTTGCCCTTTT
AAGTCTTCTAAAGTAATTCAATACATCTAATCAAAGTACAGAACTGACACGGGTTGCTTCC
TCATTTGTGACACAGCCACTCAGAAGACTGTTGATGGCCCATCAATGAAGGACTGGAGAGG
TGGAAGAGCTGCTTCTTTCAACATCATTCCCAGCAGCACCGGTGCTGCAAAGGTACCTTGA
ACTGTAAAGTTTCCTGATTACTGGTGTAGTGTGATAATAAATGCGCTCGTTTTCATTTCTT
CTGGGGTTGTTTGCCGCAGCGACATATTTTTGCATTATCTGGTGGAATTTTTGGCTATAAA
ATTCTTTTTTCTTGTATGTCGTGTGCTTTGCGAAGCTCAAGTTGATTTTGCTAAAGCATGT
GTCTGGTGCTTATAGGCCGTGGGAAAGGTGCTGCCTTCCCTCAATGGTAAGCTCACTGGAA
TGTCCTTCCGCGTTCCTACAGTTGATGTGTCAGTTGTGGATCTCACCGTCAGGCTTGAGAA
GGCGGCAACTTATGATGAGATCAAAGCTGCCATTAAGTAAGTTTTGAATAGTCTTTTGATT
TGTCTATTTAGGAGGATATTTTGTTGCACAAGCTGT

>MH177934 | *Syzygium* sp elongation factor 1-alpha gene, partial sequence
AAAGTCGACCACCACCGGACACTTGATCTATAAGCTTGGAGGAATTGACAAGCGTGTTATT
GAGAGGTTCGAGAAGGAAGCTGCTGAGATGAACAAGAGGTCTTTCAAGTATGCTTGGGTGT
TGGACAAGCTCAAGGCAGAACGCGAACGTGGTATTACTATTGACATTGCCTTGTGGAAGTT
CGAAACCACCAAGTACTACTGTACTGTCATTGATGCTCCCGGTCACCGTGACTTCATCAAG
AATATGATCACCGGTACCTCACAGGCTGACTGTGCTGTTCTGATCATTGACTCTACGACTG
GTGGTTTTGAAGCTGGTATTTCTAAGGATGGTCAGACTAGGGAGCATGCTTTGCTCGCTTT
CACTCTTGGTGTCAAGCAGATGATCTGTTGCTGCAACAAGGTAAATTCCTGGCAATCTATG
GCTAGTTAAGCATTAAAGTTTTTGCTGTTCGATGACTCTAACCTCTGTTTCTTCTTTAATT
TTTGTCTAGATGGATGCCACTACCCCTAAGTATTCCAAGGCAAGGTATGATGAAATCGTTA
AGGAAGTTTCTTCCTACCTGAAGAAGGTGGGTTATAACCCTGACAAGATTCCATTTGTCCC
CATTTCGGGCTTTGAGGGTGACAACATGATTGAGAGGTCCACAAACCTCGACTGGTATAAG
GGCCCTACACTCCTTGAGGCCCTTGACCTGATCCTGGAGCCCAAGAGGCCCACAGACAAGC
CACTCCGCCTTCCCTTACAAGATGTCTACAAGATTGGTGGTATTGGTACCGTCCCAGTGGG
ACGTGTTGAGACTGGAATCCTCAAGCCTGGTATGGTGTCACGT

>MH177935 | *Syzygium* sp beta tubulin gene, partial sequence
TGGGCCAAGGGGCACTACACTGAGGGGGCTGAACTGATCGATTCGGTCCTTGATGTCGTCC
GTAAAGAGGCAGAAAATTGTGATTGCCTTCAAGGTACACATATATAAGTTGTGATCTCATT
AGTCGTTTATTTTGAGTCAAATCTATAGGCCTAGAGAGATCGTTCTGTTCCTCAATGTTCA
TACATGCCTCCATGATCAGGGTTCCAGATTTGCCATTCCCTTGGAGGCGGGACGGGCTCGG
GGATGGGGACGCTATTGATTTCCAAGATCAGAGAAGAGTTTCCTGACCGGATGATGCTGAC
CTTCTCGGTTTTCCCCTCACCCAAGGTATCCGACACCGTGGTCGAGCCATACAACGCCACG
CTCTCCGTGCACCAGCTCGTCGAGAATGCCGACGAGTGCATGGTCCTTGATAACGAGGCCC
TCTACGACATCTGCTTCCGGACCCTCAAACTAACAAACCCAAGCTGTGAGTTTGAAAATGC
TTAAATCAGTTACTATTTCTGGTGGCTCGTTAGTTTTGTTAGTTATATGCCCCTTGTGTTC
TGGTCTAATCTGACAGTTGCAATGTATACACATTCACATGTAACAAACAGTTGGAGATCTC
AACCATTTGATCTCAACCACCATGAGTGGCGTCACTTGCTGCCTCCGGTTCCCCAGCCAGC
TCAACTCTGACCTCCGCAAGCTCGCCGTGAACCTGATCCCATTCCCGCGCCTCCACTTCTT
CATGGTGGGTTTCGCCCCGCTGACCTCCCGGGGTTCGCAGCAGTACCGCGCCCTCACAATC
CCCGAGCTCACGCAGCAGATGTGGGATGCCAAGAATATGATGTGTGCTGCCGACCCAAGGC
ACGGCCGGTACCTGACTGCCTCCGCCATGTTCCGGGGCAAGATGAGCACCAAAGAAGTGGA
TGAGCAGATGCTCAATGTCCAGAACAAGAACTCGTCCTACTTCGTCGAGTGGATCCCAAAC
AATGTGAAGTCGAGCGTGTGCGACATCCCGCCGACGGGGCTGCGCATGTCGTCGACGTTCA
TGGGGAACTCGACGTCGATCCAGGAGATGTTCCGGCGCGTGTCGGAGCAGTTCACGGTGAT
GTTCCGGCGCAAGGCCTTCCTCCACTGGTACACAGGGGAGGGCATGGACGAGATGGAGTTC
ACCGAGGC

>MH177937 | *Syzygium* sp ubiquitin gene, partial sequence
AATACTACTCCTTCTGGATGTTGTAGTCCCAGTTTTCGTGTTTCAACATTTTTTTCTCTCT
TTGTTAGTCATCTAAATTGTTAATGCAAATGAAGACATTGTGCAGCAAGAAGAAGCGTTTA
GAAAGTTGTTCTTGGGACTTCAATACCCCTTGAAAGGTGTTTTCTAAGTTCACATCATCTT
GAGAAGGGACATGCGTATCTCCTTGCTGTCATTCGTGTTGGATATAGTCCAGTAATAGACA
TGTAAGCAATGTTTTGCAGAATTTAACCCCAAAAGCTGCTCCATTTGAAGAAGAGACAAAT
CCGAATGCTTCTCAGTGGAGGACACTAAGTTGATAATCTGTCTGCAATTTGTCTGAGTTGT
TCTAGCTTCTGTTTGAGTATAATCACAATCATCATGTTCGACCCAAAAAAAAAAAAATCAT
CATGTGGGATCCCCCTTATCACAGTGTGCATCTGAAATTGCAGAGTTAGACACAATATTTA
AGGGGCTGCTACGAACTTTGTCTTTGATGTTCTTGTCTTCAGGTGGCTGGTGAACCACATG
CTGGAACTCGGTTTCTCAAAGTCCTTAGCAGAGTGGATAGGCAGCAACCTGAAGAAATCTG
GAGATCGTGAAACGTGGGCTTTTAATCTGGAAGGTGCCGTTCAGATGTTTGATTCGTACAG
GTAATTATCTGCCTTCATTTTCATGTAAGTGGGTGAATATGCACTCCTTCCCCGTGATATA
AGGTGACTTGTATACCATCAGGGAGACCTCCTATTGGTCTTTGTTGGAGGAACCACCGAAA
GCCACGGAAATAGCAATTGTACGTGCAGAGAAGAGCGATCGATGGGACCCGGACGTGATAC
AACGGCTCGAAAGACTTTCTAGTCTAGGAAGGGACCGATCCGAGGGGAAGGTTTCACTTCA
TGTTCTTCCAAATTCTGGCCACTGGGTTCATGTGGACAATCCGAAGGGACTTCTCGAGATC
GTGGCTCCAAAGTTCACATCCATCTAGCCTCACCCTACTGAATTCCGACTCCCCAGGCGCC
TGCTGGTGCGGAGCCAGTGGCTTTTCATTCTTGCCCGTAACTATTGAAATCGGGAAGTCCC
GCTTCGATACGAATAAATCTCTGTCCTATCCTGATGTTGTCTCGGCGCGATAGAGCGCCTT
ATTTATCCTCTTTTCATGTAGTGGACGGAGTTCAGATTGATTGACATTTCTTTCATTCACA
TAATCCGTCATTTTGATTCGGCGAATGCACACATTTTCATTTAGCCTCAATGGCAGGTCGT
AATGACATGTAATGGTGATTGCTCTTCGTGACGTTGCAGTGCTTTTTCTCTTTCTCTTTTT
TCGCTTAATACCGTCCTAAACCCCTAACTGGGTACACTAATGTCATATTTATCTCAAATTA
ATTTTCATGTAATAAAAAATCCTCTTATGATATATTCATCTCAAATTAAATCTCGCCTTCG
ACACGAAGAGAATGCGTGCGCGGCGATCTGAATGAAATCCGATAGTCAAGGGGGCGAAATG
AGAAATCATTTCTCTTAGGGCAAGTTTTTGAGGGCCAAAAGTGGAAAATGTGCAACAGAAT
GAAGAAATTGCAAAATGGGTCTGCTCGGCCCAACAGGCCCAAAGAAAGTTGGGGTCAAGGG
AGAGGGGACTCGCGAGTCAAAACCCTAGCTCCGGCAGAAGCCCGTATAAAAGCCCTCGAGT
TCTCTCTTCACCCCTCTCCGTTCCCTCCTCCTCGCACAGCCGAGAAGTCGCGCAGAGAGCG
AGAGAGAGAGAGAGAAGAGGAAGCGAAGATGCAGATCTTCGTGAAAACCCTAACCGGCA
AGACCATCACCCTCGAGGTCGAGAGCAGCGACACCATCGACAACGTCGAGGCCAAGATCCA
AGACAAGGAAGGTCCGTCCCCGTCCTCCTCCTCCTCGCTGGTGGAGCCTCCGTTCTC
GTTCGCCTCTGCTCCGGAACGGATCGGCGAACGATCGGCTTCGGTTTTCGCGCCGAGATTT
CCGCGATTTTGATGCGTTTGCTTTTTTGTTTTGTCGGATGTCGCTGAGCAGGAATCCCGC
CGGACCAGCAGCGGCTGATCTTCGCCGGGAAGCAGCTCGAGGATGGCAGGACTCTGGCCGA
CTACAACATCCAGAAGGAGT

Figure 2. Housekeeping gene sequences on Durik-durik (*Syzygium* sp).

BLASTn analysis showed that housekeeping genes from Durik-durik had relatively high similarities with fellow housekeeping genes from some plants, especially members of the Myrtaceae family. The identity and query cover values were very high on the BLASTn analysis based on genes encoding the 18S rRNA, actin, EF1α and β-tubulin, ranging from 86%-99% and 80%-100%, respectively. Meanwhile, although the identity values of BLASTn analysis based on the glyceraldehyde-3-phosphate dehydrogenase and ubiquitin genes were very high, ranging from 86% to 95%, but the query cover values were low, i.e. 9%-42% (Table 2). It because the sequences available in GenBank database are mRNA molecules which are consisting of exons only. These results also indicated that the exon regions of both genes were short. The length of Syz-GAPDH and Syz-UBQ genes obtained in this study were quite long, i.e. 1134 bp and 2155 bp, respectively, but the exon lengths were predicted at 456 bp and 192 bp, respectively (Table 3).

The six housekeeping genes obtained in this study can then be used as an internal control on the study of gene expression in Durik-durik plant. The first step in the study of gene expression is determining whether or not genomic DNA contaminants are present in total cDNA (Hannum *et al.,* 2010). The total cDNA molecules contaminated with genomic DNA can not be used for the study of gene expression because the expression data becomes invalid and unreliable (Laurell *et al.,* 2012).

The determination of genomic DNA contaminants can be performed using five housekeeping genes other than 18S rRNA. A simple way to detect the genomic DNA contaminants is to do PCR using a template of total DNA and cDNA separately and the primer pair flanking the introns, then observe the number and size of the PCR product from both (Table 4). If the PCR products of PCR using a template in the form of total

Table 2. BLASTn result of the six housekeeping genes of Durik-durik (*Syzygium* sp).

Description	Family	Query cover	E value	Ident	Accession
Syz-18S					
Eucalyptus coccifera	Myrtaceae	99%	0.0	99%	GU476423.1
Syzygium maire	Myrtaceae	99%	0.0	99%	GU476479.1
Syzygium claviflorum	Myrtaceae	99%	0.0	99%	GU476478.1
Syz-ACT					
Eucalyptus grandis	Myrtaceae	84%	3e-136	96%	XM_010029397.2
Hevea brasiliensis	Euphorbiacea	84%	6e-105	91%	GU270586.1
Betula luminifera	Betulaceae	84%	6e-105	91%	FJ410442.1
Syz-GAPDH					
Eucalyptus grandis	Myrtaceae	40%	5e-59	95%	XM_010027051.2
Heteropyxis natalensis	Myrtaceae	36%	2e-58	92%	AY903419.1
Punica granatum	Lythraceae	42%	6e-44	87%	KF856731.1
Syz-EF1α					
Eucalyptus grandis	Myrtaceae	88%	0.0	98%	XM_010045409.2
Betula luminifera	Betulaceae	88%	2e-157	91%	KP245811.1
Salvia divinorum	Lamiaceae	88%	5e-154	90%	KX268510.1
Syz-βTUB					
Eucalyptus pilularis	Myrtaceae	100%	0.0	92%	AB591246.1
Eucalyptus globulus	Myrtaceae	98%	0.0	92%	JX904068.1
Eucalyptus grandis	Myrtaceae	80%	0.0	95%	EF534219.1
Syz-UBQ					
Eucalyptus grandis	Myrtaceae	15%	2e-49	86%	XM_010038362.2
Fragaria vesca subsp. vesca	Rosaceae	10%	6e-42	90%	XM_004303489.2
Populus trichocarpa	Salicaceae	9%	4e-36	94%	XM_002317177.1

Table 3. Structure of the six housekeeping genes on Durik-durik (*Syzygium* sp).

Genes	Length (bp)	Prediction of Exon Position (bp)	Prediction of Exon Total Length (bp)	Number of amino acids deduction
Syz-18S rRNA	421	-	-	-
Syz-actin	679	<1...294, 402...>679	570	190
Syz-gapdh	1134	<223...373, 472...529, 622...724, 929...>1074	456	152
Syz-Ef1 alpha	836	<1...406, 498...>836	744	248
Syz-β-tubulin	1167	<1...95, 201...472, 600...>1167	936	312
Syz-ubiquitin	2155	<1798...1903, 2067...>2155	192	64

Table 4. Primer pairs and their PCR product (bp) and annealing temperature (°C) in gene expression study on Durik-durik (*Syzygium* sp).

Primer Pairs	PCR Product (bp) with template		Annealing Temperature (°C)
	DNA	cDNA	
18S_F/18S_R	421	421	55.0
Syz-act-F2/ Syz-act-R2	679	570	53.0
Syz-GAPDH-F/Syz-GAPDH-R	803	456	55.0
EF1α_F/EF1α_R	836	744	54.7
P-bTub3-F/ P-bTub4-R	1167	936	58.4
Syz-Ubq-F/Syz-Ubq-R	353	192	55.0

cDNA there are more than one DNA band and one of them has the same size as PCR products of PCR using total DNA as template, then it is concluded that total cDNA is contaminated with DNA.

The primer pairs of Syz-18S_F/Syz-18S_R, Syz-act-F2/Syz-act-R2, EF1α_F/EF1α_R and P-bTub3-F/P-bTub4-R (Table 1 and Table 4) can be directly used in the study of gene expression in Durik-durik because those are annealing in two different exon regions flanking the introns. Exon is the coding region and intron is the non coding region of a gene. Genomic DNA contains both exon and intron, whereas cDNA contains only exon (Rogozin *et al.,* 2005; Zhu *et al.,* 2009; Griffiths *et al.,* 2008). This is the basis for determining the genomic DNA of contaminants in total cDNA (Hannum *et al.,* 2010).

Meanwhile, the primer pairs of Syz-gapdh-F/Syz-gapdh-R and Gg-Ubq_F1/Ubiq_R (Table 1) can not be directly used in the study of gene expression on Durik-durik because the two pairs are attached not to the exon regions. Therefore, new primer pairs should be designed for both genes (Table 4). The newly designed primers are as follows: Syz-GAPDH-F: 5'- TGT TTT TAG GGT GGA GCT AA-3' and Syz-GAPDH-R: 5'- GAT CCA CAA CTG ACA CAT CA-3'; Syz-Ubq-F: 5'- ATG CAG ATC TTC GTG AAA AC-3' and Syz-Ubq-R: 5'- CCT TCT GGA TGT TGT AGT CG-3' with the size of its PCR product can be seen in Table 4.

In addition to the Syz-18 gene, the five housekeeping genes obtained in this study were the first reported from the *Syzygium* genus. Therefore, all five can serve as a basis for isolating housekeeping genes in other species within the genus *Syzygium*.

CONCLUSION

This research has isolated six housekeeping genes from Durik-durik plant (*Syzygium* sp), i.e. 421 bp of Syz-18S rRNA, 679 bp of Syz-ACT, 1134 bp of Syz-GAPDH, 836 bp of Syz-EF1α, 1167 bp of Syz-βTUB and 2155 bp of Syz-UBQ. Housekeeping genes from Durik-durik have relatively high similarities with fellow housekeeping genes from several plants, especially members of the Myrtaceae family. In addition to the Syz-18 gene, the five housekeeping genes obtained in this study were the first reported from the *Syzygium* genus. These six housekeepings may be used in the study of gene expression as internal control after validation and also as a marker of genomic DNA contaminants in total cDNA.

ACKNOWLEDGMENTS

This research was fully sponsored by a grant from Directorate General of Research and Community Service - Directorate General for Research and Development - Ministry of Research, Technology and Higher Education, Republic of Indonesia year 2018 under 'Penelitian Dasar Unggulan Perguruan Tinggi' with the contract number 302/UN.19.5.1.3/PP/2018 on behalf of Dr. Dewi Indriyani Roslim. Thanks to LPPM of Riau University.

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