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cDNA Actin Isolated From *Pandanus* Sp.

Dewi Indriyani Roslim[1*], and Herman[1]

[1]Departement of Biologi, Faculty Mathemathic and Science, Universitas Riau, Bina Widya Campus, Jl. HR Soebrantas, Panam, Pekanbaru 28293, Riau, Indonesia.

Abstract. Actin is one of the reference genes that is often used as an internal control in gene expression analysis. This study aimed to isolate actin cDNA from *Pandanus* sp originated from Riau. Fresh leaves *Pandanus* sp. Lake Kajuik, Langgam District, Pelalawan Regency, Riau Province. Isolation of RNA, synthesis of total cDNA, amplification of actin genes used McDowell's designed degenerate primer (PlAc46S-20/PlAc245N-20), electrophoresis, sequencing, and data analysis. Actin cDNA fragments obtained were 353 pb in size, registered at GenBank and encoded 117 amino acids. Actin cDNA fragment consists of two exons and one introne. Specific actin primers from Riau *Pandanus* sp. can be designed based on sequences obtained for the purpose of analyzing certain gene expressions.

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Keywords: actin cDNA, Lake Kajuik, *Pandanus* sp, RNA, Riau

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1. Introduction

Pandanus sp. grows on Lake Kajuik, Langgam District, Pelalawan Regency, Riau Province, Indonesia. The genus often growth submerged and sank to 1.5 m below the surface of the water and resistant to flooding stresses and certainly contains tolerant genes inundation stress. To study the expression and role of these genes a reference gene is needed as an internal control. The reference genes are genes that are expressed in all tissues and the stages of development of eukaryotic plants. The expression is abundant and not affected by external conditions (Thellin *et al*, 1999). Because of its characteristics, the reference gene after being validated is often used as an internal control for the purposes of gene expression. Some examples of reference genes that have been validated and used as internal controls are actin genes (ACT), cassava (UBQ), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and tubulin (TUB) (Volkov *et al*, 2003; Jain *et al*, 2006; Caldana *et al*, 2007).

*Corresponding author at: Universitas Riau, Bina Widya Campus, Jl. HR Soebrantas, Panam, Pekanbaru 28293, Riau, Indonesia.

E-mail address: *dewiindriyaniroslim@gmail.com*

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The actin gene encodes the actin protein which is the constituent unit of actin filaments in the cytoskeleton. This actin protein is present in all eukaryotic cells (Thellin *et al*, 1999). Actin genes have been isolated from *Arabidopsis thaliana* plants (Volkov *et al*, 2003; McDowell *et al*, 1996). *Melastoma malabathricum* (Hannum *et al*, 2010) and *Populus* (Zhang *et al*, 2010). This study aims to isolate actin cDNA from *Pandanus* sp. from Riau.

2. Materials and Methods

Fresh leaves of *Pandanus* sp. were obtained from Lake Kajuik, Langgam District, Pelalawan Regency, Riau Province. The primers used were primary degenerate as follows PlAc46S-20: 5'-ATG GTN GGN ATG GGN CAR AA-3 'and PlAc245N-20: 5'-GTD ATN ACY TGN TCN GG-3 CCR' (McDowell *et al*, 1996).

Total RNA Isolation

RNA isolation were treated with DEPC water to protect RNA from nuclease. Total RNA isolation of leaves and roots was carried out using Trizol reagents following the manufacturer's instructions (Invitrogen®, Molecular Research Center, Inc., USA). The total RNA molecules obtained were then treated with DNase enzymes to remove DNA that might contaminate.

Total cDNA synthesis

Synthesis of cDNA (reverse transcription) was carried out using Superscript First-Strand synthesis system kit (Invitrogen®, USA) and primary oligo (dT) 21. The reaction composition as follows: 5000 ng RNA, 1x first strand buffer, 1 μM primer Oligo (dT) 21, 0.5 mM dNTPs, 10 mM DTT, 40 units of enzyme RTase, 0.01% water DEPC to total reaction 20 μl. The mixture was incubated on a PCR machine with the following program: 30°C for 10 minutes, 42°C for 50 minutes, 95 °C for 5 minutes, and 20 °C for 5 minutes.

Total cDNA amplification using PCR (Polymerase Chain Reaction) Technique

Amplification was carried out with PCR components including 1x PCR buffer (plus Mg2 +), 0.1 mM dNTPs, 0.2 μM forward primer, 0.2 μM reverse primer, 1 U Dream Taq DNA polymerase enzyme (Thermo Scientific), 1 μl total cDNA and water to the total reaction volume of PCR 50 μl. The PCR program included: pre-PCR at 95 °C for 5 minutes, then followed by an amplification process of 35 cycles including stages of denaturation at 95 ° C for 45 seconds, attachment of primer at 50 °C for 45 seconds, and extension of primer or DNA synthesis at 72 ° C for 1 minute 30 seconds. After that, post-PCR at 72 °C for 10 minutes. The results of the amplification were electrophoresed and photographed.

Electrophoresis

Electrophoresis on 1.2% agarose gel in electrophoresis buffer solution in the form of 1x buffer TBE (Tris-Borate-EDTA pH 8.0), voltage 65 volts for 30 minutes. The staining of DNA bands uses 5 µg / ml of ethidium bromide. The DNA tape visualized on a UV lamp (WiseUV WUV-M20, Daihan Scientific) was then photographed using an Olympus SP500 UZ digital camera.

Nucleotide Tracing (Sequencing)

PCR products are sent to PT Genetika Science to be sequenced at 1st Base Malaysia. Sequencing was performed using a primer for PCR.

Bioinformatics Analysis

The DNA sequence data is then analyzed and aligned using the MEGA6, BLASTn (Basic Local Alignment Search Tool) program on the web site http: //www.ncbi. nlm.nih.gov/ BLAST (Altschul *et al,* 1997), and ExPASy-Translate tools on the web site http://www.expasy.ch/tools/dna.html (Gasteiger *et al*, 2003). Phylogenetic trees are made using the Neighbor Joining method with Kimura-2-Parameter model and 1000 times bootstrap. Sequences of several accessions were used to make phylogenetic trees obtained from the GenBank database.

3. Result and Discussion

The cDNA fragment of actin encoding has been obtained with a size of around 370 pb (Figure 1). Sequencing the cDNA fragment obtained 353 pb (Figure 2) and was registered with the GenBank database with accession number MG836260.

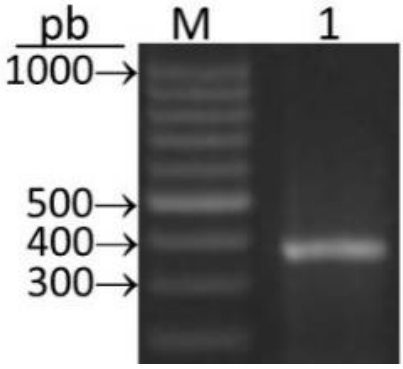


Figure 1. Fragment of cDNA actin isolated from *Pandanus* sp. M=1 kb DNA ladder;

1= cDNA fragment code for actin

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>MG836260 Pandanus sp clone Riau actin mRNA, partial sequence
GGCACCACACTTTCTACAATGAGCTTCGTGTGGCACCAGAAGAACATCCTGTTCTTCTGACAGAAGCCCC
TCTCAACCCCAAGGCCAACAGGGAGAAGATGACACAAATCATGTTTGAGACCTTCAATGTCCCCGCCATG
TATGTTGCAATTCAAGCCGTCCTTTCCCTTTATGCCAGTGGTCGTACCACAGGTATTGTGCTAGATTCTG
GTGATGGGGTCAGTCATACTGTGCCAATTTATGAGGGTTATGCGCTTCCTCATGCCATTCTCCGGCTTGA
TCTTGCGGGAAGAGACCTGACAGATTGCCTTATGAAGATCCTCACAGAGAGAGGCTACTCGTTCACAACC
ACT
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Figure 2. Sequence of cDNA actin iolated from *Pandanus* sp.

BLAST analysis in the cDNA sequence showed that cDNA sequences of actin encoding in *Pandanus* sp. had similarities up to 88% with actin cDNA sequences from several plant accessions (Table 1). But none of these accessions are members of the genus *Pandanus*. Therefore the sequences obtained in this study are cDNA sequences of actin encoders which were first reported from the genus *Pandanus*.

Table 1. BLAST analysis on cDNA sequences of actin encoding in *Pandanus* sp. plants from Riau.

Table 1. BLAST analysis cDNA sequence isolated from *Pandanus* sp.

Description	*Max score*	*Total score*	*Query cover*	*E value*	*Ident*	***Accession***
Lilium regale	437	437	99%	9e-119	88%	JX826390.1
Lilium davidii	432	432	99%	4e-117	87%	KP861871.1
Lilium hybrid	423	423	99%	2e-114	87%	KU176087.1
Tulipa fosteriana	423	423	99%	2e-114	87%	KM507834.1
Magnolia grandiflora	421	421	100%	7e-114	86%	KJ579271.1
Gagea nigra	421	421	100%	7e-114	86%	KR633144.1
Magnolia denudata	421	421	100%	7e-114	86%	AF281323.1
Tulipa gesneriana	419	419	99%	2e-113	86%	AB456684.1
Cinnamomum camphora	416	416	100%	3e-112	86%	KM086737.1
Galtonia saundersiae	414	414	99%	1e-111	86%	KM510380.1

In addition, the results of the BLAST analysis showed that the cDNA sequences obtained in this study consisted of two exons and introns. The two exons encode 117 amino acids.

Phylogenetic trees built on cDNA sequences (Figure 3) and amino acids (Figure 4) actin encoding indicate that cDNA sequences of actin encode from *Pandanus* sp. form the same group as fellow actin encoding sequences, separate from the polyubicuitin coding sequences as comparison. These results support the results of the BLAST analysis that the cDNA sequences obtained in this study are part of the actin encode.

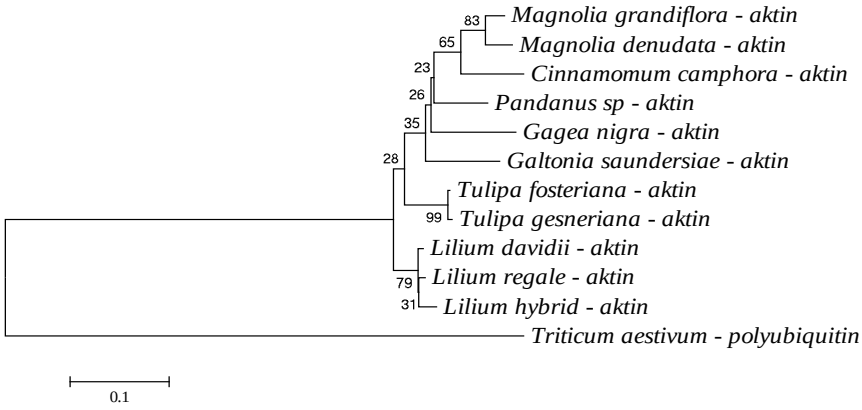


Figure 3. Phylogenetic tree based on cDNA sequence encode for actin

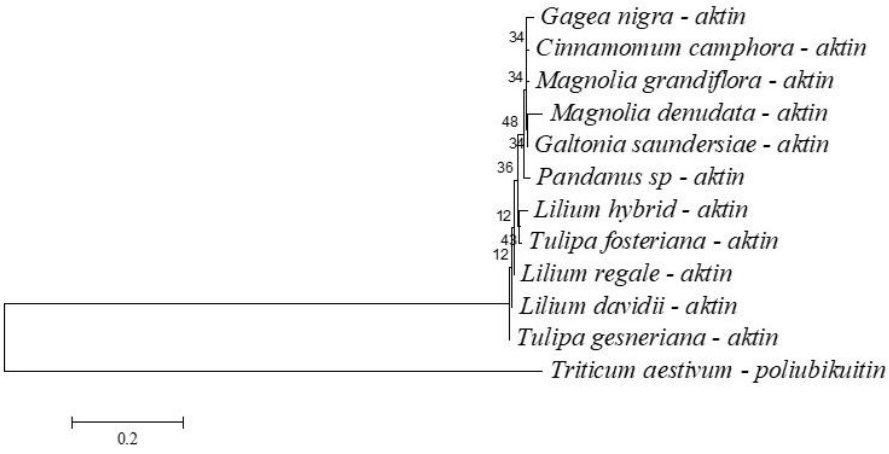


Figure 4. Phylogenetic tree based on amino acid sequence encode actin

4. Conclussion

The actin cDNA fragments obtained in this study were 353 pb in size and were registered at GenBank and encoded 117 amino acids. This actin cDNA fragment consists of two exons and one introne. Specific actin primers from Riau *Pandanus* sp. can be designed based on sequences obtained for the purpose of analyzing certain gene expressions.

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