THE DNA SEQUENCE ENCODING GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (GAPC) ENZYME ON TUNTUN ANGIN PLANT (ELAEOCARPUS FLORIBUNDUS BI)

Dewi Indriyani Roslim*, Hestia Hairima, Herman, and Wahyu Lestari

Biology Departement, FMIPA, Universitas Riau, Indonesia

Received: 09-01-2020; Reviewed: 10-01-2020; Accepted: 17-02-2020

*Corresponding author: E-mail: dewiindriyaniroslim@gmail.com

Abstract

Glyceraldehyde 3-phosphate dehydrogenase (GapC) is an enzyme involved in glycolysis. The expression of this gene tends to abundant in eukaryotic cells, so this gene is frequently used as an internal control in gene expression analysis. This research aims to isolate the DNA sequence of the GapC gene from tuntun angin (Elaeocarpus floribundus BI). Methods included a collection of the leaves from Kajuik Lake, Riau Province then the DNA extraction, electrophoresis, amplification of partial DNA sequence of GapC gene, cloning and sequencing. The DNA sequence was analyzed using the BLASTn program and MEGA6 software. The GapC sequence obtained in this study was 933 bp in size, consisting of four introns and five exons, and encoding 137 deduced amino acids. The BLASTn analysis showed that the sequence has 89.84%-90.16% similarity to other species of Cunoniaceae family, such as species from the genus of Spiraeanthemum and Codia. The partial sequence of E. floribundus GapC gene was more resemble the one of Spiraeanthemum than Codia genus. The GapC sequence obtained in this study was the first reported from the Elaeocarpaceae family. This sequence has the opportunity to serve as an internal control after validation.

Keywords: GapC gene, Elaeocarpus floribundus, internal control, Riau.

INTRODUCTION

The *Elaeocarpus floribundus* BI plant is a species of the *Elaeocarpaceae* family that grows in Lake Kajuik, Riau Province (Elvyra & Yus, 2012; Roslim et al., 2016). This plant has adapted well to the flood exposure ecosystem, that is, this plant can grow well in flooded conditions for months during the rainy season. This shows that this plant is most likely to carry inundation-tolerant genes.

To study the theory that underlies the tolerance of plants to inundation stress, it is necessary to analyze the expression of these genes. For this purpose several reference genes are needed which act as internal controls in gene expression studies (Ezin et al., 2012; Wang et al., 2017). One gene that is often used as internal control in plants is the gene coding for the enzyme glyceraldehyde 3-phosphate dehydrogenase (*GapC*) (Kozera & Rapacz, 2013). In wheat plants (*Triticum aestivum*) 4 groups of gene coding for the enzyme glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) were identified, namely *GapA / B, GapC, GapCp, and GapN* (Zeng et al., 2016).

This gene is involved in *glycolysis* and is expressed in abundant amounts in plant cells. The universal primer for amplifying this gene has been designed by *Strand* et al. (1997).

Therefore, this study aims to isolate partial DNA sequences of the *GapC* gene in *E. floribundus* plants.

MATERIALS AND METHODS

The research was conducted from January to April 2019. The research material was in the form of leaves from *Elaeocarpus floribundus* taken from Kajuik Lake in Riau Province. Molecular analysis was carried out in the Genetics laboratory, Department of Biology, FMIPA, Riau University, Indonesia. The primers used are as follows: GPDX7F: 5' - GAT AGA TTT GGA ATT GTT GAG G - 3' and GPDX9R: 5' - AAG CAA TTC CAG CCT TGG - 3' (Strand et al., 1997; Wu et al., 2007).

**Total of DNA Isolation.** The total DNA molecule from the *E. floribundus* plant was isolated using the Genomic DNA Mini Kit (Plant, Geneaid GP100). 100 mg of leaves were crushed using mortar and pestle and assisted with liquid nitrogen. The total DNA molecule obtained was then migrated to 1.2% agarose gel in a 1X TBE buffer solution to determine the success of DNA isolation.

**Polymerase Chain Reaction (PCR), Cloning and Sequencing.** The total DNA molecule is then used as a template to amplify the *GapC* gene using the primer
pair of GPDX7F / GPDX9R. The annealing temperature used is 52.3 °C. Components and PCR programs follow Roslim et al. (2018). The target gene PCR product is then inserted into a pTA2 vector (Toyobo) and then transformed into the bacterial cell *Escherichia coli* Zymo 5α (Zymo Research). Transformed colonies were amplified using T3 and T7 promoter primers. Plasmids from selected colonies were then isolated using ZR Plasmids MiniPrep (Zymo Research) and sequenced using T3 and T7 promoter primers.

**Data Analysis.** The DNA sequence data was analyzed by bioinformatics using the BLASTn program. The GapC sequence of 10 accessions that appeared in the BLASTn analysis was downloaded to create a dendrogram using MEGA6 software with Neighbor Joining Tree, Kimura-2-Parameter model and 1000 bootstraps.

**RESULTS AND DISCUSSION**

PCR products from the *GapC* gene in the *Elaeocarpus floribundus* plant have been obtained with a size of around 1000 bp (Picture 1). These results are not much different from those found in *Hibiscus tiliaceus* plants. Amplification of the *GapC* gene in *H. tiliaceus* plants produced a band of about 950 bp (Wu et al., 2007).

![Picture 1. DNA band profile of the *Elaeocarpus floribundus* *GapC* gene in 1.2% agarosa gel. pb = base pairs; M = 1 kb of DNA Ladder (Thermo Scientific); GapC = DNA band from the *GapC* gene.](image)

The *GapC* gene PCR product in this study appeared thin when electrophoresed. The thin *GapC* gene band obtained in this study shows the amount of amplification products is small. According to Olsen & Schaal (1999) and Wu et al. (2007) the *GapC* gene in plants is included in the nucleus with a low copy number. Therefore, cloning was carried out on the plasmid vector so that sequencing could be carried out properly and the DNA sequences obtained were accurate. The sequencing results of the *E. floribundus* *GapC*
The DNA sequence encoding Glyceraldehyde 3-Phosphate Dehydrogenase (GapC) enzyme on Elaeocarpus floribundus BI gene were 933 bp. The sequence has been registered in GenBank database with registration number MK942072 (Picture 2).

Picture 2. DNA sequence of the GapC gene from *Elaeocarpus floribundus*.

Using the same primer, Olsen & Schaal (1999) obtained the GapC gene in cassava (*Manihot esculenta*) with a size of 962 bp. Wu et al. (2007) have also obtained the GapC gene in *H. tiliaceus* plants with a size of 935 bp.

The *E. floribundus* GapC gene sequence obtained in this study is predicted to consist of 4 introns and 5 exons. These results are consistent with those obtained in cassava plants (Olsen & Schaal, 1999). The position of the exons in the *E. floribundus* GapC gene is in nucleotides 1-53, 136-233, 403-545, 728-811, and 898-933. The total exon is estimated to be around 409 bp and encodes 137 amino acid deductions.

The results of the BLASTn GapC *E. floribundus* sequence show that the *E. floribundus* GapC sequence has a high similarity, which is 89.84% - 90.16% with several species from the Cunoniaceae family (Table 1).

In this study, the *E. floribundus* GapC gene sequence has a high similarity with several plant species with different families because the GapC gene in all plants plays a role in the process of glycolysis.
Table 1. BLASTn analysis results on the *Elaeocarpus floribundus* GapC gene sequence.

<table>
<thead>
<tr>
<th>Species</th>
<th>Query Cover (%)</th>
<th>Identity (%)</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. densiflorum</em></td>
<td>90</td>
<td>89.84</td>
<td>Cunoniaceae</td>
</tr>
<tr>
<td><em>C. triverticillata</em></td>
<td>85</td>
<td>90.16</td>
<td>Cunoniaceae</td>
</tr>
<tr>
<td><em>C. spatulata</em></td>
<td>85</td>
<td>90.16</td>
<td>Cunoniaceae</td>
</tr>
<tr>
<td><em>C. montana</em></td>
<td>85</td>
<td>90.16</td>
<td>Cunoniaceae</td>
</tr>
<tr>
<td><em>C. microphylla</em></td>
<td>85</td>
<td>90.16</td>
<td>Cunoniaceae</td>
</tr>
<tr>
<td><em>C. mackeeana</em></td>
<td>85</td>
<td>90.16</td>
<td>Cunoniaceae</td>
</tr>
<tr>
<td><em>C. jaffrei</em></td>
<td>85</td>
<td>90.16</td>
<td>Cunoniaceae</td>
</tr>
<tr>
<td><em>C. incrassata</em></td>
<td>85</td>
<td>90.16</td>
<td>Cunoniaceae</td>
</tr>
<tr>
<td><em>C. ferruginea</em></td>
<td>85</td>
<td>90.16</td>
<td>Cunoniaceae</td>
</tr>
<tr>
<td><em>C. belepensis</em></td>
<td>85</td>
<td>90.16</td>
<td>Cunoniaceae</td>
</tr>
</tbody>
</table>

The dendrogram shows that the *E. floribundus* GapC sequence forms a group with *Spiraeanthemum densiflorum* and is separated from species of *Codia* genus (Figure 3). This result shows that the *E. floribundus* GapC sequence has more similarities with the *S. densiflorum* GapC sequence.

![Dendrogram](image)

Picture 3. Dendrogram are based on the GapC gene sequence.

The GapC gene obtained in this study further needs to be validated - one of which is the quantitative real time PCR (qRT-PCR) technique - to determine whether this gene can be used as an internal control in *E. floribundus* plants. The GAPDH gene has been used as an internal control in several plants such as dates (*Phoenix dactylifera*) (Patankar et al., 2016), pigeonpea (*Cajanus cajan*) (Sinha et al., 2015), citrus (Mafra et al., 2012) and olive (*Olea europaea*) (Ray & Johnson, 2014).

Aside from being as an internal control, the *E. floribundus* GapC gene can also function as a marker of DNA contamination in cDNA molecules isolated when gene expression studies are carried out, because the GapC
gene obtained in this study contains introns and exons. The trick is to amplify the GapC gene using a template in the form of DNA and cDNA separately. The PCR products with DNA as a template will be 933 bp while cDNA as a template will be 409 bp.

CONCLUSION

The partial GapC gene sequences obtained in this study were 933 bp in size, consisting of 4 introns and 5 exons, and encoded 137 deduction amino acids. BLASTn analysis shows that the sequences obtained in this study have similarities of 89.84% -90.16% and the query cover of 85% -90% with species from the family Cunoniaceae, namely species of the genus Spiraeanthemum and Codia. Sequences of the partial GapC gene of E. floribundus are more similar to sequences in Spiraeanthemum compared to species of the genus Codia. The partial GapC gene sequence of E. floribundus obtained in this study was the first to be reported from the Elaeocarpaceae family.

ACKNOWLEDGMENTS

Acknowledgments to the Directorate General of Research and Development - Ministry of Research, Technology and Higher Education, Republic of Indonesia, which has funded this research through the 2019 Superior Higher Education Basic Research Grant with contract number 756 / UN.19.5.1.3 / PT.01.03 / 2019.

REFERENCES


