HAYATI
Journal of Biosciences

Vol. 28 No. 2, April 2021 181-184
DOI:10.4308/hjb.28.2.181
ISSN: 1978-3019
EISSN: 2086-4094

Analysis of Genetic Potential of Banyuwangi Local Rice (*Oryza sativa* L.) Based on Relative Expression of *Homogentisate Geranylgeranyl Transferase* (*HGGT*) and *Granule-Bound Starch Synthase I* (*GBSSI*) Gene

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ARTICLE INFO

Article history:
Received October 13, 2020
Received in revised form March 5, 2021
Accepted March 12, 2021

KEYWORDS:
Oryza sativa L.,
gene expression,
qRT-PCR,
HGGT,
GBSSI

ABSTRACT

Rice nutrition including vitamin and amylose contents become important aspect for many people around the world. Rice with high amylose content (low glycemic index) is good for those with Diabetes mellitus. Tocotrienol, one precursor of Vitamin-E biosynthesis is catalyzed by enzymes encoded *HGGT*, while amylose biosynthesis is catalyzed by enzymes encoded *GBSSI*. The objective of this study was to find rice varieties with high tocotrienol and/or amylose content based on the expression of *HGGT* and *GBSSI* among eight Banyuwangi local rice varieties. Relative expression of *HGGT* and *GBSSI* was measured by qRT-PCR and analyzed using $2^{\Delta Ct}$ method. Statistical analysis resulted in the significantly different of *HGGT* and *GBSSI* relative expression among samples. Relative expression of *HGGT* from the highest to the lowest were demonstrated by Hitam Melik, Hitam Pekat, Blambangan A3, Merah Bali, Blambangan A2, Berlian, Janur Kuning, and SOJ A3, respectively; while relative expression of *GBSSI* from the highest to the lowest were demonstrated by Hitam Melik, Hitam Pekat, SOJ A3, Janur Kuning, Berlian, Merah Bali, Blambangan A3, and Blambangan A2, respectively. Based on this research we conclude that Hitam Melik potentially produces higher tocotrienol and lower glycemic index than other studied varieties.

1. Introduction

Rice, one of the food crops, is consumed by more than half of world's population, especially in Asia (Milovanovic and Smutka 2017). Rice has been developed in Indonesia, even several local rice varieties have been identified and registered officially, including Hitam Melik, Hitam Pekat, Blambangan A3, Merah Bali, Blambangan A2, Berlian, Janur Kuning, and Sunrise of Java A3. Nutritional quality of rice needs to be considered, such as the content of tocotrienols acting as strong antioxidants (Wang *et al.* 2018) and amylose affecting the rice glycemic index (Pandey *et al.* 2012).

Rice has different levels of tocotrienol in each variety (Dian *et al.* 2018). The gene that plays an important role in tocotrienol biosynthesis is *Homogentisate Geranylgeranyl Transferase* (*HGGT*) which encodes HGGT enzyme to catalyze the condensation reaction of geranylgeranyl diphosphate (GGDP) with homogenticic acid into 2-methyl-6-geranylgeranyl benzoquinol which will be converted into four tocotrienol isomers (Zhang *et al.* 2013; Comitato *et al.* 2017). Tocotrienols in rice that we consume have antioxidant activity which can neutralize free radicals before causing lipid oxidation or DNA damage (Aggarwal *et al.* 2010; Kannappan *et al.* 2012; Miyazawa *et al.* 2019) and display potent anticancer (Sylvester *et al.* 2010).

Furthermore, rice which has low glycemic index is also good for health (Pandey *et al.* 2012). Rice with low glycemic index is characterized by a higher percentage of amylose than amylopectin (Denardin *et al.* 2012; Jeevetha *et al.* 2014). Amylose biosynthesis involves *Granule Bound Starch Synthase I* (*GBSSI*) that encode

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GBSSI enzyme to catalyze the process of converting glucose-1-phosphate adenyl transferase (ADP-glucose) into amylose (Ruan *et al.* 2010; Ordonio and Matsuoka 2016).

The amount of tocotrienol in rice is influenced by the level of *HGGT* gene expression (Dian *et al.* 2018), while the amount of amylose is influenced by the level of *GBSSI* gene expression (Ordonio and Matsuoka 2016). The level of expression of both *HGGT* and *GBSSI* genes in cells can be measured using Real Time PCR technique and analyzed by the $2^{\Delta Ct}$ method (Kozera and Rapacz 2013; Šárka and Dvořáček 2017). High level of gene expression produce high end products, therefore this research aims to find rice varieties potentially produce high tocotrienol and/or amylose based on expression of *HGGT* and *GBSSI* among eight Banyuwangi local rice varieties.

2. Materials and Methods

This research was done in Biotechnology Division, Central Laboratory of Mineral and Advanced Material, Faculty of Mathematics and Natural Sciences, University State of Malang in August 2018 to January 2019. The object of this research was mature seed of eight Banyuwangi local rice varieties including Hitam Melik, Hitam Pekat, Blambangan A3, Merah Bali, Blambangan A2, Berlian, Janur Kuning, and Sunrise of Java A3 obtained from local farmer group in Singojuruh, Banyuwangi, East Java.

2.1. Total RNA Synthesis

Total RNA was isolated from 200 mg mature rice seeds ground with mortar and pestle. Total RNA isolation was done using QIAzol Kit. Total RNA quantity and quality were measured using Scientific Nanodrop Spectrophotometer 2000.

2.2. cDNA Synthesis

cDNA was synthesized from total RNA using Reverse TOYOBO Kit. Thermal cycler profile for cDNA synthesis was 37°C for 15 min, 50°C for 15 min, and 98°C for 15 min. cDNA quantity and quality was measured using Thermo Scientific Nanodrop Spectrophotometer 2000. The primers used in this research are shown in Table 1.

2.3. Gene Expression Analysis Using RealTime PCR

HGGT and *GBSSI* relative expression was analyzed from cDNA using Rotor Gene Q Series thermal cycler and SensiFAST SYBR No-Rox Kit. Thermal cycler software was set at 50 cycles for all three genes. The temperature and duration settings are shown in Table 2.

2.4. Data Analysis

Cycle threshold (Ct) values obtained from the real time PCR process were analyzed using $2^{\Delta Ct}$ method to find out the relative expression of *HGGT* and *GBSSI* (Kozera and Rapacz 2013). Both *HGGT* and *GBSSI* relative expressions were presented in bar charts using GraphPad Prism 5 software, tested statistically using JMP 6 software.

3. Results

3.1. *HGGT* Gene Expression

Based on the result of $2^{\Delta Ct}$ data, the relative expressions of *HGGT* in eight Banyuwangi local rice

Table 1. *HGGT*, *GBSSI*, and *β-Actin* primer sequences

Gene	Forward primer	Reverse primer
HGGT	5’-TTTCAAATCACCCACCGTCAG-3’	5’-TAGGAGCATACAGTTTAGAGCA-3’
GBSSI	5’-AGGCATCGAGGGTGAGGAG-3’	5’-CCATCTGGCCCACATCTCTA-3’
β-Actin	5’-CTCAGGAGGAGCAATGATC-3’	5’-GACCTGTACGCCAACACAG-3’

Table 2. Real-time PCR settings

Gene	Stage	Temperature (°C)	Duration (minutes)
HGGT	Activation	95	5
	Annealling	55	30
	Denaturation	95	10
GBSS	Activation	95	5
	Annealling	56.8	30
	Denaturation	95	10
β-Actin	Activation	95	5
	Annealling	55	30
	Denaturation	95	10

from the highest to the lowest were Hitam Melik ($2^{\Delta Ct}$ = 16.989542), Hitam Pekat ($2^{\Delta Ct}$ = 5.376001), Blambangan A3 ($2^{\Delta Ct}$ = 4.943469), Merah Bali ($2^{\Delta Ct}$ = 3.479590), Blambangan A2 ($2^{\Delta Ct}$ = 2.599881), Berlian ($2^{\Delta Ct}$ = 1.290429), and Janur Kuning ($2^{\Delta Ct}$ = 1.173945) respectively (Figure 1).

3.2. *GBSSI* Gene Expression

Based on the result of $2^{\Delta Ct}$ data, the relative expressions of *GBSSI* in eight Banyuwangi local rice from the highest to the lowest were Hitam Melik ($2^{\Delta Ct}$ = 3,333.3), Hitam Pekat ($2^{\Delta Ct}$ = 1,844.8), SOJ A3 ($2^{\Delta Ct}$ = 1,086), Janur Kuning ($2^{\Delta Ct}$ = 649.9), Berlian ($2^{\Delta Ct}$ = 571.3), Merah Bali ($2^{\Delta Ct}$ = 20.2), Blambangan A3 ($2^{\Delta Ct}$ = 2.4), dan Blambangan A2 ($2^{\Delta Ct}$ = 0.8) respectively (Figure 2).

Statistical analysis resulted on the significantly different (Chi-Square Value>Prob. Value) relative expression of *HGGT* and *GBSSI* among samples (Table 3).

4. Discussion

High relative expression of *HGGT* in Hitam Melik indicates that this variety is able to synthesize tocotrienol more than other varieties because the *HGGT* codes homogentisate geranylgeranyl transferase (HGGT) enzyme to catalyze condensation process of geranylgeranyl diphosphat (GGDP) and homogentisate acid into 2-methyl-6-geranylgeranyl benzoquinol (MGGBQ) in plastid. Furthermore, MGGBQ was changed into four isomers of tocotrienol (Mène-Saffrané and DellaPenna 2010; Zhang *et al.* 2013; Comitato *et al.* 2017).

Blambangan A3 and Merah Bali rice seed are reddish so that they commonly known as red rice. They have *HGGT* relative expression higher than the other varieties (except Hitam Melik and Hitam Pekat). The reddish colour of rice seed is suspected to be a β-caroten pigment, while the white seed has no pigment. The reddish colour corelates with total tocotrienol which is produced by the same promoting substance (refers to GGDP) from tocotrienol and β-caroten biosynthesis in mevalonate pathway (Zhang *et al.* 2013), therefore Blambangan A3 and Merah Bali potentially produce tocotrienol more than other varieties (except Hitam Melik and Hitam Pekat). Furthermore, red rice contains total vitamin E more that came from red part of rice seed caryopsis layer than white seed rice (Shammugasamy *et al.* 2015). The lowest relative expression of *HGGT* gene was found in SOJ A3. This variety is commonly called Genjah Harum which has lower total tocotrienol than Hitam Melik and Merah Harum (Blambangan A3) (Dian *et al.* 2018). When the relative expression of *HGGT* is low, the HGGT enzyme biosynthesis will decreases (Yang *et al.* 2011).

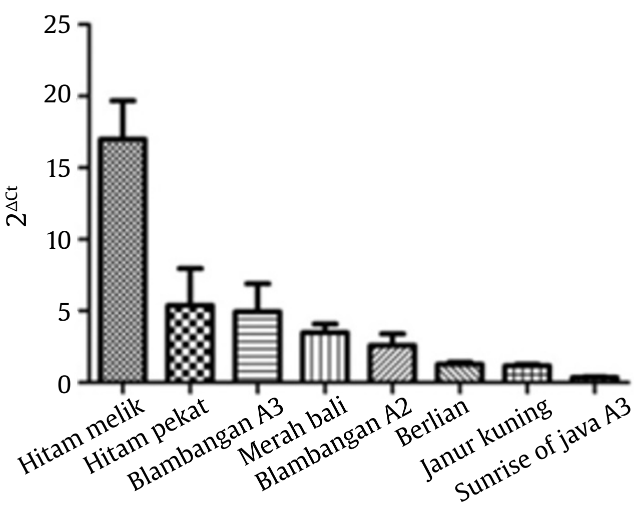

Figure 1. Relative expression of *HGGT* in eight Banyuwangi local rice varieties

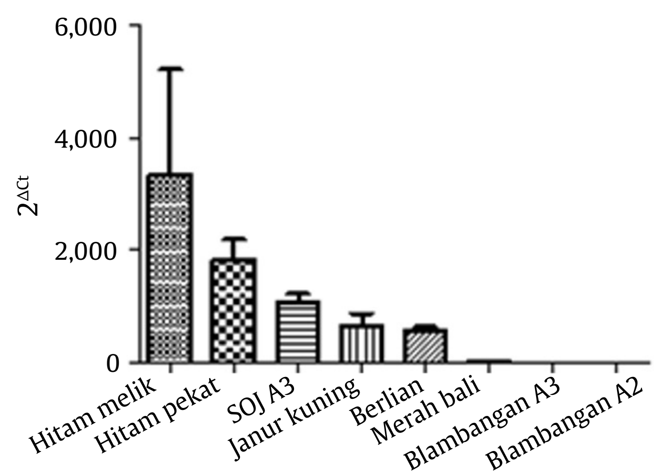

Figure 2. Relative expression of *GBSSI* in eight Banyuwangi local rice varieties

Table 3. Wilcoxon/Kruskal Wallis's statistical results

Gene	Chi-square value	DF	Prob. value
HGGT	16.0570	7	0.0246
GBSSI	20.2000	7	0.0052

Relative expression of *GBSSI* gene correlates with amylose level in rice seed (Fitzgerald *et al.* 2011; Pandey *et al.* 2012). Based on the relative expression result showed that Hitam Melik has the highest expression of GBSSI gene, but based on physicochemical test of amylose level showed that Merah Harum and Blambangan A3 have higher amylose level than Hitam Melik (Sholikhah *et al.* 2018). The difference in *GBSSI* expression and amylose level in rice endosperm is due to the repressor that affect amylose biosynthesis (Cheng *et al.* 2010; Ordonio and Matsuoka 2016). Repressors are proteins that can bind to specific DNA sequences and affect the ability of polymerase RNA in gene control. When repressor is bound to the operator, the gene cannot be transcribed by polymerase RNA then it causes the amylose level in rice seed decreases even though there are many GBSSI enzymes (Ordonio and Matsuoka 2016).

5. Conclusion

Relative expressions of *HGGT* in 8 varieties of Banyuwangi local rice from the highest to the lowest were Hitam Melik, Hitam Pekat, Blambangan A3, Merah Bali, Blambangan A2, Berlian, and Janur Kuning; while the *GBSSI* relative expression from the highest to the lowest were Hitam Melik, Hitam Pekat, SOJ A3, Janur Kuning, Berlian, Merah Bali, Blambangan A3, and Blambangan A2. Hitam Melik has the highest relative expression either the *HGGT* or *GBSSI* gene so that Hitam Melik potentially produces higher tocotrienol and lower glycemic index than other studied varieties.

Conflict of Interest

The authors claim that there is no conflict of interest. All research members had performed equal contributions.

Acknowledgements

The authors would like to extend the acknowledgements to Mr. Samanhudi, as the leader of Singojuruh Farmer Group for providing the samples and Direktorat Riset dan Pengabdian Masyarakat (DRPM) Directorate of Higher Education, Ministry of Education and Culture for supporting the funding of this research to S[1], E.S., S[2], and D.L.

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