BIOTROPIC The Journal of Tropical Biology

Vol 1. No 2. Agustus 2017

ISSN 2580-5029

CmBGI Gene Expression encoding β-glucosidase in melon (*Cucumis melo* L.) under stress condition

Yuanita Rachmawati*[1], Ganies Riza Aristya[2], Budi Setiadi Daryono[2]

[1] Universitas Islam Negri Sunan Ampel of Surabaya,

[2] Genetic Laboratory, Biologi Faculty, Universitas Gadjah Mada.

* e-mail: yuanitarhartono@gmail.com

ABSTRACT

CmBGI is the enzymatic genes encoding β-glucosidase that involved in Abscisic Acid (ABA) metabolism of Cucumis melo L. β-glucosidase promotes the accumulation of glucose, fructose, and sucrose, and it might act as a regulator that mediates melon fruit ripening both climacteric and nonclimacteric. ABA mediates adaptive responses to abiotic and biotic stresses. Agricultural Balitbang in 1997 showed that there were approximately 158.600 ha of degraded land scattered in three zones of agroecosystems in Yogyakarta (DIY). One of them is Dlingo Bantul area which has a karst type critical land area. Karst provides stress to the certain plant growth. One way to conserve critical land is making this area for agriculture. Cultivar TACAPA and TA were superior melons that have been developed by Genetic Laboratory of Biology Faculty UGM. This preliminary research was conducted to examine molecular characterization of CmBGI gene expression in cultivar TACAPA and TA which are planted in normal condition medium and in critical land medium treatment. Total RNA was extracted from leaf tissue then Reversed Transcriptase (RT-PCR) to collect cDNA library. cDNA was amplified using specific primer. Spectrophotometry was conducted in λ260 nm and electrophoresis run in 1.5% agarose gel. Control of band chosen was Cm-Actin. CmBGI gene concentration of TACAPA and TA in normal condition medium are in succession 578.5 and 579.4 µg/ml then for critical land medium treatment 743.4 and 773.5 µg/ml. CmBGI band was showed both of TACAPA and TA as ± 1258 bp. Cm-actin was showed band of DNA as ± 445 bp. CmBGI gene concentration in critical land medium treatment which is given greater stress on melons are higher than normal condition. This suggests that the CmBGI gene is expressed more in cultivar TACAPA and TA melons when they are grown under stress condition.

***Keywords**: Gene expression, CmBGI, β-glucosidase, stress condition, TACAPA and TA.*

PENDAHULUAN

CmBGI is the enzymatic genes involved in ABA metabolism of *Cucumis melo* L. The phytohormone abscisic acid (ABA) has been reported that plays an important role in plant growth, fruit development, seed maturation, dormancy, and germination as discussed by (Ren, et.al., 2010; Li, et.al., 2012; Sun, et.al., 2013). ABA mediates adaptive responses to abiotic and biotic stresses ABA induces or regulates corresponding

gene expressions in the biochemical and physiological processes during plant development (Barthe, 2000). It is known that plants mediate adaptive responses to physiological and environmental changes by fluxing the endogenous ABA level, which is controlled by the process of biosynthesis and catabolism.

ABA synthesis process in plant. ABA in higher plants is formed from xanthoxin via ABA-aldehyde by two oxidation reactions. The cis-isomers of violaxanthin and neoxanthin are cleaved to a C15 product, xanthoxin and a C25 metabolite (Li, et.al., 2012; Sun, et.al., 2013). Active ABA can be either degraded to some inactive structures in higher plants, through an irreversible pathway starting with 8'-hydroxylation and catalyzed by ABA 8'-hydroxylase (CYP707As), or stored in the bound form ABA-glucosylester (ABA-GE), catalyzed by ABA glucosyltransferase (ABA-GTase) as discussed by (Barthe, 2000). The conjugation is the simple process of ABA to either ABA-glucosyl ester (-GE) or ABA-glucosyl ether (-GS). ABA-GE and ABA-GS have been isolated from several plant species as discussed by (Xu, et.al., 2002) The glucosyl-transferase (GTase) can transfer nucleoside diphosphate-activated sugars to receptors of low molecular weight substrates.

Researches on the role of the gene encoding β-glucosidase of various crops have been widely studied. β-glucosidase 1 (*AtBG1*) was found in Arabidopsis thaliana, where it catalyzes the release of ABA-GE back into active ABA in order to rapidly adjust ABA levels as discussed by (Lee, et.al., 2006). These findings suggest a complex regulation mechanism for ABA accumulation. During grape ripening, the expression of *VvBG1* remains at high levels from coloration to fruit ripening, which indicates that ABA, produced by *VvBG1*, plays an important role in regulating the levels of ABA during the later stages of ripening (Sun, et.al., 2013). In addition, the level of ABA in plants is susceptible to the effect of environmental stress (Castellarin, 2007). Nowadays, the study of the molecular development mechanisms in climacteric fruits has made great progress. Therefore it has been suggested that ABA may participate in the regulation of watermelon ripening. However, it still remains unclear how the ABA levels are regulated by BG transcript levels during watermelon development and ripening, and what the roles of β-glucosidase are during the process of water-melon ripening.

Indonesian Agricultural Research and Development (Balitbang) in 1997 showed that there were approximately 158.600

ha of degraded land scattered in three zones of agro-ecosystems in Yogyakarta (DIY). One of them is Dlingo Bantul area which has a karst type critical land area. Karst is an area that has specific characteristics of relief and drainage because the degree of dissolution of limestone rock undergoes intensive weathering (Nahdi, dkk., 2012). The drainage system is very unique because it is dominated by subsurface water which mostly go to the underground layer in the rainy season. This condition causes the water cannot be retained on the surface and straight into the ground. Characteristics of highly specific karst ecosystem can cause biological problems, especially the plants that can live in this region is a plant that has a high adaptability to drought, and high pH as discussed by (Nahdi, dkk., 2012). Karst provides stress to the certain plant growth.

TACAPA is one of climacteric melons which developed from the plant breeding process. It is resulted from the crossing between ♀ Action 434 which is a commercial melon with a sweet taste and ♂ PI 371795 which is resistant to powdery mildew. TACAPA melon have superior as discussed by (Aristya, 2006) characteristics which is resulted from the breeding of its parent characteristics, in examples the flattened shape with fine net, green yellowish flesh, sweet taste, and resistant to powdery mildew as discussed elsewhere (Aristya, 2009; Daryono dan Qurrohman, 2009; Qurrohman, 2011). While melon TA is the result of a cross between TACAPA with Action 434. Melon TACAPA and TA are superior melons which are developed by Biology.

In this study, we conducted an analysis of *CmBGI*gene expression encoding β-glucosidase that contributes to the regulation of phytohormones ABA. Gene expression was quantitatively and qualitatively analyzed between melons which were grown in normal condition compared to melons which were grown in normal critical land medium.

MATERIAL AND METHOD

Experiments were conducted in 2014 in the *greenhouse* of KP4 UGM. Melon cultivar TACAPA and TA were used in

Table 1. Specific primers used for amplification of genes from melon

Name	Oligonucleotides	Accession
CmBGI-F	5'-ACAGAGACCCACCCATCTACATAACAGA-3'	JQ268078
CmBGI-R	5'-TCGACGTAGGTTATACCAAATCGCA-3'	
Cm-Actin	5'-CATGTTCACCACCACTGCCGA-3'	AB640865
Cm-Actin	5'-TGGCTGGAATAGAACTTCTGGGC-3'	

these experiments. These melons were developed by genetics laboratory of Biology Faculty of Gadjah Mada University (UGM). Molecular scale experiments conducted at genetics laboratory of Biology Faculty, FALITMA laboratory of Biology Faculty, and LPPT UGM.

Melon TACAPA and TA planted on 2 different growing mediums. Normal soil medium containing soil mixed with fertilizer. Critical land medium contain karst soil type that taken from Aroforestry II Critical land area of Yogyakarta, Dlingo Bantul. Coordinates land acquisition lies in BT: 110°27'56.2" LS: 07°55'58.7" with Elevation: 230.

Molecular data were analyzed qualitatively by looking at the band that formed in the process of electrophoresis. *CmBGI* genes will show a DNA band at 1258 bp. *CmActin* control genes will show a DNA band at 445 bp. Quantitative molecular data analysis done by looking at the concentration of the isolated RNA (mRNA), cDNA, and *CmBGI* genes PCR results using spectrophotometry at λ 260 nm.

Data Table 2 shows that the RNA concentration results for the melons grown in critical land medium treatment are lower than melons grown in normal condition. It occurs in both of cultivars TACAPA and TA.

cDNA library concentration results obtained after reverse transcription process shows different results. Melons growth in critical land medium treatment are having more cDNA libraries than compared to normal condition. As like as cDNA library concentration results, the concentration results of the *CmBGI* gene in critical land medium treatment which is given greater stress on melons are higher than normal condition.

RESULT AND DISCUSSION

RNA isolation was performed to compare *CmBGI* gene expression in *Cucumis melo* L. which are grown in normal condition with critical land medium treatment. Dlingo Critical land give stress on both the melon cultivars

Table 2. Genes concentration of melon cultivar TACAPA and TA

Genes	Concentration (µg/ml)			
	Control		Critical Land	
	TACAPA	TA	TACAPA	TA
Genome	186.7	108.2	131.3	102.3
cDNA	1031.5	1295.7	2524.9	2715.9
PCR Product *CmBGI* (25ρmol)	578.5	579.4	743.4	773.5

TACAPA and TA. The results showed that the concentration of RNA isolation, cDNA libraries, and the results of PCR (gene *CmBGI*) are different between melons grown in normal condition with critical land medium treatment (see Table 2).

Results of PCR using specific primers were run on 1.5% agarose gel electrophoresis. PCR results in the electrophoresis process including: *CmBGI* genes of cultivars TACAPA and TA which are grown either in normal condition or critical land medium treatment; *Cucumis sativus* RNA isolation result and then carried out reverse transcription to obtain cDNA library, cDNA obtained are amplified by using *CmBGI* gene-specific primers (as a negative control 1); *Cucumis melo* L. DNA isolation result, then amplified by using gene-specific primers *CmBGI* (as a negative control 2).

In this study the concentration of cDNA generated on cultivar TACAPA and TA melons are much larger when compared to the results of RNA isolation. This is due to in the process of reverse transcription, RNA are amplified and copied into cDNA when OligodT primer initiates elongation process. In addition, OligodT primer itself is DNA sequence.

CmBGI gene concentration resulted from the PCR process of each cultivar TACAPA and TA indicates that the melon cultivars grown on critical land medium treatment gives results greater than melons grown in normal condition. This suggests that the *CmBGI* gene is expressed more when grown in stress condition. *CmBGI* gene encodes β-glucosidase enzymes that regulate ABA. This regulation are shown in a role of the releasing of the bond between the ABA-

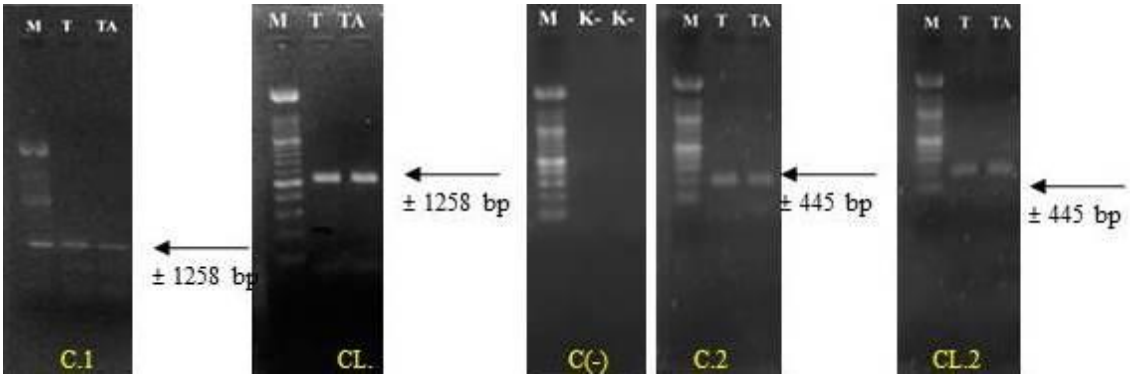

Figure.1. PCR profiles of TACAPA and TA melon. **M** - molecular marker (Vivantis, 1 kb), **T** - cultivar TACAPA, **TA** - cultivar TA, **K1(-)** negative control of *CmBGI* in *Cucumis sativus*, **K2(-)** negative control of *CmBGI* from DNA isolation result of *Cucumis melo* L., **C.1** - *CmBGI* gene of Melon in a control medium, **CL.1** - *CmBGI* gene of Melon in a critical land medium, **C (-)** negative control of *CmBGI* in *Cucumis sativus* and *CmBGI* from DNA isolation of *Cucumis melo* L., **C.2** - *CmActin* gene of Melon in a control medium, **CL.2** - *CmActin* gene of Melon in a critical land medium

glucosylester (ABA-GE) and ABA-glucosyl ether (-GS). Catalyzed by ABA glucosyltransferase (ABA-GTase) as discussed by [8]. The conjugation is the simple process of ABA to either ABA-glucosyl ester (GE) or ABA-glucosyl ether (-GS). If the bond has been detached, the ABA will be active.

CmBGI gene encodes an enzyme that regulates hormone ABA. According to (Chernys et.al.,2000; Zhu, et.al.,2002) ABA mediates adaptive responses to abiotic and biotic stresses. It is known that plants mediate adaptive responses to physiological and environmental changes by fluxing the endogenous ABA level, which is controlled by the process of biosynthesis and catabolism. The level of ABA in plants is susceptible to the effect of environmental stress (Castellarin, et.al.,2007)

Results of running gene on 1.5% agarose gels showed that *CmBGI* genes of each cultivar are expressed both of normal condition and critical land medium treatment. However, the thickness of band ± 1258 bp formed also different, which is shows qualitatively of expressed genes.

Running negative control of *Cucumis sativus* showed that in cucumber, *CmBGI* gene is not expressed. This is due to primers used are proven to be specific for *Cucumis melo* L.. While the negative control 2, *Cucumis melo* L. template for amplification from DNA isolation process, also showed no *CmBGI* gene expression. This is due to the gene is specific for DNA sequences derived from mRNA expression (from the isolated RNA).

To determine quantitatively the *CmBGI* gene expression, it is required a more comprehensive techniques such as Real Time PCR.

CONCLUSION

CmBGI gene concentration in critical land medium treatment which is given greater stress on melons are higher than normal condition. This suggests that the *CmBGI* gene is expressed more in cultivar TACAPA and TA melons when they are grown under stress condition. Running negative control *Cucumis sativus* in electrophoresis gel showed that in cucumber, *CmBGI* gene is not expressed. This is due to primer used are proven to be specific for *Cucumis melo* L. While the negative control 2, *Cucumis melo* L. template for amplification from DNA isolation process, also showed no *CmBGI* gene expression. Real time PCR is needed to measure the gene expression quantitatively.

ACKNOWLEDGEMENT

Our gratitude goes to the Ministry of Research and Technology of Indonesia (KEMENRISTEK) through the research grants from INSINAS program that have been given for this team.

DAFTAR PUSTAKA

Ren J, Sun L, Wu JF, Zhao SL,Wang CL, WangYP. 2010. Cloning and expression analysis of cDNAs for ABA8'-hydroxylase during sweet cherry fruit maturation and under stress conditions. J Plant Physiol;167:1486–1493.

Li Q, Ping L, Liang S, Yanping W, Kai J, Yufei S, Shengjie D, Pei C, Chaorui D, Ping L. 2012. Expression analysis of β-galactosidase that regulate abscisic acid homeostatis during watermelon (*Citrulus lanatus*) development and under stress condition. J Plant Physiol:169:78-85.

Sun Y, Pei C, Chaorui D, Pang T, Yanping W, Kai J, Yin H, Qian L, Shengjie D, Yan W, Hao L, Liang S, Ping L. 2013. Transcriptional regulation of gene encoding key enzymes of ABA metabolism during melon (*Cucumis melo* L.) fruit development and ripening. J Plant Growth Regulation 2013:32:233-244.

Chernys JT, Zeevaart JAD. 2000. Characterization of the 9-cis-epoxycarotenoid dioxygenase gene family and the regulation of abcisic acid biosynthesis in avocado. J Plant Physiol:124:343-353.

Zhu JK. 2002. Salt and drought stress signal transduction in plants. Annu Rev Plant Biol:53:247-273.

Koyama K, Sadamatsu K, Goto-Yamamoto N. 2010. Abscisic acid stimulated ripening and gene expression in berry skins of the Cabernet Sauvignon grape. Funct Integr Genomics:10:367-381.

Barthe P, Garello G, Bianco-Trinchant J, Ie Page-Degivry MT. 2000. Oxygen availability and ABA metabolism in *Fagus sylvatica* seeds. Plant Growth Regul:30:185–191.

Xu ZJ, Nakajima M, Suzuki Y, Yamaguchi I. 2002. Cloning and characterization of the abscisic acid-specific glucosy-ltransferase gene from Adzuki bean seedlings. J Plant Physiol:129:1285-1295.

Lee KH, Piao HL, Kim HY, Choi SM, Jiang F, Hartung W. 2006. Activation of glucosidase via stress-induced polymerization rapidly increases active pools of abscisic acid. J Cell:126:1109-1120.

Castellarin SD, Pfeiffer A, Sivilotti P, Degan M, Peterlunger E, Di Gaspero G. Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. J Plant Cell Environ 2007:30:1381-1399.

Nahdi, M. S. 2012. Konservasi Ekosistem Lahan Kritis Berbasis Kearifan Masyarakat di Kawasan Imogiri Yogyakarta. Disertasi Fakultas Biologi Universitas Gadjah Mada Yogyakarta.

Aristya, G. R. 2006. Skrining dan Pewarisan Sifat Ketahanan Tanaman Melon (*Cucumis melo* L.) terhadap Powdery Mildew (Jamur Tepung). Skripsi Fakultas Biologi Universitas Gadjah Mada Yogyakarta..

Aristya, G. R. 2009. Pewarisan dan Pemetaan Penanda Sequence Characterized Amplified Region (SCAR) Terpaut Gen Penyandi Ketahanan Powdery Mildew [*Podosphaera xanthii* (Castag.) Braun et Shiskoff)] Pada Tanaman Melon (*Cucumis melo* L.). Tesis Fakultas Biologi Universitas Gadjah Mada Yogyakarta.

Daryono BS, Qurrohman MT. 2009. Pewarisan Sifat Ketahanan Melon (*Cucumis melo* L.) Terhadap Powdery Mildew (*Podosphaera xantii* (Castag.) Braun et Shishkoff). J Perlindungan Tanaman Indonesia:15:1-6.

Qurrohman MT. 2011. Analisis Keterpautan Gen Ketahanan terhadap Powdery Mildew pada Tanaman Melon (*Cucumis melo* L.) Hasil Test Cross dengan Penanda Sequence Characterized Amplified Region (SCAR). Tesis Fakultas Biologi Universitas Gadjah Mada Yogyakarta.