Gene Expression of G-Box Binding Factor3 (GBF3) in Local Cassava (Manihot esculenta Crantz) under Drought Stress

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Abstract. Commonly, cassava could survive for a moment under drought stress. However, there are limitations to face the drought stress for the growth and productivity then affect starch synthesis. Gunungkidul is one of the biggest cassava producers in Indonesia, in meanwhile cassava production is not able to increase due to the characteristics of karst land topography and rainfed area. Tolerance of drought stress is a complex process relates to physiological and molecular responses. In molecular level, many genes revealed multi-complex responses to drought stress. One of the responsible gene assumed to correlate to drought stress is G-Box Binding Factor (GBF3). The study aims to detect the gene expression of GBF3 in local cassava cultivars of Gunungkidul under drought stress. The result revealed that at moisture levels of 50 percent and 25 percent, GBF3 (Gene Expression of G-Box Binding Factor 3) expressions are identified in the cassava cultivar Gatotkaca, which differed from the detection result of the cassava cultivar Pandesi hijau at all moisture levels. Based on the presence of phenotypic characters and gene expression, the local cultivar of Gatotkaca is assumably to be more drought tolerant than Pandesi Hijau.

1. Introduction
Starch processed products called Cassava (Manihot esculenta Crantz) Modified Cassava Fluor (MOCAF) offers consumers preference to decrease dependency of starch usage from wheat which imported from some countries. Starch content of cassava contributes to human calories for 600 million of population in developing countries which nutrient lack as one of main problems [1]. Phyco-chemistry of cassava starch has cleaner structures, high viscosity with low gelatinization temperature and low retrogradation compares to another starch material sources. Moreover, starch from cassava has been chosen for specific industrial products because of the soften texture [2].

In Indonesia, Gunungkidul Regency is one of the big producers of MOCAF. Cultivar Darawati, Genjah, and Kropak are well known as local cultivars which be able to produce high yield, however, the quality of starch content is low [4]. Thus, the local cultivars Gatotkaca, Kirik, and Pandesi Hijau were chosen to address the MOCAF production in Gunungkidul Regency. However, the productivity of cassava currently could not achieve yet the demand of MOCAF [3]. The reason is due to Gunungkidul Regency has the area which has been dominated by the karst characteristic with the yielding texture which commonly lacks nutrient for the farming system. The rainfall average is almost zero in the middle of 2018 and 479 mm in January for the maximum. The daily temperature average is 27.7 °C with a minimum temperature of 23, 3 °C and maximum temperature is up to 32, 4 °C. The relative humidity is around 80-84 % [5].

Critical environmental stress to crops that affect damage up to 70 % to the plant is drought stress. The condition is appeared due to the limited rain in the area thus the abiotic stress will affect plant productivity throughout the world [6]. Commonly, cassava could survive for a moment under drought stress. However, there is an average level of drought for growth and productivity, especially early phase of the vegetative period [7]. Tolerance of drought stress is a complex process relates to physiological and molecular responses [8]. At the physiological level, plant resistance under drought stress could be revealed too early
flowering phase, stomata conductance, and roots system development, phenotypic flexibility, water conservation, antioxidant maintenance, water conservation in tissues, plant regulation to the hormone, and osmotic regulation [9].

At the molecular level, many genes revealed multi-complex responses to drought stress [10]. For instance, cassava cultivar of DS Nyalanda Uganda cultivated under 10 days under drought stress revealed yellowish leaves than another cultivar called MH96/0686 Uganda. Along with Relative Water Content and Stomatal Conductance is then analyzed to verify the leaves respond to both varieties [11].

Many genes involved in starch biosynthesis metabolism from cassava such as Starch branching enzyme (Me-Be), Granule bound starch synthase (Me-GBSSI), Starch enzyme I (Me-SSI), Sucrose transporter I (Me-SUT I), Alpha-amylase (Me-Amy2). Also, AGPase (ADP-glucose pyrophosphorylase) has the role to regulate starch synthesis in plants, moreover, it correlates to critical biosynthesis [12]. The study of cassava genetic diversity is not only required to investigate germplasm but also adaptive potential and evolution. Thus, analysis of physiological characters and gene expression relates to starch synthesis on Cassava (Manihot esculenta Crantz) under drought stress conditions from local cultivar cultivated in Gunungkidul is important to conduct [13]. One of the genes that encode starch in cassava plants is the Transcription Factor and Regulates Alcohol Dehydrogenase (Adh) Gene Through ABA. In the condition of water deficiency, it will affect the increasing synthesis of hormones Abscisic acid (ABA) which is one of the indicators in plants when experiencing insecurity, including less water condition [14]. G-Box Binding Factor 3 (GBF3) was identified as a candidate drought stress response gene in Arabidopsis thaliana, and the function of GBF3 in drought tolerance was investigated [15]. Therefore, to detect gene Transcription Factor and Regulates Alcohol Dehydrogenase (Adh) through ABA in cassava plants, the expression of these genes in two local cultivars was investigated by integrating drought conditions.

2. Materials and Methods

2.1. Materials Preparation

Two local cultivars of Gatotkaca and Pandesi Hijau were used with the stem length around 30 centimeters cultivated in 20 Liter of polybag of regosol soils, respectively. Those specific stems were collected from Gunungkidul, Special Region of Yogyakarta in Indonesia which 32 stems cultivated in each polybag to fulfill samples need of 3 types of treatments (percentage of soil moisture for 75 %, 50 %, and 25 %) for 3 replication and 2 samples for each replication.

2.2. Drought Stress Treatments

Before 60 days after planting of cassava, all stems were watered for 2 liters every two days. After 60 days after planting, cassava plants then were watered different depend on the treatment types. For control, the percentage of soil moisture for 75 % could be represent as control, the plants were watered 1 Liter for 24 hours. This condition assumably could maintain the yield capacity. In meanwhile, 5 days after 60 days after planting then drought stress was conducted for reach percentage of soil moisture of 50 % without watering and then 5 days after 65 days after planting to reach the moisture of 25 % without any watering [13].

In this research, modification of watering was conducted to reach 75 %, 50 %, 25 % by using watering the plants after 21 weeks after planting or 294 days after planting. The microclimate in the location could not reach drought stress treatment for only 10 days after 60 days planting without watering as mentioned above.

2.3. Detection of Gene Expression Related to Starch Synthesis under Drought Stress

Fresh samples of third leaves with soft texture tissues from apical leaves were collected at 9.00-12.00 am [13], then put into an eppendorf then poured with liquid nitrogen. RNA isolation was conducted by RNAeasy kit (catalogue number 1072935; Qiagen). Quantity of RNA concentration then measured by nanovue spectrophotometry in absorbance level of A260/A280 and A260/A230. Quality of RNA then was measured by using electrophoresis gel for 1.5 % mixed with 8.75 ml formaldehyde at 50 Volt, then stained by Ethidium Bromide and visualized by UV transilluminator.

RNA products then used as template of RNA in reverse transcriptase PCR (RT PCR) by using OneStep RT-PCR Kit. Standard concentration of total RNA extracted from the plant range from 1 pg to 2 µg (catalogue no./ID:210210; Qiagen). Following primers sequence was used to amplify the target genes relates to starch synthesis on cassava.
Table 1. List of Primers Used to Amplify the Starch Synthesis Genes on Cassava

| Primer     | Sekuen ID | Gen                                  | Sekuen Primer                      | Fragment (bp) |
|------------|-----------|--------------------------------------|------------------------------------|---------------|
| Me-GBF3    | Nm180119  | Transcription factor and regulates alcohol dehydrogenase (Adh) Via ABA | F: TGC ATC AAC TGT TGG GTG CG       | 244           |
|            |           |                                      | R: ACC CAG AGC CAT GAG AAG GCT      |               |

After cDNA production by RT PCR then target of genes amplified by those primer in Table 1, the target genes were run by using 1 % of gel electrophoresis at 100 volts, stained by Ethidium Bromide, then visualized by UV transilluminator (Biorad).

3. Results and Discussion

3.1. Phenotypic Characters of Cassava under Drought Stress

The visual withering of plants can be used as a measure to assess the capacity of water in plants through the appearance of leaves in drought conditions [16]. The visual appearance term in line with the previous study of venation skeleton-based modeling of cucumber leaf wilting, which was categorized: no wilting, slight wilting, and acute wilting [17].

![Figure 1. Phenotypic characters of cassava cultivars under variant of moisture levels](image)

This figure reveals the phenotypic characters of cassava cultivars under a variant of moisture levels. At a moisture level of 75 %, there is no wilting that is a significant appearance. Meanwhile, at the moisture level of 50 %, cassava leaves turn to begin revealed slight wilting at Pandesi Hijau cultivar. Then, the phenotypic characters of cassava leaves become acutely wilted, turn to yellow, and begin to fall off under a moisture level of 25 % at both cultivars. Compared to Pandesi Hijau, Gatotkaca had slight wilting at the phenotypic characters of plants under the condition of 50 % and 25 %. Meanwhile, Pandesi Hijau's appearance was acute wilting at a moisture level of 25 %.
3.2. Detection of G-Box Binding Factor 3 (GBF3) in local cassava cultivars at moisture level of 75 %, 50 %, and 25 %

Total RNA concentrations of all samples in this study ranged from 2.30 to 11.5 ng/l with the ratio of absorbance ranging from 1.3 to 2.87 at A260/A230 and ratios ranging from 0.024 to 0.91 A260/A280. In this research, that purity of RNA samples is in a range of standard template of RNA for accurate detection of specific gene by Reverse Transcripase-PCR. Visualization of GBF2 gene expression by using Reverse Transcriptase-PCR on cassava plants under drought stress is provided in Figure 2.

Figure 2 revealed bands of fragment goal about 244 basepairs as the appearance sign of detected GBF3 in the cassava cultivar Gatotkaca at moisture levels of 50% and 25%, which differs from the detection result of the cassava cultivar Pandesi Hijau at all moisture levels. This observation showed that the GBF3 gene on cassava was expressed differently in two cultivars. This was also observed in a previous study, which found that different molecular mechanisms, such as pathogen perception and control, differ between cultivars [18].

Compared with Pandesi Hijau, Gatotkaca had bolder bands at the moisture levels of 50 % and 25 %. As shown in Figure 1, Gatotkaca has greater phenotype characters with only minor wilting than Pandesi Hijau at a moisture level of 25% under drought stress with an acute wilting appearance. Gatotkaca is assumed to be more drought tolerant than Pandesi Hijau because of its greater phenotypic characters and gene expression detection during drought stress.

4. Conclusion

GBF3 (Gene Expression of G-Box Binding Factor 3) expressions are detected in the cassava cultivar Gatotkaca at moisture levels of 50% and 25%, which differed from the detection result of the cassava cultivar Pandesi Hijau at all moisture levels. The local cultivar of Gatotkaca is assumed to be more drought-resistant than Pandesi Hijau based on the appearance of phenotypic characters and the gene expression.

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References

[1] Ihemere U, Garzon-DA, Lawrence S and Sayre R 2006 Plant Biotechnology Journal 4 453-465
[2] Raphael M, Yona B, Stephen K, Ephraim N, Patrick R, Mukasa S, Bruce H, Samuel K 2011 Journal of Plant Breeding and Crop Science 3 (19) 195-202
[3] Rosyadi M I, Toekido, Supriyanta 2014 Vegetalika 3(2) 59-71
[4] Badan Pusat Statistik 2016 Kabupaten Gunungkidul dalam Angka
[5] Badan Pusat Statistik Gunungkidul 2019 Statistik Daerah Kabupaten Gunungkidul
[6] Saini H S, and Westgate M E 1999 Advances in Agronomy 68 59-96
[7] Perez J C, Lenis J I, Calle F, Morante N, Sanchez N, Debouck D, Ceballos H 2011 Plant
Breeding 130 688-693

[8] Gong P, Zhang J, Li H, Yang C, Zhang C, Zhang X, Khurram Z, Zhang Y et al 2010 Journal of Experimental Botany 61 (13) 3563-3575
[9] Farooq M., Hussain M., Wahid A., Siddique K.H.M 2012 Springer Berlin Heidelberg 1-33
[10] Behringer D, Zimmermann H, Ziegenhagen B, Liepelt S 2015 Prasad M, ed. PLoS ONE 10 (4)
[11] Turyagyenda LF, Kizito EB, Ferguson M, et al. 2013 AoB Plants 5 (0)
[12] Vasconcelos LM, Brito AC, Carmo CD, Oliveira EJ, 2016 Genetics and Molecular Research 15 (4)
[13] Beyene D, Mukasa SB, Jansson C, et al 2013 Ethiop J. Sci. 36 (2)
[14] Lu G, Paul AL, Mc Carty DR, Feri RJ 1996 The Plant Cell 8 (847-857)
[15] Ramegowda V, Gill U S, Sivalingam PN, Gupta C., et al. 2017 Scientific Reports 7 (9148)
[16] Bettina M. J. E., M.T. Tyree & T.A. Kursar 2007. Journal of Tropical Ecology. 23(4)
[17] Lu S, Zhao C, Guo X 2009 International Journal of Computer Games Technology
[18] Silva KJP, Singh J, Bednarek R, Fei Z, Khan A 2019 Horticulture Research 6 (35)