Characterization of genes encoding key enzymes involved in sugar metabolism of matoa (*Pometia pinnata*)

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Abstract. Matoa (*Pometia pinnata* JR Forst. & G Forst.) is one of Indonesia's underutilized fruits, which can grow to giant trees up to 50 m (164 ft) and found naturally in the Asia-Pacific region, mainly in lowland tropical areas about 14°N to 20°S. Matoa fruit contains phytochemical compounds such as flavonoid, tannin, and saponin, secondary metabolite compounds derived from sugar metabolism. Sugar metabolism involved several genes that control the photosynthesis pathway. This research aimed to isolate and characterize the gene related to sugar metabolism depending on the assembled-transcriptome database genome against the UniProt database using the BLASTX program. Six gene sequences characterized 176,002 number of contigs that are nine contigs of Sucrose-phosphate synthase (SPS), four contigs of Sucrose-phosphate (SPP), 12 contigs of Sucrose synthase (SUS), 19 contigs of Alkaline/neutral invertase (INV), four contigs of Cytosolic invertase (CINV), 20 contigs of Beta-fructofuranosidase (CWINV). The research lays the foundation for a comprehensive study of biological mechanisms involved in sugar metabolism growth and the development life circle of matoa.

1. Introduction
Matoa (*Pometia pinnata* JR Forst. & G Forst.) comes from the Sapindaceae family can be found throughout the Andaman Islands, Sri Lanka, southern China, Vietnam, Malaysia, Indonesia, Philippines, Papua New Guinea, and the South Pacific Islands. Matoa adapts and spreads in warm and hot, humid to wet, in subtropical and tropical regions from 14 °N to 20 °S, with average annual rainfall from 1500 to 5000 mm [1]. Based on the skin color of matoa, the fruit is divided into three types: red skin matoa, green skin matoa, and yellow skin matoa. Based on the fruit's texture, matoa can be divided into two types, namely coconut matoa and papeda matoa. Coconut matoa is characterized by chewy and loose flesh, having a fruit diameter of 2.2-2.9 cm. Its relatively soft and sticky flesh characterizes Matoa papeda with a fruit diameter ranged 1.4-2.0 cm [2]. Matoa trees generally bloom once a year between July - October and can be harvested 3-4 months later. The matoa tree can also flower and bear fruit twice a year. If flowering in December, the fruit can be picked in February - March and generally harvested in the form of fruit picked [3].

The community's utilization of matoa trees has been used for various purposes, such as building and handicraft materials, fresh food for fruit, medicines, and ornamental plants [4]. Based on phytochemical screening, matoa leaf ethanol extract have antioxidant activity with IC₅₀ of 45.78 ppm, and ethanol extract of matoa leaf contains flavonoid compounds and tannins [5]. The content of secondary...
metabolites of matoa tree bark is thought to act as α glucosidase inhibitor that can help treat diabetes [6]. The compound of anti-HIV-1 IN activity successfully isolated from matoa leaf extract [7].

Sugar (carbohydrates) is the result of photosynthesis used by plants to form various complex compounds needed for fruit growth and production and secondary compounds [8]. Sugar plays an essential regulation in metabolism as a signaling molecule regulating various genes and affects development aspects in higher plants, including flowering [9]. Sugar metabolism is used in the formation of secondary metabolism in the cell [10].

Chemically, the main differences between DNA and RNA are (1) the hydroxyl group in RNA two carbon position in ribose sugar while in DNA it does not, (2) there is no thymine base in RNA but instead is uracil base. More specifically, RNA is composed of ribonucleotide precursors, while DNA is composed of deoxyribonucleotide precursors. It is also chemically and biologically more unstable RNA molecules than DNA molecules, especially at high temperatures and in an alkaline state [11]. The structure of RNA is similar to DNA, the main difference being the presence of sugar in the form of ribose and uracil base instead of the thymine base. RNA is generally single-stranded, although short chains of double-stranded RNA can be found in the complementary region. RNA consists of two types, namely RNA associated with gene expression and RNA that is not related to gene expression [12].

2. Materials and methods

2.1. Study site
The study was conducted in the garden of PT. Mekar Unggul Sari in Cileungsi Bogor Regency with a height of 85 meters above sea level.

2.2. Material and equipment
Matoa trees that have been planted since 1995 (approximately 24 years old) in the PT. Mekar Unggul Sari.

2.3. Methods
The assembly was performed by using the DDBJ Read Annotation Pipeline using Velvet 1.2.10 with default parameters. The assembled contigs were filtered to reduce redundancy by CAP3 (-p 90) and CD-HIT-EST (-c 0.90 -M 0 -T 0), the contigs were annotated against SwissProt and TrEMBL databases from UniProt using the BLAST program with the cut-off of $10^{-5}$ gene-related to sugar metabolism (Fig. 2).

2.4. Procedure
3. Results and discussion
The fruit flesh (aril) used are cultivars matoa with red skin fruit diameter increased until the age of 12 weeks after anthesis (Fig. 1). General ripeness indicators can be determined by looking at changes in fruit shape, skin color, skin hardness, and fruit weight [14].

Reverse transcriptase-PCR (RT-PCR) is the method used for amplifying cDNA from mRNA. RT-PCR is used to get back and copying the 5’ and 3’ threads from mRNA, resulting in a large collection of cDNA from a minimal amount of mRNA. RT-PCR can be easily used to identify mutations, polymorphism, and measure the strength of gene expression.

A concept the main thing in this technique is converting mRNA to chain shape single for cDNA prints. Six types of genes related to sugar metabolism were identified from DNA genome-assembled dataset with the number of 176,002 contigs (Table 1).
Table 1. Genes putatively involved in sugar metabolism

| Gene Name | Number of Contig | Enzyme Code (KEGG) |
|-----------|------------------|--------------------|
| Sucrose-phosphate synthase (SPS) | 9 | 2.4.1.14 |
| Sucrose-phosphate (SPP) | 4 | 3.1.3.24 |
| Sucrose synthase (SUS) | 12 | 2.4.1.13 |
| Alkaline/ neutral invertase (INV) | 19 | 3.2.1.26 |
| Cytosolic invertase (CINV) | 4 | 3.2.1.26 |
| Beta-fructofuranosidase (CWINV) | 20 | 3.2.1.26 |

Gene primer of the sucrose-phosphate synthase (SPS), Sucrose-phosphatase (SPP), Sucrose synthase (SUS), Alkaline, neutral invertase (INV), Cytosolic invertase (CINV), and beta-fructofuranosidase (CWINV) are used from DNA sequences of matoa, and genes actin is used as an internal reference gene (Table 2). Analysis of gene expression refers to [15]. Sucrose synthase (SUS), sucrose phosphate synthase (SPS), and invertase (INV) were critical enzymes which are catalyzed sugar degradation and biosynthesis in postharvest fruits determined [16].

Table 2. Genes function

| Gene | Function | Organism |
|------|----------|----------|
| SPS | Catalytic activity: D-fructose 6-phosphate + UDP-alpha-D-glucose = H⁺ + sucrose 6-phosphate + UDP Molecular: glycosyltransferase, transferase Biological: sucrose metabolic process | *Litchi chinensis* |
| SPP | Catalyzes the final step of sucrose synthesis Catalytic: H₂O + sucrose 6-phosphate = phosphate + sucrose Molecular: Mg²⁺ binding Sucrose-phosphate phosphatase Biological: sucrose biosynthetic process | *Arabidopsis thaliana* |
| SUS | molecular function: glycosyltransferase, transferase | *Aeschynomene abyssinica* |
| INV | Cleave sucrose into glucose and fructose. Catalytic: Hydrolysis of terminal non-reducing β-D-fructofuranoside residues in β-D-fructofuranosides Molecular: Glycopeptide α-N-acetylgalactosaminidase Sucrose α-glucosidase Biological: sucrose catabolic process | *Litchi chinensis* |
| CINV | Molecular: signal peptide Glycopeptide α-N-acetylgalactosaminidase | *Vitis vinifera* |
| CWINV | Molecular: sucrose alpha-glucosidase Biological: process: carbohydrate metabolic process | *Litchi chinensis* |

Source: UniProt.org

Sugar is an important character affecting matoa on fruit quality. However, the molecular basis for sugar metabolism and its regulation is poorly understood. Ripe matoa fruit has a sweet taste that providing useful material for transcriptomic to analyze sugar metabolism. A total of 176,002 contigs expressed genes sweet categorized into six DNA genetic sequence according to KEGG (Kyoto Encyclopedia of Genes and Genome) databases, respectively (Fig. 3).
Sugar changes followed the growth and development of plants in tissue such as glucose and sucrose [17]. The sugar response pathway interacted with numerous other pathways as phytochemical compounds [18]. Transport molecules, transient energy storage, signals, osmolytes, and carbon skeleton demands soluble sugar for multicellular organisms [19].

Figure 3. Starch and sucrose metabolism - reference pathway (KEGG)

Soluble sugars alteration such as sucrose and glucose have been shown affected by the growth and development in all stages of fruit development [20]. Sugar metabolism affected plant metabolism, growth and development, stress response, and gene expression complex picture of sugar-controlled regulatory and interactions with multiple signaling pathways [21]. SPS and SPP activity have shown that sucrose synthesis rapidly converts into glucose and fructose by invertase or sucrose synthase activities [22].

Sugar transport in matoa will be a subject in the exploration of sugar gene expression and matoa flavor. Genes of sugar metabolism in matoa will benefit future studies to elucidate the detailed roles from vegetative and generative stage circles in Sapindaceae crops.
4. Conclusion
Six types of genes related to sugar metabolism were identified from DNA genome-assembled dataset with 176,002 contigs will be the basic for comprehensive study of molecular mechanisms in sugar metabolism.

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References
[1] Lim, T. K. 2013. Edible Medicinal and Non-Medicinal Plants. Vol. 6. Springer Dordrecht Heidelberg New York London. p 92. DOI 10.1007/978-94-007-5628-1
[2] Papua Technology Research Centre (BPTP). 2014. Book Series Papua Typical Plants: Matoa. Jayapura (ID). Papua
[3] Wambraw, H. L. 2011. Morphological and isozyme characterization of the matoa (Pometia pinnata Forst.). [Thesis]. Bogor (ID). Faculty of Math and Science. IPB University
[4] Sada, T. J. and Rosye, T. H. R. 2010. Diversity of traditional medicinal plants in Nansfors Village-North Supiori District. Jayapura (ID). Jurnal Biologi Papua. Cendrawasih University. 2 (2)
[5] Martiningsih, N. W., Widana, G. and Kristiyanti, P. 2016. Phytochemical screening and antioxidant activity test of ethanol extract of matoa leaves (Pometia pinnata) using the DPPH method. MIPA National Seminar Proceedings. Undiksha. 332-338
[6] Mataputun, S. P., Rorong, J. A. and Pontoh, J. 2013. Activity of α-glukosidase inhibitor of the stem bark extract of matoa (Pometia pinnata. Spp.) as an antihyperglicemic agent. Jurnal Unstrat. 2 (2): 119-123
[7] Suedee, A., Tewtrakul, S. and Panichayupakaranant, P. 2013. Anti-HIV-1 integrase compound from Pometia pinnata leaves. Pharm Biol. 51 (10): 1256-1261
[8] Hamim. 2018. Plant Physiology 1: Water, Energy and Carbon Metabolism. Bogor (ID). IPB Press
[9] Bernier, G., Havelange, A., Houssa, C., Petitjean, A. and Lejeune, P. 1993. Physiological signals that induce flowering. Plant Cell. 5:1147-1155
[10] Leegood, R. C., Sharkey, T. D., and Caemmerer, S. V. 2004. Photosynthesis: Physiology and Metabolism. Vol 9. Dordrecht (Netherlands): Kluwer Academic
[11] Farrell, R. E. 1993. RNA Methodologies: A Laboratory Guide For Isolation and Characterization. San Diego: Academic Press
[12] Hartati, N. S. 2002. Sengon cDNA library construction induced by Bokor attack. [thesis]. Bogor (ID): Institut Pertanian Bogor
[13] Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods. 25(2001):402-408. DOI: 10.1006/meth.2001.1262
[14] Khandaker, M. M. and Boyce, N. N. 2016. Growth, distribution and physicochemical properties of wax apple (Syzygium samarangense). Australian journal of crop science. 10(12): 1640-1648. DOI:10.21475/ajcs.2016.10.12.PNE306
[15] Matra, D. D., Ritonga, A. W., Natawijaya, A., Poerwanto, R., Sobir, Siregar, U. J., Widodo, W. D., Inoue, E. 2019. Datasets for genome assembly of six underutilized Indonesian fruits. Data in brief. 22: 332.
[16] Ruan, Y. 2014. Sucrose metabolism: gateway to diverse carbon use and sugar signaling. Annual Review of Plant Biology. 65: 33–67
[17] Paul, M. J., Pellny, T. K. 2003. Carbon metabolite feedback regulation of leaf photosynthesis and development. Journal Experimental Botany. 54: 539-547
[18] Leon, P. and Sheen, J. 2003. Sugar and hormone connections. Trends Plant Science. 8: 110-116
[19] Chen, L. Q., Cheung, L. S., Feng, L., Tanner, W. and Frommer, W. B. 2015. Transport of sugars.
Annual Review of Biochemistry. 84: 865-894

[20] Susan and Gibson. 2005. Control of plant development and gene expression by sugar signaling. Current Opinion in Plant Biology. 8: 93-102. Department of Plant Biology. University of Minnesota. USA

[21] Rolland, F., Moore, B. and Sheen, J. 2002. Sugar sensing and signaling in plants. The Plant Cell. Department of Molecular Biology. Harvard Medical School. 14:S185-S205. doi:10.1105/tpc.010455

[22] Maloney, V. J., Park, J., Unda, F. and Mansfield, S. D. 2015. Sucrose phosphate synthase and sucrose phosphate phosphatase interact in planta and promote plant growth and biomass accumulation. Journal of Experimental Botany. Vol 66 (14): 4383-4394. doi:10.1093/jxb/erv101