Isolation and characterization genes in lobi-lobi (*Flacourtia inermis*) related to sugar metabolism

Y W Silaban¹, K Suketi², S Susanto² and D D Matra²*  

¹Study Program of Agronomy and Horticulture, Faculty of Agriculture, Graduate School of IPB University, Jl Meranti Kampus IPB Dramaga, Bogor, 16680, Indonesia  
²Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Jl Meranti Kampus IPB Dramaga, Bogor, 16680, Indonesia  

*Email: dedenmatra@apps.ipb.ac.id

**Abstract.** Lobi-lobi (*Flacourtia inermis*) is probably a native plant to the Moluccas (Maluku). In Indonesia, lobi-lobi fruit is used for traditional medicine because it has a phenolic compound. Sugar metabolism involved several genes that control the photosynthesis pathway. This research aims to isolate and characterize genes in which related to sugar metabolism in Lobi-lobi. The lobi-lobi contigs have 694 452 contigs produced by the velvet-assembled genome. The sugar-related genes were isolated from the assembled-genomes database against to SwissProt Database using the BLASTX program. The six-sugar-related-genes were characterized involved 36 contigs of Sucrose-phosphate synthase (SPS), six contigs of Sucrose-phosphatase (SPP), 46 contigs of Sucrose synthase (SUS), 36 contigs of Alkaline/neutral invertase (INV), four contigs of Cytosolic invertase (CINV), and 17 contigs of Beta-fructofuranosidase (CWINV), respectively. Comprehensive research on sugar metabolism was used in this study. Then, the data also revealed the biological functions of these genes in flowering time control in Lobi-lobi.

1. **Introduction**  
Lobi-lobi (*Flacourtia inermis*) is a plant originating from the African and Asian continents. The natural habitat of this plant is a tropical forest. This plant can grow with a minimum height of 2400 m above sea level. The lobi-lobi will survive with a daily temperature of 13-30 °C and daily rain of 500-1600 mm in tropics and subtropics. This plant can be eaten directly by a human. In Indonesia, Usually, Indonesian people are using lobi2 as a dessert called rujak (a mixture of fruits with peanut sauce) [1]. Several studies have shown that the fruit of the lobi-lobi plant contains some benefit compounds such as anti-bacterial, antifungal, anti-protozoa, and antioxidants [2][3][4]. Lobi-lobi fruit extract can be used in the process of preserving fish [5].

Contigs are fragments that map the genome to a piece of DNA. Each contig has a different function. Researchers use contig to map the role of a long DNA sequence. Contig mapping has difficulties identifying the contig's role, so a database and identification method are needed. There are many ways to identify contigs, one of which can be using Yeast Artificial Chromosomes (YAC). YAC can store up to 300-1500 kb of DNA [6]. The arrangement of contig fragments and playing an essential role in the identification process of a DNA thread, but contig has an essential function in the consistency of chromosome shape in an organism and identifying an organism's ancestral origin. Research by analyzing the T genome of parasitic worms uses the Expressed sequence tags (EST) method on The three stages of worm development and found the basis for forming the T genome sequence. The EST results have
been divided into several clusters, and these gene clusters have entered into GenBank results. Interference comparisons are used to identify the evolution that has occurred in these parasitic worms [7].

Sugar metabolism is the result of photosynthesis. These results are used in the formation of secondary metabolism in plant cells [8]. Photosynthetic sugar in fructose 6-phosphate will be partially converted into starch and converted into secondary metabolite compounds. The formed starch will be used as a food reserve [9]. The conversion of fructose 6-phosphate is an example of the function of the process of sugar metabolism. Every method of sugar refurbishment uses enzymes. The enzymes released are regulated by several genes.

Research on trehalose-6-phosphate shows that several enzymes regulate plant metabolism. The enzyme used comes from several expression genes [10]. Signaling of trehalose-6-phosphate will affect flowering in plants. The plant will experience a late fruiting period.

The composition of sugar forms in various raw ingredients of our daily food. Like plants harvested, the fruit has a higher sucrose content than other sugar forms (Fructose and glucose) [11]. Knowledge of the content of this form of sugar will help researchers in agriculture improve what factors a plant needs to maximize crop production (further increase the activation of sucrose-making enzymes). Knowledge about the formation of sugar is in line with research conducted by N’chobo et al. (1999) shows that knowing the supply of sucrose and the activity of sucrose synthesis can increase the maximum development of tomatoes [12].

2. Materials and method
This research was initiated at the Mekarsari Fruit Garden in Cileungsi, Bogor District. The analysis was conducted at the Postharvest Laboratory of the Department of AGH IPB University and Advance Laboratory, IPB University. The analytical material used is the mature leaves of the lobbies plant. Mature leaves have a length range of 10-15 cm and have a leaf width of 4-8 cm [13]. Data from the NGS is gathered from the DDBJ with DRA007395 accession and the assembled-contigs [14]. The assembled-contigs were assembled to reduce redundancy using CAP3 (-p 90) and CD-HIT-EST (-c 0.90 -M 0 -T0). The Swiss-Prot and TrEMBL databases from Uniprot were used to classify contig details using the BLAST software using 10-15 genes linked to sugar metabolism.
3. Result

The lobi-lobi contigs have 694 452 contigs produced by the velvet-assembled genome. The sugar-related genes were isolated from the assembled-genomes database against to SwissProt Database using the BLASTX program. The lobi-lobi was divided into six genes in regulating sugars. Sucrose-phosphate synthase (SPS) has 36 contigs. Sucrose-phosphatase (SPP) has five number of contigs. Sucrose-synthase (SUS) has 46 contigs. Alkaline/neutral invertase (INV) has 36 contigs. Cytosolic invertase (CINV) has four contigs. Beta-fructofuranosidase (CMINV) has 17 contigs.

Table 1. The six genes related to sugar metabolism

| Genes                           | Number of contigs |
|---------------------------------|-------------------|
| Sucrose-phosphate synthase (SPS)| 36                |
| Sucrose-phosphatase (SPP)       | 6                 |
| Sucrose synthase (SUS)          | 46                |
| Alkaline/neutral invertase (INV)| 36                |
| Cytosolic invertase (CINV)      | 4                 |
| Beta-fructofuranosidase (CMINV)| 17                |
4. Discussion
The discovery of genetic sucrose phosphate synthesis in the lobi-lobi was used to achieve these plants' yield potential. This potential yield can be improved by changing the behavior of sucrose-phosphate synthase (SPS) [15]. Analysis has shown that sucrose-phosphate synthase plays an essential function in the mechanism of photosynthesis. The increase in SPS is induced by the supply of nutrients, particularly the elemental content of nitrogen. The soybean plant reported that leaf extracts from the NO₃-supplied plants had higher sucrose phosphate synthase (SPS) activity than the N₂-dependent plants. The starch accumulation has a negative correlation with SPS on the starch proliferation stage. The action of SPS regulates the aggregation of starch. The soybean experiment showed that starch and starch accumulation is influenced by the N source [16]. The next research on lobi-lobi is needed to determine the nitrogen source and the right time in the fertilization process.

The increase in the SPS enzyme is also influenced by how long the plants get sunlight. The more plants get sunlight, and there will be an increase in the enzyme SPS compared to SUS. Plants need this SPS enzyme to break down Fructose 6 Phosphate into Sucrose, required by plant cells. Apart from being influenced by the length of the light acquisition period, the SPS activity of sugar beet is controlled by the phase of leaf development. The 19th leaf, which 50% develop, had 16.1 mmol sucrose of SPS activities compared with the 7th leaf as a source leaf, which is a leaf that completed its growth had 40.3 mmol sucrose. Analysis has shown that the action of SPS in young leaf plants was 2.5 times lower [17]. Further research is also needed for this plant to see whether it requires a long exposure time or not.

Forming sucrose into UDP Glucose (Figure 2) required a precursor in the SUS enzyme form. The formation of sucrose in the SUS pathway needed UDP to convert sucrose to fructose. The shape of Fructose 6 phosphate from sucrose needed UDP to convert sucrose to fructose. Fructose requires HK to become Fructose 6 phosphate on the SUS pathway [18]. The process of forming sucrose six phosphates from Fructose 6 phosphate requires a precursor, namely Sucrose Synthase. Any plant can convert sucrose directly into six phosphates without the enzyme sucrose. Monophyletic plants need a horizontal shift of the gene from Cyanobacteria to convert sucrose [19]. The leaves of the plant of Hevea need a 1.57-fold rise in sucrose as the plant creates new leaves [20]. Hevea plants have three stages of leaf growth. Leaves will have the highest sugar content (several types of sugar) when entering the 3rd stage.

![Figure 2. Starch and sucrose metabolism](image)
Plants in some situations, when these plants need sucrose, will synthesize sucrose in cellulose cells. The Sucrose synthesis only occurs when hydrolysis and interaction between cells biosynthesis in the process of carbon formation. The mechanism of new sucrose production is highly regulated by the enzyme sucrose phosphate synthase (SPS). This enzyme's function can convert sucrose-6 phosphatase into sucrose in mature leaves (Figure 3) [21]. The knowledge regarding the formation of this sucrose process can manipulate the environmental conditions of this plant alive. Research that has been carried out to increase the SPS enzyme activity is to continuously improve the rate of cellulose synthesis and the mechanism of cell wall deposition in cotton trees. The process of cell wall increase or cellulose synthesis is calculated by SuSy activity. SuSy activity is indicated by the microscopic distribution of immunolocalized electrons on the plasma membrane forming cotton fibers [22].

Sucrose synthase (SUS) and Alkaline / neutral invertase (INV) are the two forms of isoforms that affect changing the structure of sugar. SUS, as described above, functions to break down fructose or glucose into sucrose. INV has different properties from SUS, where INV will hydrolyze sucrose into a more effective form, namely fructose and UDP-glucose. This INV will take place in the cytosol. Sucrose overhaul is needed by plants when the plant has decreased SUS enzyme activity due to reduced plant oxygen levels. Decreased SUS activity will result in damage to some plant cells. Protection of Arabidopsis plants from reducing SUS is by activating the INV enzyme. INV enzymes will replace the SUS process to grow naturally [23]. INV is started by plants in oxygen deprivation stress conditions and when environmental conditions experience alkaline stress. Research conducted by testing Anabaena algae shows similarities in the emergence of Alkaline Invertase (A.INV) and Neutral Invertase (N.INV) enzyme activity in alkaline environmental conditions. The enzyme activity test carried out by these two enzymes will run optimally in the pH range 6.5 - 6.9 and 7.6 - 8.0 [24].

5. Conclusion
Several gene contigs regulate the lobi-lobi plants in the process of sugar formation. This is needed as necessary information as determining nutrient requirements for plants. The expressed genes when the plants are stressed can give information about determining fertilization. Breeders can use the discovery of the genes regulating the sugar-making enzymes to engineer plants to be more efficient in sugar translocation.
References

[1] Lim T K. 2013. Edible Medicinal and Non-Medicinal Plants vol 5. (New York: Springer) p 757

[2] George S and Benny PJ 2010 Anti-bacterial potency of fruit extracts of Flacourtia inermis against multidrug-resistant strains and comparison of its activity with that of standard antibiotics Int J. Pharm 1 95

[3] George G, Kuriakose S, George S and Mathew T 2011 Antifungal activity of silver nanoparticle-encapsulated β-cyclodextrin against human opportunistic pathogens Supramolecular Chemistry 23 593

[4] George S, Benny P J, Kuriakose S and George C 2011 antibiotic activity of 2,3-dihydroxybenzoic acid isolated from Flacourtia inermis fruit against multidrug-resistant bacteria Asian J Pharm Clin Res 4 126

[5] Pribadi A, Nurhamidah N and Elvinawati N 2018 Pemanfaatan ekstrak air buah Flacourtia inermis roxb. (lobi-lobi) sebagai pengawet ikan laut J. Pendidikan dan Ilmu Kimia 1 1

[6] Zhang P, Schon E A, Fischer S G, Cayanis E, Weiss J, Kistler S and Bourne P E 1994 An algorithm based on graph theory for assembly of contigs in physical mapping of DNA Bioinformatics 10 309.

[7] Chuan J, Feng Z, Brindley P J, McManus D P, Han Z, Peng J and Hu W 2010 Our Wormy World : Genomics, Proteomics and Transcriptomics in East and Southeast Asia. Vol 73 (Netherlands:Elsevier/Advance in Parasitology) p 327

[8] Leegood R C, Sharkey T D and Caemmerer S V 2004 Photosynthesis: Physiology and Metabolism vol 9 (Dordrecht/ Netherlands: Kluwer Academic)

[9] Weiner H, Stitt M and Heldt W 1987 Subcellular compartmentation of pyrophosphate and alkaline pyrophosphatase in leaves vol 893. BBA 893 13

[10] Ponnu J, Wahl V and Schmid M 2011 Trehalose 6 phosphate : connecting plant metabolism and development Front. Plant. Sci. 70 1.

[11] Shanmugavelan P, Kim S Y, Kim J B, Kim H W, Cho S M, Ki, S N, Kim S Y, Cho Y S and Kim H R 2013 Evaluation of Sugar Content and Composition in Commonly Consumed Korean Vegetables, Fruits, Cereals, Seed Plants and Leaves by HPLC-ELSD vol 380. (United Kingdom/Carbohydrate Research) p 112

[12] N’tchobi H, Dalí N, Nguyen-Quoc B, Foyer C H and Yelle S 1999 Starch synthesis in tomato remains constant throughout fruit development and is dependent on sucrose supply and sucrose synthase activity J. Exp. Bot 50 1457

[13] Verheij EWM Coronel RE 1992 Plant Resources of South-East Asia No.2 Edible Fruits and Nuts (Indonesia:Bogor/Prosea Foundation) p 277

[14] Matra DD, Ritonga AW, Natawijaya A, Poerwanto R, Sobir, Siregar U J, Widodo W D, and E Inoue. 2019. Datasets for Genome Assembly of Six Underutilized Indonesian Fruits vol 22. (Netherlands/Data in Brief) p 960

[15] Hattenbach A, Muller-Rober B. Nast G and Heineke D. 1997. Antisense repression of both adp-glucose pyrophosphorylase and triose phosphate translocator modifies carbohydrate partitioning in potato Plant Physiol 115 p 471

[16] Kerr P S Huber S C and Israel D W 1984 Effect of n-source on soybean leaf sucrose phosphate synthase, starch formation, and whole plant growth Plant Physiol 75 483

[17] Pavlinova O A, Balakhonstein E N, Prasolova M F and Turkina M V. Sucrose-phosphate synthase, sucrose synthase, and invertase in sugar beet leaves Plant Physiol 49 68

[18] Fernandez E B, Munoz F J, Saikusa T, Lopez M R, Akazawa Y and Romero J P 2003 Sucrose synthase catalyzes the de novo production of adpglucose linked to starch biosynthesis in heterotrophic tissues of plants Plant Cell Physiol 5 500
[19] Chua T K, Bujnicki J M, Tan T C, Huynh F, Patel B K and Sivaraman J 2008 The Structure of sucrose phosphate synthase from *halothermothrix orenii* reveals its mechanism of action and binding mode *The Plant Cell* **20** 1059

[20] Zhu J, Qi J, Fang Y, Xiao X, Li J, Lan J and Tang C 2018 Characterization of sugar contents and sucrose metabolizing enzymes in developing leaves of *Hevea Brasiliensis* *Front. Plant. Sci.* **9** 1

[21] Babb V M and Haigler C H 2001 Sucrose Phosphate synthase activity rises in correlation with high-cellulose synthesis in three heterotrophic systems *J. Plant Physiol* **127** 1234.

[22] Haigler C H, Datcheva M I, Hogan P S, Salnikov V V, Hwang S, Martin K, and Delmer D P 2001 Carbon partitioning to cellulose synthesis *Plant Mol. Biol* **47** 29.

[23] Barratt DHP, Derbyshire P, Findlay K, Pike M, Wellner N, Lunn J, Feil R, Simpson C, Maule AJ, and Smith AM 2009 Normal growth of arabidopsis requires cytosolic invertase but not sucrose synthase *PNAS* **106** 13124.

[24] Vargas W, Cumino A, and Salerno GL 2002 Cyanobacterial alkaline/neutral invertases. origin of sucrose hydrolysis in plant cytosol? *Int. J. Plant Biol* **216** 951.