Characterization of rambai (*Baccaurea motleyana*) genes putatively involved in sugar metabolism

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Abstract. Rambai (*Baccaurea motleyana*) is one of the underutilized fruits native to Indonesia. Rambai has high antioxidant activities containing phenolic, flavonoid, and anthocyanin compounds, secondary metabolite compounds derived from sugar metabolism. The sugar metabolism involved several related genes. This research aimed to characterize genes putatively involved in sugar metabolism in Rambai. Six sugar gene families were identified from 37 077 contigs of the assembled-transcriptome database against to UniProt database using the BLASTX program. The six sugar-related genes were characterized involved nine contigs of sucrose-phosphate synthase (SPS), three contigs of sucrose-phosphatase (SPP), 14 contigs of sucrose synthase (SUS), 19 contigs of alkaline/neutral invertase (INV), one contig of cytosolic invertase (CINV) and five contigs of beta-fructofuranosidase (CWINV). This research aims to give a comprehensive study of the sugar metabolism mechanism in *B. motleyana*. The data also revealed the genes that encoded the enzymes that were putatively involved in sugar metabolism.

1. Introduction

The tropical rainforests of Southeast Asia, especially the Indo-Malay region, have diverse plant genetic resources, including wood species, medicinal plants, and fruit trees. Many fruits are considered rare or poorly known because they have not been commercially explored, such as menteng (*Baccaurea racemosa*), rambai (*Baccaurea motleyana*), and gandaria (*Bouea macrophylla* Griffith) [1]. The commercial value of plants is obtained from that function derived by its secondary metabolites as a source of food additives, pharmaceuticals, or flavor [2]. Rambai (*Baccaurea motleyana*) belongs to the family of *Phyllanthaceae* with menteng and tampoi. Rambai is a plant native to Sumatra, Kalimantan, and Java, then spread to Peninsular Malaysia, Sumatra, Kalimantan, Java, and Bali, and other countries as Thailand and the Philippines [3]. Rambai can grow in lowlands, tropical rain forests, open scrub, and riparian forests and often cultivated in home gardens. The fruit has a size of 2.5-4 cm, oval-shaped, the fruit's color is yellowish-green when unripe and yellowish-white when ripe [4].

In Kalimantan, rambai is one of the non-timber forest products (NTFPs), which are sold at a price of Rp.10,000 / kg and commonly consumed as a table fruit [3][5]. Each 100 gram of *B. motleyana arillocde* contains 64 kcal energy, 83.7 g water, 0.4 g protein, 0.4 g fat, 14.6 total carbohydrates, 0.1 g fiber, 0.2 g ash, and variants of mineral and vitamins such as Ca, P, Fe, Na, K, vitamin C, vitamin B, niacin, and vitamin K [6]. In addition to its nutritional and vitamin content, rambai fruit also contains secondary metabolites in phenols, flavonoids, and antioxidants. The phenol content of rambai in three different
maturities was 97.23 mg/100g, 63.90 mg/100 g, 79.57 mg/100 g for unripe, mature, and ripe, respectively [7][8]. Furthermore, ethanol extract from the fruit skin showed antibacterial activity for *Bacillus cereus*, *B. subtilis*, *Staphylococcus vulgaris*, and *Escherichia coli* [9] and had a decreased blood sugar activity that caused hypoxides in mice [10].

Secondary metabolites known as the intermediate products of plant metabolism are usually described as small molecules with various functions such as cell structure, signaling, fuel, or even stimulate several enzymes responsible for having an inhibitory effect, defenses, stress response, and interactions with other organisms. Plants produce various organic compounds, most of which do not play a direct role in growth and development; these compounds are known as secondary metabolites [2]. The function of secondary metabolites in plants is closely related to plants' physiological processes in their environmental growth [11]. The expression of synthetic pathways of plant secondary metabolites can be affected by the supply of precursors, environmental and climatic conditions, and special treatment such as immobilization and biotransformation [12]. The precursor source can be derived from sugar metabolism, and this research aimed to characterize genes putatively involved in sugar metabolism in Rambai. This research aims to give a comprehensive study of the sugar metabolism mechanism in *B. motleyana*. The data also revealed the genes that encoded the enzymes that were putatively involved in sugar metabolism.

2. Materials and methods

The study was conducted in the experimental field of PT. Mekar Unggul Sari, Cileungsi Bogor. The NGS data were collected from DDBJ with accession DRA007358 and the assembled-contigs [13]. SwissProt database (UniProt) was used to annotate using the BLAST program to gene-related to sugar metabolism with the cut-off of 10⁻⁵. Gene primers of the Sucrose synthase (SUS), sucrose-phosphate synthase (SPS), Sucrose-phosphatase (SPP), Alkaline, neutral invertase (INV), Cytosolic invertase (CINV), and beta-fructofuranosidase (CWINV) are used from DNA sequences of rambai (*B. motleyana*), and genes actin is used as an internal reference gene.
3. Results and discussion

As the figure mentioned in Table 1, nine contigs of Sucrose-phosphate synthase show that this enzyme has an essential role in B. motleyana fruit metabolism. The enzyme of alkaline/neutral invertase (INV) was established as the most counted contigs, as many as 19 contigs, followed by sucrose synthase (SUS), sucrose-phosphate synthase (SPS), Beta-fructofuranosidase (CWINV), sucrose-phosphate (SPP), and cytosolic invertase (CINV) respectively.

| Gene Name                        | Number of Contig | Enzyme Code (KEGG) |
|----------------------------------|------------------|--------------------|
| Sucrose-phosphate synthase (SPS) | 9                | 2.4.1.14           |
| Sucrose-phosphate (SPP)          | 3                | 3.1.3.24           |
| Sucrose synthase (SUS)           | 14               | 2.4.1.13           |
| Alkaline/neutral invertase (INV) | 19               | 3.2.1.26           |
| Cytosolic invertase (CINV)       | 1                | 3.2.1.26           |
| Beta-fructofuranosidase (CWINV)  | 5                | 3.2.1.26           |

The enzymes involved in sucrose metabolisms such as invertase (EC 3.2.1.26), sucrose synthase (SS, EC 2.4.1.13), and sucrose phosphate synthase (SPS, EC 2.4.1.14) have a dominant factor in determining fruits sugar composition and accumulation [27]. Sucrose-phosphate synthase (SPS; EC. 2.4.1.14) has a main role in sucrose biosynthesis. SPS in plant enzyme that regulated by reversible proteins and metabolites on both photosynthetic and non-photosynthetic tissues.

This enzyme has the role of helping leaves respond to light and dark signals to produce the end product of photosynthetic [14]. Both SPS and SUS have a strong function on sucrose accumulation on some plants, such as Asian pear (Pyrus pyrifolia) and Chinese pear (Pyrus Bretschneider Rehd.). Meanwhile, SS and SPS activity increased during fruit maturation, and both of them play a strong role in sugar assimilation on fruits. A study reported that these enzyme activities were elevated four and seven times on the maturation stage, respectively [15]. In the aril of Litchi chinensis Sonn, the pattern of enzyme activities and gene expressions explained that hexose/sucrose ration was unlikely determined by SPS activity. This research also revealed that sucrose cleavage enzymes such as AI and SS more dominant than sucrose synthetic enzyme-like SPS in determining fruit's sugar content. In contrast to litchi, 'Yali' pear (Pyrus Bretschneider Rehd.) does not show an SPS and SS activities increasing during fruit development and fruit maturation [29].

The next enzyme established was Sucrose-phosphatase (SPP; EC 3.1.3.24), which counted as many as three contigs on rambai fruit flesh. SPP has a significant impact on the finalization of the sucrose synthesis pathway. Many studies revealed that most higher plants contain this enzyme on several types or isoform encoded by different genes. For instance, this enzyme is encoded by genes on chromosomes 1 (AtSPP1), 2 (AtSPP2), and 3 (AtSPP3a and AtSPP3b) on the dicotyl plant such as on Arabidopsis thaliana [17]. Another study reported that this enzyme was highly expressed on environmental stress such as cold, drought, and salinity.

In extreme conditions, SPP has catalyzed sucrose-6P-phosphate (Suc6P) production that is used to produce an SPS enzyme that finally plays a role in hydrolyzing sucrose [16] irreversibly. A different gene might encode this enzyme on each plant. For example, this enzyme's presence on Pyrus patens was encoded by a four kDa protein, which had both of HAD phosphatase and C terminal domains of SPP [17]. The enzyme codes were related to the enzyme's function in the sucrose metabolism pathway, as revealed in Figure 2.
Another enzyme established on rambai fruit flesh is Sucrose synthase (SUS/ EC 2.4.1.13), which has an essential role in sucrose assimilation during fruit ripening [18]. A study reported that this enzyme plays a dominant role in strawberry fruit ripening and will be inhibited by abscisic acid and sucrose at a specific concentration [19]. However, in some plants, SUS was responded to sucrose cleavage rather than sucrose synthesis [27]. The activity of SS (sucrose synthase) in 'Yali' fruit has a significant SS activity when at the early stage of fruit development but declined dramatically along with fruit development. This study also explained that SS activity was seasonal and leads toward sucrose cleavage, then sucrose synthase[29]. Meanwhile, in melon (Cucumis melo), the SUS role catalyzes sucrose synthesis from fructose and UDP-glucose or reversibly from sucrose to fructose and UDP-glucose. This reaction might occur after the production of sucrose-P finished by SPS [20].

The most dominant enzyme established at rambai is Alkaline-neutral-invertase (EC 3.2.1.26), with as many as 19 contigs. As many as the enzyme established, this enzyme is thought to have a strong impact on sugar metabolism. Many studies established that INV is an enzyme that has the function to catalyze the irreversible hydrolysis of sucrose into fructose and glucose. This enzyme could be determined by the optimum pH (acid or alkaline/neutral) and divided into two groups in higher plants. They are the alkaline/neutral invertases and the acid invertases. Alkaline/neutral invertases belong to glucosidases with alkaline/neutral optimum pH ranged from 6.5–8.0 and predicted to be established on cytosol or other organelles.

Meanwhile, acid invertases are called as fructofuranosidase, which have pH optimum (acidic) from 4.5–5.0. These enzymes are predicted to be located in the cell wall and vacuole [21]. Many studies have reported that most acid invertases are involved in several plant life cycle aspects such as photosynthetic assimilates partitioning, environmental condition response, cell enlargement, and plant growth and development [22][23][24].

The next enzyme that putatively on rambai sugar metabolism is cytosolic invertase (CINV/ EC 3.2.1.26) appeared at one contig. Some studies showed that CINV genes are important for Lotus japonicas and Medicago sativa growth and development [30,31]. In Arabidopsis, the CINV/UGP vital important to cellulose synthesis. Another study reported that CINV activity was affecting carbon partitioning [32].
The last enzyme analyzed to have a role in rambai’s sugar metabolism is beta-fructofuranosidase (CWINV/ 3.2.1.26) appeared at five contigs. Both of these enzymes play a strong role in producing glucose and fructose—the role of CINV and CWINV on sugar metabolism at plant cells described in Fig 3.

Figure 3. Sugar metabolism at plant cell and the role of putative enzymes [25]

Figure 3 described that CWINV from wall-cell was hydrolyzed the sucrose on apoplast to be glucose and fructose, then carried out to the cell by a monosaccharide transporter. Besides that, sucrose is delivered to other sink cells through plasmodesmata by sucrose transporter. The sucrose is then stored at the vacuole or hydrolyzed by vacuolar invertase (vINV). Furthermore, after the sucrose is stored in the cytosol, hydrolyzed by cytosolic invertase (CINV) becomes glucose and fructose. SUS can reverse this reaction to yield fructose and UDP-G [25].

4. Conclusion
There are six sugar-related genes characterized involved nine contigs of sucrose-phosphate synthase (SPS), three contigs of sucrose-phosphatase (SPP), 14 contigs of sucrose synthase (SUS), 19 contigs of alkaline/neutral invertase (INV), one contig of cytosolic invertase (CINV) and five contigs of beta-fructofuranosidase (CWINV).

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