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Transcriptome and selected metabolite analyses reveal points of sugar metabolism in jackfruit (*Artocarpus heterophyllus* Lam.)

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ABSTRACT

Artocarpus heterophyllus Lam., commonly known as jackfruit, produces the largest tree-borne fruit known thus far. The edible part of the fruit develops from the perianths, and contains many sugar-derived compounds. However, its sugar metabolism is poorly understood. A fruit perianth transcriptome was sequenced on an Illumina HiSeq 2500 platform, producing 32,459 unigenes with an average length of 1345 nt. Sugar metabolism was characterized by comparing expression patterns of genes related to sugar metabolism and evaluating correlations with enzyme activity and sugar accumulation during fruit perianth development. During early development, high expression levels of acid invertases and corresponding enzyme activities were responsible for the rapid utilization of imported sucrose for fruit growth. The differential expression of starch metabolism-related genes and corresponding enzyme activities were responsible for starch accumulated before fruit ripening but decreased during ripening. Sucrose accumulated during ripening, when the expression levels of genes for sucrose synthesis were elevated and high enzyme activity was observed. The comprehensive transcriptome analysis presents fundamental information on sugar metabolism and will be a useful reference for further research on fruit perianth development in jackfruit.

1. Introduction

Jackfruit (*Artocarpus heterophyllus* Lam.) is a fruit tree that belongs to Moraceae (mulberry family). It is native to the Western Ghats of India, but is the national fruit of Bangladesh and is now widely planted in various countries such as Brazil, Thailand, Indonesia, Malaysia and China. Jackfruit is a medium-sized monoecious tree that typically ranges from 5 to 20 m in height (Fig. 1a). The fruits are borne on the trunk and side branches, and weigh between 3.5 and 35 kg per fruit [1]. The fruits are multiple fruits, formed from hundreds of flowers on a single inflorescence, and the flesh that develops around the seed comes from the perianth of a pollinated flower (Fig. 1). The pulp (perianth hereinafter) contains various classes of compounds including carotenoids, flavonoids, volatile acids, sterols and terpenes, which give it a unique flavor [2–4].

Primary sugar metabolism begins after the synthesis of sucrose from triose phosphate, which is the product of photosynthesis. Sucrose (Suc) is the primary form of carbohydrate that is transported from source tissues (primarily mature leaves) to sink tissues including flowers, fruits, seeds and roots [5]. Once it reaches those sinks, Suc is hydrolyzed by invertase, generating glucose (Glc) and fructose (Fru), or hydrolyzed by sucrose synthase (SUSY), producing fructose and uridine diphosphate glucose (UDPG). Vacuolar acid invertase (vAINV) hydrolyzes Suc to Glc and Fru, which can be stored in the vacuole. In the apoplast, extracellular Suc is hydrolyzed to Glc and Fru by cell wall invertase (CWINV), and then hexose transporters move these sugars into parenchyma cells. Suc cleavage is the principal step in sugar signaling, because Suc must be hydrolyzed into hexoses before it can be used in various metabolic and biosynthetic processes [6–8].

The resulting Glc and Fru can be phosphorylated to glucose 1-phosphate, glucose 6-phosphate and fructose 6-phosphate by

Abbreviations: AGPase, adenosine diphosphate glucose pyrophosphorylase; AI, acid invertase; CINV, cytoplasmic invertase; COG, clusters of orthologous groups; CWINV, cell wall invertase; CYC-β, lycopene beta-cyclase; Ct, cycle threshold; DAP, day after pollination; FK, fructokinase; Fru, fructose; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GAUT, galacturonosyltransferase; GCS, glucosidase; Glc, glucose; GO, gene ontology; HK, hexokinase; KEGG, Kyoto encyclopedia of genes and genomes; KO, KEGG orthology; M, geometric stability measure; NI, neutral invertase; NR, nonredundant; NT, nucleotide; PE, pectinesterase; PSY, phytoene synthase; SBE, starch branching enzyme; SP, starch phosphorylase; SPS, sucrose phosphate synthase; SS, starch synthase; Suc, sucrose; SUSY, sucrose synthase; UGPase, UDP glucose pyrophosphorylase; vAINV, vacuolar acid invertase.

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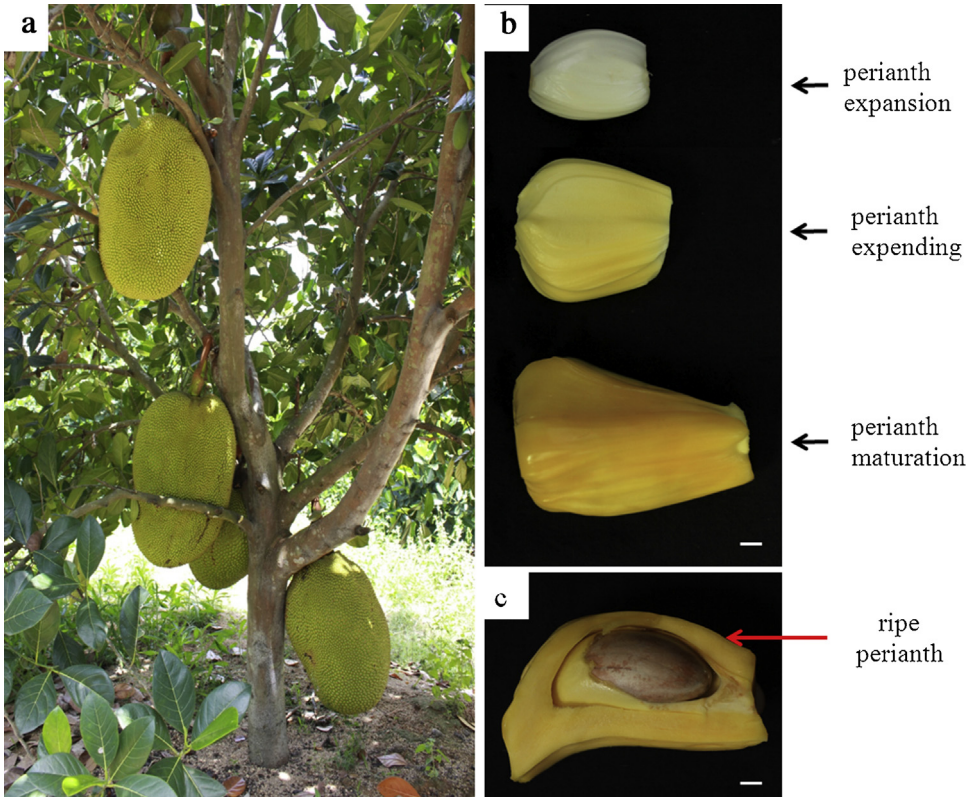

Fig. 1. Jackfruit tree and fruits. (a) The fruits on the tree. (b) Typical stages of perianth (fruit) development. (c) Ripe jackfruit perianth. (Scale bar = 1 cm).

hexokinase (HK) and fructokinase (FK). The inter-conversions among these hexoses are catalyzed by uridine diphosphate glucose pyrophosphorylase (UGPase), phosphoglucoisomerase and phosphoglucomutase [9]. Hexoses can be imported into the plastid, where they are used for starch synthesis. Starch is a stored and usable form of energy that is important during fruit ripening [7,10]. Fructose 6-phosphate and UDPG can be combined to resynthesize Suc by sucrose phosphate synthase (SPS). The Glc, Fru and Suc generated from these reactions determine the sweetness of sink tissues. The degradation and resynthesis system of Suc is known as the "futile cycle" and is a key component of primary metabolism [11]. Meanwhile, the hexoses enters glycolysis, where it used to produce adenoside triphosphate, reduced nicotinamide adenine dinucleotide (NAD) and pyruvate, which are crucial for many other processes [12]. Generally, sugar metabolism not only provides energy for plant development, but also contributes to the quality of sink tissues.

There is limited research on the characterization of sugar metabolism in jackfruit, but the jackfruit genome has not been sequenced yet. In the absence of a sequenced genome, the *de novo* assembly of RNA-Seq data is a cost-effective method to study a transcriptome. High-throughput RNA sequencing, combined with assembly tools and special bioinformatics analyses, has enabled the construction of high-quality gene libraries for research on gene cloning, sequence polymorphisms identification and novel trans-splicing analysis [13]. A perianth transcriptome was generated for jackfruit using an Illumina HiSeq 2500 platform (BGI Genomics, Shenzhen, China) to sequence as many transcripts as possible. Approximately 30 million clean reads were obtained, representing 32,495 unigenes. members of various gene families that encode key enzymes and transporters involved in sugar metabolism were identified. The points of sugar metabolism in jackfruit were described by analyzing the expression patterns of sugar-related genes, corresponding enzyme activity, and the concentrations of accumulated sugars during perianth development. Results obtained from this study will provide a foundation for further studies to gain a comprehensive understanding of jackfruit perianth development.

2. Materials and methods

2.1. Plant materials and RNA extraction

Plants of *A. heterophyllus* line XYS-11 were cultivated in an experimental field (Wanning, Hainan, China). Pistillate inflorescences were tagged on the day after pollination (DAP). Perianths were removed carefully from fruits at different developmental stages (at 50, 70, 90, 110, 117, and 120 DAP) from three different trees. Samples of pollen, roots, and leaves were also collected from these plants. Samples of the stem were collected from three-month-old plants in the field. All the samples were immediately frozen in liquid nitrogen and stored at −80 °C for subsequent analysis. Three sets of samples (biological replicates) were collected from each tree. Total RNA was extracted with a commercial plant RNA extraction kit (Spectrum™ Plant Total RNA Kit, Sigma-Aldrich, St. Louis, MO, USA). Total mRNA (5 μg) from the perianths was purified by using poly-T oligo-attached magnetic beads (Life Technologies, Carlsbad, CA, USA), and mRNA was subjected to cDNA synthesis, which was normalized for further RNA sequencing (BGI Genomics, Shenzhen, China).

2.2. RNA sequencing and analysis

The normalized cDNA pool was sequenced on an Illumina HiSeq 2500 platform (BGI Genomics, Shenzhen, China). Total raw reads from the sequencing were preprocessed to remove dirty raw reads, following Hu et al. [14]. *De novo* assembly of filtered clean reads was conducted with Trinity software (http://trinityrnaseq.sourceforge.net/). Overlapping information in clean reads was used to construct