Journal of Plant Physiology 221 (2018) 1–10

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Transcriptomic analyses reveal complex and interconnected sucrose signaling cascades in developing seeds of castor bean

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ARTICLE INFO

Keywords:
Castor bean
Sucrose signaling
Non-green seeds
Seed development
Transcriptomic analysis

ABSTRACT

Seeds are highly specific organs that strongly sink sucrose resources from leaf and stem tissues to trigger seed metabolism and development. In particular, for heterotrophic non-green seeds, the potential molecular mechanism underlying sucrose-driven seed development remains unanswered. Castor bean (*Ricinus communis* L.), a typical non-green seed, has been considered as a model plant for seed biology study in dicotyledonous plants due to its heterotrophic seeds with persistent endosperms. In the present study, the fast-developing castor bean seeds were treated with exogenous sucrose and mannitol for four hours. The global transcriptomic data were obtained by high-throughput RNA-seq technique, resulting in 468 differentially expressed genes (DGEs). Further analyses revealed that sucrose functioned as both metabolic substrates and signal molecules. Specifically, 73 DGEs involved in carbohydrate and nitrogen metabolism, 42 differentially expressed transcription factors, and 35 DGEs involved in diverse signaling pathways such as auxin, brassinosteroid, ethelyene, cytokinin, gibberellin, and calcium signals, were identified, suggesting that the sucrose signaling pathway might have complex and multi-connected cross-talks with other signals to regulate castor bean seed development. Taken together, this study provides novel data to improve understanding of the potential molecular mechanisms of sucrose in regulating non-green seed development and storage reservoir accumulation during seed development.

1. Introduction

Sucrose, derived from plant photosynthesis, is critical for integrating the functions of internal and external regulatory signals required for driving various physiological processes from embryogenesis to senescence (Li and Sheen, 2016). It is the main transporting carbohydrate in plants, generally synthesized from photosynthetically fixed carbon in the cytosol of the source tissues (e.g., leaves) and then transported into the sink tissues (e.g., seeds, roots, or fruits) where sucrose is stored or utilized (Wind et al., 2010). Once sucrose translocates via phloem from the sources into sink tissues, it can be hydrolyzed to glucose and fructose by cell wall invertase (*CWINV*) or sucrose synthase (*SUS*). The long-distance transportation of sucrose from source to sink tissues provides sufficient substrate and energy for sink tissue development (Braun et al., 2013). Synthesis, transportation, and utilization of sucrose are tightly regulated, and changes in these processes affect plant growth and development (Wind et al., 2010).

Sucrose is not only a critical component for general metabolism, but also serves as an important signaling molecule in cellular metabolism as well as in abiotic stress responses (Smeekens and Hellmann, 2014; Wind et al., 2010). Studies have shown that sucrose signaling is often involved in regulating carbon and nitrogen metabolism as well as in transporting and partitioning (Tognetti et al., 2013). For example, the expression of *BvSUT1*, which encodes a proton-sucrose symporter, is specifically repressed by sucrose signaling in beet (Vaughn et al., 2002). The translation of bZIP11 protein can be repressed by sucrose signaling via a sucrose control peptide in Arabidopsis (Rahmani et al., 2009). Also, a sucrose-specific signaling pathway can mediate anthocyanin and fructan biosynthesis, participating the regulatory processes of plant responses to biotic and abiotic stresses (Ende and El-Esawe, 2014). In addition, sugar signaling can target hormonal signaling pathways integrating plant growth and development (Ljung et al., 2015). Several studies have shown that sucrose signaling is involved in auxin biosynthesis (Lilley et al., 2012; Sairanen et al., 2012). Cytokinin and gibberellin signaling pathways are also influenced by sugar signaling (Lilley et al., 2012). However, unlike glucose signaling, which functions via the hexokinase signaling pathway, the sensor and transduction mechanisms of sucrose signaling in plants remain largely unknown

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https://doi.org/10.1016/j.jplph.2017.11.012
Received 28 August 2017; Received in revised form 29 November 2017; Accepted 29 November 2017
Available online 06 December 2017
0176-1617/

(Tognetti et al., 2013).

Seeds are highly specific organs that can acquire sucrose from source tissues to synthesize a wide array of storage reservoirs (storage proteins or lipids) during development. These storage reservoirs provide sufficient calories for human consumption. Generally, plant seeds can be grouped into photo-heterotrophic green seeds (e.g., rapeseed and soybean) or heterotrophic non-green seeds (e.g., sunflower, castor bean, and safflower), depending on the seed color during development (Borisjuk and Rolletschek, 2009; Zilkey and Canvin, 1972). For green seeds, light is essential for seed development and seed storage material accumulation, as tissues contain light-harvesting pigments and many enzymes involved in photosynthesis resulting in sucrose biosynthesis (Tschiersch et al., 2011). In the developing photo-heterotrophic green seeds, studies have shown that sucrose functions as a signaling molecule in regulating embryo cell division, differentiation, and storage reserve accumulation (Weber et al., 1996). Exogenous application of sucrose also showed increased oil accumulation in developing seeds of *Brassica napus* (Vigeolas and Geigenberger, 2004) and *in vitro* cultured rapeseed embryos (Jing et al., 2014). Recently, it has been shown that a conserved sugar-signaling kinase KIN10 can regulate lipid biosynthesis by phosphorylating WRI1, a master transcriptional activator of oil synthesis (Zhai et al., 2017). These results strongly imply that sucrose is a critical signaling molecule that controls seed development and triggers the biosynthesis of storage reservoirs in green seeds. Unlike green seeds, the non-green seeds only receive sucrose from the source tissues (such as leaf and stem). It seems that sucrose plays a special role in providing energy for seed development and metabolic substrates for the biosynthesis of storage materials in non-green seeds. Comparative proteomics analysis between green seeds (soybean and rapeseed) and non-green seeds (castor bean and sunflower) revealed the physiological divergence of carbon recapture, carbon flow, and energy (ATP and NADPH) supply for seed development (Houston et al., 2009). However, it is not clear whether sucrose is involved as a signaling molecule to control the seed development and storage reservoir accumulation in non-green seeds.

Castor bean (*Ricinus communis* L.) is often considered as a model plant for studying the seed biology in dicotyledonous plants due to its heterotrophic (non-green) seeds with persistent endosperm in the mature seed (Greenwood and Bewley, 1982; Houston et al., 2009). In particular, its seed contains up to 60% storage lipids (mainly composed of ricinoleic acids) (Houston et al., 2009). Because of the unique chemical properties of ricinoleic acids, castor bean seed oil is highly valuable in industry, particularly for production of lubricating oil and biodiesel (Ogunniyi, 2006). Also, its seed accumulates up to 35% of storage protein (Sgarbieri and Whitaker, 1982), such as the unique ricin protein, a substance whose extreme toxicity makes it a potential biological weapon and a specific immunotoxin for medicinal therapy (Chan et al., 2010). As the demand of castor oil is increasing fast in many countries, breeders have paid more and more attention to the breeding and improvement of castor beans (Qiu et al., 2010). More efforts should be made to uncover the molecular mechanism of how the seed development and storage reservoir accumulation is regulated. In this study, we found that sucrose functioned as a metabolic substrate and signal molecule, globally involved in regulating the metabolism and development of castor bean seeds, using high-throughput RNA-Seq data. This study provides novel data to improve understanding of the potential molecular mechanisms of sucrose in regulating non-green seed development and storage reservoir accumulation during seed development.

2. Materials and methods

2.1. Plant materials

Castor bean seeds var. ZB107 elite (kindly provided by Zibo Academy of Agriculture Science, Shandong, China) were grown in the research base of Kunming Institute of Botany under normal conditions from April to October 2015. To protect against cross-pollination, hand-pollination was carried out, while the inflorescences were covered with paper bags and tagged to keep records from the days after pollination (DAP). According to previous investigation (Zhang et al., 2016), castor bean seeds at 25 DAP exhibit rapid increases in seed mass and weight caused by the accumulation of both protein and oil. Importantly, the seed coat at 25 DAP is not lignified, thus guaranteeing successful uptake of sucrose for seed development. Therefore, developing capsules tagged at 25 DAP were collected for further study.

2.2. Seed culture in vitro

To test whether the sugar signaling has an influence on castor bean seed development, the young developing seeds were cultured in liquid MS medium *in vitro* and were provided with exogenous sucrose (or mannitol as a control). First, the young capsules were washed with distilled water, then sterilized with 70% ethanol and subsequently washed with ddH_2O three times. The developing seeds were isolated from their capsules and transferred to a modified liquid MS medium: basic MS medium supplied with 20% (m/v) polyethylene glycerol 4000, 50 mM MES, pH 5.8. Based on our previous study (see Zhang et al., 2016), 160 mM sucrose or mannitol (as an optimum concentration) were supplemented in the medium in order to conduct the sugar treatment experiments on caster bean seed development. Typically, a single capsule contains three seeds. One seed from a single capsule was cultured in liquid MS medium with sucrose, and another from the same capsule was cultured in liquid MS medium with mannitol as a control and incubated at 25 °C with gentle shaking at 140 rpm for 4 h. Each treatment contained three to four seeds from different capsules. Then the dissected seeds were washed with sterile distilled water and quickly dried with sterile blotting paper. Tissues were snap frozen in liquid nitrogen and stored at −80 °C for further use. Each of the experiments was repeated at least three times, totaling nearly nine capsules containing 27 seeds with different treatments being examined. Usually, physiological responses to sugar signaling can be detected within four hours when plant tissues are cultured with exogenous sugar. Therefore, we applied four-hour treatment to detect whether sucrose signaling arose in developing castor bean seeds cultured *in vivo*.

2.3. Total RNA isolation and transcriptomic sequencing

RNA was isolated from treated seeds using TRIzol (Invitrogen, Carlsbad, CA) followed by RNeasy Mini Kit purification (Qiagen, Valencia, CA) according to the manufacturer's protocol. Then the quality of RNA was checked by nanodrop spectrophotometer (ND-1000 spectrophotometer, Peqlab) and by agarose gel electrophoresis. Equal RNAs isolated from at least three treated seeds were pooled for constructing cDNA libraries. Quality cDNA libraries were constructed and Illumina sequencing was carried out at Novogene sequencing platform (Beijing, China) to generate single-end 50-bp reads. After sequencing, the clean reads were obtained by removing reads with adapter contamination, poly-N, and low-quality reads from the raw data. Single-end clean reads were then aligned to the reference genome of castor bean (http://castorbean.jcvi.org/index) using TopHat v2.0.12 (Trapnell et al., 2012), and then the expression level of each transcript was normalized to FPKM (fragments per kilobase of exon per million fragments mapped) (Trapnell et al., 2010). Analyses of differential expression between sucrose and mannitol treatment were performed using DEGSeq R package (1.20.0). The genes were selected for further GO and KEGG analysis with a P-value of 0.05, log2 fold change > 1, and FPKM > 1.

2.4. GO and KEGG pathway enrichment analysis

To provide a functional overview on DEGs between the two