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The functions of cucumber sucrose phosphate synthases 4 (*CsSPS4*) in carbon metabolism and transport in sucrose- and stachyose-transporting plants

Xin Li, Jing Du, Jinju Guo, Hongyun Wang, Si Ma, Jianguo Lü, Xiaolei Sui*, Zhenxian Zhang*

Beijing Key Laboratory of Growth and Developmental Regulation for Protected Vegetable Crops, College of Horticulture, China Agricultural University, Beijing, China

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ABSTRACT

Sucrose phosphate synthases (SPSs) are rate-limiting sucrose synthesis enzymes present in photosynthetic and non-photosynthetic tissues. The cucumber genome contains three SPSs that can be grouped into families A, B, and C. *CsSPS1* and *CsSPS2* are highly expressed in flowers and mature leaves, while the expression level of *CsSPS4* increased gradually after leaf unfolding in our study and reached its peak after 20 days. In *CsSPS4*-overexpression tobacco plants, sucrose content and sucrose/starch ratio were increased significantly and resulted in improved leaf yield. By contrast, in *CsSPS4*-overexpression (*CsSPS4*-OE) cucumber lines, contents of sucrose and starch were unchanged, and raffinose was increased in transgenic cucumber leaves. The expression of cucumber raffinose family oligosaccharide (RFO)-synthesis-related genes increased obviously in cucumber *CsSPS4*-OE plants, and the sucrose, raffinose, and stachyose contents increased significantly in the petioles of *CsSPS4*-OE lines. In *CsSPS4*-antisense (*CsSPS4*-A) cucumber lines, decreases occurred in mRNA expression, enzyme activity, sucrose content, sucrose/starch ratio, and stachyose transport, but the RFO-synthesis-related genes were nearly unchanged. Together, these results suggest that overexpression of *CsSPS4* can lead to carbon metabolism prioritizing sugar transport in cucumber, and suppression of *CsSPS4* likely promotes carbon metabolism to accumulate starch, showing a more complicated carbon distribution model than in transgenic tobacco plants.

1. Introduction

As the main form of photoassimilate in leaves, sucrose can either be directly used in glycolysis or supplied to the rest of the plant to provide carbon and energy for growth and development (Ho and Thornley, 1978; Rocher et al., 1989; Battistelli et al., 1991; Davoren et al., 2002). Starch accumulates in the leaves during the day and is used overnight (Geiger and Servaites, 1994). In leaves, the rate of sucrose synthesis can affect the availability of carbon export from the leaf (Harn et al., 1993; Chavez-Barcenas et al., 2000). It also affects the photosynthetic rate by influencing chloroplast triose phosphate production (Stitt, 1986). In addition to its role in energy metabolism and photosynthesis, sucrose is critically important for production of storage reserves (Lunn and Furbank, 1999; Zourelidou et al., 2002), as a signal compound (Ciereszko et al., 2001; Stitt et al., 2002), influencing transporter function (Vaughn et al., 2002), modulating gene expression (Krapp and Stitt, 1995; Koch, 1996; Wind et al., 2010), and responding to environmental stress (temperature, salinity, drought) (Yang et al., 2001; Strand et al., 2003; Niedzwiedz-Siegien et al., 2004).

Sucrose phosphate synthase (SPS; EC 2.4.14) is the rate-limiting enzyme in sucrose synthesis. It catalyzes the conversion of UDP-glucose (UDPG) and fructose-6-phophate (F6P) to UDP and sucrose-6-phosphate (S6P), which is subsequently hydrolyzed by sucrose-phosphatase (SPP; EC 3.1.3.24) to form sucrose. SPS and SPP actually form a complex in vivo (Huber and Huber, 1996), making the reaction irreversible.

SPS enzymes of higher plants contain an N-terminal glucosyltransferase domain and a C-terminal SPP-like domain that might allow the formation of heteropolymeric complexes. A recent study confirmed the interactions of SPS and SPP, and demonstrated that the enzyme complex impacts carbohydrate pools and promotes plant growth in

Abbreviations: ADPG, ADP-glucose; F6P, fructose-6-phophate; FBP, fructose-1,6-bisphosphate; Fru, fructose; G1P, glucose-1-phophate; G6P, glucose-6-phophate; Galac, galactinol; Glu, glucose; GolS, galactinol synthase; IC, intermediate cell; MC, mesophyll cell; Myo-iso, myo-inositol; Pi, orthophosphate; PPC, phloem parenchymal cell; Raf, raffinose; RFOs, raffinose family oligosaccharides; RS, raffinose synthetase; S6P, sucrose-6-phosphate; SE, sieve element; SPP, sucrose-phosphatase; SPS, sucrose phosphate synthases; Sta, stachyose; STS, stachyose synthase; Suc, sucrose; SUS, sucrose synthase; TP, triose phosphate; TPT, triose phosphate translocator; UDPG, UDP-glucose; UDP-Gal, UDP-galactose; VIN, vacuole invertase; WT, wild-type

* Corresponding authors.
E-mail addresses: sui-office@cau.edu.cn (X. Sui), zhangzx@cau.edu.cn (Z. Zhang).

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both transgenic *Arabidopsis* and hybrid poplar (Maloney et al., 2015). Plant SPSs can be clustered into four groups based on their amino acid sequence. Three groups were first described as the A, B, and C gene families by Langenkamper et al. (2002), while the D family is only found in the Poaceae (Castleden et al., 2004).

A number of studies have investigated the role of SPS enzymes in both photosynthetic and non-photosynthetic tissues. Most of them focused on the function of SPS in carbon metabolism and sink strength in sucrose-transporting heterologous expression plants, while the sinks may range from flowers to fruits, tubers, fiber, stem, and so on (Laporte et al., 1997; Nguyen-Quoc et al., 1999; Haigler et al., 2007; Ishimaru et al., 2008; Park et al., 2008, 2009; Seger et al., 2015). For example, overexpression of a maize *SPS* cDNA in tomato increased the sucrose to starch ratio in leaves (Worrell et al., 1991; Galtier et al., 1993, 1995). Biomass also increased with overexpression of maize *SPS* cDNA in tomato and potato plants (Foyer and Ferrario, 1994; Laporte et al., 1997; Ishimaru et al., 2008). Overexpression of maize SPS in tobacco plants increased the numbers of flowers significantly (Baxter et al., 2003). However, in Cucurbitaceae, Lamiaceae, Oleaceae, and other plant families, raffinose family oligosaccharides (RFOs), including raffinose and stachyose, are the predominant carbohydrates translocated in phloem. So far, there has been no such report on the function of SPS in RFO-transporting plants.

Cucumber, an important horticultural crop, is a typical stachyose-transporting plant. Sucrose produced in the cytoplasm of mesophyll cells is transferred to intermediate cells where it is used to synthesize raffinose and stachyose by raffinose synthetase (RS: EC 2.4.1.82) and stachyose synthase (STS: EC 2.4.1.67), respectively, which enter the phloem for transport (Fig. 1) (Turgeon, 1996; Rennie and Turgeon, 2009). Galactinol synthase (GolS: EC 2.4.1.123) catalyzes the first step in RFO biosynthesis to synthesize galactinol, and then used for RFO synthesis. At the same time, starch accumulates in chloroplasts (Fig. 1). The impact of SPS on carbon metabolism and transport has only been studied in sucrose-transporting plants; thus, our work investigates the relationship between sucrose synthesis mediated by SPS and RFO anabolism in cucumber, which transports sugars by symplastic phloem loading with the polymer trap mechanism. We cloned three *SPS* genes from cucumber and assigned them to different families. The tissue-specific expression analyses aided us in further understanding different functions of these three *CsSPSs*. Our research on *CsSPS4* transgenic plants provides the theoretical basis for further studies of carbon metabolism and sugar transport in cucumber, showing different carbon distribution models for transgenic tobacco plants.

2. Materials and methods

2.1. Plant material and growth conditions

Cucumber (*Cucumis sativus* L. Xintaimici) plants for analysis were grown in greenhouses of China Agricultural University. The samples for analyzing tissue-specific transcript abundances were collected when the first cucumber fruit grew to commodity maturity. Leaf development experiments were initiated when the plant had five or six leaves. The day 0 leaf was defined as the first unexpanded leaf from the tip; similarly, the unexpanded leaf closest to the tip was noted every five days. On the 40th day, all the leaves (marked as 0 d, 5 d, 15 d, 20 d, 25 d, 30 d, 35 d, and 40 d) were collected for *CsSPS4* transcriptional analysis. Cucumber and tobacco (*Nicotiana tabaccum* cv. Yunyan 85) plants were grown in 8 × 8 cm pots in a growth chamber at 25 °C/18 °C with a 12 h/12 h light/dark cycle.

2.2. Cloning of CsSPS family genes

Cloning of the three cucumber *CsSPS* genes was based on similarity between other known *SPS* sequences and the cucumber genome database (http://cucumber.genomics.org.cn). Total RNA was isolated from leaves of cucumber seedlings using Trizol; cDNA was synthesized with dT_{16} oligonucleotides with the superscript first-strand synthesis system. All predicted coding regions of *CsSPS* genes were amplified by polymerase chain reaction (PCR) using three primer pairs (P1q-F: AGATA CGAATCGAAGAACATAATGGCTG; P1q-R: CGTTTATAATGACCATTCG CCAA; P2q-F: ACGGACAGCAGTATTGGAAATG; P2q-R: CCGTTTAGAA AGTAAGATGTAAATCTGTTAC; P4q-F: ATGGCAGGTGGGAATGAATG; P4q-R: CTAGTAGCTCTTGATCTCAGCAACG). These fragments were cloned into pGEM-T Easy vector and sequenced.

2.3. Quantitative real-time PCR

Total RNA was extracted from various tissue samples, and one microgram of DNase-treated RNA was used for cDNA synthesis following the manufacturers' instructions. *CsSPS* transcript levels were quantified using quantitative real-time PCR (qRT-PCR) analysis by Brilliant SYBR Green QPCR Master Mix on ABI7500 Real-Time PCR System. Three pairs of *CsSPS*-specific primers (P1-F: ACACGTTTGCCAAGAATCAGTT;

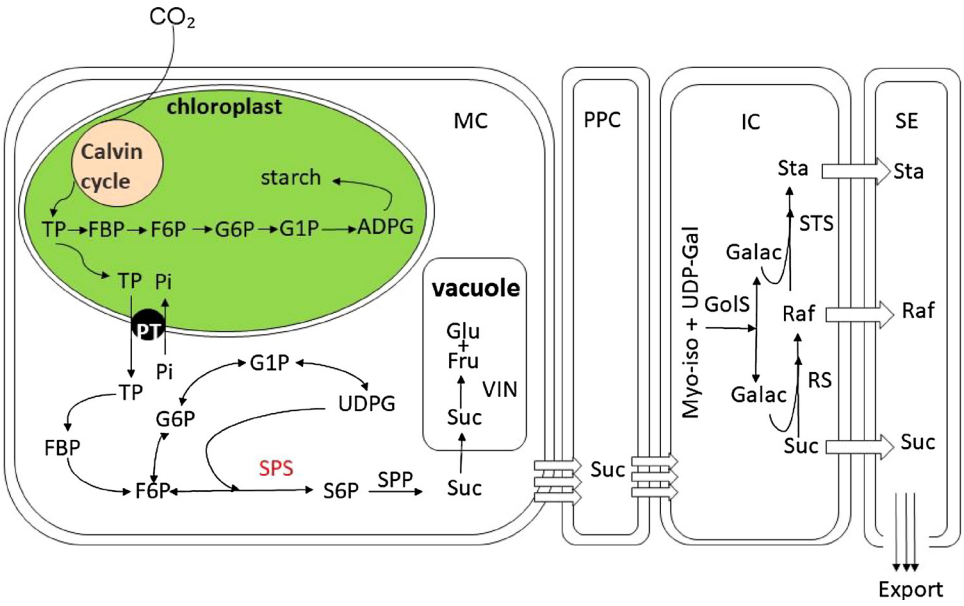

Fig. 1. An integrated model depicting synthesis of soluble sugars and phloem loading in cucumber source leaves. ADPG, ADP-glucose; F6P, fructose-6-phophate; FBP, fructose-1,6-bisphosphate; Fru, fructose; G1P, glucose-1-phophate; G6P, glucose-6-phophate; Galac, galactinol; Glu, glucose; GolS, galactinol synthase; IC, intermediate cell; MC, mesophyll cell; Myo-iso, myo-inositol; Pi, orthophosphate; PPC, phloem parenchymal cell; Raf, raffinose; RS, raffinose synthetase; S6P, sucrose-6-phosphate; SE, sieve element; SPP, sucrose-phosphatase; SPS, sucrose phosphate synthases; Sta, stachyose; STS, stachyose synthase; Suc, sucrose; SUS, sucrose synthase; TP, triose phosphate; TPT, triose phosphate translocator; UDPG, UDP-glucose; UDP-Gal, UDP-galactose; VIN, vacuole invertase.