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RESEARCH ARTICLE

Structure and expression analysis of the sucrose synthase gene family in apple

TONG Xiao-lei[1*], WANG Zheng-yang[1*], MA Bai-quan[1], ZHANG Chun-xia[2], ZHU Ling-cheng[1], MA Feng-wang[1], LI Ming-jun[1]

[1] *State Key Laboratory of Crop Stress Biology for Arid Areas (SKLCSBAA)/College of Horticulture, Northwest A&F University, Yangling 712100, P.R.China*

[2] *College of Forestry, Northwest A&F University, Yangling 712100, P.R.China*

Abstract

Sucrose synthases (SUS) are a family of enzymes that play pivotal roles in carbon partitioning, sink strength and plant development. A total of 11 SUS genes have been identified in the genome of *Malus domestica* (*MdSUSs*), and phylogenetic analysis revealed that the MdSUS genes were divided into three groups, named as SUS I, SUS II and SUS III, respectively. The SUS I and SUS III groups included four homologs each, whereas the SUS II group contained three homologs. SUS genes in the same group showed similar structural characteristics, such as exon number, size and length distribution. After assessing four different tissues, *MdSUS1s* and *MdSUS2.1* showed the highest expression in fruit, whereas *MdSUS2.2/2.3* and *MdSUS3s* exhibit the highest expression in shoot tips. Most *MdSUSs* showed decreased expression during fruit development, similar to SUS enzyme activity, but both *MdSUS2.1* and *MdSUS1.4* displayed opposite expression profiles. These results suggest that different *MdSUS* genes might play distinct roles in the sink-source sugar cycle and sugar utilization in apple sink tissues.

Keywords: apple, sucrose synthase, phylogenetic analysis, gene expression, enzyme activity

1. Introduction

Sucrose is a major photosynthetic product in leaves, which is transported to the sink organs in most plant species (Lutfiyya *et al*. 2007; Chen *et al*. 2012). In sink tissues, two key enzymes, invertase and sucrose synthase, are required to participate in the sucrose cleavage reaction (Geigenberger *et al*. 1993; Kleczkowski *et al*. 2010). Invertase catalyzes the irreversible hydrolysis of sucrose to fructose and glucose (Hirose *et al*. 2008). Sucrose synthase (SUS) is a cytosolic and reversible enzyme that catalyzes sucrose degradation and supplies ADP- and UDP-glucose for starch and cell wall polysaccharide synthesis (Yagi *et al*. 2003). Both invertase and sucrose synthase enzymes play crucial roles in plant growth, development, stress responses, and carbon partitioning in sink tissues (Sturm and Tang 1999; Koch 2004). SUS is also important for cellulose synthesis, and SUS suppression might impair cell wall integrity by reducing the UDP-glucose supply, which is the essential substrate for the synthesis of cellulose and non-cellulose cell wall compounds (Albrecht and Mustroph 2003; Fujii *et al*.

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TONG Xiao-lei, E-mail: xiaoleitong322@163.com; WANG Zheng-yang, E-mail: 691319339@qq.com; Correspondence LI Ming-jun, Tel/Fax: +86-29-87082613, E-mail: limingjun@nwafu.edu.cn
[*] These authors contributed equally to this study.

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2010). High SUS activity was closely associated with sink strength during the sink-source transition, and SUS activity determines sink strength in potato tubers (Zrenner *et al*. 1995; Strum and Tang 1999). Furthermore, SUS regulates several developmental processes, such as flowering induction (Ohto *et al*. 2001), seed development (Iraqi *et al*. 2001), cell division (Gaudin *et al*. 2000), and storage product accumulation (Rook *et al*. 2001).

The gene encoding SUS was isolated primarily from starch-storing and sucrose- or hexose-storing plants (Sturm and Tang 1999). SUS belongs to a small multi-gene family in both monocot and dicot species that contains four subunits with a molecular weight of approximately 83–100 kD. Based on the phylogenetic relationship and structural analyses, SUS genes were divided into three major groups: SUS I, SUS II, and SUS III (Zhang *et al*. 2011, 2013). Variation in the number of SUS family members has been discovered in different plant species; such as, *Arabidopsis*, *Lotus japonicus*, *Amygdalus persica* and *Hevea brasiliensis* tree all encode six distinct active SUS genes (Baud *et al*. 2004; Horst *et al*. 2007; Xiao 2014; Zhang *et al*. 2015), while the poplar genome encodes seven SUS genes (An *et al*. 2014).

Apple is an economically important fruit crop worldwide. As a predominant photosynthesis product (70–80% of the total photosynthetic product in leaves), sorbitol is transported to the sink organs in apple (Yamaki *et al*. 1992), although sorbitol only represents 3–8% of the total soluble sugar in mature fruit (Yamaki *et al*. 1986). In the sink, sorbitol is predominantly converted to fructose before entering central metabolism. However, the role of sucrose metabolic enzymes in the regulation of sink strength in apple remains unclear. In this study, we report the isolation and characterization of SUS family members in apple based on the Genome Database for Rosaceae (GDR, http:/www.rosaceae.org) and The *Arabidopsis* Information Resource (TAIR, http://www.arabidopsis.org/). Additionally, the expression level of *MdSUS* genes was detected in different apple tissues and transgenic lines in which sorbitol concentration was significantly decreased at the same time sucrose concentration was elevated. Our results would provide basic information for further functional analysis of the MdSUS genes and help elucidate whether the two metabolic systems (one for sucrose and one for sorbitol) are coordinated in response to sorbitol and sucrose utilization in sink tissues.

2. Materials and methods

2.1. Plant materials

The 9-year-old Gala apple tree was used in this study at the Horticultural Experimental Station of Northwest A&F University, Yangling, China. Young shoot tips and mature leaves were collected and used for RNA extraction. The samples were immediately frozen in liquid nitrogen and stored at –75°C. The fruits were randomly collected at 16, 34, 55, 75, 98, and 122 days after blooming (DAB), respectively, and each developmental stage had three replicates of 10 fruits. Fruit samples were mechanically peeled and cored. Pulps were cut into small sections, immediately frozen in liquid nitrogen, and stored at –75°C for RNA extraction. All the materials used in the study were the same as those in Wei *et al*. (2014).

2.2. Characterization and phylogenetic analysis of SUS genes in the apple genome

The amino acid sequences of SUS genes in *Arabidopsis* were used as a query sequence to identify homologous genes of the apple reference genome sequence (Velasco *et al*. 2010) using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), which uncovered 11 apple transcript IDs from the SUS family collected after removing transcripts with $E>e^{-10}$. Amino acid sequences were aligned using CLUSTAL X (http://www.clustal.org/), and molecular evolutionary genetics analysis (MEGA) ver. 6.06 (http://www.megasoftware.net/) was used to construct a phylogenetic tree based on the neighbor joining (NJ) method, the parameters are as follows: the model is JTT+G, the missing is set to 'complete deletion' and the check parameter Bootstrap is 1 000. The specific conserved domains in the SUS family were searched on InterProScan (http://www.ebi.ac.uk/Tools/InterProScan/), and the conserved domains were predicted by Pfam (http://pfam.xfam.org/search/sequence). The number of amino acids, molecular weight, predicted theoretical isoelectric point (pI), aliphatic index, and grand average of hydropathicity (GRAVY) were calculated using ProtParam tool (http://www.expasy.org/tools/protparam.html). Multiple EM for Motif Elictiation (MEME, http://meme.nbcr.net/meme/cgi-bin/meme.cgi) was used to identify conserved motifs shared by the MdSUS proteins. The program Clustal W2 was used to calculate the similarity/identity of amino acid/nucleotide sequences.

2.3. Quantitative real-time RT-PCR (qRT-PCR)

Total RNA was isolated using the cetyltrimethyl ammonium bromide (CTAB) method (Chang *et al*. 1993). RNA samples were treated with DNase I (Invitrogen, USA) to remove genomic DNA contamination. The quality of isolated total RNA was assessed on a 1.5% agarose gel and then adjusted to 200 ng μL^{-1} using a NanoDrop Lite spectrophotometer (ND2000, USA). One mg of total RNA per sample was used for cDNA synthesis using SYBR® PrimeScript™ RT-PCR Kit II