

Citrus sucrose transporter genes: genome-wide identification and transcript analysis in ripening and ABA-injected fruits

Mohammad Zahidul Islam^{1,2,3} · Long-Fei Jin^{1,2} · Cai-Yun Shi^{1,2} · Yong-Zhong Liu^{1,2} · Shu-Ang Peng^{1,2}

Received: 27 April 2015 / Revised: 5 August 2015 / Accepted: 28 August 2015 / Published online: 11 September 2015
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Abstract The sucrose transporter (SUT) plays a major role in the transport of sucrose apoplastically. An extensive mining of the data confirmed that at least three SUT genes (*CitSUT1*, *CitSUT2*, and *CitSUT3*) were found in the databases of the three currently available citrus genomes. The exon and intron sizes varied among the three *CitSUT* genes, and the similarities among the genes were 40–50 %. The phylogenetic analysis revealed that the *CitSUT1*, *CitSUT2*, and *CitSUT3* were distributed into groups SUT I, SUT II, and SUT III, respectively. The analysis of the spatiotemporal expression of the genes showed that the *CitSUT1*, *CitSUT2*, and *CitSUT3* were predominantly expressed in the stamens, young leaves, and fruits [juice sacs (JS) and/or segment membrane (SM)], respectively. Moreover, in addition to the significant increase in sucrose levels from 114 days after anthesis (DAA) to 160 DAA, the transcript levels of *CitSUT1* and *CitSUT3* increased significantly in the JS and decreased significantly in

the SM, whereas the *CitSUT2* transcript levels decreased significantly either in the JS or in the SM. Additionally, treatment with ABA increased the accumulations of sucrose and fructose in fruits, which were accompanied by the induction of the *CitSUT2* transcripts in fruits (JS and SM) and the *CitSUT3* transcripts in the SM and the reduction of the *CitSUT1* and *CitSUT3* transcript levels in the JS. Thus, the *CitSUT1* and *CitSUT3* might play important roles in the transport of sucrose into the fruit JS during normal fruit development; the transcript alterations of the *CitSUT2* and *CitSUT3* under the ABA treatment might contribute to the increased accumulation of sucrose.

Keywords Citrus · Exogenous ABA · Sucrose accumulation · Sucrose transporter

Communicated by C. Chen

This article is part of the Topical Collection on *Gene Expression*

Electronic supplementary material The online version of this article (doi:10.1007/s11295-015-0918-2) contains supplementary material, which is available to authorized users.

✉ Yong-Zhong Liu
liuyongzhong@mail.hzau.edu.cn

¹ Key Laboratory of Horticultural Plant Biology (Huazhong Agricultural University), Ministry of Education, Wuhan 430070, People's Republic of China

² Key Laboratory of Horticultural Crop Biology and Genetic Improvement (Central Region), Ministry of Education, Wuhan 430070, People's Republic of China

³ Present address: Bangladesh Water Development Board, Motijheel Dhaka, Bangladesh

Introduction

Citrus are woody perennial trees that are grown globally to provide important and different nutrition and antioxidants for human health. The fleshy fruits are strong, terminal reproductive sinks that are perfectly suited for the storage of sucrose (Koch and Avigne 1990). Sucrose, an important soluble disaccharide, is the principal form of transported sugar in some higher plants, including citrus (Lowell et al. 1989). Sucrose is loaded into the phloem symplastically and apoplastically (Koch and Avigne 1990). In symplastic transport, the sucrose movement from cell to cell requires the plasmodesmatal connections, whereas the apoplastic transport primarily occurs without the plasmodesmatal connections but with the facilitation of sucrose transporters (SUTs) (Lalonde et al. 1999; Rennie and Turgeon 2009).

The SUTs function in the loading of sucrose into the phloem (sieve element/companion cell) and into the sink tissues

such as seeds, flowers, and fruits (Reinders et al. 2012). Moreover, the SUTs also play indispensable roles in maintaining fruit size and pollen germination (Hackel et al. 2006; Sivitz et al. 2008) as well as the dual functions of sugar transport and sugar sensing (Lalonde et al. 1999). Additionally, Jung et al. (2015) reported that these SUTs located in the phloem parenchyma cells were critical for sucrose loading into the companion cell/sieve element complex (phloem) in *Arabidopsis*. Williams et al. (2000) also found that the sucrose concentration increased up to 1 mol L^{-1} with the cotransformation of SUT1/SUC2. Lalonde et al. (1999) demonstrated experimentally that five SUT genes showed activities on the translocation path from the source to the sink. Moreover, the plants were stunted dramatically and the carbohydrates accumulated primarily in the sources instead of the sinks when the activities of the SUTs were reduced in the phloem loading region by mutation or transcript reduction (Gottwald et al. 2000; Kühn et al. 1996; Slewinski et al. 2009).

The research in the recent decade showed that the gene encoding the SUT isoforms is in a small multigene family (Kühn 2003). For example, nine SUT genes are in the model plant *Arabidopsis thaliana* (Büttner 2007), and six SUT genes are in *Theobroma cacao* (Li et al. 2014), *Sorghum bicolor* (Milne et al. 2013), and *Zea mays* (Slewinski et al. 2009). Moreover, three SUT genes are found in potato (Riesmeier et al. 1993) and in tomato (Weise et al. 2000). The complete genome sequences and assemblies of the clementine mandarin and the sweet orange [www.phytozome.net and <http://citrus.hzau.edu.cn/orange/> (Xu et al. 2013)] are now available for comprehensive studies on the numerous known or novel small multigene families. In the previous decade, the two genes that encode SUT (*CitSUT1* and *CitSUT2*) were isolated from citrus using PCR cloning with degenerate primers (Li et al. 2003). These transporter genes are essential for sucrose loading and unloading into sinks, typically fleshy fruits during ripening, such as in citrus (Koch and Avigne 1990), tomato (Ruan and Patrick 1995), and grape (Zhang et al. 2006). Although Zheng et al. (2014) suggested that there possibly are three SUT genes in sweet orange, however, an extensive study of the SUT genes and an examination of the possible roles for the genes in the apoplastic loading of sucrose remain scarce in citrus.

In the present study, we identified the SUT genes from the currently available citrus genome databases and investigated the possible roles of these genes in sucrose transport in citrus ripening fruits. We also investigated their possible roles in ABA-treated fruits since previous researches suggested that ABA plays a central role in carbohydrate accumulation (Kempa et al. 2008) or influence sorbitol uptake in growing apple fruits (Berüter 1983). The results will provide fundamental knowledge for further investigations on the specific role of each member of the SUT gene group that was involved in the apoplastic transport of sucrose in citrus.

Materials and methods

Plant materials

The fruits of the Satsuma mandarin (*Citrus unshiu* Mark cv. Guoqing No. 1) grafted onto *Poncirus trifoliata* in the citrus orchards of the Huazhong Agricultural University, Hubei Province, China, were used in the present study. The fruits were sampled at two stages of growth: the period of rapid growth [114 days after anthesis (DAA)] and the period of ripening (160 DAA). At each sample time, three to four healthy and uniform fruits were randomly collected from the outer crown of the tree. The fruit juice sacs (JS) and the segment membrane (SM) from each fruit were separated in a pot on ice while wearing gloves. The isolated samples were mixed, ground in liquid nitrogen, and stored at $-80 \text{ }^{\circ}\text{C}$ for further use.

Additionally, young leaves (YL, the average length=2 cm) at full anthesis and mature leaves (ML, the average length=10 cm) at 44 DAA were collected independently and randomly from the *Guoqing No. 1* trees. The fully opened flowers at full anthesis were collected, and the ovary and the stamens were separated in a pot on ice with the help of forceps; the samples were treated with liquid nitrogen immediately and were stored at $-80 \text{ }^{\circ}\text{C}$ for the tissue-specific expression analyses.

ABA treatment

For the ABA treatment, more than ten pairs of Guoqing No. 1 fruits were selected. Each pair of fruits was grown on the identical fruit-bearing shoot, and the fruits were similar in size. The ABA injection was performed according to Liu et al. (2014). In each pair of fruits, one fruit was injected with an ABA solution, and one fruit, the control, was injected only with the solvent (without the ABA). Beginning 142 DAF, the ABA was injected three times into each fruit at 3-day intervals for a total of $0.2 \text{ } \mu\text{mol}$ ABA and 0.1 mL of $0.2 \text{ } \%$ (v/v) emulsifying agent (phosphoglycerate mutase/Tween 20=16:1 w/w). At 3 days after the final injection, the injected and control fruits were harvested. The JS and the SM were separated immediately, frozen in liquid nitrogen, and stored at $-80 \text{ }^{\circ}\text{C}$ until later use.

Soluble sugar assay

The soluble sugars, which included sucrose, glucose, and fructose, were analyzed by gas-liquid chromatography (Bartolozzi et al. 1997).

Sequence data mining and molecular cloning

As reported by Li et al. (2003), the amino acid sequences of the known *CitSUT1* (AY09889) or *CitSUT2* (AY098894)

were used as a query to identify the SUT family members in the three currently available citrus genome databases [the sweet orange genome database was from the Huazhong Agricultural University (HZAU), China (<http://citrus.hzau.edu.cn/orange/>) (Xu et al. 2013), and the sweet orange and clementine genome databases were from the Phytozome (www.phytozome.net)]. The filter criteria were as follows: the *E*-value was 0 or near 0, the sequence was complete, and the sequence annotation was the name of the target gene. To confirm the authenticity or for the full-length cloning of the unidentified gene (ID Cs3g16640.1), gene-specific primers (Table 1) were designed using Primer 3.0 (Koressaar and Remm 2007) based on the respective genomic sequences for the PCR amplification. The PCR amplifying conditions were 94 °C for 2 min, which was followed by 30 cycles at 94 °C for 45 s, at 60 °C for 1 min, and at 72 °C for 1 min, and then a final 10-min extension at 72 °C. The PCR was conducted with the first-strand complementary DNA (cDNA) of young leaves. The amplicons were cloned into pEASY-T1 cloning vectors (Beijing TransGene Biotech Co., China), which were transformed into *Escherichia coli* competent cells (DH5a) for sequencing. The gene structure was analyzed by the Gene Structure Display Server (GSDS, gsds.cbi.pku.edu.cn) (Guo et al. 2007).

Phylogenetic tree

The phylogenetic tree was constructed using the neighbor-joining algorithm of the MEGA 4.0 software (Tamura et al. 2011). The bootstrap analysis was performed using 1000 replicates. The gene or protein accession numbers for the citrus SUT genes and the other known SUT genes used in this study are listed in Table S2.

Quantitative real-time PCR analysis

The total RNA was isolated according to the method described previously (Liu et al. 2006). One microgram of high-quality total RNA was used for the first-strand cDNA synthesis using

a PrimeScript RT Reagent kit with genomic DNA (gDNA) Eraser (TaKaRa, DALIAN, China). The specific primers for the citrus SUT genes and the actin gene were designed using Primer 3.0 (Koressaar and Remm 2007) and are listed in Table 1. The qRT-PCR was conducted on three biological replicates and was performed in a 10- μ L reaction volume using the Thunderbird TM SYBR qPCR Mix (TOYOBO, Japan) on the LightCycler 480 Real Time System (Roche, Switzerland), following the manufacturer's protocols. The reactions began with an initial incubation at 50 °C for 2 min and then at 95 °C for 10 min, which was followed by 45 cycles at 95 °C for 15 s and at 60 °C for 60 s. The Livak method (Livak and Schmittgen 2001) was used to calculate the relative expression levels.

Statistical analyses

The data were evaluated with Duncan's multiple range tests or Student *t* tests in the ANOVA program of the SAS statistical software package (SAS Institute, Cary, NC, USA). The differences were considered significant at $P < 0.05$.

Results

Data mining and molecular characterization of SUT genes

An extensive search using the known sequences of *CitSUT1* (AY09889) or *CitSUT2* (AY098894) was performed in the three citrus genome databases. The queries with either *CitSUT1* or *CitSUT2* produced the identical results and showed at least three homologs in the HZAU orange database, two homologs in the Phytozome orange database, and three homologs in the Phytozome clementine database (Fig. S1). Based on the nucleotide sequences, we divided the homologs into three groups, CitSUT1, CitSUT2, and CitSUT3 (Fig. S1). Moreover, a mutual comparison showed that the sequences had high identity among the members in each group (Table S1). The sequences of the Cs3g22560.1 and the

Table 1 Basic information for the citrus putative SUT genes and the specific primers used in this study

Gene name	Sequence ID	ORF amino acid length	Mol. wt (kDa)	<i>pI</i>	Primer name	Sequence (5'–3')		Amplicon size (bp)
						Forward primer	Reverse primer	
CitSUT1	Cs3g22560.1	527	55.93	8.91	Z1	GGTGTGAAAGTTGGCGCTTT	TTGCAGCCATGCACCACATT	496
					Z2	CTTTATGGTCGGCGCAGTTG	TACACACACAAGCCTTGCAG	288
CitSUT2	Cs7g04100.1	606	64.92	7.58	Z3	TGGCAACCACGGCTATCATT	CCCACAGACGGGGAGAAAAA	656
					Z4	TGTATCACTTGGTGC GGTC	ACCATTGATTTCCGCAGGGT	322
CitSUT3	Cs3g16640.1	505	54.51	8.81	Z5	AATCAGGCAACAACTACTC	CTTTGAGCCTATGACTTGAT	1916
					Z6	GTCATAGGCTCAAAGGGACTCT	CCACCAAGCACAAATTCACCG	391
<i>Actin</i>	XM_006464503	–	–	–	Actin	CCGACCGTATGAGCAAGGAAA	TTCTGTGGACAATGGATGGA	200

Cs7g04100.1 from the HZAU genome database were very similar to the known citrus SUT genes, *CitSUT1* and *CitSUT2*, respectively. However, the sequence of the Cs3g16640.1 was a new sequence and was named *CitSUT3*. The PCR amplification confirmed the authenticity (Fig. S2). Moreover, the genome sequence of the Cs3g16640 had four putative transcripts (Cs3g16640.1 to Cs3g16640.4). We used primer Z5 (Table 1), which was designed based on the conserved region at both sides of the opening reading frame (ORF) of these putative transcripts, for PCR amplification. The sequencing results revealed that the Cs3g16640.1 was found in the citrus fruit. The ORF of the Cs3g16640.1 was 1518 bp in length, and the putative protein that was encoded had the predicted molecular mass and pI of approximately 54.51 and 8.81, respectively, whereas the predicted molecular mass and pI of the *CitSUT1* and *CitSUT2* were approximately 55.93 and 8.91 and 64.92 and 7.58, respectively (Table 1).

The full-length cDNA and gDNA sequences of the three citrus SUT genes were downloaded from the *Citrus sinensis* genome database (<http://citrus.hzau.edu.cn/orange/>), and the exon and intron structures (from start to stop codon) were analyzed with GSDS (gsds.cbi.pku.edu.cn) (Guo et al. 2007). The numbers and the lengths of the exons and introns were different among these three *CitSUT* genes (Fig. 1). Specifically, the *CitSUT1* contained four exons and three introns, the *CitSUT2* contained 14 exons and 13 introns, and the *CitSUT3* contained five exons and four introns. Moreover, only two exons were conserved in length among the *CitSUT* genes (Fig. 1). Additionally, the similarities among the *CitSUT1*, *CitSUT2*, and *CitSUT3* were less than 50 %, with the similarity of 49.6 % between *CitSUT1* and *CitSUT2*, 49.0 % between *CitSUT1* and *CitSUT3*, and 41.8 % between *CitSUT2* and *CitSUT3*.

To compare the relations between the *CitSUT* genes and the other plant SUT homologs, a phylogenetic tree was constructed with protein sequences from the SUTs of the different plant species (Fig. 2). These SUT homologs were clustered into three main groups: SUT I, SUT II, and SUT III. The three citrus SUT genes were evenly distributed into the three plant SUT groups; the *CitSUT1* and *CitSUT2* were in the SUT I and

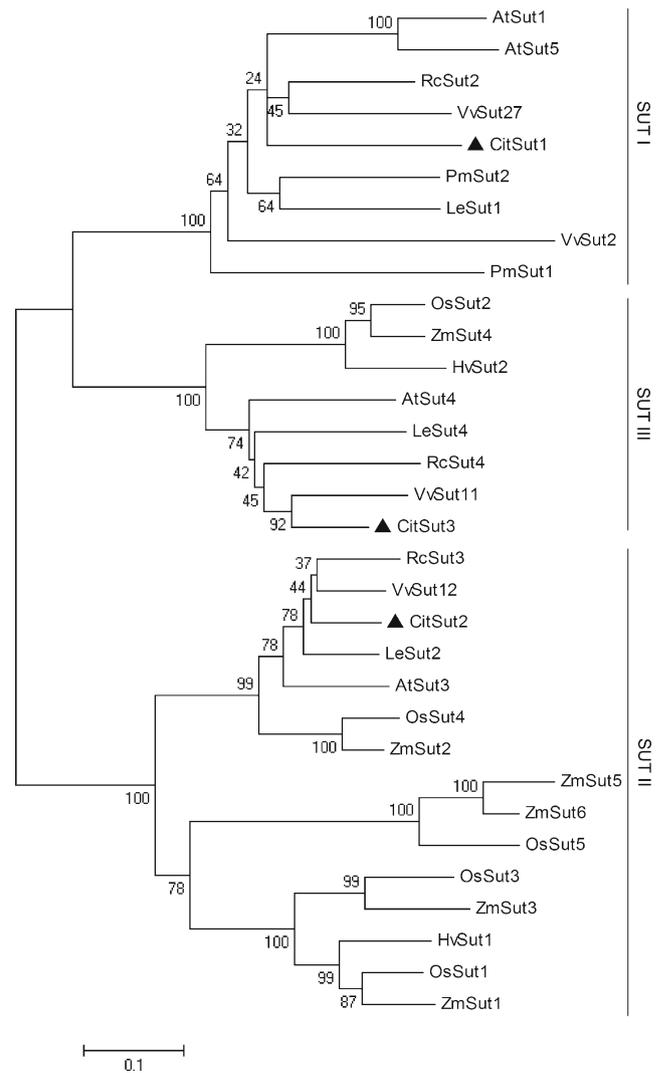


Fig. 2 Phylogenetic analysis of the *CitSUT* proteins and the other plant SUT homologs. The consensus tree was constructed by the neighbor-joining method using the MEGA 4.0 program. The SUT accession numbers are listed in Table S2. The black triangles show the positions of the three *CitSUT* isoforms

the SUT II groups, respectively, whereas the newly identified *CitSUT3* was in the SUT III group (Fig. 2).

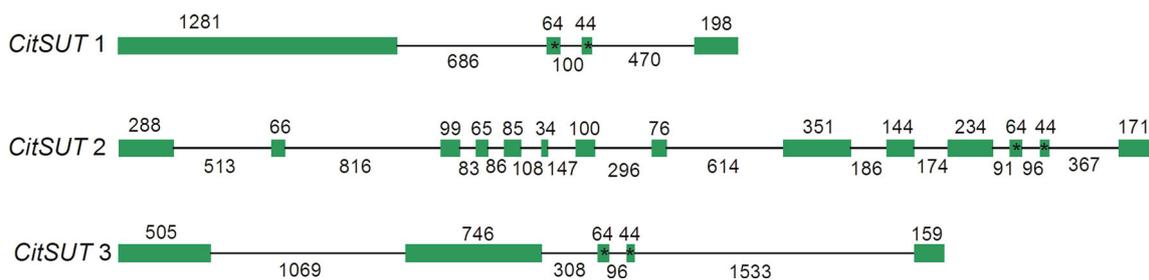


Fig. 1 Schematic representation of the structure of the three sucrose transporter genes in citrus. Boxes indicate exons, and solid lines between the boxes indicate introns. The numbers on boxes and under

lines indicate the lengths of exons and introns, respectively. Asterisks in the boxes indicate that the three *CitSUT* genes contained exons of the same size

Expression analysis of *CitSUT* genes in different tissues and sugar accumulation during fruit ripening

The three *CitSUT* genes were first examined in the different tissues, which included the JS, SM, YL, ML, ovary, and stamens (Fig. 3A-C). The *CitSUT1* transcript level was predominant in the stamens and was more than 8-, 9-, 82-, 25-, and 1.6-fold higher than that in the JS, SM, YL, ML, and ovary, respectively. The *CitSUT2* transcript level was predominant in

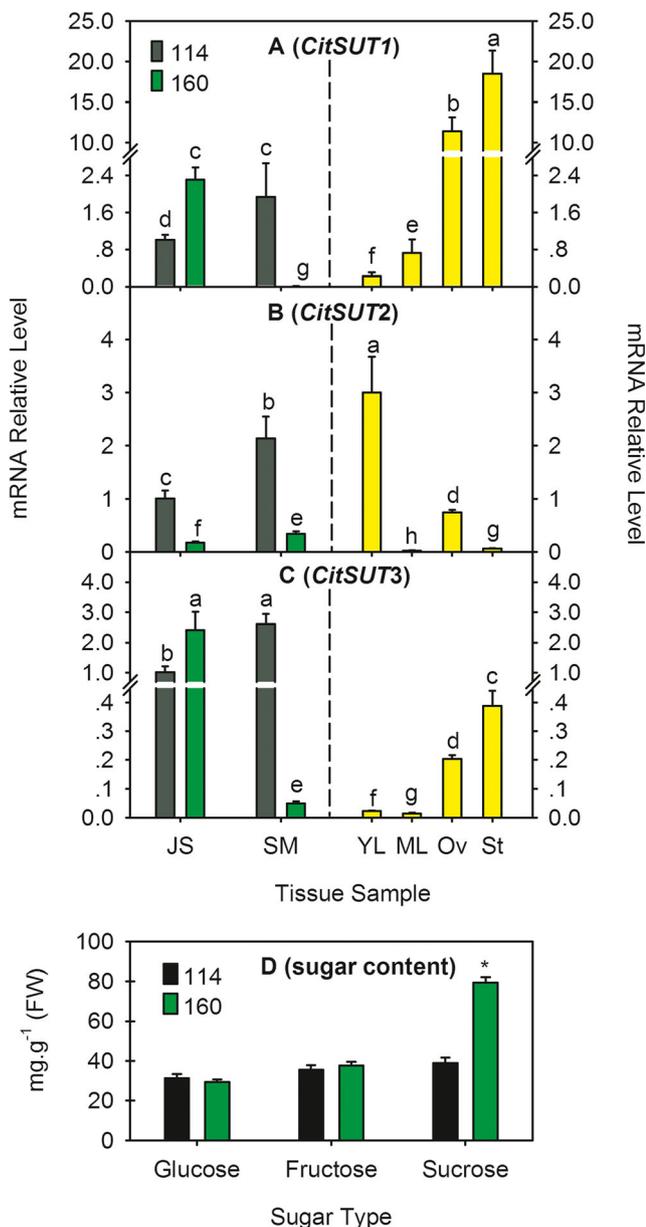


Fig. 3 a-d Analyses of the relative transcript levels of *CitSUT1-3* genes in different tissues or organs and the sugar content in juice sacs at 114 and 160 days after anthesis. The abbreviations YL, ML, Ov, St, JS, and SM are identified in “Materials and methods” section. All qRT-PCR values are the mean±SE of three replicates. The different lowercase letters on the error bar for each gene indicate a significant difference at $P<0.05$ by Duncan’s tests

the YL and was more than 3-, 1.4-, 100-, 4-, and 40-fold higher than that in the JS, SM, ML, ovary, and stamens, respectively. The expression was different for the *CitSUT3* transcripts, which was predominant in the JS and in the SM 114 DAA and was approximately 44-, 75-, 4.9-, and 2.5-fold higher than that in the YL, ML, ovary, and stamens, respectively. Moreover, the transcript profiles of the *CitSUTs* were detected in the JS and the SM as the fruit developed and ripened. In addition to the significant increase in sucrose concentration from 114 to 160 DAA (Fig. 3D), the *CitSUT1* transcript level increased significantly in the JS and decreased significantly in the SM (Fig. 3A), the *CitSUT2* transcript level decreased significantly either in the JS or in the SM (Fig. 3B), and the *CitSUT3* transcript profile was similar to the profile of the *CitSUT1* and increased significantly in the JS and decreased significantly in the SM (Fig. 3C).

Effects of ABA on sugar accumulation and expression of *CitSUTs* in citrus fruits

After the ABA injection, the sucrose and fructose contents were significantly higher in the ABA-treated fruits than those in the control fruits (Fig. 4a). However, the response of the expression of the *CitSUTs* to the ABA injection was different. Specifically, the *CitSUT1* transcript levels decreased significantly in the ABA-treated JS and SM compared with the respective control tissues (Fig. 4b), whereas the mRNA level of *CitSUT2* increased significantly in both the JS and the SM of

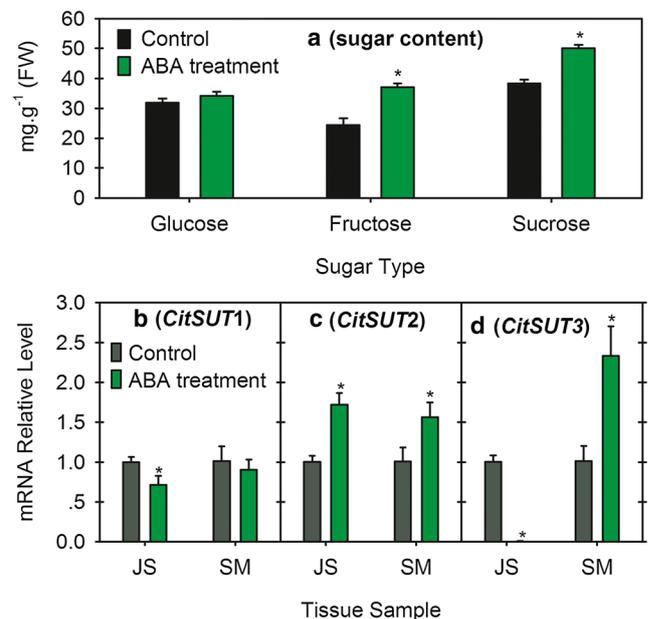


Fig. 4 a-d Analyses of the soluble sugar content in fruit juice sacs (JS) and the *CitSUT1-3* gene expression levels in the JS and fruit segment membrane (SM) in response to ABA treatment. Asterisks on the bars indicate significant differences ($P<0.05$) between fruits treated with the ABA and those of the control by *t* tests (LSD)

ABA-treated fruits and was approximately 1.8-fold higher than that in the control fruits (Fig. 4c). For the *CitSUT3*, the transcript level decreased markedly in the JS of the ABA-treated fruits compared with the control fruits for which the transcript level was undetectable, and in the SM, the transcript level increased significantly with ABA application and was 2.4-fold higher in the ABA-treated fruits than in the control fruits (Fig. 4d).

Discussion

The plant SUT encoded genes are members of a small multigene family (Büttner 2007; Li et al. 2014; Milne et al. 2013; Slewinski et al. 2009). To date, although the genomes of many citrus cultivars have been sequenced (Wu et al. 2014), only three citrus genome databases were released publicly (<http://citrus.hzau.edu.cn/orange/> and www.phytozome.net/). In this study, we found that at least three SUT genes were in the citrus genome following an extensive search of the sweet orange and the clementine genomic databases (Fig. S1 and Table 1), which were also confirmed with PCR amplification (Fig. S2). Moreover, the three SUT genes were also recently reported by Zheng et al. (2014). Among these three citrus SUT genes, the sequence IDs Cs3g22560.1 and Cs7g04100.1 had 99 and 98 % similarity to the previously identified *CitSUT1* and *CitSUT2* genes (Li et al. 2003), respectively. The new sequence (ID Cs3g16640.1) had an ORF that consisted of 505 amino acid residues of which the predicted molecular mass and pI were approximately 54.51 and 8.81, respectively (Table 1). Overall, at least three CitSUT genes were identified in the currently available citrus genomes, which was similar to potato (Riesmeier et al. 1993), tomato (Weise et al. 2000), and grape (Davies et al. 1999).

Reportedly, the SUT genes of dicot plants possessed different numbers of exons and introns and lengths of the ORF when compared with one another (Aoki et al. 2003; Davies et al. 1999). In this study, we also found that the number of exons and introns and the lengths of the ORF of these three putative CitSUT genes were completely different from one another (Fig. 1). The similarity among the *CitSUT1*, *CitSUT2*, and *CitSUT3* genes was less than 50 %. Moreover, the phylogenetic analysis revealed that the putative three CitSUT gene members were evenly divided into the three groups: SUT I, SUT II, and SUT III (Fig. 2), which was similar to the SUT isomers of other plant species, such as pear (Zhang et al. 2013), rice (Aoki et al. 2003), and grape (Davies et al. 1999).

The photosynthetic sucrose is synthesized in source leaves (mature leaves) and is transported to the sink tissues such as the early or young leaves, flowers (pollen/ovary), and fruits. Li et al. (2003) were the first to isolate two citrus SUTs (*CitSUT1* and *CitSUT2*) and to detect the expression only in

the mature and young leaves and then found that the *CitSUT1* was expressed strongly in mature leaves, which are sugar-exporting organs, and the *CitSUT2* was expressed strongly in young leaves, which are sugar-importing organs. In this study, additional organs or tissues were examined for the analyses of tissue- or organ-specific expressions. The *CitSUT1* was highly expressed in the stamens (Fig. 3A), the *CitSUT2* gene was predominantly expressed in the YL (Fig. 3B), and the *CitSUT3* was highly expressed in fruits (Fig. 3C). For the two tissues of ML and YL, the *CitSUT1* and *CitSUT2* expression profiles were consistent with the results of previous research (Li et al. 2003). As a result of more tissues examined in the present study, the *CitSUT1* also showed a predominant expression in sink organs such as in the flowers (ovary and stamens) (Fig. 3A).

The expressional divergence of the genes indicated the functional diversity. In the present study, the spatiotemporal patterns of expression of the three citrus putative SUT genes were different (Fig. 3A-C), which indicated that the SUT genes in citrus were functionally diverse. In the ovary from which the hesperidium berry develops, all the CitSUT genes were detected with relatively strong expressions (Fig. 3A-C), which were similar to the expression patterns of the *VvSuc11* and *VvSuc12* in grapes (Davies et al. 1999), suggesting that the three *CitSUTs* might cooperate in the transport of sucrose in the initiation of the development of young fruit. During the development and ripening of fruit, the accumulation of sucrose was obvious in the fruit JS (Fig. 3D), as were the increases in the transcript levels of *CitSUT1* (Fig. 3A) and *CitSUT3* (Fig. 3C) and the decrease in transcript level of *CitSUT2* (Fig. 3B), which suggested that the functional roles of the three *CitSUTs* differentiated as the fruit developed and ripened. However, Zheng et al. (2014) analyzed the three SUTs' gene expression profiles in the pulp of a sweet orange from 120 to 210 DAA and found that the transcript level of *CsSUT1* was decreased along with fruit ripening. Additionally, no symplastic connections with the surrounding tissues of the male gametophyte (pollen/anther) were found, which indicated that the pollen grain and tube organs developed as symplastically isolated organs (Lemoine et al. 1999), thus the requirement for a transporter for nutrient uptake into the pollen. In this study, we found that *CitSUT1* and *CitSUT3* (Fig. 3A, C), but not *CitSUT2* (Fig. 3B), were expressed strongly in the stamens, which indicated that *CitSUT1* and *CitSUT3* played important roles in the transport of sucrose into the stamens apoplastically. A similar observation was reported for tobacco in which a cDNA that encoded a SUT gene was highly expressed in the pollen (Lemoine et al. 1999).

The three critical periods for the development and ripening of citrus fruits are cell division, fast cell expansion, and fruit maturation (Goldschmidt and Monselise 1977). From the initiation of the maturation of fruits, sucrose synthase plays a major role in maintaining the sink strength for sucrose import

into sink cells (Islam et al. 2014; Jiang et al. 2014). In the present study, the contents of glucose and fructose were relatively constant; however, the sucrose content increased sharply as the fruit ripened (Fig. 3D). This phenomenon suggested that there would be a drastic demand for the import of sucrose into the fruit flesh during the development and ripening of the fruit. The sucrose synthesized in the source leaves would move out from the mesophyll cells and into the phloem, moving first through a symplastic cytoplasmic path caused by the concentration gradient between the source and sink; after balancing the concentration gradient, the sucrose must cross a membrane before entering into the cell via an apoplastic pathway, which required the presence of a plasma membrane SUT (Turgeon 2006). Moreover, the sucrose is moved from the cytoplasm into the vacuole by the tonoplast or the vacuole membrane transporter (Davies et al. 1999). Additionally, the flesh of citrus fruits is a strong sink tissue that is heterogeneous and is separated into two tissues: the phloem-free JS and the SM with vascular bundles (Lowell et al. 1989). The SM is composed of vascular bundles and is the only site for the entry of assimilate into the JS because the JS are symplastically isolated; therefore, the sucrose entry into the JS cells occurs primarily through the apoplastic pathway during the fruit developmental stages (Lowell et al. 1989). In this study, the transcript level of *CitSUT1–3* was strongly expressed in the SM at 114 DAA and then decreased to very low levels at 160 DAA (Fig. 3A–C); however, the transcript levels of *CitSUT1* and *CitSUT3* (Fig. 3A, C) increased significantly in the fruit JS from 114 to 160 DAA. Although a previous study suggested that sucrose synthase played an important role in sucrose entry into the citrus fruit JS during fruit development and ripening (Jiang et al. 2014), the results of this study suggested that *CitSUT1* and *CitSUT3* might also play important roles in the entry of sucrose into the JS. Moreover, phylogenetic analysis showed that the *CitSUT3* gene was in the same clade as the *Arabidopsis* homologue *AtSUT4* (Fig. 2), which was reported to be located either in the plasma membrane or in the tonoplast/vacuolar membrane (Schneider et al. 2012). Hence, the *CitSUT3* was also possibly located in the plasma membrane or the tonoplast membrane, thereby indirectly supporting the possibility that the *CitSUT3* was responsible for the sucrose transport between the vacuolar lumen and the cytoplasm.

To date, treatment with ABA has been implicated in the increase in sugar accumulation in fleshy fruits (Berüter 1983; Jia et al. 2011; Kempa et al. 2008). In this study, we also found that the concentrations of sucrose and fructose increased significantly with ABA treatment (Fig. 4a). Moreover, with ABA treatment, the transcript levels of the *CitSUT1* decreased in both the JS and the SM (Fig. 4b), the transcript levels of *CitSUT2* increased significantly in both the JS and the SM (Fig. 4c), and the expression of the transcript levels of *CitSUT3* decreased significantly in the JS and increased

significantly in the SM (Fig. 4d). Therefore, the consistency between the *CitSUT2* transcript profiles and the sucrose content indicated that the induced expression of *CitSUT2* might contribute to the increase in sugar accumulation with the ABA treatment. The SM is the only site for sucrose to be downloaded from the phloem and for sucrose entry into the JS (Lowell et al. 1989). With the ABA treatment, the increase in the transcript levels of *CitSUT3* in the SM implied that more sucrose were downloaded from the phloem and entered into the JS. Although the *CitSUT3* transcript level decreased significantly (Fig. 4d), we suggest that the change in the transcript levels of *CitSUT3* remained at least partially related to the increase in sucrose accumulation with the ABA treatment.

Conclusions

This study identified a small SUT multigene family in the citrus genome and showed that at least three SUTs (*CitSUT1*, *CitSUT2*, and *CitSUT3*) are found in the currently available citrus genome databases. The spatiotemporal expressions showed that the *CitSUT1*, *CitSUT2*, and *CitSUT3* were predominantly expressed in the stamens, young leaves, and fruits (JS and/or SM), respectively. Although the divergent patterns of expression suggested that the function of each of the *CitSUT* genes was possibly complex in the transport of sucrose to the different tissues, the *CitSUT1* and *CitSUT3* might play important roles in sucrose transport into the fruit JS during normal fruit development, and the *CitSUT2* might play an important role in the downloading of sucrose into young leaves. However, the *CitSUT2* and *CitSUT3* appeared to contribute to the increased accumulation of sucrose in fruits with the ABA treatment.

Acknowledgments The Fundamental Research Funds for the Central Universities in China (Grant No. 2013PY082) supported this work.

Competing interests The authors declare that they have no competing interests.

Authors' contributions MZI, LFJ, and CYS conducted the experiments, and MZI analyzed the data and drafted the manuscript. YZL and SAP supported the project and designed the experiment, and YZL polished the manuscript. All authors read and approved the manuscript.

Data archiving statement The three citrus SUT genes (*CitSUT1* to 3) were derived from the published genome sequences (<http://citrus.hzau.edu.cn/orange/> and www.phytozome.net), and the accession IDs or numbers of all sequences used in the present study, including the three citrus SUT genes, are listed in Fig. S1 and Table S2.

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