Systematic Analysis of Sugar Unloading And Accumulation Mechanism In Sucrose- And Hexose-Accumulating Tomato Fruits

Lulu Sun
Beijing University of Agriculture

Jianli Wang
Beijing University of Agriculture

Jian Song
Beijing University of Agriculture

Xueni Du
Beijing University of Agriculture

Wenke Liu
Beijing University of Agriculture

Wenchao Zhao
Beijing University of Agriculture

Liu Yang
Beijing University of Agriculture

Changbao Li
Beijing Academy of Agriculture and Forestry Sciences

Yong Qin
Xinjiang Agricultural University

Rui Yang (yangrui@bua.edu.cn)
Beijing University of Agriculture  https://orcid.org/0000-0002-0720-0051

Research Article

Keywords: Sucrose, Hexose, Accumulation, Acid enzyme, Plasmodesmata, Metabolism

Posted Date: July 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-666424/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

Sugar is the key factor in the formation of fruit quality. Hexose is the main form of sugar accumulated in ordinary cultivated tomatoes, while a small number of wild-type tomato varieties can also accumulate sucrose. Although several studies have focused on wild sucrose-accumulating tomatoes, the sugar accumulation mechanism in tomato is still unclear. Here, two cherry tomatoes lines that accumulated sucrose and hexose respectively were selected to analyze the assimilates unloading pathway and sugar accumulation mechanism using CF tracing, cytological observation, proteomics methods, etc. The results indicated that the later stages of fruit development were key stages for sugar accumulation, and sucrose-accumulating (S) cherry tomatoes had higher sucrose contents in the fruits, while hexose-accumulating (H) cherry tomatoes accumulated more glucose, fructose and starch. The unloading pathway of assimilates in the S cherry tomato was switched from apoplastic to symplastic during fruit development, and the opposite was true in the H type. Plasmodesmata transport may be the main means of sucrose accumulation and high activity or expression levels of acid invertase (AI) and SUT1 may be important factors in hexose accumulation in H and S cherry tomatoes, respectively. In addition to sugar metabolism, photosynthesis, fatty acid metabolism and other secondary metabolism pathways also play important roles in sugar accumulation. This study provides detailed evidence for the tomato sugar accumulation mechanism from the perspective of cell structure, physiology and molecular biology, providing a theoretical basis for the improvement of tomato quality and aiding the utilization of tomato genetic resources.

Key Message

Assimilates unloading pathway was switched from apoplastic to symplastic in sucrose-accumulating cherry tomato fruit, and the opposite was true in hexose-accumulating cherry tomato.

Introduction

Tomatoes have high economic value and are widely consumed, and the functional value of these fruits is primarily determined by their contents of sugar, lycopene, vitamin C and polyphenols(Granato et al. 2010), which are beneficial to human health and promote healthy aging by protecting against heart disease, osteoporosis, and likely certain cancers(Oude Griep et al. 2010; Tan et al. 2010). Sugars are the key components that affect tomato quality and customer preferences and are important for various aspects of plant development, such as providing energy and carbon structural components for plant growth and functioning as signaling molecules in many developmental processes(Granot et al. 2013; Lastdrager et al. 2014).

Plant photosynthesis produces sucrose, which is transported long-distance along the phloem to sink organs, such as seeds, roots, and young leaves, and then unloaded from the phloem to the sink cells although the symplastic pathway (via plasmodesmata) or apoplastic pathway (via sugar transporters), providing nutrients for the growth and development of the sink cells or being directly stored as an energy
source (Turgeon and Wolf 2009). The accumulation of sugar in the sink organs mainly depends on the input of sugar and biochemical processes such as sugar synthesis and decomposition.

Tomato cultivars are typically hexose-accumulating plants, with soluble fructose and glucose-based sugars accumulated at similar concentrations and only a small amount of sucrose accumulation (Ruan et al. 1997). Wide genetic variation in the sugar content of tomato fruits has been observed. Interestingly, genotypes that accumulate high levels of total sugars also accumulate high levels of sucrose, while fruits with genotypes that accumulate low levels of sugar do not accumulate sucrose (Balibrea et al. 2006; Yelle et al. 1988). Some genotypes, such as *Lycopersicon hirsutum*, *Lycopersicon chmielewskii* and *Lycopersicon peruvianum*, accumulate higher levels of sucrose than typical hexose-accumulating tomatoes, and in the final developmental stages, the fruit sugar contents of these types are approximately 10 times that of ordinary tomatoes and primarily include sucrose, whereas the contents of glucose and fructose are relatively lower (Balibrea et al. 2006). Genetic variations in total sugar concentrations and in the ratios among various sugars reflect variations in sugar metabolism during fruit development, which could be regulated to create more valuable tomato varieties (Baxter et al. 2005; Fridman et al. 2000).

However, only a few studies have focused on wild tomato species that accumulate sucrose, and little information exists concerning other tomato species. Previous studies on *Lycopersicon peruvianum* and *Lycopersicon esculentum* reported that the increase in sucrose levels in sucrose-accumulating fruits was associated with invertase and sucrose phosphate synthase activities. Sucrose phosphate synthase seems to play a key biochemical role in the accumulation of sucrose and the establishment of a high sugar content in tomato fruits (Dali et al. 1992; Egashira et al. 1999). A study on *Lycopersicon chmielewskii*, a wild tomato species that accumulates high levels of sucrose in its mature fruits, found that the lack of acid invertase activity in sucrose-accumulating fruit was the only significant enzymatic difference between the sucrose-accumulating and hexose-accumulating fruit, whereas sucrose phosphate synthase did not play an important role (Klann et al. 1993). Another report noted that the accumulation of hexose in cultivated tomato may be related to the accumulation of starch before fruit ripening, but the accumulation of sucrose in *Lycopersicon cheesmanii* may be related to the continuous input of sucrose in the later stage (Balibrea et al. 2006). Collectively, these studies did not thoroughly analyze the mechanism of sucrose and hexose accumulation.

Recently, we identified and developed a sucrose-accumulating cherry tomato cultivar that has not previously been reported. In this study, we compared the fruit quality, sugar content in different stages and tissues of fruit, activities of sugar metabolism-related enzymes and expression of sucrose transporter genes of sucrose-accumulating and hexose-accumulating cherry tomatoes. We systematically analyzed the sugar unloading pathway in the two types of tomato fruits. Moreover, the differences in physiology and biochemistry between them were studied through proteomics, and the sugar unloading and accumulation mechanisms in sucrose and hexose-accumulating tomato fruits were investigated. These results provide a better understanding of sucrose-accumulating mechanisms in tomato.
Materials And Methods

Plant growth

The sucrose-accumulating (S) type and hexose-accumulating (H) type cherry tomato (*Lycopersicon esculentum* Mill.) lines are stable and homozygous inbred lines. Seeds of two cherry tomato lines were supplied by Research Fellow Changbao Li (Beijing Vegetable Research Centre, Beijing Academy of Agriculture and Forestry Sciences) and were grown under greenhouse conditions.

Determination of fruit quality

The vitamin C content was spectrophotometrically determined in the UV region. The soluble sugar content was determined by the fluorenone colorimetric method (Krumbein et al. 2004). The lycopene content was quantified according to the method of (Krumbein et al. 2006). Petroleum ether was used to dissolve the lycopene, and the absorbance was determined at 502 nm. The titratable acid content was determined using the titration method (Auerswald et al. 1996). A 10 g fruit sample was ground in an ice-bath, and distilled water was added to reach a consistent volume of 100 ml. The mixture was filtered, and 2 drops of 1% (m/v) phenolphthalein were added to 20 ml of the filtrate. NaOH was used for titration, and the end point was determined when a pink color appeared and was maintained for 0.5 min.

Carbohydrate analyses

The analysis of soluble sugars (sucrose, glucose and fructose) was performed by the ethanol extraction method (Lü et al. 2017) with some modifications. The sample (1g) was immediately drenched in liquid nitrogen for grinding for 3–5 min. Then, 2–3 ml 80% (v/v) ethanol was added, and the mixture was transferred to a test tube. Then the test tube was placed in a water bath at 80°C for 1 h. Samples were centrifuged at 4000 g for 10 min, and the supernatants were collected. The pellet was resuspended in 2–3 ml 80% (v/v) ethanol, and three rounds of extraction and centrifugation steps were performed. The three supernatants were combined and evaporated to dryness. Then, the pellet was resuspended in 1 ml of deionized water, filtered through a 0.45 µm filter membrane and analyzed by high-performance liquid chromatography (HPLC). Three biological replicates for each sample and three time repeats for each replicate were conducted for all measurements.

Starch was extracted by incubating the residue left after ethanolic extraction with 10 ml of 30% (v/v) perchloric acid overnight, and the mixture was transferred as to a test tube, placed in a water bath at 80°C for 1 min, then centrifuged at 5000 g for 3 min. The supernatant and diluted to 25ml and the starch content was analyzed with anthrone-\(\text{H}_2\text{SO}_4\) reagent (Wang et al. 2014).

CFDA assay

Carboxyfluorescein diacetate (CFDA, Sigma-Aldrich) solution was introduced into tomato fruits from the pedicel as described previously (Zhang et al. 2004) with some modifications. After rubbing a few cortical cell layers of the pedicel (did not damage the xylem), the pedicel was rinsed with 2.5 mM EDTA solution, and the pedicel was enlaced by cotton thread at one end. The other end of the cotton thread was
immersed in a tube with 100–300 µL 1 mg mL\(^{-1}\) CFDA aqueous solution (the tube and pedicel were wrapped with tin foil to protect it from light). After 24 hours, the tomato fruits were picked and sliced by hand, and CF fluorescence was observed using confocal laser scanning microscopy (CLSM).

**Fruit cytological observation**

The vascular bundle and surrounding tissues of fruits at different stages were cut into small pieces of 1–3 mm\(^3\) by a blade. Tissue embedding was performed according to a previous method (Yang et al. 2017). Ultrathin sections were observed under a HITACHI-7650 transmission electron microscope. The measurement of plasmodesmal densities between SE and CC, SE and PC, CC and PC, PC and PC was performed according to a previous method (Ma et al. 2019).

**qRT-PCR analysis**

Total RNA was extracted from 100 to 200 mg of frozen fruit tissue and reverse transcribed into cDNA using an RNA extraction kit (Takara) and reverse transcription kit (Takara) according to the manufacturer’s protocol. Gene-specific primers and internal control (Actin mRNA) primers (Table S1) were used to amplify PCR products on an ABI 7500 system (Bio-Rad). qRT-PCR was performed using SYBR Premix Kit (Takara). Three biological replicates (samples from three individual plants) were performed, and relative amounts of mRNA were calculated using the 2\(^{-\triangle\triangle CT}\) method (Livak and Schmittgen 2002).

**Enzyme extraction and activity assays**

Enzyme extraction was performed according to (Zhang et al. 2015) and with some modifications. Fruit material samples (1 g) were homogenized in 10 ml 4-(2-hydroxyethyl)-1-pipera-zineethanesulfonic acid (HEPES) buffer (50 mmol\(\cdot\)L\(^{-1}\) HEPES-NaOH, 1 mmol\(\cdot\)L\(^{-1}\) ethylenediaminetetraacetic acid (EDTA), 10 mmol\(\cdot\)L\(^{-1}\) magnesium chloride (MgCl\(_2\)), 2.5 mmol\(\cdot\)L\(^{-1}\) dithiothreitol (DTT), 10 mmol\(\cdot\)L\(^{-1}\) ascorbic acid (Vc), 5% polyvinylpyrroli-done, pH = 7.5). A small amount of quartz sand was added and ground into a homogenate on ice. The homogenates were centrifuged for 20 min at 12000 r/min at 4°C. The supernatant was collected, 5.6 g ammonium sulfate was added, dissolved and the mixture was centrifuged for 30 min at 12000 r/min at 4°C. The supernatant was removed, and the precipitate was dissolved in 2–5 ml HEPES buffer. Then the solution was dialyzed with a semipermeable membrane and 10 times diluted HEPES buffer for 20 hours.

Acid invertase (AI) activity was enzymatically assayed according to (Gibon et al. 2004). Sucrose synthase (SS) and sucrose phosphate synthase (SPS) activities were determined as described previously (Zrenner et al. 1995).

**iTRAQ-based protein profiling**

Protein sample extraction, iTRAQ labeling, and LC-MS/MS analysis were performed according to (Guo et al. 2017; Liu et al. 2015) with minor modifications. Ripe fruits of sucrose-accumulating and hexose-accumulating cherry tomatoes were extracted individually and cut into small pieces, immediately frozen
in liquid nitrogen and stored at -80°C for further studies. Five biological repeats were performed, and each measurement was repeated three times.

Results

Growth and quality identification

Two cherry tomato (*Lycopersicon esculentum* Mill.) lines, including a sucrose-accumulating (S) type and hexose-accumulating (H) type were selected for this study, and these fruits were divided into 5 stages (Ⅰ, Immature; Ⅱ, Mature; Ⅲ, Green Breaker; Ⅳ, Pink; Ⅴ, Red Ripe) according to the tomato growth period standards developed by the United States Department of Agriculture (USDA) and the growth curves of fruits of the two cherry tomato genotypes (Fig. S1).

Comparing the growth of H and S cherry tomatoes, we found that there were no visible differences in general plant morphology between two kinds of cherry tomatoes with respect to plant height and the number of leaves and fruits in the whole growth cycle, while differences were noted in the number of flowers (Fig. S2).

Assays involved in testing the quality and yield of the two kinds of cherry tomatoes indicated that the S cherry tomato had the higher total soluble sugar, Vitamin C and lycopene and lower organic acid at the fruit Red Ripe (V) stage than the H cherry tomato. There were no significant differences in fruit firmness or yield. It was easy to conclude that the S cherry tomato had better quality than the H type (Fig. 1).

Carbohydrates accumulation analysis

Sucrose, fructose, glucose and starch accumulation were analyzed in the developing fruits of sucrose-accumulating and hexose-accumulating cherry tomato lines. Regardless of any stage and tissue of the fruit, the S cherry tomato accumulated more sucrose than the H type, while the accumulation of glucose, fructose and starch in the H cherry tomato was significantly higher than that in the S type (Fig. 2). Sucrose accumulation in whole fruit and different fruit tissues (exocarp, mesocarp, endocarp, placenta and septum) of S tomato showed a similar trend; the sucrose content reached a high point at Mature stage (Stage Ⅱ) first, then declined and reached its highest point at Red Ripe stage (Stage Ⅴ). The sucrose content in the S type fruit was dozens of times that in the H type fruit and there was little difference in sucrose content between different fruit tissues. The accumulation of glucose and fructose in the H type tomato fruit presented a similar trend as sucrose in the S cherry tomato and was high mainly in the later stages. It is worth mentioning that hexose also occupied a certain proportion in the S type cherry tomato, especially in the endocarp. In general, the starch content declined in early stages and slightly increased at the later stage and the H cherry tomato accumulated more starch than the S type in all tissues and stages.

Cytological path of phloem unloading of two cherry tomato fruits
The accumulation of sucrose or hexose in the fruit depends on the unloading of sugar from the phloem.
To investigate the cytological bases of the accumulation of sugar during fruit development and ripening,
we examined the phloem unloading pathway and the connection between the phloem and surrounding
cells.

The CF tracer was used to detect the assimilate unloading pathway between the phloem and the
surrounding parenchyma cells in the S cherry tomato. The results indicated that the pathway of fruit
phloem unloading was altered during tomato development. CF was strictly restricted in the phloem at the
eyl stages (Stage 1 to Stage 6), which indicates an apoplastic pathway of fruit phloem unloading, while
CF was distributed to surrounding parenchyma cells at the later stages (Stage 6 to Stage 8), which means
that the phloem unloading pathway was a symplastic pathway (Fig.3).

Observation of the plasmodesmata between the phloem and surrounding cells by transmission electron
microscopy (TEM), revealed that in the early stages of the S cherry tomato fruit (Stage 1 to Stage 6), there
were only a few plasmodesmata between the sieve element/companion cell complex (SE/CCs) and
parenchyma cells (PCs), and these plasmodesmata were blocked. However, there were many
plasmodesmata at the later stages (Stage 6 to Stage 8), implying that the phloem unloading pathway of
the S cherry tomato changed from symplastic to apoplastic during fruit development, which was
consistent with the results of the CFDA tracer (Fig.4 and Table S2).

Otherwise, the phloem unloading pathway of the H cherry tomato changed from apoplastic to symplastic
during fruit development, because there were many plasmodesmata in the early stages and few
plasmodesmata in the later stages between SE/CCs and PCs.

**Expression analysis of sugar transporters and related metabolic enzymes**

To further analyze the sugar accumulation mechanism in tomato fruits, the expression of three tomato
sucrose transporters and the activities of several sugar metabolism enzymes were studied.

The mRNA expression levels of sucrose transporter (SUT) genes in the two cherry tomatoes had no
obvious patterns (Fig.5). However, the expression of *LeSUT1* was higher in the H tomato than in the S
tomato, which implies that there may be more sucrose unloading from the phloem. The activities of
sugar-related metabolic enzymes indicated that the H cherry tomato had higher activities of acid
invertase (AI) than the S type at almost all fruit stages and tissues, and reached to the highest level at the
Pink stage (Stage IV), which means that a large amount of sucrose hydrolysis may occur during this
stage (Fig.6A).

The activity of sucrose phosphate synthase (SPS) was higher in the S cherry tomato than in the H type
(Fig.6B), indicating that more sucrose was synthesized. However, sucrose synthase (SS), a reversible
enzyme that broke down and synthesized sucrose, had higher levels in the early stages of fruits, implying
that SS played an important role in the hydrolysis of sucrose in the early stages (Fig.6C). The S type
tomato had higher SS activities than the H type tomato, but a similar trend in fruit tissues as the H type
tomato. In contrast to Al, SS had the lowest activity in Stage IV. It is possible that the hydrolysis of sucrose during this stage mainly depends on Al.

These results indicated that LeSUT1 and Al played more important roles in the H cherry tomato, responsible for unloading sucrose from the phloem and breaking down sucrose into glucose and fructose. SPS and SS may play more roles in the S tomato, responsible for maintaining the homeostasis of sucrose by hydrolysis and synthesis. It is likely that sucrose accumulation in the S tomato mainly relied on symplastic transport at later stages.

**Proteomics analysis**

To further analyze the mechanism of sugar accumulation in the S and H cherry tomatoes, the iTRAQ method for proteome sequencing of ripe fruits of two types of tomato was performed (Fig. S3). Mass spectrometry to identify differential proteins indicated that total 420 differentially expressed proteins were identified between S and H tomatoes, and 235 proteins were upregulated, while 185 proteins were downregulated (Fig.7A).

The COG analysis indicated that these differential proteins were mainly related to metabolic pathways, biosynthesis of secondary metabolism, biosynthesis of amino acids, carbon metabolism, starch and sucrose metabolism, sugar metabolism, photosynthesis, and so on (Fig.7B). Further KEGG analysis indicated that in sucrose-accumulating tomatoes, most differential proteins involved in starch and sucrose metabolism and photosynthesis were downregulated, while most differential proteins involved in fatty acid degradation were significantly upregulated (Fig. S4). These results showed that in addition to sugar metabolism, other metabolic pathways also changed accordingly with the different sugar accumulations between the two types of tomatoes. Differential proteins involved in carbon metabolism and photosynthesis were shown in Fig.7C and D in detail. It is worth noting that acid invertase was significantly downregulated in the sucrose-accumulating tomato, which was consistent with the results of the enzyme activity assay.

**Discussion**

Sugar is the most important factor affecting plant growth and fruit quality (Dai et al. 2016). Sugar is not only an important form of stored energy in many plant storage organs but also a signaling molecule that composes a complex regulatory network with other signals such as hormones and nitrogen, and regulates gene expression and plant growth through signal transduction mechanisms (Granot et al. 2013; Ruan 2014; Yoon et al. 2021).

The mature fruits of ordinary cultivated tomatoes mainly accumulate monosaccharides such as glucose and fructose, while the characteristics of high sucrose accumulation were only found in a few types of wild tomatoes, such as *Lycopersicon hirsutum*, *Lycopersicon chmielewskii* and *Lycopersicon peruvianum* (Balibrea et al. 2006; Dali et al. 1992; Krumbein et al. 2006). High sucrose accumulation may be stably inherited, increasing the sweetness of the fruit and leading to more valuable genetic
variation (Baxter et al. 2005; Chetelat et al. 1995; Fridman et al. 2000). Here, the soluble sugar and starch contents in the fruits of different tissues and developmental stages of two cherry tomatoes, that accumulated sucrose and hexose respectively, were determined. The results indicated that the carbohydrate content between various fruit tissues had no obvious difference. Sugar accumulation mainly occurred in the later stages of fruit development, and the sucrose content in S type tomato was absolutely dominant, while the contents of glucose, fructose, and starch in H type tomatoes were several times higher than that of sucrose (Fig. 1).

The unloading of the photosynthetic assimilates through the phloem was a key step in the accumulation of sugar in the sink organs. In the past two decades, extensive research has been conducted on the phloem unloading pathway. Cytology and molecular biology methods are important means with which to study the phloem unloading pathway and explore the mechanisms of fruit sugar accumulation (Hu et al. 2011; Nie et al. 2010; Viola et al. 2001).

After phloem long-distance transport, assimilates were unloaded from the SE/CC complex through the apoplastic or symplastic pathway. These two pathways can function separately or exist at the same time, and they could also be transformed into each other under certain conditions, which may be related to the development and function of sink organs. Assimilates unloading from the phloem used the apoplastic pathway in several sink organs that accumulate solute sugar at high concentrations, such as strawberry (Li et al. 2012) and apple (Zhang et al. 2004), because of the lack of plasmodesmata between SE/CCs and surrounding cells. A shift of phloem unloading from the apoplastic to symplastic pathway was involved in tuberization in potato (Viola et al. 2001). The ripening process of grape berry involves a switch from symplastic to apoplastic phloem unloading (Zhang et al. 2006). A similar switch occurred during the rapid elongation period of cotton fiber (Ruan et al. 2004). In ordinary cultivated tomato, the symplastic unloading pathway exists in young fruit storing starch, and converted to apoplastic unloading pathway in ripe fruits, which mainly accumulate hexose (Ruan and Patrick 1995). The unloading pathway of assimilates of sucrose-accumulating tomato fruit has not been reported. Here, through CF tracer analysis and cytological observations, we found that a switch from the apoplastic to symplastic pathway was involved in the development of fruit in sucrose-accumulating cherry tomato, and the opposite occurred in H type cherry tomato (Figs. 2 and 3).

In recent years, studies on many horticultural plants have demonstrated that sugar accumulation is closely related to sucrose transporters and sucrose metabolism enzymes. An apple sucrose transporter MdSUT4 was found to be significantly associated with fruit sugar accumulation (Peng et al. 2020). Sucrose synthase may play a critical role in the sugar metabolism of sucrose-accumulating grape berries (Shiraishi and Hamada 2020). SUS, SPS and AI activities in leaves and stems were important factors for regulating sucrose accumulation in high sugar varieties of sugarcane at the mature stage (Niu et al. 2019). The soluble acid invertase activity of watermelon was significantly lower in genotypes accumulating high levels of sucrose than in low-sucrose-accumulating genotypes. Conversely, the activities of SUS and SPS were higher in the high sucrose accumulating genotypes (Yativ et al. 2010). Overexpressing an invertase inhibitor gene from tomato inhibited the activity of cell wall invertase, which
increased sugar accumulation in tomato fruit (Zhang et al. 2015). Tomato SUS1 mediated reversible sucrose hydrolysis is important for maintaining the balance between sucrose and its monomers not only in fruits, but also in other tomato organs (Slugina et al. 2019). Only apoplastic invertase, but not vacuolar invertase, was present in the mature, sucrose-accumulating L. hirsutum pericarp (Miron et al. 2002). In this study, we analyzed the expression and activity of three sucrose transporters and three sucrose metabolism enzymes in detail in various tissues of fruits at different stages. The results indicated that the activity of acid invertase in the H cherry tomato was much higher than that in the S cherry tomato, and sucrose transporters may also function at the later stage of the development of the H cherry tomato fruits. At the early stages of development in the S cherry tomato fruits, SUS and SPS may be related to maintaining the balance of the sucrose concentration with high activities, while sucrose accumulation may rely on the transport of plasmodesmata at later stages.

Proteomics is an important technique for studying the function of proteins in organisms. A proteomics technology named iTRAQ (isobaric tag for relative and absolute quantification) has been widely used in quantitative plant proteomics research (Lan et al. 2011; Owiti et al. 2011; Zi et al. 2013). iTRAQ technology was used to study the changes in the proteome during rice embryo development and a total of 2165 proteins were identified, of which 867 proteins were differential proteins. iTRAQ analysis was conducted on cassava roots and identified a total of 4000 proteins (Owiti et al. 2011).

Here, we used iTRAQ technology to analyze sucrose-accumulating and hexose-accumulating cherry tomatoes. The results showed that 420 differentially expressed proteins were identified between S and H tomatoes, and 235 proteins were upregulated while 185 proteins were downregulated. These differential proteins were mainly concentrated in processes such as sugar metabolism, photosynthesis, and secondary metabolism. Sugar metabolism and photosynthesis-related proteins were significantly downregulated, while fatty acid metabolism-related proteins were mostly upregulated; results may be related to plant adaptation to the corresponding sugar accumulation mechanism.

Some researchers have proposed that excessively high concentrations of sucrose could lead to a decrease in the expression level of genes related to photosynthesis in plant-derived tissues, while in sink tissues it can increase the expression levels of genes related to plant growth, sucrose hydrolysis and respiration (Koch 1996; Smeekens and Rook 1997).

In conclusion, assimilates unloading pathway was switched from apoplastic to symplastic in sucrose-accumulating cherry tomato fruit, and the opposite was true in hexose-accumulating cherry tomato. Plasmodesmata transport may be the main element of sucrose accumulation, and high activity or expression levels of AI and SUT1 may be important factors in hexose accumulation in H and S cherry tomatoes, respectively. In addition to sugar metabolism, photosynthesis, fatty acid metabolism and other secondary metabolism pathways also play important roles in sugar accumulation.

**Declarations**

**Acknowledgements**
This research was supported by the National Natural Science Foundation of China (31301817), a scientific research quality improvement grant from Beijing University of Agriculture (GJB2013007).

Author contribution

R.Y. and Y.Q. designed the project; L.S., J.W., J.S., X.D., W.L., W.Z. and L.Y. performed research; L.S., J.W., C.L., Y.Q. and R.Y. analyzed data; L.S. and R.Y. wrote the first draft; C.L. and Y.Q. revised the manuscript.

Conflict of interest

The authors declare no competing interests.

References

1. Auerswald H, Drews M, Krumbein A (1996) The effect of different methods of cultivation on characteristics of the internal quality of greenhouse tomatoes in the course of one year. Gartenbauwissenschaft 61:77-83. 10.1002/ijc.2910150506

2. Balibrea ME, Martinez-Andujar C, Cuartero J, Bolarin MC, Perez-Alfocea F (2006) The high fruit soluble sugar content in wild Lycopersicon species and their hybrids with cultivars depends on sucrose import during ripening rather than on sucrose metabolism. Functional Plant Biology 33:279-288. 10.1071/fp05134

3. Baxter CJ, Carrari F, Bauke A, Overy S, Hill SA, Quick PW, Fernie AR, Sweetlove LJ (2005) Fruit carbohydrate metabolism in an introgression line of tomato with increased fruit soluble solids. Plant and Cell Physiology 46:425-437. 10.1093/pcp/pci040

4. Chetelat RT, Deverna JW, Bennett AB (1995) Effects of the lycopersicon-chmielewskii sucrose accumulator gene (sucr) on fruit yield and quality parameters following introgression into tomato. Theoretical and Applied Genetics 91:334-339. 10.1007/bf00220896

5. Dai Z, Wu H, Baldazzi V, van Leeuwen C, Bertin N, Gautier H, Wu B, Duchene E, Gomes E, Delrot S, Lescourret F, Genard M (2016) Inter-species comparative analysis of components of soluble sugar concentration in eshy fruits. Frontiers in Plant Science 7:10.3389/fcls.2016.00649

6. Dali N, Michaud D, Yelle S (1992) Evidence for the Involvement of Sucrose Phosphate Synthase in the Pathway of Sugar Accumulation in Sucrose-Accumulating Tomato Fruits. Plant physiology 99:434. 10.1104/pp.99.2.434

7. Egashira H, Takahashi S, Doi H, Nishizawa T, Escalante A, Takashina T, Imanishi S (1999) Genetic analysis of sucrose - Accumulating ability in Lycopersicon peruvianum. Breeding Science 49:155-159. 10.1270/jsbbs.49.155

8. Fridman E, Pleban T, Zamir D (2000) A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. Proceedings of the National Academy of Sciences of the United States of America 97:4718-4723. 10.1073/pnas.97.9.4718
9. Gibon Y, Blaesing OE, Hannemann J, Carillo P, Hohne M, Hendriks JHM, Palacios N, Cross J, Selbig J, Stitt M (2004) A robot-based platform to measure multiple enzyme activities in Arabidopsis using a set of cycling assays: Comparison of changes of enzyme activities and transcript levels during diurnal cycles and in prolonged darkness. The Plant cell 16:3304-3325. 10.1105/tpc.104.025973

10. Granato D, Branco GF, Nazzaro F, Cruz AG, Faria JAF (2010) Functional foods and nondairy probiotic food development: trends, concepts, and products. Comprehensive Reviews in Food Science and Food Safety 9:292-302.

11. Granot D, David-Schwartz R, Kelly G (2013) Hexose kinases and their role in sugar-sensing and plant development. Frontiers in Plant Science 4:10.3389/fpls.2013.00044

12. Guo X, Xu J, Cui X, Chen H, Qi H (2017) iTRAQ-based protein profiling and fruit quality changes at different development stages of oriental melon. BMC plant biology 17:28. 10.1186/s12870-017-0977-7

13. Hu L, Sun H, Li R, Zhang L, Wang S, Sui X, Zhang Z (2011) Phloem unloading follows an extensive apoplastic pathway in cucumber (Cucumis sativus L.) fruit from anthesis to marketable maturing stage. Plant Cell and Environment 34:1835-1848. 10.1111/j.1365-3040.2011.02380.x

14. Klann EM, Chetelat RT, Bennett AB (1993) Expression of acid invertase gene controls sugar composition in tomato (lycopersicon) fruit. Plant physiology 103:863-870. 10.1104/pp.103.3.863

15. Koch KE (1996) Carbohydrate-modulated gene expression in plants. Annual Review of Plant Physiology and Plant Molecular Biology 47:509-540. 10.1146/annurev.arplant.47.1.509

16. Krumbein A, Peters P, Brückner B (2004) Flavour compounds and a quantitative descriptive analysis of tomatoes (Lycopersicon esculentum Mill.) of different cultivars in short-term storage. Postharvest Biology and Technology 32:15-28. https://doi.org/10.1016/j.postharvbio.2003.10.004

17. Krumbein A, Schwarz D, Klaering HP (2006) Effects of environmental factors on carotenoid content in tomato (Lycopersicon esculentum (L.) Mill.) grown in a greenhouse. Journal of Applied Botany and Food Quality 80:160-164. http://hdl.handle.net/2078.1/151622

18. Lü J, Sui X, Ma S, Li X, Liu H, Zhang Z (2017) Suppression of cucumber stachyose synthase gene (CsSTS) inhibits phloem loading and reduces low temperature stress tolerance. Plant molecular biology 95:1-15. 10.1007/s11103-017-0621-9

19. Lan P, Li W, Wen T-N, Shiau J-Y, Wu Y-C, Lin W, Schmidt W (2011) iTRAQ protein profile analysis of Arabidopsis roots reveals new aspects critical for iron homeostasis. Plant physiology 155:821-834. 10.1104/pp.110.169508

20. Lastdrager J, Hanson J, Smeeekens S (2014) Sugar signals and the control of plant growth and development. Journal of experimental botany 65:799-807. 10.1093/jxb/ert474

21. Li CL, Fang KF, Lei H, Xing Y, Shen YY (2012) Phloem unloading follows an extensive apoplastic pathway in developing strawberry fruit. Journal of Horticultural Science & Biotechnology 87:470-477. 10.1080/14620316.2012.11512897

22. Liu J, Pang C, Wei H, Song M, Meng Y, Ma J, Fan S, Yu S (2015) Data for proteomic profiling of Anthers from a photosensitive male sterile mutant and wild-type cotton (Gossypium hirsutum L.).
Data in brief 4:500-509. 10.1016/j.dib.2015.06.022

23. Livak K, Schmittgen T (2002) Analysis of relative gene expression data using real-time quantitative PCR. Methods (San Diego, Calif) 25:402-408. 10.1006/meth.2001.1262

24. Ma S, Sun L, Sui X, Li Y, Chang Y, Fan J, Zhang Z (2019) Phloem loading in cucumber: combined symplastic and apoplastic strategies. The Plant journal : for cell and molecular biology 98:391-404. 10.1111/tpj.14224

25. Miron D, Petreikov M, Carmi N, Shen S, Levin I, Granot D, Zamksi E, Schaffer AA (2002) Sucrose uptake, invertase localization and gene expression in developing fruit of Lycopersicon esculentum and the sucrose-accumulating Lycopersicon hirsutum. Physiologia plantarum 115:35-47. 10.1034/j.1399-3054.2002.1150104.x

26. Nie P, Wang X, Hu L, Zhang H, Zhang J, Zhang Z, Zhang L (2010) The predominance of the apoplastic phloem-unloading pathway is interrupted by a symplasmic pathway during chinese jujube fruit development. Plant and Cell Physiology 51:1007-1018. 10.1093/pcp/pcq054

27. Niu J, Miao X, Wang D, Yang L, Li Y (2019) Relationship between sugar accumulation and sucrose metabolism enzymes in the high and low sugar sugarcane at maturing stage. Acta Agriculturae Universitatis Jiangxiensis 41:431-439.

28. Oude Griep LM, Geleijnse JM, Kromhout D, Ocke MC, Verschuren WMM (2010) Raw and processed fruit and vegetable consumption and 10-year coronary heart disease incidence in a population-based cohort study in the Netherlands. PloS one 5:10.1371/journal.pone.0013609

29. Owiti J, Grossmann J, Gehrig P, Dessimoz C, Laloï C, Hansen MB, Gruissem W, Vanderschuren H (2011) iTRAQ-based analysis of changes in the cassava root proteome reveals pathways associated with post-harvest physiological deterioration. Plant J 67:145-156. 10.1111/j.1365-313X.2011.04582.x

30. Peng Q, Cai Y, Lai E, Nakamura M, Liao L, Zheng B, Ogutu C, Cherono S, Han Y (2020) The sucrose transporter MdSUT4.1 participates in the regulation of fruit sugar accumulation in apple. BMC plant biology 20:10.1186/s12870-020-02406-3

31. Ruan Y-L (2014) Sucrose metabolism: Gateway to diverse carbon use and sugar signaling. Annual Review of Plant Biology 65:33-67. 10.1146/annurev-arplant-050213-040251

32. Ruan YL, Patrick JW (1995) The cellular pathway of postphloem sugar-transport in developing tomato fruit. Planta 196:434-444.

33. Ruan YL, Patrick JW, Brady C (1997) Protoplast hexose carrier activity is a determinate of genotypic difference in hexose storage in tomato fruit. Plant Cell and Environment 20:341-349. 10.1046/j.1365-3040.1997.d01-73.x

34. Ruan YL, Xu SM, White R, Furbank RT (2004) Genotypic and developmental evidence for the role of plasmodesmatal regulation in cotton fiber elongation mediated by callose turnover. Plant physiology 136:4104-4113. 10.1104/pp.104.051540

35. Shiraishi M, Hamada M (2020) Genetic and gene expression analysis of berry sugar composition in a sucrose-accumulating grapevine. Horticult J 89:216-224. 10.2503/hortj.UTD-124
36. Slugina MA, Shchennikova AV, Kochieva EZ (2019) Differences in the sucrose synthase gene SUS1 expression pattern between Solanum lycopersicum and wild tomato species. Theor Exp Plant Physiol 31:455-462. 10.1007/s40626-019-00158-6

37. Smekkens S, Rook F (1997) Sugar sensing and sugar-mediated signal transduction in plants. Plant physiology 115:7-13. 10.1104/pp.115.1.7

38. Tan H-L, Thomas-Ahner JM, Grainger EM, Wan L, Francis DM, Schwartz SJ, Erdman JW, Jr., Clinton SK (2010) Tomato-based food products for prostate cancer prevention: what have we learned? Cancer and Metastasis Reviews 29:553-568. 10.1007/s10555-010-9246-z

39. Turgeon R, Wolf S (2009) Phloem transport: Cellular pathways and molecular trafficking. Annual Review of Plant Biology 60:207-221. 10.1146/annurev.arplant.043008.092045

40. Viola R, Roberts AG, Haupt S, Gazzani S, Hancock RD, Marmiroli N, Machray GC, Oparka KJ (2001) Tuberization in potato involves a switch from apoplastic to symplastic phloem unloading. The Plant cell 13:385-398. 10.1105/tpc.13.2.385

41. Wang H, Sui X, Guo J, Wang Z, Cheng J, Ma S, Li X, Zhang Z (2014) Antisense suppression of cucumber (Cucumis sativus L.) sucrose synthase 3 (CsSUS3) reduces hypoxic stress tolerance. Plant Cell & Environment 37:795-810.

42. Yang R, Wang S, Wang J, Luo X, Zhao W, Zhang Q, Nan Z, Yang L, Wang S (2017) Comparison of manual and automatic processing of biological samples for electron microscopy. Microscopy research and technique 80:10.1002/jemt.22832

43. Yativ M, Harary I, Wolf S (2010) Sucrose accumulation in watermelon fruits: Genetic variation and biochemical analysis. Journal of plant physiology 167:589-596. 10.1016/j.jplph.2009.11.009

44. Yelle S, Hewitt JD, Robinson NL, Damon S, Bennett AB (1988) Sink metabolism in tomato fruit .3. Analysis of carbohydrate assimilation in a wild-species. Plant physiology 87:737-740. 10.1104/pp.87.3.737

45. Yoon J, Cho L-H, Tun W, Jeon J-S, An G (2021) Sucrose signaling in higher plants. Plant Science 302:10.1016/j.plantsci.2020.110703

46. Zhang LY, Peng YB, Pelleschi-Travier S, Fan Y, Lu YF, Lu YM, Gao XP, Shen YY, Delrot S, Zhang DP (2004) Evidence for apoplastic phloem unloading in developing apple fruit. Plant physiology 135:574-586. 10.1104/pp.103.036632

47. Zhang N, Jiang J, Yang Y-I, Wang Z-h (2015) Functional characterization of an invertase inhibitor gene involved in sucrose metabolism in tomato fruit. Journal of Zhejiang University-Science B 16:845-856. 10.1631/jzus.B1400319

48. Zhang X-Y, Wang X-L, Wang X-F, Xia G-H, Pan Q-H, Fan R-C, Wu F-Q, Yu X-C, Zhang D-P (2006) A shift of phloem unloading from symplasmic to apoplastic pathway is involved in developmental onset of ripening in grape berry. Plant physiology 142:220-232. 10.1104/pp.106.081430

49. Zi J, Zhang J, Wang Q, Zhou B, Zhong J, Zhang C, Qiu X, Wen B, Zhang S, Fu X, Lin L, Liu S (2013) Stress responsive proteins are actively regulated during rice (Oryza sativa) embryogenesis as indicated by quantitative proteomics analysis. PloS one 8:10.1371/journal.pone.0074229
Zrenner R, Salanoubat M, Willmitzer L, Sonnewald U (1995) Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (Solanum tuberosum L.). Plant J 7:97-107. 10.1046/j.1365-313X.1995.07010097.x

**Figures**

**Figure 1**
Analysis of the fruit quality and yield of tomato genotypes with different sugar accumulation levels at Red Ripe stage (Stage III). A, Content of soluble sugar. B, Content of titratable acid. C, Content of Vitamin C. D, Content of lycopene. E, Fruit hardness. F, Fruit yield. Student’s t test, *P < 0.05; **P < 0.01.

Abbreviations: H, hexose-accumulating cherry tomato; S, sucrose-accumulating cherry tomato.

Figure 2

Carbohydrate contents in different tissues and stages of two cherry tomato fruits. (a) Sucrose content; (b) Glucose content; (c) Fructose content; (d) Starch content. Data are expressed as mean ± SEM (n = 3). Student’s t test, *P < 0.05; **P < 0.01. Abbreviations: H, hexose-accumulating cherry tomato; S, sucrose-accumulating cherry tomato; I, Immature stage; II, Mature Green stage; III, Breaker stage; IV, Pink stage; V, Red Ripe stage.
Figure 3

Confocal laser scanning microscopy (CLSM) imaging of carboxyfluorescein (CF) unloading during the development of sucrose-accumulating tomato fruits. The treated fruit was sampled 24 h after CF loading, and the hand-sections were prepared from vascular bundle zones of the developing fruit. A, B, C, D, E, Fluorescence images of fruits from Stage I to Stage V. a, b, c, d, e, Bright-field images. I to V represent Stage I to Stage V, respectively. Abbreviations: P, phloem; X, xylem. Bars = 100 µm. I, Immature stage; II, Mature Green stage; III, Breaker stage; IV, Pink stage; V, Red Ripe stage.

Figure 4
The ultrastructure of the sieve element-companion cell complex (SE–CC) and its surrounding parenchyma cells (PCs) in developing tomato fruit. All sections were cut transversely. A-F, Sucrose-accumulating cherry tomato. a-f, Hexose-accumulating cherry tomato. A, a, The phloem of the vascular bundle, SE–CCs and PCs are shown in the images. B-F, b-f, PD (red arrows) between different cells of the phloem during developmental stages I to III. Abbreviations: SE, sieve element; CC, companion cell; PP, phloem parenchyma cell; PD, plasmodesmata; M, mitochondrion; G, Golgi apparatus; RER, rough endoplasmic reticulum; Ve, vacuole; H, hexose-accumulating cherry tomato; S, sucrose-accumulating cherry tomato.
Figure 5

The mRNA levels of sucrose transporters (SUTs) in different tissues and developmental stages of tomato fruits of different sugar-accumulating genotypes. Error bars = ± SEM (n = 3). Abbreviations: FW, fresh weight; AI, acid invertase; SPS, sucrose phosphate synthase; SS, sucrose synthase; H, hexose accumulating tomato genotype; S, sucrose accumulating tomato genotype.
Figure 6

Comparison of the enzyme activities of acid invertase (A), sucrose phosphate synthase (B), and sucrose synthase (C) in different tissues and developmental stages of tomato fruits of different sugar accumulating genotypes. Error bars = ± SEM (n = 3). Abbreviations: FW, fresh weight; AI, acid invertase; SPS, sucrose phosphate synthase; SS, sucrose synthase; H, hexose accumulating tomato genotype; S, sucrose accumulating tomato genotype.
Figure 7

Proteomics analysis of ripe fruits of two types of tomato used iTRAQ technology. A, Volcano plot of downregulated and upregulated proteins based on proteomics data from the sucrose-accumulating tomato genotype versus the hexose-accumulating tomato genotype; B, Functional categories of differentially expressed proteins in proteomics data identified by COG; C, Differentially expressed proteins involved in carbohydrate metabolism; D, Differentially expressed proteins involved in photosynthesis.

Abbreviations: iTRAQ, isobaric tags for relative and absolute quantitation; H, hexose-accumulating cherry tomato; S, sucrose-accumulating cherry tomato; FC, fold change.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.
• Supplementary information PCR.docx