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

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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>

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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-H₂SO₄ 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 $2^{-\Delta\Delta\text{CT}}$ 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-piperazineethanesulfonic acid (HEPES) buffer (50 $\text{mmol}\cdot\text{L}^{-1}$ HEPES-NaOH, 1 $\text{mmol}\cdot\text{L}^{-1}$ ethylenediaminetetraacetic acid (EDTA), 10 $\text{mmol}\cdot\text{L}^{-1}$ magnesium chloride (MgCl_2), 2.5 $\text{mmol}\cdot\text{L}^{-1}$ dithiothreitol (DTT), 10 $\text{mmol}\cdot\text{L}^{-1}$ ascorbic acid (Vc), 5% polyvinylpyrrolidone, 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 early stages (Stage I to Stage II), which indicates an apoplastic pathway of fruit phloem unloading, while CF was distributed to surrounding parenchyma cells at the later stages (Stage III to Stage IV), 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 I to Stage II), 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 III to Stage IV), 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 AI, SS had the lowest activity in Stage IV. It is possible that the hydrolysis of sucrose during this stage mainly depends on AI.

These results indicated that LeSUT1 and AI 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.

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Figures

Figure 1

Analysis of the fruit quality and yield of tomato genotypes with different sugar accumulation levels at Red Ripe stage (Stage V). 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 μ m. I, Immature stage; II, Mature Green stage; III, Breaker stage; IV, Pink stage; V, Red Ripe stage.

Figure 4

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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 V. 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 = $\pm$ 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 = $\pm$ 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.

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