Predominantly symplastic phloem unloading of photosynthates maintains efficient starch accumulation in the cassava storage roots (*Manihot esculenta* Crantz)

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Abstract

Background: It is well known that cassava (*Manihot esculenta* Crantz) highly efficiently accumulates starch in its storage roots, but how photosynthates are transported from the leaves to the phloem (especially how they are unloaded into parenchymal cells of storage roots) remains unclear.

Methods: Here, we investigated the phloem unloading pattern of sucrose and its impact on the development of cassava storage roots through microstructural and physiological analyses and carboxyfluorescein (CF) and isotope C¹⁴ tracing. Expression profiling of genes involved in symplastic and apoplastic transport was performed, and their correlations with storage root yield were determined in populations according to enzyme activity, Western blotting analysis and transcriptome sequencing.

Results: Carbohydrates are transported mainly in the form of sucrose, with more than 54.6% present in the stem phloem at any time. Sucrose was predominantly unloaded symplastically from the phloem into storage roots, but there was a shift from apoplastic to symplastic unloading accompanied by the onset of root swelling. Statistical data concerning the microstructure revealed an enrichment of plasmodesmata within sieve, companion and parenchyma cells in the developing storage roots of a cultivar but not in a wild ancestor. Tracing tests with CF verified the existence of a coplastid channel, and [¹⁴C]Suc demonstrated that the marked sucrose could rapidly diffuse into root parenchyma cells from phloem cells. The relatively high expression of genes encoding SuSy and associated proteins in the storage roots at the middle and late stages but not in the early stage of storage roots, primary fibrous roots or secondary fibrous roots and the inverse expression pattern of SUTs, CWI and SAI in these corresponding organs supported the presence of a symplastic sucrose unloading pathway. The transcriptome pattern of genes involved in symplastic unloading and their significantly positive correlation with starch yield at the population level confirmed that symplastic sucrose transport is vitally important in the development of cassava storage roots.

Conclusions: In this study, we revealed a predominantly symplastic phloem unloading pattern of sucrose in cassava storage roots. This pattern is essential for the efficient starch accumulation for sucrose transport between high-yielding varieties and low-yielding wild ancestors.

Background

Photosynthesis, transport and accumulation of carbohydrates are the three essential physiological processes of yield formation for any economically important crop [1]. However, how photoassimilates are transported from leaf sources to storage sinks is unexpectedly diverse among species. The phloem loading in leaves and unloading at the sink are two restricted steps that play a pivotal role and can significantly affect crop yield and plant productivity [2]. To date, three general sucrose unloading models, namely, symplastic, apoplastic and mixed models, have been identified in plants. Symplastic phloem unloading, in which sucrose passes through the plasmodesmata between phloem companion cells (CCs) and parenchyma cells (PCs), into sink tissues is the principal pathway for most plant species[2]. A series

of genes related to the formation of plasmodesmata have been reported via transcriptomics[1]. Sucrose synthase (SuSy) catalyzes the reversible conversion of sucrose into fructose, and uridine diphosphate glucose acts as a carrier to maintain a physiologically low concentration of sucrose in parenchymal cells. Compared with invertase (INVs), SuSy is a component of post-symplastic unloading and is more important for starch and protein synthesis[1, 3]. The regulation of symplastic transport mediated by changes in plasmodesmata has been extensively studied in many plant species[4-6].

Apoplastic unloading depends on sucrose transporters (SUTs) to take up sucrose from the phloem to parenchyma cells in storage roots. Additionally, this process is accompanied by postunloading invertases. (Here, we need describe the several kinds of INVs and their functions.) Invertases are promising candidates for mediating sink strength because they catalyzes the irreversible cleavage of sucrose to glucose and fructose. Apoplastic invertase (a cell wall-bound acid invertase), which is associated with apoplastic unloading[1, 7], enables the storage of osmotically active solutes against a concentration gradient [8]. Alternatively, the sucrose in the apoplastic space between cells can be transported via sucrose transporters (SUTs) located on the plasma membrane [9, 10].

The unloading processes can vary on the basis of sink type, developmental stage and function of genes involved [2]. The transition between two unloading pathways is closely related to the soluble sugar content and invertase activity[11, 12]. Sucrose utilization is initiated by cleavage catalyzed by SuSy and invertase within the organs involved; this cleavage in turn regulates the unloading rate through two distinct phloem unloading pathways[8, 13]. An inverse correlation between the mRNA expression of soluble acid invertase (SAI)- and SuSy-encoding genes has been found, and the balance between SuSy- and SAI-mediated sucrolytic pathways determines the type of unloading pathway and starch accumulation in potato tubers [14].

An understanding the cellular routes of phloem unloading is needed to enhance plant yield [15], but developing a direct experimental model for complex sink organs together with the substantial technical challenges associated with this process is difficult. To date, mechanistic studies of phloem unloading have focused only on fleshy fruits, such as those of grape [16], Chinese jujube [17], peach [10], blueberry [18] and kiwifruit [19], and few have been dedicated to vegetative storage sinks. Cassava (*Manihot esculenta* Crantz) is a tropical perennial root crop species that is harvested yearly, with an approximately 12-month growth cycle. Cassava ranks as the fifth most important food crop worldwide, and approximately 1 billion people rely on its starch-rich roots [20]. High-efficiency photosynthesis, tolerance to drought and an extraordinarily high starch accumulation in its storage roots are essential biological characteristics of cassava [21]. Moreover, cassava has three root types: primary and secondary fibrous roots and storage roots [22]. Storage roots develop from primary fibrous roots (PFRs) by following a distinct pattern of secondary growth, and starch can constitute up to 85% of the dry weight of storage roots [23]. However, how such a large amount of carbohydrates are loaded into the phloem in the leaves and unloaded into parenchymal cells in the storage roots is poorly understood.

In this study, we determined the general transport models of carbohydrates in cassava, clarified that sucrose is the major transported component, discovered a predominantly symplastic phloem-unloading system in the storage roots and found that there was a shift from apoplastic to symplastic transport at the beginning of the fibrous root swelling stage, which is essential for highly efficient starch accumulation in cassava. These findings are based on structural, physiological and biochemical experiments on developing storage roots, which included measurements of the activity of enzymes and expression of mRNAs of genes involved in the transport and posttransport events.

Results

Sucrose is the Primary Component of Photosynthates Transported in the Phloem of Cassava

Cassava plants were potted in a greenhouse. The biomass accumulation curve across three growth stages, the anatomical structure of the storage roots, the results of the phloem sap collection and the phloem composition are shown in Figure 1. Phloem sap was collected from the stem of plants during the development of the storage roots (approximately 60 DAPE). HPLC-based examination showed that sucrose had the largest peak, accounting for approximately 79% of the phloem sap, followed by glucose and fructose (Fig. 1D), whose concentrations in the phloem sap constituted 54.6%, 22.7% and 22.5% (Table 1). These results indicate that sucrose is the main form of carbohydrate transported from leaves to the belowground cassava storage roots.

SE-CC Complex is Symplastically Connected to Surrounding PCs through Plasmodesmata in Storage Roots

According to the biomass (fresh and dry weights) accumulation curve of the storage roots, we classified the developing storage roots into three stages: early stage (60 DAPE, initial storage roots), middle stage (180 DAPE, fast growth of storage roots) and late stage (270 DAPE, mature storage roots) (Fig. 1A). The microstructure of the developing roots in the three key stages were investigated in detail, including the control of fibrous roots (SUPPL1). Our observation particularly focused on the plasmodesmata among phloem sieve elements (SEs), CCs and PCs (Fig. 2). A mass of plasmodesmata was observed between the SEs and CCs (Fig. 2A-C), the SE-CC complex and PCs (Fig. 2D) and between PCs (Fig. 2 G-I) during the three key developmental stages. However, plasmodesmata were rarely observed in fibrous roots, particularly between the SE-CC complex and PCs (Table 2). In the expanding SEs, one SE was always accompanied by two middle- or late-stage CCs (Fig. 2 E), and the majority of the plasmodesmata were branched (Fig. 2 A-D). However, the plasmodesmata were almost always simple (nonbranched) between PCs (Fig. 2 G-I). Thick-walled SEs sometimes emerged during the late stage of development (Fig. 2 F) in the pore-plasmodesma (PD) units (PPUs) connected to CCs or PCs (SUPPL2). The plasmodesmal density in cross-sections containing several cell types was calculated (Table 2). PD numbers among the three types of storage roots cells tended to increase in the three developmental stages but were very scarce in the fibrous roots, especially in the PFRs, as the PDs were mostly present at the interface of the SE-CC complex. The number of PDs increased at the interface between two adjacent PCs compared to those

between other interfaces, even in the SFR, with limited starch storage capacity. The ultrastructure of the phloem and the distribution of plasmodesmata between the SE-CC, SE-CC complex, PCs and adjacent PCs in the developing storage roots indicate the predominance of symplastic transport and communication characteristics of cassava.

Rapid Lateral Diffusion of Carboxyfluorescein (CF) and [^{14}C]Suc Strongly Support the Predominance of Symplastic Phloem Unloading in Developing Storage Roots

Upon entering the cells, the membrane-permeable nonfluorescent 6(5)-carboxyfluorescein diacetate (CFDA) is broken down to 6(5) carboxyfluorescein (CF), which is a membrane-impermeable fluorescent dye. CF is often used as a fluorescent marker of phloem transport and symplastic phloem unloading [11]. In this study, it was injected into the stem near the base of cassava plants at early, middle and late developmental stages. After 72 h, the CF green fluorescent signals were detected immediately in the roots at the three stages via microsections. CF molecules were extensively distributed throughout the phloem (SPH) and xylem (SXY) regions of the storage roots at the three developmental stages (Fig. 3 A-I), which contrasted with the those of the control (Fig. 3 A1-F1), and the highest density of CF predominantly appeared at the middle stage (Fig. 3 D-F, K). In contrast, the CFs were restrictedly released along the cortex but were not visible in the primary phloem (PPH) or primary xylem (PXY) in the primary fibrous roots (PFRs) (Fig. 3 J). There was no distribution of CF green fluorescence in the PFRs under excitation at 405 nm (Fig. 3 J1) or bright-field microscopy (Fig. 3 J2). This CF tracing experiment clearly illustrated that there are channels allowing CFs with molecules similar to the sucrose path through the cell membrane driving fast spreading among the parenchyma cells of the xylem in the storage roots, especially at the middle developmental stage. We further utilized [^{14}C]Suc tracing to inspect the diffusion strength difference of the sucrose molecules marked across the three developmental stages. [^{14}C]Suc was fed to the upper stem of the plants, and after 72 h, we investigated its distribution and density in the roots at the three stages (Fig. 3 M). [^{14}C]Suc accumulated immediately in the phloem and xylem tissue of the developing storage roots. The [^{14}C]Suc signal was stronger in the storage roots at the middle stage than at late stage, and the signal in the early stage was very weak. This sucrose unloading trend in the roots at the three key developmental stages is in agreement with the demands of the growth and biomass accumulation characteristics of cassava plants (Fig. 1 A). The fast unloading and dynamic change in sucrose traced by [^{14}C]Suc and the existence of symplastic space identified by CF transport strongly support the idea that a symplastic phloem unloading pathway exists in cassava storage roots.

Differential Expression Level of Genes Involved in Post-phloem Transport and the Relevant Activity of Their Encoded Enzymes Further Support Symplastic unloading in Cassava Storage Roots

Sucrose molecules are broken down immediately by sucrose synthase (SuSy) following symplastic unloading or by invertase (INV) in the case of apoplastic unloading. The sucrose transporter SUT is the main regulator of apoplastic sucrose transport [10, 24]. We compared the expression levels of genes encoding SUTs, SuSy and cell wall acid invertase (CWI) and their enzyme levels in the leaves, different root types and storage roots of plants at the three developmental stages.

SuSy was highly expressed in the storage roots of plants at the three developmental stages but less expressed in the primary fibrous roots (PFRs) and secondary fibrous roots (SFRs) (Fig. 4 A), whereas CWI expression was higher in the PFRs and SFRs than in the storage roots of plants at the three developmental stages (Fig. 4B). SuSy-encoding genes, including SuSy1, SuSy3 and SuSy4, were more highly expressed than were CWI- and SUT-encoding genes in the storage roots of plants at the three developmental stages (Fig. 4 C). Even SUTs were expressed in the storage roots (Fig. 4 C), though SUT members such as SUT1, SUT2 and SUT4 were much more highly expressed in the leaves, except in the fibrous roots of plants at the early developmental stage (Fig. 4D). The protein expression levels of SuSy, CWI and SAI in the different roots and at the key developmental stages of the storage roots were measured via Western blot analysis (Fig. 4E). The expressed protein level of SuSy in storage roots of the early, middle and late stages was significantly higher than that in the PFRs and SFRs, whereas the CWI protein was markedly lower in the storage roots at the three stages than in the PFRs and SFRs. The SAI protein quantity was consistently low, with no apparent change between the tested root types. The enzyme activities were very consistent with their protein levels, and CWI was significantly active in the PFRs and SFRs but later was replaced by SuSy in the storage roots (Fig. 4F). The quantified results above show that the high expression of SuSy and low expression of SUTs and CWI in the developing storage roots as well as the opposite expression in the primary fibrous roots and secondary fibrous roots are all identifications of a sucrose symplastic unloading pathway in cassava storage roots, which developed from primary fibrous roots at the early stage of cassava plant growth.

Immunogold labeling assays revealed that CWI molecules, which are biomarkers of apoplastic transport of sucrose, were enriched in the early fibrous roots but not in the developing storage roots. The labeled CWI proteins were visible predominantly on the cell walls between the SEs and PCs, CCs and PCs, and SEs and CCs (Fig. 5A-D) of the primary fibrous roots (PFRs). However, very few CWI proteins were found in the cell wall spaces between CCs and PCs, SEs and CCs and between PCs and PCs (Fig. 5 E-H) in the storage roots at the middle or late developmental stage. No CWI molecules were observed in any control without antiserum or preimmune serum (data not shown), indicating that the antiserum was highly specific and that nonspecific labeling was negligible. In summary, the results also supported that a predominantly symplastic phloem unloading pathway exists in developing cassava storage roots.

Discussion

Cassava (*Manihot esculenta* Crantz), known as one of three of the most important tuber and root crop species, is a typical tropical crop species with a high potential for starch accumulation. It has a high photosynthesis capacity with photosynthesis characteristics between those of C3 and C4 plants. This means that a large amount of carbohydrates will drive transport from sources to the belowground sink tissues. Accession W14 of the wild cassava ancestor species, which has very low starch yield, presents very low numbers of plasmodesmata in its storage roots (unpublished data). Patrick and Offler [25] found that predominantly symplastic unloading is associated with a great transport capacity and low resistance. Plasmodesmata provide symplastic continuity between most adjacent cells in higher plants and thus substantially contribute to phloem unloading into sink tissues [26]. Our research showed that

the number of plasmodesmata increased with the expansion of storage roots, branched plasmodesmata were mainly found between SEs and CCs, and simple plasmodesmata were found between PCs and accounted for the majority of phloem unloading cells. Simple plasmodesmata are 'open', the branched plasmodesmata are associated with low conductivity [5, 27]. The cell walls thickened during the late stage of cassava storage roots (Fig 2. G), but minimal changes were observed in the amount of plasmodesmata during storage. This is different from that in apple [28] and jujube [17], wherein the plasmodesmata were blocked during the shift of the unloading pathway.

Storage roots undoubtedly develop from special fibrous roots in cassava under some conditions [22]. This induces a change in the belowground roots to becoming nutrient sinks along with intense changes in structure. The energy cost of apoplastic unloading becomes replaced by the more effective cost of symplastic unloading. Sucrose is the dominant photosynthate in cassava phloem exudates, and carbohydrates (mainly starch) rapidly accumulate during the early and middle developmental stages of cassava storage roots but slowly accumulate during the late developmental stage. This is different from the mechanism in fleshy fruits, wherein the accumulation of a high concentration of soluble sugars relies on apoplastic phloem unloading [10, 16, 29]. In other tuber or root crop species, such as potato [11], carrot [30] and radish [31], switches from apoplastic unloading to symplastic unloading occur during the development of the swelling roots. Thus, a similar switch occurs in cassava from fibrous roots to storage roots, and the switch node may be a critical developmental checkpoint for cassava storage roots.

In actuality, in terms of immediately breaking down or removing sucrose from the intracellular space, this postunloading event is extremely important for the lack of concentration gradient involved in symplastic transport of sucrose. The expression of SuSy, which is responsible for sucrose conversion into glucose and fructose, is significantly correlated with symplastic phloem unloading and driving sucrose conversion into starch, cellulose and lignin, which then accumulate in sink organs [13, 15, 32]; SuSy is considered a biomarker of storage sink capacity [33-35]. SuSy, but not CWI or SAI, was identified at the expression and protein levels and is maintained at high concentrations in storage roots at the early, middle and late developmental stages of cassava roots. This feature was also found in kiwifruit and apple [19, 36]. In contrast, SUTs, which are key players in the apoplastic transport of sucrose, were expressed at much higher levels in the leaves at all three developmental stages and were expressed more in the fibrous roots than in the storage roots of cassava at the middle and late stages. It seems that there is a predominant apoplastic loading mechanisms in the leaves of cassava. This has already been investigated in a doctoral thesis by us. For sucrose unloading in roots, SUT-dependent positive unloading plays a much greater role in the primary fibrous roots and secondary fibrous roots than in the storage roots, which is different from that of sucrose unloading in the fruits of grape, jujube and *Camellia oleifera* [16, 17]. Cell wall-bound invertase (CWI), which catalyzes the irreversible conversion of sucrose into glucose and fructose, is associated with phloem apoplastic transport. CWI is strictly expressed in dormant apical buds, but it has been isolated from symplast in potato tubers [11]. In cassava, we found that CWI was obviously less expressed in the storage roots than in the fibrous roots, and plasmodesma formation occurred in the primary structure of roots. Therefore, it is inferred that the cambium of the primary storage roots may also be symplastically isolated in cassava and functionally connected to the symplast in the

parenchyma at the onset of differentiation. Afterward, the number of plasmodesmata connected to the pericycle cells increases. Reports in *Arabidopsis* stated that CF and free green fluorescent protein (GFP) could be symplastically unloaded from phloem into cells of the root cortex and epidermis [37, 38]. We also found that CF was restricted to the endodermis in primary fibrous roots (Fig. 3J). Soluble acid invertase (SAI) is another sucrose-catabolizing enzyme that is localized in the vacuole and participates in the breakdown of sucrose inside cells, especially in soluble sugar-accumulating sinks such as sugarcane and fruits [12]. In this study, SAI exhibited a lower level and minimal changes in the cassava roots of different developmental stages. The unloading pathway is linked to concentration differences in total soluble sugars between source and sink cells [15]. As in cassava, high levels of soluble sugars could increase turgor pressure, and the steepness of the turgor gradient is the driving force for symplastic phloem unloading by means of bulk flow through plasmodesmata [2, 39]. This phenomenon is generally ensured by the subcellular compartmentalization of sucrose in sink cells and the utilization of imported metabolites for the production of high-molecular-weight storage compounds or for cell growth [1, 4]. The relatively strong correlations between the expression level of genes involved in symplastic unloading to dry matter yield of cassava cultivars confirmed that this symplastic unloading system that evolved in storage roots is essential for high potentials of starch accumulation in cassava.

Conclusions

Based on the results presented above, we summarized our hypothesis for the sucrose symplastic unloading model in cassava, as shown in Fig. 6:

(1) There are increasing numbers of plasmodesmata between the SE–CC and PCs of developing storage roots of cultivated cassava. This allows sucrose, the main form of photosynthates, to be efficiently unloaded into parenchyma cells in the storage roots. (2) This symplastic unloading process causes a change of primary fibrous roots to being storage roots, and there is a shift from apoplastic unloading to symplastic phloem unloading following the formation of plasmodesmata. (3) Post-phloem unloading events and increased SuSy activity (but not CWI or SAI activity) responsible for the sucrose degradation and biosynthesis of starch and dominant gene sets involved in the formation of plasmodesmata support the proposed link to the high yield potential of cassava.

Methods

Plant Materials

The cassava (*Manihot esculenta* Crantz) cultivars Arg7 and Ku50 and cassava (*Manihot esculenta* ssp. *Flabellifolia*) accession W14, a wild ancestor of cassava, were used in this study. W14 was donated by CIAT, KU50 originated from Argentina, and Arg7 was provided by the Royal Agricultural University of Thailand. All materials were introduced and identified by the Tropical Crop Genetic Resources Institute of the Chinese Academy of Tropical Agriculture Sciences (CATAS) and deposited as MS000581, MS000124

and MS000580, respectively, in the variety resource bed of the Tropical Crop Genetic Resources Institute, CATAS.

Arg7 and Ku50 are cultivars with high-yielding storage roots, with approximately 30% starch content in them. Compared with Arg7, W14 presents 2-5-fold lower storage root yields and less than 5% starch content in its storage roots. Comparison of the biological indexes between cultivars and the wild ancestor were made to determine the underlying features of the modern cultivar. The plants were planted in a greenhouse in early March 2015, with enough plants planted. With respect to the biomass, microstructure, and sampling performed for the different experiments, treatments were applied at the early stage (60 DAPE), at which point the cassava storage roots were forming and elongating, at the middle stage (180 DAPE), at which point the storage roots were expanding and rapidly accumulating starch, and the late stage (270 DAPE), at which point the storage roots were near their maximum weight or had the highest density of starch. All the original data were collected from three plants, with the average value taken for each genotype.

CF and [^{14}C]Suc Tracing

CFDA labeling was performed as described previously [9, 16]. The CFDA solution was introduced into the stem near the base of cassava plants. A cotton thread was placed in a tube at one end, with the other end passing through the phloem zone of the stem base of the storage roots. This method was selected for its high speed of transport and reliability. After 40 min, the plants were labeled with approximately 500 μl of 1 $\text{mg}\cdot\text{ml}^{-1}$ CFDA aqueous solution (prepared from a stock solution in acetone). The tubes were wrapped in aluminum foil to avoid loss of dye and fluorescence quenching under sunlight, and the CF was allowed to translocate for 72 h (which was selected after a time gradient experiment); approximately 1000-2000 μl of 1 $\text{mg}\cdot\text{ml}^{-1}$ CFDA aqueous solution was used for the developing storage roots. The belowground sink tissues were subsequently sectioned and examined for CF fluorescence using CLSM (FV1000, Olympus, Japan). The samples were scanned using an excitation wavelength of 350–600 nm, and the strongest fluorescence peak was observed at 488 nm excited by CF,

The strongest autofluorescence of cassava roots was detected at 405 nm. We scanned every section using 405 nm and 488 nm excitation wavelengths to determine the distribution of autofluorescence and the green fluorescence of CF.

Sucrose was labeled with the ^{14}C isotope under experimental conditions. The resulting [^{14}C]Suc was injected into the upper stem according to the same method as that used for CFDA. After 48 and 72 hours, the storage roots were sampled, and paraffin sections were made. Manually made sections were gently compressed and autoradiographed in cassettes using a Kodak XBT-1 instrument at 4 °C for 30 d. The distribution of [^{14}C]Suc in the storage roots was detected by [^{14}C]-autoradiography.

Measurement of Plasmodesmal Density

Plasmodesmal density was measured as described in [40] and as used in a previous study[17]. Five serial sections of two orientations (transverse and longitudinal) of ultrathin sections were prepared from Spurr-infiltrated samples; each group comprised sections located approximately 20 μm apart. From each group, six ultrathin sections were selected at random and placed on copper grids (100-mesh). Five samples (each consisting of phloem and surrounding PCs) were observed from each ultrathin section for transmission electron microscopy (TEM). Plasmodesmata were counted at all cell interfaces (i.e., the interfaces between SEs and CCs, SEs and PCs, CCs and PCs and PCs and PCs; PCs include both phloem parenchyma and xylem storage parenchyma cells) in each selected field. The results of the plasmodesma counting are shown as the number of plasmodesmata per micron of specific cell/cell interface length per transverse section, which is referred to as plasmodesmal density (No. plasmodesmata. μm^{-1}); half plasmodesmata were counted as one.

Histochemical Analysis and Ultrastructural Observations

Antibodies against CWI, SAI and SuSy were generated against polypeptides by ComWin Biotech Co., Ltd. (China). The gene sequences of CWI were obtained from Phytozome V12.1 (<http://www.Phytozome.com>), and we selected three isoforms of this enzyme that were expressed in cassava roots (at four different developmental stages): CWI-1, CWI-2 and CWI-5. The last two isoforms with the same amino acids have the partial sequence QPYRTSYHFQPPK, whereas the last one has the specific sequence DPKQRQVQNYAVPK. The isoform-specific partial amino acid sequence of SAI was QKGSEQTFPSRE; this sequence was generously provided by Zhang Peng (Institute of Plant Physiology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences) and was proven to be highly and specifically expressed in cassava roots. The sequence of SuSy, which was also highly expressed in cassava roots, was generously provided by Dr. Luiz (EMBRAPA Genetic Resources and Biotechnology, Brasilia, DF, Brazil); its GenBank number is AAV74405.1, and the specific sequence of this isoform is RRKESKDLEEXA (basic information about these genes is shown in Table 3

Ultrathin sections were essentially prepared as follows: the storage roots were cut into small cubes (approximately 2 mm^3) that were immediately fixed with 4% (v/v) glutaraldehyde in 100 mM precooled phosphate buffer (pH 7.4) for 4 h at 4 °C. The penetration of the glutaraldehyde buffer was performed manually by using a syringe. After an extensive rinse with the precooled phosphate buffer (pH 7.4), the tissue cubes were postfixated in 1% (w/v) OsO_4 for 1–2 h and shaken several times during that time period. Following another extensive rinse with the same buffer, the samples were dehydrated through a graded ethanol series (50%–100%). Propylene oxide was used to displace the Spurr epoxy resin, which was then infiltrated for 24 h at room temperature. Polymerization was conducted at 70 °C for 8 h. These sections (approximately 80 nm in thickness) were mounted on 100-mesh copper grids or were nickel coated with 0.25% Formvar film for ultrastructural observations using a JEM 2100 transmission electron microscope.

Sections prepared from paraffin-embedded tissues were used for histological structure observations. Small cubes (2-3 cm^3) of cassava storage roots were immediately fixed in formalin–acetic acid–alcohol (FAA) solution for 36 h at room temperature and dehydrated through a series of graded ethanol

(80%–100%). The subsequent material was processed by *n*-butyl alcohol three times and then embedded in paraffin for 12 h at 60-65 °C.

Immunogold Labeling

Immunogold labeling was conducted as described in [16]. Briefly, ultrathin sections were prepared as described above except that they were fixed with 4% glutaraldehyde. The sections were first incubated with rabbit antiserum specific for SAI, CWI or SUT prepared as described above and then incubated together with secondary antibodies (goat anti-rabbit IgG antibodies conjugated to 10 nm gold). Finally, the sections were double-stained with uranyl acetate and alkaline lead citrate and subsequently examined with a JEM-2100 transmission electron microscope. The specificity and reliability of the immunogold-labeling experiments were verified using two negative controls. In the first negative control, the antiserum was omitted to test for potential nonspecific labeling by a goat anti-rabbit IgG antibody-gold conjugate. In the second negative control, rabbit preimmune serum was used instead of rabbit antiserum prior to immunogold labeling to determine the specificity of the antiserum. At least three replicates of the control experiments were included for each sample.

Extraction of mRNA, Determination of mRNA Levels and Enzyme Activity and Western Blotting Analysis

Total RNA from cassava roots was extracted using RNeasy Plant plus Reagent (TIANGEN, China). Primers for SuSy, CWI and SAI were designed based on the sequences described above, and amplicons were detected using a real-time quantitative PCR cycler (Rotor-Gene 6000, QIAGEN, German). β -Actin was used as a reference gene.

Enzyme extraction and assays of SAI or CWI activity were performed as described previously [41]. Briefly, the extraction buffer A medium was composed of 150 mM Tris-HCl (pH 8.0), 2 mM ethylenediaminetetraacetic acid, 10 mM MgCl_2 , 0.2% (v/v)-mercaptoethanol, 0.1 mM phenylmethyl sulfonyl fluoride, 1 mM benzamidine, 10 mM ascorbic acid and 3% (w/v) polyvinylpolypyrrolidone. The slurry was passed through four layers of cheesecloth. The filtrate was subsequently centrifuged at 16,000 g for 20 min, after which the supernatant was used for SAI assays. The residue, which was used to prepare CWI, was rinsed with the same buffer without polyvinylpolypyrrolidone until the effluent was free of protein. From this material, CWI was extracted in buffer A supplemented with 0.5 M NaCl with gentle shaking for 24 h. After centrifugation, the supernatant was used for the CWI enzyme assays. All extraction procedures were performed at 4 °C. The SAI activity was assayed using soluble and insoluble fractions, and each assay consisted of 0.3 ml of 100 mM sodium acetate buffer (pH 4.8), 0.1 ml of 100 mM sucrose and 0.1 ml of enzyme sample, as described in [42].

The enzyme extractions and assays of SuSy activity were performed as described in [43]. Briefly, frozen root tissues were ground in 5 ml of media containing 50 mM HEPES buffer (pH 7.0), 10 mM 2-mercaptoethanol, 2% polyvinylpolypyrrolidone, 1% polyvinylpyrrolidone, 1 mM EDTA and 10 mM MgCl_2 . Afterward, 0.1 ml of the desalted extract was added to 0.9 ml of reaction media composed of 25 mM HEPES-NaOH buffer (pH 6.5), 125 mM sucrose, 15 mM MgCl_2 and 2 mM UDP. The enzyme activity in the

direction of sucrose cleavage was assayed at 28 °C in the presence of sucrose. The reducing sugars produced were assayed using the 3,5-dinitrosalicylic acid-base method described in [44].

Proteins were extracted according to the methods of Miron and Schaffer [45]. The extraction buffer consisted of 100 mM Tris-HCl (pH 8.9), 250 mM sucrose, 10 mM MgCl₂, 5 mM vitamin C and 3.5% crosslinking polyvinylpyrrolidone. (NH₄)₂SO₄ was used to precipitate CWI, SAI and SuSy proteins, and protein concentrations were determined using the Bradford [46] method, with bovine serum albumin used as a standard. SDS-PAGE and immunoblotting assays were performed as described by Pan [41], with slight modifications. After electrophoretic transfer from the polyacrylamide gels, the nitrocellulose membranes were blocked and incubated overnight at 4 °C in antiserum specific to SAI (1:4000), CWI (1:2000) and SuSy (1:5000), which were diluted with Tris-buffered saline (TBS; 10 mM Tris-HCl, 150 mM NaCl) +0.05% Tween 20 +3% bovine serum albumin (BSA). The membranes were then washed in TBS together with Tween 20 (TBST; 10 mM Tris-HCl, 150 mM NaCl +0.05% Tween 20) three times, incubated for 45 min at room temperature together with goat anti-rabbit IgG-alkaline phosphatase conjugate, diluted 1000-fold with TBST₂ (50 mM Tris-HCl, 150 mM NaCl +0.1% Tween 20 +1% BSA) and then treated with secondary antibodies. After being washed three times in TBST₂ and TBS, these membranes were stained with a BCIP/NBT Kit (ComWin Biotech Co., Ltd., China). β-Actin was used as a reference gene.

Collection and Analysis of Phloem Exudates

Phloem exudates were collected from the stems of 60-day-old seedlings as described by King and Zeevaart [47]. The stem was cut near the base, washed with ultrapure water, and inserted into a solution of 20 mM EDTA (pH 7.5). The detached plant was kept in darkness at 30 °C and 95% humidity for 1 to 2 h and then transferred to ultrapure water for collection for 4-5 h. Phloem exudates were lyophilized and stored at -80 °C. The sucrose, glucose, fructose, sorbitol, stachyose and raffinose contents of the exudates were evaluated by using HPLC with an evaporative light-scattering detector (ELSD). The samples were analyzed in 10 µl increments with water amide (xBridge 3.5 µm, 4.6 mm × 150 mm, USA); the mobile phase consisted of 70% (v/v) acetonitrile and 0.1% (v/v) ammonium hydroxide; the flow rate was 1 ml min⁻¹; the temperature was 25 °C; the drift tube temperature was 85 °C; the nitrogen flow rate was 2.0 l min⁻¹; and the gain value was 2.

Transcriptome Sequencing and Annotation

Ten RNA libraries generated from developing leaves and storage roots of KU50, Arg7 and W14 plants were sequenced using an Illumina High-Seq 2000 system, with approximately 100 bp reads. After preprocessing, the mRNA sequence reads from 10 samples were mapped to the draft genome sequence of AM560-2. Approximately 20,000 unigenes were annotated. The genes involved in the development of all RNA-seq reads of the 10 samples were uploaded to the NCBI SRA under the following accession numbers: SRX551093, SRX553797, SRX553799, SRX553800, SRX553801, SRX553802, SRX553803, SRX553804, SRX553805, and SRX553807.

Chemicals

Sucrose, glucose, fructose, sorbitol, stachyose, and raffinose were purchased from TCI (Japan). The protein ladder (Fermentas, Canada), SYBR Premix ExTaq (TaKaRa, Bio Inc., Japan), ReverAid First-strand cDNA Synthesis Kit (Fermentas, Canada), and glutaraldehyde (TED, Pella, Inc., USA) were obtained from the indicated suppliers. All the other chemicals used were purchased from Sigma (USA).

Abbreviations

CF, carboxyfluorescein; CC, companion cell; CFDA, nonfluorescent 6(5) carboxyfluorescein diacetate; CLSM, confocal laser scanning microscopy; CW, cell wall; CWI, cell wall acid invertase; DAPE, days after plant emergence; GFP, green fluorescent protein; HT, hexose transporter; NI, neutral invertase; SAI, soluble acid invertase; SEL, size exclusion limit; SE, sieve element; SFR, secondary fibrous root; SuSy, sucrose synthase; SUT, sucrose transporters; PC, parenchyma cell; PD, plasmodesma; PFR, primary fibrous root; PPH, primary phloem; PPU, pore-plasmodesma unit; S, starch granule; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; TBST, Tris-buffered saline with Tween 20; TEM, transmission electron microscopy; UDPG, uridine diphosphate glucose; V, vacuole; Suc, sucrose; Glc, glucose; Fru, fructose; CATAS, Chinese Academy of Tropical Agriculture Sciences

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All the data generated during this study are included within this article and its supplementary information files. **Competing interests**

The authors declare that they have no competing interests.

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Authors' contributions

WW conceived and designed the project. KP and CL performed the experiments, KP wrote the manuscript, and PN and MH participated in the preliminary preparation of the experiments. XZ analyzed the RNA-seq data, and XC revised the manuscript. All the authors have read and approved the final manuscript.

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References

1. Frommer WB, Sonnewald U: Molecular analysis of carbon partitioning in solanaceous species. *J Exp Bot* 1995, 46:587-607.
2. Patrick JW: Phloem unloading, sieve element unloading and post-sieve element transport. *Annu Rev Plant Physiol Plant Mol Biol* 1997, 48:191-222.
3. Ishimaru T, Hirose T, Matsuda T, Goto A, Takahashi K, Sasaki H, Terao T, Ishii R, Ohsugi R, Yamagishi T: Expression patterns of genes encoding carbohydrate-metabolizing enzymes and their relationship to grain filling in rice (*Oryza sativa* L.): comparison of caryopses located at different positions in a panicle. *Plant Cell Physiol* 2005, 46(4):620-628.
4. Herbers K, Sonnewald U: Molecular determinants of sink strength. *Curr Opin Plant Biol* 1998, 1:207-216.
5. Oparka KJ, Roberts AG, Boevink P, Cruz SS, Roberts I, Pradel KS, Imlau A, otlizky GK, Kotlizky G, Sauer N *et al*: Simple, but not branched, plasmodesmata allow the nonspecific trafficking of proteins in developing tobacco leaves. *Cell* 1999, 97:743-754.
6. Roberts AG, Boevink P, Roberts AG, Sauer N, Reichel C, Oparka KJ: Dynamic changes in the frequency and architecture of plasmodesmata during the sink-source transition in tobacco leaves. *Protoplasma* 2001, 218:31-44.
7. Roitsch T, Balibrea ME, Hofmann M, Proels R, Sinha AK: Extracellular invertase: key metabolic enzyme and PR protein. *J Exp Bot* 2003, 54(382):513-524.
8. Patrick JW, C.E. O: Compartmentation of transport and transfer events in developing seeds. *J Exp Bot* 2001, 52:551-564.
9. Zhang LY, Peng YB, Pelleschi-Travier S, Fan Y, Lu YF, Lu YM, Gao XP, Shen YY, Delrot S, Zhang DP: Evidence for apoplastic phloem unloading in developing apple fruit. *Plant Physiol* 2004, 135(1):574-586.
10. Zanon L, Falchi R, Santi S, Vizzotto G: Sucrose transport and phloem unloading in peach fruit: potential role of two transporters localized in different cell types. *Physiol Plant* 2015, 154(2):179-193.
11. Viola R, Roberts AG, Haupt S, Gazzani S, Hancock RD, Marmioli N, Machray GC, K..J. O: Tuberization in potato involves a switch from apoplastic to symplastic phloem unloading. *Plant Cell* 2001, 13:385-

12. Wang X, You H, Yuan Y, Zhang H, Zhang L: The cellular pathway and enzymatic activity for phloem-unloading transition in developing *Camellia oleifera* Abel. Fruit. *Acta Physiologiae Plantarum* 2018, 40(2).
13. Godt D, Roitsch T: The developmental and organ specific expression of sucrose cleaving enzymes in sugar beet suggests a transition between apoplastic and symplasmic phloem unloading in the tap roots. *Plant Physiol Biochem* 2006, 44(11-12):656-665.
14. Baroja-Fernandez E, Munoz FJ, Montero M, Etxeberria E, Sesma MT, Ovecka M, Bahaji A, Ezquer I, Li J, Prat S *et al*: Enhancing sucrose synthase activity in transgenic potato (*Solanum tuberosum* L.) tubers results in increased levels of starch, ADPglucose and UDPglucose and total yield. *Plant Cell Physiol* 2009, 50(9):1651-1662.
15. Braun DM, Wang L, Ruan YL: Understanding and manipulating sucrose phloem loading, unloading, metabolism, and signalling to enhance crop yield and food security. *J Exp Bot* 2014, 65(7):1713-1735.
16. Zhang XY, Wang XL, Wang XF, Xia GH, Pan QH, Fan RC, Wu FQ, Yu XC, Zhang DP: A shift of Phloem unloading from symplasmic to apoplastic pathway is involved in developmental onset of ripening in grape berry. *Plant Physiol* 2006, 142(1):220-232.
17. Nie P, Wang X, Hu L, Zhang H, Zhang J, Zhang Z, Zhang L: The predominance of the apoplastic phloem-unloading pathway is interrupted by a symplasmic pathway during Chinese jujube fruit development. *Plant Cell Physiol* 2010, 51(6):1007-1018.
18. Zhang HH, Li YF, Nie PX, Wang HY, Zhang LY: Phloem unloading pathway of photosynthates and sucrose-metabolizing enzymes activities in *Vaccinium corymbosum* Fruit. *Scientia Silvae Sinicae* 2016, 53:40-48.
19. Chen C, Yuan Y, Zhang C, Li H, Ma F, Li M: Sucrose phloem unloading follows an apoplastic pathway with high sucrose synthase in *Actinidia* fruit. *Plant Sci* 2017, 255:40-50.
20. Jansson C, Westerbergh A, Zhang J, Hu X, Sun C: Cassava, a potential biofuel crop in (the) People's Republic of China. *Appl Energ* 2009, 86:S95–S99.
21. Teerawanichpan P, Lertpanyasampatha M, Netrphan S, Varavinit S, Boonseng O, Narangajavana J: Influence of Cassava Storage Root Development and Environmental Conditions on Starch Granule Size Distribution. *Starch - Stärke* 2008, 60(12):696-705.
22. Medina RD, Faloci MM, Gonzalez AM, Mroginski LA: In vitro cultured primary roots derived from stem segments of cassava (*Manihot esculenta*) can behave like storage organs. *Ann Bot* 2007, 99(3):409-423.
23. Zhang P, Bohl-Zenger S, Pounti-Kaerlas J, Potrykus I, Gruissem W.: Two cassava promoters related to vascular expression and storage root formation. *Planta* 2003, 218:192-203.
24. G.B. Cabral LJCBC, B.A. Schaal: The formation of storage roots in cassava. Brazil: EMBRAPA-Genetic Resources and Biotechnology Brasilia-DF; 1998.

25. Patrick JW, Offler CE: Post-sieve element transport of photoassimilates in sink regions. *J Exp Bot* 1996, 47:1165–1177.
26. Oparka KJ, Cruz SS: The great escape, phloem transport and unloading of macromolecules. *Annu Rev Plant Physiol Plant Mol Biol* 2000, 51:323-347.
27. Maule AJ: Plasmodesmata: structure, function and biogenesis. *Curr Opin Plant Biol* 2008, 11(6):680-686.
28. Eom JS, Choi SB, Ward JM, Jeon JS: The mechanism of phloem loading in rice (*Oryza sativa*). *Mol Cells* 2012, 33(5):431-438.
29. Zhang H-p, Wu J-y, Tao S-t, Wu T, Qi K-j, Zhang S-j, Wang J-z, Huang W-j, Wu J, Zhang S-l: Evidence for Apoplasmic Phloem Unloading in Pear Fruit. *Plant Molecular Biology Reporter* 2014, 32(4):931-939.
30. Sturm A, Sebkova V, Lorenz K, . , Hardegger M, Lienhard S, Unger C: Development and organ-specific expression of the genes for sucrose synthase and three isoenzymes of acid β -fructofuranosidase in carrot. *Planta* 1995, 195:601-610.
31. Usuda H, Rouhier H, Demura T, Fukuda H: Development of sink capacity of the "storage root" in a radish cultivar with a low ratio of "storage root" to shoot. *Plant Cell Physiol* 1999, 40:1210–1218.
32. Konishi T, Ohmiya Y, Hayashi T: Evidence that sucrose loaded into the phloem of a poplar leaf is used directly by sucrose synthase associated with various beta-glucan synthases in the stem. *Plant Physiol* 2004, 134(3):1146-1152.
33. Ruan YL, Patrick JW, Bouzayen M, Osorio S, Fernie AR: Molecular regulation of seed and fruit set. *Trends Plant Sci* 2012, 17(11):656-665.
34. Katz E, Boo KH, Kim HY, Eigenheer RA, Phinney BS, Shulaev V, Negre-Zakharov F, Sadka A, Blumwald E: Label-free shotgun proteomics and metabolite analysis reveal a significant metabolic shift during citrus fruit development. *J Exp Bot* 2011, 62(15):5367-5384.
35. Moscatello S, Famiani F, Proietti S, Farinelli D, Battistelli A: Sucrose synthase dominates carbohydrate metabolism and relative growth rate in growing kiwifruit (*Actinidia deliciosa*, cv Hayward). *Scientia Horticulturae* 2011, 128(3):197-205.
36. Li M, Feng F, Cheng L: Expression patterns of genes involved in sugar metabolism and accumulation during apple fruit development. *PLoS One* 2012, 7(3):e33055.
37. Oparka KJ, Duckett CM, Prior DAM, Fisher DB: Real-time imaging of phloem unloading in the root tip of *Arabidopsis*. *Plant J* 1994, 6:759-766.
38. Stadler R, Wright KM, Lauterbach C, Amon G, Gahrtz M, Feuerstein A, Oparka KJ, Sauer N: Expression of GFP-fusions in *Arabidopsis* companion cells reveals non-specific protein trafficking into sieve elements and identifies a novel post-phloem domain in roots. *Plant J* 2005, 41(2):319-331.
39. Patrick JW: Does Don Fisher's high-pressure manifold model account for phloem transport and resource partitioning? *Front Plant Sci* 2013, 4:184.

40. Kempers R, Ammerlaan A, van Bel AJE: Symplasmic constriction and ultrastructural features of sieve element/companion cell complex in the transport phloem of apoplasmically and symplasmically phloem loading species. *Plant Physiol* 1998, 116:271-278.
41. Pan Q-H, Li M-J, Peng C-C, Zhang N, Zou X, Zou K-Q, Wang X-L, Yu X-C, Wang X-F, Zhang D-P: Absciscic acid activates acid invertases in developing grape berry. *Physiologia Plantarum* 2005, 125(2):157-170.
42. Schaffer AA, Sagee O, Goldschmidt EE, R. G: Invertase and sucrose synthase activity, carbohydrate status and endogenous IAA levels during citrus leaf development. *Plant Physiol* 1987, 69:151-155.
43. Cruz JL, Mosquim PR, Pelacani CR, Araujo WL, F.M. D: Carbon partitioning and assimilation as affected by nitrogen deficiency in cassava. *Photosynthetica* 2003, 41:201-207.
44. Miller GL: Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Anal Chem* 1959, 31:426-428.
45. Miron D, Schaffer AA: Sucrose phosphate synthase, sucrose synthase and invertase activities in developing fruit of *Lycopersicon esculentum* Mill. and sucrose accumulation in *Lycopersicon hirsutum* Humb. *Plant Physiol* 1991, 95:623-627.
46. M.M. B: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anual Biochem* 1976, 72:248-254.
47. King RW, Zeevaart JAD: Enhancement of phloem exudation from cut petioles by chelating agents. *Plant Physiol* 1974, 53:96-103.

Tables

Table 1
The sugar components of the phloem sap of cultivated Arg7 cassava

Sugars	Samples	Mean Content of sugars (mg·mL ⁻¹)	Percentage (%)
Sucrose	Arg7	5.687 ± 0.011	54.6
Glucose	Arg7	2.370 ± 0.004	22.7
Fructose	Arg7	2.342 ± 0.025	22.5

Table 2
Plasmodesmata density in the cell walls of SE-CC and PC cells in the late storage and fibrous roots of cultivated Arg7 cassava

Plasmodesmal density (Number per μm)			
SE-CC	SE-CC/PC	PC/PC	
Primary fibrous root	0.013 $\pm$ 0.021	0.007 $\pm$ 0.019	0.030 $\pm$ 0.032
Secondary fibrous root	0.008 $\pm$ 0.007	0.001 $\pm$ 0.001	0.049 $\pm$ 0.022
Elongation stage of tuberous root	0.033 $\pm$ 0.026	0.022 $\pm$ 0.015	0.095 $\pm$ 0.023
Middle stage of storage root	0.040 $\pm$ 0.024	0.027 $\pm$ 0.018	0.147 $\pm$ 0.035
Later stage of storage root	0.032 $\pm$ 0.025	0.021 $\pm$ 0.021	0.100 $\pm$ 0.065
Plasmodesmata were counted on five scopes that selected from six ultrathin sections for each sample, and the value above was the means $\pm$ SE (individuals).			

Table 3
Basic information of the cassava genes of sucrose synthase, cell wall invertase and sucrose transporters

Gene	JGI ID	Location	Transcript (bp)	CDS (bp)	Protein (aa)
	v6.1				
<i>SuSy1</i>	Manes.03G044400	Chr03:3583270..3588587 reverse	2752	2421	806
<i>SuSy2</i>	Manes.03G198900	Chr03:28057541..28064463 reverse	2598*	2241	746
<i>SuSy3</i>	Manes.01G221900	Chr01:30850003..30857026 reverse	2867	2436	811
<i>SuSy4</i>	Manes.16G090600	Chr16:24754130..24759053 forward	2750	2421	806
<i>SuSy5</i>	Manes.02G081500	Chr02:6080529..6085146 reverse	2881	2526	841
<i>SuSy6</i>	Manes.14G107800	Chr14:8748090..8752577 forward	3026	2739	912
<i>SuSy7</i>	Manes.01G123800	Chr01:24140021..24144607 reverse	2783	2526	841
<i>CWI1</i>	Manes.03G049200.1	Chr03:4223184..4226988 forward	2401	1779	592
<i>CWI2</i>	Manes.08G027200.1	Chr08:2431570..2433947 forward	1851	1719	572
<i>CWI3</i>	Manes.11G025400.1	Chr11:2236114..2240122 reverse	2079	1767	588
<i>CWI4</i>	Manes.04G140500.1	Chr04:26727894..26729547 forward	1369	1278	425
<i>CWI5</i>	Manes.008G027200.1	Chr08:2431570..2433947 forward	1851	1719	572
<i>CWI6</i>	Manes.0009G053500.1	Chr09:7064243..7068843 reverse	1719	1719	572
<i>SUT1-1</i>	Manes.18G099400.1	Chr18:8603890..8607266 forward	2193	1785	594
<i>SUT1-2</i>	Manes.02G190300.1	Chr02:15510673..15513298 forward	2189	1542	513
<i>SUT4-1</i>	Manes.18G054200.1	Chr18:4548075..4559586 forward	3720	1497	498
<i>SUT4-2</i>	Manes.05G186600.1	Chr05:25724747..25733136 forward	3144	1491	496
<i>SUT2-2</i>	Manes.05G099000.1	Chr05:8333636..8347861 reverse	3028	1827	608

Figures

Figure 1

The Biomass accumulation, anatomical structure and substance transported in the storage root of cassava A, The curves of biomass accumulation of cassava storage root in three growth stages. The value for fresh and dry weight is the mean of three replicates $\pm$ SD; B, C, Transverse section anatomy of the storage root; D, HPLC-ELSD chromatograms of the cassava phloem exudates.

Figure 2

Ultrastructure of phloem cells in storage roots of cassava shown high dense plasmodesmata A, B, C, SE and CC cells in the storage root at the early, middle and late stages respectively; B, A partial enlargement of E; D, plasmodesmata between a PC and adjacent CC cell; E, F, A larger scope in the transverse section of the phloem cell group in the storage root at the middle and late stages; arrows in F indicated thickened cell wall; G, H, I, Transverse section of the secondary xylem cells in the storage root, arrows indicated abundant plasmodesmata between two xylem PCs at the early, middle and late stages respectively. Bars in A, B, C, D, G, H, I, 0.5 μm ; E, 5 μm ; F, 10 μm .

Figure 3

Carboxyfluorescein (CF) and $[^{14}\text{C}]$ Suc tracing confirm a predominantly symplasmic phloem unloading pathway in cassava A, B, C, early stage root; D, E, F, middle stage root; G, H, I, late stage root; J, primary fibrous root; A-J, under 488nm excitation wave; A1, B1, C1, J2; bright-field microscopy; D1, E1, F1, J1, 405nm excitation wave and bright-field microscopy; A, D, G, A1, D1, cortex; B, E, H, B1, E1, secondary phloem; C, F, I, C1, F1, secondary xylem; K, CF fluorescence intensity of different development roots; L, CF fluorescence intensity of different sampling times; M, The difference of distribution density of $[^{14}\text{C}]$ Suc in cross sections of developmental roots at three stages. Arrows indicate the vascular cambium.

Figure 4

The expression and their enzymatic activities of genes involved in post phloem unloading in cassava A, B, Relative expression difference of CWI and SuSy measured by Q-PCR; C, Heat Map Comparisons of Differential Gene Expression of CWIs, SuSs and SUTs; D The expression profiling of SUTs (SUT1, SUT2 and SUT4) in leave and storage roots measured by Q-PCR; E, The expressed protein levels of SuSy, CWI and soluble acid invertase (SAI) assessed by using immunoblotting, the blot images were cropped,

uncropped images were uploaded as additional file; F, Enzymatic Activities of SuSy (triangles), CWI (squares) and SAI (circles). a, b and c denote differentially significant level at $P < 0.05$.

Figure 5

Sub-cellular location of CWI presented its enrichment in early fibrous root using immunogold particle labelling A-D, Immunogold labelling of CWI in primary fibrous root with an antibody directed against

cassava acid invertases. E-G, Immunogold labelling of CWI in storage root with an antibody directed against cassava acid invertases. Bars A, 2 μm ; E, 10 μm ; B, C, D, F, G, H: 200 nm.

Figure 6

A model for sucrose symplasmic phloem unloading in the storage root of cassava Long-distance transported sucrose (SUC) in the SE enters into the CC and PC predominantly through plasmodesmata (PD), with except of a few SUC that are transported by SUTs. These SUC molecules are primarily catabolized by SuSy into fructose and nucleoside diphosphate-glucose (UDPG), which enter starch synthesis in the amyloplast. Occasionally, SUC can be stored temporarily in vacuoles by SUT or HT in a hexose form. This structured energy-saving model accounts for the highly efficient starch accumulation observed in cassava storage roots.

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