Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Stem Vacuole-targeted Sucrose Isomerase Enhances Sugar Accumulation in Sorghum

Guoquan Liu (g.liu2@uq.edu.au)
Centre for Crop Science, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland; https://orcid.org/0000-0001-5979-785X

Yan Zhang
University of Queensland - Saint Lucia Campus: The University of Queensland

Hao Gong
University of Queensland - Saint Lucia Campus: The University of Queensland

Shan Li
The University of Queensland

Yunrong Pan
University of Queensland - Saint Lucia Campus: The University of Queensland

Christopher Davis
The University of Queensland

Hai-Chun Jing
Chinese Academy of Agricultural Sciences

Luguang Wu
The University of Queensland

Ian D. Godwin
The University of Queensland

Research

Keywords: Isomaltulose, Sorghum, sucrose isomerase, sugar accumulation, renewable energy, photosynthesis, sugarcane, genetic engineering

Posted Date: December 31st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-135798/v1

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

Version of Record: A version of this preprint was published on March 1st, 2021. See the published version at https://doi.org/10.1186/s13068-021-01907-z.

Abstract

Background: Sugar accumulation is critically important in determining sugar crop productivity. However, improvement in sugar content has been stagnant among sugar crops for decades. Sorghum, especially sweet sorghum with high biomass, has shown great potential for biofuel. In this study, sorghum was investigated as a C_4 diploid model for crops with more complicated genomes such as maize and sugarcane. To enhance sugar accumulation, the sucrose isomerase (*SI*) gene, driven by stem-specific promoters (*A2* or *LSG*) with a vacuole-targeted signal peptide, was transformed into the sorghum inbred line (Tx430).

Results: The study demonstrated that transgenic lines of grain sorghum, containing 50-60% isomaltulose, accumulated sevenfold (804 mM) more total sugar than the control Tx430 did (118 mM) in stalks. Subsequently, the elite engineered lines (A5, and LSG9) were crossed with sweet sorghum (R9188, and Rio). Total sugar contents (over 750 mM), were significantly higher in F_1, and F_2 progenies than the control Rio (480 mM). The sugar contents of the engineered lines (over 750 mM), including T_0, T_1, F_1, and F_2, are higher than that of the field-grown sugarcane (normal range 600-700 mmol/L). Additionally, physiological characterization demonstrated that the superior progenies had notably higher rates of photosynthesis, sucrose transport, and sink strength than the controls.

Conclusions: The genetic engineering approach has significantly enhanced total sugar content in grain sorghum (T_0, and T_1) and hybrid sorghum (F_1, and F_2), demonstrating that sorghum can accumulate sugar contents as high or higher than sugarcane. This research puts sorghum in the spotlight and frontier as a biofuel crop, particularly as it is a shorter duration crop. The substantial increase in sugar content would lead to enormous financial benefits for industrial utilization. This study could have a substantial impact on renewable bioenergy. More importantly, our results demonstrated that the phenotype of high sugar accumulation is inheritable and shed light on improvement for other sugar crops.

Background

Sugar yield is a key determinant of economic sustainability for sugar crops. In recent decades, improvement of sugar yield has been achieved almost entirely through increased biomass [1-3], despite the higher commercial value and higher heritability of increased sugar content [4]. Recent studies on the manipulation of plant genes, which are involved in sugar metabolism, have been unsuccessful for increasing sugar accumulation in sugar crops [5-7]. There is significant pathway redundancy in elite cultivars to buffer against increases in stored sucrose levels through the manipulation of a single gene [8]. Multiple mechanisms appear to contribute to the upper limit of sugar concentration, including regulation in signal transduction from specific (e.g. sucrose) or broad (e.g. osmotic) sensors, thermodynamic limitations (e.g. leakage of sucrose through storage compartment membranes), or energetic limitations (e.g. continuous 'futile' cycle of sucrose cleavage and synthesis within the storage pool) [9-12].

Among sugar crops, sugarcane accounts for almost 80% of global sugar production. Sweet sorghum has demonstrated the huge potential to be multiple sources of energy, food and animal feed and could be a substitute for sugarcane to produce biofuel [13, 14]. It grows quickly in adverse stress conditions of marginal lands in tropical, subtropical and temperate zones. It is a C_4, drought tolerance, high biomass, and high water use efficiency plant that produces a stalk up to five meters tall, accumulating sucrose (α-D-glucopyranosyl-1,2-D-fructofuranose). However, current sweet sorghum varieties, producing comparatively low sugar content (around 500 mmol/L), urgently requires breeders to improve sugar accumulation in stalks for biofuel [13].

Some bacteria have the ability to convert sucrose to isomaltulose (α-D-glucopyranosyl-1,6-D-fructofuranose) [15]. Unlike sucrose, isomaltulose can not be digested by invertases [16] nor be metabolized by many microbes, including the predominant oral microflora, presenting benefit in many foods as an acariogenic sweetener [17]. However, isomaltulose can be digested by humans with the same glucose/fructose as primary products and have the same final energy value as sucrose. Interestingly, the first step of digestion involves an intestinal disaccharidase rather than salivary invertase, which slows down the isomaltulose digestion. The slow process results in less fluctuation of glucose and insulin concentration in blood [18]. Therefore, isomaltulose has a growing demand as a stable, slowly digestible, acariogenic, non-hygroscopic sugar in the modern world [18-20]. Futhermore, isomaltulose has an accessible carbonyl group, which makes it attractive as a renewable starting material for manufacture [21]. The application is currently limited due to the high cost of isomaltulose production through fermentation [22, 23].

Isomaltulose can be produced through expression of the sucrose isomerase (SI) gene without any cofactor or substrate in plants[24]. Compared to sucrose, isomaltulose is very slowly metabolized and can not be transported in plants [25], hence the site of isomaltulose production becomes a storage. Exogenous application of isomaltulose triggers some plant sugar sensing mechanisms and changes gene expression profiles differently from sucrose [25, 26]. Previously, it has been demonstrated that the efficient conversion of sucrose into the non-metabolized isomer (palatinose) is disruptive or lethal for plant development [27]. The tuber-specific expression of the apoplasm-targeted *SI* allowed the partial conversion of sucrose to isomaltulose in potato, but the total non-structural carbohydrate content was decreased [28, 29]. Significant progress has been made in last two decades. Recent studies have indicated that the N-terminal pro-peptide (NTPP) fragment from sweet potato sporamin can deliver various proteins to the sugarcane vacuole, but low pH and high protease activity make the vacuole environment hostile [30]. With the availability of strong stem-specific promoters, a highly efficient *SI* gene, and silencing motifs, high concentration of isomaltulose (up to 483 mM or 81% of total sugars) has been successfully achieved in sugarcane [15, 24, 31]. To the best of our knowledge, a similar investigation has not been reported in other biomass species.

In the storage parenchyma cells of mature stems of sweet sorghum, the sugar storage vacuole occupies about 90% of the symplast and 80% of the total tissue space. The vacuole stores a correspondingly large proportion of sucrose, which can accumulate up to 500 mM fresh weight (FW). Our objective was to

determine the effect of directing *SI* activity into the sucrose-storage cell compartment to improve sugar accumulation in sorghum. We hypothesized that high isomaltulose concentration could be accumulated in stems of engineered lines and lead to high sugar content in sorghum especially in sweet sorghum. Since the efficient transformation system of grain sorghum (Tx430) has been well established in our lab [32]. We strategically avoid transforming sweet sorghum directly due to its highly recalcitrance to tissue culture and transformation [33]. However, investigation on hybrids of (grain x sweet) sorghum could provide insightful information on sugar accumulation in commercial hybrids sorghum and sweet sorghum.

Results

Accumulating substantial isomaltulose in transgenic lines

Twenty independent transgenic lines were demonstrated to contain the *sucrose isomerase* (*SI*) gene using the polymerase chain reaction (PCR) analysis. Among these lines, 16 showed detectable isomaltulose levels by high-performance liquid chromatography (HPLC) in stalk tissues (Fig. 1a). Isomaltulose was accumulated up to 472 mM in stalk juice, which was four-fold higher than the total sugar content of the untransformed Tx430. The isomaltulose concentrations were substantially variable among lines (Fig. 1b). Similar patterns were observed in two transgenic populations driven by different promoters of A1 or LSG2 (Fig. 1b).

Because the UQ68J *SI* gene is highly specific for producing isomaltulose [24], trehalulose concentrations were generally below 4% of the isomaltulose concentrations in the corresponding internodes (Table S1). Transgenic lines were morphologically similar and equivalent to the untransformed control Tx430 in the glasshouse (Fig. S1). Transgenic plants flowered at a similar time as the control Tx430 (Fig. S1).

The roots and leaves were tested from all the transgenic lines, isomaltulose concentrations were below 5 mM in roots. Isomaltulose concentration increased with age in leaves to a maximum of about 20 mM, which is consistent with the expression patterns for the ‘stem-dominant’ promoters [34, 35]. However, *SI* enzyme activity could not be detected from cell extracts of transgenic roots or leaves. The negative effect on sorghum growth was not observed due to the small amount of isomaltulose accumulation in roots and leafs (Fig. S1). Despite substantial isomaltulose accumulation in stalks, SI enzyme activity was below the detection threshold in cell extracts, indicating a short half-life of this protein after delivery into the acidic/proteolytic sucrose storage vacuoles.

Enhancing total sugar content in grain sorghum

The total sugar content has been significantly increased in 20 transgenic lines compared to the untransformed control except two lines (L2, and L24), regardless of which promoter used (A1 or LSG2) (Fig. 2). The total sugar content in internode number 4 of most lines was in a range of 600-1,000 mM, which was equivalent to five to eight folds of the untransformed control. These concentrations were

comparable or even higher than that of the field-grown sugarcane (normally around 600-700 mM). The predominant components of sugar were sucrose and isomaltulose in transgenic lines, however, their glucose and fructose contents were similar to the parent (Fig. 2).

Unexpectedly, some transgenic lines such as L4 and A2 had no detectable isomaltulose but sucrose contents were enhanced five-fold to eightfold when compared to the control Tx430 (Fig. 2), regardless of the promoter used.

Accumulating high sugar contents across internodes of transgenic stalk

Three transgenic lines, designated A2, A5 (both driven by A1 promoter) and L9 (driven by LSG2 promoter), with high-sugar content were selected for further characterization on sugar profiles in developmental stages. Lines A5 and L9 accumulated high levels of isomaltulose down the stalk up to 691 mM in juice from mature internodes (Fig. 3c, d). Compared to the control Tx430, the transgenic lines with high yields of isomaltulose did not show commensurable reduction but enhanced levels in stored sucrose concentrations in most internodes (Fig. 2).

Surprisingly, isomaltulose could not be detected in any A2 tissues including all internodes of the stalks, but sucrose content accumulated eightfold higher than the level in the control Tx430 (Fig. 3b).

Further investigation on T_1 progenies of A2, A5, and L9 has been performed and focused on heritability of high sugar content. Twelve samples of each progeny have been analysized. T_1 progenies of L9 outperformed counterparts of A2, and A5 in terms of high heritability and fertility. Because no isomaltulose was detected in A2, the phenotype of high sugar content did not transmit to the next generation. T_1 progenies of A5 did not display full fertility the same as the T_0 generation. The results of L9 T_1 progeny samples were very promising and displayed high heritability of high sugar content (up to 896 mM in stalk). Positive samples have accumulated much higher sugar content than negative samples (Nil-LG9) and the control (Fig. S4).

Real-time PCR of T_1 generation

Quantitative real-time PCR was deployed to determine the *SI* gene expression in different transgenic lines. The elite transgenic lines, accumulating high isomalutlose, and high total sugar, A5 and L9 were selected. Line L2 , with poor isomaltulose accumulation, was chosen for comparison. Non-transgenic Tx430 was used as the wild-type control. The RT-PCR results revealed that A5 and L9 displayed a relatively high levels of *SI* gene expression, which is in agreement with their high level of isomaltulose accumulation. L2 showed comparatively low levels of *SI* gene expression, which aligned with the low level of isomaltulose accumulation. As expected, no *SI* gene transcript was detected in stalks of the wild-type Tx430 (Fig. 4).

Inheriting high-sugar contents in F_1 hybrids

The elite sweet sorghum cultivar R9188, and Rio were selected as female lines for crossing due to its advantages of large biomass and high-sucrose content in stalks. Transgenic lines A5, and L9 were chosen as male line because of their superior performance on isomaltulose accumulation and high total sugar content. Crosses were performed with the male-sterile lines of R9188, and Rio. However, transgenic line L9 displayed the normal development in reproductive organs compared to transgenic line A5 which is partially sterile. Rio had stronger and taller stem than R9188 had in the glasshouse. Hybrid seeds were harvested from successful crossing.

Thirty seeds of hybrids of Rio X L9 were sown in pots along with the controls of Rio, R9188, and Tx430 in the glasshouse. The sweet sorghum cultivar R9188 is another version of Rio with an extra dwarf gene, hence almost 50 cm shorter. Germination and early plant growth were similar to the controls, except the progenies of one hybrid seed which did not germinate. Sugar profiles showed that among 29 progenies of the F_1 generation, 15 progenies were isomaltulose positive (51.7%) and 14 had no detectable isomaltulose (48.3%), close to the predicted 1:1 ratio (Fig. 5), indicating hybrid seeds inherited the *SI* gene sexually from the parent L9 to its progenies.

Within the isomaltulose positive group, three progenies (10.3%) converted almost all sucrose into IM; six (20.6%) converted more than 65% of sucrose; two (6.9%) converted about 33% of sucrose; four (13.8%) had less than 1% sucrose converted (Fig. 5). Notably, the enhancement of total sugar content was observed in most isomaltulose positive groups (Fig. 5). The increase of total sugar content in the positive group was on average of 37% when compared to the sweet sorghum Rio. The increase ranged from 484% to 932% if compared with the grain sorghum Tx430, which is in agreement with the results of the first transgenic generation (Fig. 2).

Another hybrid population of R9188 X L9 were planted as well. It showed similar pattern as the population of Rio X L9. Among 26 F_1 population, 12 of them are positive for sucrose isomerse gene gene (Fig. S5). The highest total sugar content at 764 mM was measured in F_1 LR920 line and the best isomaltulose content at 565 mM was detected in the F_1 LR99 line. By comparison, remarkably higher total sugar contents were monitored in positive SI lines (on average 538 mM) than negative *SI* lines (on average 342), which means the sugar content has been improved 57.3% because of the *SI* gene. While the average sugar content in the sweet sorghum R9188 and grain Tx430 were 261 and 93 mM respectively. The detail of results was shown in (Table S2).

Inheriting high-sugar contents in F_2 populations

Based on isomaltulose production, total sugar content, stalk biomass, and seed production, F_1 (Rio X L9) progenies LR3, 19 and 20 were selected for further characterization. With the parental controls of sweet

sorghum Rio, progeny 24, a null segregant with comparative high sugar content was also selected as a hybrid control. Seeds were produced by self-pollination of the selected progenies.

Sugar profiles of the isomaltulose positive plants showed that they inherited the phenotype of both isomaltulose production and high-sugar accumulation (Fig. 6). In all three *SI* positive progenies, isomaltulose accumulated at high levels in all internodes along the stalk, plus sucrose stored at comparable levels (total sugar content up to 812.2 mM), resulting in enhancement by up to 69% in total sugar content compared to either the parental (480.6 mM) or the hybrid control (470.9 mM) (Fig. 6).

Increasing sugar content and water content in stalk juice

Carbon partitioning into sugars and fiber was estimated in the selected F_2 progenies and controls. There was more sugar per unit fresh weight (FW) in all internodes of the tested high-sugar progenies along the stalk than the controls (Fig. 7a). In the sweet sorghum Rio and hybrid control P24, the water content was typically constant around 75% along the stalk with a slight increase in the bottom internodes, however, in the stalks of the three high-sugar progenies, water content was significantly lower at around 70% (Fig. 7b). Moreover, there were no significant changes in the fiber content among all samples, which was around 11% in internode tissues (Fig. 7). These results indicated that instead of alteration of fiber and sugar, assimilation was improved and more sugar was stored in the progenies P3, P19, and P20 than the controls. Therefore, the commercially important traits of higher sugar concentration in juice from the selected progenies are underpinned by increasing the storage of photosynthate as sugars and decreasing water content in the mature stalk.

Increasing photosynthesis in high-sugar hybrid lines

Two key physiological characteristics, including photosynthetic electron transport and CO_2 assimilation, were examined to understand the mechanisms of enhanced sugar accumulation. Rates of leaf electron transport and CO_2 assimilation of the progenies P3, P19, and P20 were higher than the controls Rio, Tx430 and hybrid P24. The increases in electron transport rates measured by chlorophyll fluorescence (reflecting photosynthetic efficiency in photosystem II) and in CO_2 assimilation rates were in the range 20% − 35% improved relative to controls at a photosynthetically active radiation (PAR) level. Light response curves from fully expanded leaf 2 are shown as an example (Fig. 8). Also, the senescence of the bottom leaves on each stalk of the high-sugar progenies was typically delayed by 2-3 weeks, resulting in leaf functional extension in photosynthesis for most of the growth period.

Improving sugar transport in source leaves and sink tissues

Rate of proton gradient-dependent sucrose transport into plasma membrane vesicles (PMV) is an indicator for sucrose uploading in the source leaves [36]. The isolated PMVs from leaf 2 and 3 of the

selected high-sugar progenies were 20% − 40% higher than that of controls (null segregant P24, parents Rio and Tx430), indicating the driving power of loading assimilation for transport was improved (Fig. 9a) in the source leaves of the high-sugar progenies.

Sorghum phloem in a stem vascular bundle is symplasmically isolated from the surrounding parenchyma cells, and the sucrose unloading is apoplasmic [37]. Cell wall invertase (CWI) activity is a determinant of sucrose gradient in the unloading area. In all tested internodes, CWI activities of the central storage parenchyma-rich zone were significantly higher in the high-sugar progenies than in the controls P24, Rio and Tx430 (Fig. 9b), but not in the peripheral vascular-rich zone (Fig. 9c). When the vascular bundles were dissected from the storage parenchyma cells in the central zone of internode 5 and assayed separately, the increased CWI activity in the high-sugar progenies was clearly restricted to the storage parenchyma (Fig. 9d), indicating the abilities on assimilate was increased within the sink tissues of the high-sugar progenies.

Discussion

The present study demonstrated that significantly higher sugar contents (over 750 mM) in transgenic grain sorghum (T_0, and T_1) and grain x sweet sorghum hybrids (F_1, and F_2), which is similar or higher than the sugar content of field-grown sugarcane (600-700 mM). The high sugar content, which were detected in T_0, T_1, F_1, and F_2 plants, displayed that the phenotype of high level of sugar accumulation was stably inheritable. This study demonstrated that sucrose isomerase can efficiently convert sucrose into isomaltulose and dramatically increase total sugar content in sorghum. In addition, the superior engineered progenies had significantly higher photosynthesis, higher sucrose transport, and higher sink strength than the controls, which could be the key drivers for higher sugar accumulation in plants. This approach provides a new perspective on the plant source-sink relationship. It would have a substantial impact on producing high-value sugar isomaltulose and have enormous potential for renewable feedstcoks for bio-energy or other high-value compounds.

Previous research on sugarcane demonstrated some similar outcomes. Firstly, sucrose depletion was avoided by targeting the *SI* enzymes into sucrose-storage vacuoles [38]. Secondly, the disturbance on normal growth/functions of other organs was circumvented by using stem-specific expression of the *SI* gene [31, 35]. Finally, the *SI* gene sequence was modified to remove the motifs that trigger silencing in plants [31, 39]. To the best of our knowledge, this could be the first report on engineering *SI* in sorghum, or any other cereal. Sweet sorghum has been considered as a biofuel and biomass crop [13]. Our results displayed that sugar content can be increased by up to 69% in hybrids compared with sweet sorghum, which will boost industrial value at large scale.

The activity of the vacuole-targeted *SI* enzyme was undetectable in cell extracts because the sucrose-storage vacuoles are highly acidic and proteolytic. Rapid degradation of vacuole-targeted *SI* presumably protects against quick sucrose running down in growing tissues. It is believed that isomaltulose accumulates gradually in the stalk during development, probably because of the followings: (i) constant

transcription of *SI* driven by the strong stem-specific *LSG2* or *ScR1MYB1* A1 promoter [31, 35]; (ii) high catalytic efficiency allowing occasional isomaltulose production before *SI* inactivation [24]; and (iii) very slow isomaltulose metabolism by plant enzymes [40]. For commercialization of this valued sugar, it is essential to achieve proper patterns of developmental expression, cell compartmentation, and enzyme stability in order to yield high isomaltulose content in stalks.

There has been an ongoing discussion as to whether current sugar crops have reached a physiological plateau with respect to sugar accumulation [41]. Compared to the sugar content of field-grown sugarcane juice (600-700 mM), high-level sugar accumulation (>1,000 mM disaccharides content), containing isomaltulose production (up to 691 mM) in stalk juice of the transgenic line in this study, sheds lights on that the assumed 'ceiling' above sugar accumulation could be exceeded.

Transgenic sorghum lines provide new insightful information on mechanisms as to how plants regulate sugar accumulation, a pivotal question in plant biology [43-46]. The phenotype of high total sugar content is attributed to delaying leaf senescence, increasing photosynthetic activity, and enhancing sucrose loading rates in source tissues, as well as higher activity in stalk storage parenchyma of CWI, which has multiple roles in sink tissues [45, 47]. Each of these activities would make a contribution to high sugar yield. Further comparative analysis of the superior lines and their parent lines could reveal key molecular and physiological control points in plant source-sink flux. As all the reported experiments were undertaken under well-watered, temperature-control glasshouse conditions, it is essential that further field trial should be undertaken, given the considerable diurnal and seasonal temperature variations, as well as water and nutrient availability.

Sweetness is an important commercial trait in many food crops. Enhancing sweetness through a slowly digested, acariogenic sugar, such as IM, can bring direct health benefits for consumers [18]. Isomaltulose is naturally present at a very low level (0.1 - 0.7%) in honey and sugarcane extracts which are too small to be extracted [18]. In this study, isomaltulose can be accumulated at a notably high level (691 mM) in transgenic sorghum lines. It could be harvested and extracted at the commercial scale in the future.

The fermentable carbohydrate content is also a key determinant of the economic and environmental feasibility of renewable biofuel production [48, 49]. Sweet sorghum is widely considered as a biofuel crop [1]. Accumulation of higher sugar content would increase the economic value of renewable energy. In the long term, sugars ultimately underpins all other biosyntheses in plants. The sugar boosting effect of the *SI* gene may be a foundation for higher sugar yields of many other bioenergy materials.

Conclusions

Our genetic engineering approach has successfully transformed the *SI* gene into sorghum and significantly improved total sugar content (up to 1000 mM) in sorghum. Remarkably, the total sugar concentration in grain sorghum increased up to sevenfold compared with the control Tx430. Furthermore, the total sugar concentration in F_1 and F_2 generations have improved 57% and 69% respectively

compared with sweet sorghum Rio. The massive increase of sugar accumulation in sorghum would boost biofuel production at the commercial scale. More importantly, the higher sugar accumulation did show not any negative effect on growth morphologically in the L9 line that was selected as a parent for crossing. These results demonstrate that sorghum has considerable potential as a highly competitive biofuel and bio-industrial crop. It could play an important role in future bio-economy.

Materials And Methods

Constructs of sucrose isomerase gene

Constructs were prepared by recombining four parts. The first part is a 1.2 Kb sugarcane *ScR1MYB1* A1 promoter (GenBank EU719199) [31] or a sugarcane *loading stem gene* promoter (*LSG2,* GeneBank JQ920356) [35]. The second part is a fragment encoding signal peptide of sweet potato sporamin NTPP as described [30, 38]. The third part is a modified gene version (GenBank KC147726) encoding the UQ68J *SI* enzyme [24, 31]. The fourth part is a terminator complex including three contiguous plant transcriptional terminator regions [31] intended to block read-through transcription in either direction (Fig. S2).

Sorghum transformation

Sweet sorghum has been considered as one of the most recalcitrant crops in terms of genetic transformation [33]. To successfully introduce the engineered *SI* construct into the large biomass sweet sorghum lines, an inbred line of grain sorghum Tx430 was first transformed. Then the Tx430 transgenic lines were used as a male partner for crossing with an elite sweet sorghum cultivar Rio as a female partner. Rio is advantageous for its large biomass and has been used as a male-sterile parent line.

Each of the constructs, with the sucrose isomerase gene driven either by *LSG2* promoter or *ScR1MYB1* A1 promoter, was co-precipitated on gold particles with *pUKN* selectable marker construct [39, 50]. Transformation protocol by particle bombardment, conditions for selection of transgenic lines, plant regeneration, and growth conditions in the glasshouse were described as GQ Liu, BC Campbell and ID Godwin [50]. Briefly, embryogenic calli derived from immature embryos (11-15 days post-anthesis) were used as explants for transformation. Transformed calli were cultured for 8-12 weeks on selective regeneration media containing 30 mg L^{-1} geneticin with subculturing onto fresh media fortnightly. Putative transgenic shoots were subsequently subcultured onto selective rooting media for 4 weeks following by a 3-day hardening off period. Details of the sorghum tissue culture system were used as described by GQ Liu, EK Gilding and ID Godwin [51].

PCR screening

Genomic DNA was extracted from the young leaves of the transgenic and non-transgenic plantlets prior to moving into the glasshouse. Extracted DNA quality and concentration were determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific). To confirm the *sucrose isomerase* (*SI*) gene, specific primer pairs were designed (Forward: 5'-AGCAACCCGATCTCAACTGG-3' and Reverse: 5'-ACGGAGTCGTTCCATTGCAT-3'). PCR screening was undertaken in 20 µl reactions each containing 20 ng of template DNA, 0.5 µM of each specific primer and 10 µl of Taq 2× Master Mix (New England BioLabs). PCR reactions were performed using a BIO-RAD T100 Thermal Cycler®. The PCR program comprised of an initial denaturation at 95 °C for 7 min, followed by 35 amplification cycles consisting of; 95 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, and a final elongation step of 72 °C for 7 min. PCR products were separated by gel electrophoresis at 120 V for 1.5 h in 1.0% agarose gels (Fig. S3).

Growth conditions and crossing

Following the hardening off period, *SI*-positive transgenic plantlets and negative controls (Healthy transgenic plantlets with *NPTII*-positive but *SI*-negative in genomic PCR) were transferred to 20-liter pots with three plantlets per pot. Pots were randomized and grown in a temperature-controlled glasshouse (18–28 °C) for around 95 days until physiological maturity. Generally, transgenic plants and the controls started flowering 60 days after moving into the glasshouse. The transgenic plants grew as healthily as the control plants and appeared to be normal in morphology (Fig. S1). Starting from the same time when the transgenic plantlets were moved to the glasshouse, seeds of the sweet sorghum Rio were sowed in the same glasshouse in different batches with a one-week interval to match the flowering of the desired transgenic line for crossing. The crossing was performed as described [52].

Measuring sugar concentrations by high-performance liquid chromatography electrochemical detection (HPLC-ED)

For stalk samples, a transverse tissue slice was taken at the mid-point of each designated internode and cut into radial sectors that were proportionately representative of the different stalk tissues by area. Sectors were placed on a support screen (Promega Spin Basket, Madison, WI) within a 1.5-mL microfuge tube, liquid nitrogen frozen for 20 min, and then thawed on ice and centrifuged at 10 000 ***g*** for 15 min at 4 °C to collect the juice. After the collected juice was boiled for 5 min to inactivate enzymes, the insoluble material was removed by centrifugation at 16 000 ***g*** for 20 min at 4 °C. In comparative tests conducted on internodes, this procedure gave sugar concentrations equivalent to the manual crushing of stalk samples to extract the juice. Moreover, it was adaptable to large scale samples. FWs were recorded before and after juice extraction and residual dry weights (DWs) were measured after 72 h at 75 °C for tissues, or 90 °C for juice samples. Water contents were measured in alternate subsamples to those used for juice extraction and analysis.

The resolution and quantification of IM, trehalulose, sucrose, glucose and fructose were achieved by isocratic HPLC at high pH (120 mM NaOH), using a Dionex BioLC system (Sunnyvale, CA) with PA20 analytical anion exchange column and quad waveform pulsed ED, with calibration against a dilution series of sugar standards for every sample batch [15, 38]. Sugar concentrations were corrected for dilutions in the procedure and presented as sucrose equivalents in juice. Total sugar contents were calculated on an FW and DW basis, taking account of the residual juice in internode tissues after centrifugation (up to 60% of total juice) and assuming 10% reduction in solute concentration in residual juice relative to first expressed juice, as typically observed in the industry [53]. For leaf samples, about 1 g FW of leaf blade without midrib was taken at one-third of the distance from the dewlap to the leaf tip. For root samples, about 0.5 g FW of young roots was taken from the interface between the soil and pot. Fluids were extracted and assayed by the freeze-thaw-centrifuge-HPLC method described above for stalk samples.

qRT-PCR of the *SI* gene expression in T_1 generation

The T_1 progeny fresh leaf samples of A5, L2, L9, and the wild-type controls were harvested from the glasshouse. Leaf samples were ground using liquid nitrogen. The DNA extraction kit (ISOLATE II Plant DNA Kit, BIOLINE Cat No. Bio-52070) was used and fellow the protocol to obtain total DNA for identifying positive progenies of *NPT*II and *SI* genes. The RNA extraction kit (ISOLATE II RNA Mini Kit, BIOLINE BIO-52072) was utilized and fellow the protocol to obtain total RNA from the fourth internode 20 days post-anthesis. For real-time PCR, the RNA was transcripted into cDNAs (GoScript™ Reverse Transcription, Promega, REF A5001). Then the GoTaq 1-Step RT-qPCR (Promega REF A6021) was deployed and was running in the Bio-RAD CFX96™ Real-Time System C1000 Touch™ Thermal cycler. SI primers (SI-forward: CGACATCAGCGACTACAGGA; SI-reverse: CCTTGGAAGATGAACGGTGT) were used to quantify the amount of *SI* transcript, which was expressed relative to the reference gene sorghum elongation factor 1-alpha (Sb02g036420; amplification of the reference gene using primers REF-forward: CCCAAGTACTCCAAGGCTCG and REF-reverse: ATGTTGTCACCCTCGAACCC). Amplification was done using a LightCycler 96 (Roche) according to the manufacturer's instructions, and data was analysed using LinReg-PCR (Ramakers et al. 2003).

Gas exchange and chlorophyll fluorescence measurements

The photosynthetic electron transport rate was estimated from the fluorescence light curve generated using a fiber-optic MINI-PAM/F (Heinz Waltz GmbH, Effeltrich, Germany) and leaf-clip holder 2030B positioned at one-tenth of the distance from the dewlap to the leaf tip. The MINI-PAM light intensity, saturation pulse intensity, saturation pulse width, leaf absorption factor and illumination time were set at 680 µmol/m2/s, 680 µmol/m2/s, 0.8 s, 0.84 and 10 s, respectively. The internal temperature of the MINI-PAM was controlled between 25 and 30 °C during measurement. An LI-6400 portable photosynthesis

system (LI-COR, Lincoln, NE, USA) was used to measure CO_2 fixation rates on the same leaves. Measurements were made on at least three replicate plants per progeny.

Plasmalemma vesicle (PMV) isolation and transport assays

The blades of the second and third leaves from the top without midribs (12.5 g FW) were homogenized in 50 mL solution which contains 240 mM sorbitol, 50 mM N-2-hydroxyethylpiperazine-N' 2-ethanesulphonic acid (HEPES), 3 mM ethyleneglycol-bis (βaminoethylether)-*N*, *N*-tetraacetic acid (EGTA), 3 mM dithiothreitol (DTT), 10 mM KCl, 0.5% bovine serum albumin (BSA), 0.6% polyvinylpyrrolidone (PVP) and 2 mM phenylmethyl sulphonyl fluoride (PMSF) (adjusted to pH 8.0 using solid Bistris propane) at 4 °C. The homogenate was filtered through four layers of cheesecloth to remove tissue debris and then centrifuged at 10 000 ***g*** for 10 min to remove mitochondria and chloroplasts. Microsomal membranes were pelleted by centrifugation at 50 000 ***g*** for 60 min. PMVs were purified from the microsomal fraction by phase partitioning [36], washed in 25 mL of sorbitol-based re-suspension buffer (SBRB) (330 mM sorbitol, 2 mM HEPES, 0.1 mM DTT, 10 mM KCl, pH 8.0 with solid Bistris propane), repelleted by centrifugation at 50 000 g for 60 min and resuspended at 3–5 mg FW mL^{-1} of re-suspension buffer. The phase-purified PMVs were layered over a 20%–50% sucrose gradient in 2 mM HEPES, 1 mM HCl and 1 mM DTT (pH 8.0 with solid Bistris propane), centrifuged for 15 h at 100 000 ***g*** and collected in 1-mL fractions. The fractions were washed in 11 mL SBRB and pelleted by centrifugation at 100 000 g for 60 min. The pellet was suspended in 0.4 mL of SBRB, checked for purity using routine tests for enzymatic activities characteristic of other cellular membrane types, and used for transport experiments.

Transport assays were conducted at 12 °C using three replicate reactions per treatment (Bush et al., 1996). Briefly, for each reaction mixture, 20 µL of resuspended PMVs were diluted into 400 µL of assay buffer (as for SBRB, except adjusted to pH 6.0 with solid 2 [*N*-morpholino ethane sulphonic acid (MES)] containing 0.2 µCi (^{14}C)sucrose and unlabelled sucrose to the desired concentration. At each time point, vesicles from one reaction mixture were collected on 0.45-µm filters and rinsed three times with 0.6 mL of assay buffer containing only unlabelled sucrose (1 mM). The accumulated radioactivity was measured by scintillation spectrometry. The difference between samples with and without 5 µM carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) was defined as ΔpH-dependent sucrose transport.

Internode tissue fractionation and enzyme assays

Transverse sections of each internode were divided into the outer rind of 2 mm thickness and two internal concentric cylinders at equal distances along the stalk radius. Of these, the central parenchyma-rich zone and the peripheral vascular-rich zone were examined for invertase activity. Furthermore, vascular bundles were separated by dissection from parenchyma tissue in the central zone for separate assays. The separated tissues were frozen immediately in liquid nitrogen for enzyme extraction, followed by the determination of CWI activity, using three replicate plants or dissected tissue subsamples per assay [54].

SI enzyme was extracted by grinding the frozen cells in a chilled mortar using three volumes of extraction buffer that contained 0.1 M Hepes-KOH buffer(pH7.5), 10 mM $MgCl_2$, 2 mM EDTA, 2mM EGTA, 10% glycerol, 5 mM DTT, 2% polyvinylpolypyrrolidone and 1x complete protease inhibitor (Roche, Mannheim, Germany). The homogenate was immediately centrifuged at 10 000 ***g*** for 15 min at 4 °C. The supernatant was immediately desalted on a PD-10 column (GE Healthcare, Buckinghamshire, UK) that was pre-equilibrated and eluted using the extraction buffer. Protein concentration was assayed by the Bradford reaction using a Bio-Rad kit (Hercules, CA, USA) with bovine serum albumin standards. *SI* activity was measured by incubating enzyme extract with 292 mM sucrose solution in 0.1 M citrate-phosphate buffer (pH 6.0) at 30 °C, and testing for isomaltulose accumulation over 80 min by HPLC-ED as described above.

Abbreviations

SI: Sucrose isomerase; FW: fresh weight; NTPP: N-terminal pro-peptide; PCR: polymerase chain reaction; qRT-PCR: quantitative real-time PCR; HPLC: high-performance liquid chromatography; PAR: photosynthetically active radiation; PMV: plasma membrane vesicles; CWI: cell wall invertase; FT: fructosyltransferase; HPLC-ED: high-performance liquid chromatography electrochemical detection; DW: dry weight; PMV: plasmalemma vesicle; HEPES: N-2-hydroxyethylpiperazine-N' 2-ethanesulphonic acid; EGTA: ethyleneglycol-bis (βaminoethylether)-N, N'-tetraacetic acid; DTT: dithiothreitol; BSA: bovine serum albumin; PVP: polyvinylpyrrolidone; PMSF: phenylmethyl sulphonyl fluoride; SBRB: sorbitol-based re-suspension buffer; MES: N-morpholino ethane sulphonic acid; CCCP: carbonyl cyanide m-chlorophenyl hydrazone.

Declarations

Acknowledgments

The authors wish to acknowledge the Q-CAS (Queensland-Chinese Academy of Sciences) collaborative science fund project, with funding from the Queensland Department of Environment and Science.

Author contributions

LW, IDG, GL, and HJ designed the experiments. LW, YP, GL, YZ, HG, SL, and CD conducted the experiments and analyzed the data. LW, GL, and IDG wrote the manuscript. LW, IDG, and HJ supervised the project. All authors reviewed, edited, and approved the manuscript.

Funding

This project is funded by the Queensland Department of Environment and Science.

Availability of data and materials

The datasets supporting the conclusions of this article are included in the article and its Additional file.

Ethics approval and consent to participate

Ethical approval and consent to participate are not required.

Consent for publication

All authors agree for the submission and publication of manuscript in the journal Biotechnology for Biofuel.

Competing interests

The authors declare no competing interests.

References

1. Mathur S, Umakanth AV, Tonapi VA, Sharma R, Sharma MK: **Sweet sorghum as biofuel feedstock: recent advances and available resources**. *Biotechnology for Biofuels* 2017, **10**.
2. Holou RAY, Stevens G: **Juice, sugar, and bagasse response of sweet sorghum (Sorghum bicolor (L.) Moench cv. M81E) to N fertilization and soil type**. *Gcb Bioenergy* 2012, **4**(3):302-310.
3. Soileau JM, Bradford BN: **Biomass and Sugar Yield Response of Sweet Sorghum to Lime and Fertilizer**. *Agron J* 1985, **77**(3):471-475.
4. Jackson PA: **Breeding for improved sugar content in sugarcane**. *Field Crop Res* 2005, **92**(2-3):277-290.
5. Lakshmanan P, Geijskes RJ, Aitken KS, Grof CLP, Bonnett GD, Smith GR: **Sugarcane biotechnology: The challenges and opportunities**. *In Vitro Cell Dev-Pl* 2005, **41**(4):345-363.
6. Manners JM: **Functional Genomics of Sugarcane**. *Adv Bot Res* 2011, **60**:89-168.
7. Jiang SY, Chi YH, Wang JZ, Zhou JX, Cheng YS, Zhang BL, Ma A, Vanitha J, Ramachandran S: **Sucrose metabolism gene families and their biological functions**. *Sci Rep-Uk* 2015, **5**.
8. Capell T, Christou P: **Progress in plant metabolic engineering**. *Curr Opin Biotech* 2004, **15**(2):148-154.
9. Moore PH: **Temporal and Spatial Regulation of Sucrose Accumulation in the Sugarcane Stem**. *Aust J Plant Physiol* 1995, **22**(4):661-679.
10. Grof CPL, Campbell JA: **Sugarcane sucrose metabolism: scope for molecular manipulation**. *Aust J Plant Physiol* 2001, **28**(1):1-12.

11. Bindon KA, Botha FC: **Tissue discs as an experimental system for metabolic flux analysis in the sugarcane culm**. *S Afr J Bot* 2001, **67**(2):244-249.
12. Gutjahr S, Clément-Vidal A, Trouche G, Vaksmann M, Thera K, Sonderegger N, Dingkuhn M, Luquet D: **Functional analysis of sugar accumulation in sorghum stems and its competition with grain filling among contrasted genotypes.** In: *Proceedings of Agro 2010 : the XIth ESA Congress: 2010; Montpellier, France*. Agropolis international: 89-90.
13. Mathur S, Umakanth AV, Tonapi VA, Sharma R, Sharma MK: **Sweet sorghum as biofuel feedstock: recent advances and available resources**. *Biotechnol Biofuels* 2017, **10**:19.
14. Stevens G, Holou RAY: **Sweet Sorghum as a Biofuel Crop**. In: *Energy Crops.* Edited by Halford NGKA, vol. 3; 2011: 56-76.
15. Wu L, Birch RG: **Characterization of Pantoea dispersa UQ68J: producer of a highly efficient sucrose isomerase for isomaltulose biosynthesis**. *J Appl Microbiol* 2004, **97**(1):93-103.
16. Wu LG, Birch RG: **Physiological basis for enhanced sucrose accumulation in an engineered sugarcane cell line**. *Funct Plant Biol* 2010, **37**(12):1161-1174.
17. Jonker D, Lina BAR, Kozianowski G: **13-week oral toxicity study with isomaltulose (Palatinose (R)) in rats**. *Food Chem Toxicol* 2002, **40**(10):1383-1389.
18. Sawale PD, Shendurse AM, Mohan MS, Patil GR: **Isomaltulose (Palatinose) - An emerging carbohydrate**. *Food Biosci* 2017, **18**:46-52.
19. Takazoe I: **Palatinose – an isomeric alternative to sucrose.** . In: *Progress in Sweeteners* Edited by Grenby TH. Barking: Elsevier; 1989: 143-167.
20. Lina BAR, Jonker D, Kozianowski G: **Isomaltulose (Palatinose (R)): a review of biological and toxicological studies**. *Food Chem Toxicol* 2002, **40**(10):1375-1381.
21. Lichtenthaler FW, Peters S: **Carbohydrates as green raw materials for the chemical industry**. *Cr Chim* 2004, **7**(2):65-90.
22. Schiweck H, Munir, M., Rapp, K.M., Schneider, B. and Vogel, M. : **New developments in the use of sucrose as an industrial bulk chemical**. In: *Carbohydrates as Organic Raw Materials* Edited by Lichtenthaler FW. Weinheim: Wiley-VCH; 1991: 57–94.
23. Buchholz K, Kasche, V., Bornscheuer, U. T.: **Biocatalysts and Enzyme Technology**: Wiley-Blackwell; 2012.
24. Wu LQ, Birch RG: **Characterization of the highly efficient sucrose isomerase from Pantoea dispersa UQ68J and cloning of the sucrose isomerase gene**. *Appl Environ Microbiol* 2005, **71**(3):1581-1590.
25. Loreti E, Alpi A, Perata P: **Glucose and disaccharide-sensing mechanisms modulate the expression of alpha-amylase in barley embryos**. *Plant Physiol* 2000, **123**(3):939-948.
26. Sinha AK, Hofmann MG, Romer U, Kockenberger W, Elling L, Roitsch T: **Metabolizable and non-metabolizable sugars activate different signal transduction pathways in tomato**. *Plant Physiol* 2002, **128**(4):1480-1489.

27. Bornke F, Hajirezaei M, Heineke D, Melzer M, Herbers K, Sonnewald U: **High-level production of the non-cariogenic sucrose isomer palatinose in transgenic tobacco plants strongly impairs development**. *Planta* 2002, **214**(3):356-364.
28. Bornke F, Hajirezaei M, Sonnewald U: **Potato tubers as bioreactors for palatinose production**. *J Biotechnol* 2002, **96**(1):119-124.
29. Hajirezaei MR, Bornke F, Peisker M, Takahata Y, Lerchl J, Kirakosyan A, Sonnewald U: **Decreased sucrose content triggers starch breakdown and respiration in stored potato tubers (Solanum tuberosum)**. *J Exp Bot* 2003, **54**(382):477-488.
30. Gnanasambandam A, Birch RG: **Efficient developmental mis-targeting by the sporamin NTPP vacuolar signal to plastids in young leaves of sugarcane and Arabidopsis**. *Plant Cell Rep* 2004, **23**(7):435-447.
31. Mudge SR, Basnayake SWV, Moyle RL, Osabe K, Graham MW, Morgan TE, Birch RG: **Mature-stem expression of a silencing-resistant sucrose isomerase gene drives isomaltulose accumulation to high levels in sugarcane**. *Plant Biotechnology Journal* 2013, **11**(4):502-509.
32. Liu GQ, Godwin ID: **Highly efficient sorghum transformation**. *Plant Cell Rep* 2012, **31**(6):999-1007.
33. Raghuwanshi A, Birch RG: **Genetic transformation of sweet sorghum**. *Plant Cell Rep* 2010, **29**(9):997-1005.
34. Mudge SR, Osabe K, Casu RE, Bonnett GD, Manners JM, Birch RG: **Efficient silencing of reporter transgenes coupled to known functional promoters in sugarcane, a highly polyploid crop species**. *Planta* 2009, **229**(3):549-558.
35. Moyle RL, Birch RG: **Sugarcane Loading Stem Gene promoters drive transgene expression preferentially in the stem**. *Plant Mol Biol* 2013, **82**(1-2):51-58.
36. Bush DR, Chiou TJ, Chen LS: **Molecular analysis of plant sugar and amino acid transporters**. *J Exp Bot* 1996, **47**:1205-1210.
37. Bihmidine S, Baker RF, Hoffner C, Braun DM: **Sucrose accumulation in sweet sorghum stems occurs by apoplasmic phloem unloading and does not involve differential Sucrose transporter expression**. *Bmc Plant Biol* 2015, **15**.
38. Wu LG, Birch RG: **Doubled sugar content in sugarcane plants modified to produce a sucrose isomer**. *Plant Biotechnol J* 2007, **5**(1):109-117.
39. Basnayake SWV, Morgan TC, Wu LG, Birch RG: **Field performance of transgenic sugarcane expressing isomaltulose synthase**. *Plant Biotechnol J* 2012, **10**(2):217-225.
40. Wu LG, Birch RG: **Isomaltulose Is Actively Metabolized in Plant Cells**. *Plant Physiol* 2011, **157**(4):2094-2101.
41. Inman-Bamber NG, Jackson PA, Hewitt M: **Sucrose accumulation in sugarcane stalks does not limit photosynthesis and biomass production**. *Crop Pasture Sci* 2011, **62**(10):848-858.
42. Nell JS: **Genetic manipulation of sucrose-storing tissue to produce alternative products.** South Africa.: The University of Stellenbosch 2007.

43. Rolland F, Moore B, Sheen J: **Sugar sensing and signaling in plants**. *Plant Cell* 2002, **14**:S185-S205.
44. Fernie AR, Roessner U, Geigenberger P: **The sucrose analog palatinose leads to a stimulation of sucrose degradation and starch synthesis when supplied to discs of growing potato tubers**. *Plant Physiol* 2001, **125**(4):1967-1977.
45. Koch K: **Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development**. *Curr Opin Plant Biol* 2004, **7**(3):235-246.
46. Fernie AR, Geigenberger P, Stitt M: **Flux an important, but neglected, component of functional glenomics**. *Curr Opin Plant Biol* 2005, **8**(2):174-182.
47. Proels RK, Huckelhoven R: **Cell-wall invertases, key enzymes in the modulation of plant metabolism during defence responses**. *Mol Plant Pathol* 2014, **15**(8):858-864.
48. De Oliveira MED, Vaughan BE, Rykiel EJ: **Ethanol as fuels: Energy, carbon dioxide balances, and ecological footprint**. *Bioscience* 2005, **55**(7):593-602.
49. Long HL, Li XB, Wang H, Jia JD: **Biomass resources and their bioenergy potential estimation: A review**. *Renew Sust Energ Rev* 2013, **26**:344-352.
50. Liu GQ, Campbell BC, Godwin ID: **Sorghum Genetic Transformation by Particle Bombardment**. *Methods Mol Biol* 2014, **1099**:219-234.
51. Liu GQ, Gilding EK, Godwin ID: **A robust tissue culture system for sorghum [Sorghum bicolor (L.) Moench]**. *South African Journal of Botany* 2015, **98**:157-160.
52. House LR: **A Guide to Sorghum Breeding**, Second edn. Patancheru, India: International Crops Research Institute for the Semi-Arid Tropics; 1985.
53. Hugot E: **Handbook of Cane Sugar Engineering**. Amsterdam Elsevier; 1986.
54. Albertson PL, Peters KF, Grof CPL: **An improved method for the measurement of cell wall invertase activity in sugarcane tissue**. *Aust J Plant Physiol* 2001, **28**(4):323-328.

Figures

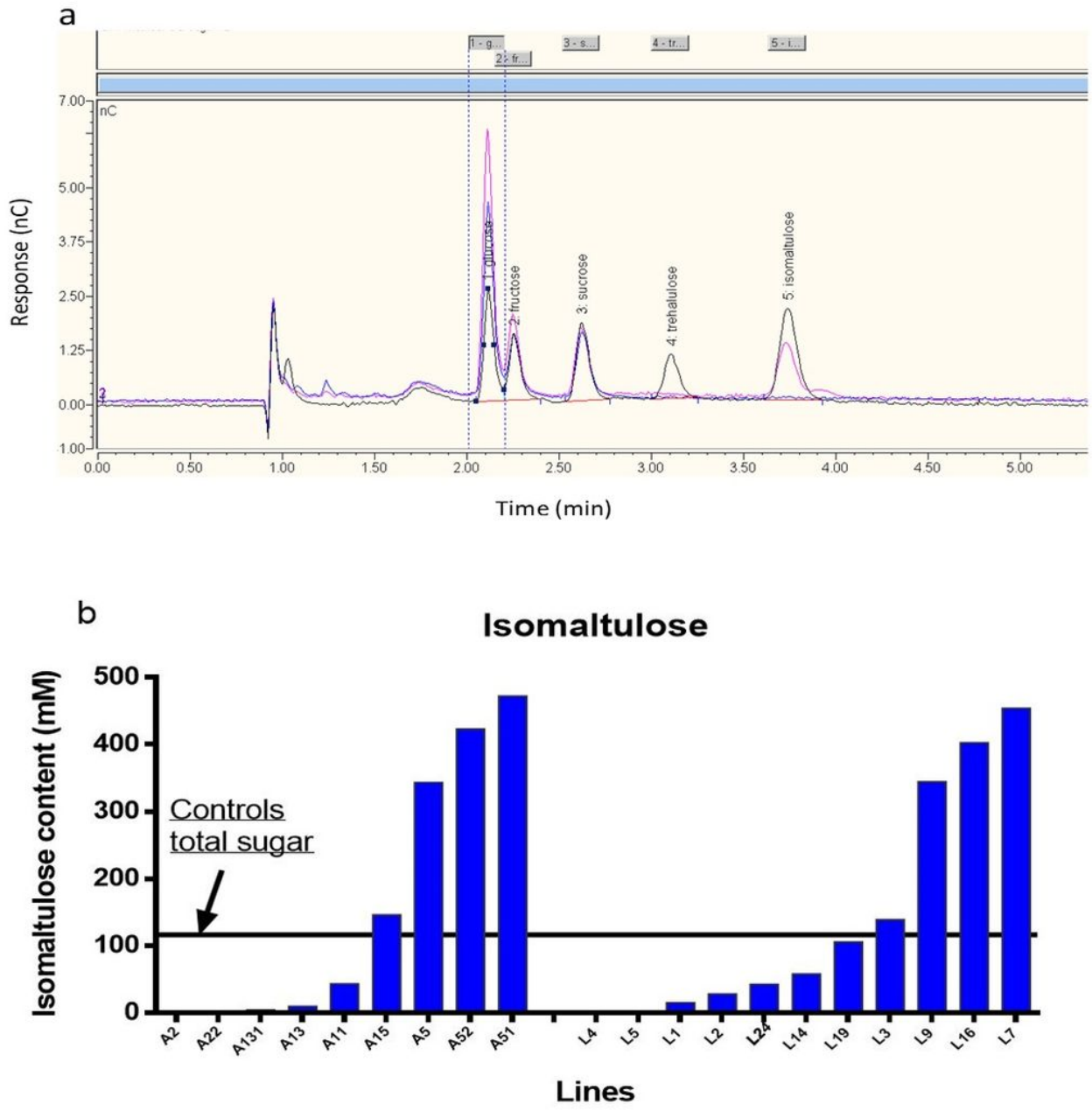

Figure 1

Screening transgenic Tx430 sorghum lines for the presence of isomaltulose (IM) in stem juice. (a) High-performance liquid chromatography (HPLC) profiles. Black curve: Standard solutions contained glucose, fructose, sucrose, trehalulose and IM; Pink curve: Diluted (16,000x) juice from transgenic sorghum stalk internode 4 showing isomaltulose (last peak #5) was accumulated beyond glucose, fructose and sucrose; Blue curve: Diluted (16,000x) juice from a parent control sorghum stalk showing no isomaltulose accumulated. (b) isomaltulose concentrations in juice from the internode 4 of the transgenic lines. The line's label starts with A driven by A1 promoter and with L driven by LSG2 promoter. The plants were the

first vegetative generation from tissue culture with around 7 internodes when sampled 20 days post-anthesis. A horizontal line was drawn on the highest total sugar content (sucrose equivalent) among the five Tx430 plants (Controls).

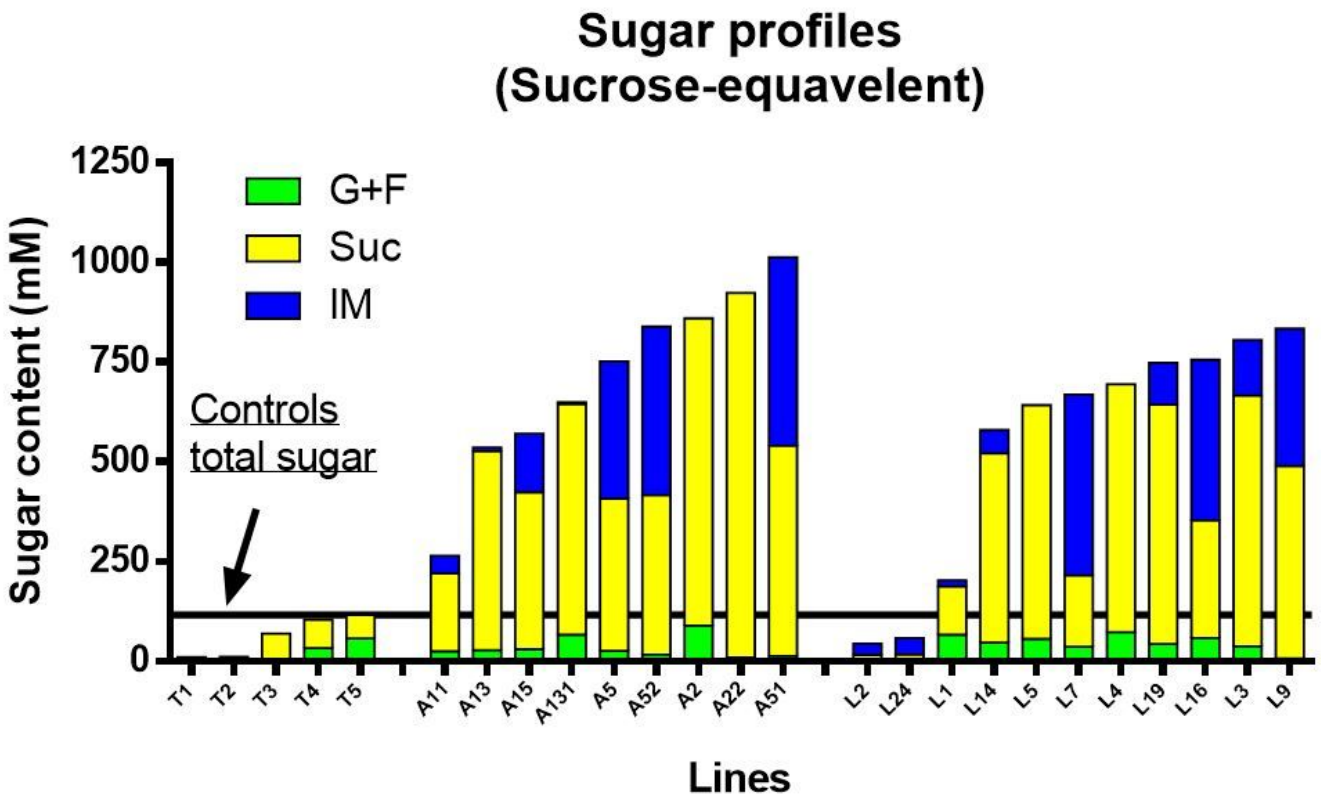

Figure 2

Total sugar profile of the internode 4 in controls Tx430 and transgenic lines. A horizontal line was drawn on the highest sugar content of the control Tx430. G+F: ½ (Glucose plus fructose); Suc: Sucrose; IM: Isomaltulose. T1 to T5: five untransformed Tx430; The line's label starts with A driven by A1 promoter and with L driven by LSG2 promoter.

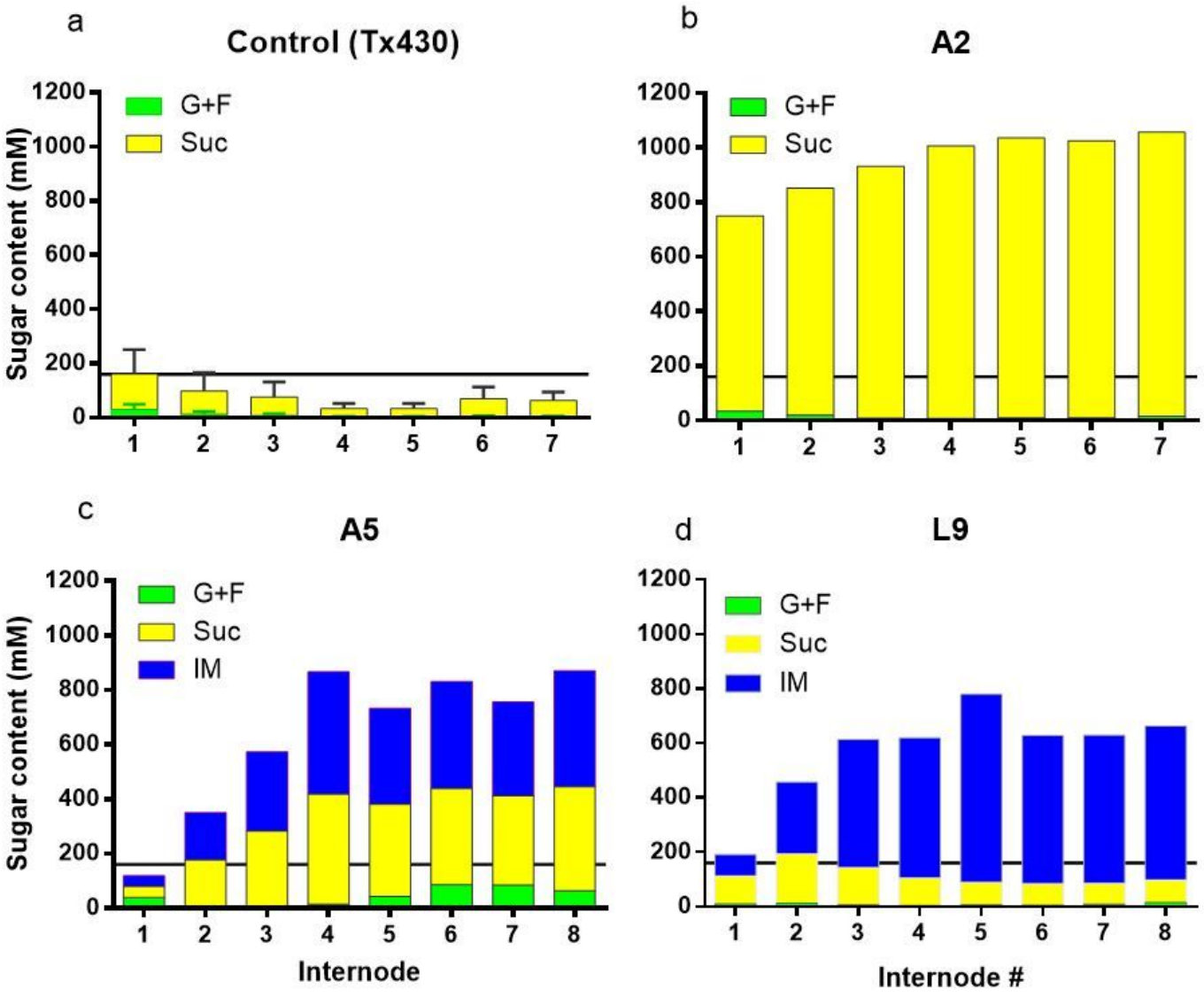

Figure 3

Sugar profile of internodes in controls Tx430 and transgenic lines. The plants were sampled 20 days post-anthesis with 7-8 internodes. G+F: ½ (Glucose plus fructose); Suc: Sucrose; IM: Isomaltulose. Results from the Tx430 controls are means of five replicates, with standard errors. A horizontal line on each panel was drawn on the highest sugar content of internode 1 of the control Tx430. (a) The controls Tx430; (b) Transgenic line A2; (c) Transgenic line A5; and (d) Transgenic line L9.

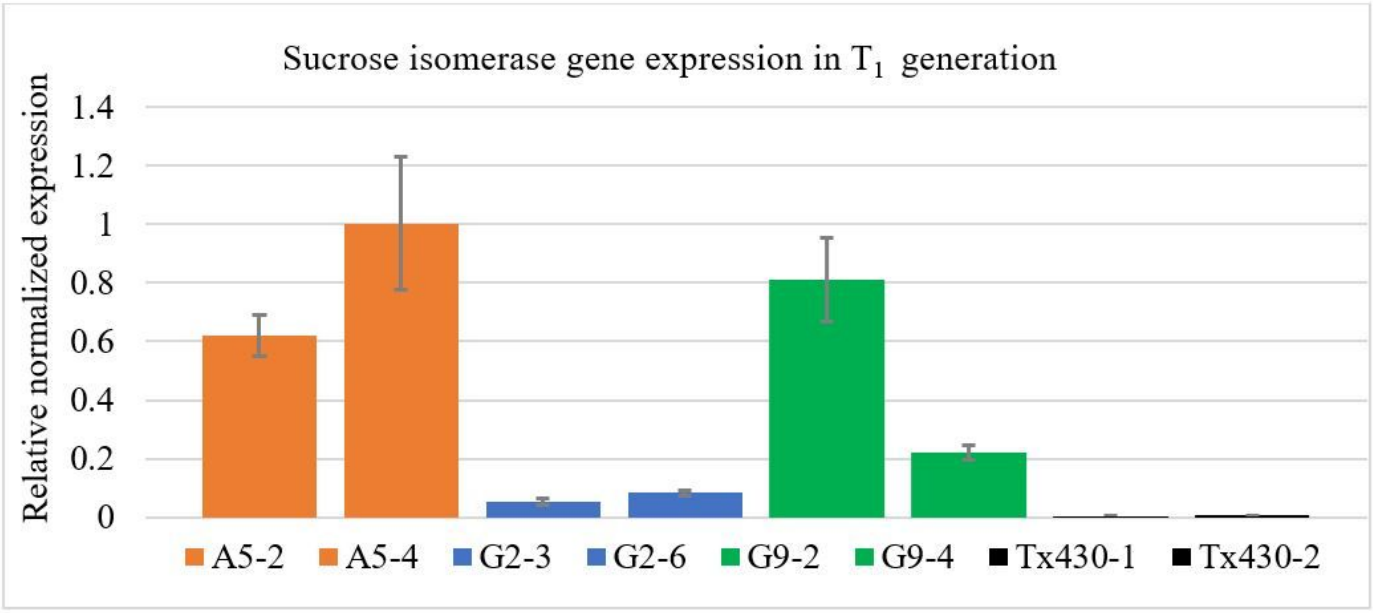

Figure 4

RT-PCR analysis of the SI gene expression in T1 progenies and Tx430. A5-2, A5-4, G2-3, G2-6, G9-2, and G9-4 are T1 positive transgenic lines; Tx430-1, Tx430-2 are non-transgenic control samples.

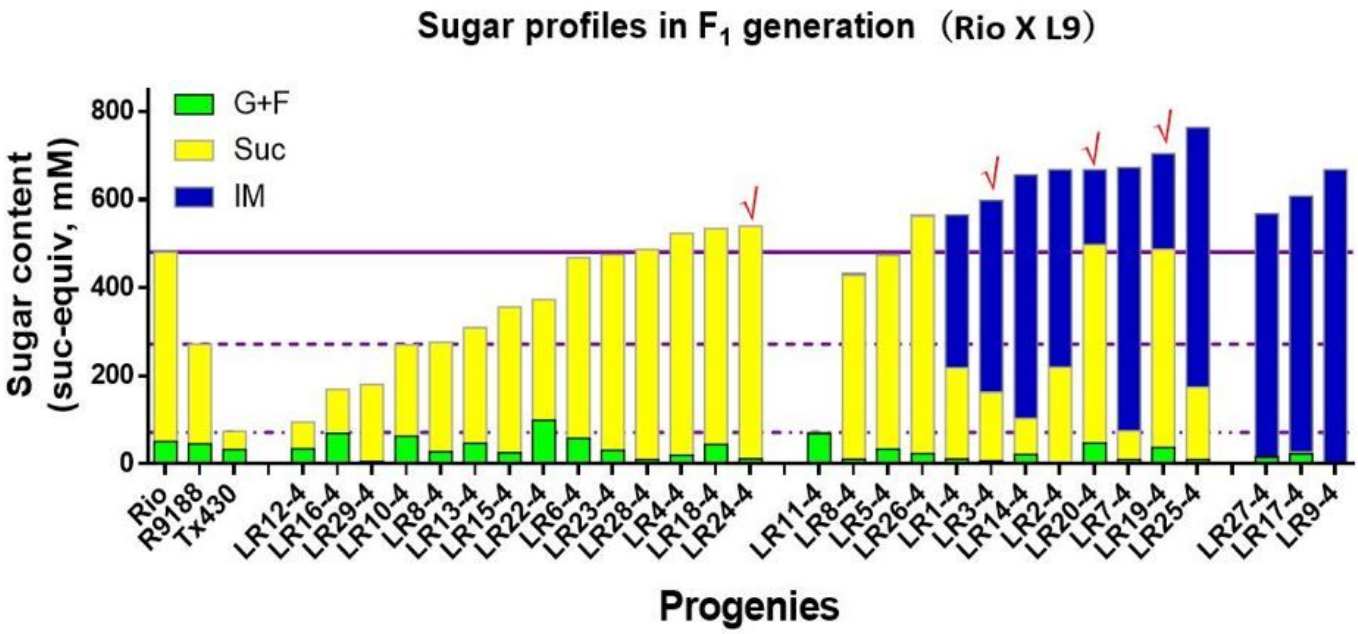

Figure 5

Total sugar content in the F1 hybrids (Rio X L9). Sugars were measured 20 days post-anthesis in the middle section of internode 4 (counted from top). L9 is the transgenic line driven by LSG2 promoter. G+F: ½ (Glucose plus fructose); Suc: Sucrose; IM: Isomaltulose. Bars of the controls Rio, R9188, and Tx430,

were means of three stalks. Three horizontal lines represent the average of the three controls respectively. The progenies with red ticks (√) were selected for further testing.

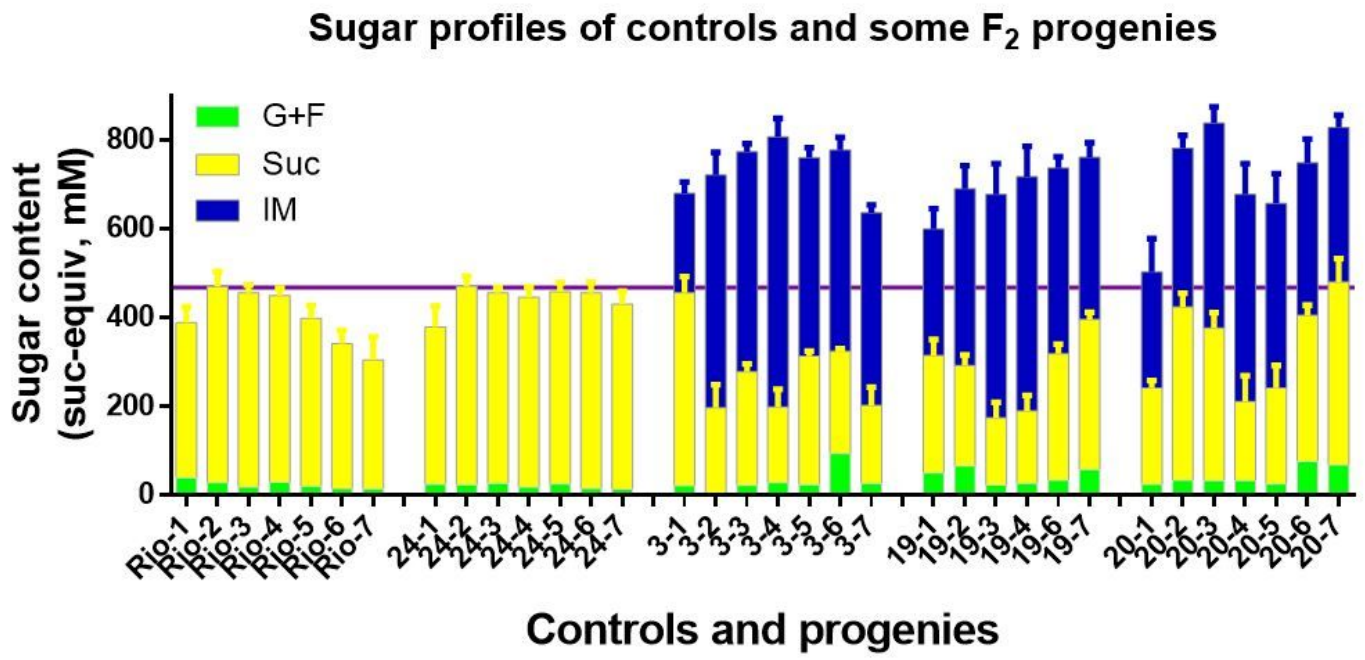

Figure 6

Sugar profile of internodes in controls and selected progenies of the F2 generation (Rio X L9). The first group represents the parent Rio control, the second group 24 (P24 from LR24) represents the transgene negative control, and the rest three groups are progenies 3 (P3 from LR3), 19 (P19 from LR19), and 20 (P20 from LR20) of positive transgenes. The last digit in the label of the X-axis is the internode number counted from the top. G+F: ½ (Glucose plus fructose); Suc: Sucrose; IM: Isomaltulose. Sugars were measured 20 days post-anthesis in the middle section of each internode. Results were means with standard errors from three replicates. The horizontal line was drawn on the highest total sugar content among all internodes of the Rio control.

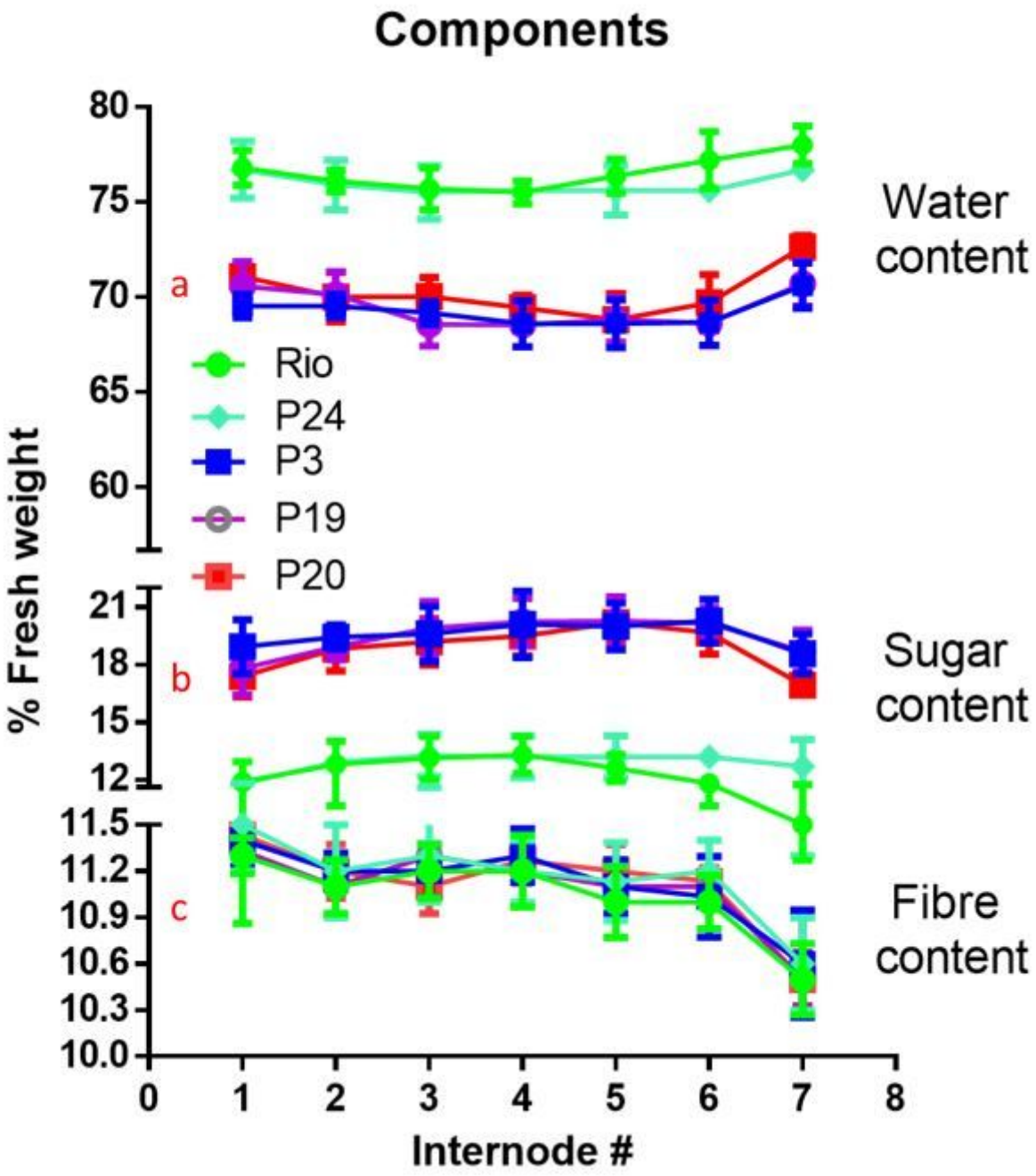

Figure 7

Total water (a), sugar (b), and fiber (c) contents in the internodes of controls and the F2 progenies. Rio was the parent control, P24 (from LR24) was transgene negative progeny as a hybrid control. P3 (from LR3), P19 (from LR19), and P20 (from LR20) were transgene positive progenies. Internodes were numbered from the top. Results were means with standard errors from three replicate plants.

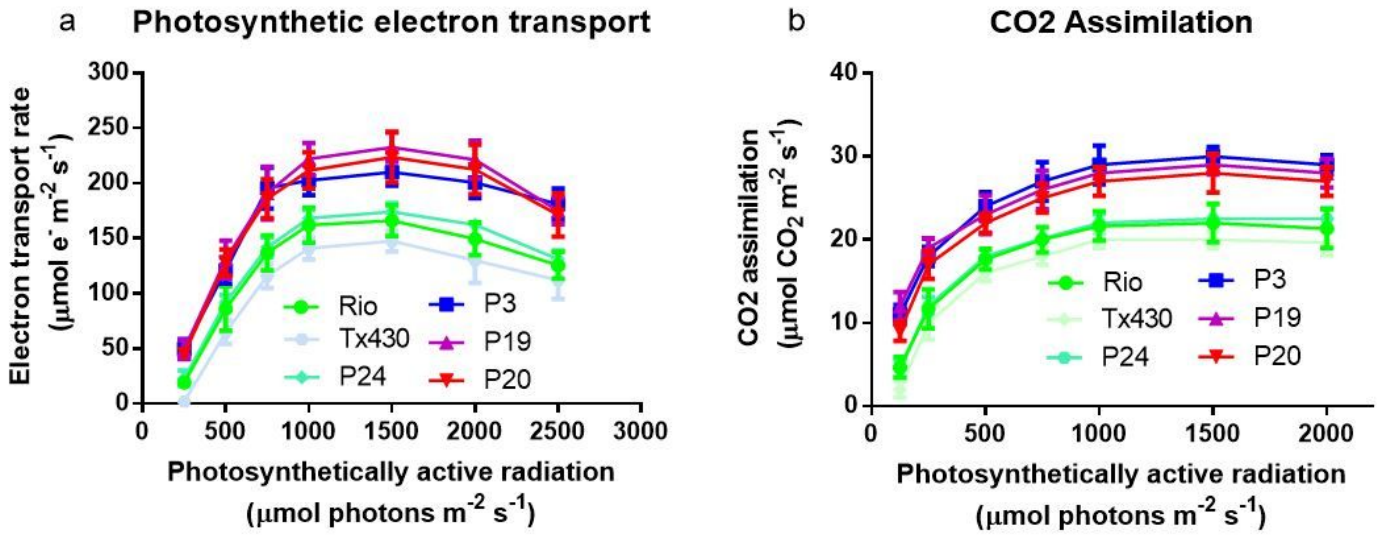

Figure 8

Photosynthetic electron transport rate (a) and CO2 assimilation (b) in controls and the F2 progenies. Three controls: Rio (parent control), Tx430 (untransformed control), and P24 (from LR24) transgene negative progeny as a hybrid control P24. Three high-sugar progenies: P3 (from LR3), P19 (from LR19), and P20 (from LR20). Photosynthesis was measured after 10-11 days of anthesis in the second leaf from the top. Results were means with standard errors from three replicates.

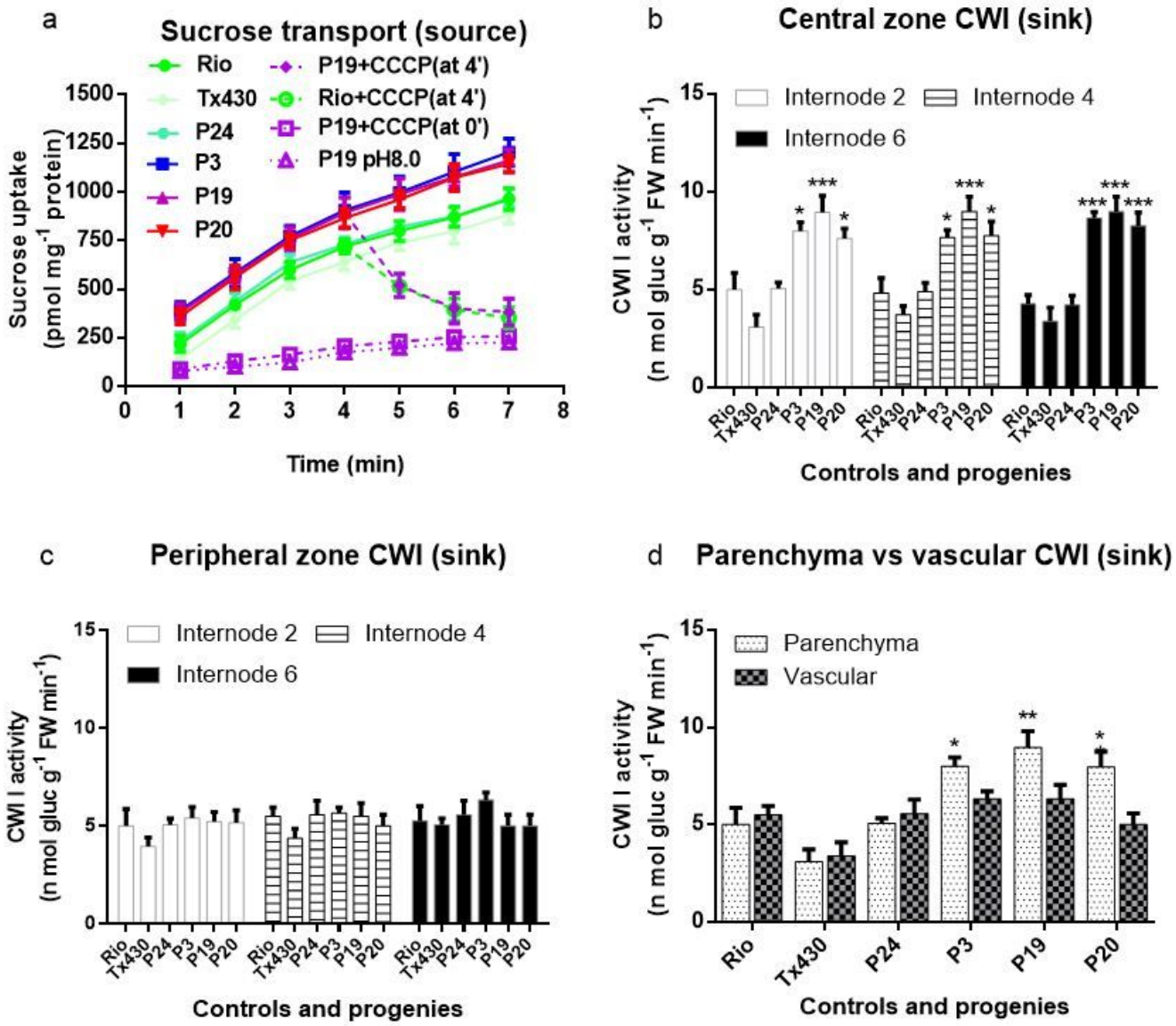

Figure 9

The relationship of source and sink in controls and the F2 progenies. (a) sucrose transport (source). (b) CWI activity was measured in the central parenchyma-rich zone (sink). (c) in the peripheral vascular-rich zone (sink). (d) in separated vascular bundles and parenchyma tissue from the central zone of internode 5 (sink). Three controls: Rio (parent control), Tx430 (untransformed control), and P24 (from LR24) transgene negative progeny as a hybrid control P24. Three high-sugar progenies: P3 (from LR3), P19 (from LR19), and P20 (from LR20). CCCP: carbonyl cyanide m-chlorophenyl hydrazone. The leaves and internodes were sampled at 20 days after anthesis. Results are means with standard errors from three replicates. Analysis of variance (ANOVA) with Bonferroni post-tests showed significant differences between any control and high-sugar progenies in the sucrose transport rates at all time points. The same statistical analysis showed significant differences between controls and high-sugar progenies in CWI activity of parenchyma cells in the central zone. *P < 0.05; **P < 0.01; ***P < 0.001.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterials.docx