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RESEARCH ARTICLE

Physiological and biochemical processes underlying the differential sucrose yield and biomass production in sugarcane varieties

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Summary
Sucrose yield in sugarcane is a complex process regulated by both environmental and endogenous factors. However, the metabolic balance driving vegetative growth and sucrose accumulation remains poorly understood. Herein, we carried out a comprehensive assessment of carbohydrate dynamics throughout the crop cycle in two sugarcane varieties varying in biomass production, evaluating the carbon metabolism in both leaves and stalks. Our data revealed that the decline in photosynthetic rates during sugarcane maturation is associated not only to accumulation of sugars in leaves but also due to stomatal and non-stomatal limitations. We found that metabolic processes in leaves and stalks were intrinsically linked. While IACSP94-2094 had higher stalk sucrose concentration than IACSP95-5000, this latter produced more biomass. Compared to IACSP95-5000, IACSP94-2094 showed higher sucrose phosphate synthase (SPS) activity in leaves and stalks, along with lower soluble acid invertase (SAI) activity in leaves during the maximum growth stage. Interestingly, IACSP94-2094 also exhibited higher stalk SPS activity and lower stalk SAI activity than IACSP95-5000 during maturation. High biomass production by IACSP95-5000 was associated with higher sucrose synthase (SuSy) and SAI activity in leaves and higher SuSy and soluble neutral invertase (SNI) activity in stalks when compared to IACSP94-2094 during the maximum growth. Despite the contrasting strategies, both varieties displayed similar total sucrose yield, a balance between sucrose concentration and biomass production. This phenomenon implies the presence of a compensatory mechanism in sugarcane, with high biomass production compensating low sucrose accumulation and vice versa.

Keywords: enzymes; metabolism; *Saccharum* spp.; sugar; source–sink regulation

Introduction

The sucrose yield in sugarcane depends on carbon assimilation and source–sink interactions, which are two key factors exhibiting significant seasonal variation (De Souza *et al.*, 2018). In subtropical conditions, photosynthesis is constrained by low temperatures and drought, limiting the availability of CO_2 due to stomatal closure and also reducing the activity of photosynthetic enzymes (Marchiori *et al.*, 2017; Silveira *et al.*, 2017; Cerqueira *et al.*, 2019). Additionally, poor light distribution within plant canopy leads to decreased CO_2 uptake by

sugarcane (Marchiori *et al.,* 2014). However, sugarcane response to these constraining conditions differs substantially among varieties. For instance, phenotypic plasticity due to water deficit varies among sugarcane genotypes as well as the photosynthetic flexibility under low light conditions (Marchiori *et al.,* 2010, 2017; Sales *et al.,* 2018).

The strength of the sink (stalks) and the accumulation of sugars in sources (leaves) have also a regulatory effect on sugarcane photosynthesis (McCormick *et al.,* 2006, 2008a; Ribeiro *et al.,* 2017). Previous studies on sugarcane plants using cold-girdling and sugar feeding techniques have demonstrated that increased leaf sugar concentrations reduce chlorophyll content, Rubisco expression and activity, and overall photosynthetic rates (McCormick *et al.,* 2008b; Lobo *et al.,* 2015; Ribeiro *et al.,* 2017). On the other hand, enhanced sink demand leads to increased photosynthetic rates and reduced leaf sugar concentration in sugarcane plants (Ribeiro *et al.,* 2017; Verma *et al.,* 2019). This suggests that increased stalk storage capacity and the decoupling of pathways mediating the feedback inhibition of sources by sinks are needed for enhancing sucrose yield in sugarcane (McCormick *et al.,* 2009). Unfortunately, most of studies have primarily focused on source tissues (Huang *et al.,* 2017).

Carbon partitioning varies between leaves and stalks, and this changes the ratio of insoluble to soluble compounds in each sugarcane organ (Mason *et al.,* 2022, 2023). The carbon allocation is also influenced by the sugarcane phenology, mainly at maturation (Mason *et al.,* 2022; García *et al.,* 2023). While sucrose is rapidly degraded and used in respiration and other biosynthetic pathways when plants are young and in active growth, there is a significant build-up of sucrose concentration in sinks during the maturation (Mason *et al.,* 2022). This process is coordinated by several enzymes, including sucrose phosphate synthase (SPS), sucrose synthase (SuSy), and invertases, which are responsible for the breakdown and synthesis of sucrose (Wang *et al.,* 2013), and play a crucial role in determining the final sugar yield (Anur *et al.,* 2020).

The invertases hydrolyse sucrose to glucose and fructose, playing an important role in the control of metabolic fluxes, phloem loading and unloading, sucrose partitioning, and plant development (Rossouw *et al.,* 2010). SPS, on the other hand, is a pivotal enzyme in leaves, controlling the flow of carbon into sucrose (Verma *et al.,* 2011). SuSy catalyses a reversible reaction, primarily cleaving sucrose in sink tissues (Mirajkar *et al.,* 2016). Additionally, the activity of these enzymes is dependent on environmental conditions (Shanthi *et al.,* 2023). For instance, acid invertase activity is reduced under low air temperature and water stress (Du and Nose, 2002). Moreover, several studies on sugarcane have revealed high genotypic variation in the expression and activity of enzymes involved in carbohydrate metabolism (Verma *et al.,* 2011; Chandra *et al.,* 2015; Huang *et al.,* 2017; Mason *et al.,* 2022).

Despite these findings, our understanding about the enzymatic balance underlying vegetative growth and sugar accumulation in sugarcane remains limited, as well as our knowledge about how varieties differ in sucrose metabolism along crop cycle (Shanthi *et al.,* 2023). Here, we argue that the differential sucrose yield among sugarcane genotypes is attributed to differences in carbon metabolism and related processes. In-depth knowledge about the factors driving sucrose yield in sugarcane can greatly benefit breeding programmes in releasing varieties with superior performance (Wang *et al.,* 2013; Misra *et al.,* 2022). In this context, we investigated the carbohydrate dynamics along the crop cycle of two sugarcane varieties differing in biomass production and considered the carbon metabolism in both leaves and stalks. Our aim was to reveal the physiological and biochemical processes underlying the differential sucrose yield and biomass production in sugarcane varieties.

Materials and methods

Plant material and field conditions

A field experiment was carried out with the sugarcane (*Saccharum* spp.) varieties IACSP95-5000 and IACSP94-2094, two commercial varieties with differential biomass production and yield

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(Silva *et al.,* 2016). IACSP95-5000 presents high yield in non-restrictive environments (Cruz *et al.,* 2021), while IACSP94-2094 is considered rustic and drought-resistant and indicated for less favourable environments (Sales *et al.,* 2013).

The plants were grown on a dystrophic red latosol in Campinas SP, Brazil (22°52′S, 47°04′W, 665 m a.s.l.). The experimental design was in randomised blocks, with four replications and plots composed of six rows of 14 m spaced by 1.5 m. The bud density in planting was 14 ± 1 buds per linear meter. The NPK fertiliser (8:28:16) was applied just before (50%) and 70 days after (50%) planting. In total, plants received 210, 135, and 195 kg ha^{-1} of N, K, and P, following a previous soil chemical analysis. The field was irrigated with a subsurface drip system, with one drip line per planting row. The drip lines were installed before planting, 0.20 m depth in the furrows. The emitters had nominal flow rate of 1.6 L h^{-1} and were spaced by 0.50 m. For soil water monitoring, we used the Enviroscan (Sentek Sensor Technologies, Stepney, Australia) and Diviner 2000 capacitance probes (Sentek Sensor Technologies, Stepney, Australia). Measurements were taken every 0.1 m down to 1.0 m depth. Three access tubes per variety were used for Diviner 2000 readings, and one access tube per variety for the EnviroScan probe, each with a length of 1.0 m and internal diameter of 0.05 m. Irrigation was meticulously managed on a daily basis to maintain soil moisture levels close to the field capacity. The water volume applied was calculated daily based on data from the Enviroscan and Diviner 2000. Field capacity was determined following the method outlined by Moraes *et al.* (1993). At 410 days after planting (DAP), irrigation was stopped to induce sugarcane maturation.

Environmental conditions were monitored using an automatic meteorological station installed inside the field plot, with sensors of air temperature (model HMP45C, Campbell, North Logan UT, USA), rainfall (model CS700, Campbell, North Logan UT, USA), wind speed (model 014A, Campbell, North Logan UT, USA), and incident photosynthetic active radiation (PAR_{IN}, model LI-190R, LI-COR, Lincoln NE, USA). The above variables were measured every minute, and data stored every five minutes in a data logger model CR1000 (Campbell, North Logan UT, USA). The climatological water balance was calculated according to Thornthwaite and Mather using the practical method described by Rolim *et al.* (1998) and considering 98 mm of soil water storage capacity.

Sampling was performed at four phenological stages, varying in terms of crop growth rate and stalk maturation: (1) maximum growth (158–362 DAP); (2) early maturation (363–397 DAP); (3) medium maturation (398–460 DAP); and (4) late maturation (461–491 DAP).

Leaf gas exchange, photochemistry, water potential, chlorophyll, and nitrogen contents

Measurements of leaf gas exchange, total chlorophyll index (Chl), and leaf water potential (ψ) were taken every ∼30 days, starting at 150 DAP and ending at the final harvest (491 DAP). Leaf gas exchange and Chl were measured in the same region on the first (+1) and third (+3) fully expanded leaves with visible dewlap of four plants in each plot. As the preliminary statistical analyses revealed no differences between leaves +1 and +3, data from both leaves were pooled.

Leaf gas exchange was evaluated with an infrared gas analyser model LI-6400XT (LI-COR, Lincoln NE, USA) equipped with a fluorometer model 6400-40 LCF (LI-COR, Lincoln NE, USA). The measurements were performed under air CO_2 concentration of 400 μmol mol^{-1} and natural variation of air temperature, relative humidity, and light intensity, with leaf exchange values being recorded after temporal stability and when the low total coefficient of variation was lower than 5%. Instantaneous measurements were taken every 2-hour intervals, predominantly on clear days, from 7h00 to 17h00. We assessed leaf CO_2 assimilation (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and the effective quantum efficiency of photosystem II (Φ_{PSII}). The instantaneous carboxylation efficiency (k) was calculated as A/C_i. From the diurnal CO_2 assimilation curves derived from these instantaneous measurements, the diurnal-integrated

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CO_2 assimilation (A_i, mol CO_2 m^{-2} d^{-1}) was estimated. Herein, g_s, k, and Φ_{PSII} are shown at 13h00, when there was the maximum light intensity.

The Chl was measured using a portable chlorophyll meter (ClorofiLOG CFL1030, Falker, Porto Alegre RS, Brazil) and considered the sum of chlorophylls *a* and *b*. ψ was measured in leaf +3 between 12h00 and 14h00, with a pressure chamber (model 3005, SoilMoisture Equipment Corp., Santa Barbara CA, USA). The leaf nitrogen concentration (LNC) was quantified in lyophilised leaves after digestion with sulphuric acid by using the colorimetric method proposed by Baethgen and Alley (1989). Such quantification was done in leaves +3 sampled at the maximum growth, and at the early and late maturation stages.

Total soluble sugars, starch, and sucrose

Total soluble sugars and sucrose were extracted from samples (0.75 g) of leaves +1 and stalks, using 10 mL of a solution composed by methanol, chloroform, and water (12:5:3, v:v:v) (Bieleski and Turner, 1966). Plant extracts were centrifuged at 600 *g* for 10 min, and the supernatants were homogenised with chloroform and water. After 24 h, the aqueous phase was used to determine the concentration of total soluble sugars and sucrose (Van Handel, 1968). Starch was measured in leaves by the enzymatic method proposed by Amaral *et al.* (2007), using 500 µL (120 U mL^{-1}) of α-amylase (EC 3.2.1.1) of thermostable *Bacillus licheniformis* (code E-ANAAM, Megazyme, Bray, Ireland) and 500 µL (30 U mL^{-1}) of amyloglucosidase (EC 3.2.1.3) from *Aspergillus niger* (code E-AMGPU, Megazyme, Bray, Ireland). The quantification of such carbohydrates was performed at the maximum growth and late maturation for leaves and at the early, medium, and late maturation for stalks. Stalks were segmented into three portions: top, medium, and bottom.

Enzymes of carbohydrate metabolism

The enzymatic analyses were performed in leaves and stalks (top, medium, and bottom portions) at the maximum growth and late maturation. The extraction of enzymes from stalks and leaves followed the method of Grof *et al.* (2007), with modifications: 0.5 g of fresh samples were macerated in a mortar with 5% polyvinylpolypyrrolidone (PVPP) in liquid nitrogen and 3.5 mL of 50 mM Hepes buffer (pH 7.5), containing 10 mM $MgCl_2$ and 1 mM EDTA. After centrifugation at 14 000 *g* for 20 min, 2.5 mL of the supernatant was collected and desalted on a Sephadex G25 column (PD-10, GE), previously saturated with the extraction buffer. The extract collected from the column after elution with the same extraction buffer was used to determine protein content and carry out enzymatic analyses. All extraction was carried out at low temperature (0–4°C), and the protein concentration in the enzymatic extract was determined following Bradford (1976).

The activities of soluble acid (SAI, EC 3.2.1.26) and neutral (SNI, EC 3.2.1.26) invertases were measured according to Zhu *et al.* (1997), using 240 mM sucrose at 37°C for 30 min. For SAI activity, the reaction was stopped by adding 2.5 M Tris and incubating at 100°C for 3 min. SNI assay reaction was stopped by incubation at 100°C for 3 min. For both SAI and SNI assays, the reducing sugars released were determined by the Somogyi–Nelson method (Nelson, 1944; Somogyi 1945, 1952). SuSy (EC 2.4.1.13) and SPS (EC 2.4.1.14) activities were evaluated according to Hubbard *et al.* (1989), with modifications suggested by Zhu *et al.* (1997). SuSy can either synthetise or hydrolyse sucrose, and its activity was measured towards sucrose synthesis. To determine the SuSy activity, the crude extracts were incubated with 50 mM of Tris-HCl (pH 7.5), containing 15 mM of $MgCl_2$, 25 mM of fructose, and 50 mM of UDP-glucose. For the SPS activity, crude extracts were incubated in 200 mM of Tris-HCl (pH 7.5), containing 10 mM of $MgCl_2$, 8 mM of fructose-6-phosphate, 40 mM of glucose-6-phosphate, 50 mM of UDP-glucose, and 2 mM of EDTA. SuSy and SPS activities were evaluated at 30°C for 0, 30, 60, and 90 minutes. Then, reactions were stopped by boiling (100 °C) for 3 min. Sucrose produced by both enzymes was assayed according to Van Handel (1968).

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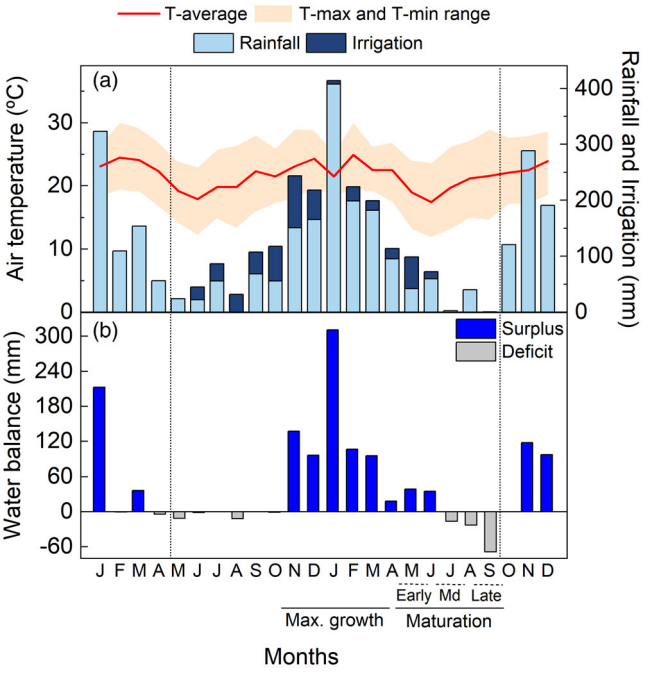

Figure 1. Monthly rainfall, irrigation, average, maximum and minimum air temperature (a), and climatological water balance (b). The experimental period is delimited by dotted lines.

Biomass production and leaf area

Evaluations of biomass were made every ~30 days (September to August) until the harvest. All plants from two linear metres of the central line of each plot were harvested for biomass quantification. Stalks and leaves were weighted to determine fresh biomass. For evaluating dry matter, fresh stalk and leaves were ground in a forage chopper and then a sample from each fraction was dried in a forced-air circulation oven at 60°C. The leaf area of all green leaves was measured with an electronic planimeter model LI-3000C (LI-COR, Lincoln NE, USA) coupled to the LI-3050C table accessory (LI-COR, Lincoln NE, USA). The leaf area index (LAI) was estimated considering all green leaves in 3 m^2.

Data analyses

The experimental design was in complete randomised blocks, with four replications. Each block was composed by two sugarcane varieties. The causes of variation were sampling time and sugarcane varieties, and data were subjected to ANOVA followed by Scott–Knott post hoc test ($P < 0.05$). The statistical analyses were performed using the software Rbio (Rbio 143, Viçosa MG, Brazil), and all graphs were created using GraphPad Prism9 (GraphPad Software Inc., San Diego CA, USA).

Results

Environmental conditions and leaf water status

During the experimental period, the accumulated rainfall was 2,039 mm, and the air temperature ranged from 12 to 30°C, with an average air temperature of 21°C (Figure 1a). Plants faced water

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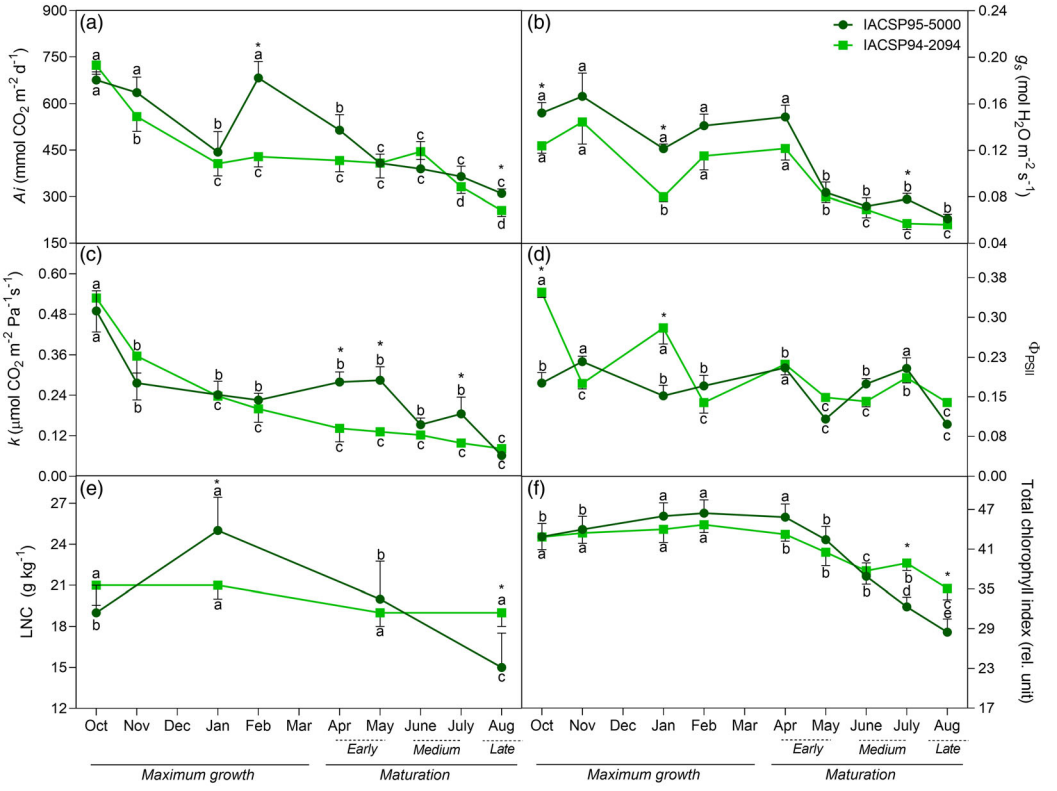

Figure 2. Diurnal-integrated leaf CO_2 assimilation (A_i, in a), stomatal conductance (g_s, in b), instantaneous carboxylation efficiency (k, in c), effective quantum yield of photosystem II (Φ_{PSII}, in d), leaf nitrogen concentration (LNC, in e), and total chlorophyll index (f) of IACSP95-5000 and IACSP94-2094 during the maximum growth and early, medium, and late maturation periods. Symbols represents mean ± SE; LNC, $n = 4$, all others $n = 8$ (pooled data from leaves +1 and +3). *Statistical difference between varieties, and distinct lowercase letters indicate statistical difference among sampling times at $P < 0.05$.

deficit at the beginning of crop cycle and during late maturation, when irrigation was stopped to induce sugarcane maturation. The maximum accumulated water deficit was 69 mm (Figure 1b).

Along the crop cycle, IACSP95-5000 and IACSP94-2094 showed similar ψ, with the lowest values found at the beginning of maximum growth stage (about −1.25 MPa), with significant recovery of ψ noticed at the rainy season. ψ decreased again at the end of maturation for both varieties (Supplementary Material Figure S1).

Leaf gas exchange, photochemistry, chlorophyll, and nitrogen contents

Diurnal-integrated leaf CO_2 assimilation (A_i), stomatal conductance (g_s), instantaneous carboxylation efficiency (k), and total chlorophyll index (Chl) exhibited a similar declining trend until the end of the maturation in both sugarcane varieties (Figure 2a–c, f). However, A_i was higher in IACSP95-5000 compared to IACSP94-2094 in February (maximum growth) and in August (late maturation), as shown in Figure 2a. The g_s followed a similar pattern of response as compared to A_i, with the highest values found during the maximum growth and then a decreasing trend from April (early maturation) in both varieties (Figure 2b). However, IACSP95-5000 had higher g_s than IACSP94-2094 during the maximum growth (October and January) period (Figure 2b). Both varieties exhibited decreases in k along the cycle, with the lowest values

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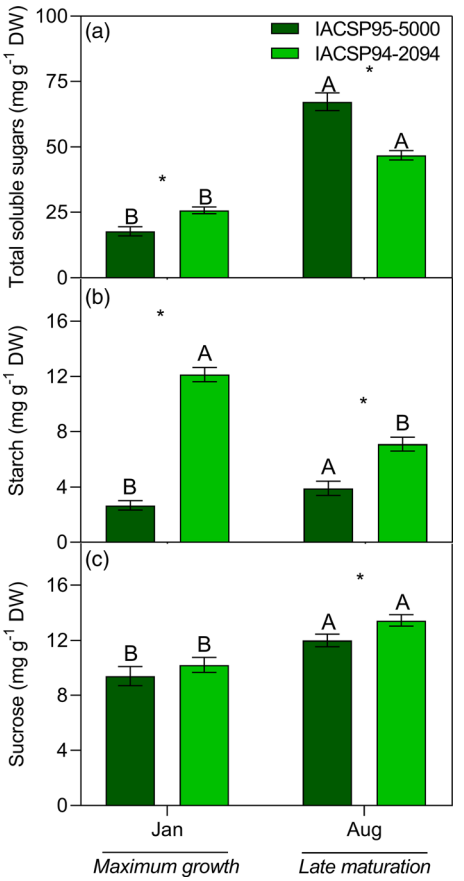

Figure 3. Concentration of total soluble sugars (a), starch (b), and sucrose (c) in leaves of IACSP95-5000 and IACSP94-2094 during the maximum growth and late maturation periods. Symbols represents mean ± SE; $n = 4$. *Statistical difference between varieties, and distinct uppercase letters indicate statistical differences among sampling times at $P < 0.05$. DW is dry weight.

occurring during the maturation period (Figure 2c). Again, IACSP95-5000 exhibited higher *k* than IACSP94-2094 during the maturation (April, May, and July) (Figure 2c). While Φ_{PSII} in IACSP94-2094 showed a decreasing trend since the maximum growth, IACSP95-5000 presented a smaller variation of Φ_{PSII} along the cycle (Figure 2d). IACSP94-2094 displayed higher Φ_{PSII} than IACSP95-5000 in October and January, that is, during the maximum growth stage (Figure 2d). Both sugarcane varieties exhibited the lowest Φ_{PSII} values at late maturation. While IACSP95-5000 presented the highest LNC at the maximum growth and the lowest LNC at late maturation, non-significant variation of LNC was found in IACSP94-2094 along the crop cycle (Figure 2e). The total chlorophyll index decreased at the maturation for both varieties. However, IACSP94-2094 exhibited Chl values higher than IACSP95-5000 at the late maturation (Figure 2f).

Carbohydrate dynamics in leaves and stalks

IACSP95-5000 and IACSP94-2094 showed higher leaf concentrations of total soluble sugars at the late maturation when compared with the maximum growth period (Figure 3a). At the maximum growth, IACSP94-2094 showed higher (1.45-fold) total soluble sugars than IACSP95-5000; however, this pattern was inverted at late maturation (Figure 3a). Starch concentration in

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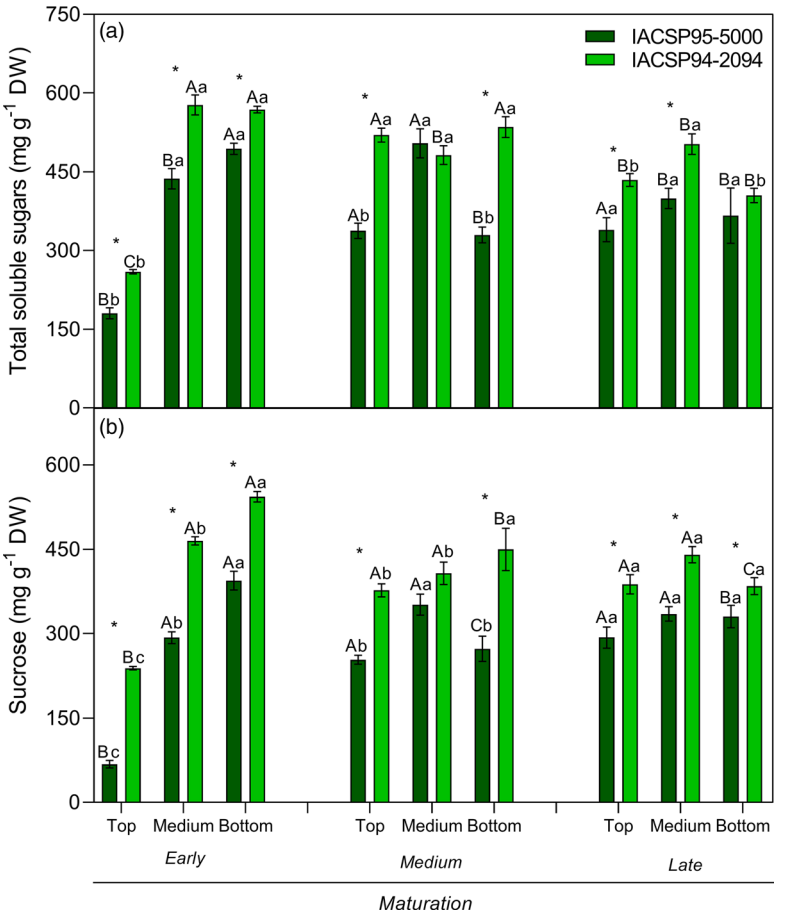

Figure 4. Concentration of total soluble sugars (a) and sucrose (b) in stalks portions (top, medium, and bottom) of IACSP95-5000 and IACSP94-2094 during the early, medium, and late maturation periods. Values are mean ± SE; $n = 4$. *Significant difference between varieties, distinct uppercase letters indicate statistical difference between sampling times, and distinct lowercase letters among stalk positions (top, medium, and bottom) at $P < 0.05$. DW is dry weight.

leaves of IACSP95-5000 was higher at late maturation than at the maximum growth, whereas IACSP94-2094 presented a significant decline at late maturation when compared to the maximum growth period (Figure 3b). Overall, IACSP94-2094 exhibited higher concentration of starch than IACSP95-5000 in both periods (Figure 3b). The sugarcane varieties exhibited similar leaf sucrose concentration during the maximum growth stage, but IACSP94-2094 presented higher sucrose concentration than IACSP95-5000 at the late maturation (Figure 3c). Both varieties exhibited higher leaf sucrose concentration at late maturation when compared to the maximum growth period (Figure 3c).

Concerning the total soluble sugars along the stalk, the top portion of both varieties showed the lowest values at early maturation, with IACSP94-2094 exhibiting higher values than IACSP95-5000 (Figure 4a). At the middle of maturation period, there was non-significant change ($P > 0.05$) in total soluble sugars along the stalk of IACSP94-2094, while IACSP95-5000 showed higher concentration in medium stalk portions (Figure 4a). IACSP94-2094 showed decreases in the total soluble sugars in medium and bottom portions at the late maturation. At that time, IACSP94-2094 had higher concentrations of total soluble sugars than IACSP95-5000 in top (1.3-fold) and medium (1.25-fold) stalk portions (Figure 4a). Overall, sucrose concentration increased gradually

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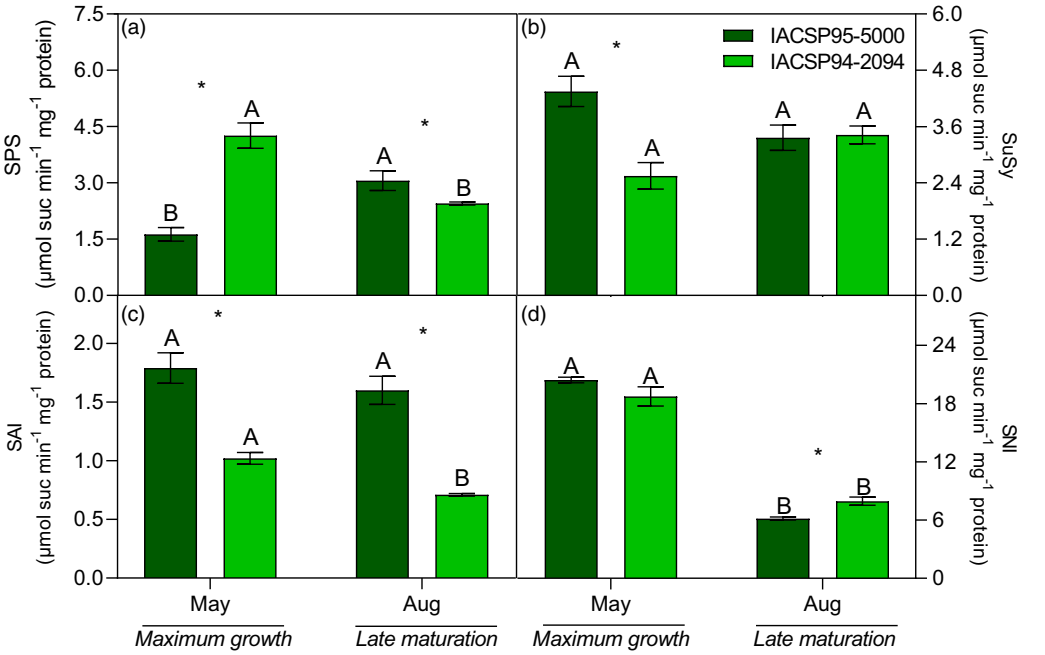

Figure 5. Activity of sucrose phosphate synthase (SPS, in a), sucrose synthase (SuSy, in b), soluble acid invertase (SAI, in c), and soluble neutral invertase (SNI, in d) in leaves of IACSP95-5000 and IACSP94-2094 during the maximum growth and late maturation periods. Symbols represents mean ± SE; $n = 4$. *Statistical difference between varieties, and distinct uppercase letters indicate statistical difference among sampling times at $P < 0.05$. suc is sucrose.

from top to bottom portions at the early maturation (Figure 4b). During the medium maturation, the highest concentration of sucrose occurred in the medium stalk portion of IACSP95-5000 and in the bottom stalk portion of IACSP94-2094 (Figure 4b). Despite the stalk portion and maturation time, IACSP94-2094 exhibited higher sucrose concentration than IACSP95-5000 (Figure 4b).

Carbohydrate metabolism: leaf enzymatic activity

The SPS activity in leaves of IACSP95-5000 increased 1.9-fold at the late maturation when compared with the maximum growth stage, while an inverse pattern was found for IACSP94-2094 (Figure 5a). Conversely, SuSy activity did not vary ($P > 0.05$) between the maximum growth and the late maturation for both varieties (Figure 5b). Leaves of IACSP95-5000 presented higher SuSy activity (1.7-fold) than ones of IACSP94-2094 at the maximum growth (Figure 5b). Leaf SAI activity was similar between periods for IACSP95-5000, which presented higher values than IACSP94-2094. This latter presented a significant decrease of leaf SAI activity at the late maturation (Figure 5c). The leaf SNI activity decreased at the late maturation of both varieties, with IACSP94-2094 exhibiting higher SNI activity than IACSP95-5000 (Figure 5d).

Carbohydrate metabolism: stalk enzymatic activity

At the maximum growth stage, stalk SPS activity of IACSP94-2094 decreased from top to bottom. When compared to IACSP95-5000, stalk SPS activity of IACSP94-2094 was higher in top (*ca.* 1.6-fold) and medium (*ca.* 1.8-fold) portions (Figure 6a). At the late maturation, IACSP94-2094 had higher SPS activity than IACSP95-5000 in top and bottom stalk portions (Figure 6a).

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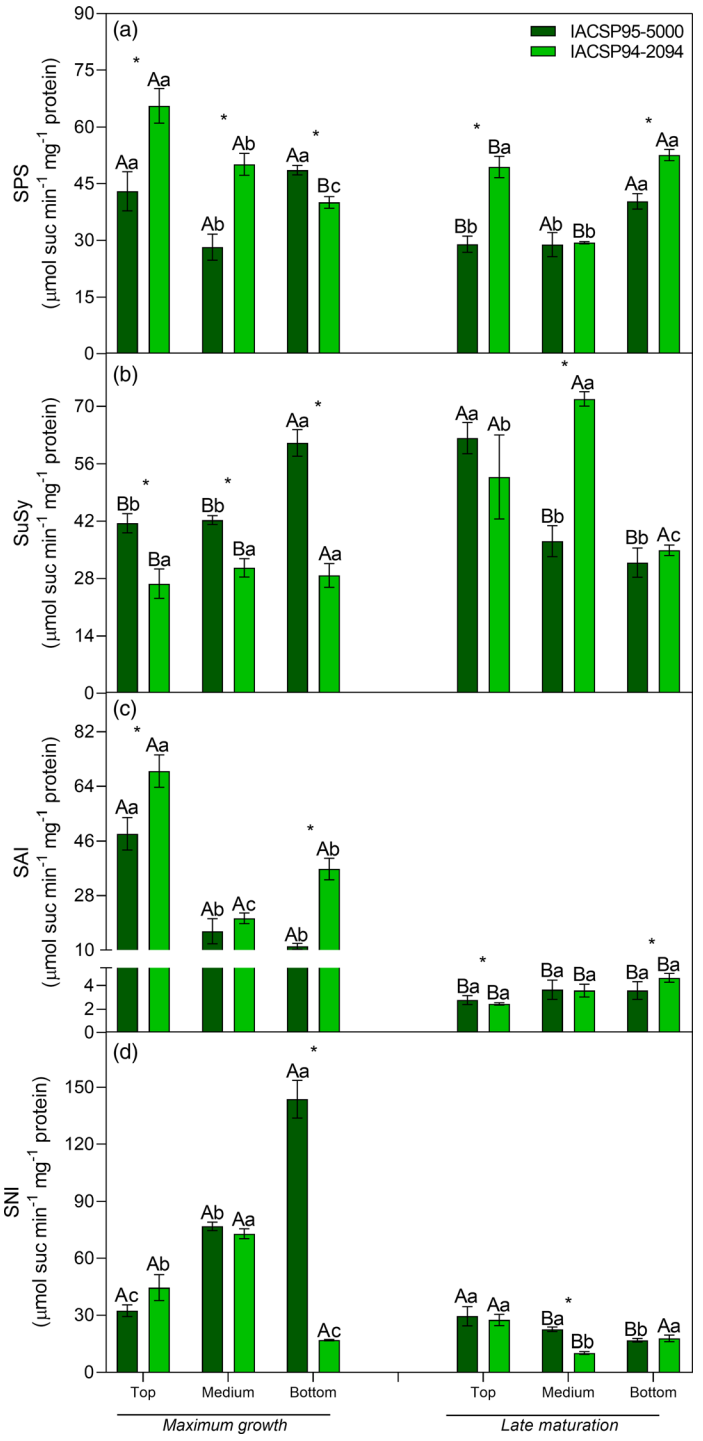

Figure 6. Activity of sucrose phosphate synthase (SPS, in a), sucrose synthase (SuSy, in b), soluble acid invertase (SAI, in c), and soluble neutral invertase (SNI, in d) in stalks portions (top, medium, and bottom) of IACSP95-5000 and IACSP94-2094 during the maximum growth and late maturation periods. Values are mean ± SE; $n = 4$. *Statistical difference between varieties, distinct uppercase letters indicate statistical differences between sampling times, and distinct lowercase letters among stalk portions (top, medium, and bottom) at $P < 0.05$. suc is sucrose.

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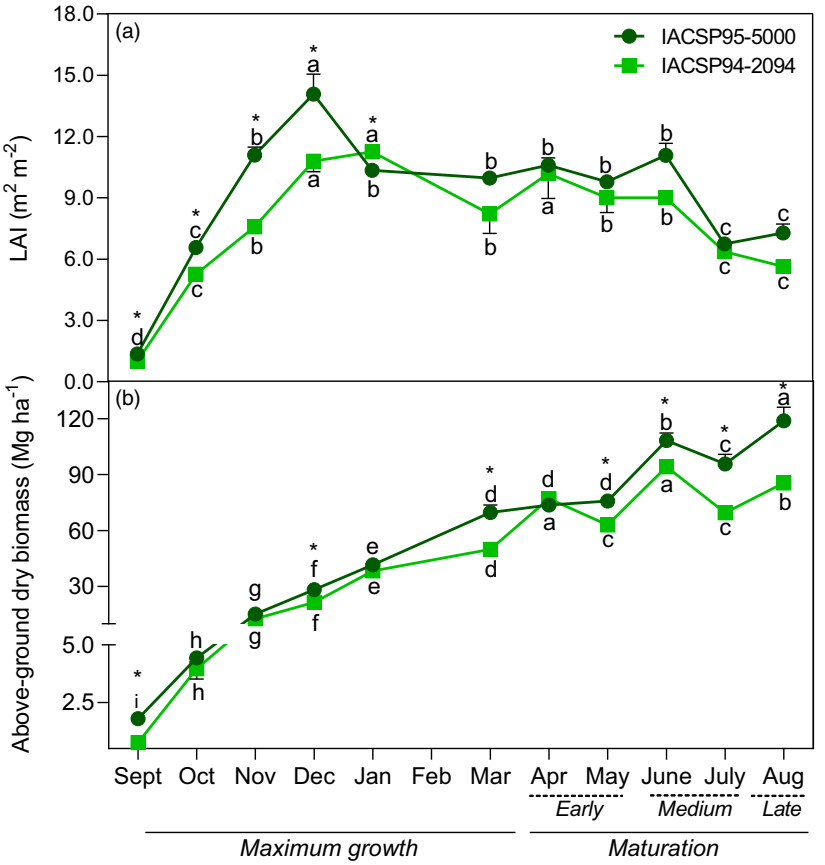

Figure 7. Leaf area index (LAI, in a) and total above-ground dry biomass (b) of IACSP95-5000 and IACSP94-2094 during the maximum growth and early, medium, and late maturation periods. Values are mean ± SE; $n = 4$. *Statistical difference between varieties, and distinct lowercase letters indicate statistical difference among sampling times at $P < 0.05$.

We measured the highest stalk SuSy activity in the bottom portion of IACSP95-5000 at the maximum growth, which presented higher SuSy activity than IACSP94-2094 (Figure 6b). At the late maturation, the highest SuSy activity was found in top portion of IACSP95-5000 and medium portion of IACSP94-2094 (Figure 6b). When comparing stalk portions at the maximum growth, the highest SAI activities were found in top portions of both varieties (Figure 6c). Stalk SAI activity in top and bottom portions was higher in IACSP94-2094 than in IACSP95-5000 (Figure 6c). IACSP95-5000 showed an increasing trend of SNI activity from top to bottom portions at the maximum growth. In bottom portion, SNI activity was 8.5-fold higher in IACSP95-5000 than in IACSP94-2094 (Figure 6d). Finally, the activities of SAI and SNI decreased at the late maturation in both varieties when compared to the maximum growth (Figure 6c, d).

Plant growth: leaf area and above-ground biomass

IACSP95-5000 exhibited higher LAI than IACSP94-2094 during the maximum growth period, with IACSP95-5000 and IACSP94-2094 reaching the highest LAI in December and January, respectively (Figure 7a). Accordingly, IACSP95-5000 produced more biomass than IACSP94-2094 at the end of crop cycle, that is, about +31% and +28% on fresh and dry bases, respectively (Figures 7b and S2).

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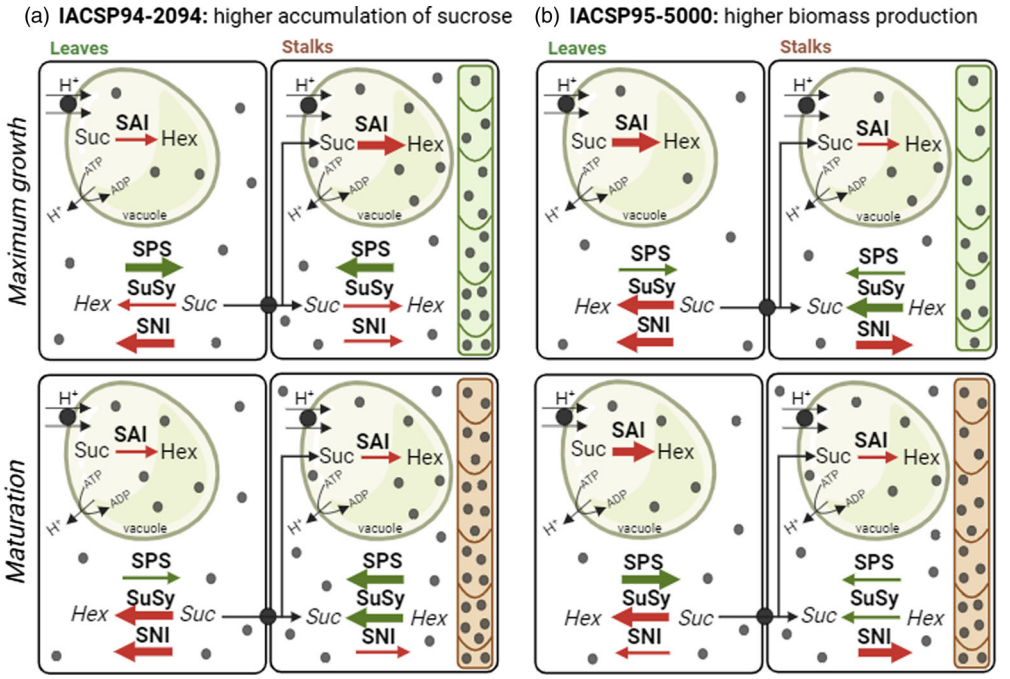

Figure 8. Scheme of sucrose accumulation and synthesis in leaves and stalks of IACSP94-2094 (a) and IACSP95-5000 (b) during the maximum growth and maturation periods. Arrow thickness denotes the intensity of responses when comparing varieties, with red arrows indicating sucrose degradation and green arrows indicating sucrose synthesis. Grey circles represent sucrose; Suc: sucrose; Hex: hexose; SPS: sucrose phosphate synthase; SuSy: sucrose synthase; SAI: soluble acid invertase; SNI: soluble neutral invertase.

Discussion

Here, we compared two sugarcane varieties with differential biomass production under field conditions. While IACSP95-5000 exhibited higher biomass production (Figures 7b and S2), IACSP94-2094 accumulated more sucrose per unit of stalk mass (Figure 4b). These differences can be explained by the carbohydrate metabolism of stalks and leaves (Figure 8). Notably and despite those contrasting strategies, both varieties displayed similar final sugar yield and then low stalk sucrose content in IACSP95-5000 was compensated by increased biomass production. Such compensatory mechanism was based on several metabolic adjustments for optimising resource allocation and promote high sugar yield.

Underlying mechanisms leading to low photosynthesis at maturation: a common response to ageing

Photosynthetic rates decreased while stalk sucrose levels increased (Figures 2a and 4b). Decline in photosynthesis of maturing sugarcane plants occurs with increasing leaf sugar concentrations (McCormick *et al.*, 2008a), with such build-up of leaf sugar being a possible consequence of impaired phloem loading and then reduced transport to sinks (stalks). Based on the source–sink relationship, one would expect stimulation of photosynthetic activity by increasing sink demand (Ribeiro *et al.*, 2017), that is, sucrose accumulation in stalks. However, this did not happen here and there is evidence that other exogenous (environment) or endogenous factors are limiting sugarcane photosynthesis at the end of plant cycle (De Souza *et al.*, 2018). Taking into account the environment, plants were clearly facing water deficit and low temperature at the maturation stage

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(Figure 1b) and such limiting condition is known to reduce stomatal conductance and photosynthesis (Sales *et al.,* 2015; Cerqueira *et al.,* 2019).

Here, our data suggest that photosynthesis is reduced during maturation due to stomatal and non-stomatal factors (Figure 2b, c). Considering stomatal aperture, previous studies revealed that sucrose accumulation in the guard cells affects the stomatal dynamics and then photosynthesis (Kelly *et al.,* 2013; Daloso *et al.,* 2015). In C4 plants, most of nitrogen is invested in the photosynthetic enzymes Rubisco and PEPC (Tazoe *et al.,* 2006), and then low LNC would justify decreases in *k* (Tofanello *et al.,* 2021) and also in total chlorophyll index with ageing, but only in IACSP95-5000 (Figure 2e, f). Even with non-significant change in LNC of IACSP94-2094 as maturation progressed, we noticed a decrease in CO_2 assimilation (Figure 2a, e). As a possible explanation, N allocation likely changed during the plant cycle and more N was driven to the synthesis of proteins not related to photosynthesis at latter phenological stages of IACSP94-2094. In addition to the water deficit and low temperature, high leaf sucrose content would be an additional factor downregulating photosynthesis (Ribeiro *et al.,* 2017).

We would argue that low Chl (Figure 2f) led to low Φ_{PSII} as maturation advanced, mainly for IACSP94-2094 (Figure 2d). Interestingly, reduction in Chl during the early and medium maturation of IACSP95-5000 did not result in low Φ_{PSII} (Figure 2d, f). In fact, photochemical activity is not defined solely by Chl (Silveira *et al.,* 2019) and decreases in photochemical activity were reported with advancing maturation, when sugarcane plants were facing water deficit (De Souza *et al.,* 2018). Here, we found evidence that plants were under water deficit only at the late maturation period, when irrigation was stopped to induce sugarcane maturation (Figure 1b). However, our data on leaf water potential did not reveal this (Figure S1). For instance, the highest and lowest diurnal-integrated CO_2 assimilation were noticed with ψ around −1.2 MPa (Figures 2a and S1). Then, low temperature seems to have an important role during the maturation period (Figure 1a), limiting photosynthesis in field-grown plants under subtropical conditions. Previously, we found sugarcane plants are sensitive to low temperature, with reduced photosynthetic rates due to stomatal, photochemical, and biochemical (low Rubisco activity) limitations (Cerqueira *et al.,* 2019).

Taken together, these findings suggest that the reduction in photosynthetic rates as sugarcane plants mature is due to ageing-related changes as an endogenous factor and also due to low temperature, both inducing decreases in carboxylation, photochemical activity, and stomatal conductance (Figure 2). Both varieties exhibited similar photosynthetic dynamics throughout the experimental period (Figure 2a), but IACSP95-5000 presented higher CO_2 assimilation (Figure 2a) and higher LAI (Figure 7a) than IACSP94-2094. As consequence, IACSP95-5000 produced more biomass than IACSP94-2094 (Figures 7b and S2). On the other hand, IACSP94-2094 accumulated more sucrose in stalks as compared to IACSP95-5000.

Stalk sucrose accumulation: an interplay between leaves and stalks

When considering biomass production (Figures 7b and S2) and sucrose accumulation (Figure 4b), differences between IACSP95-5000 and IACSP94-2094 can be explained by the carbohydrate metabolism of stalks and leaves (Figure 8). During the period of maximum growth, IACSP94-2094 showed high SPS activity (Figures 5a and 8) and low SAI activity in leaves (Figures 5c and 8). In contrast, leaves of IACSP95-5000 exhibited high SAI and SuSy activities (Figures 5b, c and 8). While increased leaf SPS activity promotes sucrose synthesis (Chandra *et al.,* 2015), low leaf SAI activity reduces sucrose degradation (Anur *et al.,* 2020). In IACSP95-5000, high leaf activities of SAI and SuSy likely accelerated sucrose hydrolysis (McCormick *et al.,* 2008a; Anur *et al.,* 2020; Mason *et al.,* 2023), a way to supply hexoses for growth respiration and then support cell division and expansion. Accordingly, leaf area was higher in IACSP95-5000 than in IACSP94-2094 (Figure 7a). Increased sucrose degradation is also an alternative to avoid the downregulation of photosynthesis induced by high leaf sucrose concentration (Ribeiro *et al.*, 2017; Anur *et al.,* 2020).

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Again, photosynthetic activity in IACSP95-5000 was significantly higher than that in IACSP94-2094 (Figures 2 and 5c).

In sink organs such as stalks, IACSP95-5000 had high activity of SuSy and SNI (Figures 6b, d and 8), two key enzymes playing an important role in controlling the rate of sucrose hydrolysis, mainly in immature tissues (Rossouw *et al.,* 2007, 2010). SuSy operates primarily towards the sucrose degradation, which is cleaved into fructose and UDP-Glu and then used in respiration and polymer (starch or cell wall constituents) biosynthesis (Wang *et al.,* 2013). For instance, low SNI activity reduces sucrose mobilisation and impairs growth in sugarcane plants due to low availability of hexoses for respiration (Rossouw *et al.,* 2007). As compared with IACSP95-5000, stalks of IACSP94-2094 showed lower SNI activity, but higher SPS and SAI activities (Figures 6a, c, d and 8). According to You-Qiang *et al.* (2009), sugarcane varieties with high sucrose accumulation exhibit higher SPS activity throughout the development stages. As stalk SAI activity provides substrates for supporting the growth of immature and expanding tissues in sugarcane (Rossouw *et al.,* 2007), a greater capacity for sucrose accumulation at maturation is expected in varieties presenting high SAI activity, as IACSP94-2094. In addition, increased SAI activity may contribute to the remobilisation of sucrose stored in vacuoles (Liu *et al.,* 2021).

Low amounts of soluble sugars in leaf tissues may indicate high sucrose transport rates to sinks rather than low photosynthesis (Mason *et al.,* 2020). At the maturation period, IACSP94-2094 presented high SPS activity and low SAI activity in stalks (Figures 6a, c and 8). In principle, these metabolic adjustments would support a greater accumulation of sucrose. In contrast, IACSP95-5000 showed an increased activity of SNI in stalks (Figures 6d and 8). Overall, the activity of SNI decreased significantly at late maturation in both varieties (Figures 6d and 8), but no impact is expected as glycolytic flux is significantly reduced at this time (Rossouw *et al.,* 2007). Furthermore, decreased SNI activity in stalks is compensated by increased SuSy activity (Rossouw *et al.,* 2010).

Our data revealed the underlying physiological mechanisms responsible for differential biomass production between the sugarcane varieties studied (Figure 8). The high-yielding variety – IACSP95-5000 – used energy and carbon skeletons to produce more biomass, which was linked to increased enzymatic degradation of sucrose in leaves and stalks. On the other hand, IACSP94-2094 demonstrated a more efficient strategy for sucrose accumulation in stalks (Figure 8). Such higher efficiency was attributed to a higher activity of SPS in leaves and stalks during the maximum growth and maturation, as well as low activity of SAI in leaves during the maximum growth and in stalks during the maturation (Figure 8). This pattern would maximise the sucrose flux to stalks and its consequent accumulation in IACSP94-2094. As mentioned before, both varieties displayed similar total sugar yield, varying between ~34 Mg ha^{-1} in IACSP95-5000 and ~29 Mg ha^{-1} for IACSP94-2094 with such difference being non-significant (t test; $P > 0.05$). Our findings indicate a compensatory mechanism between biomass production and sucrose accumulation in sugarcane, involving several metabolic adjustments for optimising resource allocation and promote high sugar yield. From a broad perspective, a potential failure in such compensatory mechanism between biomass production and sucrose accumulation or even a change in resource (carbon and nitrogen) allocation could explain the declining trend of cane and sucrose yield ratoon after ratoon, with important consequences for sugarcane management and industry.

Agronomic and industrial perspectives: maximising biomass vs. sucrose yield

Here, IACSP94-2094 showed higher and faster sucrose accumulation in stalks than IACSP95-5000, as shown here (Figures 4, 8) and evidenced by soluble solids (°Brix, data not shown). Such characteristics would favour early harvesting of IACSP94-2094 (Singh *et al.*, 2017). Additionally, the need for ripeners would be reduced in IACSP94-2094 as compared to IACSP95-5000, an interesting benefit for managing sugarcane fields. Besides the environmental impact (De Almeida *et al.*, 2022), the use of ripeners can reduce ratoon sprouting, affecting sugarcane regrowth and longevity (Mehareb *et al.*, 2016).

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Considering an industrial perspective, the choice of varieties with high biomass production or high sugar yield depends on the purpose. For conventional first-generation ethanol production, one would prefer varieties with high sucrose concentration in stalks (De Almeida and Colombo, 2023), while the best options for the second-generation ethanol would be sugarcane varieties or other species with high biomass production, such as energy cane (Dias *et al.,* 2012; Cruz *et al.*, 2021). As lower sucrose concentration is compensated by higher biomass production, IACSP95-5000 would be a better option than IACSP94-2094 when the aim is the first (from sucrose) and second (from bagasse) generation ethanol. Industrial capacity is an important aspect for such double-purpose varieties, a discussion beyond the scope of this paper. On the other hand, IACSP94-2094 is rustic and less sensitive to limiting conditions, such as water deficit (Ribeiro *et al.*, 2003; Contiliani *et al.,* 2023). As biomass production is limited not only by endogenous factors but also by environmental ones, IACSP94-2094 would be an interesting sugarcane variety to supply industry when growing areas are rainfed and present a large seasonal variation of water availability.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0014479724000061

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References

Amaral, L.I.V., Gaspar, M., Costa, P.M.F., Aidar, M.P.M. and Buckeridge, M.S. (2007). A new rapid and sensitive enzymatic method for extraction and quantification of starch in plant material. *Hoehnea* **34**, 425–431. https://doi.org/10.1590/S2236-89062007000400001.

Anur, R.M., Mufithah, N., Sawitri, W.D., Sakakibara, H. and Sugiharto, B. (2020). Overexpression of sucrose phosphate synthase enhanced sucrose content and biomass production in transgenic sugarcane. *Plants* **9**, 1–11. https://doi.org/10.3390/plants9020200.

Baethgen, W.E. and Alley, M.M. (1989). A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant kjeldahl digests. *Communications in Soil Science and Plant Analysis* **20**, 961–969. https://doi.org/10.1080/00103628909368129.

Bieleski, R.L. and Turner, N.A. (1966). Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. *Analytical Biochemistry* **17**, 278–293. https://doi.org/10.1016/0003-2697(66)90206-5.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Cerqueira, G., Santos, M.C., Marchiori, P.E.R., Silveira, N.M., Machado, E.C. and Ribeiro, R.V. (2019). Leaf nitrogen supply improves sugarcane photosynthesis under low temperature. *Photosynthetica* **57**, 18–26. https://doi.org/10.32615/ps.2019.033.

Contiliani, D.F., Nebó, J.F.C.D.O., Ribeiro, R.V., Landell, M.G.D.A., Pereira, T.C., Ming, R., Figueira, A. and Creste, S. (2023). Drought-triggered leaf transcriptional responses disclose key molecular pathways underlying leaf water use efficiency in sugarcane (*Saccharum* spp.). *Frontiers in Plant Science* **14**, 1182461. https://doi.org/103389/fpls.2023.1182461.

Chandra, A., Verma, P.K., Islam, M.N., Grisham, M.P., Jain, R., Sharma, A., Roopendra, K., Singh, K., Singh, P., Verma, I. and Solomon, S. (2015). Expression analysis of genes associated with sucrose accumulation in sugarcane (*Saccharum* spp. hybrids) varieties differing in content and time of peak sucrose storage. *Plant Biology* **17**, 608–617. https://doi.org/10.1111/plb.12276.

Cruz, L.P., Pacheco, V.S., Silva, L.M., Almeida, R.L., Miranda, M.T., Pissolato, M.D., Machado, E.C. and Ribeiro, R.V. (2021). Morpho-physiological bases of biomass production by energy cane and sugarcane: a comparative study. *Industrial Crops and Products* **171**, 113884. https://doi.org/10.1016/j.indcrop.2021.113884.

Daloso, D.M., Antunes, W.C., Pinheiro, D.P., Waquim, J.P., Araújo, W.L., Loureiro, M.E., Fernie, A.R. and Williams, T.C.R. (2015). Tobacco guard cells fix CO_2 by both Rubisco and PEPcase while sucrose acts as a substrate during light-induced stomatal opening. *Plant Cell and Environment* **38**, 2353–2371. https://doi.org/10.1111/pce.12555.

De Almeida, M.A. and Colombo, R. (2023). Production chain of first-generation sugarcane bioethanol: characterization and value-added application of wastes. *BioEnergy Research* **16**, 924–939. https://doi.org/10.1007/s12155-021-10301.

De Almeida, S.M., Véliz, J.G.E., Sartori, M.M.P. and Santos, H.L. (2022). Glyphosate applied at a hormetic dose improves ripening without impairing sugarcane productivity and ratoon sprouting. *Science of the Total Environment* **806**, 150503. https://doi.org/10.1016/j.scitotenv.2021.150503.

De Souza, A.P., Grandis, A., Arenque-Musa, B.C. and Buckeridge, M.S. (2018). Diurnal variation in gas exchange and nonstructural carbohydrates throughout sugarcane development. *Functional Plant Biology* **45**, 865–876. https://doi.org/10.1071/FP17268.

Dias, M.O., Junqueira, T.L., Jesus, C.D., Rossell, C.E., Maciel Filho, R. and Bonomi, A. (2012). Improving second generation ethanol production through optimization of first generation production process from sugarcane. *Energy* **43**, 246–252. https://doi.org/10.1016/j.energy.2012.04.034.

Du, Y.C. and Nose, A. (2002). Effects of chilling temperature on the activity of enzymes of sucrose synthesis and the accumulation of saccharides in leaves of three sugarcane cultivars differing in cold sensitivity. *Photosynthetica* **40**, 389–395. https://doi.org/10.1023/A:1022627125355.

García, J.M., Molina, C., Simister, R., Taibo, C.B., Setten, L., Erazzú, L.E., Gómez, L.D. and Acevedo, A. (2023). Chemical and histological characterization of internodes of sugarcane and energy-cane hybrids throughout plant development. *Industrial Crops and Products* **199**, 116739. https://doi.org/10.1016/j.indcrop.2023.116739.

Grof, C.P.L., Albertson, P.L., Bursle, J., Perroux, J.M., Bonnett, G.D. and Manners, J.M. (2007). Sucrose-phosphate synthase, a biochemical marker of high sucrose accumulation in sugarcane. *Crop Science* **47**, 1530–1539. https://doi.org/10.2135/cropsci2006.12.0825.

Huang, D.L., Qin, C.X., Gui, Y.Y., Zhao, L.H., Chen, Z.L., Wang, M., Sun, Y., Liao, Q., Li, Y.R. and Lakshmanan, P. (2017). Role of the SPS gene families in the regulation of sucrose accumulation in sugarcane. *Sugar Tech* **19**, 117–124. https://doi.org/10.1007/s12355-016-0454-x.

Hubbard, N.L., Huber, S.C. and Pharr, D.M. (1989). Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (*Cucumis melo* L.) fruits. *Plant Physiology* **91**, 1527–1534. https://doi.org/10.1104/pp.91.4.1527.

Kelly, G., Moshelion, M., David-Schwartz, R., Halperin, O., Wallach, R., Attia, Z., Belausov, E. and Granot, D. (2013). Hexokinase mediates stomatal closure. *Plant Journal* **75**, 977–988. https://doi.org/10.1111/tpj.12258.

Liu, G., Zhang, Y., Gong, H., Li, S., Pan, Y., Davis, C., Jing, H.C., Wu, L. and Godwin, I.D. (2021). Stem vacuole-targetted sucrose isomerase enhances sugar content in sorghum. *Biotechnology for Biofuels* **14**, 1–14. https://doi.org/10.1186/s13068-021-01907-z.

Lobo, A.K.M., De Oliveira, M.M., Lima, N.M.C., Machado, E.C., Ribeiro, R.V. and Silveira, J.A.G. (2015). Exogenous sucrose supply changes sugar metabolism and reduces photosynthesis of sugarcane through the down-regulation of Rubisco abundance and activity. *Journal of Plant Physiology* **179**, 113–121. https://doi.org/10.1016/j.jplph.2015.03.007.

Marchiori, P.E.R., Machado, E.C. and Ribeiro, R.V. (2014). Photosynthetic limitations imposed by self-shading in field-grown sugarcane varieties. *Field Crops Research* **155**, 30–37. https://doi.org/10.1016/j.fcr.2013.09.025.

Marchiori, P.E.R., Machado, E.C., Sales, C.R.G., Espinoza-Núñez, E., Magalhães Filho, J.R., Souza, G.M., Pires, R.C.M. and Ribeiro, R.V. (2017). Physiological plasticity is important for maintaining sugarcane growth under water deficit. *Frontiers in Plant Science* **8**, 1–12. https://doi.org/10.3389/fpls.2017.02148.

Marchiori, P.E.R., Ribeiro, R.V., Da Silva, L., Machado, R.S., Machado, E.C. and Scarpari, M.S. (2010). Plant growth, canopy photosynthesis and light availability in three sugarcane varieties. *Sugar Tech* **12**, 160–166. https://doi.org/10.1007/s12355-010-0031-7.

Mason, P.J., Furtado, A., Marquardt, A., Hodgson-Kratky, K., Hoang, N.V., Botha, F.C., Papa, G., Mortimer, J.C., Simmons, B. and Henry, R.J. (2020). Variation in sugarcane biomass composition and enzymatic saccharification of leaves, internodes and roots. *Biotechnology for Biofuels* **13**, 1–19. https://doi.org/10.1186/s13068-020-01837-2.

Mason, P.J., Hoang, N.V., Botha, F.C., Furtado, A., Marquardt, A. and Henry, R.J. (2022). Comparison of the root, leaf and internode transcriptomes in sugarcane (*Saccharum* spp. hybrids). *Current Research in Biotechnology* **4**, 167–178. https://doi.org/10.1016/j.crbiot.2022.02.005.

Mason, P.J., Hoang, N.V., Botha, F.C., Furtado, A., Marquardt, A. and Henry, R.J. (2023). Organ-specific expression of genes associated with the UDP-glucose metabolism in sugarcane (*Saccharum* spp. hybrids). *BMC Genomics* **24**, 1–23. https://doi.org/10.1186/s12864-023-09124-8.

Mehareb, E.M., Abou-Elwafa, S.F. and Galal, M.O. (2016). Mean performance and Ratooning ability of sugarcane promising genotypes at early clonal selection. *American-Eurasian Journal Agriculture Environmetal Science* **16**, 20–27. https://doi.org/10.5829/idosi.aejaes.2016.16.1.12850.

McCormick, A.J., Cramer, M.D. and Watt, D.A. (2006). Sink strength regulates photosynthesis in sugarcane. *New Phytologist* **171**, 759–770. https://doi.org/10.1111/j.1469-8137.2006.01785.x.

McCormick, A.J., Cramer, M.D. and Watt, D.A. (2008a). Regulation of photosynthesis by sugars in sugarcane leaves. *Journal of Plant Physiology* **165**, 1817–1829. https://doi.org/10.1016/j.jplph.2008.01.008.

McCormick, A.J., Cramer, M.D. and Watt, D.A. (2008b). Differential expression of genes in the leaves of sugarcane in response to sugar accumulation. *Tropical Plant Biology* **1**, 142–158. https://doi.org/10.1007/s12042-008-9013-2.

McCormick, A.J., Watt, D.A. and Cramer, M.D. (2009). Supply and demand: sink regulation of sugar accumulation in sugarcane. *Journal of Experimental Botany* **60**, 357–364. https://doi.org/10.1093/jxb/ern310.

Mirajkar, S.J., Suprasanna, P. and Vaidya, E.R. (2016). Spatial distribution and dynamics of sucrose metabolising enzymes in radiation induced mutants of sugarcane. *Plant Physiology and Biochemistry* **100**, 85–93. https://doi.org/10.1016/j.plaphy.2015.12.018.

Misra, V., Mall, A.K., Ansari, S.A. and Ansari, M.I. (2022). Sugar transporters, sugar-metabolizing enzymes, and their interaction with phytohormones in sugarcane. *Journal of Plant Growth Regulation* **42**, 4975–4988. https://doi.org/10.1007/s00344-022-10778-z.

Moraes, S.O., Libardi, P.L. and Dourado Neto, D. (1993). Methodological problems of the soil-water retention curve. *Scientia Agricola* **50**, 383–392. https://doi.org/10.1590/s0103-90161993000300010.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry* **153**, 375–380. https://doi.org/10.1016/s0021-9258(18)71980-7.

Ribeiro, R.V., Machado, E.C., Magalhães Filho, J.R., Lobo, A.K.M., Martins, M.O., Silveira, J.A.G., Yin, X. and Struik, P.C. (2017). Increased sink strength offsets the inhibitory effect of sucrose on sugarcane photosynthesis. *Journal of Plant Physiology* **208**, 61–69. https://doi.org/10.1016/j.jplph.2016.11.005.

Rolim, G.S., Sentelhas, C.P. and Barbieri, V. (1998). Planilhas no ambiente excel para os cálculos de balanço hídricos: normal, sequencial, de cultura e de produtividade real e potencial. *Revista Brasileira de Agrometeorologia* **6**, 133–137.

Rossouw, D., Bosch, S., Kossmann, J., Botha, F.C. and Groenewald, J.H. (2007). Downregulation of neutral invertase activity in sugarcane cell suspension cultures leads to a reduction in respiration and growth and an increase in sucrose accumulation. *Functional Plant Biology* **34**, 490–498. https://doi.org/10.1071/FP06214.

Rossouw, D., Kossmann, J., Botha, F.C. and Groenewald, J.H. (2010). Reduced neutral invertase activity in the culm tissues of transgenic sugarcane plants results in a decrease in respiration and sucrose cycling and an increase in the sucrose to hexose ratio. *Functional Plant Biology* **37**, 22–31. https://doi.org/10.1071/FP08210.

Sales, C.R.G., Marchiori, P.E.R., Machado, R.S., Fontenele, A.V., Machado, E.C., Silveira, J.A.G. and Ribeiro, R.V. (2015). Photosynthetic and antioxidant responses to drought during sugarcane ripening. *Photosynthetica* **53**, 547–554. https://doi.org/10.1007/s11099-015-0146-x.

Sales, C.R.G., Ribeiro, R.V., Hayashi, A.H., Marchiori, P.E.R., Silva, K.I., Martins, M.O., Silveira, J.A.G., Silveira, N.M. and Machado, E.C. (2018). Flexibility of C4 decarboxylation and photosynthetic plasticity in sugarcane plants under shading. *Environmental and Experimental Botany* **149**, 34–42. https://doi.org/10.1016/j.envexpbot.2017.10.027.

Sales, C.R.G., Ribeiro, R.V., Silveira, J.A.G., Machado, E.C., Martins, M.O. and Lagôa, A.M.M.A. (2013). Superoxide dismutase and ascorbate peroxidase improve the recovery of photosynthesis in sugarcane plants subjected to water deficit and low substrate temperature. *Plant Physiology and Biochemistry* **73**, 326–336. https://doi.org/10.1016/j.plaphy.2013.10.012.

Shanthi, R., Alarmelu, S., Mahadeva, S.H. and Lakshmi, P.T. (2023). Impact of climate change on sucrose synthesis in sugarcane varieties. In Verma, K.K., Rajput, V.D., Li, Y.R., Song, X.P., Solomon, S. and Rao, G.P. (eds.), *Agro-industrial Perspectives on Sugarcane Production Under Environmental Stress*. Singapore: Springer Nature, pp. 13–38.

Silva, A.L.B.O., Pires, R.C.M., Ribeiro, R.V., Machado, E.C., Blain, G.C. and Ohashi, A.Y.P. (2016). Development, yield and quality attributes of sugarcane cultivars fertigated by subsurface drip irrigation. *Revista Brasileira de Engenharia Agricola e Ambiental* **20**, 525–532. https://doi.org/10.1590/1807-1929/agriambi.v20n6p525-532.

Silveira, N.M., Marcos, F.C.C., Frungillo, L., Moura, B.B., Seabra, A.B., Salgado, I., Machado, E.C., Hancock, J.T. and Ribeiro, R.V. (2017). S-nitrosoglutathione spraying improves stomatal conductance, Rubisco activity and antioxidant defense in both leaves and roots of sugarcane plants under water deficit. *Physiologia Plantarum* **160**, 383–395. https://doi.org/10.1111/ppl.12575.

Silveira, N.M., Seabra, A.B., Marcos, F.C.C., Pelegrino, M.T., Machado, E.C. and Ribeiro, R.V. (2019). Encapsulation of S-nitrosoglutathione into chitosan nanoparticles improves drought tolerance of sugarcane plants. *Nitric Oxide* **84**, 38–44. https://doi.org/10.1016/j.niox.2019.01.004.

Singh, P., Pathak, S.K., Singh, M.M., Mishra, V. and Sharma, B.L. (2017). Impact of high sugar early maturing varieties for sustainable sugar production in subtropical India. *Sugar Tech* **19**, 368–372. https://doi.org/10.1007/s12355-016-0477-3.

Somogyi, M. (1945). A new reagent for the determination of sugars. *Journal of Biological Chemistry* **160**, 61–68. https://doi.org/10.1016/s0021-9258(18)43097-9.

Somogyi, M. (1952). Notes on sugar determination. *The Journal of Biological Chemistry* **195**, 19–23. https://doi.org/10.1016/s0021-9258(19)50870-5.

Tazoe, Y., Noguchi, K. and Terashima, I. (2006). Effects of growth light and nitrogen nutrition on the organization of the photosynthetic apparatus in leaves of a C4 plant, *Amaranthus cruentus*. *Plant, Cell and Environment* **29**, 691–700. https://doi.org/10.1111/j.1365-3040.2005.01453.x.

Tofanello, V.R., Andrade, L.M., Flores-Borges, D.N.A., Kiyota, E., Mayer, J.L.S., Creste, S., Machado, E.C., Yin, X., Struik, P.C. and Ribeiro, R.V. (2021). Role of bundle sheath conductance in sustaining photosynthesis competence in sugarcane plants under nitrogen deficiency. *Photosynthesis Research* **149**, 275–287. https://doi.org/10.1007/s11120-021-00848-w.

Van Handel, E. (1968). Direct microdetermination of sucrose. *Analytical Biochemistry* **22**, 280–283. https://doi.org/10.1016/0003-2697(68)90317-5.

Verma, I., Roopendra, K., Sharma, A., Chandra, A. and Kamal, A. (2019). Expression analysis of genes associated with sucrose accumulation and its effect on source–sink relationship in high sucrose accumulating early maturing sugarcane variety. *Physiology and Molecular Biology of Plants* **25**, 207–220. https://doi.org/10.1007/s12298-018-0627-z.

Verma, A.K., Upadhyay, S.K., Verma, P.C., Solomon, S. and Singh, S.B. (2011). Functional analysis of sucrose phosphate synthase (SPS) and sucrose synthase (SS) in sugarcane (*Saccharum*) cultivars. *Plant Biology* **13**, 325–332. https://doi.org/10.1111/j.1438-8677.2010.00379.x.

Wang, J., Nayak, S., Koch, K. and Ming, R. (2013). Carbon partitioning in sugarcane (*Saccharum* species). *Frontiers in Plant Science* **4**, 2005–2010. https://doi.org/10.3389/fpls.2013.00201.

You-Qiang, P., Hai-Ling, L. and Yang-Rui, L. (2009). Soluble acid invertase and sucrose phosphate synthase: key enzymes in regulating sucrose accumulation in sugarcane stalk. *Sugar Tech* **11**, 28–33. https://doi.org/10.1007/s12355-009-0005-9.

Zhu, Y.J., Komor, E. and Moore, P.H. (1997). Sucrose accumulation in the sugarcane stem is regulated by the difference between the activities of soluble acid invertase and sucrose phosphate synthase. *Plant Physiology* **115**, 609–616. https://doi.org/10.1104/pp.115.2.609.

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