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Exogenous sucrose supply changes sugar metabolism and reduces photosynthesis of sugarcane through the down-regulation of Rubisco abundance and activity

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ABSTRACT

Photosynthetic modulation by sugars has been known for many years, but the biochemical and molecular comprehension of this process is lacking. We studied how the exogenous sucrose supplied to leaves could affect sugar metabolism in leaf, sheath and stalk and inhibit photosynthesis in four-month old sugarcane plants. Exogenous sucrose 50 mM sprayed on attached leaves strongly impaired the net CO_2 assimilation (P_N) and decreased the instantaneous carboxylation efficiency (P_N/Ci), suggesting that the impairment in photosynthesis was caused by biochemical restrictions. The photosystem II activity was also affected by excess sucrose as indicated by the reduction in the apparent electron transport rate, effective quantum yield and increase in non-photochemical quenching. In leaf segments, sucrose accumulation was related to increases in the activities of soluble acid and neutral invertases, sucrose synthase and sucrose phosphate synthase, whereas the contents of fructose increased and glucose slightly decreased. Changes in the activities of sucrose hydrolyzing and synthesizing enzymes in leaf, sheath and stalk and sugar profile in intact plants were not enough to identify which sugar(s) or enzyme(s) were directly involved in photosynthesis modulation. However, exogenous sucrose was able to trigger down-regulation in the Rubisco abundance, activation state and enzymatic activity. Despite the fact that P_N/Ci had been notably decreased by sucrose, *in vitro* activity and abundance of PEPCase did not change, suggesting an *in vivo* modulation of this enzyme. The data reveal that sucrose and/or other derivative sugars in leaves inhibited sugarcane photosynthesis by down-regulation of Rubisco synthesis and activity. Our data also suggest that sugar modulation was not exerted by a feedback mechanism induced by the accumulation of sugars in immature sugarcane stalk.

Abbreviations: Ci, intercellular CO_2 partial pressure; ETR, electron transport rate from photosystem II; Fm′, maximum fluorescence in the light-adapted leaves; Fm, maximum fluorescence in dark-adapted leaves; Fs, steady-state fluorescence in the light-adapted leaves; g_S, stomatal conductance; MCW, methanol:chloroform:water solution; MDH, malate dehydrogenase; NI, neutral invertases; NPQ, non-photochemical quenching; PEPCase, phosphoenolpyruvate carboxylase; P_N, net CO_2 assimilation; P_N/Ci, instantaneous carboxylation efficiency; PPFD, photosynthetic photon flux density; RH, relative humidity; Rubisco, ribulose-1,5-biphosphate carboxylase:oxygenase; RuBP, ribulose-1,5-bisphosphate; SAI, soluble acid invertases; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SPS, sucrose phosphate synthase; SuSy, sucrose synthase; $\Delta F/Fm'$, effective quantum efficiency of photosystem II.

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Introduction

Photosynthesis in higher plants is up-regulated by sink demand and down-regulated by sugar accumulation (Ribeiro et al., 2012; Quentin et al., 2013). Despite these processes have been known for many years, their biochemical and molecular mechanisms are still poorly understood (Cheng, 2009). In addition, the regulatory mechanisms of photosynthesis triggered by sugars are more complex in C4 than in C3 plants as the former have two interdependent CO_2 assimilatory cycles (McCormick et al., 2009). These cycles are driven by carboxylation reactions involving phosphoenolpyruvate carboxylase (PEPCase) and ribulose-1,5-biphosphate carboxylase:oxygenase (Rubisco) enzymes, which must act in a coordinated way to reach high photosynthetic efficiency (Sage and McKown, 2006). The coordination between the C4–C3 pathways also requires the close interaction of distinct reactions in mesophyll and bundle sheath cells (Muhaidat et al., 2007).

Among C4 plants, sugarcane is an interesting species for studying the source-sink regulation of photosynthesis as its leaves and stalks exert strong source and sink activities associated with sucrose accumulation (McCormick et al., 2008a). The photosynthesis of sugarcane varies widely as sugars are accumulated in stalks and leaves in parallel with the plant aging (Allison et al., 1997). This fact has suggested that some endogenous factors should negatively modulate sugarcane photosynthesis, most likely by decreasing the sink activity, source capacity and/or down-regulating gene expression related to photosynthetic activity (Paul and Foyer, 2001; Rolland et al., 2006; McCormick et al., 2008b). This unsolved question has encouraged several investigators with the aim of understanding the modulation mechanisms to improve photosynthesis and sucrose accumulation in sugarcane stalks. However, their comprehension is far from complete (Moore, 2005).

Experimental data obtained from different methodological approaches (supplying of exogenous sugars, cold girdling, leaf shade, defoliation, *etc.*) have suggested that the accumulation of hexoses and sucrose in leaves might inhibit photosynthesis in sugarcane (McCormick et al., 2008a,b; Inman-Bamber et al., 2011) and in other species (Ribeiro et al., 2012; Quentin et al., 2013). Moreover, sugar accumulation in leaves of higher plants might trigger a down-regulation in the expression of several photosynthetic genes (Stitt et al., 2010). Some sugars down-regulate the expression of Rubisco subunits and other enzymes of the Calvin cycle (Paul and Pellny, 2003; Smith and Stitt, 2007; McCormick et al., 2008a), but the causal relation involving specific sugars and the expression of particular photosynthetic genes is still unknown (Lloyd and Zakhleniuk, 2004).

Some reports have suggested that hexose levels in leaves are more effective than sucrose in reducing sugarcane photosynthesis (McCormick et al., 2008b). However, this assumption is controversial and largely debated as there is no direct evidence of this effect, and sugar metabolism in leaves is very fast, dynamic and complex (Rolland et al., 2006). Changes in sugar metabolism may deeply alter the carbohydrate profile in leaves, and as a consequence, data interpretation becomes a difficult task (Rolland et al., 2006). In addition, these changes may affect the levels of inorganic phosphate (Pi), triose phosphates, sucrose, hexoses and starch, which may cause changes in photosynthesis by regulation of the Calvin cycle activity and sugar metabolism (Paul and Foyer, 2001).

The acid (SAI) and neutral (NI) invertases, sucrose synthase (SuSy) and sucrose phosphate synthase (SPS) are important enzymes that regulate the levels of hexoses, sucrose and Pi in both chloroplast and cytosol (Paul and Pellny, 2003). The activities of these enzymes in coordination with hexokinase can generate a futile cycle of sucrose synthesis and degradation, allowing the accumulation of hexose phosphate in the cytosol and reduction of Pi input into chloroplasts (Ruan et al., 2010). For elucidating the involvement of sucrose on photosynthesis modulation, is crucial to assess the changes induced by this disaccharide on the sugar metabolism. Moreover, the elucidation of these metabolic processes in sugarcane is essential for adding new insights on how the photosynthesis and sucrose accumulation in stalks could be improved (McCormick et al., 2008b; Inman-Bamber et al., 2011).

In this study, we tested the hypothesis that excess sucrose in leaves negatively modulates sugarcane photosynthesis direct or indirectly by changing sugar metabolism and down-regulating Rubisco and PEPCase abundance and activity. The down-regulation of photosynthesis was not associated with sucrose or hexoses (glucose and fructose) accumulation in sheath and stalk tissues, suggesting that the negative modulation of CO_2 assimilation was triggered by sucrose in sugarcane leaves *via* down-regulation in Rubisco expression and activity.

Materials and methods

Plant material and growth conditions

Four-month-old sugarcane plants (*Saccharum* spp.), cv. IACSP94-2094 supplied by the Instituto Agronomico (IAC), Brazil, were propagated by planting stalk segments with a single bud. The plants were grown in 8-L pots containing a mixture of sand:vermiculite:humus (1:1:1, v/v/v), watered daily with distilled water until reaching the substrate holding capacity. Once a week, the plants were irrigated with Hoagland and Arnon solution (Hoagland and Arnon, 1950) until reaching complete substrate saturation. The pots were exhaustively leached with distillated water to avoid substrate salinization every month. The plants were initially grown under natural conditions in a greenhouse (3°44′ S; 38°34′ W; 31 m of altitude). The average air temperature, relative humidity and the maximum photosynthetic photon flux density (PPFD) were $27 \pm 3\,^{\circ}C$, $58 \pm 5\%$ and $1100 \pm 100\,\mu mol\,m^{-2}\,s^{-1}$ respectively, with 12 h of photoperiod inside the greenhouse. All tillers were removed during the experimental period to retain only the primary stalk per pot and avoid excessive self-shading and related changes in source-sink relationships. At the beginning of the experiments, the plants exhibited a stalk diameter of 2.5 cm and five internodes per stalk.

Experiments

The first experiment was performed with leaf segments using Petri dishes to evaluate the sucrose uptake and metabolism involving hexoses in a time-course approach. Leaf segments (10 cm length), from leaves +1 and +2 (the two first fully expanded ones from the shoot top), were used. The segments were incubated in a reaction medium containing 50 mM sucrose or 50 mM mannitol (control) dissolved in 10 mM HEPES buffer (pH 6.0) for 12 h. In the time-course study, samples were collected at 0, 3, 6, 9 and 12 h after sucrose exposure. The Petri dishes were kept in a growth chamber under controlled conditions as follows: PPFD of $500\,\mu mol\,m^{-2}\,s^{-1}$, air relative humidity of 80% and temperature of 29 °C. After the incubation period, the leaf segments were immersed in 1.5 mM $CaCl_2$ solution to eliminate any residual sucrose and mannitol. Then, the leaf segments were gently dried on paper towels and after that, they were placed in liquid N_2 and stored in −80 °C for further analysis.

In the second experiment 4-month-old plants were transferred to a growth chamber with the following controlled conditions: 29/24 °C day/night; relative humidity (RH) 70%; air CO_2 partial pressure of 38 Pa, PPFD of $800\,\mu mol\,m^{-2}\,s^{-1}$ and a 12 h photoperiod. This experiment was performed to assess the role of exogenous sucrose on photosynthesis and sugar metabolism at the