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Increased sink strength offsets the inhibitory effect of sucrose on sugarcane photosynthesis

Rafael V. Ribeiro[a,*], Eduardo C. Machado[b], José R. Magalhães Filho[b], Ana Karla M. Lobo[c], Márcio O. Martins[c], Joaquim A.G. Silveira[c], Xinyou Yin[d], Paul C. Struik[d]

[a] Department of Plant Biology, Institute of Biology, University of Campinas, Campinas, SP, Brazil
[b] Laboratory of Plant Physiology "Coaracy M. Franco", Centre for Research and Development in Ecophysiology and Biophysics, Agronomic Institute, Campinas, SP, Brazil
[c] Department of Biochemistry and Molecular Biology, Federal University of Ceará, Fortaleza, CE, Brazil
[d] Centre for Crop Systems Analysis, Department of Plant Sciences, Wageningen University & Research, Wageningen, The Netherlands

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ABSTRACT

Spraying sucrose inhibits photosynthesis by impairing Rubisco activity and stomatal conductance (g_s), whereas increasing sink demand by partially darkening the plant stimulates sugarcane photosynthesis. We hypothesized that the stimulatory effect of darkness can offset the inhibitory effect of exogenous sucrose on photosynthesis. Source-sink relationship was perturbed in two sugarcane cultivars by imposing partial darkness, spraying a sucrose solution (50 mM) and their combination. Five days after the onset of the treatments, the maximum Rubisco carboxylation rate (V_{cmax}) and the initial slope of A-C_i curve (k) were estimated by measuring leaf gas exchange and chlorophyll fluorescence. Photosynthesis was inhibited by sucrose spraying in both genotypes, through decreases in V_{cmax}, k, g_s and ATP production driven by electron transport (J_{atp}). Photosynthesis of plants subjected to the combination of partial darkness and sucrose spraying was similar to photosynthesis of reference plants for both genotypes. Significant increases in V_{cmax}, g_s and J_{atp} and marginal increases in k were noticed when combining partial darkness and sucrose spraying compared with sucrose spraying alone. Our data also revealed that increases in sink strength due to partial darkness offset the inhibition of sugarcane photosynthesis caused by sucrose spraying, enhancing the knowledge on endogenous regulation of sugarcane photosynthesis through the source-sink relationship.

1. Introduction

Photosynthetic regulation by environmental changes has been extensively studied in sugarcane (*Saccharum* spp.), with plants showing large seasonal variation in leaf CO_2 uptake. For instance, cool temperatures and low water availability are commonly found during the winter season and they represent two of the most important factors leading to low photosynthetic rates in sugarcane plants growing in subtropical regions (Sales et al., 2012, 2013; Machado et al., 2013). Besides the exogenous regulation, endogenous factors as the source-sink relationship also affect photosynthetic rates of sugarcane (McCormick et al., 2008a,b, 2009). While an increase in sink demand due to active growth is able to stimulate photosynthesis in source leaves, the opposite happens when plants are dormant or when sucrose loading in phloem and export from leaves are reduced (McCormick et al., 2006, 2008a; Inman-Bamber et al., 2011).

Increases in sugarcane photosynthesis induced by increased sink demand was associated with over-expression of genes encoding for PEPC, Rubisco, and hexokinase, as well as some components

Abbreviations: A, leaf CO_2 assimilation in a given condition; A_{380}, leaf CO_2 assimilation under 380 μmol CO_2 mol^{-1} in the air and a level of photosynthetically active irradiance of 2000 μmol m^{-2} s^{-1}; Dark, partial darkness treatment; D+S, treatment combining partial darkness and spraying a sucrose solution; g_s, stomatal conductance; J_{atp}, rate of ATP production driven by electron transport; k, the initial slope of the A-C_i curve; NI, neutral invertase; NS_L, non-stomatal limitation of photosynthesis; PEPC, phosphoenolpyruvate carboxylase; R_d, day respiration or leaf respiration in the light; Ref, reference treatment; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; s', calibration factor for converting electron flux to ATP flux; SAI, soluble acid invertase; S_L, stomatal limitation of photosynthesis; SPS, sucrose-P synthase; Suc, treatment with spraying a sucrose solution; SuSy, sucrose synthase; V_{cmax}, maximum Rubisco carboxylation rate; ϕ_{PSII}, apparent operating quantum efficiency of photosystem II.

* Corresponding author.
E-mail addresses: rvr@unicamp.br, rvribe@gmail.com (R.V. Ribeiro), caruso@iac.sp.gov.br (E.C. Machado), zeroagro@yahoo.com.br (J.R. Magalhães Filho), karlamlobo@gmail.com (A.K.M. Lobo), momartins@yahoo.com.br (M.O. Martins), silveira@ufc.br (J.A.G. Silveira), xinyou.yin@wur.nl (X. Yin), paul.struik@wur.nl (P.C. Struik).

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of the mitochondrial metabolism and sugar transport (McCormick et al., 2008b). At post-translational level, our knowledge about photosynthesis regulation by sugar remains limited (Lobo et al., 2015). In general, source-sink imbalances may change the leaf levels of inorganic phosphate (Pi), triose phosphates, sucrose and hexoses, which compose the sugar sensing and signalling mechanisms in plants and then could affect photosynthesis through the regulation of Calvin cycle reactions and sugar metabolism (Paul and Foyer, 2001; Rolland et al., 2002, 2006).

Sugarcane is among the most important crop species for studying source-sink relationships due to its high accumulation of sucrose in culms and its C_4 photosynthetic metabolism. In fact, sucrose concentration in culms may reach 0.7 M in sugarcane (Chandra et al., 2011), with this species being very sensitive to the manipulation of source-sink relationship (Inman-Bamber et al., 2008, 2009, 2010, 2011; McCormick et al., 2006, 2008a,b,c, 2009). As sugarcane shows high photosynthetic rates, it has a great potential for producing and exporting sucrose from leaves to culms during ripening, when culms are the main sinks. However, ripening happens when environmental conditions (*i.e.*, cool temperature and drought) are limiting for sugarcane photosynthesis. If plants are able to maintain source activity under unfavourable conditions, we would expect higher sucrose yield in field-grown plants as photoassimilates would be partitioned to storage in culms rather than to vegetative growth (Inman-Bamber et al., 2008, 2009, 2010, 2011; McCormick et al., 2006, 2008a,b,c, 2009).

Sugarcane photosynthesis is sensitive to sucrose and/or derivative sugars: spraying a sucrose solution significantly impairs *in vitro* Rubisco activity, reduces Rubisco activation state and abundance, and decreases photosynthetic rates and stomatal conductance in four-month old plants (Lobo et al., 2015). We also know that increases in sink demand stimulate photosynthesis through increases in both carboxylation efficiency and photochemical activity (McCormick et al., 2006, 2008a, 2009) and changes in sugar concentration of immature culms have controversial effects on leaf photosynthesis (McCormick et al., 2006; Inman-Bamber et al., 2011; Lobo et al., 2015). In fact, the underlying processes driving the source-sink relationship are still poorly understood for C_4 species like sugarcane (McCormick et al., 2008a; Lobo et al., 2015).

As there is a close cooperation between C_3 and C_4 cycles, we may argue that both *in vivo* Rubisco and PEPC enzymes could be down-regulated by spraying sucrose solution on leaves. In fact, we have recently proposed such an effect of exogenous sucrose on PEPC activity based on the instantaneous ratio between CO_2 assimilation and intercellular CO_2 concentration in sugarcane plants (Lobo et al., 2015). In addition, we have at least one open question regarding the endogenous regulation of photosynthesis by source-sink perturbation: Is the increase in sink demand able to offset the inhibitory effect of spraying sucrose solution in sugarcane leaves? Previously, the inhibitory effect of hexoses on sugarcane photosynthesis was reversed by darkening leaf segments (McCormick et al., 2008a); however, such approach using leaf segments incubated with hexoses impedes any signalling between plant tissues/organs and also the transport of nutrients, which would occur in intact plants.

Herein, we report the inhibition of *in vivo* Rubisco activity and stomatal aperture and provide evidence towards inhibition of PEPC activity due to sucrose spraying on two sugarcane varieties with differential biomass production. In addition, the relative importance of sink demand for sugarcane photosynthesis was revealed through the perturbation of source-sink relationship, a neglected issue in regulation of sugarcane photosynthesis (McCormick et al., 2009). Our data confirm the inhibition of both key photosynthetic enzymes by spraying a sucrose solution and demonstrate that increased sink demand is able to offset the inhibitory effect of sucrose spraying on photosynthesis and stomatal conductance of sugarcane plants. Such results are discussed taking into account the underlying mechanisms regulating photosynthesis and also highlighting the relevance of such findings for crop production.

2. Materials and methods

2.1. Plant material and growth conditions

Sugarcane (*Saccharum* spp.) varieties IACSP94-2094 and IACSP95-5000 were grown in pots filled with 12 L of commercial substrate (Carolina Standard, Carolina Soil of Brazil, Vera Cruz RS, Brazil). IACSP94-2094 and IACSP95-5000 are commercial genotypes with differential biomass production and yield (Silva et al., 2016). Each pot containing one plant was fertilized with 3.00 g urea, 7.50 g of superphosphate and 1.95 g potassium chloride at sowing. Fifty days after sowing, each pot received additional fertilization of 1.35 g urea, 1.35 g superphosphate and 1.17 g potassium chloride. Plants were maintained under well-watered conditions through daily irrigation during the experimental period. Sugarcane varieties were grown for 90 days under greenhouse conditions, where air temperature (day/night) was set to $31.5 \pm 1.0/21.5 \pm 1.5\,^{\circ}C$. The maximum photosynthetic active irradiance measured inside the greenhouse was 1100 $\mu mol\,m^{-2}\,s^{-1}$ and photoperiod was around 13 h.

2.2. Source-sink perturbation

Source-sink perturbation was initiated when plants were three-months old. Four groups of plants (with four plants each) for each variety were formed and then subjected to partial darkness and/or spraying of a sucrose solution as follows. In one group of plants all leaves were sprayed with sucrose 50 mM solution (Suc) and another group was subjected to partial darkening by enclosing plant shoots in black plastic bags for five days (Dark). In the Dark treatment, only the youngest fully expanded leaf with visible ligule (leaf + 1) was maintained under light conditions and therefore those plants had only one source leaf. As the third treatment, sucrose solution spraying and partial darkening were combined, with the leaf + 1 receiving sucrose solution spraying (D + S). The sucrose solution was composed of sucrose 50 mM, water and Triton X (0.01%, v/v). We have previously shown that plants supplied with sucrose at 50 mM presented a significant inhibition of photosynthesis (Lobo et al., 2015). As reference (Ref), a solution of water and Triton X (0.01% v/v) was sprayed in all leaves of the fourth group of plants. The water solution was also sprayed on leaf +1 of plants subjected to partial darkening. Both sucrose and water solutions were sprayed on leaves twice a day during five consecutive days, with physiological measurements done at the first day after ending treatments.

2.3. Gas exchange and chlorophyll fluorescence measurements

Measurements of gas exchange and chlorophyll fluorescence were taken simultaneously from leaf + 1, with an infrared gas analyser LI-6400XT (Li-Cor Inc., Lincoln NE, USA) and a modulated fluorometer model 6400-40 (Li-Cor Inc., Lincoln NE, USA). During measurements, leaf temperature was maintained at 31 °C and leaf-to-air vapour pressure difference was kept between 1.5 and 2.0 kPa. Air CO_2 partial pressure was varied as suggested by Long and Bernacchi (2003) and then leaf gas exchange and chlorophyll fluorescence were measured at 380, 200, 100, 90, 80, 60, 50, 380, 600, 1300 and 2000 μbar, under constant incident photosynthetically active irradiance (I_{inc}) of 2000 $\mu mol\,m^{-2}\,s^{-1}$ and two [O_2] levels of 21% and 1.2%. Measurements of leaf CO_2 assimilation (A) and stomatal conductance (g_S) were started after 15 min of acclimation to high light and were taken after reaching steady-state (~6 min) in each CO_2 concentration. Estimations of stomatal limitation (S_L) and non-stomatal limitation ($NS_L = 1\text{-}S_L$) of photosynthesis