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Evidence of photosynthetic acclimation to self-shading in sugarcane canopies

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Abstract

Increasing the efficiency of photosynthesis in sugarcane canopies is the key for improving crop yield. Herein, we evaluated the photosynthetic performance along the canopy of ten sugarcane cultivars and three *Saccharum* species. Canopy morphological traits were evaluated, and leaf gas exchange was measured in the first (sun-exposed, +1) and the fourth (shaded, +4) fully expanded leaves and under low- and high-light conditions. Similar photosynthetic capacity was found in leaves +1 and +4 under high light in genotypes with a high leaf area index and a high fraction of the sky blocked by the foliage (> 85%). Interestingly, such canopy characteristics cause low light availability to leaves +4, suggesting the photosynthetic acclimation of these leaves to self-shading in some genotypes. We highlight IACCTC06-8126 and CTC4 as those genotypes with higher canopy photosynthetic capacity, presenting high leaf area, high photosynthetic rates in sun-exposed leaves, and high responsiveness of shaded leaves to increasing light availability.

Keywords: light; photosynthesis; plant canopy; *Saccharum* spp.

Introduction

Population growth increases the demand for food and renewable energy sources, challenging global agriculture for higher yield (Ray *et al.* 2013, Salter *et al.* 2019). Sugarcane (*Saccharum* spp.), a C_4 crop, is a promising alternative due to its high biomass and sucrose production (Waclawovsky *et al.* 2010). Increasing the efficiency of

Highlights

- Responsiveness of shaded leaves to light was evaluated in 13 sugarcane genotypes
- CO_2 uptake varied among genotypes under varying light conditions
- IACCTC06-8126 and CTC4 have high responsiveness of shaded leaves to high light

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Abbreviations: C_i – intercellular CO_2 concentration; DAP – days after planting; g_s – stomatal conductance; k – instantaneous carboxylation efficiency; L_{abs} – leaf light absorbance; LAI – leaf area index; MTA – mean tilt angle; PEPC – phospho*enol*pyruvate carboxylase; PEPCK – phospho*enol*pyruvate carboxykinase; P_N – photosynthetic rate; Q – photosynthetic photon flux density; R_D – dark respiration; Φ_{CO2} – instantaneous CO_2 quantum efficiency.

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photosynthesis in plant canopy is the key to higher crop yield, and studies dealing with canopy photosynthesis or even leaves under different light exposure are limited. A large proportion of the sugarcane canopy is under low light conditions due to self-shading (Marchiori *et al.* 2010, 2014). Photosynthetic acclimation to light-limiting conditions has been explored in sugarcane, with plants showing a shift of the main decarboxylation pathway in bundle-sheath cells towards a higher contribution of phospho*enol*pyruvate carboxykinase (PEPCK) than NADP-dependent malic enzyme to maximize the quantum efficiency of CO_2 assimilation (Sales *et al.* 2018).

In theory, about 6% of global solar radiation is converted into biomass by C_4 plants (Zhu *et al.* 2008), determining crop growth and yield (Lawlor 1995, Long *et al.* 2006, Marchiori *et al.* 2010). Light conversion efficiency in sugarcane may reach ~ 5.39 g MJ^{-1} (Cruz et al. 2021, 2022), and such efficiency is driven by canopy photosynthesis and respiration, with high photosynthetic rates and low respiration (mainly maintenance respiration) enhancing biomass production (Zhu *et al.* 2010). Light interception efficiency is determined by the speed of canopy development, light absorption by leaves, longevity, size, and architecture of the canopy (Zhu *et al.* 2010, Davey *et al.* 2017), and is dependent on genotype and planting density (Robertson *et al.* 1996, Tejera *et al.* 2007). A significant proportion of canopy CO_2 assimilation occurs under light-limitation conditions caused by clouds and wind-induced leaf and plant shading (Kromdijk *et al.* 2016). The selection of plants more efficient in intercepting and converting light into biomass would be a way to increase crop yield in breeding programs (Lawlor 1995, Long *et al.* 2006, Marchiori *et al.* 2010).

Marchiori *et al.* (2010, 2014) highlighted the lack of information about the sugarcane canopy structure in breeding programs and showed that small variations in canopy architecture cause important changes in the photosynthesis of three commercial sugarcane cultivars under field conditions. Throughout the decades, sugarcane breeding programs have focused on the development of cultivars with high yields under stressful environments and resistance to pests and diseases. However, the physiological processes underlying the light-conversion efficiency and yield remain poorly understood in field-grown sugarcane plants (Zhu *et al.* 2010, Lopes *et al.* 2011, Moore *et al.* 2014). Recently, Almeida *et al.* (2021) reported a significant variation in photosynthesis among sugarcane genotypes and identified valuable and heritable photosynthetic traits. However, Almeida *et al.* (2021) evaluated only one fully expanded and sun-exposed leaf in sugarcane plants, as done by others (Irvine 1967, 1975; Jackson *et al.* 2016, Li *et al.* 2017). Then, an intriguing question arises: would shaded leaves have a photosynthetic capacity similar to the light-exposed leaves in field-grown sugarcane plants?

Herein, we aimed to evaluate the photosynthetic performance of light-exposed and shaded leaves of several sugarcane cultivars originated from crosses between 1948 and 2006 and three *Saccharum* species (*S. officinarum*, *S. spontaneum*, and *S. robustum*), emphasizing the effects of self-shading, leaf aging and addressing the physiological bases of such variability in photosynthetic traits.

Materials and methods

Plant material and experimental conditions: A field experiment was conducted between December 2018 and May 2019, with ten sugarcane cultivars and three species, grown under rainfed conditions (dystrophic red latosol) in Campinas, SP, Brazil (22°86'S, 47°08'W, 642 m a.s.l). Sugarcane hybrids and species, with their year of hybridization, progenitors, and institution responsible for breeding are listed in the table below. The experimental design was in randomized blocks, with four replications, each composed of six rows (11 m, spaced 1.5 m) of pre-sprouted plants spaced 0.45 m. Each plant was fertilized with 80 g P_2O_5, 133 g $CaCO_3$, 28 g KCl, and 34 g $(NH_4)_2SO_4$, following van Raij *et al.* (1996). Environmental conditions were continuously monitored by a weather station close to the experimental area. The air temperature was monitored with an *HMP-45C* probe (*Vaisala*, Helsinki, Finland) and rainfall with a tipping bucket rain gauge (model *CS700*, *Campbell-Scientific*, Logan, UT, USA). Data were recorded every

Genotypes	Year	Progenitors	Institution
IAC48-65	1948	CP27108 × ?	IAC
SP70-1143	1970	IAC48-65 × ?	Copersucar
SP80-3280	1980	SP7-088 × H57-5028	Copersucar
RB83-5486	1983	L60-14 × ?	RIDESA
IAC87-3396	1987	SP70-1143 × Co 740	IAC
CTC4 (92-4221)	1992	SP83-5073 × ?	CTC
IACSP94-2094	1994	SP84-7017 × ?	IAC/Copersucar
IACSP95-5000	1995	SP84-2066 × SP80-85	IAC
IACSP01-5503	2001	IACSP95-2312 × CTC9	IAC
IACCTC06-8126	2006	IACSP95-5000 × IACSP96-6114	IAC
S. officinarum (Don Cico)	Noble species		
S. spontaneum (19-95)	Wild species		
S. robustum (NG 57-055)	Wild species		

20 min by a data logger (model *CR1000*, *Campbell-Scientific*, Logan, UT, USA). During the sugarcane growing season, the accumulated rainfall was 653 mm, and air temperature ranged from 13.3 to 36.0°C, with an average air temperature of 25.1°C (Fig. 1S, *supplement*). Leaf gas exchange and plant canopy were evaluated in four-month-old sugarcane plants 129 d after planting (DAP).

Plant canopy: Leaf area index (LAI), mean tilt angle of the foliage (MTA), and the fraction of the sky that is not blocked by the foliage (visible sky ratio – indicates the absorption of diffuse, short-wave light < 490 nm) were measured with the *LI-2000C* (*LICOR Inc*., Lincoln, NE, USA), following the manufacturer's instructions. Photosynthetic photon flux density (Q) reaching the first (sun-exposed) and the fourth (shaded) fully expanded leaves was measured between 12:00 and 13:00 h with a linear quantum sensor (model *LI-191R-BNC-2*, *LICOR*, Lincoln, NE, USA) arranged at 90° with planting line. The first and fourth fully expanded leaves are leaf +1 and leaf +4, respectively, following the Kuijper system of leaf classification (Cheavegatti-Gianotto *et al.* 2011). The number of tillers per plant was also counted.

Leaf gas exchange: Leaf gas exchange was measured using an infrared gas analyzer (*LI-6400XT*, *LICOR Inc*., Lincoln, NE, USA) in +1 and +4 leaves. Measurements were taken between 8:00 and 15:00 h, under low [200 μmol(photon) m^{-2} s^{-1}, at leaf +4] and high [2,000 μmol(photon) m^{-2} s^{-1}, at leaf +1 and leaf +4] light intensity (Q), air CO_2 partial pressure of 40 Pa, cuvette temperature of 25°C, and natural variation of air relative humidity. Data were recorded after temporal stability and when the total coefficient of variation was lower than 2%. Photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured. Leaf light absorbance (L_{abs}) was estimated by following the *LICOR* protocol 'Estimating Light Absorbance in the *6400-40* Leaf Chamber Fluorometer Using an External Quantum Technical Sensor – Note #128', https://licor.app.boxenterprise.net/s/9bgi9ayo5yx7dwjnts8c. We estimated the instantaneous carboxylation efficiency ($k = P_N/C_i$) and the instantaneous CO_2 quantum efficiency [$\Phi_{CO2} = (P_N + R_D)/(Q \times L_{abs})$] (Edwards and Baker 1993). Dark respiration (R_D) was obtained from Almeida *et al.* (2021), and we assumed that leaves +1 and +4 have similar R_D (unpublished data).

Data analyses: The data were analyzed using Bayesian statistics and mean values were compared using the Bayes factor (BF_{10}): when $1 < BF_{10} < 3$, there is weak support for the alternative hypothesis (H1); $3 < BF_{10} < 20$ indicates positive support for H1; and $BF_{10} > 20$ indicates strong support to the alternative hypothesis, following Miranda *et al.* (2021). Correlations between traits were evaluated through *Spearman*'s coefficient. All analyses were done using the *R* software (R Core Team 2021; *version 4.1.1*, *R-project*, packages 'Hmisc', 'Corrplot', and 'Readxl') and the software *JASP* (JASP Team 2021; *version 0.15*, Amsterdam, The Netherlands).

Results

Plant canopy and light availability: We found a significant variation for LAI (BF_{10} = 27.6), tillering ($BF_{10} = 1.3 \times 10^{11}$), sky ratio ($BF_{10}$ = 8.6), and light intensity reaching leaves +4 (BF_{10} = 956.6). IACCTC06-8126 presented the highest LAI (Fig. 1*A*), while *S. spontaneum* presented the highest tillering (Fig. 1*B*). For the sky ratio, IACCTC06-8126 presented lower values than that of *S. spontaneum*, *S. officinarum*, SP70-1143, IACSP01-5503, and IAC48-65 (Fig. 1*C*). The light intensity measured at leaves +1 (upper canopy) did not vary among genotypes and was 1,450 μmol(photon) m^{-2} s^{-1} (on average). The light availability for leaves +4 was the lowest in IACCTC06-8126 and the highest in IACSP01-5503 (Fig. 1*D*). For the mean tilt angle of the foliage, we found the lowest values in IACCTC06-8126 (BF_{10} = 6.60), with an overall variation between 38 and 63° (Fig. 2S, *supplement*).

Leaf gas exchange: Significant variation among genotypes was found for photosynthetic rates (P_N) measured in leaves +1 (index '+1', $BF_{10} = 7.1 \times 10^5$) and +4 (index '+4', $BF_{10} = 2.2 \times 10^5$) under high light [index 'H', Q = 2,000 μmol(photon) m^{-2} s^{-1}] and in leaves +4 ($BF_{10} = 1.9 \times 10^3$) under low light [index 'L', Q = 200 μmol(photon) m^{-2} s^{-1}]. Differences between P_N^{+1H} and P_N^{+4H} were noticed in *S. spontaneum*, *S. robustum*, *S. officinarum*, SP80-3280, IACSP01-5503, and RB83-5486 (Fig. 2*A*). We found differences between P_N^{+1H} and P_N^{+4L} for all genotypes (Fig. 3S*A*, *supplement*). In addition, g_s^{+4H} also differed between genotypes (BF_{10} = 69.9) while g_s^{+1H} and g_s^{+4L} did not (BF_{10} = 0.9 and 2.38, respectively), as shown in Figs. 2*B* and 3S*B*. Differences between g_s^{+1H} and g_s^{+4H} were found for IACSP94-2094 and SP80-3280, while between g_s^{+1H} and g_s^{+4L} for IACSP94-2094 and RB83-5486 (Figs. 2*B*, 3S*B*). Significant variation was also noticed among genotypes for Φ_{CO2}^{+1H}, Φ_{CO2}^{+4H}, and Φ_{CO2}^{+4L} ($BF_{10} = 9.88 \times 10^6$, 1.54×10^5, and 156.96, respectively), with differences in Φ_{CO2}^{H} between leaves +1 and +4 for *S spontaneum*, *S. officinarum*, SP80-3280, IACSP01-5503, and RB83-5486 (Fig. 2*C*). There was a large variation in Φ_{CO2}^{+4L} among genotypes (Fig. 3S*C*). Regarding the instantaneous carboxylation efficiency (*k*), differences between genotypes were found only on leaf +4 under high light (BF_{10} = 289.2) and variations between k^{+1H} and k^{+4H} were found in IACSP94-2094 ($k^{+4H} > k^{+1H}$) and SP80-3280 ($k^{+4H} < k^{+1H}$), with no variation in SP70-1143 for k^{+1H} and k^{+4L} (Figs. 2*D*, 3S*D*).

The ratio between photosynthetic rates measured on leaves +4 and +1 under high light ($P_N^{+4H:+1H}$, acclimation of the bottom canopy to high light) varied among genotypes (BF_{10} = 104.40), with *S. officinarum* and CTC4 presenting the lowest and the highest mean values, respectively (Fig. 3). When comparing the same leaf +4 under low- and high-light conditions (responsiveness of the bottom canopy to light fluctuation), $P_N^{+4L:+4H}$, $\Phi_{CO2}^{+4L:+4H}$, and $k^{+4L:+4H}$ also varied ($BF_{10} = 3.5 \times 10^3$, 156.96, and 63.03, respectively) among genotypes, with the highest

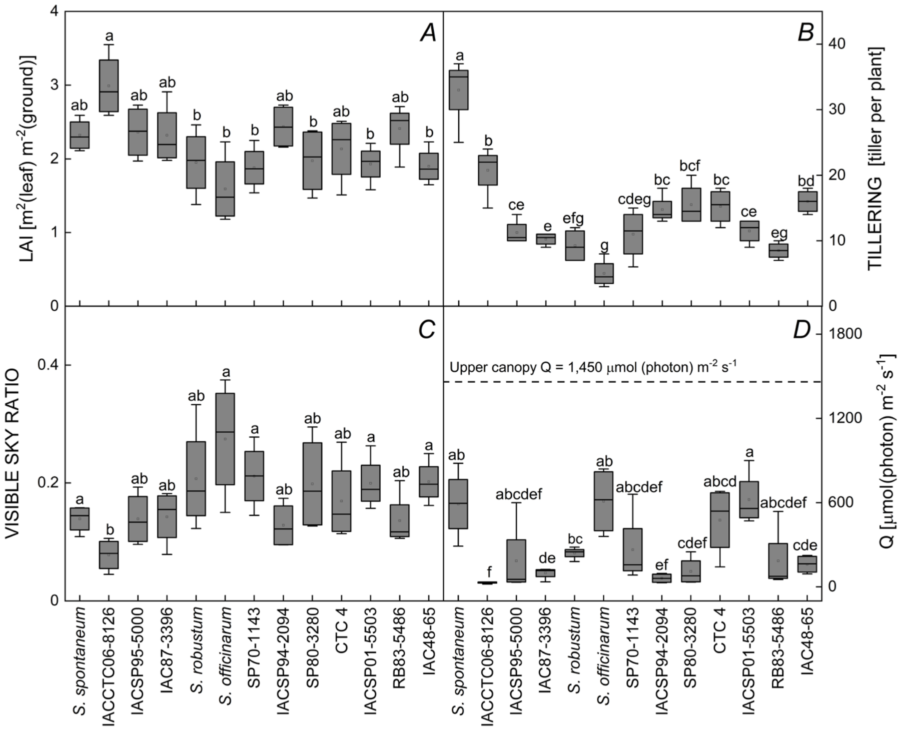

Fig. 1. Leaf area index (LAI) (*A*), tillering (*B*), visible sky ratio – fraction of the sky that is not blocked by the foliage (*C*), and photosynthetic photon flux density on leaf +4 (Q) (*D*) of thirteen sugarcane genotypes. *Different letters* indicate statistical differences between genotypes ($BF_{10} > 3$, $n = 4$).

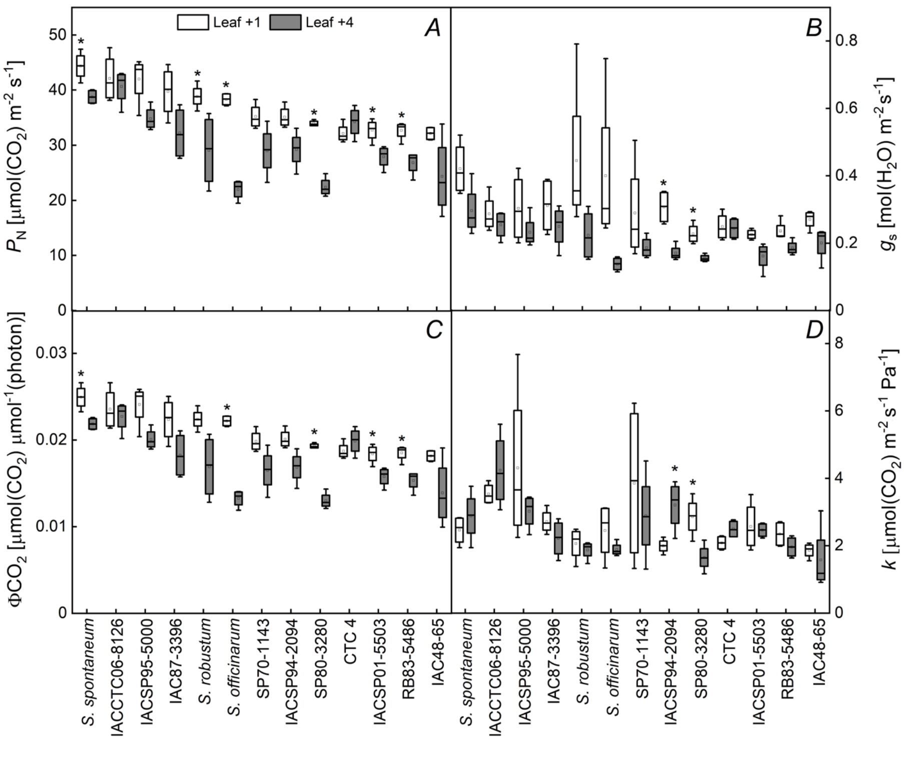

Fig. 2. Photosynthetic rate (P_N) (*A*), stomatal conductance (g_s) (*B*), instantaneous CO_2 quantum efficiency (Φ_{CO2}) (*C*), and instantaneous carboxylation efficiency (k) (*D*) in leaves +1 and +4 of thirteen sugarcane genotypes under high light [index 'H', Q = 2,000 µmol(photon) m^{-2} s^{-1}]. * indicates a difference between leaves +1 and +4 ($BF_{10} > 3$, $n = 4$).

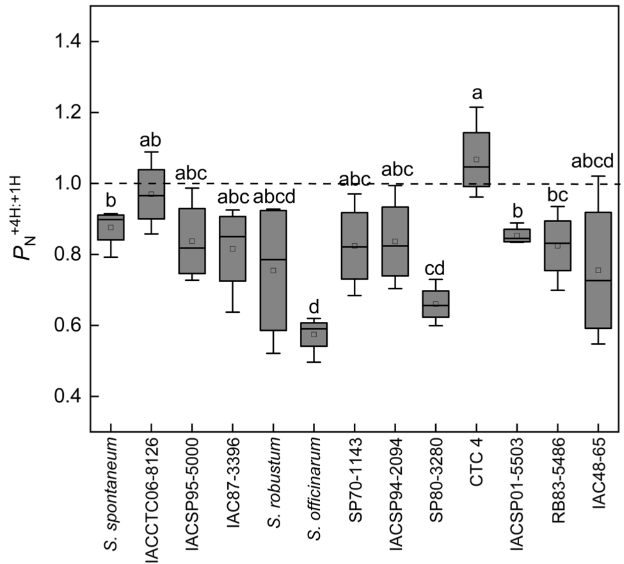

Fig. 3. Ratio of photosynthesis between leaves +4 and +1 ($P_N^{+4H:+1H}$) of thirteen sugarcane genotypes under high light [index 'H', Q = 2,000 μmol(photon) m^{-2} s^{-1}]. *Different letters* indicate statistical differences between genotypes (BF$_{10}$ > 3, *n* = 4).

values measured in SP80-3280 and the lowest ones found in *S. spontaneum*, IACCTC06-8126, and CTC4 (Fig. 4*A*,*C*,*D*). Regarding $g_s^{+4L:+4H}$, only RB83-5486 differed from *S. officinarum* and IACSP94-2094 (BF$_{10}$ = 3.31 and 6.84, respectively), as shown in Fig. 4*B*. Under natural conditions, leaves +4 receive less light than leaves +1 and we decided to compare the measurements taken considering such assumption (Figs. 3S, 4S, *supplement*). $P_N^{+4L:+1H}$ and $\Phi_{CO2}^{+4L:+1H}$ varied (BF$_{10}$ = 6.7×10^3 and 6.1×10^3, respectively) among genotypes, with SP80-3280 presenting the highest mean values and *S. spontaneum* the lowest ones (Fig. 4S*A*,*C*). IACSP01-5503 exhibited higher $g_s^{+4L:+1H}$ than *S. officinarum* and RB83-5486 (Fig. 4S*B*). IACSP94-2094 showed higher $k^{+4L:+1H}$ than *S. spontaneum*, IACCTC06-8126, and CTC4 (Fig. 4S*D*).

Correlations: P_N^{+1H} was correlated with P_N^{+4H} ($r = 0.62$), g_s^{+1H} ($r = 0.68$), and Φ_{CO2}^{+1H} ($r = 0.96$). P_N^{+4H} was positively correlated with g_s^{+4H} ($r = 0.87$), Φ_{CO2}^{+4H} ($r = 0.99$), k^{+4H} ($r = 0.76$), and LAI ($r = 0.66$), while negatively correlated with sky ratio ($r = -0.64$) and MTA ($r = -0.65$) (Fig. 5). Significant correlation was also observed between k^{+4H} and Φ_{CO2}^{+4H} ($r = 0.72$), LAI ($r = 0.59$), and sky ratio ($r = -0.60$), as shown in Fig. 5. Positive correlations were noticed for $P_N^{+4L:+4H}$ *vs.* sky ratio ($r = 0.60$) and MTA

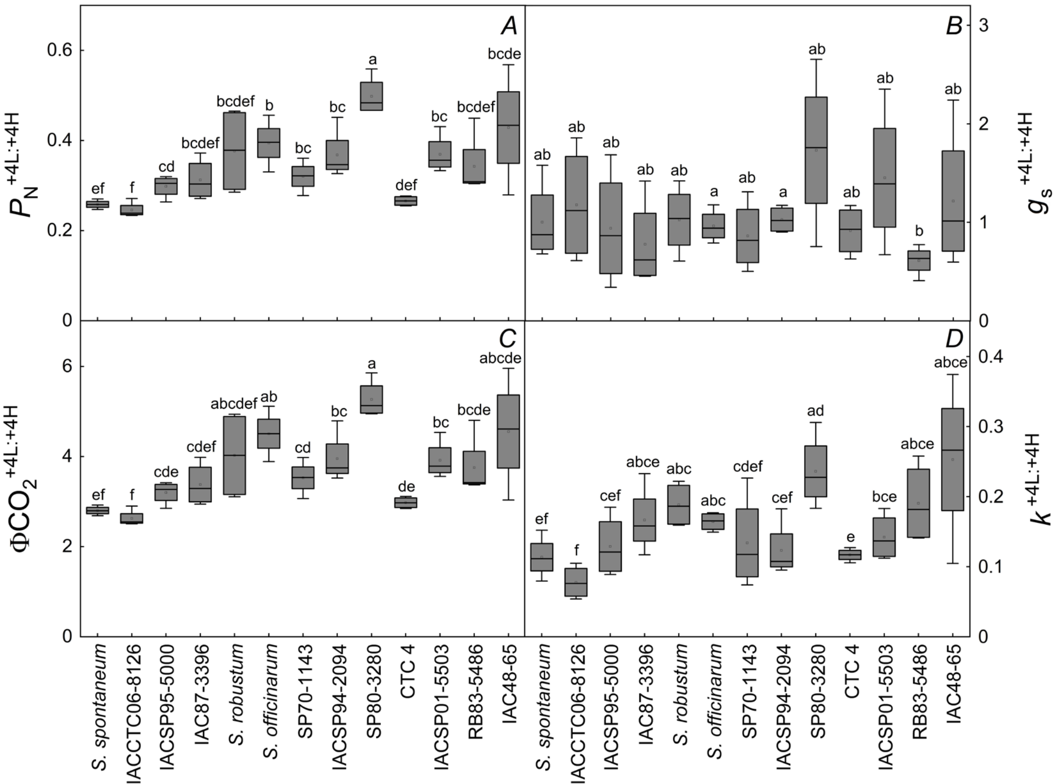

Fig. 4. Ratios of photosynthesis ($P_N^{+4L:+4H}$), stomatal conductance ($g_s^{+4L:+4H}$), instantaneous CO_2 quantum efficiency ($\Phi_{CO2}^{+4L:+4H}$), and instantaneous carboxylation efficiency ($k^{+4L:+4H}$) in leaf +4 of thirteen sugarcane genotypes under low [index 'L', Q = 200 μmol(photon) m^{-2} s^{-1}] and high [index 'H', Q = 2,000 μmol(photon) m^{-2} s^{-1}] light. *Different letters* indicate statistical differences between genotypes (BF$_{10}$ > 3, *n* = 4).

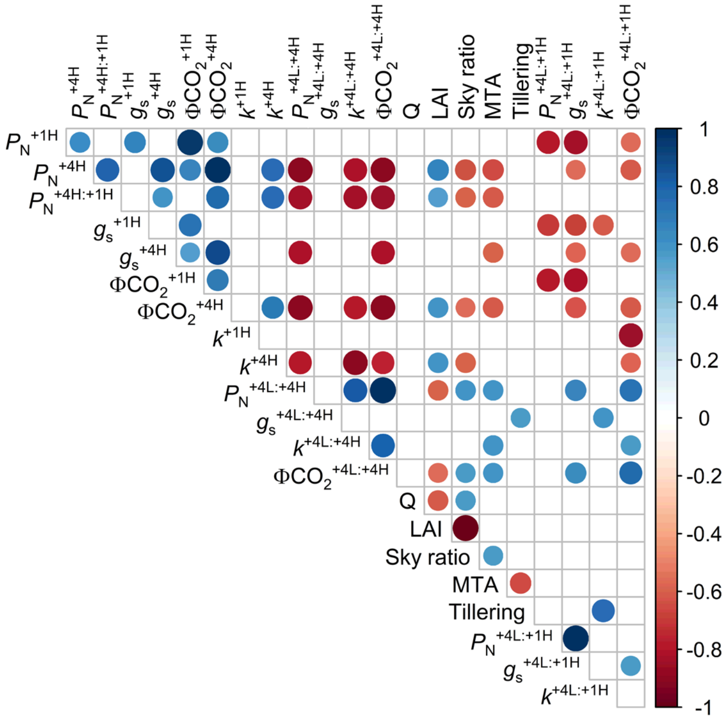

Fig. 5. Correlation of thirteen sugarcane genotypes, based on *Spearman*'s coefficient ($P<0.05$). Photosynthetic rate (P_N), stomatal conductance (g_s), instantaneous CO_2 quantum efficiency (Φ_{CO2}), and instantaneous carboxylation efficiency (k) and ratios considering leaves +1 and +4 and light level [low (L) or high (H)]; photosynthetic photon flux density (Q), leaf area index (LAI), the proportion of the sky that is not blocked by the foliage (Sky ratio), mean tilt angle of the foliage (MTA) and tillering.

($r = 0.60$). Tillering correlated negatively ($r = -0.65$) with the mean tilt angle of the foliage (Fig. 5).

Discussion

We found a high genotypic variation of photosynthesis through the sugarcane canopies, with P_N^{+1H} varying from 32 to 44 μmol(CO_2) m^{-2} s^{-1}, and P_N^{+4H} from 22 to 41 μmol(CO_2) m^{-2} s^{-1} (Fig. 2*A*). Such photosynthetic variability was already reported and ascribed to differences in leaf anatomy and morphology, stomatal conductance, leaf nitrogen content, and phospho*enol*pyruvate carboxylase (PEPC) and Rubisco abundances and activities (Irvine 1967, 1975, 1983; Marchiori *et al.* 2010, 2014; Jackson *et al.* 2016, Li *et al.* 2017, Almeida *et al.* 2021). As a novelty, our data revealed that some sugarcane canopies have similar P_N in leaves +1 and +4 (IACCTC06-8126, IACSP95-5000, IAC87-3396, SP70-1143, IACSP94-2094, CTC4, and IAC48-65) while others have leaf +1 with higher P_N than that of leaf +4 (*S. spontaneum*, *S. robustum*, *S. officinarum*, SP80-3280, IACSP01-5503, and RB83-5486) under high light (Fig. 2*A*). P_N was similar in leaves +1 and +4 in sugarcane canopies with high leaf area index and a high fraction of the sky blocked by the foliage, with planophile-like leaves (Figs. 1*A*,*C*; 2*A*, 5, and 2S). Such similar photosynthetic performance between shaded (leaf +4) and light-exposed leaves (+1) would be a consequence of increasing photosynthetic nitrogen-use efficiency, instantaneous CO_2 quantum efficiency (as shown herein, Fig. 2*C*), and the maintenance of Rubisco activity (Marchiori *et al.* 2014) in leaves +4.

While upper leaves are usually exposed to direct solar radiation, those located inside the canopy intercept diffuse light or sun flecks (short periods of direct solar radiation inside the canopy), being progressively shaded by new leaves (Bellasio and Griffiths 2014). Acclimation processes to low light would increase light-use efficiency and then photosynthesis of shaded leaves or those at the bottom canopy positions, enhancing the amount of CO_2 fixed by the entire canopy. Ideally, a canopy would have (*1*) top leaves with high photosynthetic rates, (*2*) small photosynthetic differences between top and bottom leaves under the same light intensity, and (*3*) bottom leaves photosynthesizing close to the maximum even under low light intensity. While such an ideotype looks most hypothetical, we were able to find genotypes with interesting characteristics. Under high light, *S. spontaneum*, *S. robustum*, *S. officinarum*, SP80-3280, IACSP01-5503, and RB83-5486 presented the highest P_N and Φ_{CO2} in leaves +1, *i.e.*, at the upper canopy position, as compared to leaves +4 (Fig. 2*A*,*C*). On the other hand, IACCTC06-8126, IACSP95-5000, IAC87-3396, SP70-1143, IACSP94-2094, CTC 4, and IAC48-65 had similar P_N and Φ_{CO2} in leaves +1 and +4 under high light, with IACCTC06-8126 presenting higher values for sky ratio, low light availability at leaf +4, and the lowest mean tilt angle (Figs. 1*C*,*D*; 2S). Among the biochemical changes underlying shade acclimation of photosynthetic apparatus in leaves +4, we would suggest increases in activity and abundance of PEPC and Rubisco, pyruvate orthophosphate dikinase, NADP-dependent malate dehydrogenase, and phospho*enol*pyruvate carboxykinase (Sales *et al.* 2018, Almeida *et al.* 2021, Jaikumar *et al.*

2021) – a subject to be further explored in future research. Such acclimation to low-light availability leading to the maintenance of the photosynthetic capacity of shaded leaves under high light would improve photosynthesis during sun flecks and then canopy photosynthesis (Waldron *et al*. 1967, Machado *et al*. 1982).

Photosynthetic rates in leaves +4 were correlated to Φ_{CO2} and *k* under high light (Fig. 5). In fact, previous studies identified a relationship between photosynthetic acclimation and instantaneous CO_2 quantum efficiency in C_4 grass canopies under shading (Marchiori *et al.* 2010, 2014; Pignon *et al.* 2017, Collison *et al.* 2020). While *S. officinarum* showed a substantial decrease (–43%) in P_N when comparing leaves +4 to +1 under high light, a such decrease was around 11% in *S. spontaneum* (Fig. 3). This latter presented higher tillering and likely higher canopy photosynthesis (Figs. 2*A*, 3). Therefore, our data revealed that photosynthetic acclimation due to self-shading is not necessarily related to higher photosynthetic rates per leaf area. Instead, such acclimation aims to increase canopy CO_2 assimilation due to high g_s, *k*, and Φ_{CO2} (Fig. 2*B–D*). In addition, photosynthesis of leaves +4 under high light was correlated positively with LAI and negatively with visible sky ratio and MTA (Fig. 5), canopy traits that compromise light availability at the bottom canopy layer.

While *S. officinarum* and SP80-3280 – genotypes showing the lowest photosynthesis in leaves +4 (Fig. 2*A*) – presented less responsiveness of P_N, Φ_{CO2}, and *k* to light, the most light-responsive genotypes were *S. spontaneum*, CTC4, and IACCTC06-8126 (Fig. 4*A*,*C*,*D*). This reinforces that the light acclimation of photosynthesis – which means that shaded leaves can carry on photosynthesis like sun-exposed ones if the light is available – and photosynthetic responsiveness to light are linked.

The commercial cultivars developed by breeding programs are inter- or intraspecific hybrids (crosses of *S. officinarum*, *S. spontaneum*, *S. robustum*, and *S. barberi*) derived mainly from the crossing of the noble species *S. officinarum* (~ 70–80%) and the wild species *S. spontaneum* (~ 10–20%) (Moore *et al.* 2014). As high heritability for photosynthesis and stomatal conductance in sugarcane has been reported by Jackson *et al.* (2016), Li *et al.* (2017), and Almeida *et al.* (2021) and there is significant variation in photosynthesis of both light-exposed and shaded leaves reported herein, our data indicate that there is room for improving canopy photosynthesis through breeding. Sugarcane plants with erectophile-like leaves at the upper canopy layer that allows light penetration (Marchiori *et al.* 2014), and planophile-like leaves at bottom canopy positions (Slattery *et al*. 2016, Walker *et al*. 2018), with high photosynthetic capacity due to shading acclimation, would have a phenotype favoring higher conversion of sunlight energy into biomass.

S. officinarum stands out for the high sucrose (up to 25% on stalk fresh mass) and low fiber content in the culms. On the other hand, *S. spontaneum* presents a higher photosynthetic rate, lower accumulation of sucrose (~ 2% of stalk fresh mass), vigorous growth, tillering, and higher resistance to abiotic and biotic stresses, compared to *S. officinarum* (Irvine 1975, Moore *et al.* 2014). Herein, our data highlighted the superiority of *S. spontaneum* for tillering and photosynthesis, fixing about 13% (leaf +1) to 43% (leaf +4) more CO_2 than *S. officinarum* under high light (Fig. 2*A*). Such high photosynthetic activity would boost biomass production through breeding for the development of improved sugarcane or energy cane varieties (Cruz *et al.* 2021), as the heritability of photosynthetic traits is high (Jackson *et al.* 2016, Li *et al.* 2017, Almeida *et al.* 2021).

Conclusion: This study revealed a significant photosynthetic variation in light-exposed and shaded leaves in canopies of *Saccharum* complex, indicating acclimation of shaded leaves towards the maintenance of photosynthetic performance in some sugarcane cultivars and species. This way, shaded leaves are responsive to high light and present photosynthetic rates similar to light-exposed leaves, even with light intensity commonly being less than 25% of reaching light-exposed ones. Our data highlight IACCTC06-8126 and CTC4 as those genotypes with higher canopy photosynthetic capacity due to high leaf area, high photosynthetic rate of light-exposed leaves, and high responsiveness of photosynthesis to high light in shaded leaves, contrasting with *S. officinarum* and SP80-3280.

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