Exploring intra-specific variation in photosynthesis of maize and sorghum

Rafael L. Almeida[1,*], Neidiquele M. Silveira[2], Heitor L. Sartori[1], Camila C. Carvalho[1], Maximiliano S. Scarpari[3], Aildson P. Duarte[4], Eduardo Sawazaki[4], Eduardo C. Machado[1] and Rafael V. Ribeiro[1,*]

[1]Laboratory of Crop Physiology (LCroP), Department of Plant Biology, Institute of Biology, State University of Campinas (UNICAMP), Campinas SP, Brazil

[2]Department of Biodiversity, Institute of Biosciences, São Paulo State University (UNESP), Rio Claro, SP, Brazil

[3]Center R&D in Sugarcane, IAC, Ribeirão Preto SP, Brazil

[4]Center R&D in Grain and Fibers, IAC, Campinas SP, Brazil

*Corresponding authors: rvr@unicamp.br; rafael.leonardo.iac@gmail.com

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ORCID

R. L. Almeida - https://orcid.org/0000-0002-0301-0381

A.P. Duarte -https://orcid.org/0000-0001-5963-0136

N.M. Silveira -https://orcid.org/0000-0003-2663-918X

E. C. Machado - https://orcid.org/0000-0002-6512-5408

R. V. Ribeiro - https://orcid.org/0000-0002-1148-6777

Abstract

Enhancing crop yield through improved photosynthesis is a key for feeding the global population and providing feedstock for a sustainable green economy. However, effectively linking photosynthetic performance and biomass production in C_4 species requires an integrative approach at plant canopy. This study aimed to characterize photosynthesis along the canopy of five maize (BM3069-PRO2, AG8701-PRO4, K7500-VIP3, DKB355-PRO3 and B2401-PWU) and sorghum (DKB560, Enforcer, IAC 7021, Brandelisa and Santa Elisa) cultivars, focusing on leaf gas exchange and chlorophyll fluorescence evaluations in three canopy strata: top; middle and bottom. Photosynthetic responses to increasing intercellular CO_2 concentration and light (A–C_i and A–PAR curves, respectively) were performed and key photosynthetic traits estimated. We found a significant variability in the maximum photosynthetic rates across the canopies. Modern maize cultivars exhibited high CO_2 assimilation in the top and middle canopy leaves, demonstrating physiological adjustments for increasing canopy homogeneity in terms of photosynthesis. Such adjustments included high maximum quantum efficiency of CO_2 assimilation (ϕ), stomatal conductance, carboxylation rates of PEPC and Rubisco, and leaf nitrogen content (LNC) along the canopy. In contrast, sorghum cultivars showed significant interactions between canopy strata, with DKB560 and Brandelisa standing out for their enhanced CO_2 uptake and ϕ throughout the plant canopy. Our findings highlight maize as an efficient C_4 crop, characterized by a high photosynthetic capacity and relative uniform photosynthesis across the canopy. This is attributed to reduced stomatal limitation and higher stomatal conductance, carboxylation of Rubisco (V_{cmax}) and LNC in the top and middle canopy, along with higher ϕ in middle and bottom layers. Overall, physiological adjustments such as nitrogen redistribution to upper canopy leaves and the optimization of light-

use efficiency in lower layers are key for enhancing canopy-level photosynthesis in maize and sorghum cultivars.

Keywords: light, photosynthesis, plant canopy, *Sorghum bicolor*, *Zea mays*.

Introduction

Higher crop yield is key for feeding the global population (Ray et al., 2013; Salter et al., 2019) and providing feedstock for a green economy. As we need highly efficient plants in converting sunlight energy into biomass, C_4 plants play an important role in food and bioenergy supply due to their high photosynthetic efficiency based on the CO_2 concentration mechanism (CCM). In the mesophyll cells, the phosphoenolpyruvate (PEP) carboxylase (PEPC) fixes the CO_2 into four-carbon molecule, which is transported to the bundle sheath cells and decarboxylated by a malic enzyme dependent on NADP or NAD (NADP-ME or NAD-ME) and by phosphoenolpyruvate carboxykinase (PEPCK). Such decarboxylation increases the [CO_2] in bundle sheath cells, where the ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco) refixes the CO_2 into carbohydrates through the C_3 pathway. Overall, the C_4 photosynthesis is regulated by the CO_2 diffusion from the atmosphere to the carboxylation sites, by the carboxylation reactions – PEPC and Rubisco activities – and by the regeneration of RuBP and PEP driven by ATP produced through photochemical reactions (von Caemmerer and Furbank, 2003; Yin et al., 2011; Sales et al., 2018).

Light distribution within plant canopy is an important factor limiting the photosynthetic performance in C_4 crops (Marchiori et al., 2010, 2014; Jaikumar et al., 2021; Cruz et al., 2022; Almeida et al., 2022). Architecture, size, composition and longevity of the plant canopy affect both quality and quantity of light reaching leaves, with aging and light acclimation along the canopy also changing CO_2 assimilation (Davey et al., 2017; Almeida et al., 2022). At the canopy scale, plant biomass can be enhanced by changes in plant architecture and higher efficiency of photosynthesis (Marchiori et al., 2010, 2014; Zhu et al. 2010; Slattery et al., 2018). According to Almeida et al. (2022), plant canopy should have three important features for high photosynthesis:

(1) top leaves with high photosynthetic rates (high photosynthetic capacity); (2) similar photosynthetic activity in top and bottom leaves in a given light intensity (homogeneous photosynthesis); and (3) bottom leaves photosynthesizing close to the maximum even under low light intensity (light acclimation). These features might boost photosynthesis and then increase yield through breeding, with the selection of top genotypes. However, our knowledge integrating canopy and photosynthesis is limited, mainly in C_4 species.

C_4 species present a high range of maximum photosynthetic rates, from 14 to 61 μmol m^{-2} s^{-1}, being a possible key trait in breeding programs due its high heritability (Jackson et al., 2016; Li et al., 2017; Almeida et al., 2021). For instance, most of the literature characterizes the C_4 photosynthetic responses in few genotypes or only in sun-exposed leaves, as done in energy cane (Cruz et al., 2021, 2022), maize and giant *Miscanthus* (Lee et al., 2021), sorghum (Jaikumar et al., 2021) and sugarcane (Marchiori et al., 2010, 2014; Almeida et al., 2021). In addition, no detailed information about how photosynthesis varies along the vertical canopy profile has been provided for modern cultivars of maize and sorghum, two important crops. An integrative perspective considering the canopy profile is needed for linking photosynthetic activity and biomass production in C_4 species. Here, we aim to characterize photosynthesis along the canopy profile of two 'NADP-ME type' C_4 crop species: maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.), revealing the variability within canopy (three leaf ages) and among genotypes (five cultivars) of each species.

Material and Methods

Plant growth conditions

Five cultivars of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) were studied (Table 1). Seeds were sown in plastic pots (12 L) filled with commercial substrate composed of *Sphagnum* spp., rice straw and perlite in 7:2:1 ratio (Carolina Soil of Brazil, Vera Cruz RS, Brazil). The plants were fertilized daily with nutrient solution modified from Sarruge (1975): 15 mmol N L^{-1} (7 % as NH_4^+); 4.8 mmol K L^{-1}; 5 mmol Ca L^{-1}; 2 mmol Mg L^{-1}; 1 mmol P L^{-1}; 1.2 mmol S L^{-1}; 28 μmol B L^{-1}; 54 μmol Fe L^{-1}; 5.5 μmol Mn L^{-1}; 2.1 μmol Zn L^{-1}; 1.1 μmol Cu L^{-1}; and 0.01 μmol Mo L^{-1}. Four plants per genotype of each species were maintained under greenhouse conditions throughout the experimental period, where air temperature averaged 25.5±5.4°C and air relative humidity 79.6±13.1%. To mitigate the potential impact of environmental variations within the greenhouse, the position of the plants was randomized weekly.

Photosynthesis

Leaf gas exchange and chlorophyll fluorescence were evaluated when plants were approximately two months old, corresponding to the R1 stage (Silking) in maize and the stage 6 (Blooming/Flowering) in sorghum. The measurements were taken in leaves from three canopy strata: top; middle; and bottom. For the top canopy, we focused on the flag leaf in maize plants. However, we observed some variations in growth habit among sorghum cultivars. The top canopy was defined by the flag leaf for four sorghum cultivars, except for Santa Elisa. For this cultivar, the top canopy was represented by leaf +1, which is defined as the first fully expanded leaf with visible ligule. Moving to the middle canopy, our attention was on the ear leaf in maize and the third leaf below the flag leaf (or leaf +1) in sorghum. For the bottom canopy, we evaluated the fourth leaf below the ear leaf in maize and the sixth leaf below the flag leaf (or leaf +1) in sorghum, as shown in Fig. S1.

Gas exchange measurements were taken using an infrared gas analyzer IRGA (Li-6400XT, LICOR Inc., Lincoln NE, USA) and a modulated fluorometer (6400-40LCF, LICOR Inc., Lincoln NE, USA) and recorded when the coefficient of variation was less than 2% and there was temporal stability. Measurements of leaf CO_2 assimilation (A), stomatal conductance (g_s) and the intercellular CO_2 concentration (C_i) were taken under air temperature of 28°C, with a water vapor pressure deficit lower than 1.5 kPa and varying the photosynthetically active radiation (PAR) and air CO_2 concentration.

Chlorophyll fluorescence was evaluated using signals emitted before and after a saturation pulse (λ<710 nm; PAR ~ 8,000 μmol m^{-2} s^{-1}; 0.8 s) and after the excitation of the photosystem I (PSI) by far-red light (λ=735 nm; PAR ~ 5 μmol m^{-2} s^{-1}; 3.0 s). Firstly, the leaves were acclimated in the dark for 30 minutes. Mitochondrial respiration in dark (R_d), the maximum (F_m) and minimum (F_o) fluorescence were measured and the variable fluorescence in dark (F_v=F_m–F_o) and the maximum quantum efficiency of PSII (F_v/F_m) estimated. During leaf gas exchange, we monitored the steady-state (F'), maximum (F_m') and minimum (F_o', after excitation of the PSI) fluorescence (Baker, 2008). From these data, the effective quantum efficiency of PSII [Φ_{PSII}=(F_m'-F')/F_m'] and the non-photochemical quenching [NPQ=(F_m-F_m')/F_m'] were calculated. The relative excess of energy (EXC) was estimated as: EXC=[(F_v/F_m– Φ_{PSII})/(F_v/F_m)], according to Bilger et al. (1995).

The response of photosynthesis to increasing intercellular CO_2 concentration (A–C_i curve) was evaluated after leaf acclimation (15 minutes) to air CO_2 concentration (C_a) of 400 μmol mol^{-1} and PAR of 2000 μmol m^{-2} s^{-1}. After this time, C_a inside the cuvette was changed as follows: 400, 300, 200, 120, 85, 70, 55, 400, 400, 500, 600, 800, 1200, and 1500 μmol mol^{-1}, with each step taking 3 minutes or coefficient of variation below 2%. The A–C_i curves were fitted (r^2>0.95) using the equation 1, according to Almeida et al. (2021):

$$A = A_{max} \times \left[1 - e^{-k(C_i - \Gamma CO_2)}\right] \quad (1)$$

where A_{max} is the maximum leaf CO_2 assimilation under high light and CO_2 saturation, ΓCO_2 is the CO_2 compensation point, and k is the fitting coefficient.

The apparent maximum carboxylation rates of phosphoenolpyruvate carboxylase (PEPC, V_{pmax}) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, V_{cmax}) were estimated from the fitted A–C_i curves (equation 1), following Yin et al. (2011):

$$V_{pmax} = s \times \frac{(C_i + K_p)^2}{K_p} \quad (2)$$

$$V_{cmax} = A_{max} + |R_d| \quad (3)$$

where s is the angular coefficient obtained by the initial linear part of A–C_i curves (C_i < 100 μmol mol^{-1}) and K_p is the Michaelis-Menten constant of PEPC for CO_2 (Yin et al., 2016; von Caemmerer, 2021):

$$K_p = K_{p25} \times e^{\left(\frac{1}{298} - \frac{1}{T+273}\right) \times \frac{E_{kp}}{R}} \quad (4)$$

where K_{p25} is Michaelis–Menten constant of PEPC for CO_2 at 25°C (82 μbar), T is the leaf temperature (°C) during measurements, E_{kp} is the K_p activation energy (38.3 kJ mol^{-1}), and R is the universal gas constant (0.008314 kJ K^{-1} mol^{-1}).

The stomatal limitation of photosynthesis (L_S) was estimated from A–C_i curve, following Long and Bernacchi (2003):

$$L_S = \frac{A'' - A'}{A''} \times 100 \quad (5)$$

where A'' and A' are leaf CO_2 assimilation at C_i and C_a of 400 μmol mol^{-1}, respectively.

The metabolic limitation (L_M) of photosynthesis in plant canopy was also calculated, adapted from Lawlor (2002):

$$L_M = \frac{A_H - A}{A_H} \times 100 \quad (6)$$

where A_H is the maximum and A is the minimum leaf CO_2 assimilation at C_a of 400 μmol mol^{-1} along the canopy profile, considering only rates measured under high light and CO_2 saturation (at the curve plateau).

The response of photosynthesis to light (A–PAR curve) was evaluated after leaf acclimation to light and CO_2, as done for the A–C_i curves. After leaf gas exchange reached the steady state, PAR inside the Li-6400XT cuvette was changed in the following sequence: 2000, 1500, 1000, 500, 300, 200, 100, 50 and 0 μmol m^{-2} s^{-1}, with each step taking 3 minutes or coefficient of variation below 2%. The A–PAR curves were fitted using the equation 7, adapted from Marshall and Biscoe (1980):

$$A = \frac{(\phi\,\mathrm{PAR} + A_{sat}) - [(\phi\,\mathrm{PAR} + A_{sat})^2 - (4\phi\,\mathrm{PAR}\theta A_{sat})]^{0.5}}{2\theta} - R_d \quad (7)$$

where A_{sat} is the maximum CO_2 assimilation on leaf area basis; ϕ is the maximum quantum efficiency of CO_2 assimilation; R_d is the dark respiration (when PAR = 0 μmol m^{-2} s^{-1}); and θ is the curvature factor. From *A*–PAR curves, we also estimated the light compensation point (LCPT, PAR when A = 0 μmol m^{-2} s^{-1}, from the *x*-axis intercept), according to Heberling and Fridley (2013).

Leaf area, chlorophyll index and leaf nitrogen content

After leaf gas exchange evaluations, leaf area (LA) was measured using a planimeter model Li-3000C (LICOR Inc., Lincoln NE, USA). Leaf dry matter (DML) was determined after drying all leaves in an oven at 60°C with forced-air circulation until a constant weight was achieved. From these data, the specific leaf mass (SLM = DML/LA, in kg m^{-2}) was estimated. The chlorophyll meter model CFL1030 (Falker, Porto Alegre RS, Brazil) was used to evaluate the chlorophyll *a*, *b*, *a*/*b* and *a* + *b* (total) indexes. Leaves used for photosynthetic evaluations were collected and their nitrogen concentration (LNC) was determined by the Kjedahl method (Bataglia et al., 1983).

Experimental design and data analysis

The experimental design was a randomized 3×5 factorial arrangement, with four biological replicates: three canopy levels (top, middle and bottom); and five genotypes for each of the two species studied (maize and sorghum). Normal distribution and homogeneity of variances were tested using the Shapiro-Wilk and Hartley tests, respectively. Following these preliminary tests, all variables were subjected to the analysis of variance (ANOVA). Mean values were compared by

the Tukey test (p<0.05) when statistical significance was detected. Additionally, the correlation between the photosynthetic traits was analyzed through Pearson's coefficient. All analyses were conducted using the R software (R Core Team 2024; version 4.4.0, R-project, packages 'corrplot', 'ExpDes', 'Hmisc' and 'Readxl').

Results

Leaf gas exchange

A–C_i and A–PAR curves revealed significant variation in the maximum photosynthetic rates across the canopies of maize (from 22.5 to 50.4 μmol m^{-2} s^{-1}) and sorghum (from 11.2 to 46.0 μmol m^{-2} s^{-1}, Figs. 1 and S2). To further analyze these findings, we estimated the photosynthetic canopy homogeneity index (CHI) – the ratio of photosynthesis between the top and bottom leaves of each species under high light and high air CO_2 concentration. For maize, the CHI varied between 1.04 (BM2401) and 1.44 (AG8701). In sorghum, the CHI ranged from 1.19 (DKB560) and 2.16 (Santa Elisa). In terms of metabolic limitation (L_M) of photosynthesis along the canopy profile, we observed differences among the genotypes of both species. L_M varied from 12.9% (B2401) to 30.9% (AG8701) in maize, whereas it ranged from 16.1% (DKB560) to 53.8% (Santa Elisa) in sorghum, as shown in Fig. 1.

Our study revealed no significant interaction (p>0.05) between the canopy position and the maize cultivars for photosynthesis (A_{400}) and stomatal conductance (g_{s400}) measured at C_a = 400 μmol mol^{-1} (Fig. 2), saturated CO_2 assimilation (A_{sat}), the maximum quantum efficiency of CO_2 assimilation (ϕ) and light compensation point (LCPT; Fig S3). When comparing maize cultivars,

K7500 exhibited the lowest A_{400}, A_{sat} and ϕ (Figs. 2A and S3A,C). Generally, the bottom leaves displayed lower A_{400} and A_{sat} (30.1±1.1 and 39.9±1.8 μmol m^{-2} s^{-1}) compared to the top (38.5±1.4 and 56.1±1.9 μmol m^{-2} s^{-1}) and middle leaves (37.3±1.0 and 53.7±1.6 μmol m^{-2} s^{-1}; Figs. 2B and S3B). Interestingly, this pattern was reversed for ϕ. The middle and bottom leaves had higher ϕ (0.065±0.003 and 0.067±0.003 μmol μmol^{-1}) than the top leaf (0.056±0.003 μmol μmol^{-1}; Fig. S3D). For stomatal conductance, DKB355 exhibited the highest g_{s400} (0.484±0.028 mol m^{-2} s^{-1}) among the maize cultivars (Fig. 2C). In general, bottom leaves presented the lowest g_{s400} when compared to the middle and top leaves (Fig. 2D). We found no interactions and differences among the genotypes and canopy layers for L_S, which averaged 3.1±0.3%. Regarding the LCPT, K7500 had the highest value (62±5 μmol m^{-2} s^{-1}) among maize cultivars (Fig. S3E) and it was lower in bottom leaves (44±2 μmol m^{-2} s^{-1}) in relation to the middle and top leaves (Fig. S3F).

Contrary to our findings in maize, there was significant interaction (p<0.01) between the canopy layers and the sorghum cultivars for photosynthetic parameters such as A_{400}, g_{s400}, L_S, A_{sat} and ϕ. Enforcer and Santa Elisa presented the largest variation in A_{400} and A_{sat} along the canopy. Enforcer, DKB560 and IAC7021 emerged as the superior genotypes for photosynthesis in top leaves, achieving A_{400} close to 46 μmol m^{-2} s^{-1} and A_{sat} about 55 μmol m^{-2} s^{-1} (Figs. 3A and S4A). Enforcer stood out as the genotype showing the highest ϕ in bottom leaves, with values about 0.077±0.004 μmol μmol^{-1} (Fig. S4B). For stomatal conductance, DKB560 showed no differences along the canopy profile (averaging 0.335±0.014 mol $m^{-2}s^{-1}$), while Enforcer, IAC7021, Brandelisa and Santa Elisa exhibited lower g_{s400} in the bottom leaves (Fig. 3B). The stomatal limitation (L_S) was higher in the top (19.2±3.2%) and middle (16.0±1.6%) than in the bottom (11.5±1.9%) leaves of DKB560. Conversely, Santa Elisa presented higher L_S in the bottom (12.4±2.6%) compared to the top and middle leaves (4.8±0.6 and 3.7±1.0%). Enforcer, IAC7021

and Brandelisa showed no differences in L_S throughout the canopy, averaging 5.3±0.5%, 12.0±1.3% and 8.1±0.8%, respectively (Fig. 4A). No interaction (p>0.05) between cultivars and canopy layers was found for light compensation point, with DKB560 and IAC7021 presenting the highest LCPT values (about 54 μmol m^{-2} s^{-1}; Fig. S5A). In general, the bottom leaves had LCPT of 39±2 μmol m^{-2} s^{-1}, which was about 19.0% and 31.3% lower than those of the middle and top leaves, respectively (Fig. S5B).

Mitochondrial dark respiration (R_d) did not change among the maize cultivars (p>0.05), with an average of 3.3±0.1 μmol m^{-2} s^{-1}. However, differences were noticed among canopy strata. The top and middle maize leaves (3.6±0.2 and 3.5±0.1 μmol m^{-2} s^{-1}, respectively) presented higher R_d than bottom leaves (2.9±0.1 μmol m^{-2} s^{-1}). In sorghum, we found no interaction between genotypes and the canopy strata. Herein, DKB560 and IAC7021 presented the highest R_d (about 2.7 μmol m^{-2} s^{-1}), and top leaves had higher R_d (2.9±0.1 μmol m^{-2} s^{-1}) when compared to the middle and bottom leaves (2.2±0.1 to 2.4±0.1 μmol m^{-2} s^{-1}, respectively).

Biochemistry and photochemistry

No interaction (p>0.05) between the canopy strata and maize cultivars was found for V_{pmax}, V_{cmax}, V_{pmax}:V_{cmax} ratio, Φ_{PSII} and F_v/F_m. BM3069, AG8701 and DKB355 presented higher V_{pmax} than K7500 and B2401 (Fig. 2E), whereas K7500 presented the lowest V_{cmax} among all maize cultivars (Fig. 2G). The V_{pmax}:V_{cmax} ratio ranged from 1.8 to 2.1, with AG8701 presented higher values than B2401 (Fig. 2I). When comparing canopy strata, both V_{pmax} and V_{cmax} were lower in the bottom one (Fig. 2F, H), while the lower V_{pmax}:V_{cmax} ratio was found in the top leaves (Fig. 2J). Φ_{PSII} and F_v/F_m also varied among the genotypes, with DKB355 and B2401 exhibiting the highest

288 Φ_{PSII} and F_v/F_m values, respectively (Fig. 5A,C). In general, the bottom leaves showed the lowest
289 Φ_{PSII} (0.148±0.004) and F_v/F_m (0.757±0.004) when compared to middle (0.186±0.005 and
290 0.778±0.002) and top (0.177±0.006 and 0.787±0.002) leaves (Fig. 5B,D).

291 In sorghum species, we found significant interaction ($p<0.05$) between the canopy layers
292 and cultivars for V_{pmax}, V_{cmax}, V_{pmax}:V_{cmax} ratio and F_v/F_m. Nonetheless, no interaction ($p>0.05$) was
293 observed for Φ_{PSII}. Enforcer, IAC7021 and Santa Elisa decreased V_{pmax} from top to the bottom
294 canopy, contrasting to DKB560 and Brandelisa. Regarding V_{cmax}, all genotypes presented higher
295 values in the top when compared to the bottom leaves (Fig. 3C,D), while DKB560, IAC7021 and
296 Brandelisa showed no differences for V_{pmax}:V_{cmax} ratio throughout the canopy (Fig. 3E). Regarding
297 Φ_{PSII}, no variation was found along canopy profile, which averaged 0.157±0.005. However, the top
298 (0.180±0.008) and middle (0.165±0.009) leaves were more efficient than the bottom (0.127±0.009)
299 ones. The lowest F_v/F_m was found in the bottom leaves, with Santa Elisa showing the most
300 significant reduction between the top and bottom (Fig. 4B).

301

302 *Nitrogen content, leaf area and chlorophyll index*

303

304 No interaction ($p>0.05$) was observed between canopy strata and maize or sorghum
305 cultivars for LNC. In maize, DKB355, AG8701 and B2401 exhibited higher LNC than BM3069
306 (Fig. 6A). Among sorghum cultivars, the grain-types Enforcer, DKB560 and IAC7021 showed
307 greater LNC than Brandelisa and Santa Elisa (Fig. 6C). Across both species, LNC was highest in
308 the top and middle canopy strata (Fig. 6B,D).

309 Among maize cultivars, K7500 presented the largest leaf area (1.01±0.03 m^2) and the
310 lowest specific leaf mass (SLM, ~44 g m^{-2}) when plants were about two months old (Fig. 7A,C).
311 When considering sorghum cultivars, Santa Elisa had the largest leaf area (1.74±0.05 m^2), while

Enforcer had the lowest SLM (~26 g m^{-2}, Fig. 7B,D). No interaction (p>0.05) between the canopy strata and maize cultivars was found for chlorophyll *a*, *b*, *a*/*b* ratio and total (*a*+*b*). There was no difference in chlorophyll *a* among maize genotypes. DKB355 presented higher chlorophyll *b* (20.5±1.0 *vs.* 15.2±0.7) and total (65.5±1.2 *vs.* 59.0±1.2); and lower *a*/*b* ratio (2.25±0.11 *vs* 2.93±0.12) when contrasted to B2401, as shown in Fig. S6A,C,E,G. Chlorophyll *a* remained uniform across the top, middle and bottom maize leaves. Contrarily, chlorophyll *b* and the total (*a*+*b*) chlorophyll were higher in middle as compared to top leaves, while *a*/*b* ratio was higher in the top ones (Fig. S6B,D,F,H).

In sorghum, DKB560 showed higher chlorophyll *a* than IAC7021 (Fig. S7A). However, chlorophyll *a* did not show any significant variation (p>0.05) across canopy strata, averaging 40.3±0.2 (Fig. S7B). There were significant interactions (p<0.01) between the canopy strata and sorghum cultivars when considering chlorophyll *b*, *a*/*b* ratio and total (*a*+*b*). The top leaves of Enforcer had higher chlorophyll *b* and total than the bottom leaves, while the opposite was true for the *a*/*b* ratio. Furthermore, IAC7021 differed from Enforcer for chlorophyll *b*, *a*/*b* ratio and total in both top and bottom leaves (Fig. S8). Unfortunately, chlorophyll data of Brandelisa and Santa Elisa cultivars are missing.

Correlations

A_{400} was correlated with g_{s400} (r=0.61 and 0.78), V_{pmax} (r=0.84 and 0.73), V_{cmax} (r=0.998, and 0.97), ϕ_{PSII} (r=0.87 and 0.94), NPQ (r=-0.82 and -0.73), EXC (r=-0.84 and -0.93) and LNC (r=0.66 and 0.67) across maize and sorghum canopies. Notably, both species exhibited a positive correlation between LNC and V_{cmax} (r=0.65 and 0.69), as well as between LNC and F_v/F_m (r= 0.70

335 and 0.67). Additionally, NPQ and EXC were well correlated in maize (r=0.75) and sorghum
336 (r=0.81) canopies (Figs. S9 and S10).
337
338 **Discussion**
339
340 *Variation of photosynthesis along the maize canopy profile: physiological adjustments for*
341 *increasing homogeneity*
342
343 Our data revealed physiological adjustments and no variation in photosynthetic traits when
344 comparing the top and middle canopy leaves in maize plants. The decline in A_{400} and V_{cmax} in the
345 bottom leaves are potentially linked to aging and decreases in leaf nitrogen content (LNC; Fig. 6B).
346 In this context, we believe that the primary N source for remobilization in bottom leaves might be
347 the protein Rubisco, as evidenced by the strong relationship of LNC with A_{400}, V_{cmax} and F_v/F_m
348 (Fig. S9). This process would redistribute nitrogen to upper leaves, sustaining their ability to
349 perform high photosynthetic rates (Yin et al., 2011; Niinemets et al., 2015; Jaikumar et al., 2021).
350 Interestingly, we found an increase in V_{pmax}:V_{cmax} ratio and ϕ in the middle and bottom leaves (Fig.
351 4J). According to Hikosaka and Terashima, (1995), N remobilization did not decrease ϕ when light
352 is not a limiting factor, as found in our study and in giant *Miscanthus* (Pignon et al., 2017). This
353 observation suggests the photosynthetic acclimation along the maize canopy to optimize the
354 photosynthetic capacity in the top and middle leaves, as found in sugarcane (Almeida et al., 2022).
355 Simultaneously, such acclimation enhances the quantum efficiency of CO_2 assimilation in the
356 bottom leaves which are several weeks older than top and middle leaves. Decreases in LCPT were

also observed in the bottom leaves (Fig. S3), suggesting again a physiological strategy to optimize light-use efficiency.

V_{pmax} is a physiological index associated with the capacity of PEPC and subsequently with the ability to increase [CO_2] in bundle-sheath cells (Bailey et al., 2000). High activities and abundance of PEPC, Rubisco, pyruvate orthophosphate dikinase (PPDK), phosphoenolpyruvate carboxykinase (PEPCK) and NADP-dependent malate dehydrogenase are crucial for maintaining photosynthesis within the deeper canopy, where leaves are shaded (Vu et al., 2006; Sales et al., 2018, Jaikumar et al., 2021). The efficiency of the CO_2 concentrating mechanism (CCM) plays a significant role in this context. If CCM is effective, photorespiration is inhibited, leakiness is low and the quantum efficiency of CO_2 assimilation is high (Farquhar, 1983; Kromdijk et al., 2008; Sales et al., 2018). Particularly, high V_{pmax}:V_{cmax} ratio and a small response (3.0±0.3%) of photosynthesis when changing C_a from 400 to 1500 μmol mol^{-1} (Figs. 1A-E and 2J) provide compelling evidence of high CO_2:O_2 ratio around the active sites of Rubisco in the middle and bottom leaves. Overall, maize photosynthesis has a similar pattern among genotypes and canopy strata, which is likely a consequence of rigorous selection pressure in breeding programs. Such programs might have inadvertently selected genotypes with higher and homogeneous canopy photosynthesis while aiming for higher yield (Lee and Tollernaar, 2007; Andorf et al., 2019; Muntean et al., 2022).

Underlying factors changing the photosynthetic activity along the sorghum canopy

In contrast to maize, sorghum genotypes exhibited significant variation of photosynthetic traits across the canopy. The source of this variability in sorghum can be traced back to breeding

purposes, such as grain yield, sugar accumulation in stalks and biomass/forage production (Table 1, Fig. 11B). Under identical light and [CO_2] conditions, we identified three distinct patterns of photosynthesis across the canopy of sorghum genotypes: #1- uniform photosynthetic activity across top, middle and bottom leaves, as found in DKB560 and Brandelisa; #2- photosynthetic activity in top and middle leaves that differed from the bottom leaves, as in IAC7021; and #3- photosynthetic activity varying among the top, middle and bottom leaves, as observed in Enforcer and Santa Elisa.

Regarding the pattern #1, DKB560 exhibited high stomatal limitation (L_S) in the top (19.2±3.2%), middle (16.0±1.6%) and bottom (11.5±1.9%) leaves as compared to other cultivars, without any difference in V_{pmax} across its canopy. Brandelisa also exhibited a uniform CO_2 assimilation and V_{pmax} throughout the canopy, but with V_{cmax} (31.9±1.3 μmol m^{-2} s^{-1}) and L_S (8.1±0.8%) lower than those found in DKB560 (Figs. 3C,D and 4A). However, high A_{400} and low metabolic limitation (from 16 to 21%; Fig. 1G,I) found here for DKB560 and Brandelisa indicate an enhanced capacity for CO_2 fixation across the canopy. Both cultivars presented higher ϕ values in the bottom than the top leaves, a response similar to one found in maize.

When considering the patterns #2 and #3, there was a pronounced reduction in photosynthesis along the canopies of Enforcer, IAC7021 and Santa Elisa. This decrease could be primarily attributed to the N remobilization from bottom leaves to upper ones (Figs. 3A and 6D). Given that a substantial portion leaf N is allocated to photosynthetic enzymes (Sage and Pearcy 1987; Sage 2002), we would speculate that such remobilization may lead to a reduction in both Rubisco abundancy and activity. If this is true, this phenomenon seems to occur without any decrease in ϕ, consistent with previous findings in maize (Hikosaka and Terashima, 1995). In this study, A_{400}, V_{cmax}, F_v/F_m and LNC exhibited remarkably similar patterns across the sorghum canopy

(Figs. 3A,C,D and 6C,D), suggesting a strong interrelation among these traits, supported by the correlation analyses (Fig. S10).

Breeding for enhancing photosynthesis: Is there room to boost canopy efficiency?

While maize genotypes had low variability for photosynthesis and leaf area, sorghum genotypes presented high variability for photosynthetic traits along the canopy profile. Such variation in sorghum indicates the existence of a broad genetic diversity that could be explored to enhance crop yield, as high heritability is found for photosynthesis and leaf area in C_4 plants (Jackson et al., 2016; Li et al., 2017; Almeida et al., 2021). As several studies have shown a strong link between high photosynthetic activity and yield (Yoon et al., 2020; Ainsworth et al., 2021; De Souza et al., 2022; Wei et al., 2022), photosynthetic traits might be a tool for selecting and screening sorghum genotypes with high yield potential. In maize, the low variability found here is in accordance with the low genetic variation within the maize germplasm, which poses difficulties for enhancing the photosynthetic capacity through breeding (Richards, 2000; Ahmadzadeh et al., 2004).

The biomass-producing sorghum genotypes Santa Elisa and Brandelisa exhibited a large leaf area (Fig. 7B). This phenotype not only enhances the visual appeal of the plant but also suggests a prolonged functional longevity of canopy in terms of CO_2 uptake and sustained chlorophyll content (Thomas and Howarth, 2000). Notably, Brandelisa exhibited a uniform CO_2 uptake and V_{pmax} throughout the canopy, along with a higher ϕ in the bottom canopy (Figs. 3A and S4B). This pattern might be associated with its ability to maintain chlorophyll content, as observed in DKB560 (Fig. S8). This strategy optimizes the photosynthetic performance in the middle and

bottom layers, representing an adaptive mechanism to enhance light-use efficiency (Almeida et al., 2022) with potential to enhance crop productivity. These findings open avenues for future research and breeding programs aimed at enhancing crop productivity in an era of climate change and growing food demand.

Conclusion

Our study has provided a comprehensive analysis of the variation of photosynthetic traits across maize and sorghum canopies. We highlighted maize as an efficient C_4 crop, showing high photosynthetic capacity and relative uniform photosynthesis throughout the canopy. This is attributed to reduced stomatal limitation and higher stomatal conductance, Rubisco activity (V_{cmax}) and LNC in the top and middle canopy, along with higher ϕ in middle and bottom layers. In contrast, sorghum cultivars presented significant variability, with DKB560 and Brandelisa standing out as cultivars with enhanced CO_2 uptake and ϕ through the canopy. The CO_2 uptake, ϕ and leaf area variability in sorghum are important traits for crop improvement. Overall, physiological adjustments such as nitrogen redistribution to upper canopy leaves and the optimization of light-use efficiency in lower canopy layers are key for improving canopy photosynthesis in maize and sorghum.

Declarations

No conflict of interests

Authors' contributions

RLA, ECM and RVR conceptualized and designed the experiment. APD and ES provided maize and sorghum seeds. RLA, NMS, HLS, MSS and CCC conducted the experiment. RLA and RVR wrote the manuscript, with all authors contributing to data interpretation, discussion and refining the final version of the manuscript.

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Tables

Table 1. List of maize and sorghum cultivars, type and the institution responsible for breeding.

Species	Cultivars	Type	Institution
Sorghum	Santa Elisa	biomass	IAC
	Brandelisa	sweet/biomass	IAC
	IAC7021	grain	IAC
	ENFORCER	grain	Nuseed
	DKB560	grain	Dekalb
Maize	BM3069 PRO2	grain/biomass	Biomatrix
	AG8701 PRO4	grain/biomass	Agroceres
	K7500 VIP3	grain	KWS
	DKB355 PRO3	grain	Dekalb
	B2401 PWU	grain	Brevant

Figures

Figure 1

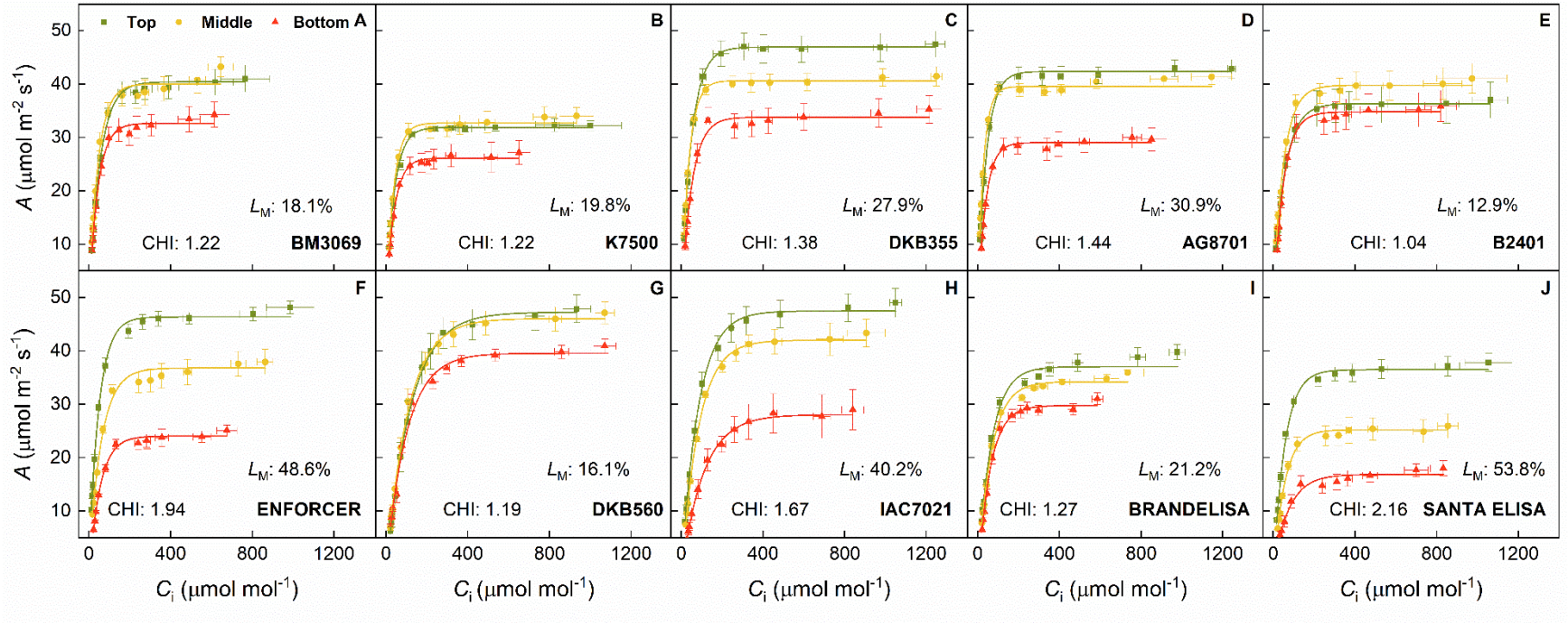

Fig. 1. Response curves of leaf CO_2 assimilation rate (A) to intercellular CO_2 concentration (C_i) in top (green), middle (yellow) and bottom (red) leaves of maize (A-E) and sorghum (F-J) cultivars, with their respective canopy homogeneity index (CHI) and metabolic limitation (L_M). Each symbol represents the mean±standard error (n=4).

Figure 2

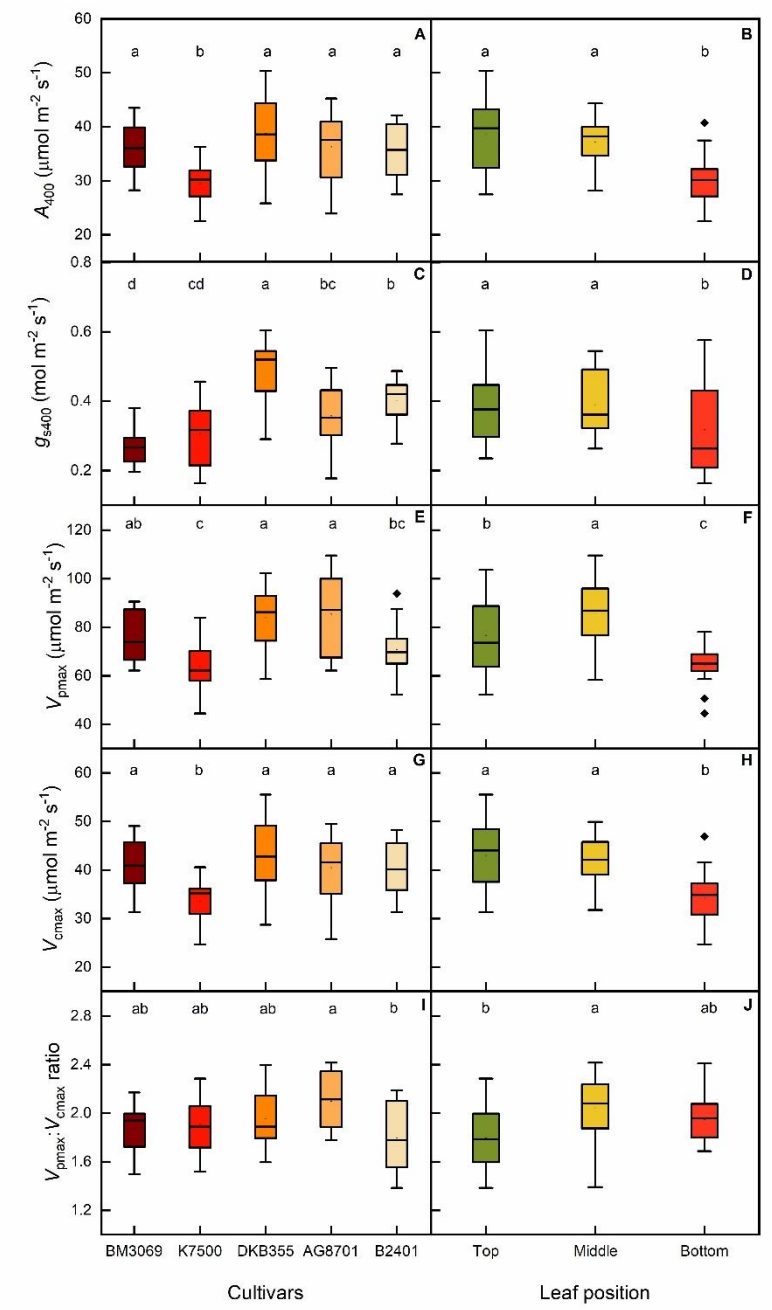

Fig. 2. Leaf CO_2 assimilation (A_{400}, A and B) and stomatal conductance (g_{s400}, C and D) at partial air CO_2 pressure (C_a) of 400 μmol mol^{-1}, *in vivo* maximum carboxylation rates of PEPC (V_{pmax}, E and F) and Rubisco (V_{cmax}, G and H) and V_{pmax}:V_{cmax} ratio (I and J) in five maize cultivars and three canopy layers. Different letters indicate statistical differences among cultivars (in A, C, E and G, Tukey p<0.05, n=12), and canopy layers (in B, D, F and H, Tukey p<0.05, n=20).

Figure 3

Figure 3

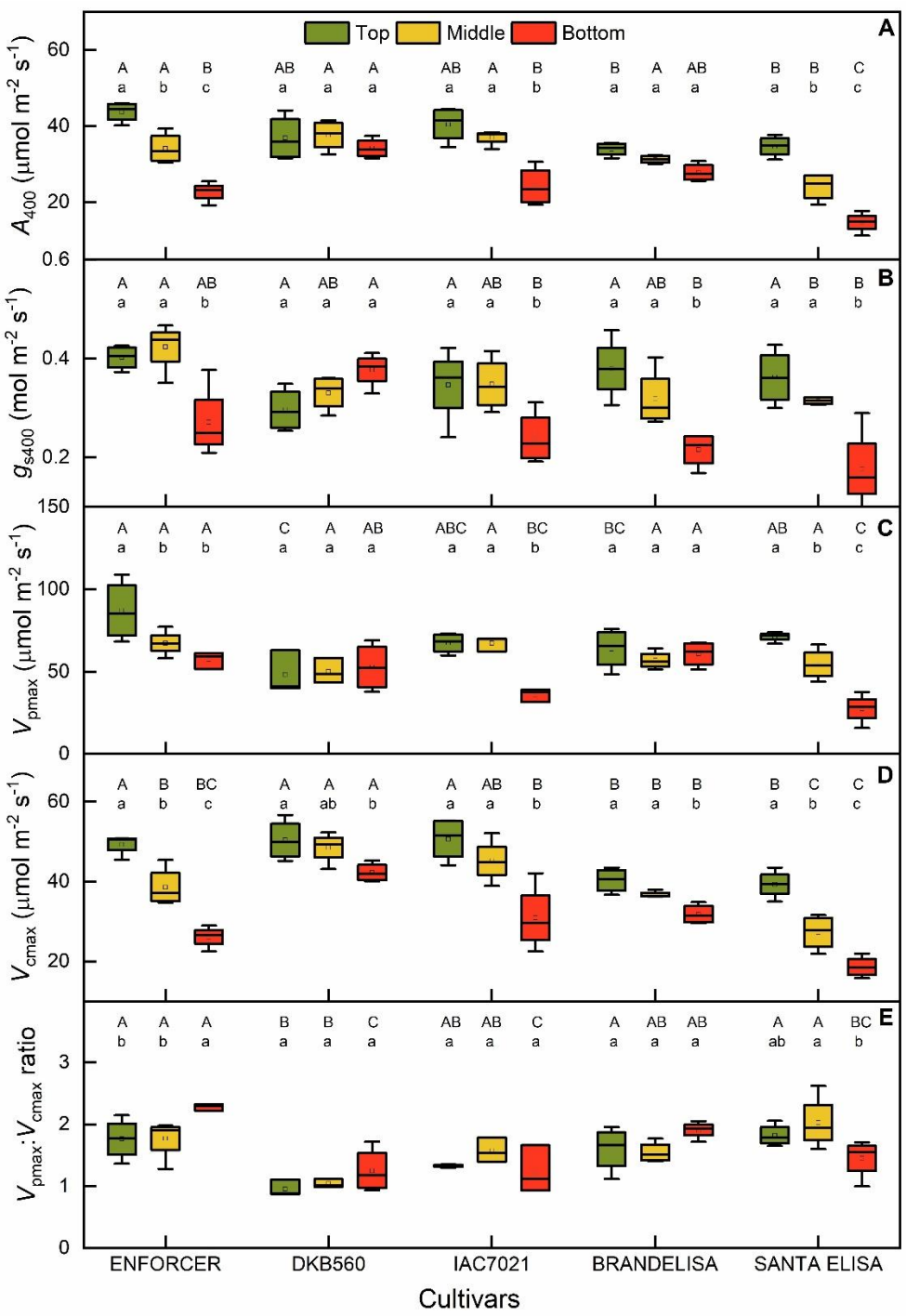

Fig. 3. Leaf CO_2 assimilation (A_{400}, in A) and stomatal conductance (g_{s400}, in B) at partial air CO_2 pressure (C_a) of 400 µmol mol^{-1}, *in vivo* maximum carboxylation rates of PEPC (V_{pmax}, C) and Rubisco (V_{cmax}, D) and V_{pmax}:V_{cmax} ratio (E) in top (green), middle (yellow) and bottom (red) leaves of five sorghum cultivars. Different capital and lowercase letters indicate statistical differences among cultivars and canopy layers, respectively (Tukey p<0.05, n=4).

Figure 4

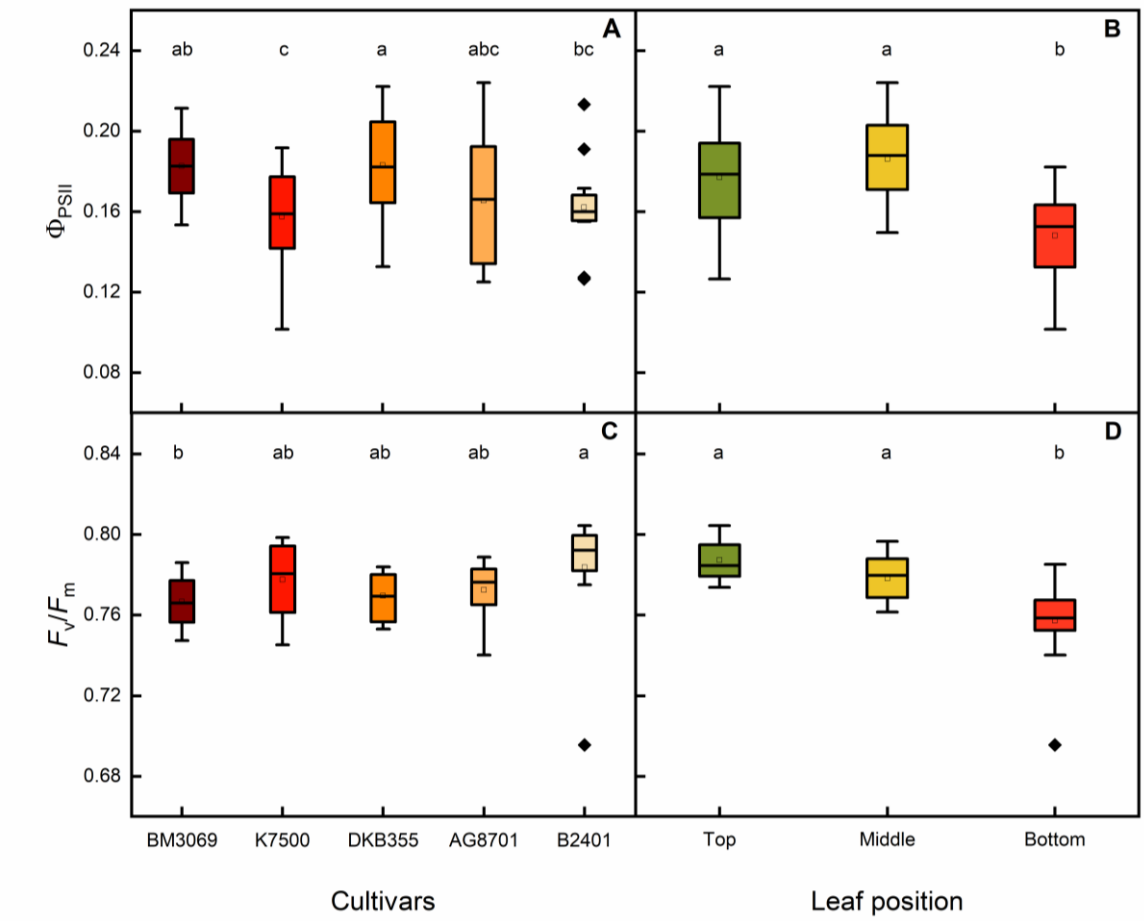

Fig. 4. Effective (Φ_{PSII}, A and B) and maximum (F_v/F_m, C and D) quantum efficiency of PSII in five maize cultivars and three canopy layers. Different letters indicate statistical differences among cultivars (A and C, Tukey $p<0.05$, $n=12$) and canopy layers (B and D, Tukey $p<0.05$, $n=20$).

Figure 5

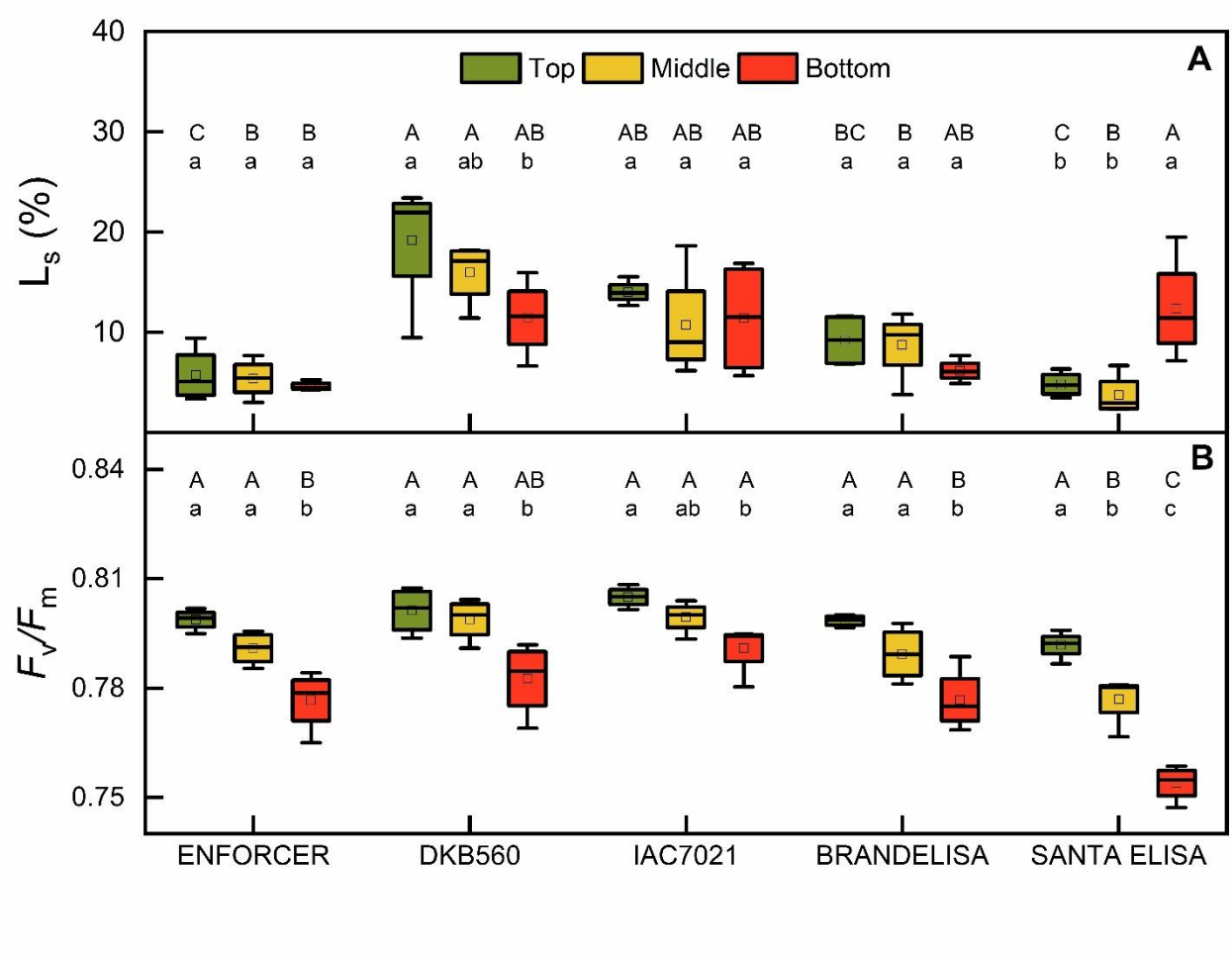

Fig.5. Stomatal limitation of photosynthesis (L_s, in A) and maximum quantum efficiency of PSII (F_v/F_m, in B) in top (green), middle (yellow) and bottom (red) leaves of five sorghum cultivars. Different capital and lowercase letters indicate statistical differences among cultivars and canopy layers, respectively (Tukey $p<0.05$, $n=4$).

Figure 6

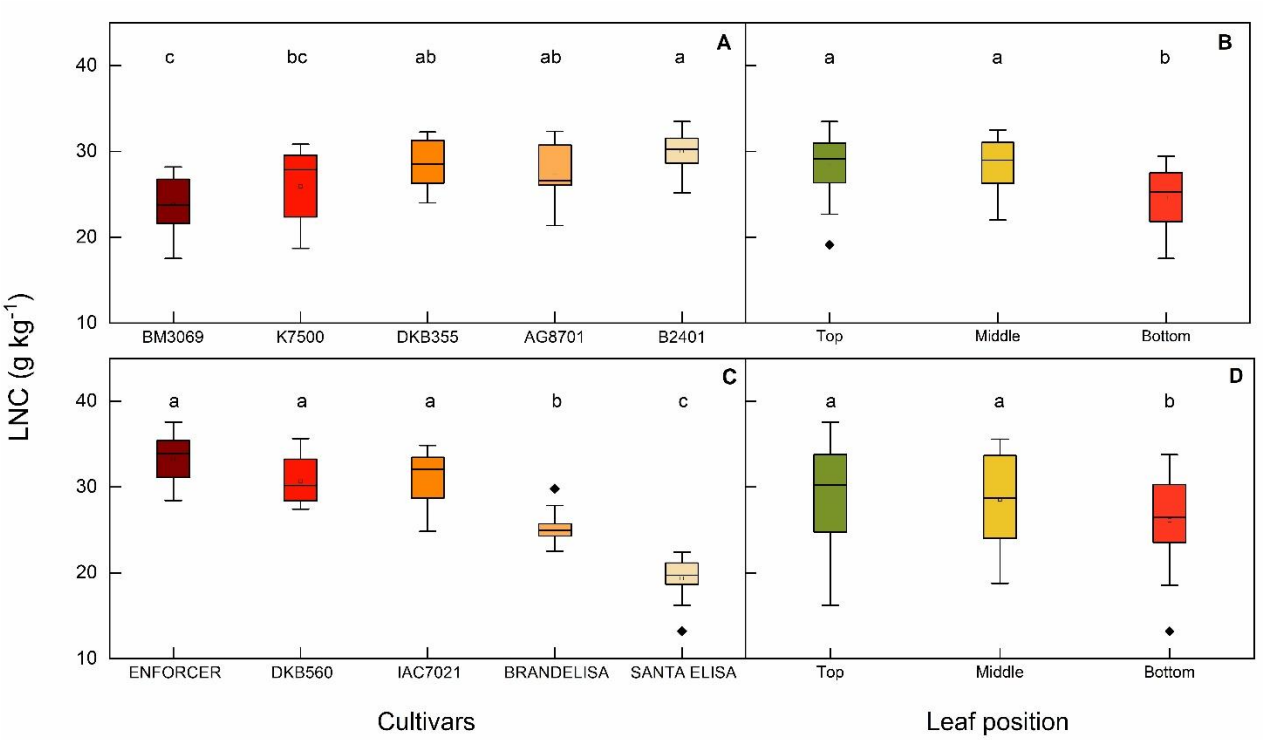

Fig. 6. Leaf nitrogen content (LNC) in five maize (A and B) and sorghum (C and D) cultivars and three canopy layers (B and D). Different letters indicate statistical differences among cultivars (A and C, Tukey $p<0.05$, $n=12$) and canopy layers (B and D, Tukey $p<0.05$, $n=20$).

Figure 7

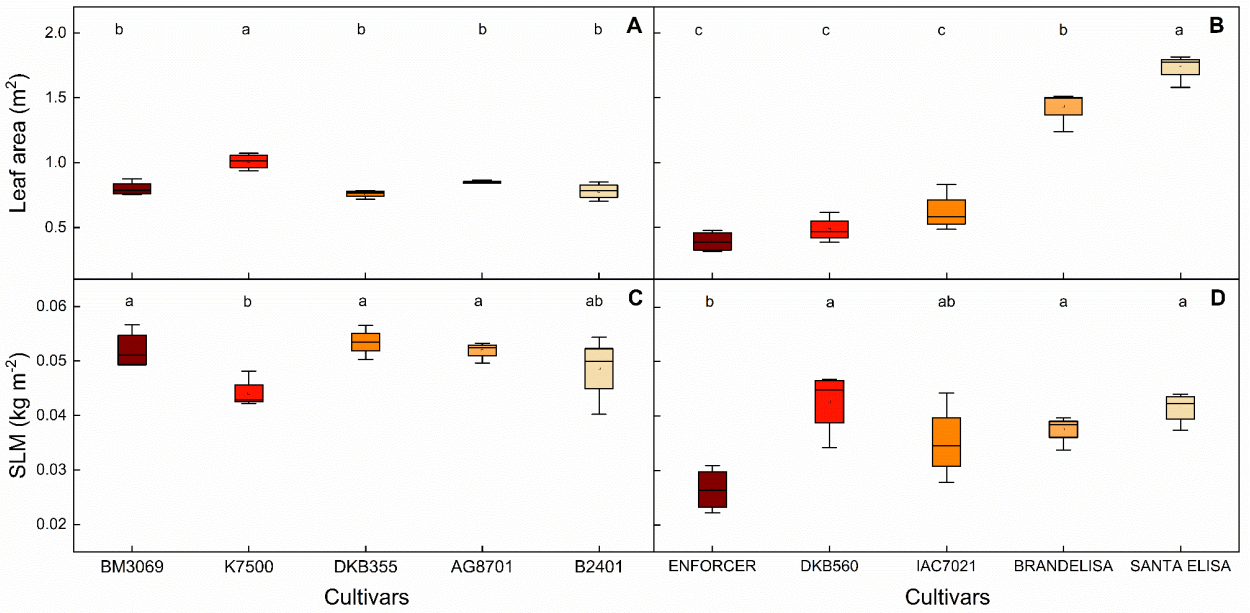

Fig. 7. Leaf area (A and B) and the specific leaf mass (SLM, in C and D) in five maize (A and C) and sorghum (B and D) cultivars. Different letters indicate significant differences among cultivars of each species (Tukey, $p<0.05$, $n=4$).