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Gas exchange and time to reach maximum rate of photosynthetic rate and their relationship with whole-plant traits in sugarcane in water abundant Louisiana, USA

P.Z. ELLSWORTH+, P.M. WHITE JR., and J. TODD

USDA-ARS Sugarcane Research Unit, 70360 Houma, LA, USA

Abstract

Variety development of sugarcane (*Saccharum* spp. hybrids) is necessary to continue improving sugar yields and selecting photosynthetic traits can improve sugar production through increased carbon inputs. In this study, gas exchange and whole-plant measurements were made on 55 sugarcane genotypes in Louisiana. Variation in the relationship between photosynthetic rate and stomatal conductance suggests that sugarcane exhibits variation in both photosynthetic capacity and CO_2 substrate availability. Genotypes that reached maximum photosynthetic rate (TRMPR) in the gas-exchange cuvette more quickly had greater CO_2 assimilation during transitory periods. Temporary shading and fluctuating light are common transitory conditions in the field, so increasing TRMPR can improve photosynthesis in water-abundant regions. Canopy leaf area was positively correlated with stalk mass, but gas-exchange traits were not correlated with whole-plant traits. A better understanding of the relationship between leaf and whole-plant traits is necessary to identify physiological traits that lead to increased genetic gain.

Keywords: gas exchange; phenotyping; photosynthesis; stomatal conductance; stomatal propensity to remain open; sugarcane.

Introduction

Sugarcane (*Saccharum* spp. hybrids) produces nearly 80% of sugar globally, is a major source of ethanol, and is the largest crop by biomass in the world (ISO 2023). In Louisiana, it is the second largest agricultural crop covering 251,000 ha, and valued at over $3.9 billion to the Louisiana economy in 2020 (ASCL 2023). The current efforts of the USDA-ARS Sugarcane Research Unit, Louisiana State University AgCenter, and the American Sugar Cane League are focused on producing new sugarcane cultivars that improve aboveground biomass, early season sugar accumulation, total recoverable sugar, disease resistance, and resistance to sugarcane borers (Hale *et al.* 2022). These efforts have increased sugar recovery from 5.8 to 11.7% from 1890 to 2010 and increased average tons of sugar per hectare from 2.5 to 8.8 over the same time period (Hale *et al.* 2022). Further progress in sugarcane variety development can be made by discovering additional physiological traits and high throughput phenotyping methods. Phenotyping of traits, especially physiological traits, requires, first, identifying specific traits that have a clearly defined role in improving sugarcane, are heritable, and exhibit genetic variation within the breeding population (Araus *et al.* 2014). Second, a preferably high throughput phenotyping method needs to be developed that is accurate, repeatable, and rapid. Selection of simple traits improve selection efficiency because it improves the relationship between genetic gain and phenotyping where genetic gain is the increase in yield or performance over time through the efforts of artificial selection and is a function of selection intensity,

Highlights

- Substantial variation in gas-exchange traits in sugarcane
- Short time to reach maximum rate of photosynthetic rate increases photosynthesis
- Canopy leaf area and stalk mass positively correlated

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+Corresponding author
e-mail: patrick.ellsworth@usda.gov

Abbreviations: C_a – ambient CO_2 concentration; C_i – intercellular CO_2 concentration; E – transpiration rate; g_s – stomatal conductance; P_N – net photosynthetic rate; TMPR – time to reach maximum photosynthetic rate.
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selection accuracy, and genetic variance relative to the years per breeding cycle (Araus *et al.* 2018). For example, greater genetic gains can be made through identifying photosynthetic traits that have high phenotypic variation and are under genetic control because of the potential of increasing the carbon available for sucrose production.

Photosynthesis has long captured the attention of plant physiologists and breeders alike as a source of improved plant productivity (von Caemmerer and Furbank 2003, Long *et al.* 2006, von Caemmerer *et al.* 2009, Evans 2013, Ort *et al.* 2015, Lawson *et al.* 2018). Most progress in increasing yield has focused on increasing carbon allocation to the harvested portion such as increasing early sucrose production in Louisiana and increasing aboveground biomass (harvest index) (Araus *et al.* 2014, 2018). Harvest index and carbon allocation to sucrose will reach their maximum threshold where the infrastructural needs of leaves, roots, and stems cannot be reduced further without negative feedback effects on sugar production. In contrast, increasing carbon inputs through improved photosynthesis has not undergone direct selection and remains a potentially major source of improvement to sugarcane growth and sugar production. To select for photosynthetic traits in Louisiana sugarcane such as leaf gas-exchange traits, phenotypic variability across genotypes must be present. Leaf gas exchange characterizes the movement of CO_2 and H_2O through leaf stomata where CO_2 diffuses into the leaf for photosynthetic assimilation and H_2O vapor exits the leaf. Gas-exchange measurements quantify CO_2 assimilation in photosynthesis and transpiration water loss and stomatal conductance at the leaf level and provide insight into leaf behavior that influences photosynthesis (Long and Bernacchi 2003). For example, gas exchange can shed light on stomatal responses to various biochemical and environmental controls and the environmental and physiological factors that influence components of photosynthesis such as enzyme concentrations and activity (von Caemmerer 2000). Because these traits represent the only influx of carbon through photosynthesis and the major source of water loss in the plant, there is an inherent relationship between leaf-level photosynthetic traits and whole-plant growth and water-loss traits.

Relationships between leaf and whole-plant traits provide insight into how leaf-level photosynthesis and transpiration influence whole-plant behavior. Leaf-level photosynthesis is the source of all carbon assimilated into plant tissue and the source of most water loss through transpiration, but it is also how carbon is used and retained in the plant that determines crop productivity and yields. Therefore, it is necessary to understand both basic photosynthesis and transpiration as leaf traits and their interaction with whole-plant traits such as canopy area and stalk traits that interconnect photosynthesis and transpiration with yield and productivity (Farquhar *et al.* 1989). However, the complex nature of whole-plant traits often obscures the role of leaf-level gas exchange in whole-plant traits, especially in perennial and woody species, which makes direct connections between leaf and whole-plant scales difficult (Medrano *et al.* 2015). Nonetheless, multiple studies have found relationships between gas-exchange traits and growth and water use at the whole-plant level (Ellsworth *et al.* 2017, 2020; Feldman *et al.* 2018, Leakey *et al.* 2019).

In this study, 55 sugarcane genotypes were measured for various leaf-level traits including net photosynthetic rate (P_N) and transpiration (E), stomatal conductance (g_s), leaf area, and time to reach maximum photosynthetic rate (TRMPR) after being placed in the gas-exchange cuvette, and whole-plant traits such as cane biomass volume and canopy leaf area. We hypothesized that because of the large genetic variation that is found in sugarcane genotypes, these leaf-level traits would have substantial phenotypic variation across genotypes and that significant relationships would be present between leaf and whole-plant level photosynthetic and water loss traits. The objectives were to (*1*) measure the phenotypic variation of these traits and to determine if there is a significant genotypic effect, (*2*) develop relationships between gas exchange and whole-plant traits and compare gas exchange and whole-plant traits between commercial cultivars and genotypes in the breeding program.

Materials and methods

Study site and sugarcane genotypes: The plants used in this study were grown in the field on the USDA-ARS Ardoyne farm in Louisiana, USA. All sugarcane plants were in plant cane crop and were grown in the same field on 1.8 m-spaced rows in a Cancienne silty clay loam soil under rainfed conditions. Plots were 2 adjacent, 7.6-m long row sections. A total of 55 sugarcane genotypes were used in this study (listed in Table 1S, *supplement*). The genotypes were either commercially released cultivars (16) or mid-stage genotypes in the breeding program (39) whose parents were a combination of commercially released cultivars and late-stage breeding genotypes from the USDA-ARS variety development program. These genotypes were chosen because they represented the major commercial genotypes and an entire cohort of new genotypes, some of which may be released as a commercial cultivar.

Gas exchange: Stalks from the field plots were cut at the base and immediately placed in a bucket of water and taken to the lab. Once in the lab, the cut end of the stalk was re-cut while submerged in water. Gas-exchange measurements were made with *LI-6800* (*LI-COR*, Lincoln, NE, USA). For gas-exchange measurements, the youngest, fully expanded leaf was placed in a 3 cm by 3 cm gas-exchange cuvette (*6800-12A*). The conditions in the cuvette were the following: 1,500 μmol(photon) m^{-2} s^{-1} of PAR (90% red and 10% blue light), 30°C leaf temperature, VPD of 1.12 ± 0.16 kPa, flow was set at 600 μmol s^{-1}, mean relative humidity of 74.3 ± 3.6%, and sample CO_2 concentration of 400 μmol mol^{-1}. The midsection of the leaf including the midvein was placed in the cuvette. Measurements were logged every minute during the entire time that the leaf was in the cuvette, and the sample and reference infrared gas analyzers were matched every 10 min. The leaf was removed once P_N and g_s remained constant for at least 4 min.

To verify that measuring gas exchange on leaves from cut stalks did not affect the measurements, gas-exchange measurements were made on 25 potted sugarcane plants from a total of nine genotypes. Leaves were inserted in the gas-exchange cuvette and placed under the same conditions stated above. Once P_N and g_s remained constant for 4 min, the leaves were removed from the cuvette. At this point the stalk was cut, and the cut end was placed in a bucket of water then recut under water. The same leaf but in a different location was placed in the gas-exchange cuvette again and remeasured under the same conditions. These paired measurements were compared to determine if P_N, E, and g_s changed after cutting the stalk and more importantly if this change varied with genotype. In a two-way repeated measures *ANOVA* with factors genotype and treatment (uncut *vs*. cut stalk), genotype was significant for P_N, E, and g_s, and the interaction was not significant (P>0.05). From these results, we conclude that cutting the stalk did not affect gas-exchange measurements and especially did not affect genotypes differentially or systematically. The model II regression slope of the line between gas exchange values of leaves from uncut (x-axis) and cut stalks (y-axis) was 1.1 for P_N, 0.94 for g_s, and 0.83 for E, showing that the gas-exchange values were similar before and after cutting the stalk. For time to reach maximum net photosynthetic rate (TRMPR) in the two-way repeated measures *ANOVA*, interaction was not significant (P>0.1), meaning that the response in TRMPR was not dependent on genotype. Therefore, measuring gas exchange of leaves from cut stalks was considered appropriate.

TRMPR was measured as the time when the leaf was placed in the gas-exchange cuvette until the leaf reached the maximum P_N and remained constant for 4 min without increasing further. Cumulative CO_2 assimilation [μmol(CO_2) m^{-2}] and transpiration [mmol(H_2O) m^{-2}] represented the total CO_2 assimilation and water loss per m^2 during the time the leaf was reaching maximum photosynthesis. Each minute log was assumed to represent the entire minute, so the sum of one-minute logs of P_N and E from the time that the leaf was placed in the cuvette until it reached its maximum P_N was multiplied by 60 to calculate them over the entire minute time frame.

Whole-plant measurements: Stalks were harvested by cutting them flush with the soil surface. The cut ends of the stalks were immediately placed in a bucket of water and stored in water until they were processed. The photosynthetically active, non-senesced leaves were removed from each stalk, and leaf area was measured using a portable leaf area meter, *LI-COR 3000C* (*LI-COR*, Lincoln, NE, USA). Then the leaves were placed in a paper bag and weighed in aggregate for the fresh mass and put in a drying oven at 60°C until their mass did not change further. At that point, the dry mass was recorded. The number of nodes were counted on the stalk, and total length and diameter at the basal end and middle of the stalk were measured. The stalk was weighed prior to being placed in a drying oven at 60°C and again once the mass was no longer decreasing. Cane volume was calculated as the volume of a cylinder using the mean cane diameter and cane height.

Statistical analysis: All statistics were done in *R* (R Core Team 2021). Genotype effect was determined by one-way *ANOVA* of each trait. Differences between commercial cultivars and mid-stage genotypes in the breeding program were tested using *t*-tests of the genotype means. Type II linear regressions were used to calculate relationships between leaf and whole-plant traits. Using *lmodel2* (*v. 1.7-2*) package, model II regression analysis (standard major axis) was used as a more appropriate regression analysis than ordinary least squares regression for all linear regressions because neither independent nor dependent variables were controlled, both varied naturally with their own associated error, and the physical units of both variables were not the same.

Results

Gas-exchange traits: Gas-exchange measurements [P_N, g_s, E, ratio of intercellular to ambient CO_2 concentrations (C_i/C_a), C_i] were consistent across replicates in each genotype in that standard errors were only 4–6% of the measured values, showing that these traits were reliable at the genotype level. All traits were significantly different across genotypes, showing a significant genotype effect (Table 1, Fig. 1). Variation across sugarcane genotypes was relatively high in gas-exchange traits, in that maximum values were 1.37 to 1.78 times that of the minimum values (Table 1). The commercial cultivars and unreleased genotypes in the breeding program did not differ in P_N and E, but slight differences were found in C_i/C_a and g_s, where commercial cultivars were slightly higher in both (P<0.01 and 0.05, respectively; Table 1). The mean maximum P_N reached in each individual leaf was 30.5 ± 2.2 (SD) μmol(CO_2) m^{-2} s^{-1} with 38.9 (L 17-428) being the highest value measured. E and g_s were rather high for a C_4 grass, reaching 3.51 mmol(H_2O) m^{-2} s^{-1} and 0.356 mol(H_2O) m^{-2} s^{-1} (L 17-428), respectively, and averaging 2.68 ± 0.26 mmol(H_2O) m^{-2} s^{-1} and 0.253 ± 0.029 mol(H_2O) m^{-2} s^{-1}, respectively.

Across genotypes mean P_N was correlated with mean g_s as expected, suggesting an increase in P_N by increasing CO_2 substrate availability, but there was considerable variation across genotypes in this relationship in that R^2 was only 0.51 (Fig. 2*A*). While most genotypes followed the expected positive relationship, a few genotypes fell off the regression line considerably. L 01-428, Ho 17-738, and HoCP 18-878 had high P_N and low g_s, suggesting higher photosynthetic capacity instead of increased CO_2 substrate availability, and HoCP 20-527, HoCP 20-548, and L 14-267 had low P_N and high g_s, suggesting lower photosynthetic capacity. Nearly all genotypes followed a general positive relationship between g_s and C_i/C_a followed a similar relationship (C_i/C_a = 1.034 g_s + 0.215, P<0.0001, R^2 = 0.61). L 01-428, Ho 17-738, and HoCP 18-878 had 7–12% lower C_i/C_a than expected based on g_s, further indicating increased photosynthetic capacity, and HoCP 20-527, HoCP 20-548, and L 14-267 had 7–11% lower C_i/C_a than expected, further indicating lower

Table 1. *ANOVA* and *t*-test table. 55 genotypes were measured including 16 commercial cultivars and 39 unreleased genotypes. Differences across genotypes were calculated using one-way *ANOVA*s. *t*-tests were used to determine if commercially released cultivars and mid-stage genotypes in the USDA-ARS breeding program were significantly different. Min. and Max. represent minimum and maximum values measured in the study. Canopy P_N, E, and g_s represent these traits scaled to the entire canopy leaf area. $^{*}g_s$ was greater in commercial cultivars (0.266) than unreleased genotypes (0.249). $^{\dagger}C_i/C_a$ was greater in commercial cultivars (0.50) than unreleased genotypes (0.47). $^{\ddagger}C_i$ was greater in commercial cultivars (198.8) than unreleased genotypes (188.2).

				One-way *ANOVA* (genotype)		*t*-test (type)	
Factor	Min.	Max.	Mean ± SE	$F_{ndf, ddf}$	*P*	T_{df}	*P*
P_N [µmol m^{-2} s^{-1}]	26.23	38.90	30.46 ± 0.29	$3.253_{54, 287}$	< 0.0001	$1.966_{1, 287}$	0.16
Canopy P_N [µmol s^{-1}]	5.15	16.39	8.75 ± 0.27	$6.220_{47, 190}$	< 0.0001	$1.130_{9.19}$	0.29
E [mmol m^{-2} s^{-1}]	2.19	3.51	2.68 ± 0.035	$2.279_{54, 287}$	< 0.0001	$0.028_{1, 287}$	0.87
Canopy E [mmol s^{-1}]	0.500	1.480	0.810 ± 0.026	$6.804_{47, 190}$	< 0.0001	$1.408_{10.41}$	0.19
g_s [mol m^{-2} s^{-1}]	0.199	0.356	0.253 ± 0.004	$2.300_{54, 287}$	< 0.0001	$4.459_{1, 287}$	0.04*
Canopy g_s [mol s^{-1}]	0.047	0.150	0.072 ± 0.002	$7.496_{47, 190}$	< 0.0001	$0.060_{10.90}$	0.95
C_i/C_a	0.39	0.55	0.47 ± 0.01	$2.398_{54, 286}$	< 0.0001	$-2.803_{27.29}$	0.01†
C_i	157.9	219.9	190.7 ± 2.2	$2.479_{54,289}$	< 0.0001	$-2.539_{27.78}$	0.02‡
Leaf area [m^2]	0.18	0.50	0.28 ± 0.01	$5.105_{47, 190}$	< 0.0001	$1.244_{9.14}$	0.24
Fresh cane mass [g]	678.7	2,051.9	1,230.5 ± 35.2	$4.998_{47, 192}$	< 0.0001	$0.466_{7.65}$	0.65
Dry cane mass [g]	190.2	574.6	343.1 ± 10.2	$4.373_{47, 192}$	< 0.0001	$0.774_{7.66}$	0.46
Fresh leaf mass [g]	48.9	156.4	88.6 ± 2.8	$7.142_{47, 192}$	< 0.0001	$0.901_{9.33}$	0.39
Dry leaf mass [g]	13.8	51.0	29.2 ± 0.9	$5.285_{47, 189}$	< 0.0001	$1.089_{9.52}$	0.31
Cane height [cm]	186.2	341.9	269.9 ± 3.9	$12.43_{47, 189}$	< 0.0001	$1.350_{7.14}$	0.22
Cane diameter [cm]	2.01	2.93	2.51 ± 0.03	$4.080_{47, 192}$	< 0.0001	$-0.680_{7.08}$	0.52
Base cane diameter [cm]	2.31	3.20	2.76 ± 0.03	$2.876_{47, 192}$	< 0.0001	$-1.041_{7.87}$	0.33
Mid cane diameter [cm]	1.98	2.80	2.40 ± 0.03	$5.855_{47, 192}$	< 0.0001	$-0.384_{6.84}$	0.71
Cane volume [L]	1.005	2.265	1.48 ± 0.04	$4.118_{47, 189}$	< 0.0001	$0.223_{7.98}$	0.83
Number of nodes	13.8	23.8	19.0 ± 0.3	$5.013_{47, 192}$	< 0.0001	$-0.009_{11.31}$	0.99
Number of green leaves	6.0	10.8	8.3 ± 0.2	$5.901_{47, 192}$	< 0.0001	$-0.935_{7.70}$	0.38

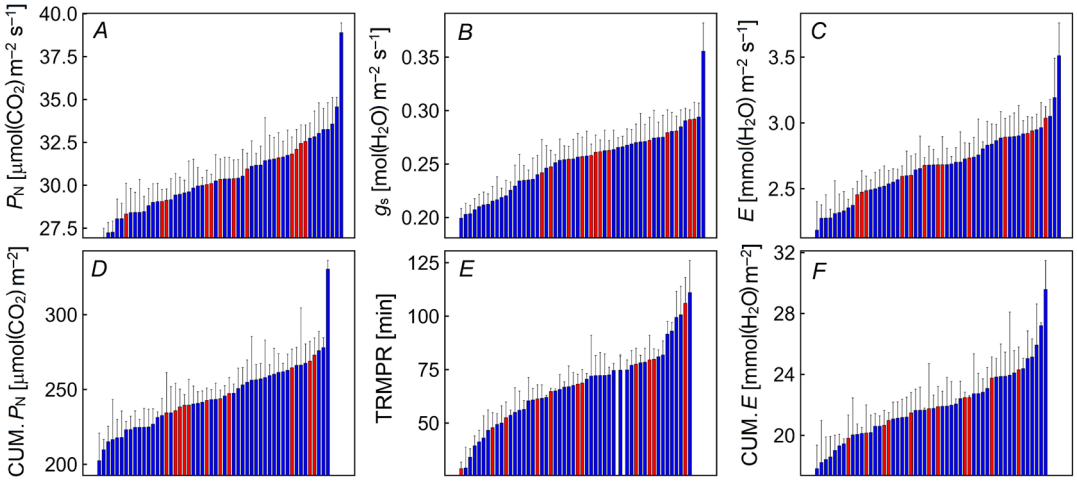

Fig. 1. Rank ordered plots of leaf gas-exchange traits. Genotypes (x-axis) are ranked by ascending order for each trait separately. Cumulative CO_2 assimilation and transpiration represent total CO_2 assimilation and transpiration that occurred during the time to reach maximum photosynthetic rate (TRMPR). *Blue* represents mid-stage genotypes currently in the USDA-ARS breeding program in Houma, LA, USA, and *red* represents commercially released cultivars. E – transpiration rate; g_s – stomatal conductance; P_N – net photosynthetic rate.

photosynthetic capacity. The top commercial cultivar by acreage planted in Louisiana, L 01-299, was in the quadrant of low P_N and g_s along with HoCP 20-532, HoCP 17-701, and HoCP 20-534. Commercial cultivars had slightly higher g_s, C_i, and C_i/C_a, indicated similar photosynthetic enzyme activities and mesophyll conductance. This relationship strengthened considerably when scaled to the entire canopy (Fig. 2*B*; $R^2 = 0.82$, $P<0.0001$).

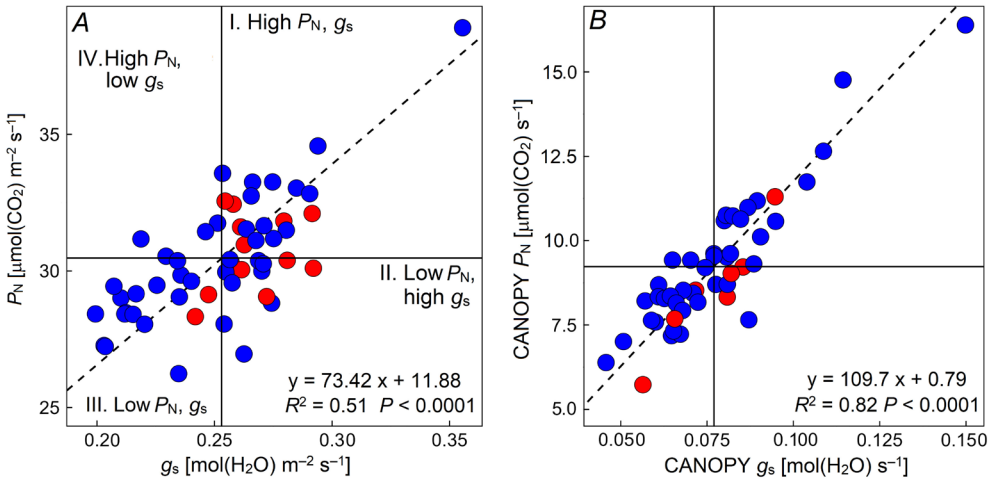

Fig. 2. Net photosynthetic rate (P_N) at the leaf level (*A*) and canopy level (*B*) *vs*. stomatal conductance (g_s) at the leaf and canopy levels, respectively of 55 sugarcane genotypes. *Blue* represents mid-stage genotypes currently in the USDA-ARS breeding program in Houma, LA, USA, and *red* represents commercially released cultivars. *Horizontal* and *vertical lines* represent the mean g_s and P_N, respectively. Quadrant I through IV represent combinations of high and low P_N and g_s. Canopy level gas exchange was calculated as gas exchange for the entire canopy leaf area, assuming uniform gas exchange over the entire canopy.

Stomatal propensity to remain open varied substantially across genotypes as expressed by genotypic variation in TRMPR, showing a significant genotype effect. TRMPR ranged from 28.7 to 111 min, showing a nearly fourfold difference across genotypes. The trait was somewhat consistent among replicates of the same genotype with standard errors between 1.5 to 34% (mean 10.9%) of the TRMPR value. TRMPR was not significantly correlated to *E* or g_s, but P_N and TRMPR formed a significant positive correlation, meaning that genotypes with large maximum P_N also reached maximum P_N more quickly (P<0.05). Further, the regression between maximum P_N and the cumulative CO_2 assimilation [µmol m^{-2}] that took place while the leaf was reaching maximum P_N was significant with an R^2 of 0.79 (Fig. 3*A*; P<0.0001). When the cumulative CO_2 assimilation was regressed on TRMPR, the regression was significant (R^2 = 0.36), meaning that TRMPR accounted for 21–36% of the variation in the cumulative CO_2 assimilation. Greater cumulative photosynthesis took place when the leaf reached maximal P_N more rapidly (Fig. 3*B*). In contrast, *E* and g_s regressed on the cumulative *E* and g_s, respectively, resulting in R^2 of 0.89 and 0.90, respectively, while TRMPR was not significantly correlated to cumulative *E* and g_s, showing that TRMPR did not affect that total water loss from the leaves (Fig. 3*C*,*D*). However, TRMPR was significantly negatively correlated with the initial g_s when the leaf was first placed in the gas-exchange cuvette (R^2 = 0.21, P = 0.0006), where short TRMPR was associated with high initial g_s. This relationship increased in strength over the first 10 min where the correlation between g_s and TRMPR reached a plateau at R^2 of 0.3–0.35 (P<0.0001). Genotypes, HoCP 20-556, HoCP 18-829, Ho 18-878, L 17-428, and HoCP 14-885 had the shortest TRMPR, while HoCP 20-541, Ho 13-739, HoCP 20-532, HoCP 20-535, and HoCP 20-529 had the longest TRMPR (Table 1S). The sugarcane genotypes that ranked high in P_N and TRMPR were L 17-428, Ho 17-738, HoCP 20-505, HoCP 14-885, and L 12-201, while HoCP 20-532, HoCP 20-529, HoCP 20-501, HoCP 20-535, and HoCP 20-527 were the lowest (Table 1S).

Whole-plant traits: Whole-plant traits exhibited more variation across genotypes than gas-exchange traits in that maximum values were 1.46 to 3.70 times that of the minimum values. This was particularly true with mass traits and leaf area (3.2×) with less variability in cane height and diameter and number of nodes (1.67×). Like gas-exchange traits, the traits were consistent across replicates of each genotype, where standard errors were 2–8% of the measurement values. Mass traits had greater variation with SE representing 7–8% of the measurement, while cane diameter, height, and number of nodes were only 2–4% of the measurement. All whole-plant traits were significantly different across genotypes, showing a significant genotype effect (Fig. 4). The commercial cultivars and unreleased genotypes in the breeding program did not differ in any whole-plant trait (P>0.05; Table 1, Fig. 4). A positive relationship was found between stalk and canopy traits, where stalks with greater leaf area (r = 0.61) and leaf mass (r = 0.65) tended to be heavier (Fig. 5; P<0.0001). No relationship was found between gas-exchange traits and whole-plant traits.

The sugarcane genotypes that ranked highest in every whole-plant trait were HoCP 20-541, HoCP 20-523, HoCP 20-538, HoCP 14-885, and L 17-428. HoCP 18-829, HoCP 20-525, L 14-267, HoCP 20-502, Ho 18-878, and HoCP 20-548 consistently ranked low. Overall, in both gas exchange and whole-plant traits, the highest ranked genotypes were L 17-428, L 12-201, HoCP 20-523, HoCP 14-885, and HoCP 20-556, while the lowest ranked genotypes were HoCP 18-829, HoCP 20-502, HoCP 17-701, L 01-299, and HoCP 20-878. The rankings for gas exchange and whole-plant traits were not correlated, even

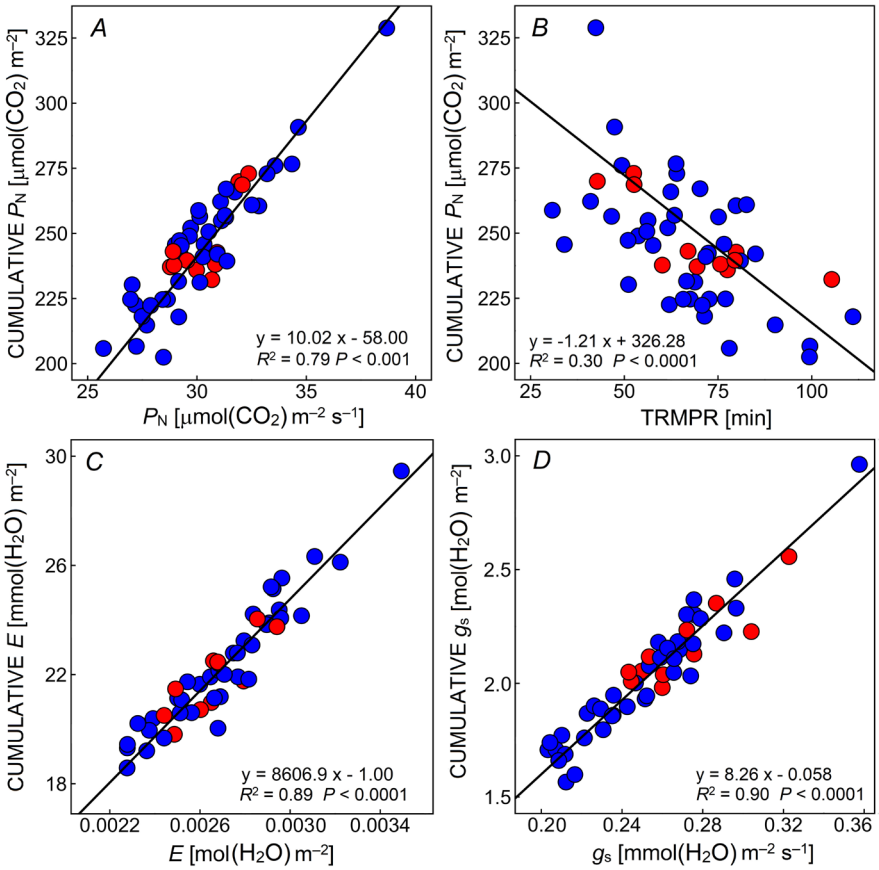

Fig. 3. Relationship of cumulative photosynthesis during the time to reach maximum photosynthetic rate (TRMPR) with P_N (*A*) and TRMPR (*B*) and cumulative *E* and g_s during TRMPR *vs.* *E* (*C*) and g_s (*D*) at TRMPR, respectively. *Blue* represents mid-stage genotypes currently in the USDA-ARS breeding program in Houma, LA, USA, and *red* represents commercially released cultivars. TRMPR and either *E* or g_s at TRMPR were not significantly correlated. *E* – transpiration rate; g_s – stomatal conductance; P_N – net photosynthetic rate.

though several genotypes were found at the top of each list. Multiple genotypes had low stalk mass but large canopies both in leaf area and mass such as HoCP 20-510 and HoCP 20-563 or large cane mass but small canopies such as HoCP 20-504 and HoCP 20-512 (Table 2S, *supplement*).

Discussion

Gas-exchange traits exhibited relatively large variation even in this highly selected group of commercial cultivars and mid-stage genotypes in the breeding program. Breeding for early sucrose accumulation, sugar and fresh aboveground biomass yield per hectare, and disease resistance has substantially increased sugar production over the previous 120 years (Hale *et al.* 2022). In sugarcane, similar to many other crops, substantial yield improvements have been made by selecting for increased carbon allocation to harvestable biomass and increased harvest index (Long *et al.* 2006, Zhu *et al.* 2010). The fact that other traits such as gas-exchange traits are not directly selected and still exhibit significant variation across genotypes indicates that multiple different phenotypic and genetic scenarios can result in a relatively good sugarcane cultivar. A lack of difference between commercial and unreleased mid-stage genotypes further indicates that likely photosynthetic traits are not being selected indirectly either. Therefore, selecting genotypes with high P_N or short TRMPR that increase carbon inputs along with the traditional breeding targets that select for increased carbon allocation to sugar production can result in greater potential yields than selecting for increased carbon allocation to sugar alone. Genetic manipulation and redesigning crop plants are often considered necessary avenues to increase potential yields through improvements in plant physiology (Zhu *et al.* 2010, Long *et al.* 2015, Ort *et al.* 2015), but substantial variability in gas-exchange traits in sugarcane indicates that traditional selection can still increase genetic gain. Even slight improvements in photosynthesis of 2–3% can produce noticeable yield increases by harvest time (Lefebvre *et al.* 2005, Lawson and Blatt 2014).

Variation in the interaction between gas-exchange traits, P_N and g_s, suggests that sugarcane exhibits variation in both photosynthetic capacity and CO_2 substrate availability. Other studies have also recorded variation in photosynthetic capacity and g_s in sugarcane, indicating an opportunity to select for improved photosynthesis (Inman-Bamber *et al.* 2011, Hoffman *et al.* 2018, Zhao *et al.* 2019, Singels *et al.* 2021). Differences in

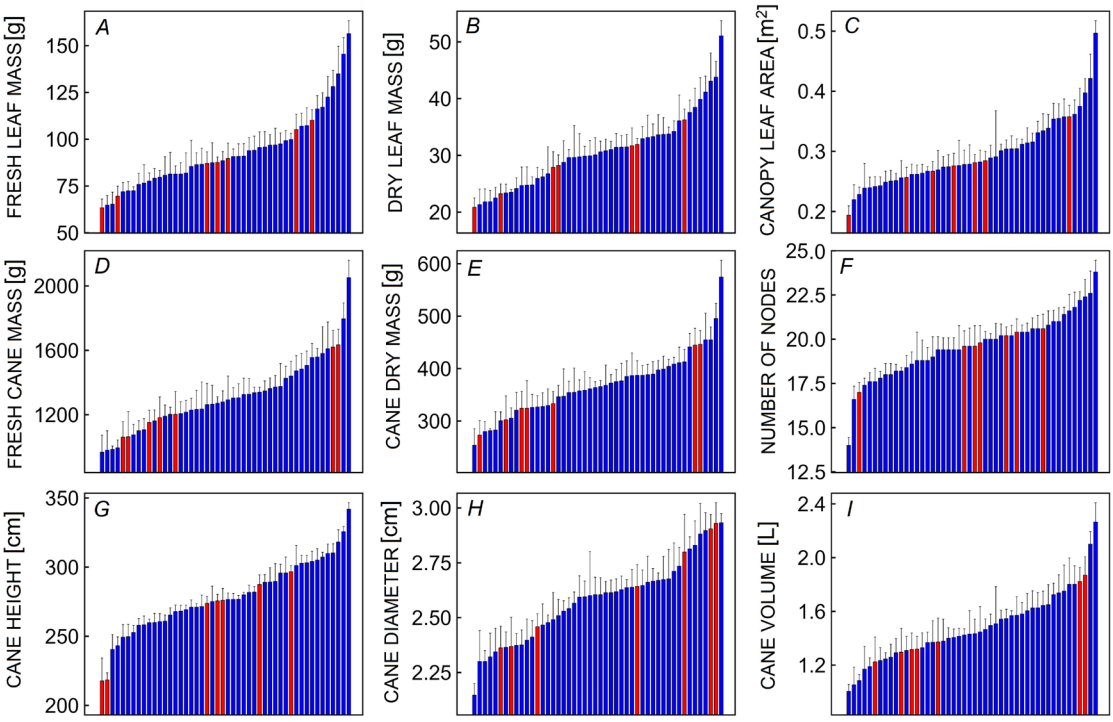

Fig. 4. Rank ordered plots of whole-plant traits. Genotypes (x-axis) are ranked by ascending order for each trait separately. Cane diameter is the mean of diameters measured at base and mid distance on the cane. Fresh and dry cane mass represent the mass of the stalk only without leaves. *Blue* represents mid-stage genotypes currently in the USDA-ARS breeding program in Houma, LA, USA, and *red* represents commercially released cultivars.

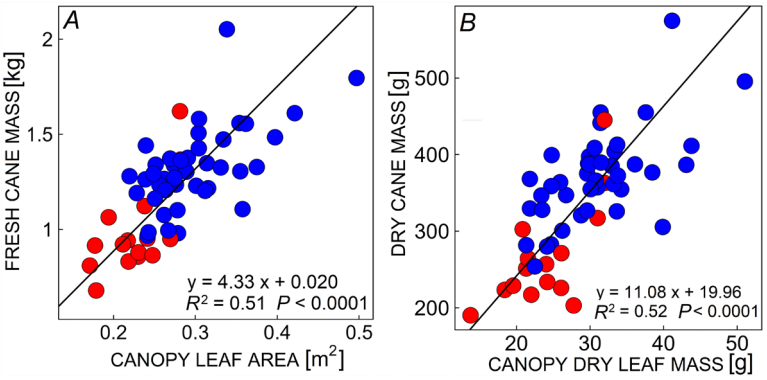

Fig. 5. Cane mass *vs.* canopy leaf area (*A*) and mass (*B*). *Blue* represents mid-stage genotypes currently in the USDA-ARS breeding program in Houma, LA, USA, and *red* represents commercially released cultivars.

photosynthetic capacity were identified as genotypes that had high P_N and low C_i/C_a compared to g_s, which could indicate a physiological difference such as modified bundle-sheath leakiness or higher mesophyll conductance or PEPC and Rubisco activities (Kromdijk *et al.* 2014, Li *et al.* 2017, Lawson *et al.* 2018). C_i and C_i/C_a are heritable in sugarcane and have been considered a proxy for increased water-use efficiency (P_N/g_s) because they indicate increased photosynthetic capacity (and, therefore, P_N) relative to g_s, but these proxies may provide a heritable screen of photosynthetic capacity, especially when coupled with increased P_N (Ghannoum 2016, Jackson *et al.* 2016). Increased substrate availability is defined as having increased CO_2 diffusion into the stomata (high g_s), resulting in higher P_N (Li *et al.* 2017). For example, Ho 17-738 appears to have high photosynthetic capacity, while L 17-428 had very high g_s to increase CO_2 substrate availability to fuel the highest P_N measured in the study and relatively low C_i/C_a, potentially having both increased photosynthetic capacity and substrate availability. The current top cultivar by total acreage planted in Louisiana, L 01-299, has multiple ideal traits such as ratooning ability that have maintained its importance in the sugarcane industry, yet it had rather low P_N and g_s (Gravois *et al.* 2011). Incorporating improved photosynthetic traits in an excellent cultivar could lead to greater carbon inputs that can be converted to sugar production. Increasing substrate availability by greater g_s has the additional consequence of increased water loss (Lawson and Vialet-Chabrand 2019), but despite that

higher substrate availability is an important trait because sugarcane seldom lack sufficient water in water-abundant south Louisiana (Ellsworth and White Jr. 2022).

Increased CO_2 assimilation during transitory periods such as fluctuating light can improve total photosynthetic output even if accompanied with increased water loss. Theoretically, stomata are only open when conditions are right for photosynthesis, attempting to adjust stomatal activity with photosynthetic demands to reduce water loss and maximize water-use efficiency (Brodribb *et al.* 2009, McAusland *et al.* 2016, Lawson and Vialet-Chabrand 2019). Considering that photosynthesis during disturbance and fluctuating light conditions account for at least 35% of all fixed carbon, closing stomata under fluctuating light and other disturbances is a major limiting factor for photosynthesis and increasing photosynthesis during transitory periods through increasing the propensity of stomata to remain open can increase photosynthetic output beyond the effect of greater photosynthetic capacity alone (Leakey *et al.* 2002, Lawson *et al.* 2018, Condon 2020, Yamori *et al.* 2020, Wang *et al.* 2021). Genotypes with the shortest TRMPR values had the highest initial g_s, so TRMPR trait represents the propensity of stomata to remain partially open during disturbance and to quickly reopen completely after disturbance. TRMPR could lead to increased transpiration, but it does not appear that TRMPR affects cumulative E as much as it affects cumulative P_N during transitory periods. Considering that fluctuating light is common for much of the leaf area in the canopy, increasing TMPR could improve photosynthesis outputs in sugarcane in Louisiana where water abundance and high humidity do not necessitate water conservation.

Combining the selection of leaf- and whole plant-level traits such as photosynthesis and canopy traits can create a synergistic effect. For example, the photosynthetic output of the whole canopy across genotypes was strongly influenced by canopy size, leading to a stronger relationship between P_N and E, where some large-canopied varieties had high canopy P_N or E, even though leaf-level P_N or E were low. This potentially shows variation in the hydraulic constraints on gas exchange and the need to understand photosynthetic traits across scales (Meinzer and Grantz 1990, Sperry 2000). The fact that increased canopy leaf area and mass was correlated with increased stalk mass and volume indicates that coupling high photosynthesis with large canopy leaf area could greatly increase aboveground biomass and sugar production. Interestingly, this relationship between leaf area and stalk size appears to contradict the concept of an ideal canopy size where optimal canopy leaf area is less than maximal canopy size (Srinivasan *et al.* 2017). For example, biomass productivity of *Miscanthus* × *giganteus* in the Midwest USA exceeded maize (*Zea mays*) because it reached 90% canopy closure about four weeks before maize (Dohleman and Long 2009). Sugarcane grows slowly and takes longer to reach canopy closure, resulting in inefficient light interception for the first part of the growing season (Allison *et al.* 2007, Inman-Bamber 2014, Dias *et al.* 2020). Therefore, an ideal sugarcane canopy would include vigorous initial growth and large size to reach canopy closure quickly and leaves with high photosynthetic capacity and low TRMPR to increase photosynthetic output throughout the growing season, leading to greater biomass and sugar yields (Zhu *et al.* 2010).

No relationship was found between gas exchange and whole-plant traits, but that does not indicate that leaf-level traits do not influence whole-plant traits; rather it shows the complexity of factors that hinder the scaling of traits. Because CO_2 assimilation and water loss from the stomata are essentially the only source of C gain and water loss, there is an implicit relationship between leaf-level gas exchange and whole-plant growth and water loss (Farquhar *et al.* 1989, Singels *et al.* 2021). Nonetheless, this relationship can be obscured because of fixed carbon losses such as non-leaf respiration and differences in carbon use such as allocation to roots. Therefore, sink strength often is stronger than source strength (photosynthesis), so that sugar transport and accumulation limit yield more than photosynthesis (McCormick *et al.* 2006, 2008, 2009; Evans 2013, Singels *et al.* 2021). Additionally, single point measurements of gas exchange do not necessarily represent mean photosynthetic rate and water loss for the whole canopy, and comparisons across genotypes conflate gas exchange differences with genotypic whole plant and leaf variation (Long *et al.* 2006). However, a clear correlation between P_N and g_s with yield was observed in sugarcane in Florida, USA, and in other C_4 Panicoid grass species (Zhao *et al.* 2015, 2019; Ellsworth *et al.* 2017). As a stronger connection between leaf-level photosynthetic traits and whole-plant traits becomes better understood, we will be better able to identify suitable traits, leading to better phenotyping and increased genetic gain.

Conclusion: Improving photosynthetic traits is one of the underutilized areas in plant breeding, and they possess great potential to increase carbon inputs. Gas exchange is a powerful tool to measure multiple traits related to CO_2 assimilation and water loss. Nonetheless, more work needs to be done to make better connections between leaf- and whole plant-level traits, so that explicit relationships can be defined and used as a basis for selection. In humid, water-abundant agroecosystems, stomatal propensity to remain open (short TRMPR) has potential to improve photosynthesis through increasing CO_2 availability in frequently occurring transitory periods. Coupled with greater photosynthetic capacity, this stomatal propensity to remain open can substantially increase photosynthesis in sugarcane. High-throughput phenotyping is problematic in that these measurements are time-consuming, so these measurements may be most useful in a more advanced stage of selection in the breeding program.

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