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Assessing diversity in canopy architecture, photosynthesis, and water-use efficiency in a cowpea magic population

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Abstract

Optimizing crops to improve light absorption and CO_2 assimilation throughout the canopy is a proposed strategy to increase yield and meet the needs of a growing population by 2050. Globally, the greatest population increase is expected to occur in Sub-Saharan Africa where large yield gaps currently persist; therefore, it is crucial to develop high-yielding crops adapted to this region. In this study, we screened 50 cowpea (*Vigna unguiculata* (L.) Walp) genotypes from the multi-parent advanced generation inter-cross (MAGIC) population for canopy architectural traits, canopy photosynthesis, and water-use efficiency using a canopy gas exchange chamber in order to improve our understanding of the relationships among those traits. Canopy architecture contributed to 38.6% of the variance observed in canopy photosynthesis. The results suggest that the light environment within the canopy was a limiting factor for canopy CO_2 assimilation. Traits favoring greater exposure of leaf area to light such as the width of the canopy relative to the total leaf area were associated with greater canopy photosynthesis, especially in canopies with high biomass. Canopy water-use efficiency was highly determined by canopy photosynthetic activity and therefore canopy architecture, which indicates that optimizing the canopy will also contribute to improving canopy water-use efficiency. We discuss different breeding strategies for future programs aimed at the improvement of cowpea yield for the Sub-Saharan African region. We show that breeding for high biomass will not optimize canopy CO_2 assimilation and suggest that selection should include multiple canopy traits to improve light penetration.

KEYWORDS
breeding, canopy architecture, canopy photosynthesis, LAI, MAGIC, stem angle, WUE

Highlight: Architectural traits favoring light distribution within the cowpea canopy contribute to greater canopy CO_2 assimilation and water-use efficiency.

1 | INTRODUCTION

Populations in most Sub-Saharan African countries are expected to double by 2050 (FAO, 2017) further threatening food security in regions where approximately 23% of the population is undernourished (FAO, 2015). There are significant yield gaps in Sub-Saharan African countries (van Ittersum et al., 2016) caused by complex constraints from abiotic, biotic, socioeconomic, and management factors (Waddington, Li, Dixon, Hyman, & de Vicente, 2010). The use of unimproved or unsuitable varieties of legumes contributes to production constraints and yield gaps in Sub-Saharan Africa (Waddington et al., 2010). Therefore, it is crucial to identify strategies that sustainably enhance yield in crops that are widely grown by African farmers and contribute to food security.

Global cowpea (*Vigna unguiculata* (L.) Walp) production is estimated to be 4.5–6.5 million tons/year, with approximately 80% of production in West Africa. Nigeria, alone, is responsible for 45% of the world's cowpea production (Jayathilake et al., 2018; Langyintuo et al., 2003; Sprent, Odee, & Dakora, 2009). Although flowers and leaves are consumed in some regions, cowpea is mainly produced for beans, which provide a high-quality plant protein source. Seeds contain 23%–32% protein, 50%–60% carbohydrate, and 1% fat (El Naim & Jabereldar, 2010; Jayathilake et al., 2018). Cowpea residues also serve as a high-quality fodder for animals during the dry season in West Africa (Singh, Ajeigbe, Tarawali, Fernandez-Rivera, & Abubakar, 2003; Tarawali, Okike, Kristjanson, Singh, & Thornton, 2005). In addition, inclusion of cowpea in the cropping system can contribute to restoration of soil fertility through the fixation of atmospheric nitrogen (Fatokun, Tarawali, Singh, Kormawa, & Tamò, 2002). Yet productivity of cowpea in Africa is low with average yields ranging 100–400 kg/ha (Kamara et al., 2017). For comparison with another dry bean, the average yield in the USA for soybean was 3,500 kg/ha in 2018 (USDA National Agricultural Statistics Service, 2019).

Enhancement of the photosynthetic process is often cited as a strategy to increase yield in crops (Bailey-Serres, Parker, Ainsworth, Oldroyd, & Schroeder, 2019; Long, Zhu, Naidu, & Ort, 2006; Simkin, López-Calcagno, & Raines, 2019; Weber & Bar-Even, 2019; Wu, Hammer, Doherty, von Caemmerer, & Farquhar, 2019; Zhu, Long, & Ort, 2010). Among the different possible pathways to improve canopy photosynthesis, alteration of the canopy architecture has been shown to contribute to yield enhancement in different crops including soybean (Srinivasan, Kumar, & Long, 2016) and maize (Liu et al., 2015). Those results are indications that canopy architecture can still be optimized in a way that benefits yield. An optimized canopy ideally allows improved distribution of radiation and reduces excess light saturation maximizing canopy CO_2 assimilation over the course of a day (Long et al., 2006; Sheehy & Mitchell, 2013). It should

also optimize the size of the vegetative reservoir of nitrogen that is later relocated to the grains (Sheehy & Mitchell, 2013; Sinclair & Sheehy, 1999).

Water is a key driver of crop productivity (Mateos & Araus, 2016), and historically, breeding for greater plant biomass and seed production has inadvertently selected for increased stomatal conductance (Roche, 2015). However, the development of varieties with abundant water demands may be unfit for cultivation in Africa where access to fresh water, its distribution and/or its usage, is still challenging especially for smallholder farmers (Burney, Naylor, & Postel, 2013). Indeed, most of the crops in Sub-Saharan Africa are currently rain-fed (Burney et al., 2013) contributing to the large gap between the yields that are currently obtained and what could be potentially achieved under a scenario where water is non-limiting (Rosa et al., 2018). For these reasons, optimization of water-use efficiency is a key part of any strategy to develop new varieties for Africa.

The multi-parent advanced generation inter-cross (MAGIC) cowpea population has been developed by inter-mating eight founder parent lines for several cycles (Huynh et al., 2018). The founders were genetically diverse and were selected based on their ability to produce high yield under drought conditions, along with other relevant agronomic traits (e.g., abiotic and biotic stress resistance and seed quality). To date, data assessing how canopy architecture affects canopy CO_2 assimilation and water-use efficiency in cowpea are scarce. As photosynthesis is an important factor determining the yield potential of a crop and its water consumption, we have investigated the association of canopy architectural traits and photosynthetic traits in 50 lines from the MAGIC cowpea population. We addressed the following questions: (a) what diversity exists in canopy architecture and canopy photosynthesis within the MAGIC germplasm collection; (b) how do canopy architectural features influence canopy photosynthesis; (c) which traits exert the most influence on cowpea water use efficiency (WUE)? Answers to these questions can inform efforts to develop cowpea cultivars with high yield potential and high water-use efficiency.

2 | MATERIALS AND METHODS

2.1 | Cowpea genotypes and field design

Fifty lines from a cowpea MAGIC population (8 founder parents plus 42 recombinant inbred lines, Supporting information 1) were planted at the University of Illinois Energy Farm Facility in Urbana, IL (40.06°N, 88.21°W) on June 26th, 2019. Inbred lines with contrasting canopy architectural traits were selected based on previous phenotyping in Puerto Rico (data not shown). Each line was planted in a single 133.5 cm row oriented North-South. Spacing between plants within a row was 3.8 cm (considered high-density for cowpea) while the spacing

between rows was 152.4 cm, which avoided competition for light between rows and maximized interception of incoming radiation within a single row. The cowpea lines were planted in a 5 × 10 grid with soybean planted in the periphery to limit potential border effects. Plants were occasionally watered during prolonged period of hot and dry days during the vegetative stage prior to phenotyping. No fertilization or pesticide treatments were applied. All measurements were performed when the genotypes had reached the reproductive developmental stage between R1 (early bloom) and R3 (early pod set).

2.2 | Phenotyping

Three days prior to gas exchange measurements, cowpea lines were phenotyped for leaf length and width using a ruler ($n = 5$ leaves from different plants), greenness using the SPAD value of a chlorophyll-meter ($n = 5$ leaves from different plants; SPAD-502, Konica Minolta Sensing), number of nodes ($n = 4$), stem angle ($n = 10$) using a digital protractor smartphone application (Sensors multitool v1.3.2 for Android), canopy width using measurements of the width of the canopy at different position in the row ($n = 5$), and the height of the canopy ($n = 5$). Leaf measurements were performed on the last fully developed non-senescing central leaflet at the fifth to seventh node down from the top of the canopy depending on cowpea line. After gas exchange measurements (described below), plants within the footprint of the canopy chamber were harvested. Leaves were separated from stems; total leaf area (LA) was measured with a leaf area meter (LI-3100C Area Meter, Licor), stem length was measured for 4 plants per line; and then leaves and stems were dried separately in an oven at 60℃ for three weeks. Subsequently, samples were weighed for total leaf mass (g) and total shoot mass (g). The total biomass (g) was obtained from the sum of total leaf mass and total shoot mass. A parameter was introduced to describe the amount of leaf area exposed to solar irradiance (leaf area exposure) and was estimated as Canopy width (cm) ÷ Total LA. A high value for this parameter indicated a wide canopy that exposed a large proportion of its leaf area while a low value indicated a narrow, self-shaded canopy. The LAI (leaf area index) was estimated as the total LA of harvested leaves (m^2) ÷ enclosed canopy footprint (m^2). The canopy footprint was calculated based on the averaged Canopy width ($n = 5$) on 1 m length. All raw values are provided in Supporting information 1.

2.3 | Gas exchange measurements

Gas exchange measurements were made on two consecutive clear days 62 days after planting. Midday (between

11:00 and 15:00) leaf photosynthetic activity was measured in the field using a four portable gas exchange systems (Li-6800, Licor) with an external light source (leaf net CO_2 assimilation; A_{leaf}) and a clear-top chamber ($A_{leaf,CT}$). Measurements ($n = 4$) were performed on a mature central leaflet fully exposed to the sun within each cowpea line. Cuvette conditions within the instrument were as follows: $[CO_2] = 420$ µmol CO_2 m^{-2} s^{-1}, flow = 400 µmol m^{-2} s^{-1}, and fan speed = 1,000 RPM. The relative humidity (RH), photosynthetic photon flux density (PPFD), and temperature (T) conditions inside the leaf chamber were set to match ambient conditions (RH = 73%–75% v/v, T = 31–33°C and when measurements were performed with the external light source PPFD = 1900 µmol m^{-2} s^{-1}). Leaf intrinsic water-use efficiency (iWUE$_{leaf}$, µmol CO_2 mol^{-1} H_2O) was estimated as A_{leaf} ÷ stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}). Immediately after the completion of leaf-level gas exchange measurements (less than 5 min), canopy-level photosynthetic activity was assessed once for each cowpea line using a closed-system, portable canopy chamber.

The canopy chamber consisted of aluminum framing (1.2 × 1 × 1 m; h × l × w) covered with thin polycarbonate panels. Air inside the chamber was sampled at a 1 liter per minute and analyzed by a CO_2/H_2O gas analyzer (LI-7000, Licor) for gas concentration measurements. Conditions inside the chamber were monitored by a temperature/relative humidity probe (HMP60, Vaisala), quantum sensor (LI-190R, Licor), barometric pressure sensor (SB-100, Apogee Instruments), and IR radiometer (SI-121-SS, Apogee Instruments). The cowpea canopy (17 ± 2 plants) was enclosed for 3 min inside the chamber in order to measure gas exchange. The canopy CO_2 assimilation was calculated as a function of the rate of CO_2 drawdown inside the chamber (dCO_2/dt, µmol $mol^{-1}$$s^{-1}$). A quadratic regression was used to describe the CO_2 depletion rate following the methods described by Pérez-Priego, Testi, Orgaz, and Villalobos (2010):

$$[CO_2] = a + bt + ct^2$$

$$\frac{dCO_2}{dt} = b + 2ct$$

The quadratic regression method is usually preferred over a linear regression method to describe the rate of concentration change as the gradient of CO_2 declines over time inside a closed chamber (Steduto, Çetinkökü, Albrizio, & Kanber, 2002). Canopy CO_2 assimilation rate ($A_{canopy,ground}$, µmol m^{-2} s^{-1}) of the enclosed vegetation was then calculated following the equation published by Song and Zhu (2018):

$$A_{canopy,flux} = \frac{dCO_2}{dt} \cdot \frac{PV}{SRT}$$

where V (m^3) is the volume of the chamber, P (kPa) is the air pressure in the chamber, S (m^2) is the ground area that the canopy occupied, R is the universal gas constant (8.3×103 m^3 kPa/mol K^{-1}), and T (K) is the air temperature in the chamber. In the same way, the canopy conductance was calculated based on the transpiration rate (E, mmol H_2O m^{-2} s^{-1}) in the canopy chamber, following a quadratic regression:

$$\frac{dE}{dt} = b + 2ct$$

The canopy conductance (g_C, mmol H_2O m^{-2} s^{-1}) was then estimated by the following equation based on Pérez-Priego et al. (2010):

$g_C = \frac{dE}{dt} \cdot \frac{\gamma}{\rho C_P} \cdot \frac{\lambda}{VPD}$ where γ is the psychometric constant (kPa/K), C_P the specific heat capacity of air at constant pressure (KJ kg^{-1} K^{-1}), ρ the air density (kg/m^3), λ is the specific heat of vaporization (MJ/kg), and VPD the vapor pressure deficit (kPa).

The intrinsic water-use efficiency of cowpea (iWUE$_{canopy}$, μmol CO_2/mmol H_2O) was calculated as $A_{canopy,ground} \div g_C$. iWUE was also scaled to biomass by dividing iWUE$_{canopy}$ by total biomass (iWUE$_{biomass}$, g/mmol H_2O m^{-2} s^{-1}).

The first 8 s of logged measurements after canopy chamber closure was ignored in order to allow sufficient time for air mixing and stability inside the chamber. Then, canopy CO_2 assimilation was calculated using a 30 s time window every 2 s. The median of $A_{canopy,ground}$ values calculated this way for the first minute of measurement was used as the estimate for the canopy CO_2 assimilation rate. In order to assess how efficiently CO_2 was assimilated by the canopy relative to the total leaf area, $A_{canopy,ground}$ was normalized by total LA ($A_{canopy,ground} \div LA = A_{canopy,LA}$, μmol CO_2 m^{-2} s^{-1}). Canopy photosynthesis was measured once per cowpea line. The same canopy chamber was used for measurement of all cowpea lines.

In order to assess the impact of taking measurements on two days, we conducted the study by either analyzing the two days separately or by applying a corrective factor to gas exchange measurements based on the difference between the two medians. Because the results from the two approaches did not change the main conclusions, measurements from the two days were pooled together for subsequent analyses.

2.4 | Statistical analysis

Pearson's correlation coefficient was calculated to assess correlations among all measured traits. General linear regression (GLM) models were then used to assess the portion of variance explained by individual traits. In the next stage, linear regression models including all canopy traits (with the exception of canopy-level gas exchange measurements and LA/LAI because of their high correlation with total leaf mass) were tested for the prediction of the canopy CO_2 assimilation (ground flux and LA-based ground flux) and water-use efficiency (canopy and biomass-based). The best minimum adequate model was selected using a stepwise algorithm based on the Akaike's information criterion (AIC). The total variance explained by the models (R^2) was then decomposed to evaluate the relative variance explained by each predictor.

All traits related to canopy architecture (with the exception of LA and LAI) were entered as variables in a principal component analysis (PCA) based on a correlation matrix (i.e., appropriate method when variables have different scales) followed by hierarchical clustering on principle components using the Ward's criterion (HCPC). The average for all traits was calculated inside each defined cluster, and the Tukey's honest significant difference test (Tukey's HSD) was used to compare the means. In order to create diverse groups of cowpea lines with different canopy architectures of sufficient size and with a high homogeneity within each cluster, five clusters were defined with this analysis (inertia = 0.5). Similar results were obtained with fewer clusters (see Supporting information 4–6).
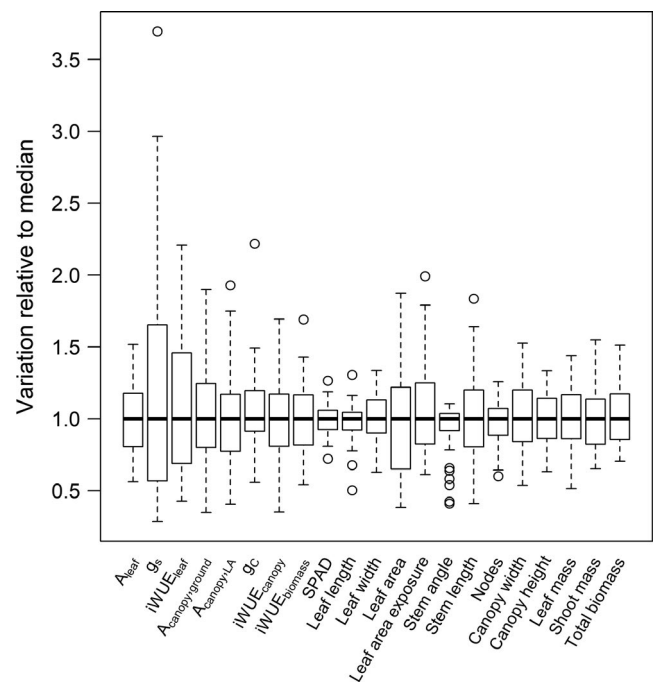

FIGURE 1 Boxplot showing the variability of phenotype traits measured for all cowpea genotypes ($n = 50$). In order to allow comparison of the variability among traits, measured values were normalized by dividing each value by the median value for that trait. The y axis, thus, represents the distribution of normalized values for each trait, where the normalized (N) value j for the trait i is equal to $N_{i,j} = \frac{x_{i,j}}{\bar{x}_i}$. A_{leaf}, net leaf CO_2 assimilation; g_s, stomatal conductance; iWUE$_{leaf}$, leaf intrinsic water-use efficiency; $A_{canopy,ground}$, canopy CO_2 assimilation ground flux; $A_{canopy,LA}$, leaf area-normalized canopy CO_2 assimilation ground flux; g_C, canopy conductance; iWUE$_{canopy}$, canopy intrinsic water-use efficiency; iWUE$_{biomass}$, biomass-based intrinsic water-use efficiency

An ANCOVA was performed with the previously defined clusters in order to assess if the linear relationship between $A_{\text{canopy,ground}}$ and $A_{\text{canopy,LA}}$ was the same among clusters with contrasting architectures. In addition, linear regressions between canopy CO_2 assimilation (ground flux and LA-based ground flux) and all measured traits were performed within each cluster to assess which traits explained the most variance in canopy CO_2 assimilation.

All statistical analyses were performed using RStudio version 1.1.453 (RStudio team, 2015), with the following R package: "FACTOMINER" (Lê, Josse, & Husson, 2008), "CAR" (Fox et al., 2016), and "RELAIMPO" (Groemping, 2006).

3 | RESULTS

3.1 | Variation in photosynthetic and canopy traits

Among the different phenotypes measured, leaf and canopy gas exchange traits (g_s, iWUE$_{\text{leaf}}$, $A_{\text{canopy,ground}}$), total LA, and leaf area exposure showed the greatest variation among the different cowpea lines (Figure 1). There was little variation among cowpea line in the number of nodes, stem angle, leaf length, and estimated chlorophyll content (SPAD). On

average, the range of variability observed in other traits including stem length, canopy width, leaf and shoot mass was ± 50% relative to the median (Figure 1).

3.2 | Correlations among canopy gas exchange traits

There was a significant positive linear relationship between $A_{\text{canopy,ground}}$ with canopy conductance, g_C ($R^2 = .27$, p-value < .001; Figure 2a). The correlation between A and g_s at the leaf-level was stronger than at the canopy-level (Supporting information 1). As expected, greater total LA was also associated with greater $A_{\text{canopy,ground}}$ ($R^2 = .32$, p-value < .001; Figure 2c). When A_{canopy} was expressed on a leaf area basis, not ground area basis, there was a negative linear relationship between $A_{\text{canopy,LA}}$ and total LA ($R^2 = .27$, p-value < .001; Figure 2d), which may be indicative of increasing self-shading in lines with greater leaf area.

Leaf-level photosynthetic measurements were not predictive of $A_{\text{canopy,ground}}$ but exhibited a positive linear relationship with $A_{\text{canopy,LA}}$ (Figure 3). Leaf photosynthesis measurements performed with a clear-top chamber or a saturated-light source performed equally at predicting A_{canopy} (Figure 3), probably because measurements were
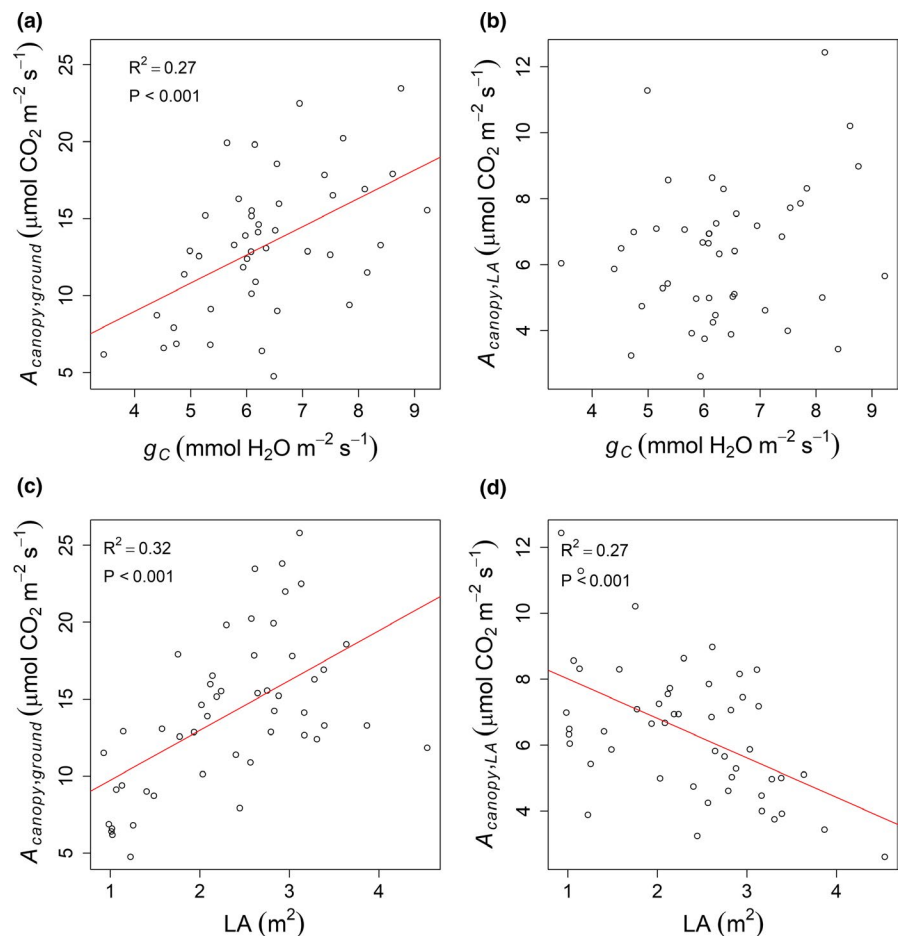

FIGURE 2 Linear regression between A_{canopy} and canopy conductance (g_C) and leaf area (LA)
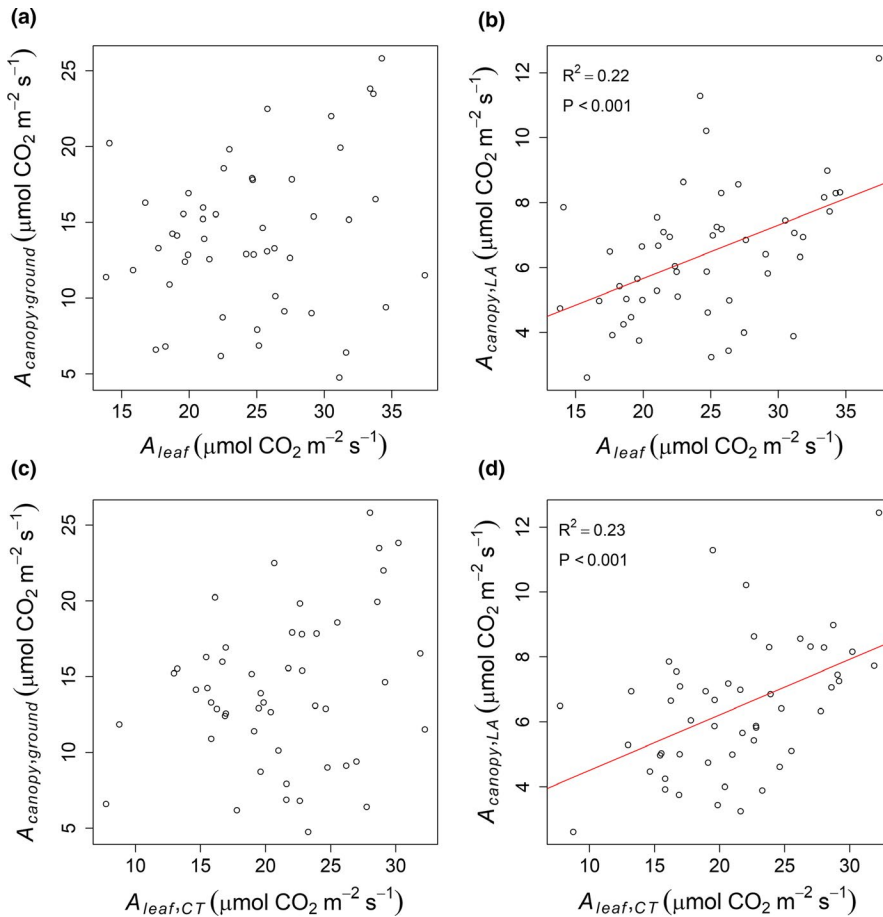
FIGURE 3 Linear regression between A_{canopy} and leaf photosynthesis measured with a light source (A_{leaf}) or with clear-top chamber ($A_{leaf,CT}$)

performed at noon when ambient light was saturating (i.e., ~1,500 μmol m^{-2} s^{-1}). For this reason, only measurements of leaf photosynthesis performed with a light source were used in subsequent analysis.

3.3 | General linear model to explain observed variability in canopy gas exchange

In a second analysis, the best minimum adequate model to predict both ground flux and LA-normalized canopy CO_2 assimilation was selected using a stepwise algorithm based on the AIC. This allows identification of the major predictors for A_{canopy} and traits with a considerable influence on canopy carbon assimilation. The best model predicting $A_{canopy,ground}$ had a R^2 = .76 (p-value < .001) while the best model predicting $A_{canopy,LA}$ had a R^2 = .66 (p-value < .001). Both models included leaf-level photosynthesis, SPAD, leaf mass, canopy width, stem angle, and leaf area exposure.

The total variance explained by the models was then decomposed in order to evaluate the relative variance explained by each predictor variable (Figure 4). For both models, plant architecture (e.g., stem angle, number of nodes, canopy width and leaf area exposure) and A_{leaf} played

comparable roles in the determination of canopy carbon assimilation. Traits related to the plant architecture explained 38.6 and 37.0% of the variance observed for $A_{canopy,ground}$ and $A_{canopy,LA}$, respectively. Leaf photosynthetic activity explained 12.3 and 18.2% of variance in $A_{canopy,ground}$ and $A_{canopy,LA}$, respectively. Biomass-related traits had a more predominant role in $A_{canopy,ground}$, by explaining 14.9% of total variance compared to 7.6% of variance in $A_{canopy,LA}$. This indicates that canopy CO_2 assimilation is not only determined by the amount of biomass and the A_{leaf} but also by traits defining the plant architecture.

3.4 | Determination of groups with contrasted canopy architecture

Five clusters with contrasting canopy architectures were defined by PCA-HCPC (Figure 5). The average trait values for the defined clusters are given in Table 1. The first principal component (PC1) explained 46.5% of the variance observed, with stem length, total leaf mass, canopy height, and canopy width as the main contributors. The clusters were mainly separated on the PC1. PC2 was mostly determined by leaf length, the number of nodes, and leaf area exposure. Leaf length was also an important contributor to PC3, along with
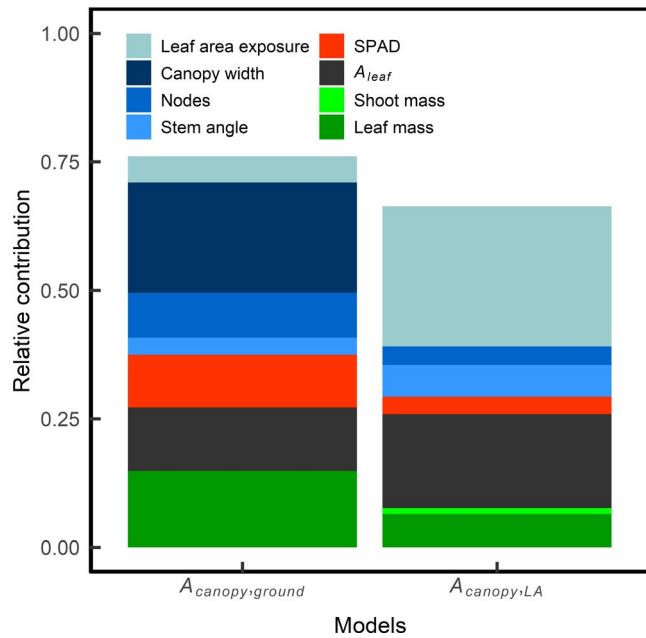
FIGURE 4 Relative contribution of the different parameters used in the minimum adequate models to explain the variation in (right) canopy CO_2 assimilation ground flux and (left) leaf area-normalized canopy CO_2 assimilation ground flux. Parameters in green are related to the canopy biomass. Parameters in blue are related to the canopy architecture

SPAD and the shoot mass. PC3, however, did not allow clusters to segregate. Clusters 1 and 2 were located in the third quadrants characterized by high LA, SPAD value, and a low node number (Figure 5). Cowpea lines located in cluster 1 showed a phenotype that strongly differed from the parents and was characterized by significantly lower leaf mass (p-value < .001, Table 1).

Clusters 3, 4, and 5 did not differ significantly in leaf and canopy photosynthesis despite showing significant differences in biomass (i.e., shoot, leaf and total biomass) (Table 1). Increasing LA in those clusters did not translate into an increase in canopy photosynthesis, which suggests differences in the efficiency of light interception with different canopy architectures. This is supported by the non-linear relationship between $A_{canopy,ground}$ and LAI (Figure 6). Leaf area exposure was also not significantly different among clusters 3, 4 and 5.

3.5 | Relationship between $A_{canopy,ground}$ and $A_{canopy,LA}$ under different canopy architecture

The correlation between $A_{canopy,ground}$ and $A_{canopy,LA}$, differed among clusters with contrasting canopy architecture (Figure 7). Comparison of the slopes from the linear regression revealed a significant difference between cluster 1 and clusters 3, 4,

and 5. This may be explained by the very low LA for lines in cluster 1 that led to a strong relationship between $A_{canopy,ground}$ and $A_{canopy,LA}$ ($R^2 = .93$, p-value < .001). This relationship was weaker in the other clusters, except in cluster 5 with very high leaf area canopies ($R^2 = .89$, p-value < .001).

The linear regressions between canopy traits with either $A_{canopy,ground}$ or $A_{canopy,LA}$ were explored within each cluster to assess if traits determining canopy CO_2 assimilation varied with differences in canopy architecture. Because of the similarities between the clusters 1 and 2 and the small size of the two clusters, they were pooled for this analysis. Most of the traits explaining observed variation in A_{canopy} were significant for only one cluster (Table 2), which could be explained by low variation of traits within each cluster. Increase in LA strongly contributed to increase in $A_{canopy,ground}$ ($R^2 = .45$, p-value < .01) in clusters 1 and 2 as expected for low leaf area cowpea lines. Total LA, however, showed a negative relationship with $A_{canopy,LA}$ ($R^2 = .61$, p-value < .01) in cluster 5, which was characterized by high biomass. SPAD values were also negatively correlated with $A_{canopy,ground}$ ($R^2 = .51$, p-value < .05) and $A_{canopy,LA}$ ($R^2 = .49$, p-value < .05) in cluster 5, which may suggest that light green leaves contributed to improved light diffusion within dense canopies. Leaf area exposure significantly contributed to the variation in $A_{canopy,ground}$ ($R^2 = .42$, p-value < .05) and $A_{canopy,LA}$ ($R^2 = .60$, p-value < .01) in cluster 5. This emphasizes the importance of light distribution for canopy CO_2 assimilation in dense cowpea canopies.

3.6 | Linear regression of intrinsic water-use efficiency with canopy traits

Variability in $iWUE_{canopy}$ could not be explained by canopy conductance (Figure 8). However, $iWUE_{canopy}$ showed a positive linear relationship with canopy CO_2 assimilation ($R^2 = .55$, p-value < .001). Genotypes from clusters 4 and 5, characterized by the highest $A_{canopy,ground}$, also showed the highest $iWUE_{canopy}$ values. Positive linear relationships between $iWUE_{canopy}$ and total leaf area and biomass ($R^2 = .20$, p-value < .01 and $R^2 = .22$, p-value < .01, respectively) were also observed and may be explained by the positive contribution these traits can have on canopy CO_2 assimilation. In contrast with $iWUE_{canopy}$, $iWUE_{biomass}$ was negatively correlated to canopy conductance ($R^2 = .52$, p-value < .001; Figure 9a). $iWUE_{canopy}$ also showed a positive linear relationship with total biomass ($R^2 = .28$, p-value < .001; Figure 9f). Interestingly, $iWUE_{canopy}$ only explained 10.7% (p-value < .05) of $iWUE_{biomass}$ variance suggesting that the ability to produce more biomass per unit of water was not explained by a better efficiency to assimilate CO_2.
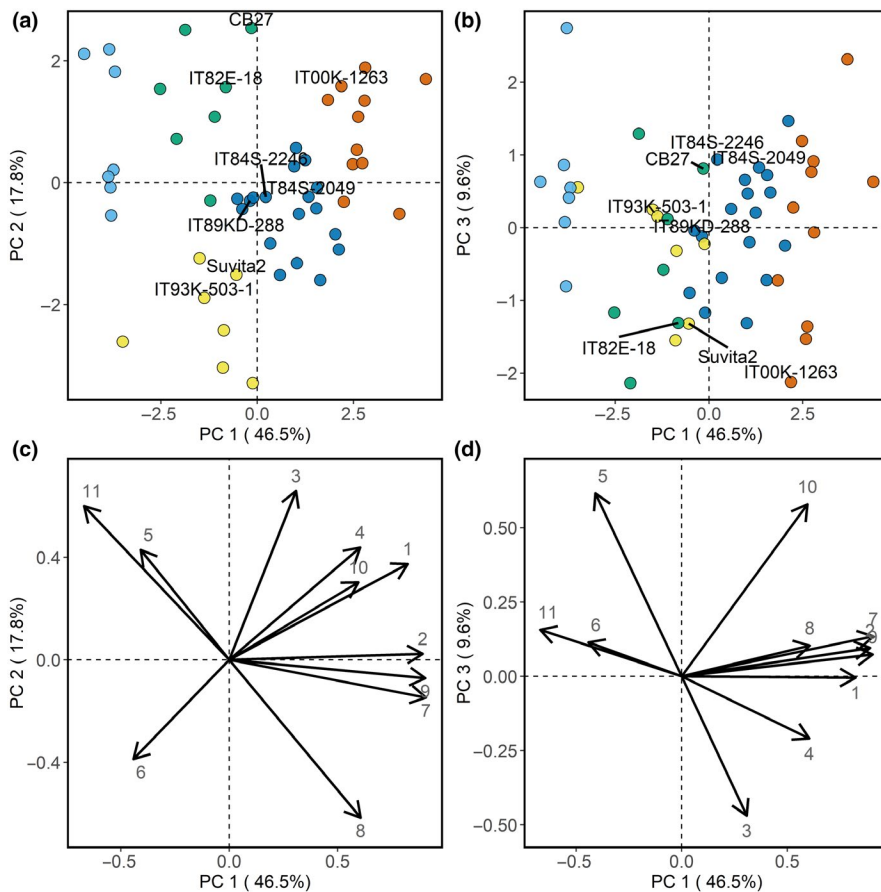
FIGURE 5 Clusters of similar and contrasted canopies. Determination of clusters with contrasted phenotypes was obtained by principal component analysis (PCA) followed by clustering. Distribution according to the principal components 1–2 (a) and 1–3 (b) and the correlation circles with the variables loaded on the principal components 1–2 (c) and 1–3 (d) are represented. The clusters have been highlighted by different colors with clusters 1, 2, 3, 4, and 5 in light blue, green, yellow, dark blue, and red. Canopy traits entered as variables in the PCA were as follows: 1—canopy width; 2—canopy height; 3—leaf length; 4—leaf width; 5—SPAD; 6—stem angle; 7—stem length; 8—nodes; 9—leaf mass; 10—shoot mass; 11—leaf area exposure

3.7 | General linear model to explain observed variability in intrinsic water-use efficiency

The best minimum adequate model to predict iWUE$_{canopy}$ both on a CO_2 assimilation and biomass basis was selected using a stepwise algorithm based on the AIC. Only traits related to the canopy architecture and A_{leaf} were added to the model in order to assess their contribution to the variance observed iWUE$_{canopy}$. The best model for the prediction of iWUE$_{canopy}$ had a $R^2 = .56$ (p-value $< .001$) while the best model predicting iWUE$_{biomass}$ had a $R^2 = .64$ (p-value $< .001$). Models only shared canopy width in common.

The partitioning of the relative contribution of each predictor variable in the models revealed that the two models had different drivers. About 45% of the variation observed in iWUE$_{canopy}$ was explained by canopy architectural traits including stem angle, stem length, canopy width, and leaf area exposure (Figure 10). In contrast, the model for iWUE$_{biomass}$ was mainly determined by plant biomass (33.5%). A_{leaf} also had strong predictive power for iWUE$_{biomass}$ by explaining 25.3% of the variance observed but was not selected in the linear model for iWUE$_{canopy}$. However, most of the traits included in the iWUE$_{canopy}$ model were also included in the A_{canopy} model.

4 | DISCUSSION

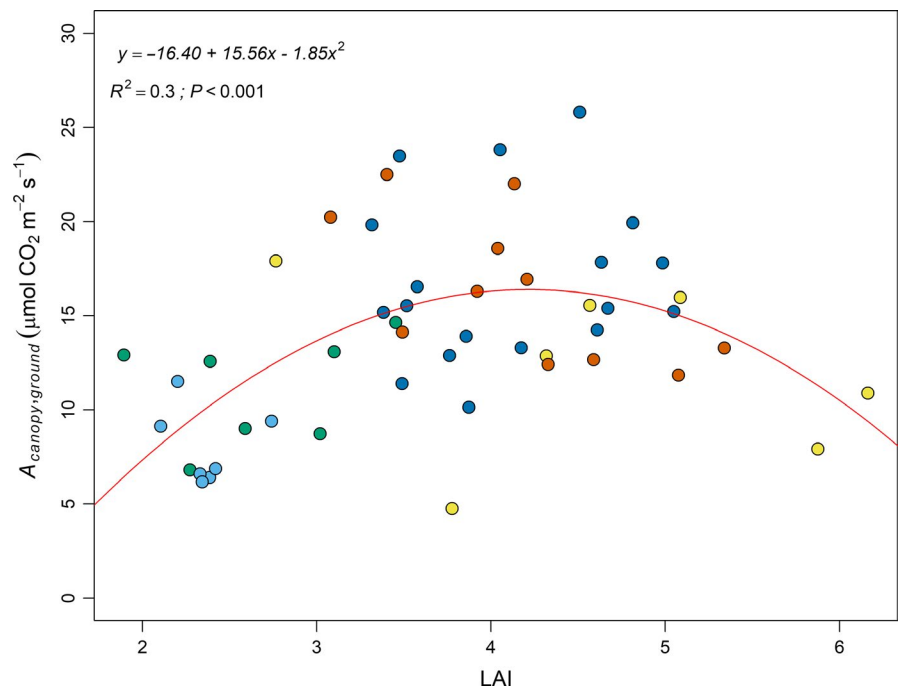### 4.1 | Drivers of canopy CO_2 assimilation

Significant variability was observed in canopy CO_2 assimilation (4.8–25.8 µmol CO_2 m^{-2} s^{-1}) among the 50 cowpea genotypes. This variation could be partly explained by leaf mass (14.9%), A_{leaf} (12.3%), chlorophyll content (SPAD value; 10.2%) and canopy architecture (38.6%). Our study highlights the diversity in canopy traits among a cowpea MAGIC population and emphasizes the multifactorial nature of canopy photosynthesis. The importance of canopy architecture for canopy photosynthesis has been highlighted in soybean (Song, Srinivasan, Long, & Zhu, 2019). Using a 3D canopy model and ray-tracing, the canopy structure was estimated to account for 4.8%–20% of the variation in canopy photosynthesis depending on the growth stage and ambient light conditions (Song et al., 2019). The greater contribution of canopy architecture to canopy photosynthesis observed in our study could be explained by the variety of phenotypes used in our study and the uniformity of environmental conditions when estimated canopy photosynthesis, while Song et al. (2019) modeled canopy CO_2 through the entire growing season, but for a single soybean cultivar.

TABLE 1 Average (+- *SD*) in the different clusters defined in Figure 5

Traits	Cluster 1 (*n* = 7)	Cluster 2 (*n* = 7)	Cluster 3 (*n* = 7)	Cluster 4 (*n* = 18)	Cluster 5 (*n* = 11)	*p*-values
A_{leaf}	27.95 ± 7.02 a	23.81 ± 3.47 a	22.83 ± 4.43 a	25.38 ± 6.49 a	20.75 ± 4.64 a	ns
g_s	0.76 ± 0.45 a	0.42 ± 0.18 a	0.39 ± 0.31 a	0.48 ± 0.29 a	0.29 ± 0.17 a	ns
iWUE$_{leaf}$	45.67 ± 19.14 a	66.05 ± 30.59 a	75.37 ± 26.39 a	65.84 ± 25.93 a	87.64 ± 30.61 a	ns
$A_{canopy,ground}$	8.01 ± 2.03 c	11.1 ± 2.9 bc	12.27 ± 4.72 abc	16.34 ± 4.36 a	15.88 ± 3.65 ab	***
$A_{canopy,LA}$	7.88 ± 2.24 a	7.38 ± 1.97 ab	5.92 ± 2.44 ab	6.45 ± 1.59 ab	4.84 ± 1.62 b	*
g_c	5.76 ± 1.75 a	5.57 ± 0.81 a	6.83 ± 1.56 a	6.86 ± 2.13 a	6.92 ± 0.95 a	ns
iWUE$_{canopy}$	1.43 ± 0.27 c	2.01 ± 0.52 abc	1.79 ± 0.54 bc	2.43 ± 0.5 a	2.32 ± 0.54 ab	***
iWUE$_{biomass}$	45.49 ± 18.67 a	48.4 ± 7.44 a	38.64 ± 11.75 a	46.87 ± 11.78 a	52.73 ± 10.02 a	ns
SPAD	73.71 ± 5.95 a	68.3 ± 6.01 ab	60.41 ± 7.46 b	64.64 ± 4.93 b	63.51 ± 7.17 b	**
Leaf length	8.77 ± 1.95 ab	9.96 ± 0.48 a	7.64 ± 0.86 b	9.47 ± 0.52 a	10.12 ± 1.15 a	***
Leaf width	4.17 ± 0.71 b	5.8 ± 0.7 a	4.41 ± 0.35 b	5.33 ± 0.7 a	5.92 ± 0.62 a	***
Leaf area	1.02 ± 0.06 d	1.52 ± 0.3 cd	2.11 ± 0.53 bc	2.56 ± 0.41 b	3.4 ± 0.52 a	***
Leaf area exposure	42.61 ± 3.46 a	38.8 ± 8.21 a	22.94 ± 6.82 b	25.46 ± 3.51 b	24.78 ± 4.36 b	***
Stem angle	78.46 ± 1.87 a	75.82 ± 4.18 a	81.39 ± 5.66 a	75.53 ± 11.55 a	57.44 ± 16.07 b	***
Stem length	27.07 ± 3.28 d	38.32 ± 7.81 cd	50.11 ± 10.39 c	62.92 ± 10.24 b	76 ± 14.04 a	***
Nodes	13.04 ± 1.93 b	14.75 ± 1.94 b	18.39 ± 1.27 a	18.38 ± 1.68 a	17.52 ± 1.98 a	***
Canopy width	43.43 ± 3.36 d	57.49 ± 8.35 bc	46.51 ± 11.16 cd	64.35 ± 8.44 b	82.74 ± 8.08 a	***
Canopy height	33.76 ± 4.46 c	42.7 ± 7.51 b	41.26 ± 4.82 bc	50.91 ± 5.25 a	56.2 ± 4.56 a	***
Leaf mass	94 ± 12.21 d	124.43 ± 13.43 c	129.86 ± 20.92 c	150.8 ± 19.6 b	175.7 ± 18.1 a	***
Shoot mass	141.71 ± 20 bc	142.71 ± 38.65 bc	119.71 ± 16.11 c	153.33 ± 22.28 ab	182.3 ± 31.3 a	***
Total biomass	235.71 ± 22.63 c	267.14 ± 42.94 bc	249.57 ± 25.57 c	304.13 ± 38.65 b	358 ± 43.99 a	***

Note: Statistical differences among clusters are assessed by *p*-values from the linear models where ns, *, **, and *** indicate *p*-values > .05, <.05, <.01, and < .001. Different letters indicate significant differences among the clusters (Tukey's HSD, α = 0.05). In the Figures, the clusters are represented by different colors with clusters 1, 2, 3, 4, and 5 shown in light blue, green, yellow, dark blue, and red.

FIGURE 6 Relationship between $A_{canopy,ground}$ and leaf area index (LAI). The best fit was obtained using a second order polynomial regression (red line). This model was used to estimate the LAI value for which $A_{canopy,ground}$ was maximum. The points are colored based on the different clusters previously defined. The same color code was been used, with clusters 1, 2, 3, 4, and 5 shown in light blue, green, yellow, dark blue, and red

$y = -16.40 + 15.56x - 1.85x^2$

$R^2 = 0.3 ; P < 0.001$

FIGURE 7 Relationship between $A_{canopy,ground}$ and $A_{canopy,LA}$ with different canopy traits. Labels show (a) leaf area, (b) leaf area exposure represented by $\frac{canopy\ width}{leaf\ area}$ and (c) leaf photosynthesis. Isoline values (blue lines) provide visual support for the distribution of the different parameters and were produced by local polynomial regression for Trait $= f\left(A_{canopy,ground}, A_{canopy,LA}\right)$ using the Loess R function. The points are colored based on the different clusters previously defined. The same color code was been used, with clusters 1, 2, 3, 4, and 5 shown in light blue, green, yellow, dark blue, and red
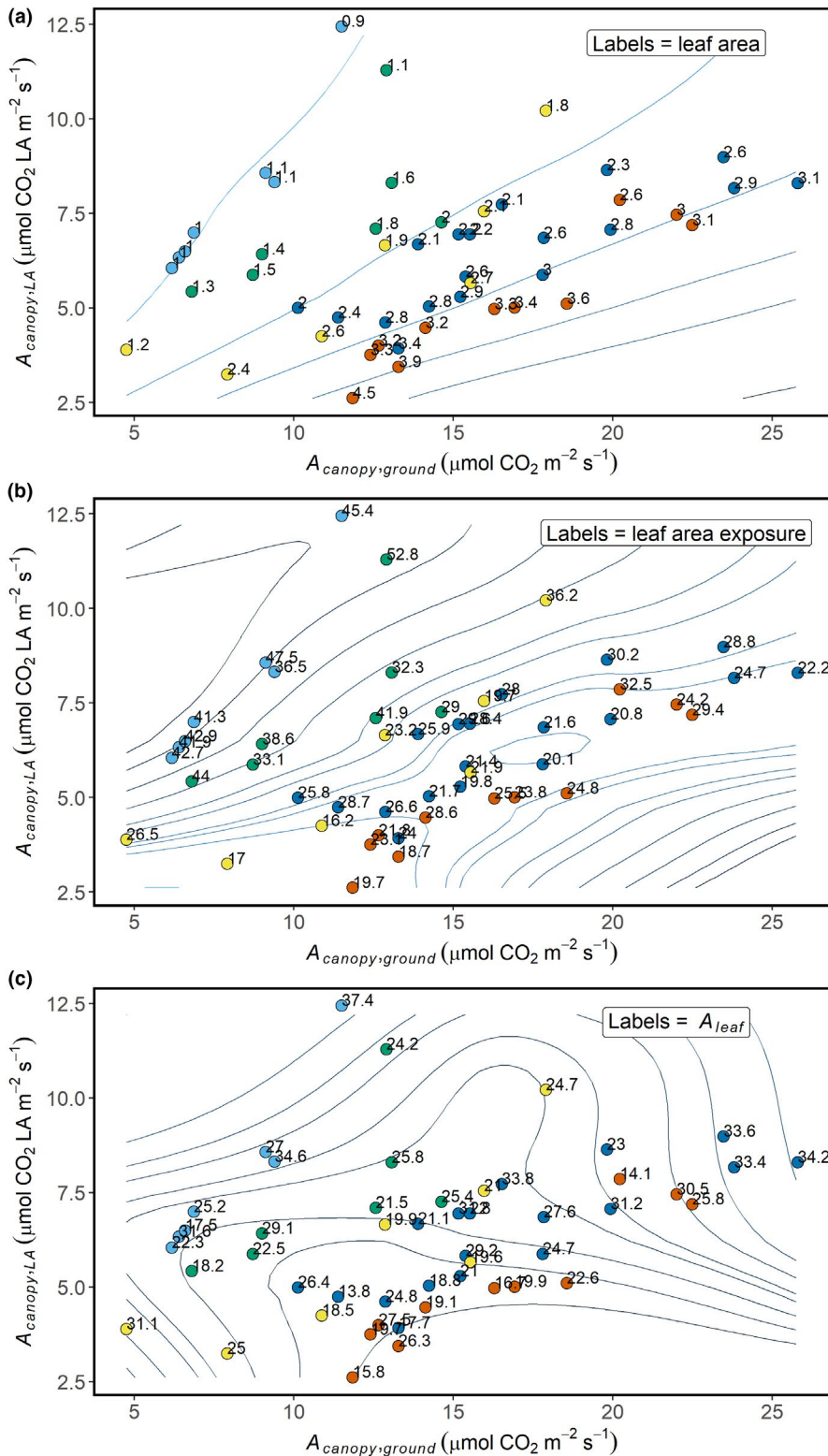
4.2 | Efficiency of canopy photosynthesis

Genotypes with canopies characterized by a high leaf area and/or covering more ground area expressed the highest values for $A_{canopy,ground}$ as they were able to intercept more incident radiation (Table 1, Figure 2, Supporting information 1). Our analysis suggests that light penetration was a limiting factor in canopy photosynthesis, especially for genotypes with high biomass. This finding supports other studies that showed an advantage of erect leaves on canopy carbon assimilation, especially in canopies with high LAI, because erect leaves allowed greater light penetration (Loomis & Williams, 1969;

TABLE 2 Linear regression of A_{canopy} with other traits within each cluster

Clusters	Traits	$A_{canopy,ground}$			$A_{canopy,LA}$		
		Slopes	R^2	*p*-values	**Slopes**	R^2	*p*-values
1&2 (*n* = 14)	$A_{canopy,ground}$	-	-	-	0.39	.31	*
	$A_{canopy,LA}$	0.79	.31	*	-	-	-
	$iWUE_{canopy}$	4.29	.54	**	0.98	.06	ns
	A_{leaf}	0.1	.04	ns	0.2	.32	*
	Leaf area	5.81	.45	**	−1.43	.05	ns
	Canopy width	0.18	.35	*	0	0	ns
1 (*n* = 7)	$A_{canopy,ground}$	-	-	-	1.06	.93	***
	$A_{canopy,LA}$	0.87	.93	***	-	-	-
	g_c	0.93	.65	*	0.96	.56	ns
	Leaf mass	−0.14	.67	*	−0.15	.72	*
2 (*n* = 7)	$A_{canopy,ground}$	-	-	-	0.44	.42	ns
	$A_{canopy,LA}$	0.95	.43	ns	-	-	-
	$iWUE_{canopy}$	4.75	.71	*	2.66	.49	ns
3 (*n* = 7)	$A_{canopy,ground}$	-	-	-	0.44	.73	*
	$A_{canopy,LA}$	1.65	.73	*	-	-	-
	$iWUE_{canopy}$	7.06	.65	*	2.89	.40	ns
	Canopy width	0.34	.65	*	0.15	.45	ns
4 (*n* = 18)	$A_{canopy,ground}$	-	-	-	0.29	.69	***
	$A_{canopy,LA}$	2.37	.69	***	-	-	-
	g_c	1.51	.54	**	0.38	.26	*
	A_{leaf}	0.47	.46	**	0.17	.49	**
	g_s	11.05	.64	***	3.34	.48	**
	$iWUE_{leaf}$	−0.12	.48	**	−0.03	.31	*
	Leaf length	−3.49	.22	*	−0.65	.06	ns
	Stem angle	0.13	.11	ns	0.08	.30	*
	Nodes	1.37	.27	*	0.38	.17	ns
	Shoot mass	0.11	.30	*	0.02	.08	ns
	Total biomass	0.06	.26	*	0.01	.04	ns
5 (*n* = 11)	$A_{canopy,ground}$	-	-	-	0.42	.89	***
	$A_{canopy,LA}$	2.14	.89	***	-	-	-
	$iWUE_{canopy}$	5.78	.72	**	2.25	.55	*
	SPAD	−0.41	.51	*	−0.18	.49	*
	Leaf area	−4.40	.33	ns	−2.63	.61	**
	Leaf area exposure	0.61	.42	*	0.32	.60	**

Note: Only traits with a *p*-value < .05 for the linear regression are shown. ns, *, **, and *** indicate a > 0.05, <0.05, <0.01, and < 0.001 *p*-value for the linear regression. Additional reported values are slope and coefficient of determination (R^2). In the Figures, the clusters are represented by different colors with clusters 1, 2, 3, 4, and 5 shown in light blue, green, yellow, dark blue, and red.

Pepper, Pearce, & Mock, 1977; Sinclair & Muchow, 1999). Thus, traits favoring the exposure of greater leaf area to ir-radiance (i.e., a wider canopy relative to its total leaf area

and lighter green leaves) may contribute to improving over-all canopy CO_2 assimilation. Greater canopy photosynthetic activity with improved light distribution in canopies has been
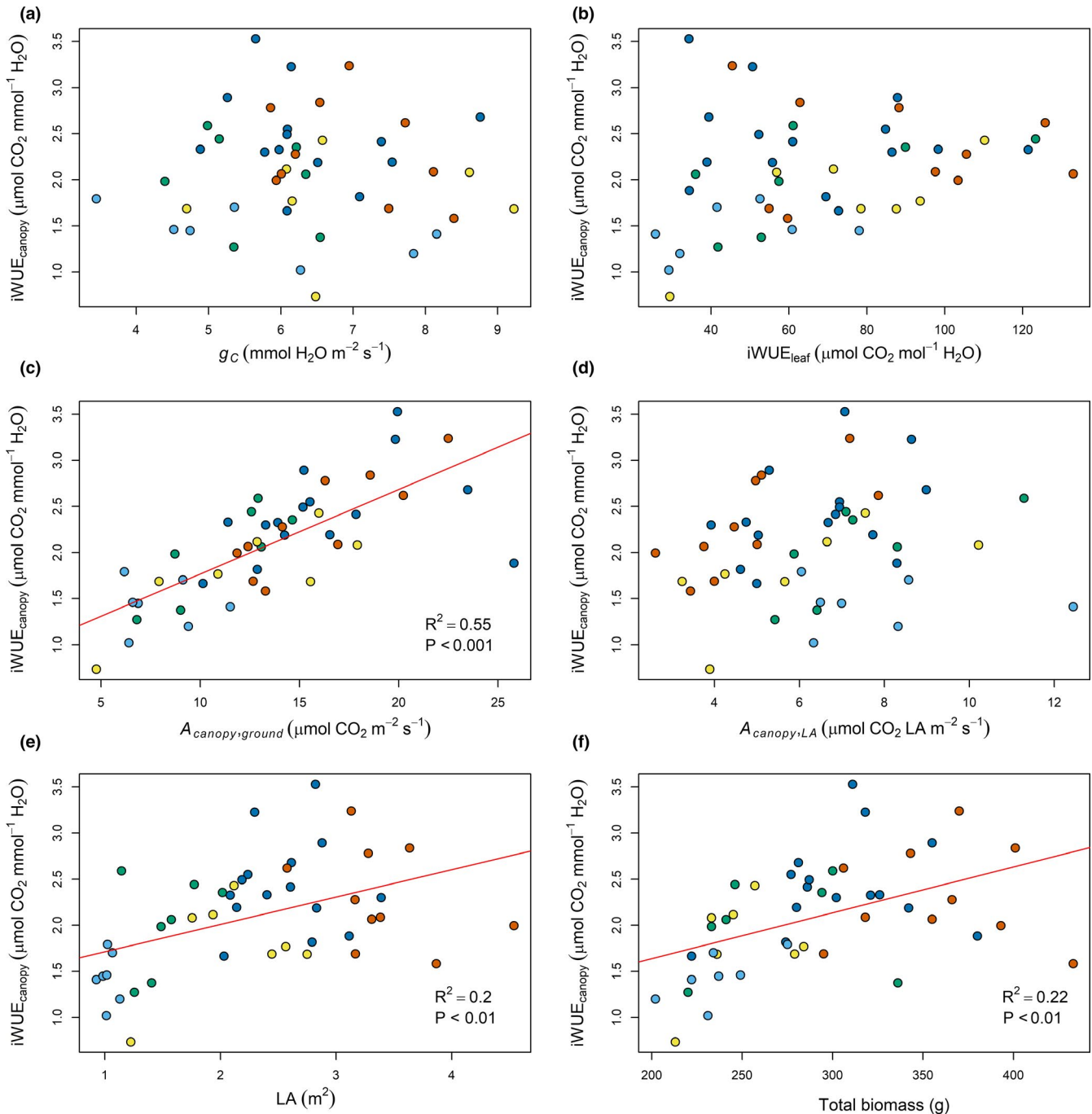
FIGURE 8 Linear regression of canopy intrinsic water-use efficiency ($iWUE_{canopy}$) with varied traits. Genotypes are colored based on clustering (Figure 5) with clusters 1, 2, 3, 4, and 5 shown in light blue, green, yellow, dark blue, and red

previously reported by several studies (Burgess, Retkute, Herman, & Murchie, 2017; Li et al., 2014; Song, Zhang, & Zhu, 2013), but has not been studied in cowpea. Better light penetration within the canopy may also contribute to delayed senescence of leaves located in the lowest layer of the canopy (Liebsch & Keech, 2016) contributing to the maintenance of leaf area later during the season eventually leading to higher yield (Koester, Skoneczka, Cary, Diers, & Ainsworth, 2014; Liu et al., 2015).

We found that canopies with significantly lower LA (e.g., cluster 4 versus cluster 5; Table 1) had similar canopy CO_2 assimilation to canopies with greater leaf area, probably because the canopy leaf area was more efficiently used (Table 1, Figure 7) with less self-shading. Lower leaf area canopies may also have lower respiratory activity compared to higher biomass canopies, as maintenance respiration cost is linearly dependent on plant biomass (Amthor, 2000; Ryan, 1991). Similar results were found in situ for soybean where a 4% reduction in
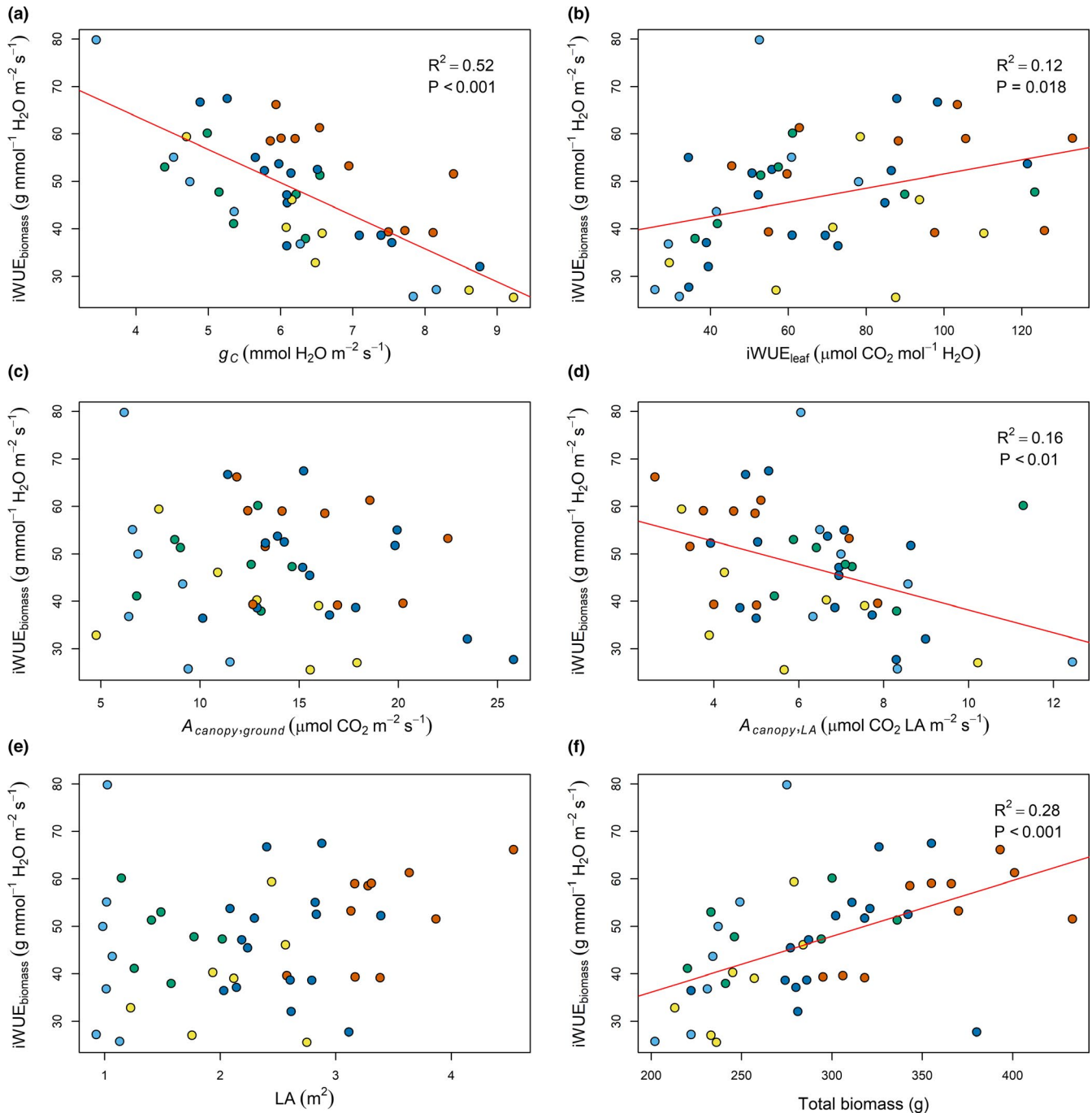
FIGURE 9 Linear regression of canopy biomass-based intrinsic water-use efficiency ($iWUE_{biomass}$) with varied traits. Genotypes are colored based on clustering (Figure 5) with clusters 1, 2, 3, 4, and 5 shown in light blue, green, yellow, dark blue, and red

leaf area by excision did not lead to a decline in CO_2 assimilation, presumably due to a reduction in canopy respiration and self-shading (Srinivasan et al., 2016). In maize, the removal of two leaves above the ear leaf led to an increase of canopy photosynthesis up to 14% (Liu et al., 2015). Both studies saw an increase in seed yield in response to the reduction in leaf area. The lowest leaf area index (LAI) for which the maximum net primary productivity and yield were achieved in soybean was 4.0 (Srinivasan et al., 2016), while in maize reported LAI

values for maximum yield ranged from 2.6 to 6.9 at maturity (Liu et al., 2017). In our study, cowpea CO_2 assimilation started to plateau in cluster 4 when an increase in LA did not result in greater $A_{canopy,ground}$. The optimum LAI value for cowpea for net canopy photosynthesis in our study was 4.19 (Figure 6). Additional studies are needed assess if this range also translates into maximum bean yield.

Light green leaves were associated with greater canopy photosynthesis (Supporting information 2), and leaf greenness
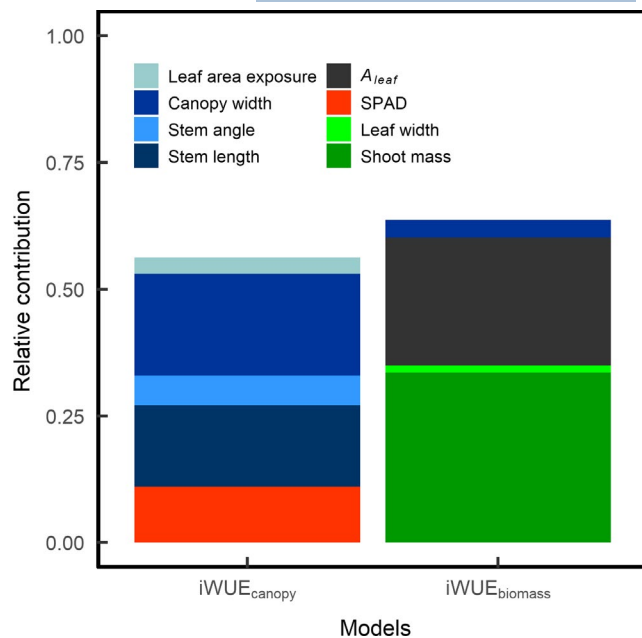
FIGURE 10 Relative contribution of the different parameters used in the minimum adequate models to explain the variation in (left) canopy intrinsic water-use efficiency and (right) biomass-based intrinsic water-use efficiency. Parameters in green are related to the canopy biomass. Parameters in blue are related to the canopy architecture

explained about 50% of the variation in $A_{\text{canopy,ground}}$ and $A_{\text{canopy,LA}}$ in genotypes in cluster 5, which were characterized by high LA (Table 2). Using published relationships between SPAD values and chlorophyll content for coffee plants (Torres Netto, Campostrini, De Oliveira, & Bressan-Smith, 2005), citrus cultivars (Jifon, Syvertsen, & Whaley, 2005), soybeans (Markwell, Osterman, & Mitchell, 1995), *Castanopsis carlessi* (Wang et al., 2009), *Spathiphyllum* Schott (Wang, Chen, & Li, 2004), and cowpeas (Murillo-Amador et al., 2004), the reduction in leaf greenness associated with the increase in canopy photosynthesis observed in the cluster 5 could have represented a $30 \pm 7\%$ reduction in leaf chlorophyll content ($\mu mol/m^2$). The resulting enhanced canopy photosynthesis could be explained by increased transmission of light to the lower canopy, due to a reduced leaf absorbance. This hypothesis is supported by a previous modeling study in rice that predicted a moderate increase in canopy photosynthesis in response to a reduction in canopy chlorophyll content (Song, Wang, Qu, Ort, & Zhu, 2017). A modeling study with soybean, however, did not show an increase in canopy photosynthesis in response to a reduction in chlorophyll content (Walker et al., 2017). Instead, canopy photosynthesis remained constant after a ~ 50% reduction in chlorophyll content (depending on the assumptions made by the model), and only after greater reductions in chlorophyll was photosynthesis reduced. The decline in canopy CO_2 assimilation was explained by an increase in leaf reflectance leading to a reduction in light absorbance by the canopy. This

contrasting result may be caused by different reasons. One possible explanation could be the 100–500 μmol/m^2 range of chlorophyll content assumed in soybean study (Walker et al., 2017). Based on the relationship performed by Murillo-Amador et al. (2004) in 60 cowpea cultivars, the range of chlorophyll content observed in the cluster 5 is estimated at 537–656 μmol/m^2, which is within the range of values commonly reported for cowpea (Jemo et al., 2017; Singh & Raja Reddy, 2011). The soybean study also assumed that chlorophyll declined from the top to the bottom of the canopy (Drewry et al., 2010; Walker et al., 2017), which may not be representative of field observations (Ciganda, Gitelson, & Schepers, 2008; Kong et al., 2017; Winterhalter, Mistele, & Schmidhalter, 2012). Our results complement previous studies by emphasizing that, in canopies of high leaf area and high chlorophyll content, canopy photosynthesis can benefit from a reduction in chlorophyll content. Further studies are needed to clarify the range of reduction in chlorophyll that can lead to an increase in canopy photosynthesis as well as how leaf area can affect the canopy response.

4.3 | Cowpea water-use efficiency

A cowpea MAGIC population was developed by inter-crossing lines that were able to produce high yields under drought conditions (Huynh et al., 2018). While there was no significant linear relationship between iWUE$_{\text{canopy}}$ and g_c, we found a strong positive linear relationship between iWUE$_{\text{canopy}}$ and A_{canopy} (Figure 8). Different adaptation mechanisms may exist in different cowpea lines in response to drought events. Drought avoidance responses include developing a more efficient root system to maximize water uptake (Munjonji, Ayisi, Boeckx, & Haesaert, 2018) or reducing the leaf area and transpiration surface, which contributes to lower water demand by the canopy (Anyia & Herzog, 2004; Bastos, Nascimento, Silva, Freire Filho, & Gomide, 2011). These strategies allow maintenance of high leaf g_s and transpirational cooling under drought (Munjonji et al., 2018). Drought tolerance in cowpeas can also be achieved by reduction of stomatal conductance, allowing maintenance of a high relative water content in leaves (Anyia & Herzog, 2004; Bertolli, Rapchan, & Souza, 2012). The MAGIC population studied here was developed to assess genes involved in drought response in high-yielding cultivars and consists of wide range of strategies to mitigate against drought stress, including early flowering (Huynh et al., 2018). The latter strategy enables completion of the reproductive cycle before the occurrence of a late drought event. This strategy was prevalent in cowpea lines with low biomass and low node number from clusters 1 and 2 where 100% and 43% of the lines were forming pods at the time of measurement. In contrast, only 14% and 28% of the lines in clusters 3 and 4, and no line in cluster

5 were forming pods at the time of measurement. This shows that early developing lines tended to have lower biomass, and that development may be associated with canopy architecture and photosynthesis. Even though there was no evidence of drought in this field experiment, the strong linear relationship observed between $iWUE_{canopy}$ and A_{canopy} (Figure 8) also suggests that the water-use efficiency of the population is partly explained by its ability to maintain high canopy photosynthesis without proportionally changing canopy conductance. This does not exclude a more predominant role of stomata in $iWUE_{canopy}$ under drought conditions. While leaf iWUE is mainly determined by the interaction between leaf physiology and the environment, plant iWUE is determined by the combination of architectural and physiological characteristics of the crop along with energy exchange at the soil surface (Hatfield & Dold, 2019). Spreading growth habit has been described as a drought-tolerant strategy and contributed to enhanced iWUE by providing better ground coverage, reducing the amount of radiation intercepted by the soil surface and thus, reducing soil evaporation (Sennhenn, Njarui, Maass, & Whitbread, 2017). We also found a positive correlation between the width of the canopy and $iWUE_{canopy}$ (Pearson's correlation coefficient = 0.56, p-value < .001; Supporting information 2), although this was found in the absence of drought stress.

4.4 | Breeding strategies

Kamara et al. (2017) reported that increased cowpea total biomass contributed to greater seed yield, based on the positive correlations between canopy height and fodder yield with seed yield. Positive correlations between canopy height and plant biomass with canopy CO_2 assimilation were found in our study (Pearson's correlation coefficient = 0.48, p-value < .001; Supporting information 2), which may indicate that improvement in canopy photosynthesis may contribute to increased seed yield. Improvement of photosynthesis has been widely proposed as a key target for increasing crop yield (Simkin et al., 2019; Weber & Bar-Even, 2019; Wu et al., 2019). Evidence of the benefit of increased photosynthetic activity for seed yield is supported by experiments in elevated CO_2 for diverse crops (Ainsworth & Long, 2005) and legumes such as soybean (Morgan, Bollero, Nelson, Dohleman, & Long, 2005; Sanz-Sáez et al., 2017). More studies are needed to determine the effectiveness of breeding for high A_{canopy} and its translation to high yield.

Potential yield increases as a result of increased canopy photosynthesis may however be mitigated by drought events in rain-fed agricultural production systems, which is a common practice in sub-Saharan Africa (Dingkuhn, Singh, Clerget, Chantereau, & Sultan, 2006; FAO, 2006; van Ittersum et al., 2016). Indeed, it was pointed that the decline in photosynthetic activity mediated by stomatal closure in response to drought events could be one of the factors responsible for the yield reduction in some cultivars (Munjonji et al., 2018; Rivas et al., 2016; Singh & Raja Reddy, 2011). Therefore, traits for improved CO_2 canopy assimilation must be selected jointly with traits for high canopy water-use efficiency. Breeding programs should also favor drought responses that allow the maintenance of high canopy CO_2 assimilation in order to maximize the benefit of high canopy photosynthesis for seed yield. This is especially crucial as selections for lines with high CO_2 canopy assimilation will also select for lines with higher canopy transpiration (Figure 2a). However, breeding for both drought tolerance and high photosynthetic capacity may be challenging, as Rivas et al. (2016) have observed a trade-off between those two traits. This trade-off may however be partly lifted by introducing more water-use efficient canopy architecture such as phenotypes with a better ground coverage (Sennhenn et al., 2017), which would also benefit the overall canopy CO_2 assimilation by exposing more leaves to solar radiation.

Traits such as canopy width, leaf mass, stem length, and canopy height were more strongly correlated with canopy photosynthesis than leaf-level measurements of photosynthesis (Supporting information 2) and may represent better proxies for selection of lines with high canopy photosynthetic capacity. Those traits also showed a stronger correlation coefficient with $A_{canopy,ground}$ (>0.7, Supporting information 2) than the total biomass (0.48, Supporting information 2). Total biomass alone only explained 28% of the variance observed in $A_{canopy,ground}$ (p-value < .001, Supporting information 7) compared to the 75% explained when several traits were used as predictor in the GLM (Figure 4). This may partly result from limited light penetration in high biomass canopies from the cluster 5. As shown in this study, canopy photosynthesis is a complex trait and is predicted by a combination of traits, including leaf photosynthesis. Targeting phenotypes with a LAI close to 4.2 at the reproductive stage may also allow the selection of canopies with maximum CO_2 assimilation without overinvesting in leaf development or vegetative biomass, which may lessen canopy respiration, transpiration and improve light penetration, thus improving yield (Liu et al., 2017; Srinivasan et al., 2016).

Interestingly, $iWUE_{canopy}$ and $iWUE_{leaf}$ did not show a significant linear relationship with each other (Figure 8) emphasizing that leaf-level measurements are not the best proxy for $iWUE_{canopy}$. This observation may not hold true in a more water-limited environment where $iWUE_{canopy}$ and $iWUE_{leaf}$ could be more tightly correlated. Further experiments would be needed to determine how leaf-level measurements related to the water-use efficiency can be used as proxies for the overall canopy. Selection of lines within this MAGIC population with high canopy photosynthetic

activity will likely contribute to the water-use efficiency of the canopy, due to the strong linear relationship observed between the two traits.

5 | CONCLUSION

Canopy architecture was shown to explain a significant part of the variance observed in canopy photosynthesis. Our analysis suggests that 50 MAGIC genotypes can be grouped into 5 general canopy architectural types. In low biomass canopies, the major limitation to canopy photosynthesis is leaf area. However, in higher biomass canopies, the light environment within the canopy became an increasingly limiting factor for canopy photosynthesis. While the negative effects of self-shading on canopy photosynthesis due to excess leaves have been shown in defoliation experiments (Liu et al., 2015; Srinivasan et al., 2016) and modeling studies (Shuting, 1994), we report a similar response across a cowpea diversity panel. The iWUE$_{canopy}$ in this MAGIC population was mainly explained by canopy photosynthetic activity, and not canopy conductance. Enhancement in canopy photosynthesis is therefore likely to improve the water-use efficiency of lines from this MAGIC population. Our results provide new insights for breeding program improvement and offer a better understanding of how canopy architecture affects photosynthesis and WUE. As the target environment for cowpea production typically faces water limitation, future analysis could explore how drought alters the relationship between canopy architecture, photosynthesis, WUE, and seed yield. Optimizing canopy architecture under different planting densities also deserves further study.

CONFLICT OF INTEREST
None declared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.