The influence of leaf anatomical traits on photosynthetic in coffee leaves in Yunnan of China

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Abstract

Leaf photosynthesis is largely determined by anatomical features. This work aimed to reveal the quantitative effects of *Coffea arabica* leaf anatomical structure on photosynthesis. Pearson's correlation and path analysis was used to explore the correlation between leaf structure and photosynthesis. The comprehensive evaluation value of the correlation between leaf anatomical traits and photosynthetic parameters is calculated by using the Criteria Importance Though Intercrieria Correlation (CRITIC) method to obtain the objective weight of each photosynthetic parameter. Most of the leaf's anatomical traits significantly (P<0.05) to highly significantly (P<0.01) correlated with photosynthetic parameters, suggesting that anatomical traits greatly affected photosynthetic capacity in *Coffea arabica* leaf. Likewise, path coefficient analysis revealed that all the leaf anatomical traits influenced photosynthetic capacity directly and indirectly. The results of the comprehensive evaluation showed that leaf thickness (LT) and stomatal density (d) are the anatomical characteristics most closely related to photosynthesis. These results may help pinpoint anatomical features required to gain higher photosynthesis in coffee leaves.

Keywords: *Coffea arabica* L., leaf anatomy, photosynthesis, correlation and path coefficient, CRITIC method, comprehensive evaluation

Introduction

Coffee (*Coffea arabica*) considered an important tropical crop is one of the most traded agricultural products in the world. Under suboptimal growing conditions, shade trees, which could reduce the stress of coffee by ameliorating adverse climatic conditions, have been recommended (Beer *et al.*, 2001; Franck and Vaast, 2009a). Yunnan province, located in Southwest China, is a predominantly coffee-growing region (ICO, 2015). Although the mountainous landscape and mild climate in this region are well-suited to coffee production, many areas are suboptimal for coffee cultivation (ICO, 2015; Ma *et al.*, 2019). Thus, agroforestry systems have been recommended for these areas, especially at altitudes below 1000m (Ma *et al.*, 2019). However, the physiological trait characteristic of shade-adapted coffee is the lower photosynthetic capacity when compared to C_3 woody plants grown in tropical climates (Ayalew, 2018; DaMatta, 2004). Leaves have evolved as photosynthetic organs in land plants (Oguchi *et al.*, 2018) so the leaf of coffee is the main organ of photosynthesis.

Because leaves are specialized organs that enable plants to intercept light and uptake CO_2 through the stomata for photosynthesis (Lambers and Oliveira, 2019), light and CO_2 availability in the mesophyll of plant leaves largely determine the net carbon

assimilation rate (A) of leaves (Franck and Vaast, 2009b). To analyze the response of photosynthesis to irradiance, the light response curves (LRCs) were used to describe the relationship between photosynthesis and light intensity (Herrmann *et al.*, 2020). It is an important method to elucidate the response mechanism of photosynthesis and evaluate photosynthetic efficiency by fitting of light-response model (Lambers and Oliveira, 2019). Often, LRCs are described mathematically using the empirical model. The operation state of plant photosynthetic apparatus and photosynthetic parameters, such as the information of the maximum photosynthetic capacity (P_{nmax}), intrinsic and apparent quantum yield (IQY and AQY), light compensation and saturation point (LCP and LSP), and dark respiration rate (R_D), can be obtained from the LRCs. In addition, mechanistic information about the key biochemical parameters of carbon assimilation can be estimated by fitting *A/C*i curves with the mechanistic Farquhar-von Caemmerer-Berry (FvCB) biochemical model described by Farquhar et al. (1980) (Farquhar *et al.*, 1980; Sun *et al.*, 2014; Wang *et al.*, 2017). This model has been widely used to simulate CO_2 assimilation and applied for decades (Farquhar *et al.*, 1980; GU *et al.*, 2010). It elegantly explains the physiological properties of photosynthesis of intact leaves (Lambers and Oliveira, 2019), and the parameters reflecting biochemical properties of leaves were easier to estimate from this model with gas exchange measurements (Wang *et al.*, 2017).

The structural organization of plant leaves could facilitate or sometimes constrain the photochemical reactions of the photosynthetic process (Boom *et al.*; Oguchi *et al.*, 2018). Therefore, the leave structural changes may affect photosynthetic efficiency (Akhkha *et al.*, 2001; Herrmann *et al.*, 2020). However, although a variety of ecological studies have revealed a correlation between leaf structural parameters and photosynthetic performance (Bolhar-Nordenkampf and Draxler, 1993; James and Bell, 2001; Vogelman *et al.*, 1996), there were contradictory and not universal differences between foliage structural and functional variables in the previous work (Niinemets, 1999). To understand the effects of leaf structure on each photosynthetic performance parameter, it is necessary to analyze the relationship between leaf structure and leaf photosynthesis and comprehensively evaluate the importance of each component in leaf photosynthesis. Because the evaluation of the relationship between leaf structure and leaf photosynthesis could provide ecophysiological evidence for understanding the structural properties of leaves how to influence photosynthetic performance. Even though a number of studies analyze the effects of leaf structure on photosynthetic performance (Bolhar-Nordenkampf and Draxler, 1993; Oguchi *et al.*, 2018), there are few works synthetically analyzing the correlation between leaf structural parameters and photosynthetic performance (Boom *et al.*). In particular, a few works were related to coffee. Therefore, the aims of this study were to explore the relationship between leaf structural parameters and photosynthetic performance, clarify the importance of the correlation between leaf structural parameters and photosynthetic performance by using a comprehensive evaluation method. Gaining these results may provide useful information for future coffee breeding and plantation management.

2 Materials and methods

2.1 Study site characterization and Plant material

The experiment was conducted in the shade coffee plantations of Dehong Tropical Agriculture Research Institute of Yunnan (DTARI), Ruili, southwest China. The plantations are located at 890 meters of altitude, at 24.025 south latitude and 97.855 west longitude, with an average temperature of 22.2 ℃ and precipitation of 1260 mm in the studies conducted years (2017-2018). The soil in the plantation is characterized as acidic lateritic red soil.

The coffee trees of the cultivar are 'Catimor', a hybrid derived from the cross between two cultivars: Caturra (*Coffea arabica* L.) and Timor (*C. arabica* × *C. canephora* Pierre ex Froehner). The coffee trees were approximately five years old and spaced 1×2 m. Shade trees (*Dimocarpus longan* Lour.) planted at 12×4 m, East to West rows-oriented.

A completely randomized design was the following: six coffee plants per treatment combination and used as replicates. All the sampling and measurement leaves used the third or fourth completely expanded leaf pair from the apex of the plagiotropic (lateral) branches located in the upper third of the plant. Nine leaves in different directions on each tree were selected to measure the chlorophyll content values and leaf gas exchange parameters.

2.2 Gas exchange measurements

The leaf gas exchange parameters (net CO_2 assimilation rate (A), stomatal conductance to water vapor (g_s), transpiration rate (E), internal CO_2 concentration (C_i), vapor pressure deficit (VPD), and leaf water use efficiency (WUE=A.E^{-1})) and environmental indicators(leaf temperature (TL), relative humidity (RH) and ambient temperature (TA)) were determined simultaneously by using a portable photosynthesis system CIRAS 3 (PP Systems, USA). Leaf water use efficiency (WUE), representing the units assimilated CO_2 per unit water lost through transpiration, is following the user manual version 1.06. All measurements were taken on sunny days from 09:00 to 11:30 and 15:00 to 18:00. The reference CO2 concentration was set at 390 ppm using a buffer bottle, the temperature at 27 ◦C, flow rate at 300 μmol.s^{-1}, relative humidity at 50 ± 10%, and irradiance at 800 μmol m^{-2}.s^{-1}. The leaf of photosynthetic light response (A/PPFD) and internal CO_2 response (A/C_i) curves were constructed by using leaf gas exchange. To collect data for building A/PPFD curves, net photosynthesis was measured at 0, 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800, 1000, and 1200 μmol m^{-2}.s^{-1} (PAR) with a cuvette environment that maintained leaf temperature at 27 ℃, reference CO2 concentration at 400 ppm. After the leaf tissue was clamped in the leaf chamber, the gas exchange rates usually stabilized within approximately 3–4 min. Data to construct A/C_i curves were collected from the same sample leaves immediately following A/PPFD measurements. P_n was measured at CO_2 concentrations of 400, 300, 200, 100, 150, 250, 350, 450, 550, 650, 750, 850, 950, 1050, 1250, 1450 and 1650 μmol.m^{-2}.s^{-1} at photosynthesis saturating irradiance 800 μmol m^{-2}.s^{-1}, which light intensity is sufficiently high to saturate the photosynthetic machinery without causing photoinhibition. A total of 41 A/PPFD and 42 A/C_i curves were generated, respectively.

2.3 Measurement of leaf anatomical traits

Following gas exchange measurement, five sample leaves of each plant were

harvested, sealed in polyethylene bags, and immediately brought back to the laboratory. Two segments (3 mm × 5 mm) were cut from each leaf halfway between the leaf apex and base. Then, the material was dehydrated on sucrose solutions, followed by the inclusion of Gum Arabic aqueous solution. Leaf transverse sections (7–10 μm) thickness cut using a rotary microtome (Leica CM3050S, Germany) and stained with toluidine blue. Surface sections of the leaves were taken manually and stained with safranine aqueous solution (0.1%). For each leaf, five transversal sections and surface sections were selected systematically to measure the leaf thickness (LT), upper cuticle thickness (UCT), upper epidermal thickness (UET), palisade tissue thickness (PT), spongy tissue thickness (ST), lower epidermal thickness (LET), lower cuticle thickness (LCT), stomatal density (*d*), and guard cell length (*l*) and width (*w*), using a microscope binocular with 10 × and 40 × objectives (Leica DM5000 B). Subsequently, five photographs of microscope fields per section were taken with a photomicroscope equipped with a Leica DFC500 camera (Leica Microsystems, Rijswijk, The Netherlands). Images were analyzed with Leica LAS X software.

2.4 Calculations and statistical analysis

A/PPFD curves were fitted by applying a modified rectangular hyperbola model using Photosynthetic Calculation Software 4.1.1(Ye, 2007; Ye, 2010; Ye and Yu, 2008). The equation of the modified rectangular hyperbola model is described by:

$$P_{\mathrm{n}}(I) = \alpha\frac{1-\beta I}{1+\gamma I}I - R_{\mathrm{d}} \qquad (1)$$

where P_n is the net photosynthetic rate (μmol·CO_2 m^{-2} s^{-1}), I is the photosynthetic photon flux density (μmol·m^{-2} s^{-1}), I_c is compensation irradiance, β and γ are the coefficients which are independent of I, and α is the absolute value of slope between $I = 0$ and $I = I_c$.

For $I = 0$, the quantum yield at this point is defined as intrinsic quantum yield (IQY), which is given by:

$$\mathrm{IQY} = \alpha(1 + (\gamma + \beta)I_{\mathrm{c}}) \qquad (2)$$

The apparent quantum yield (AQY) ignored the Kok effect is obtained by:

$$\mathrm{AQY} = \alpha\frac{1+(\gamma-\beta)I_{\mathrm{c}}-\gamma\beta I_{\mathrm{c}}^2}{(1+\gamma I_{\mathrm{c}})^2} \qquad (3)$$

For $I = 0$, the rate of dark respiration (R_d) is:

$$R_{\mathrm{d}} = -P_{\mathrm{n}}(I_0) = -\alpha I_{\mathrm{c}} \qquad (4)$$

The saturation irradiance I_{sat} is obtained by:

$$I_{\mathrm{sat}} = \frac{\sqrt{(\beta+\gamma)/\beta}-1}{\gamma} \qquad (5)$$

The maximum photosynthetic rate P_{nmax} is given by:

$$P_{\mathrm{nmax}} = \alpha\left(\frac{\sqrt{\beta+\gamma}-\sqrt{\beta}}{\gamma}\right)^2 - R_{\mathrm{d}} \qquad (6)$$

A/Ci curves were fit by uploading the datasets to the website (http://Leafweb.ornl.gov) to estimate the parameters. In this study, A/C_i curves were at saturating light levels. Thus, the rate of electron transport (*J*) was to assume the maximum rate of electron

transport (J_{max}).

The total stomach pore area (TSP) was calculated as $d \times 1/4\pi \times l \times w$ (7), and by assuming that the stomatal pore was an ellipse(Batos *et al.*, 2010; James and Bell, 2001; Patricia *et al.*, 2014). Where d is the stomatal frequency calculated by dividing the stomatal count by the area of the field of view, l is the guard cell length, and w is the guard cell width.

The relationships of photosynthetic capacity with leaf anatomical traits were analyzed by Pearson's correlation. The possible direct and indirect effects of each leaf anatomical trait on the photosynthetic parameter were further identified by path analysis(Zhang *et al.*, 2005). For path analysis, direct and indirect effects were obtained from partial least squares (PLS) regression of leaf anatomical traits on photosynthetic parameter and Pearson's correlations between leaf anatomical traits(Guohua *et al.*, 2017). To combine effect leaf anatomical traits on photosynthetic parameters, a multiple criteria decision analysis (MCDA) has been utilized in this study. The objective weight of each photosynthetic parameter (w_j) is obtained by using the Criteria Importance Though Intercrieria Correlation (CRITIC) method, which was a correlation method using a standard deviation of column and correlation coefficients of all paired columns(Diakoulaki *et al.*, 1995). The comprehensive evaluation of the effects of leaf anatomical traits on photosynthetic parameters was calculated by using the following equation: $Q_i = \sum_{j=i}^{n}(w_j \times r_{ij})$(8), where r_{ij} was the correlation coefficient between leaf anatomical trait i and photosynthetic parameter j. All statistical analyses were conducted with IBM SPSS Statistics 24.

Results

Results showed the photosynthetic light-response curve (Fig.1) fits very well with the modified rectangular hyperbola model (R^2=0.945±0.006) described by Ye (2007) (Ye, 2007). The P_n increased with I below the I_{sat}. When I was above I_{sat}, the P_n decreased as I increased, which means the photoinhibition phenomenon. The calculated AQY would be 0.028±0.003 if the Kok effect were ignored. This value was lower than α (0.031±0.003) and the IQY (0.033±0.003). The maximum P_n values calculated by modified rectangular hyperbola model at I_{sat} of 621.762±37.358 μmol(photon) $m^{-2}s^{-1}$ was 5.131±0.478 μmol(CO_2)$m^{-2}s^{-1}$ for coffee leaf. The values of I_c and R_D were 60.238±11.332μmol(photon)$m^{-2}s^{-1}$ and 0.930±0.098μmol(CO_2)$m^{-2}s^{-1}$, respectively.

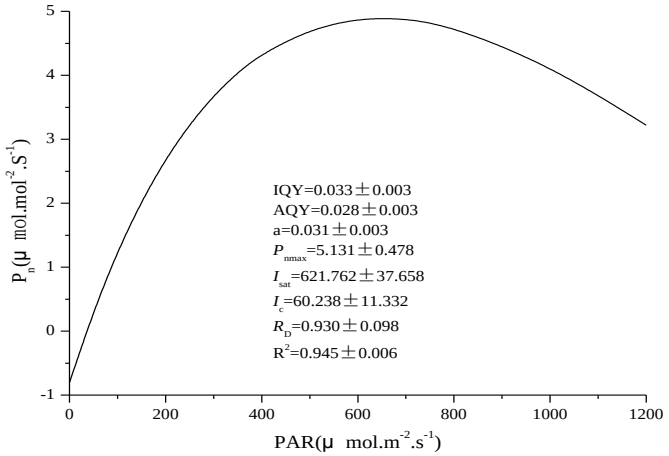


Fig.1 Photosynthetic light-response curve of coffee plants. Data shown in the figure indicate mean ± stand error. IQY–intrinsic quantum yield; AQY–apparent quantum yield; α–the absolute value of slope between $I = 0$ and $I = I_c$; P_{nmax}–maximum photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]; I_{sat} – light saturation point [μmol (photon) m^{-2} s^{-1}]; I_c –light compensation point [μmol (photon) m^{-2} s^{-1}]; R_D– dark respiration [μmol(CO_2) m^{-2} s^{-1}].

Based on the FvCB model structure, the A/C_i curve was fitted with the interactive website (http://www.leafweb.ornl.gov)(Gu and Sun, 2014). Figure 2 shows the response of A to C_i for coffee leaves. The parameters of values for V_{cmax}, J_{max}, T_p, R_d, Γ*, g_i, and K_{co} were 61.749±5.394, 80.721±17.963, 5.316±0.576, 3.225±0.441, 3.309±0.333, 1.101±0.287, 103.096±11.721, respectively. The transition between the Rubisco- and RuBP regeneration-limited states (C_{i_CJ}) occurs around 30 Pa intercellular CO_2 and the TPU-limited state (C_{i_JP}) is around 90 Pa (Fig 2.).

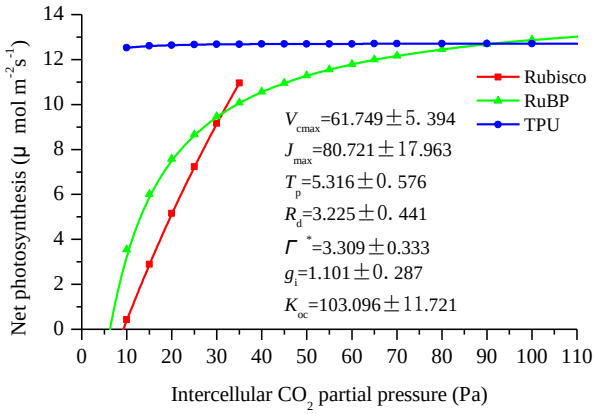


Fig 2. A/C_i curves for coffee plants. Data shown in the figure indicate mean ± stand error. C_i–Intercellular CO_2 concentration (μmol mol^{-1}); V_{cmax}–Maximal Rubisco carboxylation rate (μmol m^{-2} s^{-1}); J_{max}–Maximal electron transport rate (μmol m^{-2} s^{-1}); T_p–Rate of triose phosphate export from the chloroplast (μmol m^{-2} s^{-1}); R_d–Day respiration (μmol m^{-2} s^{-1}); Γ*–CO_2 compensation point in the absence of dark respiration (Pa); g_i– Internal (mesophyll) conductance to CO_2 transport (μmol m^{-2} s^{-1} Pa^{-1}); K_{oc} –A composite parameter (Pa): $K_{oc} = K_c\left(1 + {}^{O}/_{K_o}\right)$.

Interrelationships with correlation analysis between photosynthetic capacity and leaf anatomical traits were presented in Table 1. There were many significant differences between photosynthetic capacity and leaf anatomical traits. The UCT

correlated negatively with IQY, AQY, α, I_{sat}, and g_i ($p<0.05$). The UET appeared to be positively related to AQY, α, and P_{max} ($p<0.05$), and negatively related to I_c, R_d, C_i, and VPD ($p<0.05$) (Table 1). The relationship between PT and IQY, AQY and αwas significantly negative ($p<0.05$). The LET had a significantly negative effect on the I_c was significantly negative ($p<0.05$). The LCT was important to I_{sat}, and the Pearson correlation coefficient was significantly negative ($p<0.05$). The guard cell width (w) significantly affected I_c and R_d. The guard cell length (l) correlated negatively with IQY, AQY, α, and I_{sat}, and positively with J_{max}. A strongly positive ($p<0.01$) correlation relationship between stomatal density (d) and J_{max} was found in this study. Based on Pearson correlation analysis, however, LT and ST had relatively little effect on the photosynthetic capacity.

Path analysis was performed to reveal the direct and indirect effects of leaf anatomical traits on photosynthetic capacity. The results indicating the effects were shown in Fig 3. Although correlation analysis suggested that there were no significant correlations between LT and photosynthetic capacity variables ($p>0.05$), its direct effects on IQY (0.488), α (0.429), R_d (0.390), and AQY(0.381) were relatively high. In addition, there were high negative direct effects of LT on I_{sat} (-0.431) and Γ* (-0.379). The negative direct effect of UCT on IQY (-0.756), α (-0.720), and AQY (-0.679) was found to be higher than the other photosynthetic capacity variables. UET has a high negative direct effect on R_d (-0.785), TPU (-0.520), and a high positive direct effect on E (0.563), g_s (0.451), and P_{max} (0.448). PT exhibited an impressive negative direct effect on TPU (-0.518), AQY(-0.410), α (-0.410), and IQY (-0.401), while a high positive direct effect was shown between PT and E (0.406). The leaves of ST presented a high positive direct effect on I_{sat} (0.583) and WUE (0.578). In addition, a similar high negative direct effect was also observed between ST and VPD (-0.588). LET presented high positive direct effects on WUE (0.514), V_{cmax} (0.483), and TPU (0.391). However, a relatively high negative direct effect was observed between LET and R_D (-0.362), VPD (-0.359), and C_i (-0.321). There was a direct effect with a relatively high negative magnitude of LCT on V_{cmax} (-0.478), I_c (-0.416), J_{max} (-0.371), and VPD (-0.342), while the LCT has a higher positive direct effect on the Γ* (0.335) than photosynthetic capacity variables. The magnitude of negative direct effect guard cell width (w) on I_{sat} (-0.461) was observed. Also, a large relatively negative direct effect of guard cell length (l) on I_{sat} (-0.422) and g_i (-0.364) was found. However, l also observed high positive direct effects on C_i (0.369). The stomatal density (d) exhibited large negative direct effects on g_i (-0.379) and high positive direct effects on J_{max} (0.350) was found. The direct effect of TSP on the photosynthetic capacity variable was low, and the value range was from -0.179 to 0.185.

Table 1. The correlation between leaf anatomical traits and photosynthetic parameter

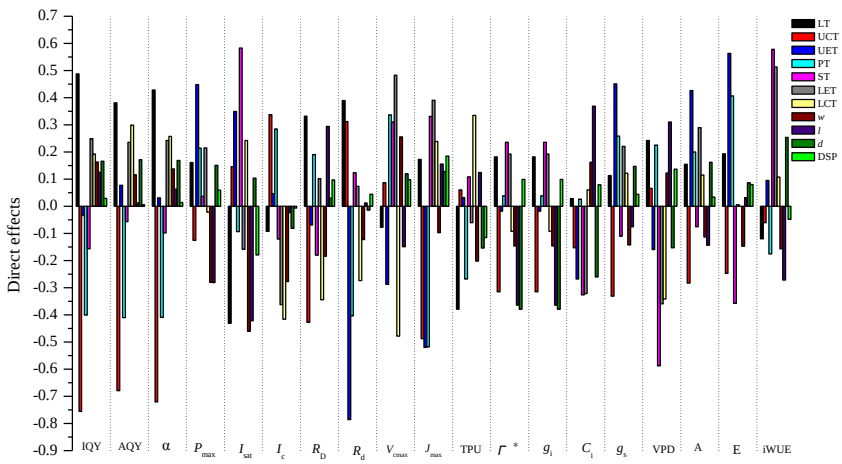
Parameters	LT	UCT	UET	PT	ST	LET	LCT	*w*	*l*	*d*	TSP
IQY	-0.044 ns	-0.336*	0.296 ns	-0.343*	-0.010 ns	0.000 ns	-0.232 ns	0.175 ns	-0.315*	0.232 ns	0.103 ns
AQY	-0.024	-0.322	0.386*	-0.394	0.012	0.039	-0.224	0.197	-0.358	0.206	0.068

	ns	*		*	ns	ns	ns	ns	*	ns	ns
α	-0.033	-0.332	0.351*	-0.375	0.005	0.022	-0.230	0.189	-0.342	0.217	0.082
	ns	*		*	ns	ns	ns	ns	*	ns	ns
P_{max}	0.178	-0.108	0.324*	-0.036	0.103	0.100	-0.065	-0.037	-0.233	0.143	-0.003
	ns	ns		ns	ns	ns	ns	ns	ns	ns	ns
I_{sat}	-0.057	-0.359	0.157	-0.278	0.189	-0.202	-0.377	-0.227	-0.460	0.031	-0.313
	ns	*	ns	ns	ns	ns	*	ns	**	ns	*
I_c	-0.064	-0.143	-0.324	0.164	-0.008	-0.336	-0.174	-0.323	-0.071	0.017	-0.150
	ns	ns	*	ns	ns	*	ns	*		ns	ns
R_D	0.025	-0.234	-0.195	0.204	0.057	-0.190	-0.150	-0.243	-0.005	0.156	0.036
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R_d	0.097	0.161	-0.477	0.101	0.003	-0.129	0.071	-0.354	0.133	-0.043	-0.114
	ns	ns	**	ns	ns	ns	ns	*	ns	ns	ns
V_{cmax}	-0.021	-0.097	0.009	0.106	0.188	0.019	-0.161	0.172	-0.003	0.139	0.200
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
J_{max}	-0.086	-0.169	-0.104	-0.047	-0.035	-0.188	-0.183	-0.003	-0.212	0.403*	0.246
	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
TPU	-0.033	-0.145	-0.209	0.012	0.045	-0.008	0.015	-0.162	0.045	0.245	0.183
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Γ^*	-0.210	0.181	-0.086	-0.159	-0.239	0.228	0.119	-0.163	0.104	-0.285	-0.276
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
g_i	0.207	-0.433	0.311	-0.092	0.371	-0.071	-0.420	-0.006	-0.301	-0.149	-0.301
	ns	*	ns	ns	ns	ns	*	ns	ns	ns	ns
C_i	-0.066	0.108	-0.396	0.257	-0.125	-0.164	0.187	-0.053	0.311*	-0.080	0.077
	ns	ns	**	ns	ns	ns	ns	ns		ns	ns
g_s	0.140	-0.111	0.278	0.073	0.044	0.146	0.053	0.005	-0.085	0.174	0.116
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
VPD	-0.023	0.071	-0.372	0.281	-0.143	-0.230	0.106	-0.063	0.221	0.052	0.148
	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
A	0.157	-0.079	0.346*	0.007	0.049	0.203	0.047	0.048	-0.117	0.149	0.094
	ns	ns		ns	ns	ns	ns	ns	ns	ns	ns
E	0.196	-0.057	0.224	0.191	0.031	0.068	0.106	0.002	0.003	0.164	0.158
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
WUE	0.049	-0.115	0.350*	-0.230	0.184	0.226	-0.142	0.041	-0.216	0.074	-0.038
	ns	ns		ns	ns	ns	ns	ns	ns	ns	ns

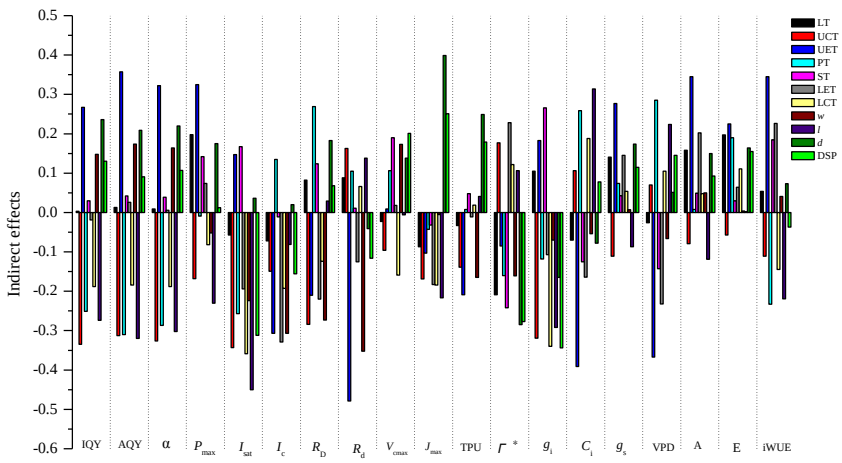
LT–leaf thickness, UCT–upper cuticle thickness, UET–upper epidermal thickness, PT–palisade tissue thickness, ST–spongy tissue thickness, LET–lower epidermal thickness, LCT–lower cuticle thickness, *w*–guard cell width, *l*–guard cell length, *d*–stomatal density, TSP–total stomach pore area. Ns = non-significant. *, ** significant significance difference at 5 % and 1 % probability levels, respectively.

The estimates of each indirect effect of leaf anatomical trait on photosynthetic capacity via other leaf anatomical traits were presented in Fig 3(b). According to this figure, the values of the indirect effect of LT and ST on photosynthetic capacity variables through other anatomical traits were lower than 0.300. UCT revealed a

negative indirect effect on IQY (-0.335), I_{sat} (-0.343), α (-0.326), g_i (-0.319), and AQY (-0.313) through other anatomical traits. UET indirectly exerted relatively high positive effects on AQY (0.357), A (0.345), P_{max} (0.325), and α (0.322) via other anatomical traits. Conversely, UET exhibited relatively high negative indirect effects on R_d (-0.479) and VPD (-0.367) via other anatomical traits. The indirect effects of PT on g_i via other anatomical traits were negative and high (-0.329). There were indirect effects with a high negative magnitude of LCT on I_{sat} and g_i via other anatomical traits(-0.359 and -0.340, respectively). The R_d and I_c were influenced indirectly with a relatively high negative magnitude by *w* via other anatomical traits (-0.352 and -0.307, respectively). There was a relatively high positive indirect effect of *l* on C_i (0.314) and negative on I_{sat} (-0.450), AQY (-0.320) and α (-0.302) through other anatomical traits. In addition, a highly positive indirect effect of *d* on J_{max} (0.399) was observed. The TSP presented a relatively high negative indirect effect on I_{sat} (-0.312) and g_i (-0.344) through other anatomical traits.



(a)



(b)

Fig 3. Direct (a) and indirect (b) effects of each leaf anatomical traits through other traits on photosynthetic capacity.

To identify the combined effect of leaf anatomical traits on photosynthetic parameters, a comprehensive evaluation was analyzed. Based on the evaluation values (Q_i), the ranking of the comprehensive scores of relationships between photosynthetic capacity and leaf anatomical traits was shown in Table 2. The highest comprehensive score of direct effect was obtained by the LT followed by LET, *d*, TSP, ST, *L*, LCT, UET, PT, *W*, and UCT. For the comprehensive score of indirect effect on of leaf anatomical traits photosynthetic capacity, the *d* held the highest comprehensive score, and ST was the second followed by LT, UET, TSP, PT, LET, *W*, LCT, *L*, and UCT. Similarly, for Pearson's correlation of comprehensive evaluation analysis, the results showed that the first comprehensive score was *d*, and the second was also ST. Then, the ranking order after the third was UET, LT, TSP, LET *W*, PT, LCT, *L*, and UCT.

Table 2. Comprehensive ordering of the effects of leaf anatomical traits on photosynthetic capacity with CRITIC method.

Anatomical traits	Direct effects		Indirect effects		Pearson's correlation	
	Comprehensive score	Ranking	Comprehensive score	Ranking	Comprehensive score	Ranking
LT	0.123	1	0.009	3	0.007	4
UCT	-0.168	11	-0.125	10	-0.137	11
UET	-0.034	8	0.002	4	0.027	3
PT	-0.056	9	-0.021	5	-0.049	8
ST	0.022	5	0.032	2	0.033	2
LET	0.098	2	-0.049	6	-0.042	6
LCT	-0.020	7	-0.079	8	-0.102	9
W	-0.062	10	-0.060	7	-0.045	7
L	-0.008	6	-0.085	9	-0.104	10
d	0.048	3	0.081	1	0.076	1
TSP	0.039	4	0.002	4	-0.007	5

LT–leaf thickness, UCT–upper cuticle thickness, UET–upper epidermal thickness, LET–lower epidermal thickness, PT–palisade tissue thickness, ST–spongy tissue thickness, LCT–lower cuticle thickness, *w*–guard cell width, *l*–guard cell length, *d* –stomatal density, TSP–total stomach pore area.

Discussion

Several studies involving gas exchange measurements of coffee leaves have been performed. These studies have pointed out that the photosynthetic capacity of coffee leaves varied with a variation environment (Ayalew, 2018; DaMatta, 2004; DaMatta and Ramalho, 2006; Kumar and Tieszen, 1980). For single coffee leaves, the saturating irradiance is between 300 and 700 mmol photons m^{-2} s^{-1}, with shade leaves showing a lower value than lower sun leaves (DaMatta, 2004; MATTA). This study found that the saturating irradiance of sun leaves was about 620 mmol photons m^{-2} s^{-1}, which was consistent with the result of the above mention. Quantum yields revealed the relationship between a given light-dependent product and the number of absorbed photons (Taiz and Zeige, 2006). Quantum yield is 0 when none of the light energy is used in photosynthesis. Quantum yield is 1 when all the absorbed light is used. Based

on the results of YE (2007), the quantum yield decreased with *I* increasing (Ye, 2007). Thus, the IQY was higher than AQY. In the past, there were several pertinent points about maximal photosynthetic rates of coffee leaves. For example, Kumar and Tieszen (1980) and Cannell (1985) have pointed out that the maximal photosynthetic rates of sun leaves of coffee are around 7 mmol CO_2 m^{-2} s^{-1} and 8.8 mmol CO_2 m^{-2} s^{-1}, respectively (Cannell, 1985; Kumar and Tieszen, 1980). However, Bote et al. (2018) reported that the maximal photosynthetic rates of sun leaves of coffee were lower than 7 mmol CO_2 m^{-2} s^{-1} with a larger N supply (Bote *et al.*, 2018). In this study, the maximal photosynthetic rates were lower than observations of Kumar and Tieszen (1980) and Cannell (1985) and consistent with the result of Bote et al. (2018). For coffee leaves, light saturation would lead to an excess of electron transport capacity (Martins *et al.*, 2014). Therefore, higher J_{max} was observed under light saturation. In addition, the values of V_{cmax} and J_{max} were larger than Martins et al. (2014) reported result.

The correlation analysis is a great utility method for understanding the relationships between variables. In this study, significant correlations between photosynthetic capacity and leaf anatomical traits were observed, indicating that leaf anatomical traits could affect photosynthetic capacity. Despite the correlation coefficients could provide a good way to understand the relationships between variables, the cause and effect relationships between variables couldn't be estimated (Gonzalez-Paleo and Ravetta, 2018). Path analysis, which investigates the cause and effect relationship, can provide a detailed knowledge of positive and negative correlations between traits (Gonzalez-Paleo and Ravetta, 2018). The result of the path analysis was shown in Fig 3, indicating that there were high-level direct and indirect effects of leaf anatomical traits on photosynthetic capacity. This may explain the importance of leaf structure in photosynthesis. In order to quantitatively evaluate the effect of each structure of leaf on photosynthesis, the comprehensive evaluation values are calculated by using the formula (8) introduced in the methods. The ordering comprehensive score showed that leaf thickness (LT) and stomatal density (*d*) were the most important leaf anatomical traits for coffee leaf photosynthesis. For Arabica coffee, the photosynthetic is greatly limited by CO_2 diffusion (DaMatta *et al.*, 2007; Franck and Vaast, 2009b). The CO_2 diffusion from the air to the leaf mesophyll is mainly modulated by the stomatal conductance (g_s), which is associated with stomatal density (*d*) (Lambers and Oliveira, 2019; Rodrigues *et al.*, 2018). In fact, higher stomatal density will increase g_s by increasing the same total pore area (Rodrigues *et al.*, 2018). This may partly explain why the stomatal density (*d*) had higher comprehensive evaluation values in the effect of coffee leaf photosynthesis.

The leaf thickness strongly positively correlated with the fraction of intercellular air space in the leaves (Niinemets, 1999). Given that the CO_2 diffusion in the gaseous phase is about three orders of magnitude larger than in the liquid (Oguchi *et al.*, 2018)(108). Thus, the diffusive resistance should not necessarily increase considerably with increasing leaf thickness (Smith *et al.*, 1997). Moreover, due to the chloroplasts adhering to the inner surface of exposed mesophyll cell walls, thicker leaves provide space for more chloroplasts per unit leaf area (Lambers and Oliveira,

2019) and possess more chloroplast surface area, which results in a substantial increase in carbon dioxide absorption because of a larger surface area for diffusion (Smith *et al.*, 1997). Furthermore, thicker leaves may have higher total contents of Rubisco and leaf N per unit area (Niinemets and Sack, 2006). In this study, thick leaves have the highest most important direct effects on photosynthetic capacity as compared with other anatomical traits by using the CRITIC method. In addition to the result of path analysis, the relatively high direct effects of thick leaves (TL) on IQY, α, R_d, and AQY were also observed.

The structure of the leaf cuticle consists of epicuticular wax, birefringent wax embedded in cutin, cutin matrix, and pectic substance (Lee and Graham, 1986). The epicuticular wax can decrease light by scattering (Bote *et al.*, 2018). The most cutin matrix in the cuticle is heterogeneous, and the areas of embedded waxes exhibit birefringence in polarized light (Lee and Graham, 1986). This heterogeneous structure of the leaf cuticle may affect epidermal focusing, allowing actinic light to penetrate deeper into the mesophyll (Bote *et al.*, 2018). These findings most likely explained the significant relationship between cuticle thickness and photosynthetic gas exchange parameters. In addition, the cuticle thickness would increase resistance to CO_2 diffusion (Miranda *et al.*, 1981), and the negative relationships between UCT and LCT and photosynthetic parameters were also observed in this study.

The epidermal cell usually has a large transparent central vacuole and lacks mature chloroplasts, thus the light can directly pass from these cells into the mesophyll (Bote *et al.*, 2018). However, these epidermal cells have various shapes, such as plano-convex, spherical, conical, or some other convex shape (Jackson *et al.*, 2012). The curved outermost epidermal cell wall can affect the focal point within the leaf (Bote *et al.*, 2018). However, the result of Brodersen and Vogelmann (2007) showed that there was no relative between the convexity of epidermal cells and the absorptance of diffuse light (Brodersen and Vogelmann, 2007). Although two contradictory hypotheses have been proposed with regard to the impacts of epidermal cells on photosynthesis, this study examined epidermal lens effects on many photosynthetic parameters and elucidated cells property plays an important role in photosynthetic capacity by using the CRITIC method.

When intrinsic quantum yields are high, light capture and utilization are most efficient(Herrmann *et al.*, 2020). However, structural changes of a leaf may affect light absorption (Herrmann *et al.*, 2020). For example, thicker mesophyll would enhance light absorptance in leaves, due to greater internal light scattering (Lambers and Oliveira, 2019). Because photon irradiance absorptance varied with variation chlorophyll concentration of leaf (Lambers and Oliveira, 2019), the quantum yield would be affected by chlorophyll concentration. Most chlorophylls were contained in the palisade cells. This could explain the significant relationship between quantum yield and PT. In addition, measurements of light gradients in leaves showed that palisade tissue (PT) facilitates the penetration of direct sunlight (Vogelman *et al.*, 1996). The result of this study also indicated that a large relatively negative direct effect of PT on IQY, AQY, and J_{max} were observed.

The concentration of CO_2 in the mesophyll is affected by air temperature, and each

degree rise in temperature above 24 ℃ results in a 20 ppm rise in CO_2 concentration (Nunes *et al.*, 1968). However, Khairi and Hall's (1976) studies of citrus photosynthesis showed that mesophyll conductance to CO_2 decreased with temperature increase when the temperature increased from 22 ℃ to around 40 ℃ (Khairi and Hall, 1976). In the analysis of effects of leaf anatomical traits on photosynthetic capacity, the spongy tissue thickness (ST) had larger indirect effects and Pearson's correlation comprehensive score, suggesting that spongy tissue thickness (ST) plays an important role in the photosynthetic process. In addition, Martins et al. (2014) showed that mesophyll thickness and stomatal attributes related to water flux and gas exchange per leaf area (Martins *et al.*, 2014), which is consistent with the result of this study. On the other hand, the irregular shaped spongy cells alternating with air spaces increases light absorption due to the optical path length through a leaf increase (Oguchi *et al.*, 2018)(105), and these absorbed light will be advanced for photosynthesis (Brodersen and Vogelmann, 2007; Cannell, 1985). Although there was little relationship between spongy tissue thickness (ST) and photosynthetic parameters, the high direct spongy tissue thickness (ST) effects upon I_{sat}, WUE, and VPD was observed.

Leaf structure plays a key role in determining the conductance of CO_2 through the leaf because changes in leaf structure will change gas diffusion within the leaves (Evans *et al.*, 1994). Moreover, the CO_2 diffusion conductance is also influenced by the density and arrangement of the stomata (Oguchi *et al.*, 2018). For the coffee leaf, the net carbon assimilation rate (A) greatly is governed by diffusive conductances of CO_2, thus the photosynthesis of the coffee leaf was largely limited by a diffusive factor(Araujo *et al.*, 2008). Traditionally, stomata have been considered as one of the major roles in controlling CO_2 diffusive (Araujo *et al.*, 2008) and were a key parameter in the photosynthesis of *C. arabica*(Ayalew, 2018). Among the various stomatal characters, stomatal density plays a major role in gas exchange and photosynthesis (Pompelli *et al.*, 2010). In this study, it is shown that stomatal density had the highest comprehensive scores compared with other anatomical traits in indirect effects and Pearson's correlation comprehensive evaluation. In addition, structural modification of the stomata is one of the key factors affecting stomatal conductance (Taiz and Zeige, 2006). The result of this study also showed that the guard cell length (l) significantly correlated positively with C_i.

For light absorption, although the result of Martins et al. (2014) showed that the guard cell length did not change in response to light treatments (Martins *et al.*, 2014), there was a significant correlation with IQY and AQY. The coordination between increased initiation of stomata cells and expansion of epidermal cells was observed in coffee leaves, implying that there was an optimization of the trade-off between transpirational costs and CO_2 assimilation (Martins *et al.*, 2014). In addition, stomatal size and density largely controlled the leaf's diffusive conductance to CO_2, and the balance between the CO_2 required for photosynthesis and the level of water availability was also determined by stomatal traits (Rodrigues *et al.*, 2018). Thus, lower epidermal stomatal density had a significant correlation with photosynthetic parameters or direct effects and indirect effects on photosynthetic parameters.

Moreover, these traits also had larger comprehensive scores in a comprehensive evaluation. This means that stomatal density was a key parameter in the regulation photosynthesis of *C. arabica*.

Conclusion

Many leaf anatomical traits significantly affected photosynthetic parameters. This result provided holistic relationships between leaf anatomical traits and photosynthetic parameters in coffee. It is tempting to suggest that these relationships might to some extent explain the photosynthetic behaviour of coffee (*Coffea arabica* L.). The relationships between leaf anatomical traits and photosynthetic parameters emphasize the importance of leaf anatomy in determining photosynthesis at the tissue level. Moreover, these correlations between leaf anatomical traits and photosynthetic parameters were useful to explore the impact of different anatomical features on photosynthetic efficiency and accordingly provide some information to design leaf anatomy for enhanced photosynthetic efficiency. To a certain extent, path analysis and comprehensive evaluation could indicate interrelationship between photosynthetic capacity and leaf anatomical traits. However, the causal interrelationship between photosynthetic parameters and leaf anatomical traits is less clear. In spite of this, the result of the study also suggests that improvements of the photosynthetic performance of coffee through selecting thinker leaf and higher stomatal density that might support higher photosynthetic capacity could be a useful strategy to facilitate the selection of promising genotypes with enhanced coffee growth and production.

Author contributions

Z.X. and G.M. conceptualization and research design; X.H. and J.L. project administration;M.Z.and L.S. investigation and fieldwork, Z.X. analysed the data and wrote the manuscript; H.M. and H.Z. resources; all theauthors read and edited the manuscript, and approved the final version.

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