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Non-destructive and accurate method for predicting cowpea leaflet area

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Abstract: Leaf area (LA) is one of the most important measures for evaluating plant growth, as it directly influences photosynthetic activities, transpiration, and is closely correlated with dry matter accumulation. Destructive and non-destructive methods can be used to evaluate this. In this context, this work aimed to formulate and fit allometric equations to estimate leaflet area in cowpea accessions as a function of leaflet linear dimensions. This study was carried out at the Federal Rural University of the Semi-Arid Region, Mossoró, RN, Brazil. A total of two thousand leaflets from ten cowpea accessions were evaluated. Length (L), width (W), and area of leaflets were measured, and the derived variables $L \times W$, L^2 , and W^2 were subsequently calculated. Descriptive analysis was used to calculate the maximum, mean, and minimum values of the data. The criteria for choosing the best equations to estimate the leaflet area in individual and pooled analysis of cowpea accessions were: coefficient of determination, Akaike's information criterion, Willmott's agreement index, and root mean square error. In addition, principal component analysis was also performed. The leaflet area of cowpea can be estimated using a non-destructive method based on biometric measurements from images. Using the product $L \times W$, the leaflet area of cowpea accessions can be estimated by the equation $\hat{y} = 0.6685 \times LW$.

Keywords: *Vigna unguiculata*, leaflet dimensions, mathematical models.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is grown predominantly by low-income farmers in the North and Northeast regions of Brazil. However, over the last decade, it has expanded its agricultural frontier into the Midwest, where it is cultivated as a

succession crop (Freire Filho et al., 2011). In addition to having a production chain different from that of common beans, cowpea stands out for its tolerance to water stress, good adaptation to high temperatures and saline soils, among other characteristics sought by producers (Salinas-Gamboa et

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al., 2016; Merwad et al., 2018; Carvalho et al., 2019).

In addition to the characteristics listed, which confer resilience to the species, cowpea is characterized as an important ally in the fight against malnutrition, since it is present in the daily diet of Brazilians, and can complement or even meet the protein and mineral needs of a population (Gonçalves et al., 2020; Honaiser et al., 2022). However, it is important to note that cowpea cultivation in Brazil does not follow a specific cultivar rule, and both improved and regional cultivars are used. This variability in cultivar types results in devaluation and hinders flow when supply is high.

Therefore, there is a need to deepen knowledge of the species, particularly given that the information available in the scientific literature cannot cover most of the cultivars used. Among the various tools that can deepen this knowledge, growth analysis can help evaluate a cultivar's responses in a specific environment. For this, several techniques are available, which can be more or less efficient according to the evaluation condition.

Among the parameters used to evaluate growth is the leaf area, as it is closely related to the photosynthetic rate. It can be assessed either in the field or in the laboratory, through destructive or direct methods. The direct method requires removing the leaves from the plant, while non-destructive (indirect) method, preserve leaf integrity and allow repeated measurements during the crop cycle (Taiz et al., 2017; Hara et al., 2019; Zhang, 2020).

Leaf area estimation models are useful for monitoring physiological processes, growth, and responses to biotic and abiotic stresses because they are practical, rapid, and non-destructive (Macário et al., 2020).

The estimation of leaf area using allometric models has been carried out in several species, such as *Theobroma cacao* (Schmidt et al., 2017), *Salvia hispanica* (Goergen et al., 2021), *Eustoma grandiflorum* (Dias et al., 2022a), and

Manilkara zapota (Ribeiro et al., 2023), among others, in an accurate, simple, and fast way and without necessarily destroying the leaves (Pinheiro et al., 2020). Thus, studies that corroborate the growth aspect and physiology of cowpea are essential, due to the socioeconomic importance of this species.

However, existing allometric equations may be insufficient for the evaluated accessions, as morphological variation in leaf shape and size among regional genotypes can significantly reduce the accuracy of generalized models. Therefore, the objective of this work was to formulate and fit allometric equations to estimate leaflet area in cowpea accessions as a function of leaflet linear dimensions, aiming to obtain a pooled equation.

Materials and Methods

The research was carried out at the Rafael Fernandes experimental farm belonging to the Center for Agrarian Sciences (CCA), Federal Rural University of the Semi-Arid Region (UFERSA), Rio Grande do Norte, Brazil (5° 03' 37" S, 37° 23' 50" W, and altitude of 72 m). The climate is classified as BSh, considered semi-arid, with a rainy season during the dry, very hot summer (Alvares et al., 2013). The mean temperature of the region is 27.4°C, and the annual rainfall is around 677 mm (Bezerra et al., 2014). The soil of the experimental area was classified as an Argissolo in the Brazilian Soil Classification System (Santos et al., 2025).

Ten accessions of *Vigna unguiculata* were used: Ac1, Ac2, Ac4, Ac64, Ac65, Ac86, Ac93, Ac99, Ac102, and Ac130, belonging to the cowpea germplasm collection of CCA/UFERSA. Each accession was evaluated in a single field plot with 75 plants arranged in rows. To ensure representativeness, statistical independence of the measurements, and avoid pseudoreplication, 200 expanded leaflets per accession were sampled evenly across the plants within the usable area of the plot (excluding borders). The sampling

encompassed different canopy positions (lower, middle, and upper thirds) of the plants. All selected leaflets were free from damage by biotic or abiotic factors. The selected leaflets had different sizes to test the model’s generality and to introduce greater variability in the sample data. The selected leaflets had different sizes to test the model’s generality and to introduce greater variability in the sample data. Immediately after collection, the samples were packed in plastic bags and transported to a controlled environment at 24°C to keep the leaves turgid for analysis.

Length (L, in cm), width (W, in cm), and leaflet area (LA, in cm²) were measured, and the derived variables L × W, L², and W² were subsequently calculated. These characters (Figure 1) were obtained by processing individual images using ImageJ (National Institutes of Health, USA), following the methodology described by Ribeiro et al. (2018).



Figure 1. Linear dimensions (length – L and width – W) used to estimate the leaflet area of *Vigna unguiculata*.

Each leaflet flattened and scanned at 300 dpi using a flatbed scanner (model HP Deskjet 3050 J610 series). During image digitization, centimeter-graduated rulers were included as indicators of the reference scale for analyses of each leaflet, and calibration in ImageJ was performed using

the “Set Scale” tool based on the known ruler distance.

Descriptive statistics were used to calculate the maximum, mean, and minimum values for each accession. The coefficient of variation was calculated. To formulate the allometric equations, linear and non-linear regression models (linear, linear without intercept (0.0), quadratic, power, and exponential) were tested (Table 1).

Table 1. Models and descriptions of the regression models used to estimate cowpea leaflet area.

Models	Descriptions
Linear	$\hat{y} = \beta_0 + \beta_1 * x + \epsilon_i$
Linear (0.0)	$\hat{y} = \beta_1 * x + \epsilon_i$
Quadratic	$\hat{y} = \beta_0 + \beta_1 * x + \beta_2 * x^2 + \epsilon_i$
Power	$\hat{y} = \beta_0 * x^{\beta_1} + \epsilon_i$
Exponential	$\hat{y} = \beta_0 * \beta_1^x + \epsilon_i$

\hat{y} – leaflet area; x – linear dimensions; β_0 , β_1 , and β_2 – model coefficients; ϵ_i – random error.

The observed LA was considered the dependent variable (\hat{y}), while the linear dimensions L, W, L × W, L², and W² were set as the independent variables (x). The criteria for choosing the best equations to estimate LA in individual and pooled analysis of cowpea accessions were: coefficient of determination (R²) (Equation 1), Pearson’s linear correlation coefficient (r) (Equation 2), Willmott agreement index (d) (Equation 3), Akaike’s information criterion (AIC) (Equation 4), and root mean square error (RMSE) (Equation 5).

$$R^2 = 1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y'_i)^2} \quad (1)$$

$$r = \frac{\sum_{i=1}^n (y_i - \bar{y})(x_i - \bar{x})}{\sqrt{\sum_{i=1}^n (y_i - \bar{y})^2 \sum_{i=1}^n (x_i - \bar{x})^2}} \quad (2)$$

$$d = 1 - \frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{\sum_{i=1}^n (|\hat{y}'_i| + |y'_i|)^2} \quad (3)$$

$$AIC = -2 \ln L (\hat{\theta}) + 2(p) \quad (4)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n}} \quad (5)$$

Where: \hat{y}_i – estimated values of the leaflet area; y_i – values of the observed leaflet area; \bar{y} – mean of the observed values; $\hat{y}'_i = \hat{y}_i - \bar{y}$; $y'_i = y_i - \bar{y}$; $L (\hat{\theta})$ – maximum likelihood function; p – number of model parameters; n – number of observations; x_i and y_i – observations of variables y and x ; \bar{y} and \bar{x} – mean of the variables y and x .

To justify pooling the data from all ten accessions into a single generalized model, principal component analysis (PCA) was performed to evaluate the multivariate grouping of leaf characteristics among the

genotypes. A paired-sample Student’s t-test ($p \leq 0.01$) was used to compare the observed (OLA) and estimated (ELA) leaflet area values in order to assess the presence of systematic bias. Data analysis was performed using R v.4.1.2 software (R Core Team, 2022).

Results and Discussion

Descriptive analysis revealed that cowpea characters and accessions showed variation based on leaflet image data (Figure 2). The characteristics of product (length – $L \times$ width – W) (Figure 2C), L^2 (Figure 2D), W^2 (Figure 2E), and leaflet area (Figure 2F) showed the most significant data dispersion. This variation is related to the use of a large number of leaves of varying sizes, which is evident when comparing the minimum and maximum values.

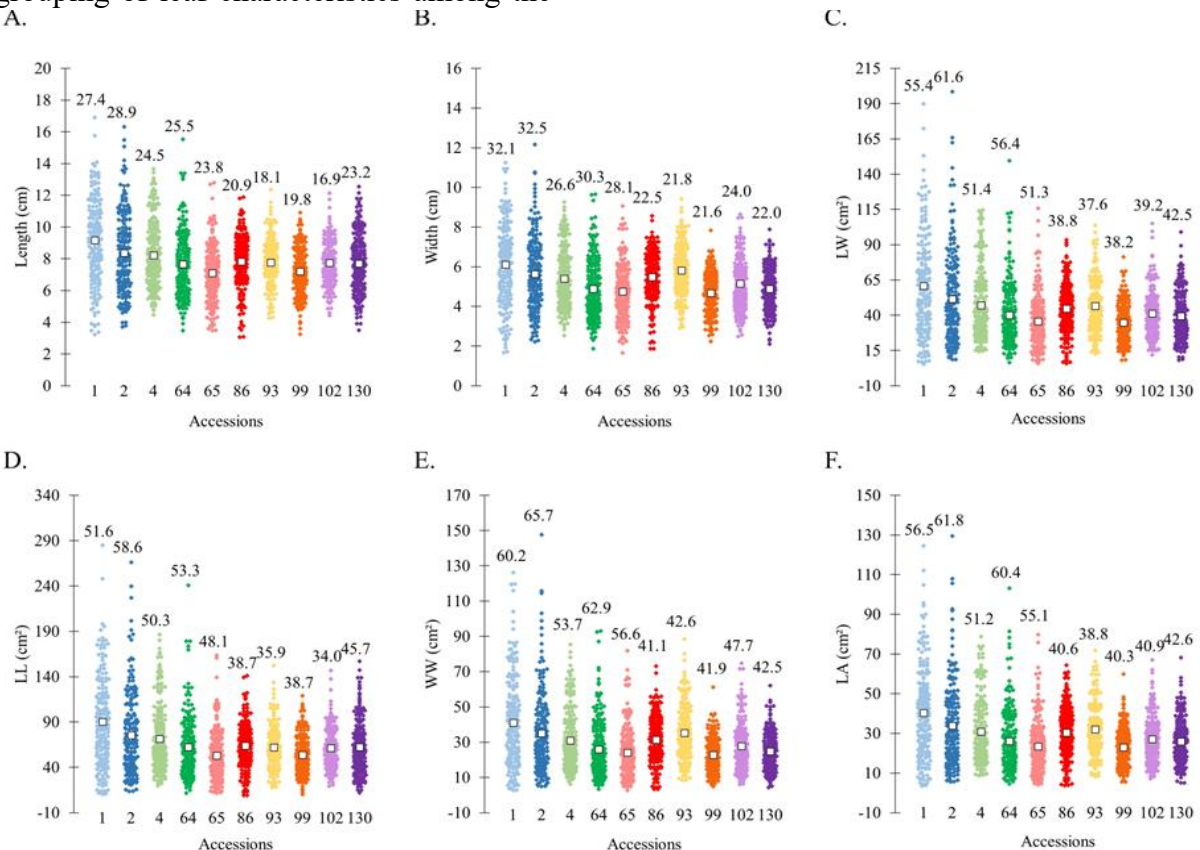


Figure 2. Descriptive analysis of length – L (A), width – W (B), LW (C), LL (D), WW (E), and leaflet area – LA (F) in cowpea accessions. The distribution of top and bottom points represents extreme values (maximum and minimum) of the data set. The symbol (□) within the data distribution represents the means in each genotype. The numbers above the points refer to the coefficients of variation (in %).

The coefficient of variation quantifies the variation in the data set; thus, collecting leaves at different developmental stages and using various accessions enabled the collection of a representative sample for estimating leaflet area in cowpea. The wide variability in the evaluated traits is essential for generating mathematical models that can be used in leaves of different sizes (Cargnelutti Filho et al., 2012).

In peanuts, Ribeiro et al. (2022) reported slight variation in the data between the six cultivars evaluated by means of imaging, in obtaining biometric measurements, enabling the construction of a single model

to estimate the leaflet area of this species, allowing researchers to make non-destructive measurements or repeat measurements on the same leaves or plants during the crop cycle.

The scatter plots between the biometric characteristics of L , W , $L \times W$, L^2 , W^2 , and LA , indicated distinct relationships (Figure 3), proposing adjustment in the linear and nonlinear regression models. As observed in this work, Dias et al. (2022b) suggested adjustments to linear and nonlinear models for estimating leaf area in *Congea tomentosa*.

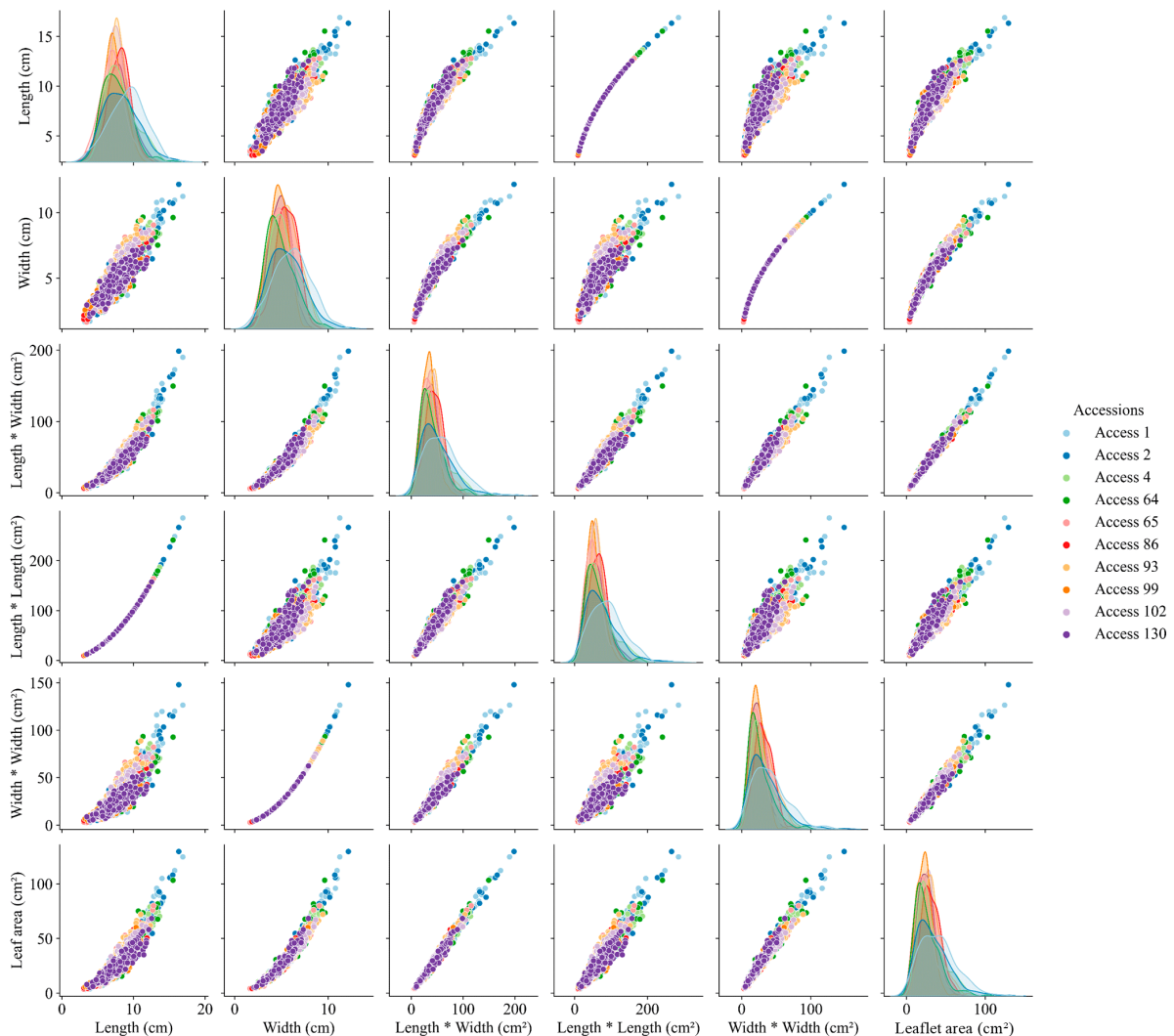


Figure 3. Histograms and scatter plots between length, width, length \times width, length \times length, width \times width, and leaflet area in 2,000 leaves used to generate models for estimating the leaflet area of cowpea accessions.

Based on the characteristics evaluated to estimate the allometric equations, linear and nonlinear regression models were used to estimate the leaflet area in cowpea (Table 2). Linear and nonlinear models are reliable for estimating leaf area across various species, as shown in *Vitis vinifera* (Teobaldelli et al., 2020), cultivars of *Arachis hypogaea* L. (Ribeiro et al., 2022), and *Dendranthema grandiflora* (Silva et al., 2023).

For most characteristics, the equations obtained from linear, quadratic, potential, and exponential models had high coefficients of determination (R^2) > 90%. The R^2 quantifies the dispersion of the observed data relative to the model; thus, the R^2 values obtained were sufficient to indicate a high-quality model with little data dispersion. Ribeiro et al. (2020) reported

that high R^2 values indicate that the variations in the actual leaf areas of the species studied are explained by the corresponding equations based on biometric data obtained from the leaf blades.

From the results in the Table 2, the Willmott agreement values were satisfactory, with values close to 1, indicating proximity between the observed and estimated values. The agreement index (d) provides a descriptive analysis of the data, indicating the precision of the estimated values of the dependent variable based on the values of the independent variable, to demonstrate whether these equations are really accurate (Willmott, 1981). Several mathematical models were evaluated to estimate the leaflet area of cowpea based on different linear dimensions (Table 2).

Table 2. Statistical models, equation, coefficient of determination (R^2), Akaike information criterion (AIC), root mean squared error (RMSE, in cm^2), and Willmott agreement index (d) for estimation of leaflet area in cowpea accessions as a function of leaflet dimensions.

Models	x	Equations	Criteria			
			R^2	AIC	RMSE	d
Linear	L	$\hat{y} = -30.61 + 7.62 \times L$	0.8675	12854.5	5.8114	0.9634
Linear	W	$\hat{y} = -25.20 + 10.35 \times W$	0.9378	11327.0	3.9824	0.9837
Linear	LW	$\hat{y} = -0.44 + 0.67 \times LW$	0.9840	8603.3	2.0310	0.9958
Linear (0.0)	LW	$\hat{y} = 0.6685 \times LW$	0.9963	8553.1	2.0039	0.9960
Linear	LL	$\hat{y} = -0.22 + 0.45 \times LL$	0.8934	12416.4	5.2144	0.9711
Linear	WW	$\hat{y} = 2.15 + 0.90 \times WW$	0.9664	10080.9	2.9259	0.9913
Quadratic	L	$\hat{y} = -1.69 + 0.35 \times L + 0.42 \times L^2$	0.8934	12417.2	5.2129	0.9712
Quadratic	W	$\hat{y} = -0.77 + 1.07 \times W + 0.81 \times W^2$	0.9668	10060.2	2.9096	0.9914
Quadratic	LW	$\hat{y} = -1.16 + 0.70 \times LW - 0.0002 \times LW^2$	0.9842	8584.3	2.0205	0.9959
Quadratic	LL	$\hat{y} = -0.45 + 0.45 \times LL - 0.00003 \times LL^2$	0.8933	12418.0	5.2138	0.9711
Quadratic	WW	$\hat{y} = 1.51 + 0.94 \times WW - 0.0004 \times WW^2$	0.9667	10066.3	2.9139	0.9914
Power	L	$\hat{y} = 0.44 \times L^{2.00}$	0.8933	12416.9	5.2151	0.9710
Power	W	$\hat{y} = 1.29 \times W^{1.86}$	0.9667	10062.3	2.9125	0.9914
Power	LW	$\hat{y} = 0.65 \times LW^{1.00}$	0.9839	8599.4	2.0281	0.9959
Power	LL	$\hat{y} = 0.44 \times LL^{1.00}$	0.8933	12416.9	5.2151	0.9710
Power	WW	$\hat{y} = 1.23 \times WW^{0.93}$	0.9667	10062.3	2.9125	0.9914
Exponential	L	$\hat{y} = 5.63 \times 1.22^L$	0.8667	12910.5	5.8925	0.9604
Exponential	W	$\hat{y} = 6.42 \times 1.31^W$	0.9370	11458.0	4.1136	0.9816
Exponential	LW	$\hat{y} = 16.01 \times 1.01^{LW}$	0.9370	13301.5	4.1136	0.9816
Exponential	LL	$\hat{y} = 15.68 \times 1.00^{LL}$	0.8484	13922.3	6.4909	0.9478
Exponential	WW	$\hat{y} = 16.74 \times 1.01^{WW}$	0.7856	13436.2	7.5687	0.9263

x – leaf dimensions; L – length; W – width; LW – length \times width product; LL – length \times length product; WW – width \times width product.

Initially, the best equations for each independent variable were selected based

on the highest R^2 values, Willmott index close to one, and the lowest values for both

the Akaike information criterion (AIC) and root mean square error (RMSE). These included equations such as $\hat{y} = -1.69 + 0.35 \times L + 0.42 \times L^2$ (for length) and $\hat{y} = -0.77 + 1.07 \times W + 0.81 \times W^2$ (for width). However, among all alternatives, the linear model passing through the origin using the product of length and width ($\hat{y} = 0.6685 \times LW$) was chosen as the final recommended equation. This specific model was selected because it demonstrated the best overall statistical performance, with the highest coefficient of determination ($R^2 = 0.9963$) and agreement index ($d = 0.9960$), and the lowest AIC (8553.1) and RMSE (2.0039 cm²).

Developing allometric equations to estimate leaf area enables these models to be applied in future studies without detaching leaves from the plant. Researchers can use a ruler or a similar tool to directly measure the leaf blade's linear dimensions in the field (Schmidt et al., 2017). It is worth noting that removing or destroying leaves is necessary only during the initial phase of calibrating these allometric relationships. The use of non-destructive methods for estimating leaf area is highly advantageous for understanding plant variability and continuously monitoring growth, as direct, destructive measurements are both time-consuming and limit repetitive evaluations on the same plant (Silva et al., 2023).

The equation proposed to estimate leaflet area in cowpea accessions showed a high fit of the data to the line, with $R^2 = 0.9963$ (Figures 4A and 4B). This value indicates little data dispersion, normal distribution, and homogeneous residual variance.

The linear regression model for estimating leaflet characteristics is suitable for accurately reproducing the data. Parmar et al. (2015) and Goergen et al. (2021) reported that the image processing method, in addition to high accuracy, takes less time and is economical for estimating leaf area, as observed in this work.

In the principal component analysis, two components were extracted, accounting for 99.39% of the total variability in the data (Figures 5A and 5B). The first principal component accounted for 98.51% of the variability, while the second component accounted for only 0.88%.

The cowpea accessions showed similar behavior in leaf characteristics, remaining close within their respective groups. This multivariate grouping showed a high degree of overlap among the genotypes, justifying the integration of all data into a single generalized equation. According to these results, Ribeiro et al. (2022) reported that when the principal components group distinct cultivars into a single group, based on allometric analysis to estimate leaf area, they allow the creation of a generalized model covering all the individuals evaluated.

The concentration of the characteristics in the first principal component (Figure 5B) confirms that the data had little variation. The data analyzed are satisfactory for this type of analysis. Cruz et al. (2014) reported that when the first principal components explain more than 70% of the total variation, the results are satisfactory, allowing evaluation of the importance of each characteristic studied relative to the available variation, as observed in this study.

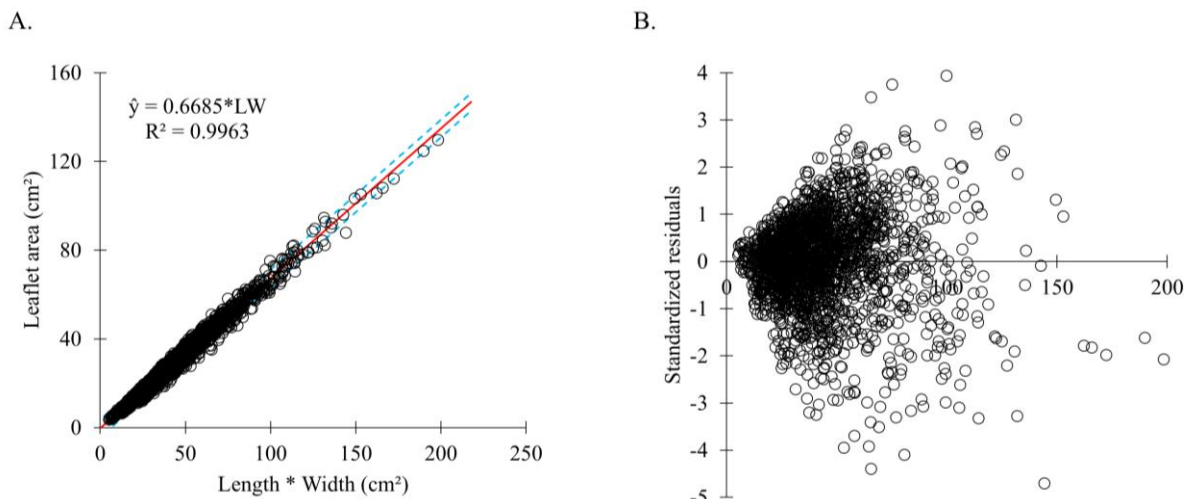
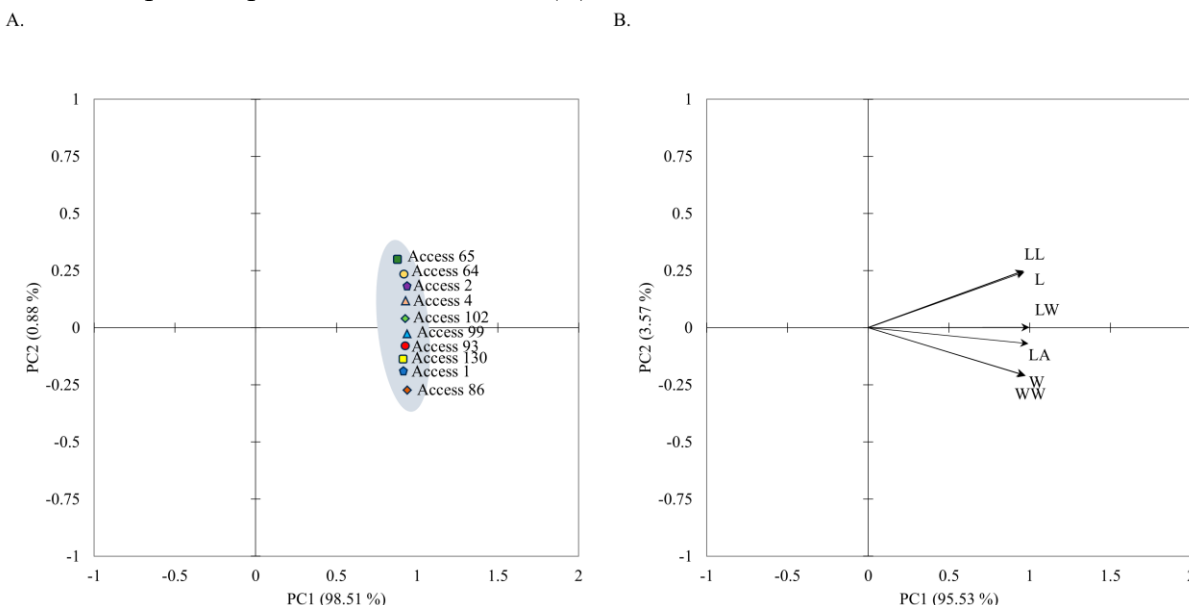


Figure 4. Relationship between the observed leaflet area and the product (length – L × width – W) for cowpea accessions (grouped data), based on the model $LA = 0.6685 \times LW$ (A), along with the dispersion pattern of the residuals (B).



In figure A, the large circle represents the grouping formed by the Euclidean distance of dissimilarity. L – length; W – width; LW – length × width product; LL – length × length product; WW – width × width product; LA – leaflet area.

Figure 5. Principal component analysis (PCA) showing the distribution of cowpea accessions (A) and the leaflet parameters (B).

The observed and estimated leaflet areas did not differ significantly by Student’s t-test (Figure 6A), indicating the absence of systematic bias and that the mean difference between the equations’ estimates and the measured values is not significant. The

leaflet area estimated by the proposed equations was positively correlated with the actual leaflet area, with a coefficient of determination (R^2) of 0.9840 (Figure 6B), indicating a high fit and little data dispersion (Figure 6C).

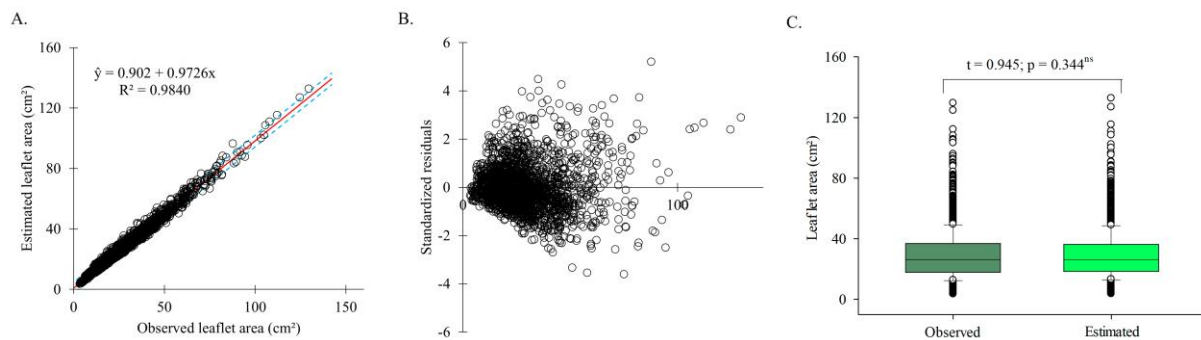


Figure 6. Relationship between observed and estimated leaflet area (LA) in cowpea accessions using the model based on the product of length – L and width – W ($LA = 0.6685 \times LW$) (A), dispersion pattern of the residuals (B), and comparison between observed and estimated LA by paired Student's t-test (C).

Several researchers have developed equations that relate leaflet length, width, or both for different species, such as *Urochloa mosambicensis* (Leite et al., 2017), *Congea tomentosa* (Dias et al., 2022b), and *Ocimum gratissimum* (Ribeiro et al., 2023), confirming the efficiency of this technique and the absence of variation between the observed and estimated values.

Conclusions

The leaflet area of cowpea can be estimated using a practical and non-destructive method based on its linear dimensions. The equation $\hat{y} = 0.6685 \times LW$ demonstrated excellent goodness-of-fit and is recommended for estimating leaflet area using the product of length and width. While further validation across independent datasets is necessary to establish broad predictive validity, this model demonstrates high internal accuracy and serves as a reliable tool for non-destructive evaluation of the studied accessions.

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