

Leaf area of red guava through image analysis

Área foliar de la guayaba roja mediante análisis de imagen

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ABSTRACT

Red guava is a species that has been relatively little studied; despite this, it is widely distributed throughout Brazil. To understand the domestication of the species, it is essential to understand the leaf area of red guava. In this regard, this study aimed to estimate the leaf area of red guava through image analysis software. Red guava leaves were collected from the Fruit Orchard, and characteristics such as length, width, leaf mass, and leaf area were evaluated. The latter was estimated after the leaves were digitized and processed using ImageJ software. Additionally, the mass of leaf disks, detached from the basal portion of the leaves, was evaluated. A completely randomized design was employed. Data were analyzed descriptively and correlated using Pearson's correlation. Red guava leaves showed significant variability in mass, linear measurements, and leaf area. Leaf area estimation through image analysis software is cost-effective, easily executable, and an efficient methodology for evaluating this characteristic.

Keywords: biomass accumulation; biometrics; canopy; crop physiology; imageJ; *Psidium cattleianum* Sabine

RESUMEN

La guayaba roja presenta una amplia distribución en el territorio brasileño, a pesar de ser una especie aún relativamente poco explorada. Es fundamental conocer el área foliar de la guayaba roja para comprender la domesticación de la especie. En este sentido, el objetivo de este estudio fue estimar el área foliar de la guayaba roja mediante software de análisis de imágenes. Se recolectaron hojas de guayaba roja en el Huerto Frutal y se evaluaron características como longitud, anchura, masa foliar y área foliar. Esta última se estimó después de que las hojas fueron digitalizadas y procesadas utilizando el software ImageJ. Además, se evaluó la masa de los discos foliares, desprendidos de la porción basal de las hojas. Se adoptó un diseño completamente aleatorizado.

Los datos se analizaron mediante análisis descriptivo y se correlacionaron mediante la correlación de Pearson. Las hojas de guayaba roja presentan una variabilidad significativa en masa, medidas lineales y área foliar. La estimación del área foliar a través de un software de análisis de imágenes es rentable, fácilmente ejecutable y demuestra ser una metodología eficiente para evaluar esta característica.

Palabras clave: acumulación de biomasa; biometría; dosel; fisiología del cultivo; imageJ; *Psidium cattleianum* Sabine

INTRODUCTION

Red guava (*Psidium cattleianum* Sabine) is a species that is little explored, but has an extensive distribution in Brazilian territory (Zandoná *et al.*, 2020). It has white, melliferous flowers and can be a cultivation option for small rural properties (Rau *et al.*, 2021).

Red guava plants have a botanical origin in the southern region of Brazil and have nutritional potential, especially their antioxidant content (Vanin *et al.*, 2021; Cavalcanti *et al.*, 2011). The fruit has a tangy-sweet taste, well accepted by consumers, with high levels of nutrients and vitamins (Neri-Numa *et al.*, 2013). Its fruits can be consumed fresh or through the preparation of sweets, jellies, and juices (Pereira *et al.*, 2020).

Evaluating leaf area is crucial for comprehending the interactions between plant growth and environmental factors (Ribeiro *et al.*, 2025). Leaf area is closely associated with phenology, biomass accumulation, metabolism, and crop yield via photosynthesis (Hu *et al.*, 2025). Therefore, its estimation is fundamental, since the architecture of the canopy and the effects of interception of solar radiation by the foliage interfere with the productivity and composition of the fruits (Zhang *et al.*, 2025).

Leaves are responsible for water loss, gas exchange, and photosynthesis, the latter of which depends on absorbing and converting light energy (Morgado *et al.*, 2013). To this end, these structures are a gateway to determining plant growth and development simulation models, light interception, biomass production, water consumption, CO₂ fixation, and comparison between species and cultivars (Taiz & Zeiger, 2017). Knowledge of the leaf area is necessary to expand research with the species; for this reason, understanding growth and vegetative development makes it possible to implement cultural management strategies such as pruning, fertilization, planting density, and application of pesticides (Lucena *et al.*, 2011).

It is essential to establish non-destructive study methods to preserve both the plant and the species, and to make rational use of time. An alternative is the method adopted for the species under study, in which the analysis can be carried out in the field using a ruler, caliper, or software, by plucking a few leaves or estimating by the equation that best represents it (Ribeiro *et al.*, 2025). Once the results have been analyzed, they will be compared with other methodologies to determine the best form of non-destructive analysis for the red guava.

The use of digital image analysis for estimating leaf area has been reported for several species, such as *Urochloa mosambicensis* (Lucena *et al.*, 2018), *Anacardium occidentale* L. (Matos *et al.*, 2019), *Anacardium humile* A. St.-Hil (Gomes *et al.*, 2020), *Nicotiana tabacum* (Zhang *et al.*, 2023), and grape (*Vitis vinifera* L.) (Sautchuk *et al.*, 2024). This methodology is simple and quick, eliminating the need for excessive use of sophisticated devices (Matos *et al.*, 2019). There are

no reports in the literature to determine the leaf area for red guava through the analysis of digital images; therefore, this study aimed to estimate the leaf area of red guava using image analysis software, using non-destructive methods.

MATERIALS AND METHODS

Leaves were collected from twenty-five red guava plants, sampling the middle third and the four quadrants of each plant, selecting only those without distortions, damage, or symptoms of disease or pest infestation. The plants were located in the Fruticulture Orchard of the Federal University of Jataí, at 17°55'33.3"S and 51°42'46.9"W. According to the Köppen classification, the region's climate is rainy tropical (Aw), with an average annual temperature of 18 to 32 °C and average annual precipitation around 1700 mm.

A total of 500 leaves were collected following the methodology of Gomes *et al.* (2020). Specifically, five leaves were taken from each quadrant of the 25 plants ($5 \times 4 \times 25 = 500$). After collection, they were packed in LDPE bags and transported to the Fruit Growing Laboratory, located approximately 500 meters from the orchard.

The leaves were then analyzed to obtain length, width, leaf mass, disc mass, and leaf area. A completely randomized design was used, in accordance with the approach used by Gomes *et al.*, (2020). The 500 leaves were evaluated individually and compared at the end of the study.

The length and width variables were measured using a caliper, with the length taken along the leaf's main vein and the maximum width measured at the median portion of the leaves. The results were recorded in centimeters. By individually weighing each leaf on a digital scale with an accuracy of 0.01 g, the variable mass of the leaf was obtained, with the fresh mass values expressed in grams.

The leaf disks were obtained using a 34.40 mm, where disks were detached from the basal portion of the leaf with only fine veins, obtaining one disc per leaf. Subsequently, the mass of the disks was determined using weighing on a precision scale of 0.01 g, whose values were expressed in grams.

Using image analysis software, the leaf area was assessed. Each leaf was individually scanned in its natural color with an HP Deskjet model F4280® scanner at 300 dpi resolution, alongside a graduated ruler for dimension reference to configure the software. The scanned images were then processed in the program, and the leaf area analysis results were expressed in cm².

Data relating to red guava leaves were subjected to descriptive analysis and correlated using Pearson's correlation at a 5% level of significance. The data for length, width, fresh mass, and leaf area were analyzed using AgroEstat statistical software (Barbosa & Maldonado, 2015). Leaf area calculations were performed with ImageJ® software version 1.52a, and the graphs were created using Origin 8.5.1 SR1 software.

RESULTS

The red guava leaves evaluated (Table 1) showed considerable variation in length and width, ranging from 47 to 62 mm and 97 to 125 mm, respectively. Fresh leaf mass varied considerably, ranging from 0.38 to 1.56 g, while the mass of the disks varied between 0.12 and 0.28 (Table 1).

Table 1. *Biometric parameters of red guava leaves*

Characteristic	Width (mm)	Length (mm)	Leaf mass (g)	Mass of the disc (g)	Leaf Area (cm ²)
Lowest value	47.90	97.13	0.38	0.12	34.38
Highest value	62.84	125.28	1.56	0.28	82.01
Average	54.73	109.86	0.92	0.19	46.18
Standard deviation	3.87	7.20	0.25	0.03	7.88
CV (%)	7.08	6.55	27.03	16.37	17.77

The leaf area of red guava was measured using image analysis software, with values ranging from 34.38 to 82.01 (Table 1).

Leaf width demonstrated a positive and significant correlation with both leaf length and area. Similarly, leaf area exhibited a positive and significant correlation with leaf length. Although no significant correlations were found between growth parameters and the mass of leaves and disks, a positive and significant correlation was found between the fresh mass of leaves and leaf disks (Table 2).

Table 2. *Pearson correlation coefficients (r) between biometric characteristics and estimated leaf area of red guava leaves*

	Width/ ¹	Length/ ¹	Sheet mass/ ²	Disc mass/ ²
Length	0.96**	-	-	-
Leaf mass	-0.12 ^{NS}	-0.15 ^{NS}	-	-
Disk mass	0.11 ^{NS}	0.10 ^{NS}	0.73**	-
Leaf area/ ³	0.93**	0.95**	-0.16 ^{NS}	0.07 ^{NS}

** Significant at 5% (P < 0.05); NS Not significant; /1 (mm); /2 (g); /3 (cm²).

The correlation between leaf area and the length and width of red guava leaves was adjusted to the linear model. The correlation between leaf area and leaf length showed a low estimate of the standard error and a high coefficient of determination, with the correlation between leaf area and leaf width showing similar behavior (Table 3).

Table 3. *Statistical models, regression parameters, and estimate of the leaf area as a function of the linear dimensions (length, width, and length x width) of 100 leaves of red guava*

Model	Parameters	Standard error estimate	R ²	F _{calc}	Leaf area estimate
AF _{LF} = $\beta_0 + \beta_1 X$	$\beta_0 = -58.18^*$ $\beta_1 = 1.90^*$	3.90	0.8785	716.83	AF _{LF} = -58.18 + 1.90X
AF _{WF} = $\beta_0 + \beta_1 X$	$\beta_0 = -68.99^*$ $\beta_1 = 1.04^*$	3.49	0.9165	1087.84	AF _{WF} = -68.99 + 1.04X
AF _{LW} = $\beta_0 + \beta_1 X$	$\beta_0 = 35.07$ $\beta_1 = 0.53$	2.89	0.9486	16005.94	AF _{LW} = 35.07 + 0.53X

* Significant at 5%.

The high coefficient of determination is linked to a decrease in the standard error estimate, which results from an increase in the F-calculated value and a reduction in the mean squares of the residuals. The greater the F-calculated value, the higher the coefficient of determination (Fialho *et al.*, 2011).

The simple linear regression model was the most precise in explaining the correlations between leaf area and leaf length and width, and it had the highest coefficient of determination (R^2) (Figure 3).

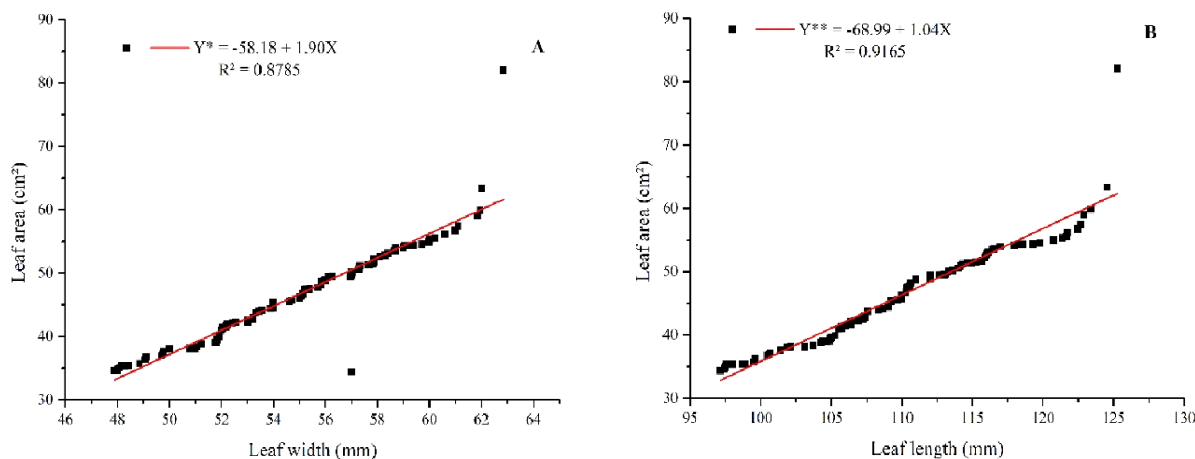


Figure 3. Correlation between leaf area and length (A) and between leaf area and width (B) of red guava leaves using linear regression models

For the leaf area of red guava plants, $AFlw = \beta_0 + \beta_1 X$ (Table 3), an increase in the values estimated by the model that used the measurements of length and width between the leaf sides ($L \times W$) together. The product model obtained results with lower standard errors (2.89), again confirming those of Ribeiro *et al.* (2025) in studies with apple cultivars, where models based on a single measure of length or width showed lower R^2 and standard errors than models with both dimensions.

DISCUSSION

The measurement of leaf area is crucial for understanding plant growth, photosynthesis, and overall health. An image processing technique is presented here to obtain accurate and efficient leaf area measurement (Li *et al.*, 2008).

Following the methodology outlined in the study, utilizing linear dimensions offers a reliable estimate of leaf area (Morgado *et al.*, 2013). These characteristics are extremely important in the assessment of plant growth, cultivation conditions, and photosynthetic efficiency of plants, with leaf dimensions being crucial for the productive potential and architecture of plants (Toebe *et al.*, 2012).

The variation of leaves has been considered, as reported by Moraes *et al.* (2013), who evaluated leaves of red guava (*P. cattleianum*) from an area with two light conditions in the state of Santa Catarina, obtaining a length of approximately 11 cm and a width of approximately 7 cm. While few studies relate length, width, and fresh mass values to leaf area, it may not be possible to establish a direct relationship between these characteristics (Cunha *et al.*, 2010). It is noteworthy that obtaining the fresh mass of leaf disks may be highly accurate in certain crops and may be inefficient in other crops (Lucena *et al.*, 2011).

The leaf area allows evaluating the ecological adaptation of species, competition with other species, and the type of management, in addition to providing information regarding the estimation of productivity, and plant growth and development (Moraes *et al.*, 2013).

Since leaves act as the main photosynthetic organ of plants, being essential in the production of carbohydrates, lipids, and proteins, knowledge of the leaf area is extremely important in understanding the processes of photosynthesis and transpiration, in addition to nutritional and water requirements (Zeist *et al.*, 2014).

The positive Pearson correlation values obtained in the present work indicate the intensity of the association between linear measurements and leaf area (Francisco *et al.*, 2014). The length and width of the leaves are the measurements that most contribute to the precision in obtaining the leaf area (Sousa *et al.*, 2015), which may explain the high values of the correlation coefficient in the present work between these leaf area variables.

The correlation between leaf area and its estimation by image is a topic of great interest in agricultural and biological research. By using image processing techniques, an accurate and inexpensive estimation of leaf area can be obtained.

According to Rincón *et al.* (2012), digital cameras (from cell phones, webcams, and semi-professional cameras) were used in conjunction with the free ImageJ software to analyze leaf photographs. The results showed correlation coefficients greater than 0.99 between the actual area values and the values estimated from the photographs. This suggests that digital photographs can be an effective tool for leaf area measurement.

The most accurate models are those that present a higher coefficient of determination and, consequently, a lower estimate of the standard error of the mean (Morgado *et al.*, 2013). It is noteworthy that linear measurements and their variations have a strong influence on model prediction, being decisive characteristics in obtaining more accurate models (Hara *et al.*, 2019).

The leaf area index (LAI) is a fundamental biophysical parameter in crop science, as it governs light interception, photosynthetic efficiency, and ultimately biomass accumulation and grain yield. In maize, LAI has been accurately estimated using hyperspectral imagery, enabling robust predictions of biomass and yield performance (Kayad *et al.*, 2022). Furthermore, in double-cropping systems, extending the duration of leaf area has been shown to significantly enhance biomass production compared to monocropping systems (Heggenstaller *et al.*, 2009).

Recent advances in phenotyping have introduced fully automated, Python-based tools for rapid leaf area quantification from scanned images, markedly improving efficiency and reproducibility in high-throughput workflows. These tools outperform conventional approaches such as ImageJ, achieving processing speeds over 1,600 times faster while maintaining near-perfect correlation with manual measurements across diverse citrus species (Suarez *et al.*, 2025). Similarly, image scaling techniques and Java-based applications have demonstrated superior accuracy and computational efficiency in leaf area analysis (Parmar *et al.*, 2016; Horgan *et al.*, 2015).

Leaf area image analysis has evolved significantly with advancements in digital imaging and processing techniques. These methods offer high accuracy, efficiency, and versatility, making them essential tools in plant physiological and ecological studies. The choice of method depends on the specific requirements of the study, such as the need for non-destructive measurement, the complexity of the plant structure, and the available resources.

CONCLUSIONS

Red guava leaves vary greatly in terms of fresh mass and size, given through linear measurements. Likewise, the leaf area presents high variability. It is noteworthy that the use of software for image analysis was efficient in obtaining the red guava leaf area, being a low-cost and easy-to-use methodology. This method appears to be promising, providing an accessible and efficient tool for leaf analysis in red guava. The model for estimating leaf area with length x width (AFLW= $35.07 + 0.53X$) presented the lowest standard error (2.89), being a practical alternative for its estimation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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