

Allometric models for estimating *Moringa oleifera* leaflets area

Modelos alométricos para estimativa de área de foliólulos de *Moringa oleifera*

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ABSTRACT

Moringa oleifera is a species of great economic, social and environmental importance, being employed for multiple purposes. Thus, the objective of this study was to fit regression models for estimating leaflets area as non-destructive method from linear measurements of leaflets of *M. oleifera* seedlings. The study was carried out at the Center for Agrarian and Environmental Sciences of the Paraíba State University. Three hundred leaflets of *M. oleifera* were collected and measured to determine length "L" and width "W" and, subsequently, leaflets area was quantified through ImageJ® software. Using 200 leaflets, the univariate regression models were fitted, adopting length, width or the product of these dimensions "LW" and a bivariate model based on length and width as predictor variables of the observed leaflets area as dependent variable. The remaining 100 leaflets were used to evaluate the relationship between the observed leaflet area "OLA" and the estimated leaflets area "ELA", based on Pearson's correlation "r"; Willmott's index of agreement "d" and index of confidence "c"; and root mean square error "RMSE". It was found that allometric models can be used with high accuracy and performance to estimate the leaflets area of *M. oleifera* as non-destructive method, and recommended model is $ELA = 0.035 + 0.720 * LW$. Future research is suggested for fittings of multivariate models to estimate the leaf area of *M. oleifera* from varying leaflet sizes, complete leaves, leaf fresh and dry weights, history of life and age of plants.

Index terms: Linear measurements; empirical modeling; model validation.

RESUMO

A *Moringa oleifera* é uma espécie de grande importância econômica, social e ambiental, sendo empregada para múltiplos usos. Assim, objetivou-se ajustar modelos de regressão para estimativa de área de foliólulos por método não-destrutivo a partir de medidas lineares de foliólulos de mudas de *M. oleifera*. A pesquisa foi realizada no Centro de Ciências Agrárias e Ambientais da Universidade Estadual da Paraíba. Foram coletados 300 foliólulos de *M. oleifera*, nos quais foram realizadas medidas para determinação do comprimento "L" e da largura "W" e, posteriormente, a área de foliólulos foi quantificada por meio do software ImageJ®. Utilizando-se de 200 foliólulos, os modelos de regressão univariados foram ajustados adotando-se comprimento, largura ou o produto destas dimensões "LW" e um modelo bivariado por meio do comprimento e largura como variáveis preditoras da área de foliólulos observada como variável dependente. Com os 100 foliólulos remanescentes, foi realizada a avaliação da relação entre a área de foliólulos observada "OLA" e a estimada "ELA", correlação de Pearson "r"; índice de concordância de Willmott "d" e de confiança "c"; e erro médio quadrático "RMSE". Verificou-se que modelos alométricos podem ser usados com elevada precisão e performance para estimativa de área de foliólulos de *M. oleifera* por método não-destrutivo, sendo recomendado o modelo $ELA = 0,035 + 0,720 * LW$. Pesquisas futuras são sugeridas para ajustes de modelos multivariados para estimativa de área foliar de *M. oleifera* a partir de tamanhos de foliólulos variados, folhas completas, massa fresca e seca das folhas, histórico de vida e idade das plantas.

Termos para indexação: Medidas lineares; modelagem empírica; validação de modelos.

INTRODUCTION

Population growth, urbanization, economic development, technological advancement and climate change have generated worldwide demand for water, food and energy (Ravar et al., 2020). Brazil is one

of the world's leading food producers and exporters, which has increased climate change due to the intensification of agriculture, highlighting the need for crop diversification for efficient use of water, production of food and energy (Piedra-Bonilla; Cunha; Braga, 2020).

The above-mentioned scenario highlights the *Moringa oleifera* Lamarck crop, notably due to its high yield and its multiple possible uses, with emphasis on its food, nutritional and medicinal potential, since its leaves are rich in proteins, vitamins, minerals and bioactive compounds, with antioxidant, antibacterial and anti-inflammatory activity (He et al., 2020).

This specie belongs to the Moringaceae family, native from India, and widely cultivated in the tropics across the globe. It was introduced in Brazil in the 1950s as an ornamental plant (Rivas et al., 2013). It has rapid growth and can survive in dystrophic soils and require little cultural treatment, in long periods of drought, as is the case in the Northeastern Brazilian semi-arid (Oyeyinka; Oyeyinka, 2018).

Leaves are composed, divided, with seven small leaflets. They are deciduous and alternate with lateral leaflets in an elliptical shape and the terminals slightly larger than the lateral ones, longitudinally distant from 30 to 70 cm and may or may not have a stipple. They are about 20 cm wide and light green, with 4 to 6 pairs of leaflets each, giving rise to two pairs of opposite leaflets and one at the apex, with glands between the leaflets and the leaflets, which measure 0.9 to 1.8 cm in length by 0.5 to 1.5 cm in width (Souto; Maior Júnior, 2018).

Phytochemicals present in *M. oleifera* leaves include tannins, steroids, triterpenoids, flavonoids, saponins, an thraquinones, alkaloids, niazimicin, moringine, and reducing sugars (Omotoso et al., 2018). Despite the importance of its leaves, allometric models for estimating leaflet area of *M. oleifera* are scarce. This suggests the creation of these models to facilitate using leaflet area in the monitoring of important processes associated with physiology, growth, biotic and abiotic stresses, and their relationship with plant production.

Several fundamental physiological processes, such as photosynthesis and transpiration are influenced by leaf morphology, so the morphometric characterization of leaflets and modeling of leaflets area are important tools for estimating crop yield in agroecosystems (Teobaldelli et al., 2020a). Measurements of length and width and the product of these dimensions can be used to estimate leaf area with non-destructive methods (Teobaldelli et al., 2020b).

Estimates of leaf area and leaf area index as non-destructive methods can be performed by capturing aerial images using unmanned aerial vehicles equipped with spectral sensors (Hussain et al., 2020). However, these technologies can be expensive, laborious and time-consuming (Apolo-Apolo et al., 2020), which justifies the creation and availability of models that use the linear

dimensions of the leaflets to estimate the area, mainly due to the ease, practicality, and speed to obtain the results.

For the creation of these models, it is necessary to collect leaves or leaflets from plants (destructive method) to determine the area of these structures by planimetry or image processing. However, after their creation, the models can be used to determine the area by the non-destructive method (without removal of leaves or leaflets), since linear measurements can be obtained directly from the leaves on the plants (Richter et al., 2014).

After determining the length and width of the leaves or leaflets, a linear regression, between these two variables, must be adjusted to allow estimating the quotient between the real area and the product of length by width. From this quotient, called the "correction factor", one can estimate the area of any other leaf or leaflet of the specie, based on the product of its linear dimensions (Fagundes; Streck; Kruse, 2009). Thus, the objective of this study was to fit and validate allometric models to estimate the leaflets area of *M. oleifera* as non-destructive method from linear dimensions of leaflets.

MATERIAL AND METHODS

The study was conducted between October 2018 and March 2019 at the Microbiology Laboratory of the Center for Agrarian and Environmental Sciences (CCAA) of the Paraíba State University (UEPB), located in the municipality of Lagoa Seca - PB, Brazil, at the coordinates of 7° 09' S Latitude, 35° 52' W Longitude and altitude of 634 m (Soares; Silva; Silva, 2017). According to Köppen's classification, the local climate is As' (humid tropical), with an average annual temperature of 22 °C, with a minimum of 18 and a maximum of 33 °C, rainfall of 800 mm and relative humidity of 80% (Silva et al., 2020).

A total of 300 leaflets of *M. oleifera*, collected from six-month-old seedlings grown in a screened environment with a 15% reduction in the original luminosity were used. The cultivation was carried out in 2-dm³ polyethylene containers filled with substrate consisting of sand and bovine manure in the proportion of 3:1. Morphometric analyses were performed to determine the length (L, cm) and width (W, cm) of the leaflets according to Wang et al. (2019). Leaflet width corresponded to the longest distance perpendicular to its length, and the length was considered as the distance between the distal base of the petiole and the end of the terminal one (Figure 1).

After the morphometric measurements were performed, the leaflets were distributed on a white-colored contrasting surface and scanned using Epson® EcoTank

L375 multifunctional printer, adopting a ruler graduated in millimeters as a measurement reference in the image. Actual leaflets area measurements were performed using ImageJ® software, available free of charge via the internet (<http://rsbweb.nih.gov/ij/>). This software captures the image of the leaflets from each full leaflets and, through color-contrast procedures, calculates the actual total area (Holguín et al., 2019).

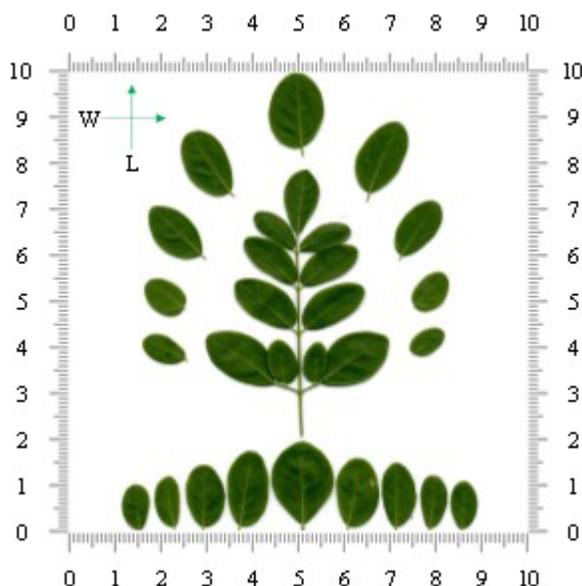


Figure 1: *M. oleifera* leaf and leaflets of varying sizes including small, medium and large. L and W indicate length and width, respectively.

With these data of morphometric analysis and leaflets area, the leaflets were classified according to their length, width and area (small, medium and large). The small and large classes were based on the first and third quartiles of the length, width and leaflets area frequency distribution curves. The leaflets whose measurements of length, width and area were equidistant between large and small were considered as medium (Souza et al., 2019).

To avoid loss of accuracy in the estimates of the regression coefficients, before fitting the allometric models between the observed leaflet area and the measurements of leaflet length and width, the degree of collinearity between the measurements of L and W was evaluated. For this, the variance inflation factor (VIF) was calculated by method of Marquardt (1970), described by Wang et al. (2019), using Equation 1.

$$VIF = \frac{1}{1 - r^2} \quad (1)$$

Where

VIF: variance inflation factor; and,

r: Pearson's coefficient of correlation between L and W.

It is worth pointing out that, if the VIF value is greater than 10, this indicates that L and W have multicollinearity and, therefore, should be disregarded for the fitting of empirical models to predict leaflet area; if the IVF value is lower than 10, the multicollinearity between L and W is negligible, so that these leaflet measurements can be maintained in the fitting of the models.

Once the assumption of no multicollinearity was met, the relationship between leaflet area and the linear dimensions of length (L, cm) and width (W, cm) of the leaflets or the product between these variables (LW, cm²) was modeled, using the models described in Equations 2, 3, 4 and 5.

$$OLA = \beta_0 + \beta_1 * L \quad (2)$$

$$OLA = \beta_0 + \beta_1 * W \quad (3)$$

$$OLA = \beta_0 + \beta_1 * LW \quad (4)$$

$$OLA = \beta_0 + \beta_1 * L + \beta_2 * W \quad (5)$$

Where:

OLA: observed leaflet area and represents the dependent variable;

β_0 , β_1 and β_2 : parameters to be estimated;

L: independent variable length;

W: independent variable width; and,

LW: independent variable represented by the product between leaflet length and width (Morgado et al., 2013).

Of the 300 leaflets collected, 200 were used to fit the models and 100 to evaluate and validated the statistical performance of the fitted models, based on quality, measured by adjusted coefficient of determination “ $R^2_{adjusted}$ ”, precision, measured by Pearson's correlation coefficient “r”; accuracy, measured by Willmott's index of agreement “d” and index of confidence “c”; and the overall performance of the model measured by the root mean square error “RMSE”, and these measurements are estimated using Equations 6, 7, 8, 9, 10 and 11 (Toebe et al., 2012; Toebe et al., 2019; Wang et al., 2019).

$$R_{\text{adjusted}}^2 = 1 - \left(\frac{N-1}{N-2} \right) \left(1 - \frac{\sum_{i=1}^N (S_i - \bar{S}) M_i^2}{\sum_{i=1}^N (S_i - \bar{S})^2 \sum_{i=1}^N (M_i - \bar{M})^2} \right) \quad (6)$$

$$r = \frac{\sum S_i M_i - \frac{\sum S_i \sum M_i}{N}}{(N-1) S_{Si} S_{Mi}} \quad (7)$$

$$d = 1 - \left[\frac{\sum_{i=1}^N (S_i - M_i)^2}{\sum_{i=1}^N (|S_i'| + |M_i'|)^2} \right] \quad (8)$$

$$\text{RMSE} = \sqrt{\frac{1}{N} \sum_{i=1}^N (S_i - M_i)^2} \quad (9)$$

$$c = \left[\frac{\sum S_i M_i - \frac{\sum S_i \sum M_i}{N}}{(N-1) S_{Si} S_{Mi}} \right] * \left\{ 1 - \left[\frac{\sum_{i=1}^N (S_i - M_i)^2}{\sum_{i=1}^N (|S_i'| + |M_i'|)^2} \right] \right\} \quad (10)$$

$$\text{SD} = (S_i - M_i) * \frac{100}{M_i} \quad (11)$$

Where:

S_i : simulated values;

M_i : measured values; and,

N: number of observations for all statistical indices.

Descriptive statistical analyses were performed to calculate the sample arithmetic mean, sample standard deviation, maximum and minimum values, and the class intervals were estimated. These procedures were performed using the software programs Microsoft Excel v. 2016 (Winston, 2016) and Sisvar 5.6 (Ferreira, 2014).

RESULTS AND DISCUSSION

Based on the summary of the descriptive analyses (Table 1), it was found that *M. oleifera* leaflets have a mean length of 1.595±0.474 cm, with values ranging from 0.695 cm to 2.831 cm and coefficient of variation percent of 29.717, so that leaflets with length lower than or equal to 1.407 cm are considered small, those with length greater than 1.407 cm and smaller than or equal to 2.119 cm are medium, and those with length greater than 2.119 cm are large.

The mean width of the leaflets was 1.142±0.329 cm, ranging from 0.443 to 1.950 cm, with a coefficient of variation percent of 28.840, and leaflets with width smaller than or equal to 0.945 cm were considered small, those with width greater than 0.945 cm and smaller than or equal to 1.448 cm were considered medium and those with width greater than 1.448 cm were considered large (Table 1).

Regarding the leaflet area, it was found that the leaflets had a mean area of 1.445±0.800 cm², with values ranging from 0.253 cm² to 3.602 cm², with a coefficient of variation percent of 55.365. Leaflets with an area smaller than or equal to 1.369 cm² were classified as small, those with an area larger than 1.369 cm² and smaller than or equal to 2.486 cm² were classified as medium and those with an area larger than 2.486 cm² were considered large (Table 1).

The mean values of leaflet length and width found in this study were similar to the values of 0.9 to 1.8 cm of length and 0.5 to 1.5 cm of width reported by Souto and Maior Júnior (2018). In the present study, the occurrence of leaves larger than those reported by these researchers may be related to the lower availability of light in the screened environment with 15% reduction in luminosity because, under conditions of light restriction, plants under full sun adapt by increasing the leaf area and reducing the thickness of leaf blade, a phenomenon that commonly occurs due to a reduction in the thickness of the palisade and spongy parenchyma (Rossato et al., 2010; Santos et al., 2014).

The increase in leaflet size occurred as a strategy of plants to optimize radiation capture under the condition of 15% reduction in luminosity, which contributed to improving the absorption of radiation by the mesophyll tissues, due to the increase of photosynthesizing tissues. This contributes to mitigating possible damage to events related to plant metabolism or development that depend on luminosity conditions (Ptushenko et al., 2016; Araújo et al., 2019).

The morphometry of *M. oleifera* leaflets is fundamental to understand the dynamics of dimensions and area, due to the importance of leaf length and width variables in studies with plant species (Chagas et al., 2008), especially to guarantee greater precision in leaf area estimation as non-destructive method. Indeed, the contribution of this study lies in the fact that no limits were reported in the literature for classifying the size of *M. oleifera* leaflets. Souza et al. (2019), working with heirloom bean seeds, estimated classes of seed sizes and reported that this information is essential for selecting seeds for cultivation.

The evaluation of the variance inflation index (VIF) showed no collinearity between leaflet length and width, since the value of VIF (4.574) was lower than 10, an indispensable condition for using the leaflet dimensions for model fitting (Wang et al., 2019). The results of the analyses and fittings of univariate and bivariate regression models from the observed leaflet area and linear dimensions of *M. oleifera* leaflets are presented in Table 2. All fitted models had a high adjusted coefficient of determination ($R^2_{\text{adjusted}} \geq 0.896$), which indicates that the length and width of the leaflets provide satisfactory estimation of the leaflet area of this species.

It was observed that, for each cm of increment in leaflet length, there is a significant increase of 1.598 ± 0.039 cm² in leaflet area, and leaflet length explains 90% of leaflet area. Leaflet width explains 93% of leaflet area, and each cm of width estimates an area of 2.340 ± 0.047 cm². The product between leaflet length and width explains 98% of leaflet area, and for each cm² of the product an area of 0.720 ± 0.007 cm² is estimated. Considering the bivariate model, each cm in length increases leaflet area by 0.737 ± 0.045 cm² and each cm in width increases leaflet area by 1.402 ± 0.065 cm², and these dimensions (L and W) together explain 97% of leaflet area (Table 2).

Table 1: Summary of descriptive analysis and estimate of size classes from the linear dimensions and area of *Moringa oleifera* leaflets.

Parameters	Linear dimensions and area of leaflets used to fit models		
	Length (cm)	Width (cm)	Leaflet area (cm ²)
Sample arithmetic mean	1.595	1.142	1.445
Sample standard deviation	0.474	0.329	0.800
Maximum	2.831	1.950	3.602
Minimum	0.695	0.443	0.253
Coefficient of variation (%)	29.717	28.840	55.365
Estimate of classes	Intervals of classes		
Class of small leaflets	≤ 1.407	≤ 0.945	≤ 1.369
Class of medium leaflets	$> 1.407 \leq 2.119$	$> 0.945 \leq 1.448$	$> 1.369 \leq 2.486$
Class of large leaflets	> 2.119	> 1.448	> 2.486
	Linear dimensions and area of leaflets used to validate models		
Sample arithmetic mean	1.895	1.342	1.943
Sample standard deviation	0.436	0.305	0.789
Maximum	2.831	1.950	3.602
Minimum	0.944	0.597	0.456
Coefficient of variation (%)	23.008	22.706	40.607

Table 2: Coefficients \pm standard errors, calculated F value and adjusted coefficient of determination of the regression models between observed leaflet area and linear dimensions of *Moringa oleifera* leaflets.

Models	Coefficients and statistics of the models				
	β_0	β_1	β_2	F _{Calculated}	R ² _{Adjusted}
ELA = $\beta_0 + \beta_1 * L$	-1.104 \pm 0.064	1.598 \pm 0.039**	-	1712.93	0.896
ELA = $\beta_0 + \beta_1 * W$	-1.227 \pm 0.055	2.340 \pm 0.047**	-	2531.21	0.927
ELA = $\beta_0 + \beta_1 * LW$	0.035 \pm 0.015	0.720 \pm 0.007**	-	12136.55	0.984
ELA = $\beta_0 + \beta_1 * L + \beta_2 * W$	-1.331 \pm 0.037	0.737 \pm 0.045**	1.402 \pm 0.065**	3094.80	0.969

ELA - estimated leaf area; β_0 - linear coefficient of the regression; β_1 and β_2 - angular coefficients of the regression; L - leaflet length; W - leaflet width; F - calculated values of Fisher test; R² - adjusted coefficient of determination.

These results indicate that the product between leaflet length and width has higher predictive capacity and lower dispersion, due to the higher adjusted coefficient of determination ($R^2_{\text{adjusted}} = 0.98$) and lower estimated standard deviation. This information is ratified by several authors (Keramatlou et al., 2015; Tondjo et al., 2015; Cai; Di; Jin, 2017; Liu et al., 2017), who studied the fitting of regression models to estimate leaf area of plant species from leaf length and width measurements. Wang et al. (2019) report that the LW product is the best variable for predicting leaf area, but

these researchers point out that plant age and history of life can influence the quality of the models, evidencing the need for complementary studies involving these factors.

Based on residual analysis, the feasibility of the regression models fitted for estimating leaflet area from leaflet length and width measurements was evaluated. It was found that the residuals are usually well distributed around the mean and located within the upper and lower limits (mean \pm 3SD) of the residuals, as can be observed in the residual dispersion illustrated in Figure 2.

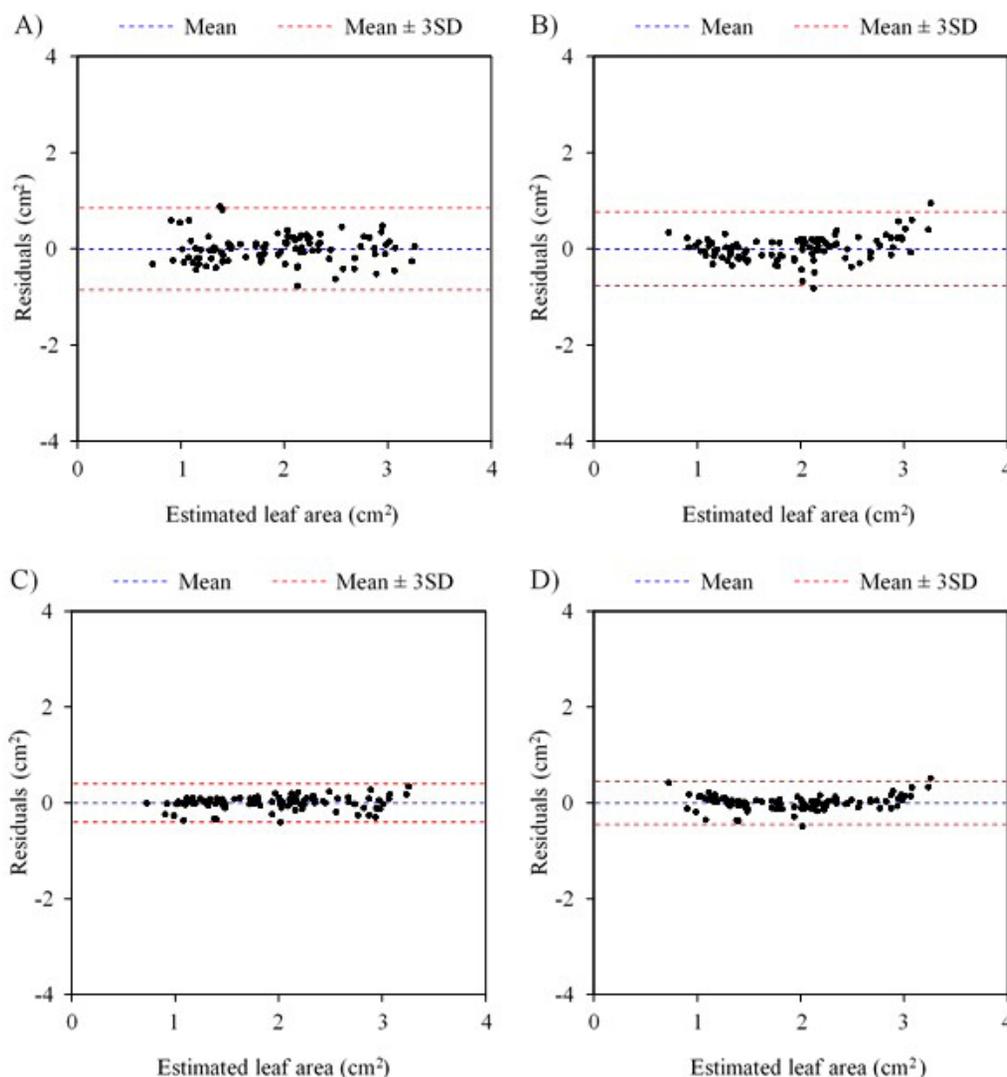


Figure 2: Residual dispersion as a function of the leaflet area estimated by the univariate models from length (A), width (B), product between length and width (C) and by the bivariate model from length and width (D). SD indicates standard deviation.

For validation and evaluation of the statistical performance of the adjusted models, based on the results of the regression analysis, it was found that the observed leaflet area had a significant correlation ($P < 0.01$) with the leaflet area estimated by the fitted models, which had high predictive capacity, with R^2 values ranging from 0.83 to 0.97, the latter being obtained from the estimate that considers the product between leaflet length and width (Figure 3).

The significant results associated with the high coefficients of determination indicate that the fitted models can be used to estimate the leaflet area of *M. oleifera*, and

the model that relates the product between leaflet length and width is the most indicated for this purpose. These results are consistent with those obtained by Morgado et al. (2013) in species of passion fruit (*Passiflora alata*, *P. coccinea*, *P. gibertii*, *P. ligularis*, *P. misera*, *P. mucronata*, *P. nitida*, *P. setacea*), by Wang et al. (2019) in broadleaf species, by Oliveira et al. (2019) in 'caruru' (*Talinum triangulare*) and 'beldroega grande' (*Talinum paniculatum*), by Hara et al. (2019) in common beans (*Phaseolus vulgaris*) and by Teobaldelli et al. (2020b) when fitting allometric models in the estimation of the leaf area of *Ocimum basilicum* L., *Mentha* Spp., and *Salvia* Spp.

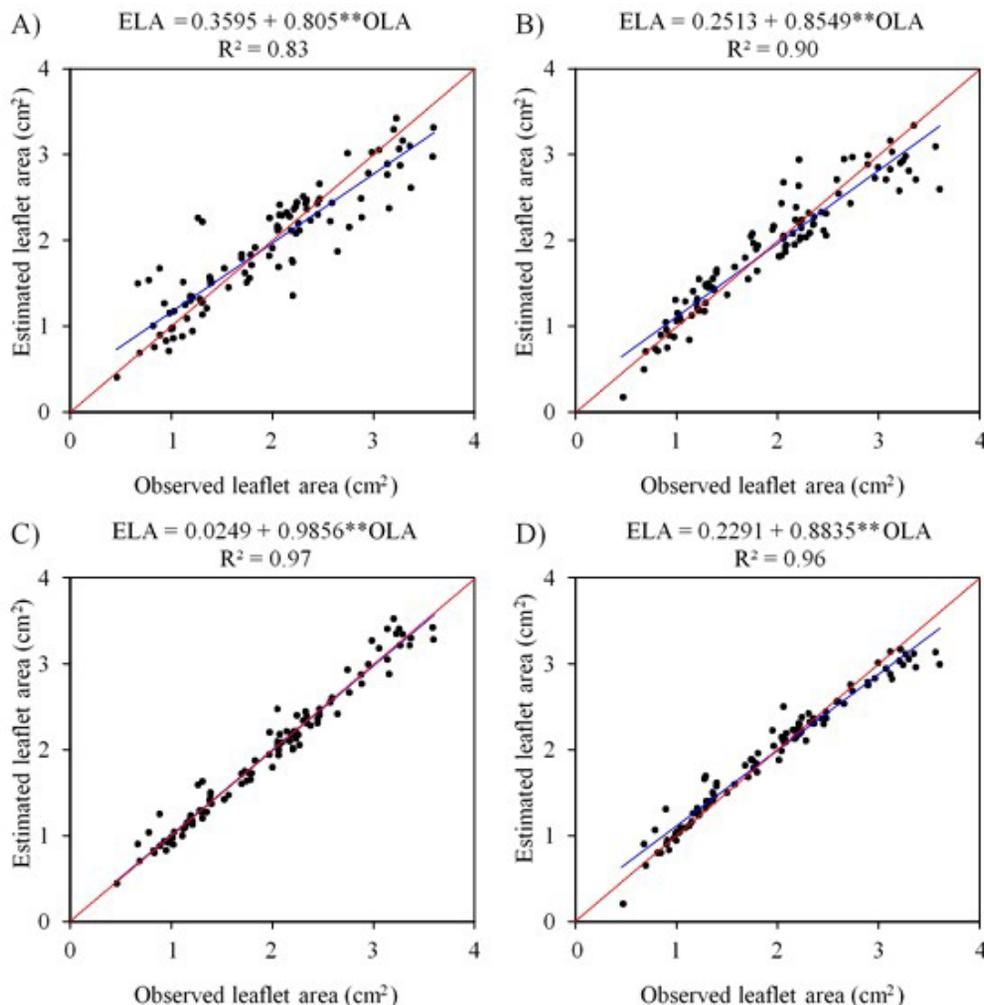


Figure 3: Relationship between observed leaflet area (OLA) and leaflet area estimated (ELA) by the univariate models as a function of length (A), width (B), product between length and width (C) and by the bivariate model as a function of length and width (D). ** indicate significant line slope ($p < 0.01$). The red diagonal line represents the 1:1 line.

When the precision of the model was evaluated through Pearson's correlation coefficient "r", it was possible to note that the models $ELA = \beta_0 + \beta_1 * LW$ and $ELA = \beta_0 + \beta_1 * L + \beta_2 * W$ had the best performance, with values higher than 0.98. These models also showed greater accuracy, with indices of agreement higher than 0.99 and confidence indices of 0.98 and 0.97. Regarding the overall performance, these models showed high performance, since they had low root mean square error, with RMSE of 0.13 and 0.16 for the respective models (Table 3).

Table 3: Performance indices of the univariate and bivariate regression models for estimating *Moringa oleifera* leaflet area as non-destructive model from linear dimensions of leaflets.

Models	Performance indices			
	r	d	c	RMSE
$ELA = \beta_0 + \beta_1 * L$	0.912	0.950	0.866	0.324
$ELA = \beta_0 + \beta_1 * W$	0.946	0.969	0.917	0.258
$ELA = \beta_0 + \beta_1 * LW$	0.985	0.993	0.978	0.134
$ELA = \beta_0 + \beta_1 * L + \beta_2 * W$	0.982	0.988	0.970	0.162

ELA - estimated leaflet area; β_0 - linear coefficient of the regression; β_1 and β_2 - angular coefficients of the regression; L - leaflet length; W - leaflet width; r - Pearson's correlation coefficient; d - index of agreement; c - index of confidence and RMSE - root mean square error.

The measurements of statistical performance of the fittings show that models that consider the product between leaflet length and width are more suitable for estimating the leaflet area of *M. oleifera*. In a study to fit models for common bean, Hara et al. (2019) recorded "r" and "d" values of 0.99 and "c" value of 0.98 for the fitted models, and recommend these models to estimate the leaf area of this crop due to their quality and greater simplicity in operation.

Wang et al. (2019), when fitting regression models to estimate leaf area in broadleaf species, found RMSE values ranging from 4.04 to 4.63 for the model fitted for the product of leaf length by width, and these values were higher than those found in the present study. The researchers highlight that the history of life and age of plants have a substantial influence on the performance of the models, which justifies the high performance obtained with *M. oleifera* leaflets, since they were collected from seedlings of the same age and at the same time of the year.

CONCLUSIONS

Allometric models can be used with high performance to estimate *Moringa oleifera* Lamark leaflet area as non-destructive method from linear measurements of leaflet length and width. The leaflet area of six-month-old *M. oleifera* seedlings can be estimated by the model $ELA = 0.035 + 0.720 * LW$, where ELA is the estimated leaflet area and LW is the product between leaflet length and width. Future research is suggested for fitting multivariate models to estimate the leaf area of *M. oleifera* from varying leaflet sizes, complete leaves, leaf fresh and dry weights, history of life and plant age.

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