Non-destructive method for estimating leaf area of *Ocimum gratissimum* L. using leaf dimensions

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10.1590/0034-737X202370050008

ABSTRACT

Basil (*Ocimum gratissimum* - Lamiaceae), is a sub-shrub plant species with great economic importance for several regions, and studies on its growth, physiology, and reproduction become needed. The aimed research was to obtain a regression equation to estimate leaf area of *O. gratissimum*. 250 basil leaves were collected and the linear dimensions (length and width) and real leaf area of each leaf were measured. From these data, the products between length and width, length and length were calculated. Equations were obtained using regression models: linear, linear without intercept, quadratic, cubic, power, and exponential. The best equation was selected based on determination coefficient, Pearson's correlation coefficient, Willmott's agreement index and CS index, Akaike information criterion, mean absolute error and root mean square error. All equations proposed using the product between length and width (LW) can be used to predict the leaf area of *O. gratissimum*. However, the equation $LA=0.54*LW^{1.03}$ (power model) is the most recommended to estimate the leaf area of this species.

Keywords: allometric equations; basil; lamiaceae; leaf blades; regression models.

INTRODUCTION

Ocimum gratissimum L. (Lamiaceae), popularly known as "basilicão", "alfavacão", "alfavaca", "alfavaca-de-vaqueiro" and "manjericão-cheiroso", is a sub-shrub plant species native to Asia and Africa, naturalized in America, and occurring in all regions of Brazil (Grin-Global, 2016; Antar, 2020). In addition to its potential source of essential oils used in the perfume, cosmetics, and pharmaceutical industries, the plant shows antibacterial and antifungal properties (Cruz & Bezerra, 2017). It is widely used in culinary and medicinal purposes for the treatment of several diseases such as cancer, inflammation, urinary tract, gastrointestinal infections, cholesterol, influenza, and secretions (Bitu *et al*., 2015; Santana *et al*., 2016; Oyelakin *et al*., 2020). Also, the plant was proven to have diuretic, hypoglycemic, antimicrobial, and antioxidant activities (Akpan *et al*., 2014; Hzounda *et al*., 2016; Monga *et al*, 2017; Monteiro *et al*., 2021).

Due to the importance of the species, studies related to its growth, production, physiology and reproduction are of great relevance. Leaf rea measurement is one of the most important analyzes since leaves hold numerous functions, such as light interception and absorption, net CO_2 assimilation, evapotranspiration, stomatal opening and closing, and biomass accumulation (Taiz *et al*., 2017).

Leaf area can be measured by methods classified as direct and indirect, destructive and non-destructive (Marshall, 1968; Peksen, 2007; Sousa *et al*., 2015). Destructive methods are simple and precise, but they are laborious

Submitted on May 10th, 2022 and accepted on March 20th, 2023.
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besides leading to the destruction of all the plant biomass, making long-term research unfeasible (Mota *et al*., 2014). Non-destructive methods based on leaf area estimation through regression equations, provide precise and fast analyzes in addition to allowing successive evaluations of plants at different growth stages (Ribeiro *et al*., 2020a; Santos *et al*., 2021).

Regression equations from linear dimensions of leaf blades have been used to prediction leaf area of other plant species belonging to the same botanical family of *O. gratissimum*, such as *Tectona grandis* Linn. f. (Tondjo *et al*., 2015), Plectranthus ornatos Codd. (Silva *et al*., 2017), *Mentha piperita* L. (Daramola *et al*., 2018), Mesosphaerum suaveolens (L.) Kuntze (Ribeiro *et al*., 2020a), *Sesamum indicum* L. (Ribeiro et al., 2023a), *Dendranthema grandiflora* Tzevele (Silva *et al.*, 2023), *Ocimum basilicum* L., *Mentha* spp. e *Salvia* spp. (Teobaldelli *et al*., 2020), and *Salvia hispanica* L. (Goergen *et al*., 2021). Likewise, this work aimed to obtain a regression equation to estimate leaf area of *O. gratissimum* through linear dimensions of leaves.

MATERIAL AND METHODS

The experiment was carried out under a greenhouse at the Federal University of Paraíba, municipality Areia, Paraíba state, Northeastern Brazil. The climate of the region is classified as As that is tropical with rains during the summer (Alvares *et al.*, 2013). Altitude ranges from 400 to 600 m, annual rainfall is around 1,400 mm, and temperature of 22 °C (Ribeiro *et al.*, 2018a). The average temperature inside the greenhouse was 28.5 °C and air relative humidity was 54% during the experiment, which was monitored using a digital thermo-hygrometer.

Basil seeds were sown in plastic pots with 5 dm^3 capacity, filled with a substrate composed of vegetable soil and cattle manure. The substrate had the following chemical attributes: 6.3 pH (H_2O); 10.5 and 294.6 mg dm^{-3} of P and K^+, respectively; 0.22, 3.2, 0.72, 2.8, 1.48, 5.8, and 5.7 cmolc dm^{-3} of Na^+, H^++Al^{+3}, Al^{3+}, Ca^{2+}, Mg^{2+}, bases sum, and cation exchange capacity, respectively; and 28.7% organic matter.

At 150 days after sowing, 250 leaf blades were randomly collected from the middle, lower and upper thirds of each plant, selecting healthy leaves, without damages caused by biotic and abiotic factors. The leaves were transported to the Laboratory of Plant Ecology Laboratory at the Federal University of Paraíba, Areia, Paraíba state, Brazil. Length (L) and width (W) of each leaf blade were measured using a millimetric ruler (Figure 1). The product of length by width (LW), length by length (LL), and width by width (WW) were calculated. Also, real leaf area (LA) was determined through digital images. For this, each leaf was scanned in a flatbed scanner (380 model, Epson) and the images were processed individually using the ImageJ® software (Ribeiro *et al*., 2018b).

A descriptive analysis was performed with data, calculating maximum and minimum values, mean, amplitude, median, standard deviation, standard error, coefficient of variation, asymmetry, and kurtosis coefficient. To determine the most suitable equation to estimate basil leaf area (LA) as a function of linear dimensions of leaves, equations were adjusted using the linear, linear without intercept, quadratic, cubic, power, and exponential regression models.

The best equation was selected following the criteria: determination coefficient (R^2) (Equation 1), Pearson's linear correlation coefficient (r), Willmott agreement index (d) (Willmott *et al*., 1981) (Equation 2), and CS index (Camargo & Sentelhas, 1997) (Equation 3) closestto one; Akaike information criterion (AIC) (Akaike, 1974) (Equation 4), mean absolute error (MAE) (Equation 5), and root mean square error (RMSE) (Janssen & Heuberger, 1995) (Equation 6) closestto zero. Statistical analyses were performed in R software v.4.0.2 (R Core Team 2020).

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

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

$$CS = r \times d \quad (3)$$

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

$$MAE = \frac{\sum_{i=1}^{n}|\hat{y}i - yi|}{n} \quad (5)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}i - yi)^2}{n}} \quad (6)$$

where: $\hat{y}_i$: estimated leaf area; y_i: observed leaf area; $\bar{y}_i$: mean of observed values; $\hat{y}'_i = \hat{y}_i - \bar{y}$; $y'_i = y_i - \bar{y}$; $L(x\setminus \theta)$: maximum likelihood function, defined as the product of density function; p: number of model parameters; and n: number of observations.

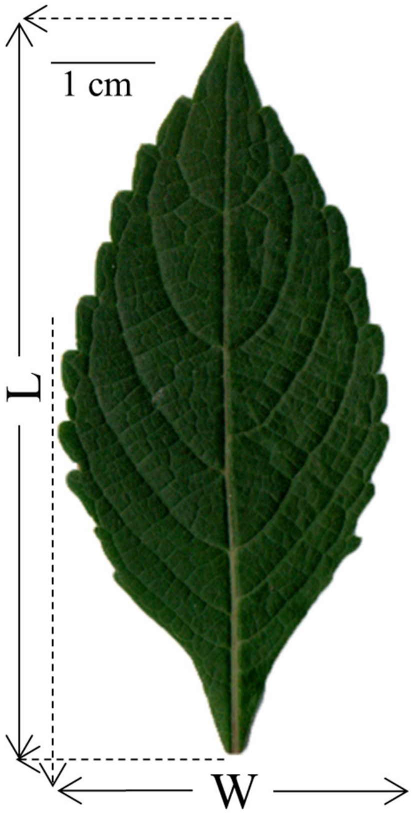

Figure 1: Linear dimensions [length (L) and width (W)] used to estimate the leaf area of *Ocimum gratissimum*.

RESULTS

Descriptive analysis of L, W, LW, LL, WW, and LA of 250 leaf blades of basil is shown in Table 1. L ranged from 0.645 to 16.349 cm, 6.887 cm on average, while W varied from 0.259 to 9.244 cm, 3.412 cm on average. In turn, LW was 3.412 cm² on average, varying from 0.416 to 267.290 cm²; LL was 15.677 cm² on average, ranging from 0.067 to 85.452 cm²; and WW was 31.403 cm² on average, with values from 0.167 to 147.802 cm². LA ranged from 0.098 to 91.503 cm², 18.999 cm² on average (Table 1).

Regarding data variability, the linear dimensions L and W showed the lowest coefficients of variation, 58.28 and 58.98% respectively, while the highest coefficients of variation were found for the LW (101.8%), LL (106.25%), WW (103.39%), and LA (104.32%) (Table 1). Also, L and W showed the lowest coefficients of asymmetry and kurtosis (L: 0.460 and 2.195; W: 0.577 and 2.553) as compared to LW (1.204 and 3.624), LL (1.518 and 5.152), WW (1.325 and 4.169) and LA (1.437 and 4.673) that presented high values for these coefficients (Table 1).

Scatterplots between L, W, LW, LL, WW, and LA indicated different patterns suggesting adjustments to linear and non-linear models (Figure 2). There was linear relationship between LW and LA, LL and LA, and WW and LA, and non-linear between L and LA, and W and LA (Figure 2).

Regarding percentage distribution of LA size classes of 250 basil leaves, it was found that 47.18% of the leaf area was between 0.50 and 10.00 cm², and 22.07% was between 30.01 and 92.00 cm², showing that most of the leaves in this species are small (Figure 3).

Table 2 shows the regression models and equations obtained from the relationship between the leaf linear dimensions and real leaf area. Power model, adjusted using with the product of length by width showed the highest R^2 (0.9974), r (0.9980), *d* (0.9990), and CS index (0.9969), and the lowest AIC (773.8), MAE (0.853), and RMSE (1.264) (Table 2).

Therefore, the equation $LA = 0.54*LW^{1.03}$ is the most suitable for prediction basil leaf area through dimensions of leaves, since there was low data dispersion to the model fit ($R^2 = 0.9974$) (Figure 4A). The leaf area estimated by the indicated equation had a high positive correlation with the actual leaf area, with a high determination coefficient ($R^2 = 0.9958$) (Figure 4B).

Table 1: Descriptive statistics on *Ocimum gratissimum* leaf data

Descriptive statistics	L	W	LW	LL	WW	LA
Minimum	0.645	0.259	0.416	0.067	0.167	0.098
Maximum	16.349	9.244	267.290	85.452	147.802	91.503
Amplitude	15.704	8.985	266.874	85.385	147.635	91.405
Mean	6.887	3.412	63.475	15.677	31.403	18.999
Median	6.211	2.986	38.577	8.916	18.493	11.112
Variance	16.110	4.051	4175.512	277.445	1054.235	392.790
Standard deviation	4.014	2.013	64.618	16.657	32.469	19.819
Standard error	0.264	0.132	4.252	1.096	2.136	1.304
CV (%)	58.28	58.98	101.8	106.25	103.39	104.32
Assimmetry [a]	0.460	0.577	1.204	1.518	1.325	1.437
Kurtosis + 3 [b]	2.195	2.553	3.624	5.152	4.169	4.673

[a] Asymmetry differs from zero by the t-test at 5% probability;
[b] Kurtosis differs from three by the t-test at 5% probability;

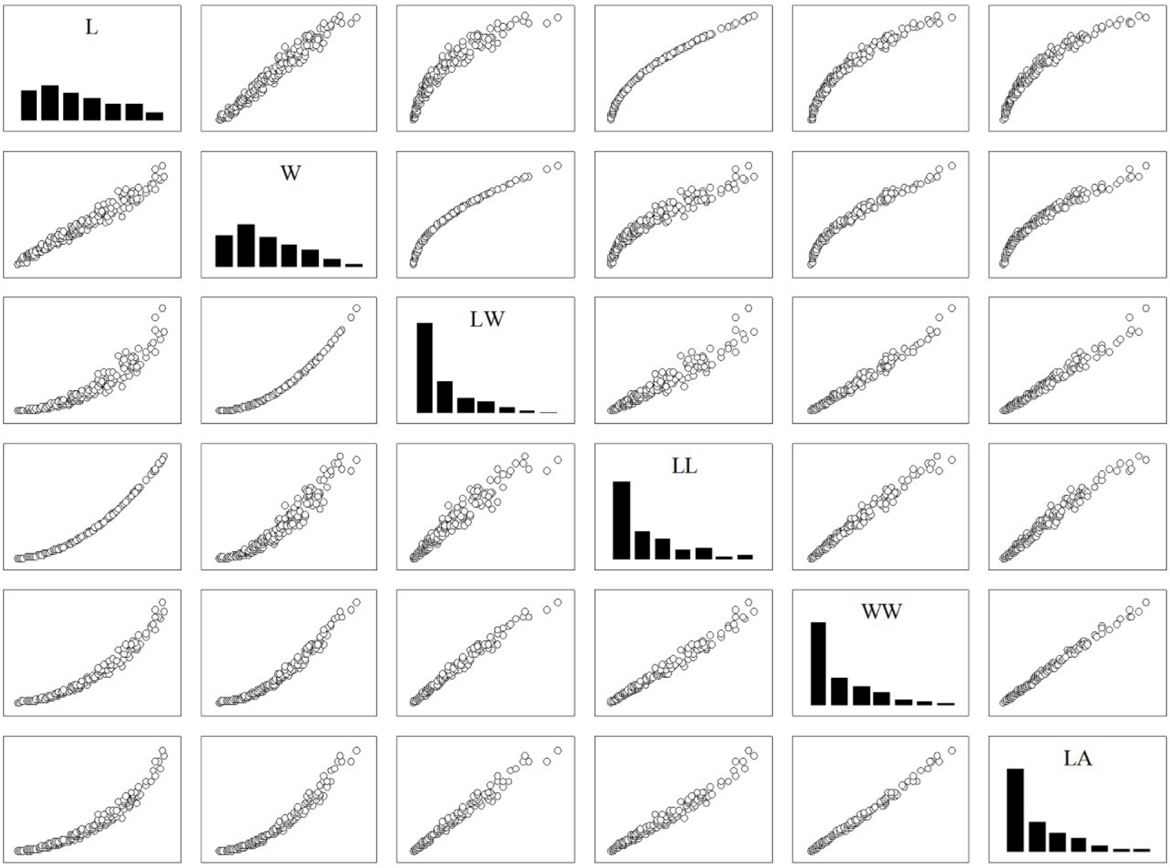

Figure 2: Histogram and scatter plots between leaves dimensions [length, width (W), product of length by width (LW), product of length by length (LL), product of width by width (WW)], and real leaf area (LA) of 250 *Ocimum gratissimum* leaves.

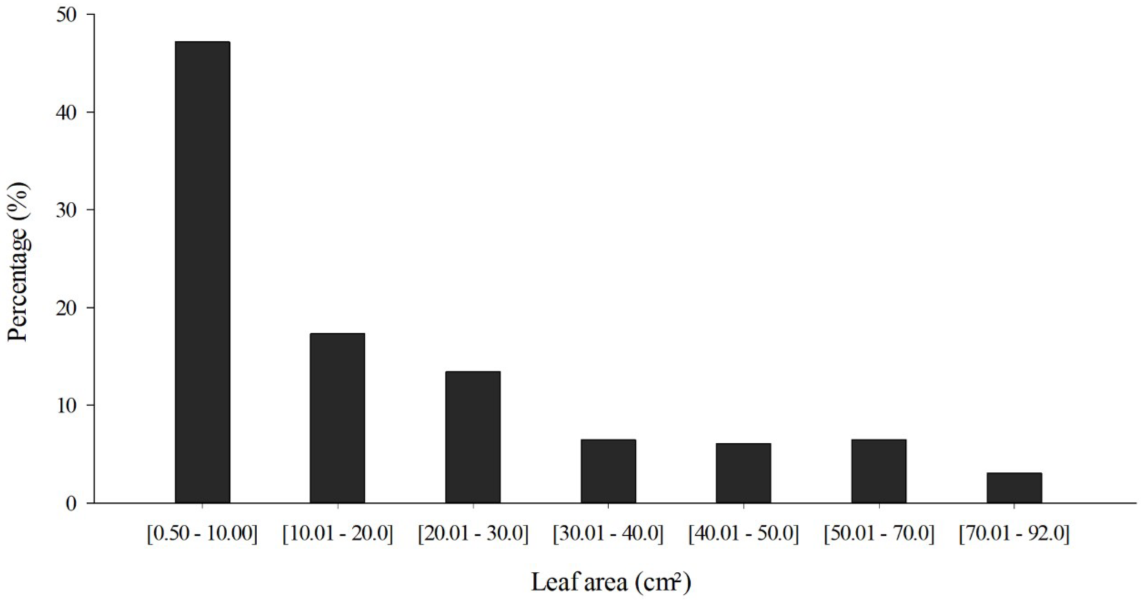

Figure 3: Percentage of real leaf area size classes of 250 *Ocimum gratissimum* leaves.

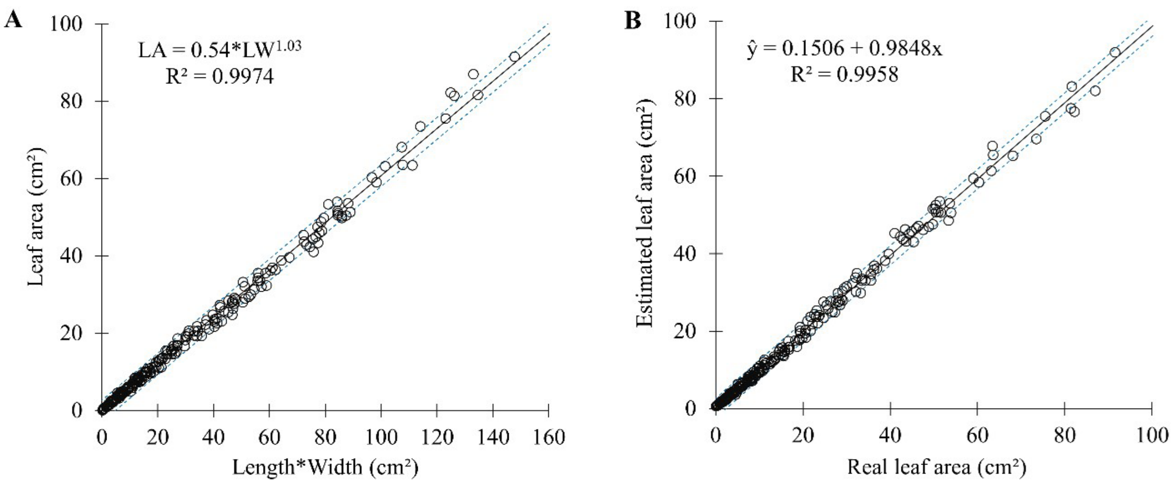

Figure 4: (A) Real leaf area and product of length by width by the proposed equation for estimating *Ocimum gratissimum* leaf area. (B) Relationship between real leaf area and leaf area estimated by the proposed equation ($LA = 0.54*LW^{1.03}$).

Table 2: Regression and equations, determination coefficient (R^2), Pearson's correlation coefficient (r), Willmott agreement index (d), CS index (CS), Akaike information criterion (AIC), mean absolute error (MAE), and root mean square error (RMSE) of 250 Ocimum gratissimum leaves

Model	x	R^2	r	*d*	CS	AIC	MAE	RMSE	Equation
Linear	L	0.9023	0.9501	0.9738	0.9252	1502.1	4.597	6.168	LA = - 13.31 + 4.69*L
Linear	W	0.9245	0.9617	0.9801	0.9425	1442.7	4.267	5.424	LA = - 13.31 + 9.47*W
Linear	LW	0.9950	0.9975	0.9987	0.9963	815.9	0.905	1.397	LA = - 0.12 + 0.61*LW
Linear (0.0)	LW	0.9958	0.9975	0.9987	0.9962	814.8	0.880	1.399	LA = 0.61*LW
Linear	LL	0.9780	0.9890	0.9944	0.9834	1158.2	1.794	2.930	LA = - 0.25 + 0.30*LL
Linear	WW	0.9828	0.9914	0.9957	0.9871	1100.4	1.630	2.585	LA = 0.50 + 1.18*WW
Quadratic	L	0.9791	0.9896	0.9947	0.9844	1147.3	1.839	2.849	$LA = 1.87 - 0.69*L + 0.34*L^2$
Quadratic	W	0.9833	0.9917	0.9958	0.9875	1095.0	1.609	2.545	$LA = - 0.85 + 0.85*W + 1.08*W^2$
Quadratic	LW	0.9954	0.9977	0.9988	0.9965	801.2	0.919	1.353	$LA = 0.46 + 0.56*LW + 0.0004*LW^2$
Quadratic	LL	0.9811	0.9906	0.9952	0.9858	1124.2	1.774	2.710	$LA = 0.93 + 0.25*LL + 0.0002*LL^2$
Quadratic	WW	0.9838	0.9919	0.9959	0.9879	1088.0	1.574	2.506	$LA = - 0.11 + 1.28*WW - 0.002*WW^2$
Cubic	L	0.9816	0.9909	0.9954	0.9863	1118.2	1.727	2.664	$LA = - 2.42 + 1.87*L - 0.03*L^2 + 0.01*L^3$
Cubic	W	0.9837	0.9919	0.9959	0.9879	1090.5	1.578	2.509	$LA = 0.68 - 0.91*W + 1.58*W^2 - 0.04*W^3$
Cubic	LW	0.9959	0.9979	0.9990	0.9969	777.4	0.898	1.279	$LA = 0.22 + 0.59*LW - 0.0003*LW^2 + 0.000004*LW^3$
Cubic	LL	0.9824	0.9913	0.9956	0.9869	1107.6	1.668	2.603	$LA = 0.01 + 0.32*LL - 0.0006*LL^2 + 0.000002*LL^3$
Cubic	WW	0.9841	0.9921	0.9960	0.9882	1084.0	1.567	2.474	$LA = 0.33 + 1.15*WW + 0.003*WW^2 - 0.00004*WW^3$
Power	L	0.9795	0.9897	0.9948	0.9846	1145.4	1.826	2.850	$LA = 0.23 * L^{2.11}$
Power	W	0.9836	0.9918	0.9958	0.9876	1091.8	1.591	2.537	$LA = 1.39 * W^{1.92}$
Power	LW	0.9974	0.9980	0.9990	0.9969	773.8	0.853	1.264	$LA = 0.54 * LW^{1.03}$
Power	LL	0.9796	0.9897	0.9948	0.9846	1145.4	1.826	2.850	$LA = 0.23 * LL^{1.05}$
Power	WW	0.9836	0.9918	0.9958	0.9876	1091.8	1.591	2.538	$LA = 1.39 * WW^{0.96}$
Exponential	L	0.9695	0.9846	0.9911	0.9758	1255.6	2.959	3.617	$LA = 3.35 * 1.23^{L}$
Exponential	W	0.9428	0.9710	0.9815	0.9531	1412.0	3.947	5.075	$LA = 4.56 * 1.42^{W}$
Exponential	LW	0.9428	0.9710	0.9815	0.9531	1556.5	3.947	5.075	$LA = 9.77 * 1.02^{LW}$
Exponential	LL	0.8896	0.9432	0.9633	0.9086	1513.8	6.001	6.939	$LA = 8.89 * 1.01^{LL}$
Exponential	WW	0.9062	0.9519	0.9704	0.9238	1667.7	5.408	6.327	$LA = 11.20 * 1.03^{WW}$

DISCUSSION

Leaf linear dimensions (length and width) showed less variability than the LW, LL, WW, and LA. High data variability is important for generating regression models aimed at estimating leaf area using linear dimensions of leaves, allowing multiple analyzes in different plants developmental stages. Therefore, the number of samples (250 leaves) used in this study was sufficient to build allometric equations to estimate the basil leaf area. High variation in LW, LL, WW, and LA were also recorded in other studies (Macário *et al*., 2020; Donato *et al*., 2020; Ribeiro *et al*., 2020b; Toebe *et al*., 2021).

Scatter plots fitted between the analyzed variables showed linear and non-linear relationships, which was observed by other studies (Carvalho *et al*., 2017; Cargnelutti Filho *et al*., 2021).

The determination coefficients (R^2) of the equations were above 0.88, showing that at least 88% of the variations in basil leaf area were explained by the models obtained through linear dimensions. As compared to the equations fitted using L or W, those equations adjusted using the LW showed the best criteria for estimating leaf area (Bezerra *et al*., 2020; Cargnelutti Filho *et al*., 2021; Lucena *et al*., 2021; Toebe *et al*., 2021), except for the exponential, which showed best indexes when using leaf length (L).

The power model using LW was most suitable to estimate leaf area of other species, such as *Urochloa mosambicensis* ($LA = LW^{0.968}$) (Leite *et al*., 2017), *Erythroxylum citrifolium* ($LA = 0.5966*LW^{1.0181}$) (Ribeiro *et al*., 2019a), *Psychotria carthagenensis* ($LA = 0.6373*LW^{0.9804}$), *Psychotria hoffmannseggiana* ($LA = 0.6235*LW^{0.9712}$) (Ribeiro *et al*., 2019b), *Psychotria colorata* (Ribeiro *et al.*, 2021), *Arachis hypogaea* (Ribeiro *et al.*, 2022a), *Ocimum basilicum* (Ribeiro *et al.*, 2022b), *Erythrina velutina* (Ribeiro *et al.*, 2022c), and *Manilkara zapota* (Ribeiro *et al.*, 2023b).

CONCLUSIONS

The equations proposed using the LW can be used to estimate the leaf area of *O. gratissimum*.

The equation LA = 0.54*LW1.03 (power model) is the most suitable to meaningfully estimate leaf area of *O. gratissimum*.

ACKNOWLEDGEMENTS, FINANCIAL SUPPORT AND FULL DISCLOSURE

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

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