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# Non-destructive method for estimating chrysanthemum leaf area[1]

## Método não destrutivo para estimar a área foliar do crisântemo

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*HIGHLIGHTS:*

*Chrysanthemum leaf area can be estimated using a non-destructive method based on allometric equations.*
*Models that use leaf length (L) and leaf width (W) are the best criteria for estimating leaf area.*
*The equation $\hat{y} = 0.6611*LW^{0.9490}$ using the LW product accurately estimates chrysanthemum leaf area.*

**ABSTRACT:** Chrysanthemum (*Dendranthema grandiflora*) is the second most produced and commercialized ornamental plant in the world. Measuring leaf area through non-destructive methods is fundamental for studies on its growth and production. The estimation of leaf area by linear dimensions of the leaves can be a strategy for this purpose. The objective of this study was to find allometric equations to estimate the leaf area of chrysanthemum. The linear, linear without intercept, quadratic, cubic, power, and exponential regression models were used for the analysis. The choice of equations was based on the highest coefficients of determination. The non-destructive method using allometric models has accuracy for estimating the leaf area (LA) of chrysanthemum from the product between leaf length (L) and leaf width (W). The LA of chrysanthemum can be estimated using the equation $\hat{y} = 0.6611*LW^{0.9490}$ (L – leaf length; W – leaf width). This equation will allow researchers and producers to determine leaf area non-destructively.

**Key words:** *Dendranthema grandiflora* Tzevelev, linear models, biometrics, allometric equations

**RESUMO:** O crisântemo (*Dendranthema grandiflora*) é a segunda planta ornamental mais produzida e comercializada no mundo. A medição da área foliar por métodos não destrutivos é fundamental para estudos sobre seu crescimento e produção. A estimativa da área foliar por dimensões lineares das folhas pode ser uma estratégia para este fim. O objetivo deste estudo foi encontrar equações alométricas para estimar a área foliar do crisântemo. Os modelos de regressão linear, linear sem intercepto, quadrático, cúbico, potência e exponencial foram utilizados para a análise. A escolha das equações foi baseada nos maiores coeficientes de determinação. O método não destrutivo por meio de modelos alométricos tem acurácia para estimar a área foliar (AF) do crisântemo a partir do produto entre o comprimento da folha (C) e a largura da folha (L). A AF do crisântemo pode ser estimada pela equação $\hat{y} = 0{,}6611*CL^{0{,}9490}$ (C – comprimento da folha; L – largura da folha). Essa equação permitirá que pesquisadores e produtores determinem a área foliar de forma não destrutiva.

**Palavras-chave:** *Dendranthema grandiflora* Tzevelev, modelos lineares, biometria, equações alométricas

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## Introduction

Chrysanthemum (*Dendranthema grandiflora* Tzevelev – Asteraceae) is one of the most popular ornamental plants worldwide (Schroeter-Zakrzewska & Pradita, 2021), being well known and cultivated as cut flowers, vase flowers, and garden plants (Bandurska et al., 2022). This species is the second largest in the commercial flower industry, second only to rose (Su et al., 2019).

Leaf area is directly related to transpiration and photosynthetic rates. A larger leaf area promotes greater interception of solar radiation, which, under appropriate temperature and water availability conditions, results in increased production of photoassimilates used in flowering, thus enhancing the formation of visually appealing flowers (Liu et al., 2017; Sabouri & Sajadi, 2022).

Leaf area can be determined by destructive (direct, e.g., pruning, defoliation, and shaping) and non-destructive (indirect, e.g., allometric models) methods (Zhang, 2020). Destructive methods are simple and accurate, but require more time to perform, and the plant is killed (Salazar et al., 2018). The non-destructive method by allometric equations from the length and width of the leaves is as efficient as the direct methods, besides being more practical and precise, and allows successive evaluations of the same plant with speed and precision (Pinheiro et al., 2020).

Digital processing methods are feasible, accurate, and economical tools for determining linear models for leaf area estimation (Sauceda-Acosta et al., 2017). The importance of studying chrysanthemum leaf area lies in its direct impact on growth and productivity, contributing to the understanding of plant development and the economic value of chrysanthemum production worldwide. Studies on non-destructive measurement of chrysanthemum leaf area have been conducted in the past (Wulfsohn et al., 2010; Fanourakis et al., 2021). However, studies on non-destructive measurement of leaf area in cut chrysanthemums are still in their early stages, especially under cultivation conditions in Brazil. This study is pioneering in the measurement of leaf area in 'Sunny Reagan' chrysanthemums cultivated in Brazil. Thus, the objective of this study was to find allometric equations to estimate the leaf area of chrysanthemum.

## Material and Methods

The study was performed at the Universidade Federal de Viçosa, Minas Gerais state, Brazil (20° 45' S 42° 52' W, and altitude of 690 m). Plants were grown from February to March 2021. Eight hundred leaves of varying sizes were collected from 100 chrysanthemum plants (var. 'Sunny Reagan'). The leaves were healthy and had no deformities due to pests or diseases. The leaves were collected from plants at the beginning of the blooming stage, with at least one well-developed flower bud.

Leaf length (L) and leaf width (W) (Figure 1) were measured from images scanned on a flatbed scanner (Epson model L395, Tokyo city, Japan) with a reference scale. From these data, the product of length by width (LW) was calculated. The leaf area measurements were made using ImageJ® software (National Institutes of Health, USA), with contrasted images to facilitate the measurements.

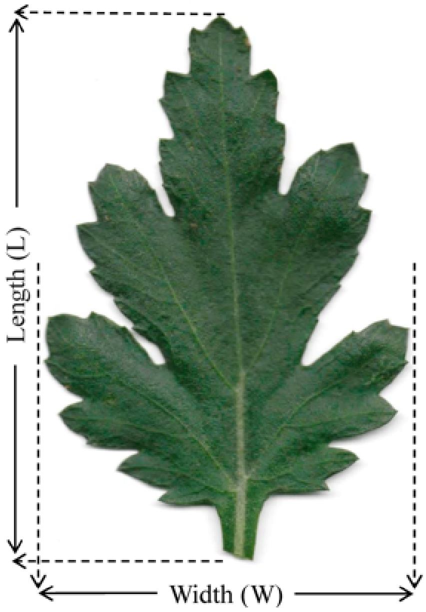


**Figure 1.** Maximum length (L) and width (W) of leaf of chrysanthemum used to estimate leaf area

A regression analysis was performed to obtain equations for the calculation of chrysanthemum leaf area. The statistical equations: linear ($\hat{y} = \beta_0 + \beta_1{*}x + \varepsilon_i$); linear without intercept ($\hat{y} = \beta_1{*}x + \varepsilon_i$); quadratic ($\hat{y} = \beta_0 + \beta_1{*}x + \beta_2{*}x + \varepsilon_i$); cubic ($\hat{y} = \beta_0 + \beta_1{*}x + \beta_2{*}x + \beta_3{*}x + \varepsilon_i$); power ($\hat{y} = \beta_0 * x^{\beta 1} + \varepsilon_i$); and exponential ($\hat{y} = \beta_0 * \beta_1^{x} + \varepsilon_i$) were used for the analysis.

A descriptive analysis of the leaf area data measured through the ImageJ® software was used to obtain the maximum and minimum values, mean, median, variance, standard deviation, standard error and coefficient of variation, and the coefficients of skewness and kurtosis were also determined. Regression analyses were performed to choose the equation for estimating chrysanthemum leaf area. The value of ŷ estimated the leaf area (LA) as a function of x, whose values were represented by the linear variables of the leaves (L - length, W - width, and LW). The best equations were chosen based on the highest coefficient of determination ($R^2$, Eq. 1), Pearson's correlation coefficients (r, Eq. 2) and Willmott index (d, Eq. 3, Willmott et al., 1985), and lowest Akaike's information criterion (AIC, Eq. 4, Akaike, 1974), and root mean square error (RMSE, Eq. 5). The Willmott index measures forecast accuracy by comparing predicted values to observed data, ranging from 0 to 1, for no correlation to perfect correlation, and is used in various fields to evaluate forecasting performance. AIC is a statistical measure used to compare the relative quality of different statistical models. It balances the goodness of fit of a model with its complexity, aiming to find the model that best balances these two factors. The lower the AIC value, the better the model is considered to be in terms of accurately representing the data while keeping model complexity in check.

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

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

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

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

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

where:

$\hat{y}_i$ - estimated values of LA;

$y_i$ - observed values of LA;

$\bar{y}_i$ - average of observed values;

$\hat{y}'_i = \bar{y}'_i - \bar{y}'$;

$\bar{y}'_i = y_i - \bar{y}$;

$L(x\setminus\theta)$ - maximum likelihood function;

p - number of model parameters;

n - number of observations;

$x_i$ and $y_i$ - observations of the variables y and x, respectively; and,

$\bar{y}$ and x - means of variables y and x, respectively.

Statistical analyses were performed using R software (R Core Team, 2022).

## Results and Discussion

The dataset displayed a significant degree of variability, showing a wide array of leaf sizes. Leaf length (L), leaf width (W), product of length by width (LW), LL, WW, and actual leaf area (LA) exhibited diverse ranges, highlighting the wide array of leaf dimensions explored in this study (Table 1). The size and shape of a leaf directly impact the exchange of energy and mass, as the thickness of the boundary layer restricts the transfer of heat, water vapor, and carbon (Verwijst & Wen, 1996). Consequently, studying leaf variability becomes essential, given the significant variations that can occur even when leaves are cultivated under similar conditions. For this investigation, a total of eight hundred 'Sunny Reagan' chrysanthemum leaves were used to determine their respective leaf areas.

Wide data variability is critical in regression model studies to estimate plant leaf area (LA) (Gomes et al., 2020), since the high variability of the data allows the estimation of more representative models and more accurate equations that can be applied to leaves of different shapes and sizes (Cargnelutti Filho et al., 2021). Direct collection of LA data necessitates a considerable number of leaf measurements, making it an expensive, time-consuming, destructive, and equipment-intensive endeavor. Consequently, the use of an accurate and non-destructive model for LA estimation would eradicate these costs and time requirements, making it more commercially viable (Montelatto et al., 2021).

There was high variability of data, ranging from 24.21 (L) to 53.07% (WW). The standard deviations of leaf length and width were low (1.781 and 1.179, respectively), while for LW the standard deviation was considered high (17.306), and this is related to the varied leaf sizes (Silva et al., 2017). The asymmetry of the LW, LL, WW, and LA data indicated higher frequency of leaves with values near the minimum and lower frequency of those with values near the maximum values, confirming the normality of the data (Ribeiro et al., 2022a). The kurtosis coefficients (k) of L and W had a leptokurtic distribution ($k < 3.26$), while those of LW, LL, WW and LA had a platykurtic distribution, flatter than the normal distribution ($k > 3.26$). All variables evaluated had normal distribution ($p \geq 0.05$), characterizing an adequate fit of the data.

This study was carried out with the analysis of 800 chrysanthemum leaves from different parts of the plants, being a sample considered ideal for the construction of models that estimate LA from linear measurements of the leaves (Ribeiro et al., 2022b), since using a small sample size to produce allometric models can lead to the generation of biased equations with low reliability to estimate LA.

Patterns of linear and non-linear association between the values of L, W, LW, LL, WW, and LA were observed in the data set used to construct the predicted regression models for LA estimation (Figure 2). Linear patterns were observed between W and LA, LW and LA, LL and LA, and WW and LA, while nonlinear patterns were evident between L and LA, indicating the need for different regression models to fit and validate the data for leaf area estimation.

The models had coefficients of determination ($R^2$) between 0.85 and 0.99, indicating that at least 85% of the variation in chrysanthemum leaf area was explained by the proposed equations (Table 2). The equations that used the product between length and width (LW) had the best criteria for estimating the LA of this species, having the best fits of the regression models (Goergen et al., 2021). However, the exponential model had different results, and the best criteria were observed in the equation where the leaf length (L) value

**Table 1.** Descriptive statistics of chrysanthemum leaves

| Descriptive statistics | L | W | LW | LL | WW | LA |
|---|---|---|---|---|---|---|
| Minimum | 2.802 | 2.073 | 7.706 | 7.851 | 4.297 | 4.670 |
| Maximum | 13.527 | 9.010 | 121.878 | 182.980 | 81.180 | 59.333 |
| Median | 7.240 | 4.379 | 31.229 | 52.418 | 19.171 | 17.477 |
| Mean | 7.356 | 4.498 | 34.997 | 57.283 | 21.623 | 19.167 |
| Standard error | 0.063 | 0.042 | 0.612 | 0.973 | 0.406 | 0.325 |
| CV (%) | 24.210 | 26.210 | 49.450 | 48.020 | 53.070 | 47.950 |

L - Leaf length; W - leaf width; LW - Product of length by width; LL - Product of length by length; WW - Product of width by width; LA - Actual leaf area

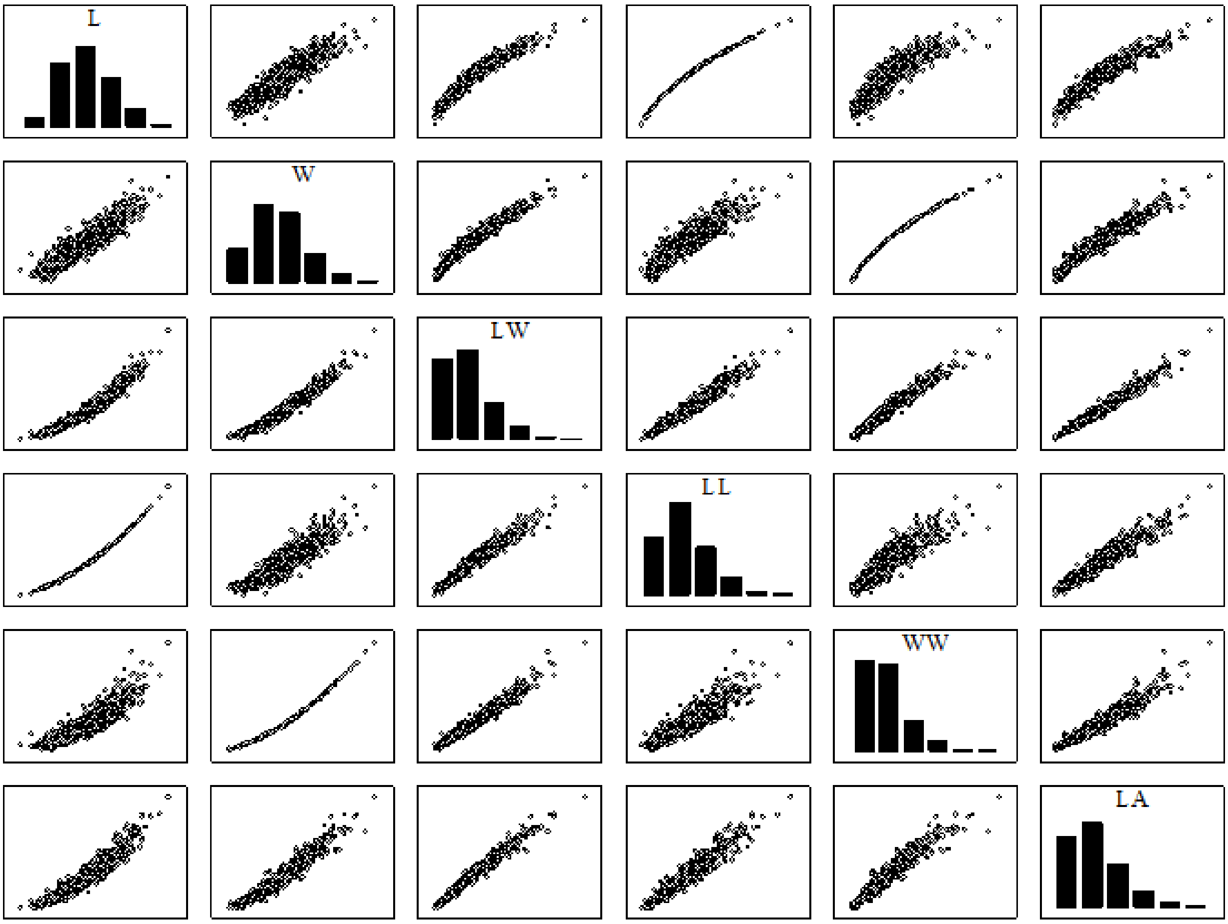


**Figure 2.** Frequency histograms (diagonal) and data dispersion between the length (L), width (W), product of length and width (LW), product of length and length (LL), product of width and width (WW), and leaf area (LA) of 800 chrysanthemum leaves used to build equations for estimating leaf area

**Table 2.** Models, coefficient of determination ($R^2$), Pearson's linear correlation coefficient (r), Willmott agreement index (d), index closest to one (CS), Akaike information criterion (AIC), mean absolute error (MAE), root mean square error (RMSE), and equations for estimating the leaf area (LA) of chrysanthemum as a function of linear leaf dimensions (length and width)

| Model | x | $R^2$ | r | d | CS | AIC | MAE | RMSE | Equation |
|---|---|---|---|---|---|---|---|---|---|
| Linear | L | 0.9027 | 0.9502 | 0.9739 | 0.9253 | 3959.4 | 2.198 | 2.863 | $\hat{y} = -16.908+4.904*L$ |
| Linear | W | 0.9223 | 0.9604 | 0.9794 | 0.9407 | 3779.6 | 1.935 | 2.559 | $\hat{y} = -14.506+7.486*W$ |
| Linear | LW | 0.9681 | 0.9840 | 0.9919 | 0.9759 | 3066.6 | 1.202 | 1.639 | $\hat{y} = 0.878+0.522*LW$ |
| Linear (0.0) | LW | 0.9690 | 0.9840 | 0.9917 | 0.9758 | 3108.5 | 1.233 | 1.684 | $\hat{y} = 0.542*LW$ |
| Linear | LL | 0.9205 | 0.9595 | 0.9789 | 0.9393 | 3797.4 | 1.949 | 2.587 | $\hat{y} = 0.802+0.320*LL$ |
| Linear | WW | 0.9229 | 0.9607 | 0.9796 | 0.9412 | 3773.2 | 1.850 | 2.549 | $\hat{y} = 2.527+0.769*WW$ |
| Quadratic | L | 0.9205 | 0.9595 | 0.9789 | 0.9393 | 3799.1 | 1.949 | 2.587 | $\hat{y} = 0.173+0.170*L+0.309*L^2$ |
| Quadratic | W | 0.9290 | 0.9639 | 0.9813 | 0.9460 | 3708.2 | 1.789 | 2.444 | $\hat{y} = -5.961+3.679*W+0.396*W^2$ |
| Quadratic | LW | 0.9695 | 0.9847 | 0.9922 | 0.9770 | 3046.7 | 1.177 | 1.602 | $\hat{y} = -0.330+0.591*LW-0.0006*LW^2$ |
| Quadratic | LL | 0.9205 | 0.9595 | 0.9790 | 0.9393 | 3798.9 | 1.949 | 2.587 | $\hat{y} = 0.582+0.328*LL-0.00005*LL^2$ |
| Quadratic | WW | 0.9311 | 0.9650 | 0.9819 | 0.9476 | 3684.5 | 1.770 | 2.408 | $\hat{y} = -0.017+0.996*WW-0.003*WW^2$ |
| Cubic | L | 0.9204 | 0.9595 | 0.9790 | 0.9393 | 3800.8 | 1.949 | 2.587 | $\hat{y} = 2.309-0.726*L+0.429*L^2-0.005*L^3$ |
| Cubic | W | 0.9314 | 0.9652 | 0.9820 | 0.9479 | 3681.7 | 1.760 | 2.401 | $\hat{y} = 8.163-5.812*W+2.395*W^2-0.132*W^3$ |
| Cubic | LW | 0.9695 | 0.9844 | 0.9920 | 0.9765 | 3033.3 | 1.187 | 1.619 | $\hat{y} = 0.019+0.561*LW-0.0001*LW^2-0.000004*LW^3$ |
| Cubic | LL | 0.9204 | 0.9595 | 0.9790 | 0.9393 | 3800.7 | 1.949 | 2.586 | $\hat{y} = 0.853+0.314*LL+0.0001*LL^2-0.0000008*LL^3$ |
| Cubic | WW | 0.9312 | 0.9651 | 0.9820 | 0.9477 | 3684.3 | 1.765 | 2.405 | $\hat{y} = 0.576+0.917*WW-0.001*WW^2-0.00002*WW^3$ |
| Power | L | 0.9206 | 0.9595 | 0.9790 | 0.9393 | 3797.4 | 1.949 | 2.588 | $\hat{y} = 0.395*L^{1.919}$ |
| Power | W | 0.9283 | 0.9635 | 0.9809 | 0.9450 | 3717.7 | 1.794 | 2.462 | $\hat{y} = 1.371*W^{1.728}$ |
| Power | LW | 0.9937 | 0.9847 | 0.9922 | 0.9770 | 3032.5 | 1.175 | 1.601 | $\hat{y} = 0.6611*LW^{0.9490}$ |
| Power | LL | 0.9206 | 0.9595 | 0.9790 | 0.9393 | 3797.4 | 1.949 | 2.588 | $\hat{y} = 0.3953*LL^{0.9598}$ |
| Power | WW | 0.9283 | 0.9635 | 0.9809 | 0.9450 | 3717.7 | 1.794 | 2.462 | $\hat{y} = 1.3711*WW^{0.8639}$ |
| Exponential | L | 0.9049 | 0.9513 | 0.9733 | 0.9259 | 3951.7 | 2.204 | 2.849 | $\hat{y} = 3.377*1.253^{L}$ |
| Exponential | W | 0.8912 | 0.9440 | 0.9680 | 0.9139 | 4071.2 | 2.314 | 3.070 | $\hat{y} = 4.339*1.372^{W}$ |
| Exponential | LW | 0.8912 | 0.9440 | 0.9680 | 0.9139 | 4199.9 | 2.314 | 3.070 | $\hat{y} = 9.440*1.019^{LW}$ |
| Exponential | LL | 0.8756 | 0.9357 | 0.9608 | 0.8991 | 4313.2 | 2.513 | 3.328 | $\hat{y} = 9.000*1.012^{LL}$ |
| Exponential | WW | 0.8534 | 0.9238 | 0.9547 | 0.8820 | 4539.3 | 2.758 | 3.572 | $\hat{y} = 10.451*1.027^{WW}$ |

* Significant at $p \leq 0.05$

was used (Ribeiro et al., 2020). The selection of the optimal model should not rely solely on $R^2$ and RMSE, but should also consider the use of an accuracy measure (Salazar et al., 2018).

The criteria used to choose the best equations for estimating chrysanthemum LA using linear leaf dimensions confirmed that the power model built with LW values was the most accurate. This model had the highest coefficient of determination ($R^2$) (0.9937), Pearson's correlation coefficient (r = 0.9847) and Willmott index (d = 0.9922) and the lowest Akaike's information criterion (AIC = 3032.5) and root mean square error (RMSE) values (1.601) (Table 2). The power models were also the best fit for estimating the LA of *Tectona grandis* (Tondjo et al., 2015), *Manihot esculenta* (Trachta et al., 2020) and *Arachis hypogaea* (Ribeiro et al., 2022c). In their study on chrysanthemum varieties, Fanourakis et al. (2021) emphasized the importance of considering both leaf length (L) and width (W) when estimating leaf area (LA). They found that incorporating both dimensions led to a more precise estimation of LA compared to relying on a single leaf dimension. The fact that changes in L and W are typically not proportionate across duplicated leaves, combined with additional variations in leaf shape, compromises the accuracy of the LA estimate when using a single leaf dimension (Verwijst & Wen, 1996).

The proposed equation to estimate the LA of chrysanthemum had a high fit of the data ($R^2$ = 0.99), in which the residual variance was homogeneous, with little data dispersion (Figure 3A). The LA data estimated from the constructed equation had positive correlations with the observed values (measured from the digital images), with a coefficient of determination ($R^2$) greater than 0.97 (Figures 3A and B). With this, the equation $\hat{y} = 0.6611*LW^{0.9490}$ is the best fit to accurately (> 99%) estimate chrysanthemum LA by linear leaf dimensions.

The model identified ($\hat{y} = 0.6611*LW^{0.9490}$) is relevant for studies of ornamental plants such as chrysanthemum, since from the knowledge of leaf area it is possible to analyze growth, development and photosynthetic rates, in addition to conducting studies of shading, landscape capacity and ecology of the species (Dias et al., 2022). Therefore, using accurate equations to estimate chrysanthemum leaf area by measuring leaf area using nonlinear models is a methodology as efficient as destructive methods. In addition, the model studied can be even more efficient, since it is not necessary to destroy the plant to estimate its leaf area. The use of the product between length and width (LW) to estimate leaf area was more efficient for chrysanthemum as well as for *Solanum melongena* (Hinnah et al., 2014), *Brassica napus* (Cargnelutti et al., 2015), *Bambusa vulgaris* (Montelatto et al., 2021), *Thunbergia grandiflora* (Mela et al., 2022) and *Eustoma grandiflorum* (Dias et al., 2022). The development of mathematical equations for leaf area estimation provides a crucial and convenient method for quickly determining leaf area, which can be easily adapted for field use and allows for multiple evaluations of the same plants throughout their growth cycle (Goergen et al., 2021).

In this study, the innovation in measuring the leaf area of chrysanthemum plants is highlighted as crucial for understanding variability and developing accurate estimation models. Direct measurements of leaf area are expensive, time-consuming, and destructive, making the need for non-destructive alternatives important. Among the models tested, the power model using the product of leaf length and width (LW) values provided the best fit. It showed the highest $R^2$, correlation coefficient, and agreement index, as well as the lowest AIC and root mean square error values. Incorporating both leaf length and width in the estimation resulted in more accurate predictions compared to using a single leaf dimension. The proposed equation ($\hat{y} = 0.6611*LW^{0.9490}$) achieved over 99% accuracy in estimating chrysanthemum leaf area. This innovative approach to leaf area estimation has various applications in plant analysis, including assessing growth, development, and photosynthetic rates. It offers a cost-effective and non-destructive alternative to direct measurement methods, providing researchers and growers with a valuable tool for studying and understanding chrysanthemum plants.

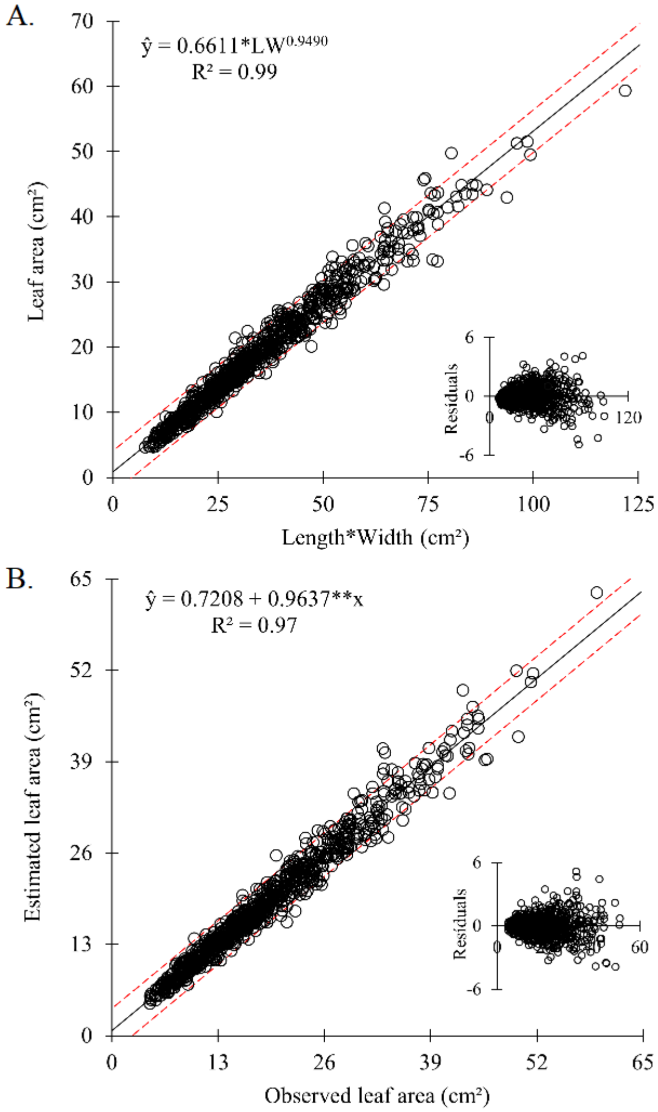


**Figure 3.** Relationship between leaf area (LA) and length × width (LW) (A) and relationship between estimated digital leaf area and observed leaf area (B)

## Conclusions

1. The non-destructive method using allometric models has accuracy for estimating leaf area (LA) of chrysanthemum from linear leaf dimensions.

2. The LA of chrysanthemum can be estimated by the equation $\hat{y} = 0.6611*LW^{0.9490}$ using the product between length (L) and width (W).

3. This equation will allow researchers and producers to determine leaf area non-destructively.

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