ISSN 1807-1929
Revista Brasileira de Engenharia Agrícola e Ambiental
Brazilian Journal of Agricultural and Environmental Engineering
v.25, n.5, p.305-311, 2021
Campina Grande, PB – http://www.agriambi.com.br – http://www.scielo.br/rbeaa

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v25n5p305-311

Allometric relationship and leaf area modeling estimation on chia by non-destructive method[1]

Relação alométrica e modelagem da estimativa da área foliar em chia por método não destrutivo

Patrícia C. H. Goergen[2*], Isabel Lago[2], Natalia T. Schwab[2], Alexandre F. Alves[2], Charles P. de O. Freitas[2] & Verlaine S. Selli[2]

[1] Research developed at experimental area of the Universidade Federal de Santa Maria/Departamento de Ciências Florestais, Santa Maria, RS, Brazil
[2] Universidade Federal de Santa Maria, Santa Maria, RS, Brazil

HIGHLIGHTS:

The model based on the length and width dimensions is the most appropriate to estimate leaf area in chia crop.
Leaf area estimated by a non-destructive method has high precision and allows continuous evaluations in chia plants.
It is possible to estimate the leaf area in chia crop by counting the number of leaves on the main stem.

ABSTRACT: This study aimed to obtain equations to estimate leaf area from linear leaf dimensions and establish the allometric relationship between leaf area and the number of leaves on the main stem of chia (*Salvia hispanica* L.) at different sowing times. The experiment was conducted in the agricultural year 2016/2017 on five sowing times in Santa Maria, RS, Brazil, in a randomized block design with four repetitions. In each plot, ten random plants were marked weekly during the vegetative phase to determine the number of leaves (NL) in the main stem, and three of these for the determination of leaf area (LA). A total of 70 leaves of different sizes were used to calibrate the model. Another 106 leaves were used to test the predictive capacity of the equations by various statistical indices. The length (L) and the largest leaf width (W) were measured. Leaf collection was carried out during the cycle, in all sowing times to represent all leaf sizes. The linear, quadratic, exponential, and potential models were adjusted. The non-destructive method, through the linear dimensions of the leaf, is appropriate for estimating the leaf area in chia. The general equation LA = 0.642 (L x W) can be used to estimate the leaf area of the chia plants without loss of precision. The potential model is appropriate to characterize the allometric relationship between leaf area evolution and the number of leaves accumulated in the main stem of chia at different sowing times.

Key words: *Salvia hispanica* L., leaf area, mathematical models, linear dimensions, plant growth

RESUMO: Objetivou-se neste estudo obter equações para estimar a área foliar a partir de dimensões lineares da folha e estabelecer a relação alométrica entre a área foliar e o número de folhas da haste principal de chia (*Salvia hispanica* L.) em diferentes épocas de semeadura. O experimento foi conduzido no ano agrícola 2016/2017 em cinco épocas de semeadura em Santa Maria, RS, Brasil, em delineamento de blocos ao acaso com quatro repetições. Em cada parcela, dez plantas ao acaso foram marcadas para determinação semanal do número de folhas (NF) na haste principal durante a fase vegetativa, e três para determinação da área foliar (AF). Um total de 70 folhas de diferentes tamanhos foram usadas para calibrar o modelo e outras 106 folhas foram usadas para testar a capacidade preditiva das equações por vários índices estatísticos, nas quais foram medidos o comprimento (C) e a maior largura da folha (L). A coleta de folhas foi realizada durante o ciclo de desenvolvimento de todas as épocas de semeadura para ter representação de todos os tamanhos de folhas. Os modelos linear, quadrático, exponencial e potencial foram ajustados. O método não destrutivo, através das dimensões lineares da folha, é adequado para estimar a área foliar em chia. A equação geral AF = 0.642 (L x W) pode ser usada para estimar a área foliar das plantas de chia sem perda de precisão. O modelo potencial é adequado para caracterizar a relação alométrica entre a evolução da área foliar e o número de folhas acumuladas na haste principal de chia em diferentes épocas de semeadura.

Palavras-chave: *Salvia hispanica* L., área foliar, modelos matemáticos, dimensões lineares, crescimento de planta

• Ref. 228412 – Received 08 Sept, 2019
* Corresponding author - E-mail: patygoergen@hotmail.com
• Accepted 03 Feb, 2021 • Published 05 Mar, 2021
Edited by: Hans Raj Gheyi

Introduction

Chia (*Salvia hispanica* L.), a plant of the Lamiaceae family, is considered a nutraceutical food (Jamboonsri et al., 2012). During the vegetative phase, the leaf growth increases leaf area (LA) (Pérez Brandán et al., 2019). Leaf area determination is essential in evaluating plant photosynthetic efficiency and growth analysis studies because it is an excellent determinant of the yield (Zanon et al., 2015).

Destructive or non-destructive methods can accomplish the LA quantification. Methods that require leaf removal are termed destructive and have the disadvantage that it is not applicable in studies where the number of samples is limited (Adami et al., 2008). On the other hand, non-destructive methods are based on measurements carried out on plants, with the advantage that sampling can be performed on the same plant throughout the development cycle, reducing the experimental error (Bakhshandeh et al., 2011). Studies have shown the viability of LA estimation from linear measures of the leaf (length, width, or its product) in crops (Fagundes et al., 2009; Maldaner et al., 2009; Schwab et al., 2014; Richter et al., 2014).

An indirect empirical method of LA estimation is the allometric relationship between LA and the number of leaves. This relationship has already been established in several crops (Sinclair et al., 2004; Pivetta et al., 2007; Okami et al., 2012; Rosa et al., 2013).

In the literature, there are no studies with LA estimation models for chia from linear leaf dimensions. With the increasing cultivation and the demand for studies that aim to explain the growth of this plant, it is necessary to estimate non-destructive models that estimate the LA of chia. This study aimed to obtain equations to estimate leaf area from linear leaf dimensions and establish an allometric relationship between leaf area and the number of leaves on the main stem of chia at different sowing times.

Material and Methods

The experiment was conducted in 2016/2017 in the experimental area of the Plant Science Department at the Federal University of Santa Maria (UFSM), Santa Maria, RS, Brazil (29° 43' S, 53° 43' W and 95 m a.s.l.).

According to the Köppen classification, the regional climate is Cfa-type humid subtropical with no dry season defined and hot summers (Kuinchtner & Buriol, 2001). The local soil is classified as Ultisol (USDA, 2014).

The experiment occurred on five sowing times concerning the most suitable sowing time (October/November) (Migliavacca et al., 2014): September 22, 2016 (early), October 28, 2016 (recommended), January 03, 2017 (late), February 08, 2017 (late), and March 24, 2017 (late). The experimental design was a randomized block with four replicates. Each experimental unit consisted of five rows measuring 3.0 m in length, spaced 0.70 m between rows, for a total area of 10.5 m^2. The plants of the three central lines were evaluated, excluding the extremities.

Management practices, such as pH correction and soil fertilization, were performed according to the soil analysis, based on recommendations for the mint (*Mentha arvensis* L.), a closely related species, since there is no recommendation for chia.

The chia seeds were acquired from local suppliers. The sowing was done manually, 1 cm deep, because the seeds are small and with a low reserve amount. After emergence, excess plants were thinned, maintaining 5 to 6 cm between plants in the line, establishing 20 plants per linear meter (Migliavacca et al., 2014).

To estimate the equation that relates the individual leaf's area and the linear dimensions (L, W, L x W), 70 leaves of different sizes and positions were collected throughout the crop development cycle. Leaf collection was carried out during the life cycle of all sowing times to represent all leaf sizes. After that, the biggest leaf length (L) and width (W) were measured. Then, each leaf was scanned in a 300-dpi scanner. The individual leaf area (LA) was calculated using the software Quant, version 1.0.1 (2003). The relationship between the leaf area and its linear dimensions was adjusted to the linear, quadratic, potential, and exponential models considering the linear dimensions, individually, and its product by the equations:

Linear:

$$LA = a + b(L) \tag{1}$$

$$LA = a + b(W) \tag{2}$$

$$LA = a + b(L \times W) \tag{3}$$

where:

LA - leaf area, cm^2;
L - leaf length, cm;
W - largest leaf width, cm; and,
a - the linear coefficient and b - the angular coefficient.

Potential:

$$LA = a(L)^b \tag{4}$$

$$LA = a(W)^b \tag{5}$$

$$LA = a(L \times W)^b \tag{6}$$

where:

a - the form coefficient and b - the power coefficient.

Quadratic:

$$LA = a(L)^2 + b(L) + c \tag{7}$$

$$LA = a(W)^2 + b(W) + c \tag{8}$$

$$LA = a(L \times W)^2 + b(L \times W) + c \tag{9}$$

where:

a, b and c - the quadratic coefficients.

Exponential:

$$LA = a\ e^{(L)} \quad (10)$$

$$LA = a\ e^{(W)} \quad (11)$$

$$LA = a\ e^{(L \times W)} \quad (12)$$

where

a - the form coefficient and e - the exponential coefficient.

To test the predictive capacity of Eqs. 1 - 12, 106 leaves were collected from plants sown at five sowing times during the 2016/2017 harvest, and leaf collection was carried out during the cycle of all sowing times to have a representation of all leaf sizes in the growing season. The performance of Eqs. 1 to 12 was evaluated by mean absolute error (MAE) - Eq. 13; root mean square error (RMSE) - Eq. 14; BIAS index - Eq. 15; concordance index (d) - Eq. 16; and modified concordance index (d1) - Eq. 17:

$$MAE = \frac{\sum_{i=1}^{N} |Si - Oi|}{N} \quad (13)$$

$$RMSE = \sum_{i=1}^{N} \frac{(Si - Oi)^2}{N} \quad (14)$$

$$BIAS = \frac{\sum_{i=1}^{N} Si - \sum_{i=1}^{N} Oi}{\sum_{i=1}^{N} Oi} \quad (15)$$

$$d = 1 - \left[\frac{\sum_{i=1}^{N} (Si - Oi)^2}{\sum_{i=1}^{N} (|Si'| + |Oi'|)^2} \right] \quad (16)$$

$$d1 = 1 - \left[\frac{\sum_{i=1}^{N} |Si - Oi|}{\sum_{i=1}^{N} (|Si'| + |Oi'|)} \right] \quad (17)$$

In Eqs. 13, 14, 15, 16, and 17, Si represents the estimated values, Oi the observed values, and N the number of observations, where Si' = Si - Ō and Oi' = Oi - Ō.

The MAE and the RMSE represent the magnitude of the error produced by the model, therefore closer to zero, the better is the model. MAE is less sensitive to extreme values than RMSE since it does not square the difference between estimated and observed values (Schwab et al., 2014). The BIAS index expresses the mean deviation of the estimated values from the observed values, thus indicating the tendency of the model to overestimate or underestimate the estimated values and, thereby, the closer to zero this statistic, the smaller systematic error magnitude of the model (Leite & Andrade, 2002).

The d index is a measure of how much the model is error-free; the closer to 1, the less error this estimate presents (Willmott, 1981). The d index values range from 0 (no agreement) to 1 (perfect agreement). The d1 index (Eq. 17) is a modification of the d index (Eq. 16) suggested by Willmott et al. (1985), since the use of quadratic function can result in high values of this index and, therefore, the proposed modification tends to result in a more rigorous index. The d1 index vary from 0 (no agreement) to 1 (perfect agreement).

Afterward, regression analyses were performed between the dependent variables (leaf area or total leaf area per plant obtained from the scanner) and independent variables [width (W), length (L), product (L x W) and the number of leaves (NL) per plant], adjusting for the linear, quadratic, potential and exponential equations. The Excel software, Version 2013®, was used to adjust the regression equations. With the best equation, the allometric relationships between LA and NL were established.

For NL and LA estimation, 10 plants in each plot (totaling 40 plants at each sowing date) were marked with colored wire in which the number of visible leaves on the main stem were counted weekly. In three of these marked plants, the length (L) and width (W) of the visible leaves were measured to estimate the photosynthetically active leaf area. Each leaf was considered visible when it had more than 2 cm in length.

To establish allometric relationships between LA and NL, the potential non-linear model $y = aX^b$ was adjusted to the accumulated LA data (cm^2) on the main stem (Y) and NL (X) on the main stem, being "a" and "b" adjusted coefficients representing respectively the area of the first leaf (cm^2) and the rate of accumulation area for each additional leaf ($cm^2\ cm^{-2}$) on the main stem. The potential model was chosen because it is realistic from the ecophysiological point of view since it describes the evolution of the leaf area with the number of leaves accumulated in a non-linear way, having been previously used in other crops (Maldaner et al., 2009; Aquino et al., 2011; Cargnelutti Filho et al., 2012; Rosa et al., 2013; Schwab et al., 2014). The data used to adjust the potential model as a model of the allometric relationships between NL and LA were collected in the leaf emission period.

The data were analyzed by R software (R CORE TEAM, 2020), with metan (Olivoto & Lucio, 2020) and ggplot2 (Wickham, 2016) packages.

Results and Discussion

The chia plants had leaves of different sizes throughout the experiment for each of the five sowing times, as shown in Figure 1.

The wide variability of leaf size, verified by the different lengths, widths, and leaf areas, is essential for the models' generation since it includes the use of a small, medium, and large leaves. Therefore, this dataset of length (L), width (W),

Figure 1. Leaves of chia plants of different linear dimensions (length - L and width - W) grown on five sowing times in the 2016/2017 harvest

product (L x W), and leaf area (Y) determined by 106 leaves measurements are adequate for the proposed study (CV = 63%).

In Figure 2, leaf area (LA) versus length (L), width (W), and product of length and width (L x W) were plotted, and the adjusted equations for each model (Eqs. 1 - 12, respectively) is shown in Table 1. When only one of the linear dimensions (L or W) is used as a predictor, the b coefficient is greater than 1, indicating a non-linear relationship between the two variables, but without saturation in the range of the measured values. When the product of length and width (L x W) is used as a predictor, the relationship becomes linear, with a higher coefficient of determination (Table 1), that is, to estimate leaf area of chia, it is more appropriate the use of two leaf dimensions instead only one of them.

The 1:1 plots with the performance of Eqs. 1 - 12 are shown in Figure 3, and the statistics of the performance of equations are listed in Table 1. The dots are much closer to a 1:1 line when the product (L x W) was used (Figure 3).

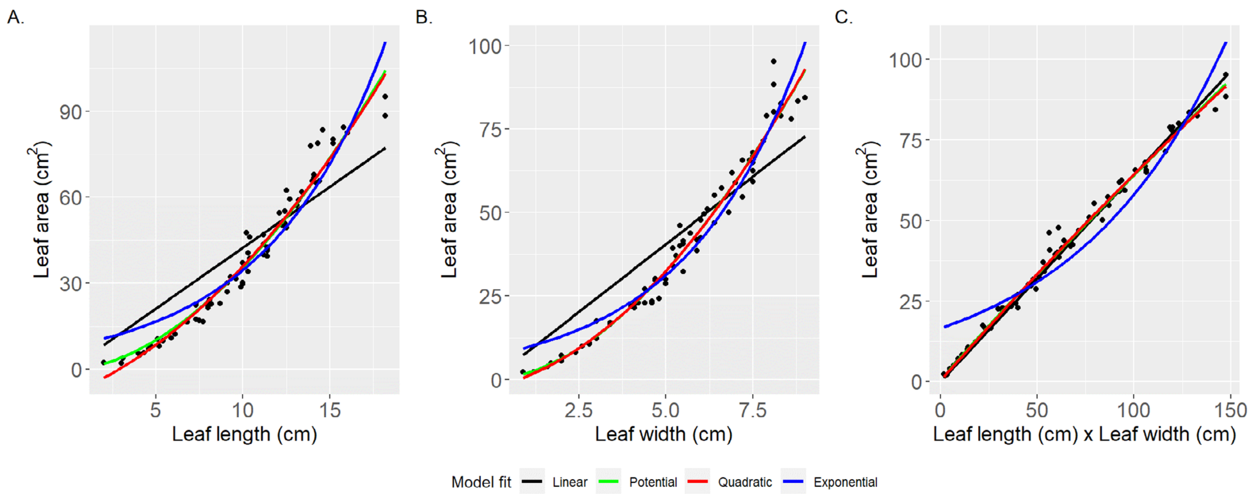
According to the analyzed data, the best equation, which presents MAE closest to zero, was the linear equation LA = 0.642 (L x W), and the worst equation was the linear equation LA = 4.256 (L). For the RMSE, the linear equation LA = 0.642 (L x W) was the best equation, and the worst was the exponential equation LA = 3.2962 $e^{0.4141(W)}$. For the BIAS index, the result closest to zero was found for the potential equation LA = 0.3842 $(L)^{1.9497}$, which is the best equation for this statistic, and the farthest from zero was found for the linear equation LA = 8.1185 (W), which is the worst one.

The equations with the highest d index are the linear equation LA = 0.642 (L x W) and the potential equation LA = 0.8567 $(L \times W)^{0.9349}$, while the equation that presented the lowest d index was the exponential equation LA = 3.2962 $e^{0.4141(W)}$. The d1 index values range from 0 (no agreement) to 1 (perfect agreement). The best equation (which presented d1 closest to one) was the linear equation LA = 0.642 (L x W), and the worst was the linear equation LA = 6.4691 (L). Except for the BIAS index, the equation that presented the best performance in all the statistics was LA = 0.642 (L x W).

From this, the equation LA = 0.642 (L x W) can be used to estimate the leaf area (cm^2) of chia leaf, where L is the length (cm) and W the width (cm) of the leaf. The linear model, using the product of the linear dimensions was also the best prediction of leaf area for *Raphanus sativus* L., soybean cultivars, gladiolus, and sunflower plants respectively by Cargnelutti Filho et al. (2012), Richter et al. (2014), Schwab et al. (2014), and Aquino et al. (2011).

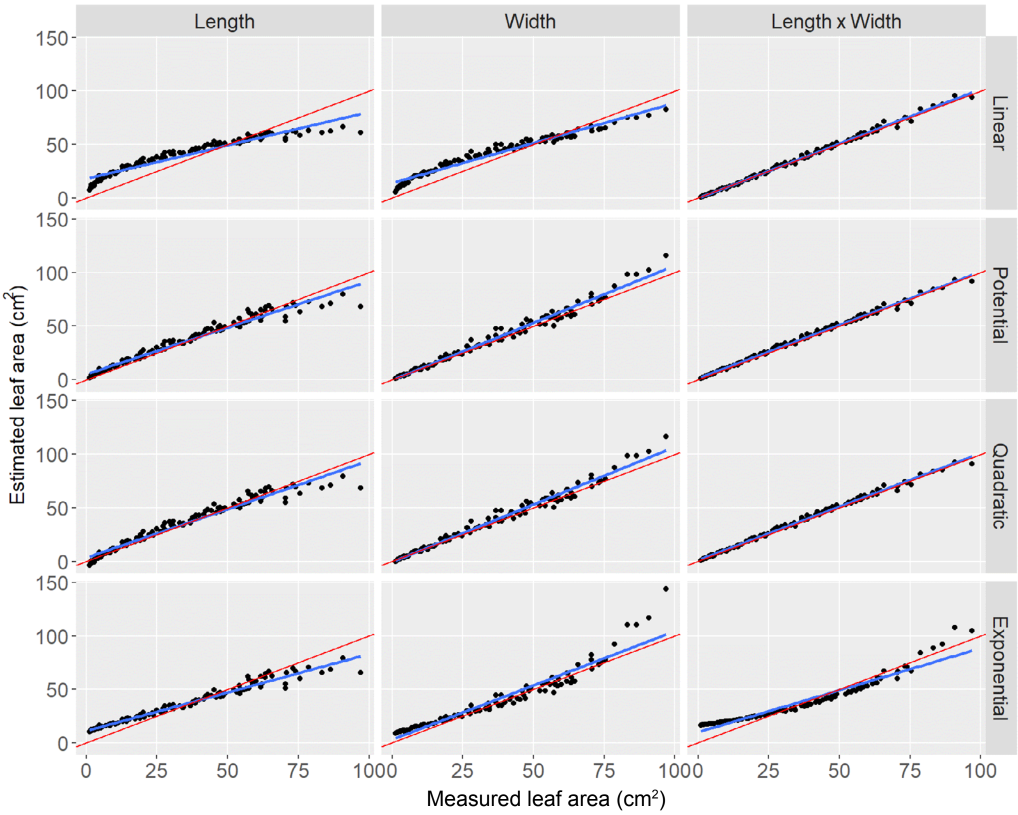
The visual analysis of the data dispersion around the 1:1 line in Figure 3 indicates an optimal predictive capacity of the equation for estimating leaf area over a wide range of chia leaves size. This is evidenced by the fact that the trend line of the estimated leaf area crosses practically over the 1:1 line. These results corroborate with those observed by Richter et al. (2014) for soybeans cultivars.

As the format of the chia leaves is likely to be similar to the leaf format of the plants used in this study, it will not be necessary to estimate the coefficient of the general equation so frequently, in agreement with the results found by Richter et al. (2014) and Bakhshandeh et al. (2011) for soybean crop, and by Aquino et al. (2011), for the sunflower crop. The use of linear dimensions to estimate the leaf area is inconvenient because it requires labor to carry out the measurements. In the chia's case, which emits several leaves, the need to measure all the plant leaves (in the main stem and other stems) requires a longer time to estimate the leaf area than species that produce few leaves as corn and gladiolus. However, the results of this study indicate, as others carried out for soybean crops (Bakhshandeh



The adjusted curves in each panel are models indicated in Eqs. 1 - 12 and presented in Table 1

Figure 2. Relationship between chia's leaf area and its linear dimensions, length (A), width (B), and the product (C) for linear, potential, quadratic, and exponential models



The blue line is the fitted curve, and the red line in each plot is the 1:1 line

Figure 3. Calculated leaf area (cm^2) by the linear, potential, quadratic, and exponential equation from the length, width, and the product (length x width), respectively, versus leaf area measured by the scanner

Table 1. Regression equations and coefficient of determination (R^2) obtained for the relationship between the measured chia's leaf area and its linear dimensions, and statistical indices of the predictive ability of the equations (MAE, RMSE, Statistics bias, d and dl)

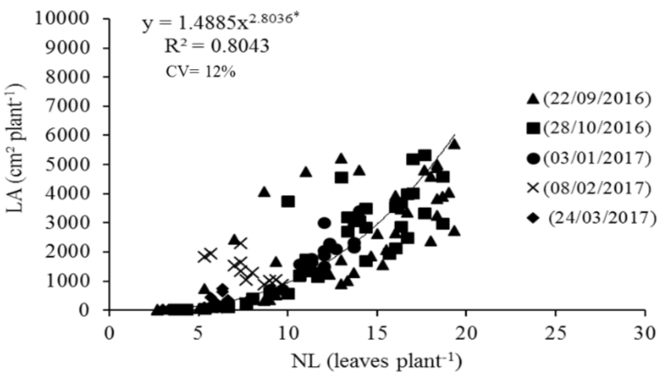
Equations' adjusting		Statistics indices				
Equation	R^2	MAE	RMSE	Statistics bias	d	d1
Linear						
LA = 0.642** (L x W)	0.99	1.11	1 .62	-0.01	0.99	0.97
LA = 4.256** (L)	0.82	10.00	11.54	-0.15	0.92	0.72
LA = 8.1185** (W)	0.84	8.11	9.01	-0.16	0.96	0.79
Potential						
LA = 0.8567** (L x W)$^{0.9349**}$	0.99	1.35	1.81	-0.02	0.99	0.97
LA = 0.3842** (L)$^{1.9497**}$	0.98	3.00	4.88	0.00	0.99	0.93
LA = 1.8704** (W)$^{1.7694**}$	0.99	2.63	4.05	-0.05	0.99	0.94
Quadratic						
LA = 0.004** (L x W)^2 + 0.6856** (L x W) + 0.2254	0.99	1.50	2.04	-0.04	0.99	0.96
LA = 0.2023** (L)^2 + 2.4491** (L) - 8.6264	0.96	3.57	5.37	-0.05	0.99	0.91
LA = 0.9037** (W)^2 + 2.4765** (W) + 2.4693	0.97	2.87	4.51	-0.06	0.99	0.93
Exponential						
LA = 8.5338** e$^{0.0202** (L x W)}$	0.81	8.11	14.34	-0.03	0.93	0.82
LA = 2.9166** e$^{0.2281** (L)}$	0.91	4.40	5.80	0.02	0.97	0.90
LA = 3.2962** e$^{0.4141** (W)}$	0.92	8.13	19.15	-0.13	0.90	0.83

MAE - Mean absolute error (cm^2 leaf^{-1}); RMSE - Root mean square error (cm^2 leaf^{-1}); BIAS - BIAS index; d - Concordance index; d1 - Modified concordance index; ** all estimated parameters were significant by the t test, at $p \leq 0.05$

et al., 2011; Richter et al., 2014), that the measurement of linear dimensions from L x W is ideal to have a reasonable estimate of the leaf area.

Figure 4 shows the allometric relationship between leaf area (LA) and the number of accumulated leaves (NL) on the main stem of chia plants for the five sowing times of the 2016/2017 harvest. The R^2 was 0.80, indicating an adequate adjustment of the potential model to characterize the allometric relationship between LA and NL in the main stem. Similar results were observed for tomato plants (Pivetta et al., 2007) and strawberry plants (Rosa et al., 2013). It was possible to adjust a single equation for the five sowing times due to the morphological similarities in the analyzed leaves, as well as for the gladiolus crop (Schwab et al., 2014).

This study indicates an alternative to estimate the LA in the chia plant from the NL since it is easier to count leaves than to measure them, as observed in strawberry (Rosa et al., 2013). Several authors have employed allometric relationships for



* - significant at p ≤ 0.05 by Ftest

Figure 4. Allometric relationship between leaf area (LA) and number of accumulated leaves (NL) on the main stem in chia at five sowing dates in the 2016/2017 harvest

estimation of leaf area in the sunflower (Maldaner et al., 2009), grape (Malagi et al., 2010), and apple (Bosco et al., 2012). These relationships are determined empirically, establishing the form and significance between two or more biological variables by regression analysis (Niklas, 1994). The mathematical equations for leaf area estimation were developed to obtain an easy and fast method to determine leaf area. This methodology is essential because it is easily adapted to field use, and evaluations can be performed several times on the same plants throughout their development cycle.

Leaf area is a growth variable recognized for its importance to plant productivity since the photosynthesis depends on the light interception by the canopy and its conversion into chemical energy. Therefore, it is important to estimate an equation indicating the leaf area and obtain the allometric relationship between NL and LA. The non-destructive method developed in this paper will allow for the monitoring of the vegetative growth of chia, as well as its floral induction, indicating the transition to the reproductive phase.

Conclusions

1. The non-destructive method, through the linear leaf dimensions, is suitable for estimating the chia's leaf area.

2. The general equation LA = 0.642 (L x W) can be used to estimate chia's leaf area without losing accuracy.

3. The potential model is appropriate to characterize the allometric relationship between the leaf area and the number of leaves accumulated in the main stem of chia at different sowing dates.

Acknowledgements

To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Universidade Federal de Santa Maria.

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