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Leaf Area Estimation of Garden Boldo From Linear Dimensions

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Abstract

The objective of this work was to determine a mathematical equation using linear measures that allows estimating a leaf area of the specie *Plectranthus barbatus* Andrews, a plant with medicinal properties popularly known as garden boldo. For this was performed a direct measurement of the leaf blade considering the length (L) along the midrib and the maximum width (W) perpendicular to the midrib of 500 leaves of different specimens and the observed foliar area (OLA), which were obtained by digitized images. A regression study with linear, quadratic, potential and exponential models was performed using a random sample of 400 from the evaluated leaves using OLA as a function of L, W or LW and then obtaining the estimated leaf area (ELA) of each model. From the remaining 100 leaves a validation of the tested models was performed using ELA as a function of OLA in a simple linear regression. From the residues between ELA and OLA the root-square-mean error and Willmot index (d) was obtained and the normality was verified. The parameters used for validation were: statistically linear and angular coefficient equal to zero and one respectively; coefficient of determination closest to the unit; RQME closer to zero; d index closest to the unit; normal distribution of residues. The equation that best represents the estimated leaf area of the garden boldo is ELA = 0.1389 + 0.6779 (LW).

Keywords: medicinal plant, non-destructive method, *Plectranthus barbatus* Andrews, regression

1. Introduction

The specie *Plectranthus barbatus* Andrews, commonly known as Boldo-de-jardim, boldo, boldo-do-reino, alum, among others (Lorenzi & Matos, 2008), belong to Lamiaceae family (synonymy Labiateae), which have about 300 species widely distributed throughout tropical Africa, Asia and Australia, and some are well adapted in Brazil.

P. barbatus Andrews is an herbaceous or sub-shrub, aromatic, perennial, erect plant when young and decumbent after 1-2 years, slightly branched, up to 1.5 meters in height. With leaves opposite, simple, oval of jagged edges, hairy, measuring 5 to 8 cm long and very bitter taste, flexible even when dry, being thicker and juicy when fresh. Blue flowers arranged in apical racemous inflorescences. It originated in India, probably brought to Brazil in the colonial period (Lorenzi & Matos, 2008).

Among the utilities of the garden boldo are the ornamental and medicinal properties (Lukhoba, Simmonds, & Paton, 2006).

The medicinal properties found in this species are possibly related to the presence of diterpenoids, essential oils and phenolic compounds (Abdel-Mogib, Albar, & Batterjee, 2002). In popular medicine, *P. barbatus* Andrews is indicated in cases of abdominal colic (Dubey, Srimal, & Nityanand, 1981), gastrointestinal diseases such as constipation, gastritis, intestinal spasms; hepatic and dental diseases, in addition, to respiratory diseases such as asthma, bronchitis and pneumonia (Lukhoba et al., 2006).

Considering the beneficial effects of *P. barbatus* Andrews, it is important to perform researches on aspects related to the growth, development and propagation of this species. In most of these studies, the knowledge of the leaf area is critical and perhaps the most important characteristic of vegetative growth. Ribeiro, Conceição, Aoyama and Furlan (2017) report the existence of several species known as Boldo including of different genera. Concerning the use in popular medicine, Milaneze-Gutierre, Famelli, Capel, and Romagnolo (2007) report that the incorrect botanical recognition of a specie can cause serious consequences such as phytotherapeutic innocuity and poisoning.

The leaves are responsible for important functions in the plant such as interception and absorption of light, photosynthesis, gas exchange and transpiration (Taiz & Zeiger, 2009). Zhang and Liu (2010) report the influence of leaf area in the light interception, and therefore plant growth and productivity, becoming one of the key traits in ecophysiological and agronomic studies. An easy economical and accurate estimate of the leaf surface area is a recurrent interest of scientists (Pandey & Singh, 2011), and mathematical equations have been used to determine the leaf area with high accuracy (Carvalho, Bianco, Galati, & Panosso, 2011). Estimating the leaf area in a plant where the leaves are the most interesting product is very important.

There are several methods to measure with accuracy the leaf area, being classified in direct and indirect methods (Olfati, Peyvast, Shabani, & Nosratie-rad, 2010). The indirect methods usually involve the use of regression equations, allow for successive evaluations in the same plant and rapidity in these evaluations, preserving the leaves for later studies, as well as not causing damage to plants (Zanetti, Pereira, Sartori, & Silva, 2017). The regression equations are obtained from modeling studies involving the leaf area observed as a function of length and width measurements of the leaf blade.

Leaf area modeling has been performed for medicinal species such as juazeiro (*Zizyphus joazeiro* Mart) (Maracajá, Madalena, Araújo, Lima, & Linhares, 2008), mofumbo (*Combretum leprosum* Mart) (Candido, Coelho, Maia, Cunha, & Silva, 2013), *Bauhinia monandra* Kurz (Schmildt, Schmildt, Alexandre, Fernandes, & Czepak, 2016), Valerian (*Valeriana jatamansi* Jones) (Walia & Kumar, 2017) and boldo (*Plectranthus ornatus*) (Silva, Pereira, Cabanez, Mendonça, & Amaral, 2017). However, we did not find in the literature any equation to estimate the leaf area of garden boldo (*Plectranthus barbatus* Andrews). In this way the objective of this work was to estimate the modeling of the leaf area of this species through the linear dimensions of the leaf blade.

2. Material and Methods

The garden boldo samples (*Plectranthus barbatus* Andrews) were loaned by the Residents Association of Nova Esperança in São Mateus (South Latitude 18°40′32″, West Longitude 39°51′39″ and with an average elevation of 37.7 m). The collect of material and measurements were performed on the same day in November 2014. The Köppen weather classification of the region is AW, presenting rain in summer and dry in winter (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2014).

In the sampling 500 leaves of a population of adult garden plants were collected. In each plant were harvested leaves at all stages of development in the four cardinal points that did not present damage or attack of diseases or plagues as recommended by Oliveira, Silva, Costa, Schmildt, and Vitória (2017). The leaves were harvested, properly packed in plastic bags and quickly transferred to the Laboratory of Plant Breeding of the Postgraduate Program in Tropical Agriculture of the Centro Universitário Norte do Espírito Santo (CEUNES/UFES), where the allometric measurements were performed.

The length (L) and width (W) dimensions of the leaves were measured with a ruler in centimeters. The length was defined as the distance between the insertion point of the petiole in the leaf blade and the opposite end of the leaf and the width as the largest dimensions perpendicular to the axis of the length, as can be seen in Figure 1. The leaf petiole was removed with scissors. With the data of length and width, the product was also determined between L and W (LW, in cm^2). After these measurements the direct measure of the observed foliar areas was determined, (OLA, in cm^2) using scanned images using the open source ImageJ® Software (Schindelin, Rueden, Hiner, & Eliceiri, 2015). The 500 leaves were scanned using an HP® C4280 multifunctional scanner and the images saved in tif format and 75 dpi resolution.

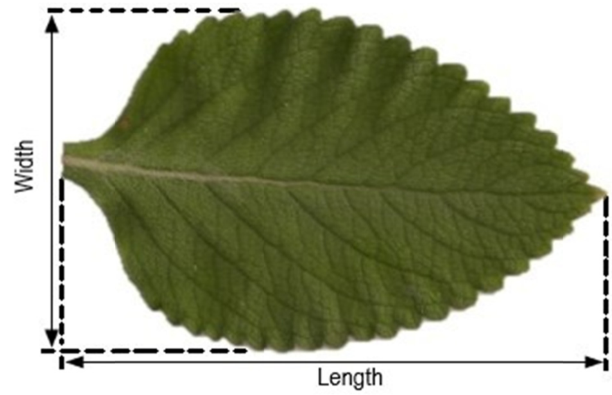


Figure 1. Representation of the predominant format of leaves of *Plectranthus barbatus* Andrews
(L: length measured along the midrib of the leaf blade; W: greatest width of the leaf blade)

Then, among the 500 leaves evaluated, two groups, one with 400 leaves and the other with 100 leaves, were randomly selected, which were used respectively to obtain regression equations and for validation. In both groups for L, W and LW of leaf blade and OLA, measures of central tendency and variability were calculated.

For the estimation of the equations from the 400 leaves sample, linear regression models were used in the parameters (linear and quadratic) and non-linear in the parameters (power and exponential). For these estimates, OLA was used as dependent variable, depending on L, W or LW as independent variables (x), where the estimated leaf area models (ELA) of the models linear, quadratic, power and exponential represented by Equations 1, 2, 3 and 4 respectively.

$$ELA = \hat{\beta}_0 + \hat{\beta}_1 x \quad (1)$$

$$ELA = \hat{\beta}_0 + \hat{\beta}_1 x + \hat{\beta}_2 x^2 \quad (2)$$

$$ELA = \hat{\beta}_0 x^{\hat{\beta}_1} \quad (3)$$

$$ELA = \hat{\beta}_0 \hat{\beta}_1^{x} \quad (4)$$

Twelve estimated equations, as well as the respective coefficients of determination, were obtained. The parameters β_0 and β_1 were estimated by the method of minimum squares, being performed, early, the linearization of the power and exponential functions.

The validation of the 12 equations of estimated leaf area was performed based on the estimated values by the model (ELA) and the observed values (OLA) of the sample of 100 leaves separated for such finality. First, for each equation of each model, a simple linear regression ($y = \hat{\beta}_0 + \hat{\beta}_1 OLA$) was adjusted and obtained the coefficient of determination. The simple linear regression was adjusted using the method of minimum squares. The following hypothesis were tested: $H_0{:}\beta_0 = 0$ *versus* $H_0{:}\beta_0 \neq 0$ and $H_0{:}\beta_1 = 1$ *versus* $H_0{:}\beta_1 \neq 1$, through Student's t-test at 5% probability of error. Then, the root-mean-square error (RMSE) was determined (Equation 5) and Willmott's index of agreement (Willmott, 1981) (Equation 6).

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(ELA_i - OLA_i)^2}{n}} \quad (5)$$

$$d = 1 - \left[\frac{\sum_{i=1}^{n}(ELA_i - OLA_i)^2}{\sum_{i=1}^{n}(|ELA_i - \overline{OLA}| + |OLA_i - \overline{OLA}|)^2}\right] \quad (6)$$

Where, ELA_i are the estimated values of leaf area from i leaves; OLA_i are the observed values of leaf area from i leaves; $\overline{OLA}$ is the mean of the observed values; n is the sample size for validation, being n = 100, in the present work.

In addition, the normality of the residues for validation was verified obtaining the difference between the ELA_i and the OLA_i, by Shapiro-Wilk test, as suggested by Schmildt et al. (2016).

All the validation statistics were represented graphically, together with the line 1:1, according to Bosco, Bergamaschi, Cardoso, Paula, and Casamali (2012).

The best equation to estimate the leaf area as a function of L, W and LW was chosen by objectives criteria of validation based on the linear coefficient (β_0) no different than zero, angular coefficient (β_1) no different than one, RMSE closer to zero, Willmont index (d) closer to one and residues showing normal distribution. The statistics analysis were performed using R software (R Core Team, 2018) and the graphics using Microsoft Office Excel (Levine, Stephan, & Szabat, 2017).

3. Results and Discussion

The minimum, maximum, mean, standard deviation and coefficient of variation (CV) values of the allometric measures of length (L), width (W), product of the length by the width (LW) and observed leaf area (OLA) of the set of leaves used for both adjust and validation of the equations are shown in Table 1.

The minimum and maximum values of L, W, LW and AFO used in validation showed interval indices with those detected in the leaves used in the equation estimation. This is an interesting confirmation, because according to Levine et al. (2017), using regression model for estimation, the values of the independent variable, which wants to estimate, must not extrapolate the values used in the construction of the regression equation. The mean found for L, in both modeling and validation, was similar to the found by Milaneze-Gutierre et al. (2007).

Table 1. Minimum, maximum, average, standard deviation and coefficient of variation in length along the midrib (L, cm), maximum width (W, cm), length of the product by the maximum width (LW, cm^2) and observed leaf area (OLA, cm^2) of *Plectranthus barbatus* Andrews leaves

Variable	Minimum	Maximum	Average	Standard deviation	Coefficient of variation
400 leaves in modeling					
L	2.6000	14.3000	7.9146	2.8424	35.9136
W	1.6000	8.6000	4.6338	1.6993	36.6726
LW	4.1600	118.6900	41.3472	27.0400	65.4000
OLA	2.8600	80.3200	28.1687	18.3200	65.1500
100 leaves in validation					
L	2.2000	13.6600	7.6581	2.1355	27.8850
W	1.6000	7.8600	4.5184	1.3931	30.8315
LW	3.5200	107.3676	37.3926	19.6488	52.5474
OLA	3.5200	72.78	25.4476	13.4474	52.8436

In relation to variability measured by CV, in the leaves sample used in the adjust of the regression equations, all the allometric measures (L, W, LW and OLA) showed values of CV very high (Table 1), according to Pimentel-Gomes' (2009) criteria. According to Pezzini et al. (2018) the high value of CV is important for models generation, because it can be explained by the collection of leaves in several growth stages, characterizing the growth of the plants. In the 100 leaves used for validation, the variability found in the measures of L, W, LW and OLA was also considered high, according to Pimentel-Gomes' (2009) criteria, showing suitable for this type of analyze, as also stood out Schmildt, Hueso, Pinillos, Stellfeldt, and Cuevas (2017).

The 12 equations obtained for estimated leaf area (ELA), as well as the respective coefficient of determination (R^2), are shown in Table 2. In general, a good adjust is verified between the OLA and the allometric models, with R^2 higher than 0.82. Among the adjusted equations (Table 2) observe that the highest R^2 was obtained using LW as an independent variable for linear (Equation 3), quadratic (Equation 6) and power (Equation 9) models, showing R^2 higher than 0.99. A good adjust was not verified for exponential model, differently from the observed by Silva et al. (2017) in modeling of another boldo species, the *Plectranthus ornatus*.

However, a model must not be selected only for the high value of R^2, during the modeling, but by the interpretation of all the statistical measures of validation from an independent sample of that used for modeling (Bosco et al., 2012; Fascella, Darwich, & Rouphael, 2013; Schmildt et al., 2016; Walia & Kumar, 2017).

Table 2. Regression models for the estimation of *Plectranthus barbatus* Andrews leaf area (ELA, cm²) with the respective coefficients of determination (R^2)

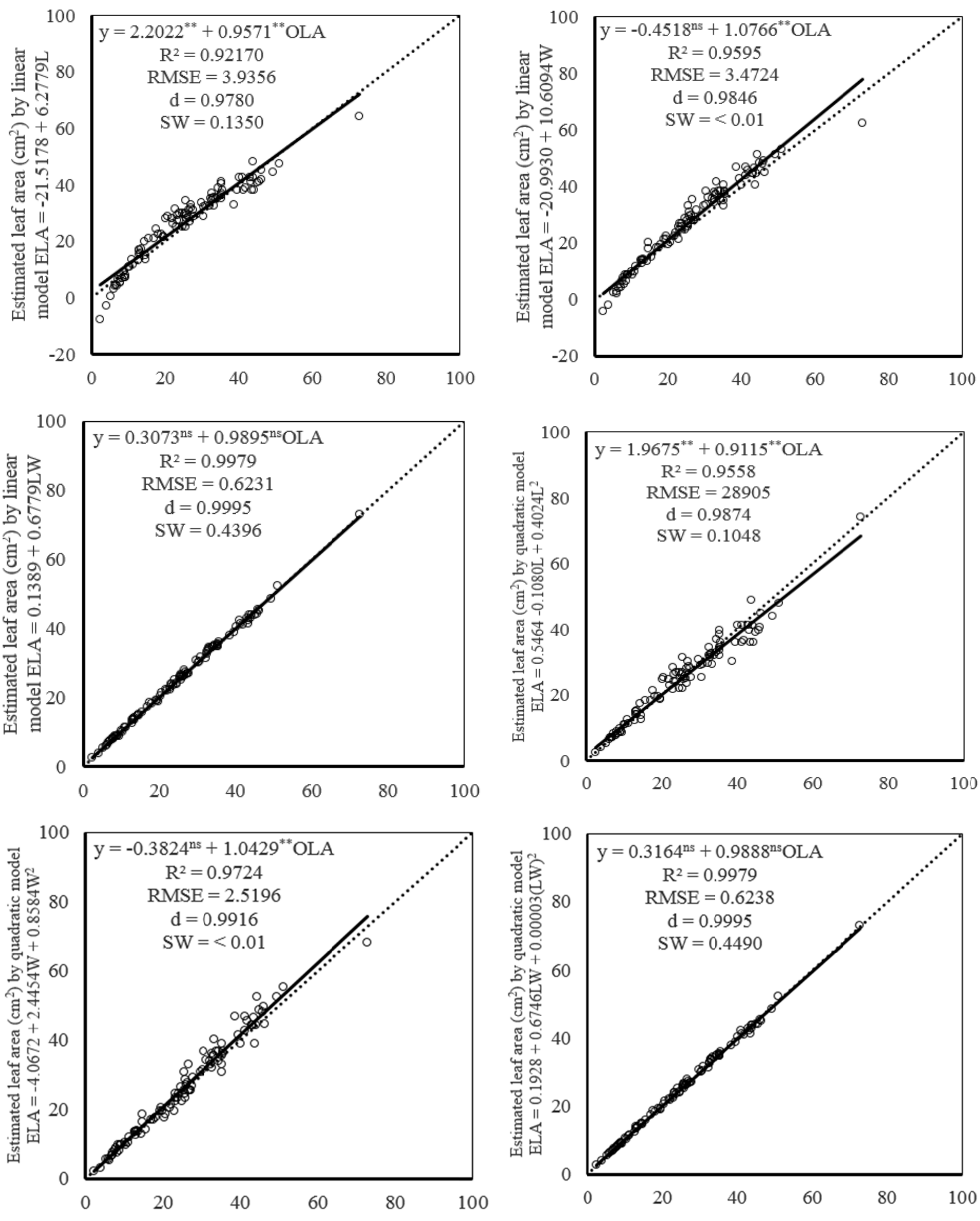
Model	Equation	R^2
1) Linear	ELA = -21.5178 + 6.2779(L)	0.9454
2) Linear	ELA = -20.9930 + 10.6094(W)	0.9651
3) Linear	ELA = 0.1389 + 0.6779(LW)	0.9978
4) Quadratic	$ELA = 0.5764 - 0.1080(L) + 0.4024(L)^2$	0.9734
5) Quadratic	$ELA = -4.0672 + 2.4454(W) + 0.8584(W)^2$	0.9821
6) Quadratic	$ELA = 0.1928 + 0.6746(LW) + 0.00003(LW)^2$	0.9978
7) Power	$ELA = 0.4473(L)^{1.9446}$	0.9733
8) Power	$ELA = 1.2637(W)^{1.9476}$	0.9812
9) Power	$ELA = 0.7189(LW)^{0.9859}$	0.9978
10) Exponential	$ELA = 2.5693(1.3092)^{L}$	0.9261
11) Exponential	$ELA = 2.7207(1.5648)^{(W)}$	0.9161
12) Exponential	$ELA = 7.0133(1.0277)^{(LW)}$	0.8257

The validation made from the sample of garden boldo leaves verified that from the 12 adjusted equations, only those that used LW as an independent variable in the linear, quadratic and power models are suitable, according to the criteria of statistically linear coefficient equal to zero and statistically angular coefficient equal to one (Figure 2). These three equations showed the highest values of R^2 and are the same ones that showed the highest values of R^2 in modeling (Table 2), as also observed by other researches (Schmildt, Hueso, & Cuevas, 2014b; Tartaglia et al., 2016). The three equations also showed suitable for use, when it analyzes the other criteria of validation, showing values of RMSE closer to zero and Willmott's index d (1981) closer to one, when it compares with the other obtained equations, and showed a normal distribution of residues. According to Figure 2, the less adjusted model to the objectives of this work was the exponential, differently from the observed by Silva et al. (2007) for another boldo species (*Plectranthus ornatus*), which claim to have found the best adjust in exponential model using the width.

In practice, the use of linear model equations based only on one dimension of the leaves is preferable due to simplicity of application, mainly on the field (Tsialtas & Maslaris, 2005), as performed by Schmildt et al. (2016), who indicated the use of length of leaves in *Bauhinia monandra*, and by Tartaglia et al. (2016), who indicated the use of width of leaves in canola. However, in relation to garden boldo leaves, none of the models was suitable using only one allometric measure, as can be seen in Figure 2.

These results point out the need of suitable use of the criteria of validation, and must also be interpreted together, as observed by Schmildt, Amaral, Schmildt, and Santos (2014a) in the determination of leaf area in different cultivars of Arabic coffee.

Thus, considering the ease of interpretation, the equation ELA = 0.1389 + 0.6779(LW) (Table 2) is recommended for use, whose criteria of validation are seen in Figure 2. In this figure can be also observed the good adjust of the simple linear equation through straight 1:1.


y = 2.2022** + 0.9571**OLA
R² = 0.92170
RMSE = 3.9356
d = 0.9780
SW = 0.1350
Estimated leaf area (cm²) by linear model ELA = -21.5178 + 6.2779L
y = -0.4518ns + 1.0766**OLA
R² = 0.9595
RMSE = 3.4724
d = 0.9846
SW = < 0.01
Estimated leaf area (cm²) by linear model ELA = -20.9930 + 10.6094W
y = 0.3073ns + 0.9895nsOLA
R² = 0.9979
RMSE = 0.6231
d = 0.9995
SW = 0.4396
Estimated leaf area (cm²) by linear model ELA = 0.1389 + 0.6779LW
y = 1.9675** + 0.9115**OLA
R² = 0.9558
RMSE = 28905
d = 0.9874
SW = 0.1048
Estimated leaf area (cm²) by quadratic model ELA = 0.5464 -0.1080L + 0.4024L²
y = -0.3824ns + 1.0429**OLA
R² = 0.9724
RMSE = 2.5196
d = 0.9916
SW = < 0.01
Estimated leaf area (cm²) by quadratic model ELA = -4.0672 + 2.4454W + 0.8584W²
y = 0.3164ns + 0.9888nsOLA
R² = 0.9979
RMSE = 0.6238
d = 0.9995
SW = 0.4490
Estimated leaf area (cm²) by quadratic model ELA = 0.1928 + 0.6746LW + 0.00003(LW)²

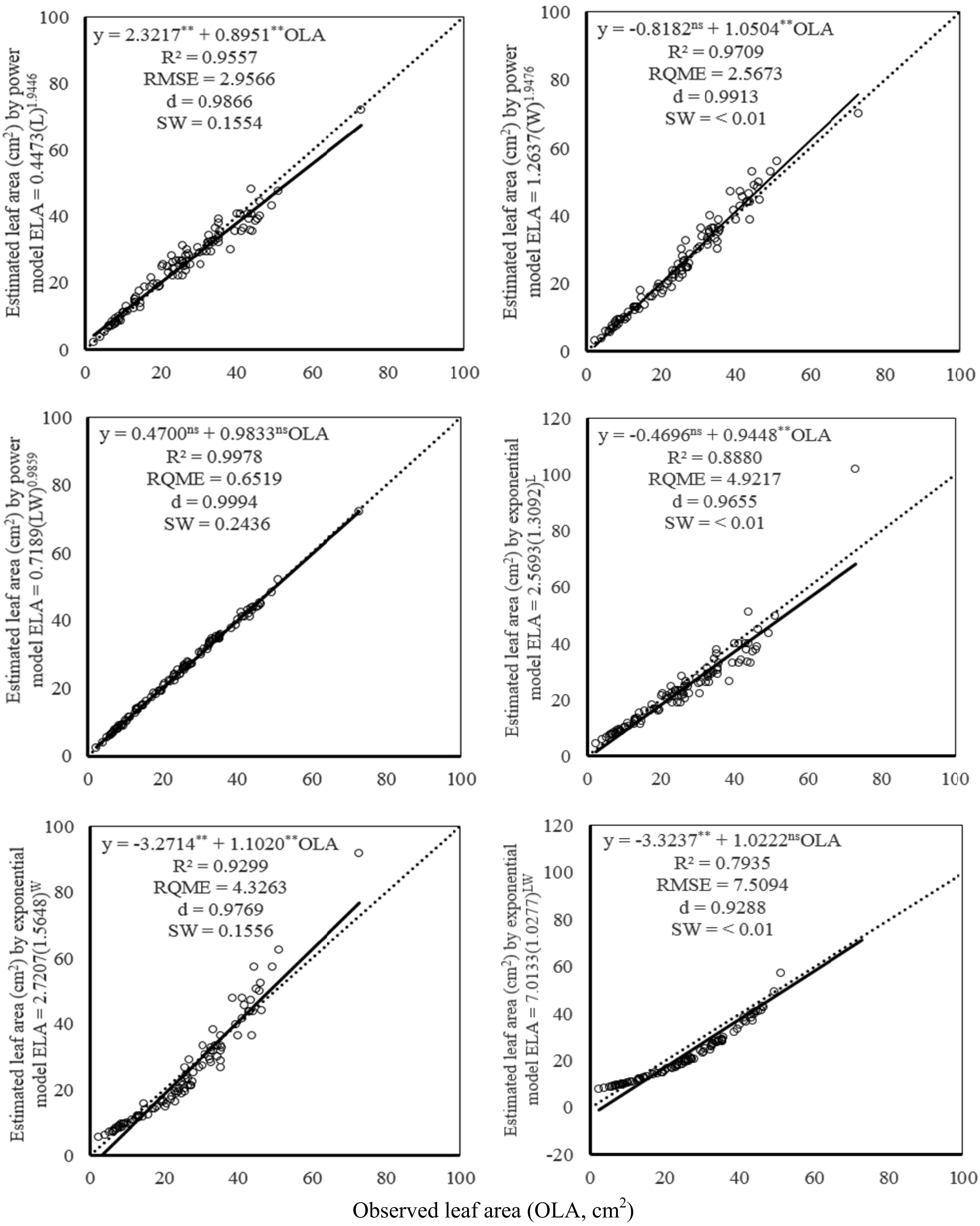


Figure 2. Adjusted equation (y), coefficient of determination (R^2), root-mean-square error (RMSE), Willmott's index of agreement (d) and p-value of the normality test of the errors by Shapiro-Wilk (SW) obtained from the relationship between estimated leaf area by different models (ELA) and observed leaf area (OLA) of *Plectranthus barbatus* Andrews (In adjusted equation $y = \hat{\beta}_0 + \hat{\beta}_1 OLA$, the hypotheses are: $H_0{:}\beta_0 = 0$ vs $\beta_0 \neq 0$ and $H_0{:}\beta_1 = 1$ vs $\beta_1 \neq 1$, where, ^{ns} is non-significant ($p > 0.05$) and ** is significant ($P < 0.01$) by the t test; dashed line is line 1: 1)

4. Conclusions

The leaf area of garden boldo can be estimated with accuracy through non-destructive methods using measures of the dimensions of length (L) and width (W) of leaves in different mathematical models.

The equation $ELA = 0.1389 + 0.6779(LW)$ provided the highest accuracy for the estimation and simplified the calculations.

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