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Allometric model for estimating leaf area of ora-pro-nobis genotypes

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ABSTRACT

The planting of unconventional vegetables has increased in Brazil. This is mainly due to the demand of nutritionally rich foods grown in sustainable crops. In this scenario, ora-pro-nobis stands out, as, although being part of the meal of large part of the Brazilian population, it has been cultivated despite the lack of scientific knowledge. Thus, this study aimed to adjust a leaf area estimation model for ora-pro-nobis, using the measurements of the leaves. For the model adjustment and validation, 4000 leaves were collected in four different genotypes, being 1000 in each of them. The regression models were adjusted using 2000 independent and random observations, using 500 leaves per genotype. Thus, the observed leaf area was studied as a linear function of the dimensions of the leaf blade: length (*L*), width (*W*) and product (*LW*). The remaining 2000 observations, also 500 per separate genotype, were used only in the statistical validation study. The adjusted allometric model based on the product (*LW*) proved to be precise and accurate for estimating the leaf area of ora-pro-nobis, being applicable to any of the four genotypes analyzed regardless of the leaf size: $\widehat{LA}_{LW} = 0.9294 + 0.6815 \times \mathrm{LW}$.

Keywords: *Pereskia aculeata*, allometric model, non-destructive method, leaf dimension.

RESUMO

Modelo alométrico para estimativa da área foliar de genótipos de ora-pro-nobis

O plantio de hortaliças não convencionais tem ganhado espaço no cenário nacional, o que se relaciona, principalmente, com a procura por alimentos nutricionalmente ricos advindos de cultivos sustentáveis. Neste cenário, destaca-se o ora-pro-nobis, que embora presente na mesa de muitos brasileiros, tem sido cultivado frente a escassez de conhecimento científico sobre a espécie. Assim, objetivou-se ajustar um modelo de estimação de área foliar (AF) em ora-pro-nobis, usando medidas de suas folhas. Para o ajuste dos modelos e validação do selecionado coletou-se 4000 folhas em quatro genótipos distintos, sendo 1000 em cada um deles. Os modelos de regressão foram ajustados a partir de 2000 observações independes e aleatórias, utilizando-se 500 folhas por genótipo. Deste modo, a área foliar observada foi estudada como função linear das dimensões do limbo foliar: comprimento (*C*), largura (*L*) e do produto (*CL*). As demais 2000 observações, também 500 por genótipo em separado, foram utilizadas apenas no estudo de validação estatística. O modelo alométrico ajustado baseado no produto (*CL*) mostrou-se preciso e acurado para a estimação da área foliar em ora-pro-nobis, sendo aplicável a qualquer um dos quatro genótipos analisados independentemente do tamanho de suas folhas: $\widehat{AF}_{CL} = 0.9294 + 0.6815 \times \mathrm{CL}$.

Palavras-chave: *Pereskia aculeata*, modelo alométrico, método não-destrutivo, dimensões foliares.

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The eating habits of the world's population have changed, and consuming foods which offer greater food security, as well as other levels of nutrients, is an increasing tendency. In this context, non-conventional vegetables stand out (HortPANC's). These vegetables are nutrient-rich and easy-grown crops, as they demand few cultural practices, which also contribute to include these plants in sustainable production systems. Among the aforementioned vegetables, ora-pro-nobis, although being part of the meal of large part of the Brazilian population, has been cultivated in an empirical way, concerning to the phytotechnical aspects of the crop (Garcia *et al*., 2019; Izzo & Domene, 2021).

Ora-pro-nobis (*Pereskia aculeata*) belongs to cactaceae family. It originates from South America and is found in several regions of Brazil (Pinto & Scio, 2014). Its succulent leaves are the main organ consumed, since they are rich in proteins, minerals, fibers, vitamins A and C, and folic acid (Souza *et al*., 2016).

This vegetable is considered a high-nutritional-quality food, and its consumption has been increasing, as well as the demand for its production. Morpho-phisiological studies on leaves have aroused phytotechnical interest, mainly concerning light interception and photosynthesis, which is directly related to plant productivity capacity (Reis *et al*., 2013).

In relation to studies on ora-pro-nobis, scientific information on growing and development of the plant is rare and that is why further studies are essential. One of the essential morphological measures in inferring plant growth is the leaf area, determining the leaf area index (IAF) which is useful in agronomic studies (Schmildt *et al.*, 2014; Taiz & Zeiger, 2017) and essential to evaluate the cultural practices such as planting density, fertilization, irrigation, pruning and pesticide application (Favarin *et al.*, 2002).

Leaf area can be determined directly by the use of leaf area integrators, considering to be, in most cases, a destructive method, or indirectly, using allometric model, by measuring the leaf blade to estimate the leaf area.

The indirect method, when the adjustment is good, is considered an accurate method, at lower cost, and easy to be used, non-destructive and applicable to different stages of crop development (Pedrosa *et al.*, 2020; Cabral *et al.*, 2020). This method also allows to evaluate plant growth and development at field level, following the evolution of the same leaf over time as its blade undergoes changes in size.

In this sense, considering the advantages of the indirect method, allometric models have been recommended for several crops, such as coffee (Silva *et al.*, 2011), sunflower (Aquino *et al.*, 2011) and yacon (Erlacher *et al.*, 2016). Such interest is also for ora-pro-nobis crop, mainly, due to the demand of studies related to the development of technologies for cultivating the crop.

Given the above, this study aimed to adjust, validate and recommend an allometric model for an indirect method for estimating the leaf area of ora-pro-nobis, measuring its leaf blade.

MATERIAL AND METHODS

The experiment was carried out at Universidade Federal do Espírito Santo, Alegre-ES campus (21°S, 42°W), from June to September, 2021.

The climate in the south region of Espírito Santo is Aw, according to Köppen. Two seasons, well defined during the year, being one hot and rainy, from October to March, and the other cold and dry, from April to September, with annual average temperature of 23ºC, and annual rainfall about 1,200 mm (Pezzopane *et al.*, 2012).

The soil of the experimental area was classified as Red-Yellow Latosol, medium texture (Santos *et al.*, 2013), collected at 0-20 cm depth. The soil sample was submitted to the laboratory analysis, showing the following chemical characteristics: pH= 6.63, 17.22 mg/dm^3 P, 224.00 mg/dm^3 K, 6.00 mg/dm^3 Na, 2.99 cmol$_c$/dm^3 Ca, 1.12 cmol$_c$/dm^3 Mg, 0.00 cmol$_c$/dm^3 Al, and 70.05% basis saturation. No correction was needed, since the soil conditions were appropriate for ora-pro-nobis cultivation (Madeira *et al.*, 2016).

The plants used in the experiment are from the germplasm bank of Departamento de Agronomia (DAG), UFES, which is composed of four genotypes. These genotypes were identified by their origins, the genotype Vale do Jequitinhonha-MG (A), the regional genotypes from Alegre-ES (B), and Alegre-ES/Rio Itapemirim (C) and the genotype from Southern Brazil (D). All genotypes two years after planting and in full vegetative growth.

In order to obtain adjusted models, 4000 leaves were collected, being 1000 of each genotype. The authors highlight that the four evaluated genotypes showed elliptical and alternate leaves (Figure 1). Thus, the authors decided to study these genotypes in the same database trying to build only one allometric model to represent them, regardless of their origin.

The leaves were collected in May 2021 and taken to Laboratório de Análises Vegetais, of UFES, being measured (cm) according to length (L), along the main vein from the leaf base to the apex, disregarding the petiole; and width (W) measured perpendicularly to the main rib from one end to the other, considering the highest value, with the aid of a millimeter ruler (Figure 1). The leaf area observed in each leaf (LA_{obs}) was obtained using the leaf area integrator LI-3100 Area Meter, LI-COR®, inc. Lincoln. Nebraska USA. Aiming to obtain representative data without distortion, the authors excluded the leaves at early developmental stage, shorter than three cm, and those ones which presented any kind of mechanical damage or pest attack or disease.

At a statistical level, before adjusting the regression models, the descriptive biometric characterization of the variables L, W and LA_{obs} of the evaluated genotypes was carried out.

The models were adjusted using 2000 leaves picked up independently and randomly, which were obtained using 500 leaves of each genotype. The leaf area observed (LA_{obs}) was studied in relation to the linear function of leaf dimensions: L, W and LW, and the other observations of each genotype (n=500) were saved and used to validate the study on the most promising model in each genetic material separately.

In order to choose the best regression model, the authors used the following statistics a) analysis of the variance of

Figure 1. Leaves of Vale do Jequitinhonha-MG (A), regional Alegre-ES (B), Rio Itapemirim/Alegre-ES (C) and Southern Brazil (D) genotypes. Length and width measurements with dimensions of the leaf blade. Alegre, UFES, 2021.

regression: F test significant for regression (α=0.01); b) Student t test significant for parameters of the model under the hypothesis: H_0: $\beta_i = 0$ vs. H_a: $\beta_i \neq 0$ (α=0.01); c) coefficient of determination adjusted for the degrees of freedom of the residue (R_a^2); d) dispersion pattern of the residues (Antunes *et al.*, 2008) and; e) standard error of estimate ($\widehat{SEE}$) obtained through the following equation (1):

$$\widehat{SEE} = \sqrt{\frac{\Sigma(Y - \hat{Y})^2}{n - 2}} \qquad (1)$$

In which Y represents the leaf area observed in this study, determined by LI-3100 Area Meter; $\hat{Y}$, the leaf area estimated by the adjusted allometric model; and n represents the sample size.

In order to validate the selected model, the authors used 500 observations reserved for each genotype separately. In this stage, the adjusted allometric model was applied to estimate the values of the leaf area in each sample, then, a new simple linear regression model without intercept (SLRWI) was adjusted, relating the leaf area values measured by the leaf area integrator (LA_{obs}) with the ones estimated by the model ($\widehat{LA}_{est}$), using the equation (2):

$$Yi = \beta Xi + \varepsilon i \qquad (2)$$

In which: Y_i represents the area of the i^th^ leaf obtained by the chosen regression model $\widehat{LA}_{est}$; β, the angular coefficient or regressor, X_i corresponds to the area of the i^th^ leaf- $LA_{obs.}$, and ε_i, the random error associated with each observation. β was estimated using the ordinary least-squares method. For SLRWI, the authors tested the hypothesis: H_0: $\beta_i = 1$ vs. H_a: $\beta_i \neq 1$ (α=0.01), which corresponds to an equivalence between leaf area measurements obtained by the chosen equation and those observed (measured in the laboratory). The existence of a linear association between observed and estimated values was investigated using Pearson's correlation (ρ).

Agreement between the estimates of the chosen model and the observed leaf area values was verified using Willmott equation ($\hat{d}$), in which the values found ranged from zero (no agreement) to "one" (perfect agreement) (Willmott *et al.*, 1985). The value of ($\hat{d}$) is calculated using the following equation (3):

$$\hat{d} = 1 - \frac{\sum_{i=1}^{n}(LA_{est} - LA_{obs})^2}{\sum_{i=1}^{n}(|LA_{est} - \overline{LA_{obs}}| + |LA_{obs} - \overline{LA_{obs}}|)^2} \qquad (3)$$

Where: $\hat{d}$= Willmott's concordance index; $\widehat{LA}_{est}$= leaf area estimated by the chosen regression model; LA_{obs}=observed leaf area and $\overline{LA_{obs}}$= general average of leaf areas observed in the study (Fialho *et al.* 2011).

The error inherent to the chosen model was evaluated as "average relative error" (ARE%), which is the average of the value of the differences (in module) between the values of leaf area estimated and observed expressed in percentage.

Statistical analyses were performed using SigmaPlot softwares (Scientific, 1986), "Table Curve 2D v5.0 trial" (Scientific, 1991) and "Genes" - computational application in genetics and statistics v. 2015.5.0 (Cruz, 2013).

RESULTS AND DISCUSSION

The biometric characterization of the ora-pro-nobis genotypes A, B, C and D in relation to their leaf variables are shown in Table 1. The length ranged from 3.5 cm (A) to 15 cm (D) with general average of 8.93 cm, width from 2.5 cm (A) to 10.2 cm (A) with general average of 4.02 cm, leaf area from 12.4 cm^2 (A) to 73.8 cm^2 (D) with general average of 25.69 cm^2. These results are in accordance with Duarte & Hayashi (2005) who reported that the ora-pro-nobis leaves, in general, measure about 7-cm long and 3-cm wide.

The authors also verified average and high variability in the studied variables which showed average coefficient of variation of 14.15%, 16.07% and 27.12% for *L*, *W* and LA_{obs}, respectively. These results showed that the sampling used in the experiment was effective, since using a high sampling variability is important in this kind of study, since the purpose is to find an adjustment of an efficient model to estimate the leaf area in several dimensions.

Several models were adjusted between *L*, *W* and LA_{obs}, to determine the leaf area of the crop. However, the authors showed the three models which the best satisfied the pre-established statistics. Two of them using *L* and *W* separately and a third model using only the product *LW*; (Box 1).

The highest adjusted coefficient of determination for the degrees of freedom of residue - R_a^2 (0.9545), smallest standard error estimates - $\widehat{SEE}$ (1.6118), smallest residual sum of squares - RSS (5190.47) and the highest calculated *F* value - F_{calc} (41952.88**) was obtained using the model which uses the product *LW* as an independent

Table 1. Minimum (Min) and maximum (Max) values, average ($\overline{X}$), standard deviation ($\hat{\sigma}$) and coefficient of variation [CV(%)] for length, width and leaf area of ora-pro-nobis genotypes: A, B, C and D. Alegre, UFES, 2021.

Genotype	Variable	Min	Max	$\overline{X}$	$\hat{\sigma}$	CV(%)
A (n =1000)	Length (cm)	3.50	13.50	8.71	1.40	16.11
	Width (cm)	2.50	10.20	3.86	0.76	19.72
	Leaf area (cm^2)	12.40	71.30	23.81	7.77	32.62
B (n =1000)	Length (cm)	6.00	13.50	8.79	1.28	14.54
	Width (cm)	2.80	7.00	3.90	0.60	15.47
	Leaf area (cm^2)	13.60	56.80	24.88	6.83	27.46
C (n =1000)	Length (cm)	5.90	12.50	8.74	1.06	12.16
	Width (cm)	2.60	6.20	4.00	0.59	14.88
	Leaf area (cm^2)	13.40	49.80	24.89	6.01	24.13
D (n =1000)	Length (cm)	5.70	15.00	9.50	1.31	13.82
	Width (cm)	2.80	9.50	4.31	0.61	14.22
	Leaf area (cm^2)	14.90	73.80	29.18	7.08	24.26
General average (n =4000)	Length (cm)	5.28	13.63	8.93	1.26	14.15
	Width (cm)	2.68	8.23	4.02	0.64	16.07
	Leaf area (cm^2)	13.58	62.93	25.69	6.92	27.12

n: sample size.

variable. Considering this model the most accurate and precise one (Box 1).

In this sense, the authors noticed that regardless of genotype and leaf size, the leaf area of ora-pro-nobis can be estimated using the model based on the *LW* product. Similar results were verified for the crops of yacon, coffee and cassava (Antunes *et al.*, 2008; Erlacher *et al.* 2016; Guimarães *et al.* 2019).

In relation to the residue distribution along the independent variables of the contrasted models (Figure 2A, B and C), the authors verified that the best standard was also obtained for the model which estimates the leaf area using the *LW* product (LA_{LW}) (Figure 2C), where a tendency of normal distribution of the residues was observed (homocedastic errors). Similar results were reported by Erlacher *et al.* (2016) also for the model which uses the *LW* product to estimate the leaf area in yacon.

Thus, considering the lowest $\widehat{SEE}$, highest $\widehat{SEE}$, significant regression parameters and tendency to normal distribution of the residues, the model chosen to study the validation was LA_{LW} (Box 1).

The statistical study to validate the model LA_{LW} showed to be extremely reliable, precise and accurate, regardless of the adopted genotype, considering that for all the cases, the angular coefficients estimated in the SLRWI did not differ from the unit by Student's t-test ($\alpha = 0.01$): $\hat{\beta}^{ns}_{1's}$ (Figure 3).

We also observed strong positive linear dependency between the observed and estimated data using LA_{WL}, Pearson correlation coefficients ($\hat{\rho}'^{s}$) close

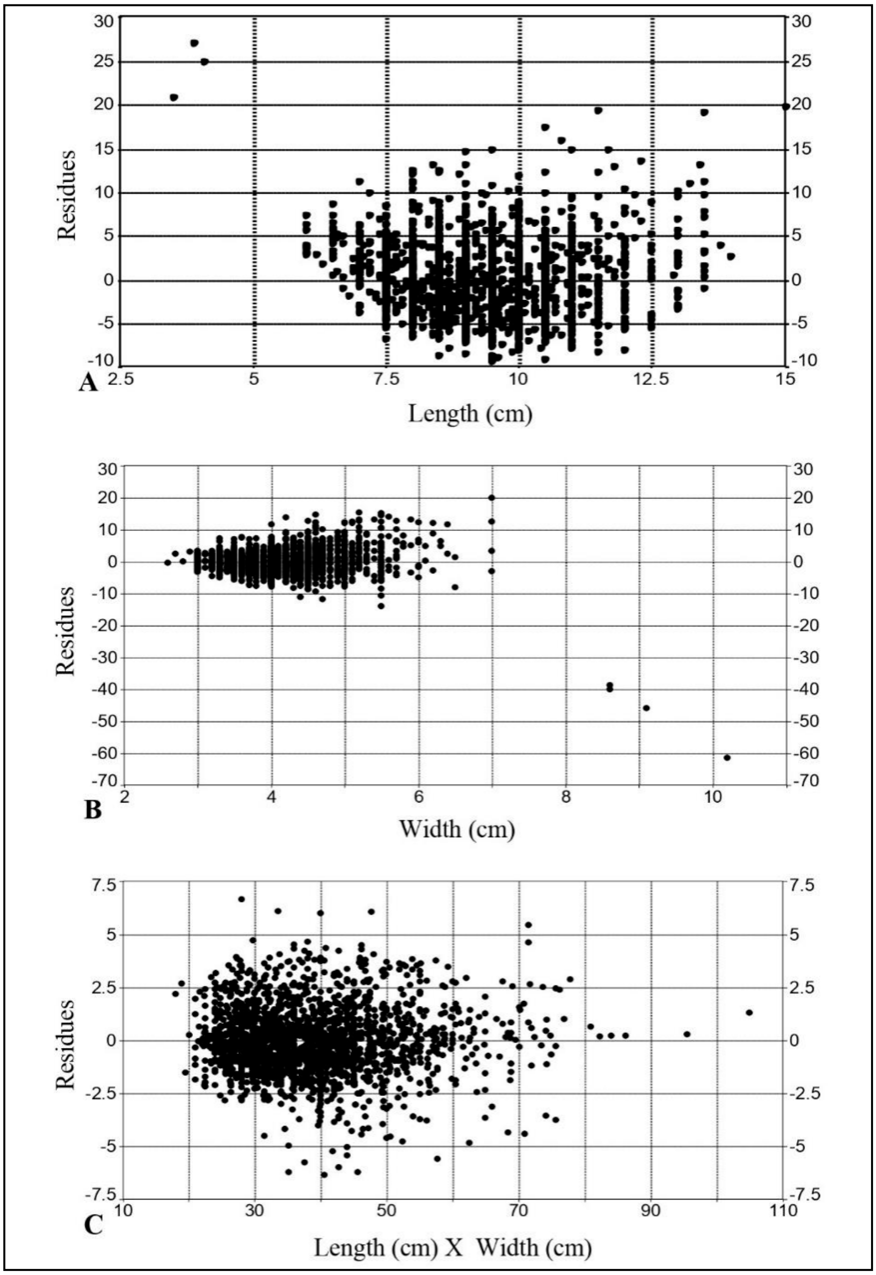

Figure 2. Analysis of the dispersion pattern of the residues for the models A = Length (*L*); B = width (*W*) and C= product (*LW*). Alegre, UFES, 2021.

Model¹	Coefficient	SEE	R^2_a	g.l.-R	SSR	F_{calc}	Estimator
$LA_L = \beta_0 + \beta_1 x$	$\hat{\beta}_0 = -15.1203^{**}$ $\hat{\beta}_1 = 4.6104^{**}$	4.1043	0.7049	1999	33657.57	4779.84**	$\widehat{LA}_L = -15.1203 + 4.6104L$
$LA_W = \beta_0 + \beta_1 x$	$\hat{\beta}_0 = -11.1638^{**}$ $\hat{\beta}_1 = 9.2710^{**}$	4.1419	0.6995	1999	34276.54	4657.45**	$\widehat{LA}_W = -11.1638 + 9.2710W$
$LA_{LW} = \beta_0 + \beta_1 x$	$\hat{\beta}_0 = 0.9294^{**}$ $\hat{\beta}_1 = 0.6815^{**}$	1.6118	0.9545	1999	5190.47	41952.88**	$\widehat{LA}_{LW} = 0.9294 + 0.6815LW$

Box 1. Statistical models, regression coefficients, standard error of estimates (SEE), coefficient of determination adjusted for the degrees of freedom (R^2_a), degrees of freedom of the residue (g.l.-R), sum of squares of the residues (SSR), F - Calculated (Fcalc) and leaf area estimators related to the sum of the linear dimensions of the leaves length (L); width (W); product (LW) of the ora-pro-nobis genotypes. **Coefficients - significant by the t-test (α= 0.01). **Fcalc. significant by the F test (α= 0.01). Alegre, UFES, 2021.

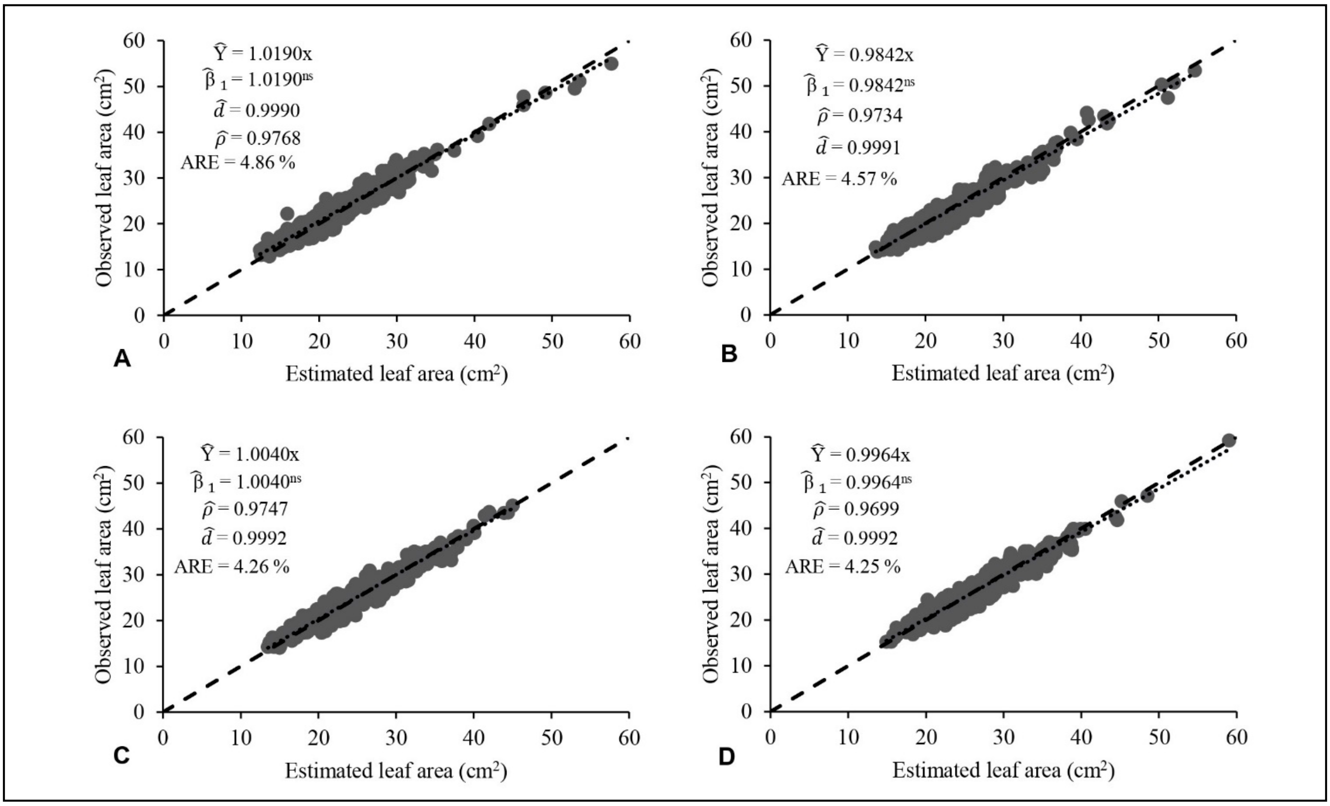

Figure 3. Relationship between observed and estimated leaf area for A, B, C and D genotypes, using the model based on the product (*LW*) of the ora-pro-nobis leaves. [1]The results of the statistics used in the validation of the model for the four genotypes are shown in the figure: *t-test* for β under hypothesis H0: βi = 1 vs. Ha: βi ≠ 1 (α=0.01); Pearson's correlation coefficient ($\hat{\rho}$); Average relative error in percentage of observed ARE values (%) and the Willmott agreement index ($\hat{d}$). Alegre, UFES, 2021.

to unity for all genotypes. That was also corroborate by high Willmott concordance ($\hat{d}$) (Figure 3), reaffirming previous statistics.

Low$\widehat{ARE}$'s values found can be explained by the high variability of the leaves used in this study: 4.86%, 4.57%, 4.26% and 4.25%; for genotypes A, B, C and D, respectively (Figure 3). These results are even smaller than the ones reported by Fialho *et al*. (2011): 5.06% and Erlacher *et al*. (2016): 7.42%; for leaf area estimation models in zucchini and yacon, respectively.

Thus, the adjusted allometric model based on product *LW* showed to be precise and accurate for estimating the leaf area in ora-pro-nobis, being applicable in any of the four evaluated genotypes A, B, C and D regardless of their leaf size: $\widehat{LA}_{LW} = 0.9294 + 0.6815 \text{ x } \mathrm{LW}$.

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