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Estimation of common bean *(Phaseolus vulgaris)* leaf area by a non-destructive method

Estimativa da área foliar de feijão comum *(Phaseolus vulgaris)* por método não destrutivo

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Highlights

The general equation can be used for both irrigated and non-irrigated cultivars.
The general equation facilitates the estimation of LA using only leaflet length.
The general equation can be used as a tool that optimizes assessments.

Abstract

The aim of this study was to develop mathematical models to estimate the leaf area of common bean *(Phaseolus vulgaris)* in irrigated and non-irrigated water regimes from linear dimensions. An experiment was carried out in a completely randomized design with a 3×2 factorial arrangement (three cultivars: Triunfo, Garapiá and FC 104; two water regimes: irrigated and non-irrigated) with 25 replicates each. A total of 523 trifoliates were collected throughout the crop cycle. The length (L, cm) and width (W, cm) of the central leaflet of the trifoliate were measured and their product (LW) (cm²) calculated. Then, the leaf area of these trifoliates was determined by digital photography methods using ImageJ® software, and using leaf discs. The number of samples required to estimate the leaf area of a trifoliate was determined to define which method is the most accurate to be used as the real leaf area in generating equations to estimate the leaf area in common bean. The relationship between area by digital photographs and the dimensions of the central leaflet of the trifoliate (L, W and LW) was fitted by linear, quadratic and power models. Subsequently, the predictive capacity of the equations was assessed by the root mean square error (cm^2 $trifoliate^{-1}$), mean absolute error (cm^2 $trifoliate^{-1}$), index of agreement and Pearson's correlation coefficient. Sample size varied between cultivars, water regimes and evaluation methods. It is more appropriate to use the leaf area provided by ImageJ® as real for comparison purposes in generating models to estimate leaf area from

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linear measurements, in common bean. The general equation LA = $1.092L^{1.945}$ can be used in the tested regimes without accuracy losses.
Key words: Digital photographs. Leaflet length. Mathematical model. *Phaseolus vulgaris*.

Resumo

O objetivo desse trabalho foi determinar modelos matemáticos para estimar a área foliar de feijão comum *(Phaseolus vulgaris)* em regime hídrico irrigado e não irrigado, a partir de dimensões lineares. Para isso, foi realizado um experimento em delineamento inteiramente casualizado em esquema fatorial 3x2 (três cultivares: Triunfo, Garapiá e FC 104; dois regimes hídricos: irrigado, não irrigado) com 25 repetições cada. Foram coletados 523 trifólios ao longo do ciclo da cultura, mensurando-se o comprimento (C) (cm), largura (L) (cm) e calculado o seu produto (CL) (cm²) do folíolo central do trifólio. Na sequência, a área foliar desses trifólios foi determinada pelos métodos de fotos digitais com auxílio do software ImageJ® e por discos foliares. Foi investigado o número de amostras necessárias para estimar a área foliar de um trifólio, e assim, determinar qual método é o mais preciso para ser usado como área foliar real na obtenção de equações para estimar a área foliar em feijão. A relação entre a área por fotos digitais e as dimensões do folíolo central do trifólio (C, L, CL) foram ajustadas por modelos lineares, quadráticos e de potência e a capacidade preditiva das equações foi avaliada através da raiz quadrada média do erro (cm^2 $trifólio^{-1}$), erro médio absoluto (cm^2 $trifólio^{-1}$), índice de concordância e coeficiente de correlação de Pearson. O tamanho de amostra variou entre as cultivares, regimes hídricos e métodos de avaliação. É mais adequado utilizar a área foliar calculada pelo ImageJ® como real para fins de comparação na determinação de modelos para estimar a área foliar por medidas lineares em feijão. A equação geral AF = $1{,}092C^{1{,}945}$ pode ser utilizada nos regimes testadas sem perda na precisão.
Palavras-chave: Comprimento do folíolo. Fotos digitais. Modelo matemático. *Phaseolus vulgaris.*

Introduction

Common bean *(Phaseolus vulgaris)* is one of the most important grain crops, which is produced in all regions and consumed daily by most part of the population. It represents a protein source for the world population, especially in the neediest regions. Despite its importance, the average yield of 1,014.6 kg ha^{-1} obtained in the last harvests (Companhia Nacional de Abastecimento [CONAB], 2021) is below its productive potential of approximately 3,000 kg ha^{-1}, in irrigated crops (Justino et al., 2019). Stress due to water deficit stands out as one of the factors responsible for decreased yields in common bean (Schwerz et al., 2017). Water deficiency lowers cell turgor, which, in turn, reduces leaf expansion, induces stomatal closure and reduces plant physiological processes, ultimately compromising grain production (Taiz, Zeiger, Moller, & Murphy, 2017).

Leaf area is one of the most important parameters in the evaluation of plant growth, since it is interconnected with photosynthetic rate (Taiz et al., 2017). This measurement can be obtained by destructive and non-destructive methods, in the field or in the laboratory (Hara, Gonçalves, Maller, Hashiguti, & Oliveira, 2019). Destructive methods require the removal of the leaves from the plant, which render them limiting in experiments with few samples or which require these leaves to be maintained until the end of the cycle. Non-destructive methods, on the other hand, preserve the integrity of the leaf, allowing repeated measurements to be made during the crop development cycle (Bakhshandeh, Kamkar, & Tsialtas, 2011; Richter et al., 2014).

Among the non-destructive methods, the use of mathematical models is considered simple, easy, reliable and does not require sophisticated equipment (Lakitan, Widuri, & Meihana, 2017; Hara et al., 2019). In common bean, reports on the topic exist since the 1970s, with cultivars of the *carioca* group. The first studies were developed by Benincasa, Benincasa, Latanze and Jenquetti (1976) and continued with cultivar UEL-2 of green bean (Queiroga, Romano, Souza, & Miglioranza, 2003), cultivar Pérola (Figueiredo, Santos, & Garcia, 2012) and cultivar IPR Tangará (Hara et al., 2019).

In the aforementioned studies with common bean, there was variability in the equations found. Models must be developed for a greater number of cultivars, since they have characteristic leaf morphological patterns (Toebe et al., 2019). In this respect, it is important to stress that extrapolating specific models to other cultivars is a common practice. Therefore, in addition to the fitting of specific models, the fitting of general models has been used as an alternative that facilitates practical application, since new cultivars are released on the market every year (Richter et al., 2014; Schwab et al., 2014).

Moreover, water deficits can change the leaf morphology, as demonstrated in soybean (Gonçalves, Silva, Pereira, Gasparino, & Martins, 2017). Because the common bean species is sensitive to this stress, the prediction of long droughts periods due to decreased rainfall and greater evaporative demand (Vicente-Serrano, Quiring, Peña-Gallardo, Yuan, & Domínguez-Castro, 2020) justify the approach. Therefore, the present study was undertaken to develop mathematical models to estimate the leaf area of common bean *(Phaseolus vulgaris)* in irrigated and non-irrigated water regimes from linear dimensions.

Materials and Methods

Experimental design

The experiment was conducted in a 150-m² shelter covered with 200-μm low-density polyethylene, with side walls lined with anti-aphid screen, located in the Department of Plant Science at the Federal University of Santa Maria, RS, Brazil (29°43’ S, 53°43 W, 95 m). The climate of the region is a Cfa type (humid subtropical) with hot summers and no defined dry season (Kuinchtner & Buriol, 2001).

To determine the models, an experiment for the collection of the trifoliates was established with sowing in August 2019 (regular crop) and conducted until the harvest in December 2019. Another experiment to collect the trifoliates to validate the developed equation was established with sowing in January 2020 (off-season crop) and conducted until April 2020. Thus, the use of trifoliates from the off-season experiment to validate an equation developed from trifoliates obtained from the regular crop indicates that the mathematical model is valid for all common-bean sowing times in the southern region of Brazil.

The leaf area was determined from an experiment conducted in a completely randomized design with a 3×2 factorial arrangement (three common-bean cultivars: Triunfo, Garapiá and FC 104; and two water regimes: irrigated and non-irrigated), with 25 replicates per treatment. The cultivars Garapiá *(Phaseolus vulgaris)* and FC104 *(Phaseolus vulgaris)* belong to the *carioca* grain group, and Triunfo *(Phaseolus vulgaris)* to the black grain group. Each experimental unit consisted of a pot with one plant. Each pot had a capacity of 8 L and was filled with typic Bruno-grayish Ultisol soil (Santos et al., 2018). Basal fertilization and inoculation with nitrogen-fixing bacteria were performed according to the technical recommendations of the crop (Comissão de Química e de Fertilidade do Solo RS/SC [CQFS], 2016).

The water regimes were implemented at the pre-flowering stage of common bean, by the methodology of the fraction of transpirable soil water (FTSW). At the beginning of the implementation, all the pots were saturated and left to drain for 24 h, to reach field capacity. After 24 h of drainage, the initial weight of each pot was determined. From that day on, the pots under the non-irrigated water regime did not receive any more irrigation until the plants in them reached 10% of the transpiration of those from the irrigated regime. The weight of all pots was measured daily, from 15h30, on an electronic scale with 50-kg capacity. On the occasion, each pot without water deficit was irrigated with the amount of water that was lost by the daily transpiration of the plant, which was calculated as the difference in daily weight, in grams, subtracted from the initial weight of each pot, using a beaker (Sinclair & Ludlow, 1986).

Analyzed variables

The trifoliates were collected every two weeks, since plant emergence. All trifoliates without mechanical damage or disease spots were removed from three plants per treatment, throughout the crop cycle. In total, 523 trifoliates were sampled from six collections. These were collected early in the morning, to avoid wilting, and immediately analyzed for maintenance of turgor. The largest length (L) (cm) and the largest width (W) (cm) of the central leaflet were measured with a millimeter ruler, considering the space between the ends of the petiole insertion and the end of the central nerve for L and the largest measurement perpendicular to the central nerve for W. Then, their product, LW (cm^2) was calculated (Figure 1) (Richter et al., 2014; Hara et al., 2019).

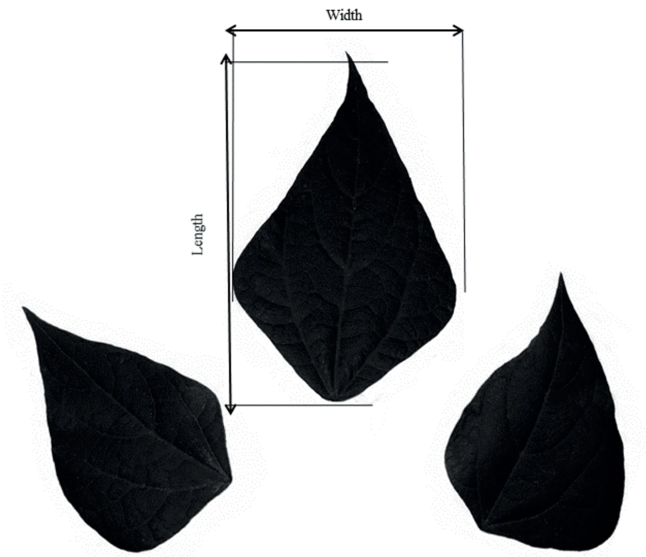

Figure 1. Linear measurements of length and width of a Phaseolus vulgaris trifoliate.
Source: Developed by the authors.

Leaf area as measured by ImageJ® software (Image Processing and Analysis in Java) was determined after photographs were taken with a 13-megapixel camera, which was positioned perpendicularly to the exposure of the trifoliates, at an approximate height of 30 cm that was measured with a millimeter ruler. The camera was held by the same person. Color contrast was applied to the trifoliates present in the photos to make them darker for analysis.

For further comparison, the area of each trifoliate was also determined by the leaf disc methodology. Using a 0.785-cm² cutter, 10 discs or the maximum possible number of discs were extracted from the blade, including the nerves. Afterward, these circular areas and the remaining trifoliates were oven-dried (65 ºC) until reaching constant weight and weighed. The trifoliate area was determined by multiplying the number of discs by the cutter area (0.785 cm²), then by the disc fresh matter, and dividing the result by the disc dry matter (Benincasa, 2003), as shown in the equation below:

$$LA = \frac{(0.785 \times \text{N}) \times \text{TFM}}{DDM} \quad (1)$$

where LA is the leaf area; N is the number of discs; TFM is the trifoliate fresh matter; and DDM is the disc dry matter.

Sample size

The L, W and LW data were subjected to analysis of variance. Means were compared by applying Scott Knott's test at the 0.05 error probability level, using Sisvar software (Ferreira, 2011).

To determine the method to be adopted as standard for fitting the equations, the precision of the methods was evaluated based on the number of trifoliate samples needed to estimate the trifoliate area, using ImageJ® and leaf discs. With the L, W and LW data of the 523 collected trifoliates, an iterative process was carried out with 2,000 resamples, with replacement, using different sample sizes (n), starting with 2 and adding 1 in each iteration up to the maximum size of 1,000 readings. In this way, 2,000 means are obtained for each of the 999 sample sizes used (Ferreira, 2009).

The following statistics were estimated from the obtained means: minimum value, 2.5% percentile, average, 97.5% percentile, maximum value and confidence interval ($CI_{95\%}$). The sample size for estimating the average was considered the number of plants from which the 95% confidence interval ($CI_{95\%}$) was equal to 10, 15, 20, 25, 30, 35 and 40% of the average estimate (Schoffel, Koefender, Camera, Golle, & Horn, 2019). Analyses were performed using R software (R Core Team [R], 2020).

Data analysis and validation

The equations for the estimation of leaf area (dependent variable) were determined by the regression of leaf area as obtained by ImageJ® on the independent variables, namely, L, W and LW, which were measured using a millimeter ruler. Linear (y = bx), quadratic ($y = bx + cx^2$) and power (y = axb) models were fitted. The linear and quadratic models were generated with the intersection at the origin, as it is the most appropriate procedure from the biological point of view (Schwab et al., 2014). The significance of the model parameters was assessed using Student's t-test at the 0.05 error probability level.

For the validation of the equations, 20 independent trifoliates were randomly collected per water regime and cultivar in a second experiment conducted in January 2020, under the same conditions as the other above-mentioned experiment. The performance of the equations in estimating the leaf area of common-bean cultivars was evaluated based on the statistics of root mean square error (RMSE), mean absolute error (MAE), Willmott's index of agreement (d) (Willmott, 1981) and Pearson's correlation (r), using the hydroGOF package (Zambrano-Bigiarini, 2020) of R software (R, 2020). The best equation was chosen considering the highest coefficients of determination (R^2), adjusted R^2 (R^2a), d and r and the lowest RMSE and MAE values. Root mean square error and MAE express the magnitude of the error produced by the model, where values close to zero indicate better models. The d index indicates the agreement between estimated and observed data, where the value of 1 expresses perfect agreement. Finally, the r index indicates the degree of dispersion and association of the simulated data in relation to the observed data, where higher values denote better models.

Results and Discussion

The sample size necessary to estimate the leaf area in the cultivars under each water regime showed greater variability in a 10% confidence interval of the average estimate, ranging from 469 to 698 in the data from digital photographs by ImageJ®, and from 572 to 911 in the data from leaf disc dry matter (Table 1). For all cultivars and water regimes evaluated, 27 trifoliates are sufficient to estimate the leaf area in a 95% confidence interval equal to 40% of the average estimate by ImageJ® and 33 trifoliates for the leaf disc method, which were the highest values observed in each approach. This information allows researchers to dimension the sample size considering the number of plants available and the desired accuracy.

Table 1

Sample size, in number of trifoliates, to estimate the average leaf area in common-bean cultivars Triunfo, Garapiá and FC 104 in the irrigated (I) and non-irrigated (NI) water regimes for confidence intervals shorter than 10, 15, 20, 25, 30, 35 and 40% of the average estimate for the digital photograph method by ImageJ® and leaf discs

	Triunfo		Garapiá		FC 104	
	I	NI	I	NI	I	NI
			Digital photographs - ImageJ®			
10%	515	548	698	691	469	480
15%	235	252	320	313	207	214
20%	131	131	174	171	121	122
25%	88	83	115	105	77	76
30%	55	63	77	76	52	53
35%	32	31	45	41	29	30
40%	22	20	27	25	19	18
			Leaf discs			
10%	744	646	911	884	572	600
15%	322	283	383	382	234	260
20%	181	162	219	221	132	149
25%	113	101	136	133	85	97
30%	80	69	96	100	61	64
35%	42	38	54	54	33	38
40%	29	26	33	33	21	22

Due to the greater accuracy of digital photographs for evaluating the entire trifoliate and not just its parts through disks, smaller sample sizes were required by ImageJ® at all accuracy levels tested. This proves that determining the equation based on digital photographs is an appropriate method, as demonstrated by Lopes et al. (2007) and by Padrón et al. (2016). In a study with common bean (Phaseolus vulgaris), Martin et al. (2013) found that the method considered standard to determine the real leaf area, the leaf area meter (LI3100 LI-COR), a high-cost instrument, can be replaced by ImageJ® software. In green beans (Phaseolus vulgaris), the leaf disc method underestimates the leaf area because there is a difference between the weight of the nerves of the rest of the leaf, which can result in errors in the estimation of leaf area (Toebe, Cargnelutti, Loose, Heldwein, & Zanon, 2012). The smaller sample size by ImageJ® is proportional to the lower variability of the data and inversely proportional to the estimation error. In addition to its greater precision, ImageJ® also has the advantages of being a non-destructive method. Thus, in determining the equation to estimate the leaf area through linear measurements in common bean, it is more appropriate to use the leaf area provided by ImageJ® as real for comparison purposes.

Table 2 shows the fitted equations of leaf area as determined by digital photographs versus length (L), width (W) and their product (length × width = LW) through power, quadratic and linear models, together with the respective coefficient of determination (R^2) and adjusted R^2 (R^2a), and for the different water regimes. The models had satisfactory fits, with R^2 above 0.65 in all cultivars and water regimes; however, the worst performances occurred in the linear models that used L and/or W with R^2 and R^2a lower than 0.80. The other models showed R^2 and R^2a above 0.90, indicating that any of these could be used to estimate the leaf area (Yuan, Peng, & Li, 2017). The best-fit equations were those with the LW product, with R^2 and R^2a above 0.94.

Table 2

Specific equations per cultivar and water regime, coefficient of determination (R^2) and adjusted R^2 (R^2a) developed from the relationship between the area of the common-bean trifoliate as obtained from digital photographs and the linear dimensions of length (L), width (W) and their product (LW) as independent variables (Xi), and statistics of the performance of these empirical models of trifoliate area estimation for the common-bean cultivars Triunfo, Garapiá and FC 104 in two water regimes

Model	X_i	Equation	R^2	R^2a	RMSE	MAE	d	r
		Irrigated Triunfo						
Power	L	$y = 1.503*L^{1.789*}$	0.90	0.90	7.28	6.02	0.95	0.91
Power	W	$y = 2.461*(W)^{1.912*}$	0.94	0.94	9.65	8.15	0.93	0.93
Power	LW	$y = 1.647*(LW)^{0.964*}$	0.96	0.96	6.89	5.88	0.96	0.94
Quadratic	L	$y = 1.046*(L)^2+0.478^{ns}L$	0.92	0.92	10.64	8.84	0.91	0.92
Quadratic	W	$y = 2.009*(W)^2+0.367^{ns}W$	0.93	0.93	8.84	7.46	0.94	0.93
Quadratic	LW	$y = 0.000^{ns}(LW)^2+1.432*LW$	0.96	0.96	6.68	5.60	0.96	0.94
Linear	L	$y = 9.075*L$	0.74	0.74	17.25	15.59	0.71	0.90
Linear	W	$y = 13.46*W$	0.74	0.74	20.06	18.61	0.69	0.92
Linear	LW	$y = 1.453*LW$	0.96	0.96	7.14	6.16	0.96	0.94
		Non-irrigated Triunfo						
Power	L	$y = 1.424*(L)^{1.810*}$	0.93	0.93	11.40	8.86	0.95	0.95
Power	W	$y = 2.228*(W)^{1.941*}$	0.96	0.96	17.12	14.70	0.92	0.97
Power	LW	$y = 1.645*(LW)^{0.957*}$	0.96	0.96	13.31	11.58	0.95	0.97
Quadratic	L	$y = 0.872*(L)^2+0.945^{ns}L$	0.94	0.94	12.99	10.80	0.94	0.95
Quadratic	W	$y = 2.790*(W)^2–0.713^{ns}W$	0.96	0.96	43.74	38.84	0.75	0.97
Quadratic	LW	$y = 0.001^{ns}(LW)^2+1.367*LW$	0.97	0.97	15.66	13.43	0.93	0.97
Linear	L	$y = 8.617*L$	0.78	0.78	18.94	16.29	0.82	0.94
Linear	W	$y = 12.490*W$	0.77	0.77	19.08	17.18	0.85	0.95
Linear	LW	$y = 1.402*LW$	0.97	0.97	14.27	12.34	0.94	0.97
		Irrigated Garapiá						
Power	L	$y = 0.588*(L)^{2.252*}$	0.93	0.93	21.20	15.21	0.89	0.93
Power	W	$y = 2.413*(W)^{1.921*}$	0.95	0.95	12.77	10.25	0.94	0.92

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Power	LW	$y = 1.128*(LW)^{1.067*}$	0.97	0.97	15.18	11.50	0.93	0.96
Quadratic	L	$y = 0.819*(L)^2–0.466^{ns}L$	0.89	0.89	11.06	8.69	0.95	0.94
Quadratic	W	$y = 2.272*(W)^2+0.124^{ns}W$	0.94	0.94	19.20	15.04	0.89	0.92
Quadratic	LW	$y = 5E-05(LW)^{2ns}+1.455*LW$	0.95	0.95	13.28	10.65	0.94	0.96
Linear	L	$y = 9.948*L$	0.68	0.68	21.93	19.94	0.78	0.95
Linear	W	$y = 14.330*W$	0.74	0.73	19.89	17.17	0.81	0.93
Linear	LW	$y = 1.516*LW$	0.95	0.95	16.14	13.29	0.92	0.96
Non-irrigated Garapiá								
Power	L	$y = 0.881*(L)^{2.050*}$	0.94	0.94	7.91	6.70	0.99	0.99
Power	W	$y = 1.882*(W)^{2.050*}$	0.96	0.96	10.78	8.51	0.98	0.97
Power	LW	$y = 1.197*(LW)^{1.046*}$	0.97	0.97	8.58	7.19	0.99	0.98
Quadratic	L	$y = 1.004*(L)^2–0.193^{ns}L$	0.93	0.93	8.19	6.94	0.99	0.99
Quadratic	W	$y = 3.620*(W)^2–2.252*W$	0.94	0.94	48.44	44.16	0.80	0.97
Quadratic	LW	$y = 0.003*(LW)^2+1.283*LW$	0.97	0.97	8.39	6.70	0.99	0.98
Linear	L	$y = 8.320*L$	0.71	0.71	24.74	17.50	0.80	0.97
Linear	W	$y = 12.030*W$	0.70	0.69	25.37	16.98	0.79	0.94
Linear	LW	$y = 1.472*LW$	0.96	0.96	9.69	8.29	0.98	0.98
Irrigated FL 104								
Power	L	$y = 1.085*(L)^{1.936*}$	0.90	0.90	10.16	7.59	0.96	0.93
Power	W	$y = 3.773*(W)^{1.722*}$	0.92	0.92	17.08	12.77	0.82	0.81
Power	LW	$y = 1.732*(LW)^{0.965*}$	0.97	0.97	11.11	8.64	0.94	0.90
Quadratic	L	$y = 0.515*(L)^2+1.633*L$	0.86	0.86	15.30	12.69	0.87	0.93
Quadratic	W	$y = -0.151*(W)^2+6.967*W$	0.85	0.84	37.12	30.81	0.25	0.81
Quadratic	LW	$y = -0.001^{ns}(LW)^2+1.609*LW$	0.95	0.95	10.76	8.29	0.91	0.90
Linear	L	$y = 8.720*L$	0.72	0.72	16.38	13.95	0.84	0.93
Linear	W	$y = 13.710*W$	0.78	0.78	16.59	13.07	0.78	0.81
Linear	LW	$y = 1.510*LW$	0.95	0.95	11.19	8.73	0.94	0.90
Non-irrigated FL 104								
Power	L	$y = 1.400*(L)^{1.842*}$	0.87	0.87	12.24	9.16	0.97	0.96
Power	W	$y = 2.989*(W)^{1.894*}$	0.96	0.96	10.64	7.41	0.97	0.97
Power	LW	$y = 1.748*(LW)^{0.977*}$	0.96	0.96	10.15	7.66	0.98	0.97
Quadratic	L	$y = 0.968*(L)^2+1.188^{ns}L$	0.88	0.88	9.91	7.54	0.98	0.96
Quadratic	W	$y = 2.610*x(W)^2+0.917^{ns}W$	0.95	0.94	9.32	7.04	0.98	0.97
Quadratic	LW	$y = 0.000^{ns}(LW)^2+1.629*LW$	0.95	0.95	9.46	7.31	0.98	0.97
Linear	L	$y = 8.863^{ns}L$	0.71	0.70	17.80	14.68	0.89	0.93
Linear	W	$y = 14.000^{ns}W$	0.75	0.75	19.26	15.74	0.87	0.94
Linear	LW	$y = 1.604^{ns}LW$	0.95	0.95	9.97	7.63	0.98	0.97

* Significant at 0.05 error probability by the t-test. ns = not significant. RMSE = root mean square error (cm^2 $trifoliate^{-1}$); MAE = mean absolute error (cm^2 $trifoliate^{-1}$); d = index of agreement; r = Pearson's correlation coefficient.

Water deficit affected the size of the central leaflet of the common-bean trifoliate. The leaflet L showed a significant difference for water regime ($F [1; 517] = 7.934$, $p = 0.005$). The same result occurred for W ($F [1; 517] = 5.860$, $p = 0.016$) and LW ($F [1; 517] = 7.941$, $p = 0.005$). The leaflets under water deficit showed lower L, W and LW due to the limitation of their growth, which was caused by the lack of water to maintain cell turgor and leaf expansion and perform the biochemical processes to obtain photoassimilates (Taiz et al., 2017). The non-significance of the cultivar factor indicates that its effect can be disregarded and general equations can be made for the water regimes.

Coupled with this, the small variation in the coefficients at x for the linear equations of LW (1.604 to 1.402); in the coefficients at x^2 for the quadratic equations of LW (0.003 to 0.00) and L (1.046 to 0.515); and in the coefficient at xb for the power equations of LW (1.067 to 0.957) and L (2.252 to 1.789), as compared with the other equations, indicates a potential to use a general equation for the three cultivars and two water regimes (Table 2). In addition, the mean errors of these same equations for RMSE and MAE were the smallest, whereas their d and r were the highest (Table 2). Thus, a general equation was developed based on the data from the irrigated trifoliates, another on the non-irrigated trifoliates and another on all data (Table 3) for the aforementioned equations. The quadratic equations of LW were an exception, since the coefficient at x^2, which delimits the parabola concavity and represents the quadratic behavior, is close to zero and did not differ significantly ($p>0.05$) (Table 2). The equations LA = 1.092L1.945 (R^2 = 0.911) and LA = 0.932L2 + 0.376L (R^2 = 0.896), developed using all data (cultivars and water regimes) (Table 3), exhibited the lowest RMSE and MAE for most of the cultivars and water regimes, including the general equations of each water regime. Therefore, they have the best fit.

Table 3

General equations for the irrigated and non-irrigated regimes and with all data, developed from the relationship between the common-bean trifoliate area as obtained from digital photographs and the linear dimensions of length (L), width (W) and their product (LW) as independent variables (Xi), and statistics of the performance of these empirical models for estimating the trifoliate area of the common-bean cultivars Triunfo, Garapiá and FC 104 in two water regimes

Model	RMSE	MAE	d	r	RMSE	MAE	d	r
	General equations for irrigated leaflets							
	LA = 1.494*LW				$LA = 0.965^{*}(L)^2+0.242^{ns}L$			
Irrigated Triunfo	8.20	7.26	0.95	0.94	7.23	5.97	0.95	0.92
Non-irrigated Triunfo	18.74	16.57	0.91	0.97	13.09	10.95	0.94	0.95
Irrigated Garapiá	15.03	12.27	0.93	0.96	15.88	12.10	0.92	0.94
Non-irrigated Garapiá	10.07	8.63	0.98	0.98	8.78	7.60	0.99	0.99
Irrigated FC 104	11.39	8.95	0.94	0.90	12.44	9.12	0.94	0.93
Non-irrigated FC 104	12.89	9.91	0.96	0.97	12.16	9.16	0.97	0.96

continue...

contuation...

	LA = 1.481*(LW)$^{0.998*}$				LA = 1.008*(L)$^{1.983*}$			
Irrigated Triunfo	7.64	6.71	0.95	0.94	6.99	5.65	0.95	0.92
Non-irrigated Triunfo	17.68	15.53	0.92	0.97	12.38	10.10	0.95	0.95
Irrigated Garapiá	13.96	11.28	0.94	0.96	14.47	10.75	0.93	0.94
Non-irrigated Garapiá	9.73	8.33	0.98	0.98	8.62	7.09	0.99	0.99
Irrigated FC 104	11.70	9.26	0.93	0.90	11.42	8.52	0.95	0.93
Non-irrigated FC 104	13.53	10.43	0.96	0.97	13.00	9.73	0.96	0.96
General equations for non-irrigated leaflets								
	LA = 1.482*LW				LA = 0.907*(L)^{2}+0.673^{ns}L			
Irrigated Triunfo	7.86	6.93	0.95	0.94	7.25	5.95	0.95	0.92
Non-irrigated Triunfo	18.13	15.96	0.91	0.97	13.14	11.00	0.94	0.95
Irrigated Garapiá	14.42	11.70	0.93	0.96	14.80	11.47	0.93	0.94
Non-irrigated Garapiá	9.84	8.44	0.98	0.98	9.28	7.85	0.98	0.99
Irrigated FC 104	11.57	9.13	0.93	0.90	11.88	8.68	0.94	0.93
Non-irrigated FC 104	13.26	10.22	0.96	0.97	12.39	9.29	0.97	0.96
	LA = 1.510*(LW)$^{0.992*}$				LA = 1.209*(L)$^{1.898*}$			
Irrigated Triunfo	7.48	6.55	0.95	0.94	7.13	5.81	0.95	0.91
Non-irrigated Triunfo	17.19	15.08	0.92	0.97	12.32	9.98	0.95	0.95
Irrigated Garapiá	13.42	10.88	0.94	0.96	13.44	10.43	0.94	0.94
Non-irrigated Garapiá	9.73	8.29	0.98	0.98	9.83	7.74	0.98	0.99
Irrigated FC 104	11.81	9.35	0.93	0.90	11.07	8.14	0.95	0.93
Non-irrigated FC 104	13.80	10.61	0.95	0.97	13.12	9.88	0.96	0.96
General equations with all data								
	LA = 1.490*(LW)				LA = 0.932*(L)^{2}+0.376^{ns}L			
Irrigated Triunfo	8.08	7.15	0.95	0.94	7.06	5.75	0.95	0.92
Non-irrigated Triunfo	18.53	16.36	0.91	0.97	12.60	10.37	0.95	0.95
Irrigated Garapiá	14.82	12.08	0.93	0.96	14.47	10.95	0.93	0.94
Non-irrigated Garapiá	9.99	8.57	0.98	0.98	8.90	7.37	0.98	0.99
Irrigated FC 104	11.45	9.00	0.93	0.90	11.52	8.53	0.95	0.93
Non-irrigated FC 104	13.02	10.01	0.96	0.97	12.79	9.58	0.96	0.96
	LA = 1.491*(LW)$^{0.996*}$				LA = 1.092*(L)$^{1.945*}$			
Irrigated Triunfo	7.52	6.59	0.95	0.94	7.03	5.71	0.95	0.92
Non-irrigated Triunfo	17.38	15.25	0.92	0.97	12.28	9.96	0.95	0.95
Irrigated Garapiá	13.64	11.03	0.94	0.96	13.90	10.53	0.94	0.94
Non-irrigated Garapiá	9.70	8.29	0.98	0.98	9.15	7.37	0.98	0.99
Irrigated FC 104	11.77	9.33	0.93	0.90	11.19	8.31	0.95	0.93
Non-irrigated FC 104	13.71	10.55	0.96	0.97	13.09	9.83	0.96	0.96

* Significant at 0.05 error probability by the t-test. ns = not significant. RMSE = root mean square error (cm^2 $trifoliate^{-1}$); MAE = mean absolute error (cm^2 $trifoliate^{-1}$); d = index of agreement; r = Pearson's correlation coefficient.

By comparing the statistics of the predictive capacity of the general equations (LA = $1.092L^{1.945}$ and LA = $0.932L^2 + 0.376L$), obtained from all data (shown in Table 3, with the specific equations in Table 2), we observe that RMSE ranged from 6.68 to 48.44 cm² trifoliate^{-1} in the specific equations (Table 2) and from 7.03 to 14.47 cm² trifoliate^{-1} in the general equations (Table 3). The maximum RMSE of the general equations (7.03 to 14.47 cm² trifoliate^{-1}) was also lower than that of the general equations for the irrigated (6.99 to 18.74 cm² trifoliate^{-1}) and non-irrigated (7.13 to 18.13 cm² trifoliate^{-1}) regimes. The RMSE values of the general equations were close to the 6.48 to 16.92 cm² leaf^{-1} found in soybean (Richter et al., 2014) and lower than the 12.56 to 39.94 cm² trifoliate^{-1} described in green beans (Toebe et al., 2012). There was a similar estimation error between the statistics of the specific and general equations, with better values occurring in the latter than in some specific equations in each cultivar and water regime.

Although the equations based on the LW variable had the highest R² and R²a and were among those with the lowest estimation error (Table 2, 3), the power L and quadratic L equations also stood with low RMSE and MAE values. The use of only one independent variable in this case, L is beneficial, as it requires less time for data collection, resulting in a lower margin of error in data collection and in the estimation of leaf area (Padrón et al., 2016). Queiroga et al. (2003) observed a similar result in green beans *(Phaseolus vulgaris)* and concluded that a power equation with only L data was sufficient to estimate the leaf area.

The fit of the two general equations (LA = $1.092L^{1.945}$ and LA = $0.932L^2 + 0.376L$) to the 1:1 line is similar (Figures 2). Thus, the criterion for selecting the best equation consists of the smallest error (RMSE, MAE) and greatest accuracy of the estimate (d, r), for which equation LA = $1.092L^{1.945}$ stood out with an average RMSE of 11.11 cm² trifoliate^{-1}, an average MAE of 8.62 cm² trifoliate^{-1} and r from 0.96 to 0.95. As shown in Figure 2, when the general equation (Figures 2 A) was compared with the specific equations (Figures 2 B) for cultivars and water regimes, the behavior was similar, with little discrepancy, since the trifoliates have similar shapes. This result warrants the use of the general equation, which can be applied to other cultivars already available on the market or that will be released and for which specific equations have not been developed. The opposite was observed in soybean, where water restriction altered leaf morphology (Gonçalves et al., 2017). However, studies have shown that even in soybean cultivars with variable leaf shapes, the general equation can be used in place of specific ones (Richter et al., 2014).

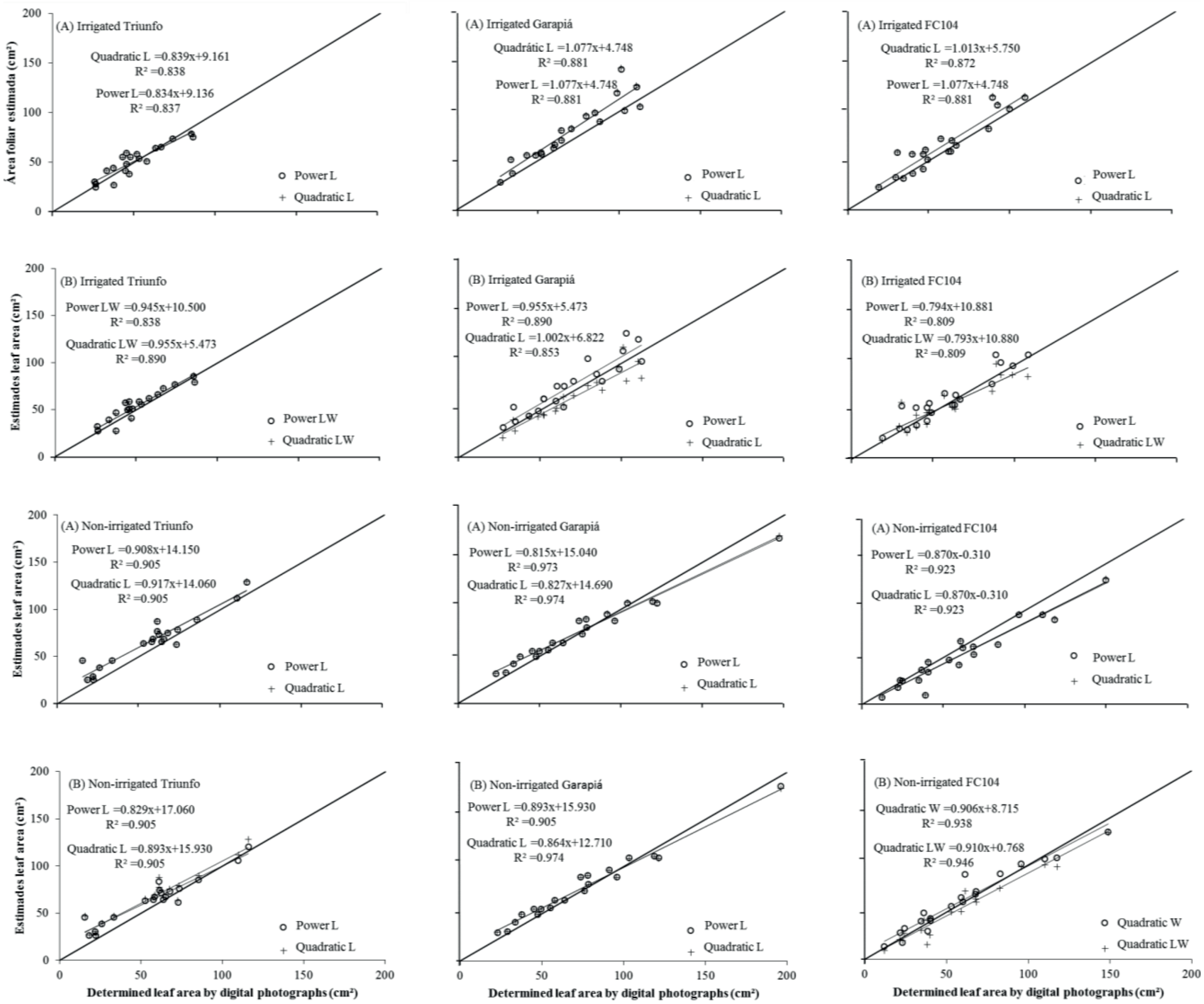

Figure 2. Leaf area as estimated by the general (cm²) power L (LA = $1.092{*}L^{1.945}$) and quadratic L (LA = $0.932{*}L^2 + 0.376{*}L$) (A) equations and the specific equations with the best performance (B) versus leaf area as determined by digital photographs (cm²) of common-bean cultivars Triunfo, Garapiá and FC104 in irrigated and non-irrigated water regimes. Table 2 describes the specific equations fitted for each situation. The central line represents the 1:1 ratio.

The differences in estimates between the data of the general equation LA = $1.092L^{1.945}$ and the best specific equation for each water regime and cultivar (Table 3) were (in cm² trifoliate^{-1}) 0.35 for irrigated cv. Triunfo; 0.88 for non-irrigated cv. Triunfo; 2.84 for irrigated cv. Garapiá; 1.24 for non-irrigated cv. Garapiá; 1.03 for irrigated cv. FC 104; and 3.77 for non-irrigated cv. FC 104. Thus, the low average RMSE value of 1.69 cm² trifoliate^{-1} reinforces the possibility of using the general equation for all cultivars and water regimes. The other MAE parameters, d and r, accompanied this behavior. Determining a general equation is important due to the scarcity of specific equations for all cultivars available on the market, provided that the morphological characteristics of the leaves are similar (Richter et al., 2014).

This study provides considerable information on the leaf area of common bean cultivars in irrigated and non-irrigated water regimes, which can assist in the research and management of crops. The presented findings suggesting the need for only L data to estimate leaf area in common bean are conflicting with those described in studies with green beans *(Phaseolus vulgaris).* For instance, researchers Toebe et al. (2012) and Queiroga et al. (2003) observed that only L data from the central leaflet of the trifoliate are sufficient to estimate the leaf area, from quadratic and power equations and power equations, respectively.

Based on the information given in Table 1, the user has the alternative of sampling the appropriate number of trifoliates from their experimental unit to accurately estimate the trifoliate leaf area. The equation to determine leaf area based on the L of the central leaflet of the trifoliate ($LA = 1.092L^{1.945}$) provides accurate data with a low estimation error. Figure 3 illustrates the adequacy of the digital photograph method to estimate the leaf area of the common-bean trifoliate, using only with the central leaflet length data.

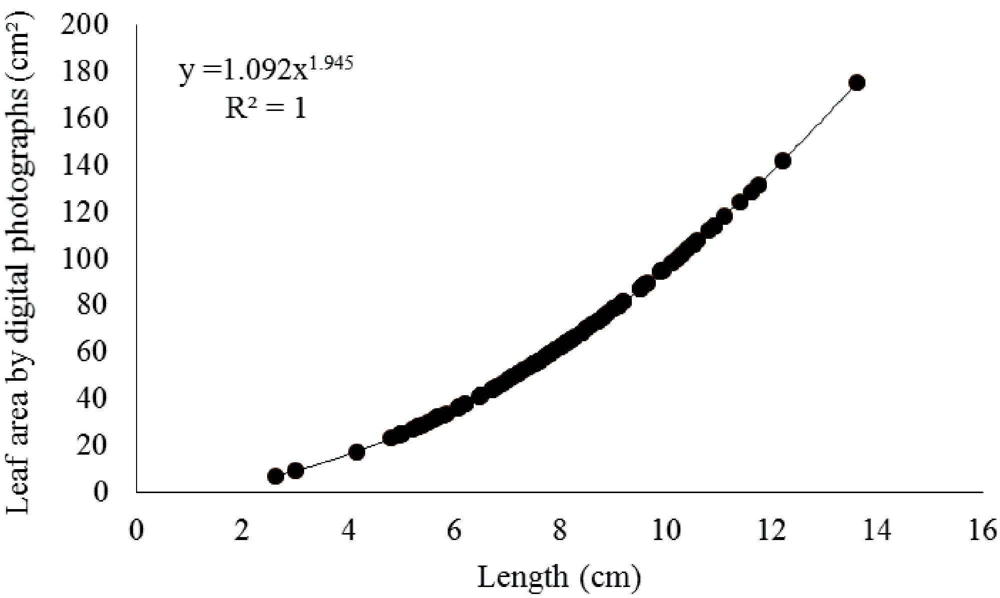

Figure 3. Power model of the leaf area of the trifoliate (three leaflets) (y) of common bean *(Phaseolus vulgaris)* as obtained from digital photographs, as a function of the maximum central leaflet length (x).

Conclusion

The non-destructive method based on the linear dimensions of the central leaflet of common bean is suitable for estimating leaf area. The general equation $LA = 1.092L^{1.945}$ can be used for cultivars Triunfo, Garapiá and FC 104 in both the irrigated and non-irrigated water regimes, without accuracy losses.

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