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ORIGINAL PAPER

Calibration and validation of regression models for individual leaf area estimation of cauliflower grown in a hydroponic system

Calibração e validação de modelos de regressão para estimativa da área foliar individual da couve-flor em sistema hidropônico

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Abstract: Leaf area (LA) is an important parameter in many studies to evaluate plant growth, but is generally estimated by a destructive measure, i.e., requires leaves to be removed. In this context, non-destructive methods have been used based on linear measurements such as leaf length (L) and/or width (W) to determine individual LA. In this study, models were developed using linear measurements (L, W or L×W) of leaf for individual LA estimation of cauliflower grown in a hydroponic system. Two experiments were conducted, one in the autumn-winter 2019 (three cauliflower cultivars 'Piracicaba de Verão', 'Sabrina', and 'SF1758' for calibration and validation) and the other in the spring-summer 2019-2020 (only cultivar 'SF1758' for validation). In the autumn-winter, the relationships between individual LA (dependent variable) and L, W or L×W (independent variables) were adjusted using the linear, exponential and power models. These models were developed individually for each cultivar, as well as for the three cultivars together (universal models). In the validation between observed and estimates values, the best estimates of individual LA of cauliflower were obtained when the product L×W was used as an independent variable, being recommended the linear (LA = -14.424 + 0.843L×W) or potential [$LA = 0.551(L\times W)^{1.057}$] models developed for 'Piracicaba de Verão' and only a linear model (LA = -22.610 + 0.928L×W) for 'SF1758'. For cultivar 'Sabrina', universal models either linear (LA = -13.770 + 0.833L×W) or potential types [$LA = 0.578(L\times W)^{1.050}$] are recommended. These models as well can be employed for cultivars 'Piracicaba de Verão' and 'SF1758'.

Keywords: *Brassica oleracea* var. *botrytis*, linear measurements, non-destructive method, L×W product.

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Resumo: A área foliar (AF) é um importante parâmetro em muitos estudos para avaliar o crescimento das plantas, sendo geralmente uma medida destrutiva, ou seja, exige que as folhas sejam removidas. Nesse contexto, métodos não destrutivos têm sido usados a partir de medidas lineares como comprimento (C) e/ou largura (L) da folha para determinar a AF individual. No presente estudo, modelos foram desenvolvidos usando medições lineares da folha (C, L ou C×L) para estimativa da AF individual da couve-flor cultivada em sistema hidropônico. Foram conduzidos dois experimentos, o primeiro no outono-inverno de 2019 (com três cultivares de couve-flor 'Piracicaba de Verão', 'Sabrina' e 'SF1758' para calibração e validação) e outro na primavera-verão de 2019-2020 (apenas com a cv. 'SF1758' para validação). No outono-inverno, as relações entre AF individual (variável dependente) e as variáveis independentes (C, L ou C×L) foram ajustadas usando os modelos do tipo linear, exponencial e potencial. Esses modelos foram desenvolvidos separadamente por cultivar, como também para as três cultivares agrupadas (modelos universais). Na validação entre os valores observados e estimados, as melhores estimativas da AF individual da couve-flor foram obtidas quando o produto C×L foi usado como variável independente, recomendando-se os modelos linear (AF = -14,424 + 0,843C×L) ou potencial [$AF = 0{,}551(C\times L)^{1{,}057}$] desenvolvidos para 'Piracicaba de Verão' e apenas o modelo linear (AF = -22,610 + 0,928C×L) para 'SF1758'. Para cultivar 'Sabrina', os modelos universais do tipo linear (AF = -13,770 + 0,833C×L) ou potencial [$AF = 0{,}578(C\times L)^{1{,}050}$] são recomendados. Esses modelos também podem ser empregados para as cultivares 'Piracicaba de Verão' e 'SF1758'.

Palavras-chave: *Brassica oleracea* var. *botrytis*, medições lineares, métodos não destrutivos, produto C×L.

Introduction

Leaf area (LA) is an important parameter in many studies to evaluate plant growth, so its measurement is extremely important. There are several methods for determining the LA of a plant, which are classified as destructive (direct) and non-destructive (indirect). Direct methods, despite being the most precise, are destructive (requires leaf excision) (Cirillo et al., 2017; Ribeiro et al., 2018), which prevents temporal measurements on the same leaf of the plant over time, and restricts its applicability in studies with limited number of plants or with limited leaves on the same plant (Yeshitila & Taye, 2016; Salazar et al., 2018).

Due to all these limitations of direct methods, the development of models based on regression analysis using linear measurements of leaves (length - L and width - W) has been recurrent to estimate the individual LA of different plant species (Tartaglia et al., 2016; Fernandes et al., 2017).

The application of these mathematical models has advantages over the use of destructive methods, mainly because they do not require plant destruction, thus allowing measurements on the same leaf during the plant growth period (José et al., 2014; Zanetti et al., 2017). Despite the ease of linear measurements in leaves, for cauliflower (*Brassica oleracea* var. *botrytis*), no study of this type is found in the literature, although it is a widely studied crop. However, some studies for crops of the same species *Brassica oleracea*, such as kale (Marcolini et al., 2005), cabbage (Olfati et al., 2009; Yeshitila & Taye, 2016) and broccoli (Olfati et al., 2010), stand out.

Given this lack of information on the LA of cauliflower, the objective of this study was to evaluate different regression models developed from linear measurements such as length and width of leaves of this species.

Material and Methods

Study site and experimental conditions

The study was conducted in a greenhouse in the experimental area of the Post Graduate Program in Agricultural

Engineering of the Federal University of Recôncavo of Bahia, Cruz das Almas, Bahia, Brazil (12º 40’ 19” S, 39º 06’ 23” W, and at an altitude of 220 m).

Two experiments were carried out in a randomized blocks design with six replications, the first between April and July 2019 (autumn-winter) with three cauliflower cultivars (‘Piracicaba de Verão’, ‘Sabrina’, and ‘SF1758’) and the second between October 2019 and January 2020 (spring-summer) with only the cv. ‘SF1758’. In both experiments, the plants were grown under different electrical conductivities of the nutrient solution (ECsol), prepared in saline waters by addition of NaCl to public-supply water (ECw 0.3 dS m^{-1}), using the following salt concentrations: 0.714, 1.377, 1.888, 2.399, and 3.153 g L^{-1}.

The seeds of the three cauliflower cultivars (‘Piracicaba de Verão’, ‘Sabrina’, and ‘SF1758’) were sown in phenolic foam (2 x 2 x 2 cm) on April 3 and of the cv. ‘SF1758’ on October 10, 2019, for autumn-winter and spring-summer experiments, respectively.

At 5 and 8 days after sowing (DAS) for the autumn-winter and spring-summer experiments, respectively, the seedlings were transferred to a nursery in NFT (Nutrient Film Technique) system, where they received a nutrient solution (Furlani et al., 1999) at 50% concentration for 23 and 21 days, respectively. At 28 and 29 DAS for autumn-winter and spring-summer experiments, respectively, the cauliflower seedlings were transplanted to the hydroponic channels, totaling three seedlings of each cultivar per channel in autumn-winter and nine seedlings only of the cv. ‘SF1758’ in spring-summer, spaced by 0.56 m.

The cauliflower plants were grown in a NFT system, in hydroponic channels (6 m long PVC pipes of 0.075 m in diameter), installed with a 3% slope. More details of the experimental structure can be seen in Costa et al. (2020), including the crop conduction and nutrient solution management.

Data collection

In both experiments, one leaf per plant (sixth fully expanded leaf, from bottom to top) was previously identified in each cultivation channel, according to the procedure adopted by Oliveira et al. (2017) for kale. The selected leaves showed no symptoms of mineral deficiency or toxicity that could be attributed to salinity, or damage caused by pests and/or diseases.

In the autumn-winter experiment, one plant of each cultivar was selected for measurements of leaf length (L), leaf width (W) and leaf area (LA). Always on the same leaf, measurements were performed the growth period at 15, 25 and 35 days after transplantation (DAT) and between 49 and 65 DAT (harvest of inflorescences). In spring-summer experiment only with the cv. ‘SF1758’, the measurements of L, W and LA were performed only at the harvest of inflorescences (between 48 and 58 DAT), in leaves of three plants per cultivation channel.

In both experiments, the L measurement was performed parallel to the direction of the midrib from the apex of the lamina to the base of the petiole; W was measured at the widest point perpendicular to the main axis of the leaf (Figure 1).

In autumn-winter experiment, at 15, 25 and 35 DAT, a digital camera was used to capture images of the leaves individually. For each photo, the individual cauliflower leaf was placed on a 21 × 29.7 cm (A4) white sheet of paper together with a ruler, which served as a scale (Figure 1). These photos were always taken from a fixed position (maintaining same height and angle). Subsequently, individual LA was measured using ImageJ software version 1.5.2a (National Institute of Mental Health, Bethesda, Maryland, USA). At the harvest of inflorescences, the leaves were detached and LA was measured using a portable leaf area meter model CI202 (CID Bio-Science, Inc., Washington, USA).

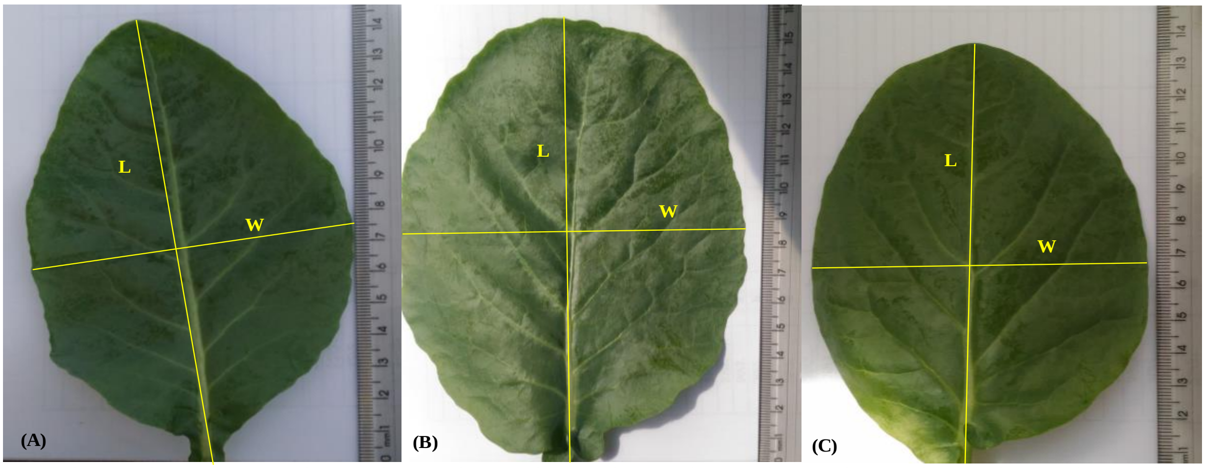


Figure 1: Leaves of cauliflower cultivars ‘Piracicaba de Verão’ (A), ‘Sabrina’ (B) and ‘SF1758’ (C) in the autumn-winter experiment.

Despite the use of two methodologies to determine LA, we believe that there was no interference in the generation of models. This fact can be supported by results of earlier studies that demonstrate that LA estimates using images processed in software were similar to those obtained directly with the leaf area integrator method (Adami et al., 2008; Martin et al., 2013).

Calibration of the models

Before developing the models based on data from the autumn-winter experiment, the L/W ratio was calculated. For any measuring period evaluated, there was no significant difference in the L/W ratio, so salinity did not influence the shape of the leaves, and the different models could be fitted to estimate the LA of cauliflower.

A total of 134, 138 and 135 measurements of L, W and LA (in all periods evaluated) of the individual leaves of the cauliflower cultivars ‘Piracicaba de Verão’, ‘Sabrina’ and ‘SF1758’, respectively, were used in the calibration of the models. The models were fitted based on the measurements of individual cultivar and also for the grouped cultivars (universal models). L, W and LA measurements were randomized, 80% were used in the calibration of the models and the remaining 20% for their validation.

The relationships between LA (dependent variable) and L, W or L×W (independent variables) were fitted using linear ($y = a + bx$), exponential ($y = ae^{bx}$) and power ($y = ax^b$) models; where: ‘y’ is the measured LA, ‘x’ are the independent variables (L, W or L×W) and ‘a’ and ‘b’ are the parameters of the models.

Before fitting the models, the degree of collinearity between the L and W measurements was analyzed. The variance inflation factor ($VIF = 1/1-r^2$) was calculated as described by Wang et al. (2019); r is the correlation coefficient. If the VIF values are less than 10, then problems of collinearity between L and W are considered insignificant and, therefore, these parameters can be included in empirical models. In the present study, there were no problems of collinearity between L and W, with VIF values lower than 10; thus, the L×W product could be used in the development of the models.

The best models were selected based on significance by the F test of the analysis of variance and their parameters using the Student’s t-test and, additionally, based on the combination of the highest values of the coefficient of determination (R^2) and lowest values of the root mean square error (RMSE) and coefficient of variation. RMSE was calculated as described by Wang et al. (2019).

Validation of the models

The validation phase of the models used 20% of the measurements (L, W and LA) that were not used in the development phase in the autumn-winter experiment (dependent data). In the spring-summer experiment with only the cauliflower ‘SF1758’ (independent data), a total of 108 measurements were used in the validation.

The universal models (three cultivars together) were validated with the complete set of data of each cultivar in the autumn-winter experiment and in the spring-summer experiment with only the cv. ‘SF1758’. In addition to the validation using the models developed for each cultivar individually and together, the models for a respective cultivar were also validated with the complete set of data of another cultivar; for example, the models developed for cv. ‘SF1758’ were validated with data set of the cv. ‘Sabrina’, and vice versa. In all combinations, the mean values of observed leaf area (OLA) and estimated leaf area (ELA) were compared using the Student’s t-test.

For each selected model, a simple linear regression was performed (ELA = a + bOLA). The hypotheses H_0: a =0 *versus* H_1: a ≠ 0 and H_0: b =1 *versus* H_1: b ≠ 1 were tested using Student’s t-test. The same statistical indicators used in the selection of models were used in the validation. Mean bias (differences between OLA and ELA) and relative bias [(OLA-ELA)/OLA] were also calculated. The limits of the mean (differences between OLA and ELA) ± 3SD (standard deviation) were also calculated as described by Rouphael et al. (2010).

Additionally, the model (LA = 0.82012 + 0.71913L×W) developed by Marcolini et al. (2005) for kale was tested. This model was developed for leaves with a maximum L of 30 cm. Therefore, the autumn-winter experiment data were used in this model in two ways: complete data set (without leaf L restriction) and L data set of leaves < 30 cm. In spring-summer experiment, the complete set of data was used, but only a few leaves had L > 30 cm.

Statistical analysis

Statistical analysis was performed using Microsoft Office Excel® application and R-statistical software version 3.6.3 (R Development Core Team, 2020).

Results and Discussion

In both experiments, a descriptive analysis (minimum, maximum, mean ± standard deviation and coefficient of variation) was performed for the measurements of L, W, L×W and LA, as shown in Table 1. From the high amplitude of the data (L, W and LA) in the autumn-winter experiment, it was possible to model the LA of cauliflower for a wide range of leaf sizes and shapes; therefore, the models developed can be used to estimate LA in different periods of crop development. Other authors reinforce the use of a database with wide variability, to ensure the development of models that may have a wide utility (Cargnelutti Filho et al., 2015; Toebe et al., 2019).

Developed models

The 36 fitted models to estimate the LA of three cauliflower cultivars (individually and jointly) based on the input variables L, W or L×W were significant by the F test of the analysis of variance. For all models, the parameter ‘b’ was significant by t-test. In general, the L×W product was the independent variable that best explained most variations in LA in comparison to individual measurements (L or W) (Table 2). Reinforcing these results, for other plant species, such as bell pepper (Padrón et al., 2016) and *Erythroxylum simonis* (Ribeiro et al., 2018), the best estimates of LA were obtained from the L×W product.

With cauliflower, in the literature there is only one study conducted by Sadik et al. (2011) comparing different methodologies for estimating the individual LA of this crop. In that study, regression analysis was a methodology when only leaf width was used as independent variable (model not presented). Contrasting results have been found in other studies with crops of the species *Brassica oleracea*, such as Olfati et al. (2010), who found better precision of the models developed having as independent variables the L×W product (for broccoli) and W^2 (for cabbage). For cabbage, Yeshitila & Taye (2016) obtained the best fit using the L×W product.

While in these studies with crops of the species *Brassica oleracea*, only linear models were tested, in the present study, in addition to linear models, exponential and power models were also tested. Testing of various types of models in the calibration phase is important, as previously calibrated models may not have the same validation performance. In the present study, better estimates of LA with the L×W product were obtained with linear (numbers 3, 9, 15 and 21) and power (numbers 6, 12, 18 and 24) models compared to the exponential model, with higher values of R^2 (between 96.12 and 98.60%) and lower values of RMSE and CV (Table 2). For this reason, the statistical indicators of that last model are not shown.

Table 1: Summary of descriptive statistics for leaf length (L, cm), leaf width (W, cm), L×W product (cm^2) and observed leaf area (LA, cm^2) of cauliflower grown in NFT hydroponics

Cultivars	Variables	Min	Max	Mean ± SD	CV (%)	*P*-value$^{(1)}$ S-F
				Autumn-winter experiment		
‘Piracicaba de Verão’ (*n* = 134)	L	6.90	66.00	20.95 ± 14.27	68.12	>0.05
	W	5.10	35.00	13.66 ± 7.75	56.76	>0.05
	L×W	37.26	2187.50	387.42 ± 499.50	128.93	>0.05
	LA	29.05	2085.86	311.37 ± 428.77	137.70	>0.05
‘Sabrina’ (*n* = 138)	L	7.60	49.50	17.99 ± 9.40	52.22	>0.05
	W	5.30	28.00	12.29 ± 4.62	37.60	>0.05
	L×W	42.40	1113.75	258.92 ± 245.16	94.69	>0.05
	LA	29.15	869.63	186.80 ± 174.10	93.20	>0.05
‘SF1758’ (*n* = 135)	L	5.30	43.00	16.70 ± 8.41	50.33	>0.05
	W	4.40	26.00	12.53 ± 5.24	41.84	>0.05
	L×W	23.32	1032.00	251.33 ± 238.10	94.74	>0.05
	LA	17.47	908.42	210.45 ± 222.36	105.66	>0.05
				Spring-summer experiment		
‘SF1758’ (*n* = 108)	L	18.50	31.00	23.70 ± 2.37	10.00	>0.05
	W	14.00	23.00	18.06 ± 1.78	9.85	>0.05
	L×W	266.00	713.00	431.55 ± 83.00	19.23	>0.05
	LA	225.42	660.19	382.67 ± 79.95	20.89	>0.05

n - number of measurements; Min - minimum value; Max - maximum value; SD - standard deviation; CV - coefficient of variation; $^{(1)}$ not significant at p = 0.05 by Shapiro-Francia (S-F) t-test, i.e., the data follow a normal distribution.

Based on the best statistical indicators obtained with the L×W product, eight models were used in the validation. Of these selected models, the values of CV and RMSE varied between 14.01 and 24.60% and between 33.10 and 89.01 cm^2, with the lowest and highest values obtained for the cauliflower ‘Sabrina’ and ‘Piracicaba de Verão’, respectively (Table 2).

Validation of the models

In the validation of the models developed individually for the cultivars ‘Piracicaba de Verão’, ‘Sabrina’ and ‘SF1758’, and the universal models validated with individual data of the respective cultivars, the values of RMSE and CV were lower than 21 cm^2 and 18%, respectively. The lowest values of R^2 (~83%) were verified for the cv. ‘Sabrina’, both in the validation for models 9 and 12 developed individually for this cultivar and using the universal models 21 and 24. For the other combinations with the individual models and/or universal models, the values of R^2 were between 87.13 and 96.05% (Table 3).

Table 2: Models for individual leaf area estimation of cauliflower ‘Piracicaba de Verão’, ‘Sabrina’ and ‘SF1758’ grown in NFT hydroponics in autumn-winter experiment

Models No.	Variables	Fitted models[1]	*P*-value F-test	R^2 (%)	RMSE (cm^2)	CV (%)
			‘Piracicaba de Verão’			
1	L	LA = -287.667 + 28.757L	<0.001	91.12	138.01	37.87
2	W	LA = -388.080 + 51.558W	<0.001	85.36	177.23	48.63
3	L×W	LA = -14.424 + 0.843L×W	<0.001	96.25	89.68	24.60
4	L	$LA = 1.526L^{1.690}$	<0.001	96.60	135.27	37.11
5	W	$LA = 1.215W^{2.06}$	<0.001	95.02	165.78	45.48
6	L×W	$LA = 0.551L{\times}W^{1.057}$	<0.001	97.12	89.01	24.48
			‘Sabrina’			
7	L	LA = -140.149 + 18.128L	<0.001	94.33	44.81	21.46
8	W	LA = -233.081 + 34.139W	<0.001	77.93	88.44	43.15
9	L×W	LA = 3.491 + 0.701L×W	<0.001	96.12	37.09	18.08
10	L	$LA = 2.019L^{1.537}$	<0.001	96.30	45.91	22.40
11	W	$LA = 1.675W^{1.854}$	<0.001	91.58	92.10	44.93
12	L×W	$LA = 0.773L{\times}W^{0.986}$	<0.001	97.81	37.09	18.09
			‘SF1758’			
13	L	LA = -221.694 + 25.896L	<0.001	93.40	56.55	23.93
14	W	LA = -306.377 + 41.495W	<0.001	91.69	54.64	23.12
15	L×W	LA = -22.610 + 0.928L×W	<0.001	97.93	33.10	14.01
16	L	$LA = 1.001L^{1.847}$	<0.001	97.74	57.22	24.21
17	W	$LA = 0.392W^{2.388}$	<0.001	96.88	41.92	17.74
18	L×W	$LA = 0.499L{\times}W^{1.088}$	<0.001	98.60	35.69	15.10
			Universal models			
19	L	LA = -240.236 + 25.676L	<0.001	88.86	90.79	33.93
20	W	LA = -356.152 + 46.234W	<0.001	83.43	112.71	42.12
21	L×W	LA = -13.770 + 0.833L×W	<0.001	95.90	57.58	21.52
22	L	$LA = 1.401L^{1.703}$	<0.001	96.14	102.31	38.23
23	W	$LA = 0.804W^{2.142}$	<0.001	94.58	115.62	43.20
24	L×W	$LA = 0.578L{\times}W^{1.050}$	<0.001	98.08	65.65	24.53

(1) Parameter ‘b’ ($y = a + bx$ or $y = ax^b$) significant at $p = 0.05$ by Student’s t-test.

Table 3: Regression equations and performance indices of the observed leaf area (OLA) and estimated leaf area (ELA) by different models

Models No.	Validated for	Regression analysis	R^2 (%)	RMSE (cm^2)	CV (%)
		Autumn-winter experiment			
3	‘PV’	ELA = -7.9241 + 1.0758**OLA	92.81	13.95	12.97
6	‘PV’	ELA = 0.3536 + 0.9880**OLA	92.79	12.48	11.61
3	‘Sabrina’	ELA = -14.3767 + 1.1573**OLA	97.41	45.22	24.30
6	‘Sabrina’	ELA = -13.0212 + 1.1291**OLA	97.38	40.78	21.92
3	‘SF1758’	ELA = 9.4160 + 0.8935**OLA	97.97	39.23	18.64
6	‘SF1758’	ELA = 11.6565 + 0.8662**OLA	98.01	43.56	20.70
9	‘Sabrina’	ELA = -4.1775 + 0.9566**OLA	83.51	18.37	15.92
12	‘Sabrina’	ELA = -6.4914 + 0.9707**OLA	83.64	18.82	16.30
9	‘PV’	ELA = 11.4162 + 0.9711**OLA	96.88	75.91	24.46
12	‘PV’	ELA = 25.1553 + 0.8009**OLA	96.85	111.85	36.04
9	‘SF1758’	ELA = 23.3158 + 0.7430**OLA	97.97	68.93	32.75
12	‘SF1758’	ELA = 22.4529 + 0.7434**OLA	97.94	69.24	32.90
15	‘SF1758’	ELA = -25.7610 + 1.2409**OLA	96.03	17.22	16.10
18	‘SF1758’	ELA = -7.2095 + 1.0278**OLA	95.90	11.08	10.36
15	‘PV’	ELA = 5.8960 + 1.0690**OLA	96.88	91.71	29.55
18	‘PV’	ELA = 0.4699 + 1.1163**OLA	97.01	104.53	33.69
15	‘Sabrina’	ELA = -22.3450 + 1.2736**OLA	97.41	66.19	35.57
18	‘Sabrina’	ELA = -21.7530 + 1.2703**OLA	97.32	66.02	35.48
21	‘PV’	ELA = -7.3463 + 1.0630**OLA	92.81	13.67	12.71
24	‘PV’	ELA = 1.0151 + 0.9942**OLA	92.79	12.52	11.64
21	‘Sabrina’	ELA = -22.8852 + 1.1368**OLA	83.51	20.74	17.97
24	‘Sabrina’	ELA = -13.2348 + 1.0601**OLA	83.01	19.07	16.52
21	‘SF1758’	ELA = -17.2173 + 1.1231**OLA	96.20	13.06	12.21
24	‘SF1758’	ELA = -7.5092 + 1.0454**OLA	96.20	10.83	10.12
		Spring-summer experiment			
3	‘SF1758’	ELA = 28.5780 + 0.8376**OLA	91.53	41.12	10.74
6	‘SF1758’	ELA = 22.8250 + 0.8194**OLA	91.46	52.40	13.69
9	‘SF1758’	ELA = 39.2550 + 0.6965**OLA	91.53	82.15	21.47
12	‘SF1758’	ELA = 40.4891 + 0.6947**OLA	91.48	81.86	21.39
15	‘SF1758’	ELA = 24.7320 + 0.9221**OLA	91.53	23.73	6.20
18	‘SF1758’	ELA = 14.6043 + 0.9233**OLA	91.44	27.57	7.20
21	‘SF1758’	ELA = 28.7230 + 0.8277**OLA	91.53	44.27	11.57
24	‘SF1758’	ELA = 25.0930 + 0.8181**OLA	91.46	50.87	13.29

‘PV’ - ‘Piracicaba de Verão’; ** significant at p = 0.01 by Student’s t-test; RMSE - root mean square error; CV - coefficient of variation.

In the combinations of the models using data from one cultivar validated in models of another cultivar, in general, the values of R^2 were above 96% (Table 3). Despite the high values of R^2, the values of RMSE in all combinations were always higher compared to the validations with the own models of each cultivar, that is, with a model developed and validated for the same cultivar. Among the possible combinations, the best results were found for models 3 and 6 developed for the cauliflower ‘Piracicaba de Verão’ and validated with data of the cultivars ‘Sabrina’ and ‘SF1758’. The RMSE and CV values varied between 39.23 and 45.22 cm^2, and between 18.64 and 24.30%, respectively.

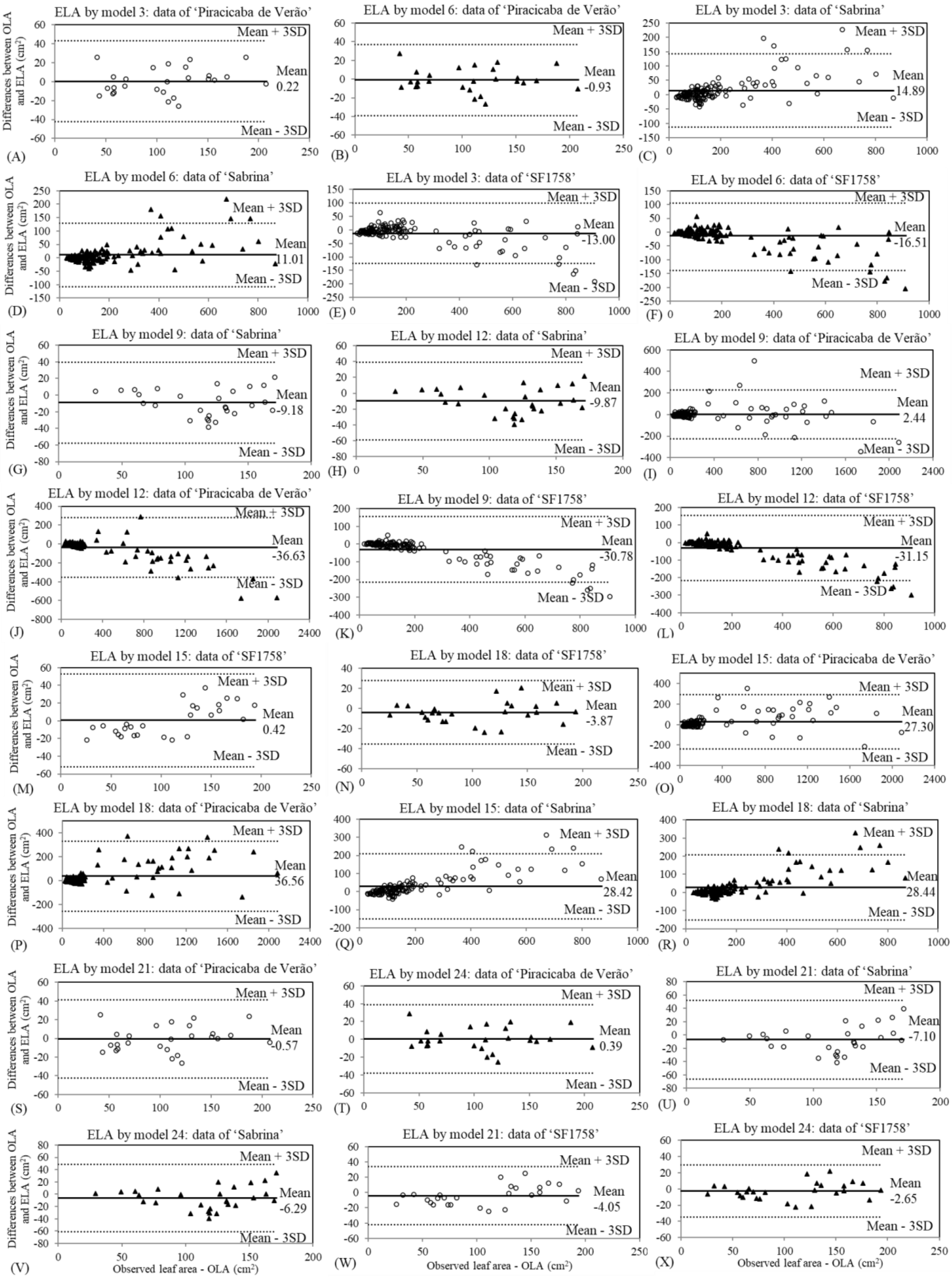
In the indication of one or more than one model for estimating the LA of cauliflower, in addition to statistical indicators (with higher values of R^2 and lower values of RMSE), the dispersion of the differences between OLA and ELA was jointly analyzed (Figures 2 and 3), helping to better understand the distribution of deviations produced by the developed models. This procedure was performed in earlier studies to validate the LA of different crops, such as *Calendula officinalis* L., *Dahlia pinnata*, *Dianthus barbatus* L., *Pelargonium* × *hortorum*, *Petunia* × *hybrida*, and *Viola wittrockiana* (Giuffrida et al., 2011), *Prunus armeniaca* L. (Cirillo et al., 2017), and chicory (Fernandes et al., 2017).

When assessing bias, that is, this indicator discriminates whether the models underestimate or overestimate the OLA, the differences between OLA and ELA found with models 3 and 6 developed for 'Piracicaba de Verão' were virtually insignificant, with overestimation and underestimation of only 0.22 cm^2 (Figure 2A) and 0.93 cm^2 (Figure 2B), respectively. Similar results were found with the universal models 21 and 24 validated with the data of this same cultivar, with underestimation and overestimation of 0.57 cm^2 (Figure 2S) and 0.39 cm^2 (Figure 2T), respectively. These underestimates or overestimates did not exceed 1% in comparison to the observed LA values.

Additionally, model 9 developed for the cauliflower 'Sabrina' proved to be adequate to estimate the LA of 'Piracicaba de Verão' (Figure 2I), with overestimation (2.44 cm^2) that did not exceed 1% in comparison to OLA. In the other combinations, model 12 developed for 'Sabrina' underestimated the LA of 'Piracicaba de Verão' by approximately 12% (Figure 2J), while models 15 and 18 developed for 'SF1758' (Figures 2O and 2P, respectively) overestimated LA by approximately 9 and 12%, respectively.

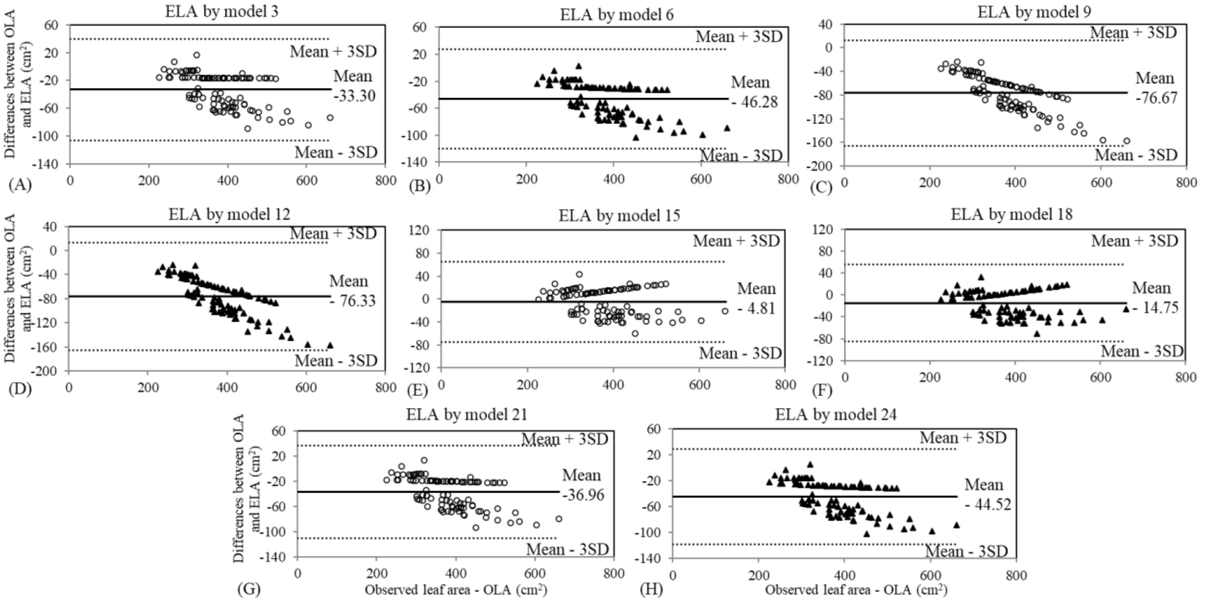
According to the dispersions between the OLA and ELA values of the cauliflower 'Sabrina', both the individual models 9 and 12 (Figures 2G and 2H, respectively) and the universal models 21 and 24 (Figures 2U and 2V, respectively) underestimated the LA of this cultivar, and these underestimates occurred basically in leaves with LA between 120 and 125 cm^2. Lower underestimates were found with the universal models (5.5-6.0%) compared to the individual models (8.0-8.5%). Models 3 and 6 developed for 'Piracicaba de Verão' produced errors in the estimation of LA of 'Sabrina' equal to or lower than those found with the models developed individually for this cultivar, but with different behaviors (overestimated the LA of 'Sabrina'). Model 6 (Figure 2D) was slightly better than the model 3 (Figure 2C), with overestimates of 5.92 and 8.00%, respectively. Models 15 and 18 developed for 'SF1758' overestimated the LA of 'Sabrina' by approximately 15% (Figures 2Q and 2 2R).

For the cauliflower 'SF1758', the linear model 15 (overestimation of 0.39%, Figure 2M) was slightly better than the power model 18 (underestimation of 3.62%, Figure 2N) in the validation with dependent data (autumn-winter experiment). In the validation with independent data (spring-summer experiment), the superiority of model 15 (underestimation of 1.26%, Figure 3E) continued in comparison to model 18 (underestimation of 3.85%, Figure 3F). There were underestimates of the LA of 'SF1758' on the order of 3.78 and 2.48% with universal models 21 (Figure 2W) and 24 (Figure 2X) using dependent data. On the other hand, using independent data, there were greater underestimates of LA with the universal models, of approximately 10 and 12% for models 21 (Figure 2G) and 24 (Figure 2H), respectively.



Solid lines are the means of the differences between OLA (observed) and ELA (estimated). The broken lines are the limits of agreement, calculated as mean ± 3SD (standard deviation).

Figure 2: Analysis of dispersion pattern of differences between observed leaf area (OLA) and estimated leaf area (ELA) using different individual and universal models for three cauliflower cultivars ('Piracicaba de Verão', 'Sabrina' and 'SF1758') grown in NFT hydroponics in autumn-winter experiment.



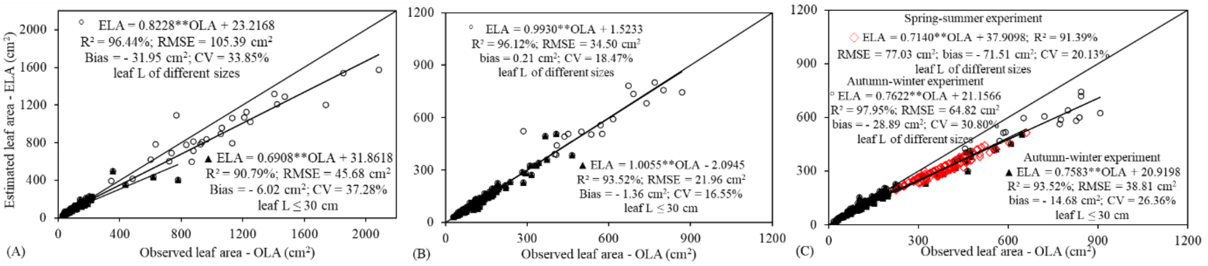
Solid lines are the means of the differences between OLA (observed) and ELA (estimated). The broken lines are the limits of agreement, calculated as mean ± 3SD (standard deviation).

Figure 3: Analysis of dispersion pattern of differences between observed leaf area (OLA) and estimated leaf area (ELA) using different individual and universal models for cauliflower 'SF1758' grown in NFT hydroponics in spring-summer experiment.

When evaluating the model of Marcolini et al. (2005) developed for kale, there was no significant difference by the t-test at $p = 0.05$ between the OLA and ELA means of cauliflower, regardless of the cultivar and leaf L class. In the validation for 'Piracicaba de Verão' using data within the same range ($L \leq 30$ cm) in which the model was conceived, there were lower errors in LA estimation, with underestimation that did not exceed 5% in comparison to OLA, while with the complete set of data (without leaf L restriction) the underestimation exceeded 10% (Figure 4A). For 'Sabrina', regardless of the leaf L class, the model was adequate to estimate the LA of this cultivar, with overestimation (without L restriction) and underestimation ($L \leq 30$ cm) that did not exceed 1% (Figure 4B).

With dependent data of the cauliflower 'SF1758' (autumn-winter experiment), there was an improvement in LA estimation using data with $L \leq 30$ cm (underestimation ~ 10%) in comparison to the set with L of different sizes (underestimation ~ 14%), while with independent data (spring-summer experiment), the underestimation was higher (~ 19%) (Figure 4C).

In summary, the LA of the cauliflower 'Piracicaba de Verão' can be estimated with the linear or power models individually or universally. For 'Sabrina', in the estimation of its LA, it is preferable to use the universal models (linear or power) instead of individual models. For 'SF1758', the linear model was better in both the autumn-winter experiment (dependent data) and the spring-summer experiment (independent data). In autumn-winter, the LA underestimates using universal models were low and compatible with that found for the individual power model for this cultivar. However, in spring-summer the use of universal models caused high underestimates of the LA of 'SF1758'.



** significant at p = 0.01 by Student's t-test; RMSE - root mean square error; CV - coefficient of variation; L - leaf length.

Figure 4: Observed leaf area (OLA) and estimated leaf area (ELA) using the model of Marcolini et al. (2005) developed for kale with validation using data of 'Piracicaba de Verão' (A), 'Sabrina' (B) and 'SF1758' (C) grown in NFT hydroponics in autumn-winter experiment.

These results found for 'SF1758' show that the cultivation seasons influenced the growth patterns of the leaves of this cultivar. In both experiments, the maximum W values of the leaves were similar (26 and 23 cm for the autumn-winter and spring-summer experiments, respectively). However, in autumn-winter there was greater growth in L, that is, more elongated leaves, while in spring-summer the leaves had a more circular shape. This can be confirmed by the L:W ratio, with mean values of the order of 1.45 and 1.31 for the autumn-winter and spring-summer experiments, respectively. The closer this ratio is to 1, the closer the L and W are to one another, and vice versa. In autumn-winter this ratio was obtained with data only from the harvest period, a condition for them to be compatible with those of the spring-summer experiment.

According to Dutra et al. (2017), in the literature there are different types of mathematical models developed to estimate the LA of various plant species and leaf types; however, they point out that usually the models are restricted to specific species and leaf shapes. In this context, as done in the present study, designing robust models involving more than one cultivar of the same species and with different leaf shapes is paramount, thus avoiding biased models for a given cultivar.

Thus, the models called universal in the present study can be used to estimate the LA of other cauliflower cultivars, unless the leaf morphology of these cultivars differs considerably from that of the cultivars used in this study. This is reinforced by other studies with different crops, such as citrus (Mazzini et al., 2010) and coffee (Schmildt et al., 2015), which developed universal models to estimate LA of these species.

Additionally, the model of Marcolini et al. (2005) developed for kale (Figure 4), proved to be adequate to estimate the LA of cauliflower, but within the limits of its conception (L ≤ 30 cm). This result was already expected because, as pointed out by Schmildt et al. (2016), once a model for LA estimation has been developed, its use should not include values outside the range used in its conception.

Similarly, Olfati et al. (2010) developed a universal model using three hybrids of red cabbage (*Brassica oleracea* var. *cappitata* L. f. *rubra*), eight hybrids of green cabbage (*Brassica oleracea* var. *cappitata* L. f. *alba*) and six hybrids of broccoli (*Brassica oleracea* var. *italica* L.). According to the authors, this model can be used to estimate LA for crops of the species *Brassica oleracea*, provided that it is validated.

Conclusions

It is possible to estimate the LA of cauliflower from measurements of L and W. Very close ratios were found between the LA values observed and estimated using two dimensions (L×W) as an independent variable compared to one dimension (L or W).

For LA estimation in studies involving the same cultivars as the present study, the linear (LA = -14.424 + 0.843L×W) or power [LA = $0.551(L\times W)^{1.057}$] individual models for 'Piracicaba de Verão' and linear model for 'SF1758' (LA = -22.610 + 0.928L×W) are suggested. For the cauliflower 'Sabrina', universal models (based on data set of three studied cultivars) of the linear (LA = -13.770 + 0.833L×W) or potential types [LA = $0.578(L\times W)^{1.050}$] are recommended. These universal models are also recommended for estimating the LA of the cultivars 'Piracicaba de Verão' and 'SF1758'.

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