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A simple model for nondestructive leaf area estimation in bedding plants

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Abstract

Measurement of leaf area is commonly used in many horticultural research experiments, but it is generally destructive, requiring leaves to be removed for measurement. Determining the individual leaf area (LA) of bedding plants like pot marigold (*Calendula officinalis* L.), dahlia (*Dahlia pinnata)*, sweet William (*Dianthus barbatus* L.), geranium (*Pelargonium* × *hortorum*), petunia (*Petunia* × *hybrida*), and pansy (*Viola wittrockiana*) involves measurements of leaf parameters such as length (L) and width (W) or some combinations of these parameters. Two experiments were carried out during spring 2010 (on two pot marigold, four dahlia, three sweet William, four geranium, three petunia, and three pansy cultivars) and summer 2010 (on one cultivar per species) under greenhouse conditions to test whether a model could be developed to estimate LA of bedding plants across cultivars. Regression analysis of LA versus L and W revealed several models that could be used for estimating the area of individual bedding plants leaves. A linear model having LW as the independent variable provided the most accurate estimate (highest R^2, smallest mean square error, and the smallest predicted residual error sum of squares) of LA in all bedding plants. Validation of the model having LW of leaves measured in the summer 2010 experiment coming from other cultivars of bedding plants showed that the correlation between calculated and measured bedding plants leaf areas was very high. Therefore, these allometric models could be considered simple and useful tools in many experimental comparisons without the use of any expensive instruments.

Additional key words: calibration; leaf area; models; regression analysis; validation.

Introduction

Leaf area (LA) is a key variable for most physiological, horticultural and agronomic studies involving plant growth, development rate, radiation use efficiency and water and nutrient use (Williams and Martinson 2003, Rouphael and Colla 2005a,b; Rouphael *et al.* 2010a, Spann and Heerema 2010). Therefore, leaf area strongly influences crop growth and productivity, and estimation of leaf area is a fundamental component of crop growth models (Lizaso *et al.* 2003).

Leaf area can be measured by destructive or non-destructive measurements. Many methods have been devised to facilitate the measurement of leaf area. However, these methods, including those of tracing, blue-printing, photographing, or using a conventional planimeter, require the excision of leaves from the plants. It is therefore not possible to make successive measurements of the same leaf. Plant canopy is also damaged, which might cause problems to other measurements or experiments. A modeling approach involving linear relationships between leaf area and one or more dimensions of the leaf (length and width) is an inexpensive, rapid, reliable, and nondestructive method for measuring leaf area and would be more advantageous than many of the methods mentioned above (Tsialtas and Maslaris 2005, Rouphael *et al.* 2007, Tsialtas *et al.* 2008).

Various models relating length and width to area

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Abbreviations: GLM – general linear model; L – leaf midvein length; W – maximum leaf width; LA – individual leaf area; LW – product of leaf length and width; L:W – leaf shape; MSE – mean square error; MSPR – mean squared prediction error; OLA – observed leaf area; PLA – predicted leaf area; PRESS – prediction sum of squares; SSE – error sum of squares; T – tolerance values; VIF – variance inflation factor.

have been developed for fruit trees (Demirsoy and Demirsoy 2003, Demirsoy *et al.* 2004, Serdar and Demirsoy 2006, Cristofori *et al.* 2007, 2008; Mendoza-de Gyves *et al.* 2007, 2008) and vegetable crops (Schwarz and Kläring 2001, De Swart *et al.* 2004, Salerno *et al.* 2005, Rouphael *et al.* 2006, Cho *et al.* 2007, Rivera *et al.* 2007, Rouphael *et al.* 2010b), whereas information on the estimation of several bedding plants such as pot marigold, dahlia, sweet William, geranium, petunia, and pansy is still lacking. However, in many studies, the adequacy of the model assumptions for estimating leaf area has not been carefully examined, since in the above studies the models have been chosen based only on values obtained for the coefficient of determination (R^2) between observed and predicted values and the standard error of estimates, but this is an inappropriate method (Bland and Altman 1986), because high correlations do not mean that the predicted values agree with the observed ones. A simple and effective method for detecting model deficiencies in regression analysis is the examination of residual plots. Moreover, the accuracy of the predictions is dependent on the variation of leaf shape between cultivars. Since leaf shape (L:W ratio) may vary among different genetic materials (Stoppani *et al.* 2003), we needed a good model of nondestructive leaf area estimation to use in physiological study of bedding plants independently of the genetic materials.

Therefore, the aims of this study were (*1*) to develop a model for leaf area prediction from linear measurements of leaf length and width in different bedding plants that was able to accommodate the effect of changes in leaf shape between cultivars and which could be used for pot marigold, dahlia, sweet William, geranium, petunia, and pansy plants of all accessions without recalibration and (*2*) to assess the robustness of the selected models on an independent set of data from other cultivar.

Materials and methods

Data collection: Three pot marigold (*C. officinalis* L.), five dahlia (*D. pinnata*), four sweet William (*D. barbatus* L.), five geranium (*Pelargonium* × *hortorum*), four petunia (*Petunia* × *hybrida*), and four pansy (*V. wittrockiana*) cultivars collected from private farms were used to develop the leaf-area prediction models. Wide varieties of fully expanded leaf samples were used. Leaves varied in size from large to small for each cultivar and were selected randomly from different levels of the canopy and during different growth stages (vegetative stage, first appearance of the flowers, and full blooming) in the spring and summer growing seasons in 2010.

Calibration experiment: A total of 395 pot marigold, 819 dahlia, 580 sweet William, 816 geranium, 560 petunia, and 583 pansy leaves (about 200 leaves per cultivar) were measured for LA, L, and W in the preliminary calibration experiment (spring growing season 2010) coming from nineteen cultivars: Fiesta Gitana light yellow, and Fiesta Gitana orange for pot marigold; Blanca, Emily, Margareth, and Tessy for dahlia; Coral, Diamond pink, and Diamond white for sweet William; Altair, Libra, Choking violet, and Sidonia for geranium; Duo, Hurrah, and Velvet for petunia; Power white blotch, Power purple blotch, and Power ocean blotch for pansy grown under greenhouse conditions at the private farm of FlorGuarino (Sicily, Italy). These cultivars were selected as a representative sampling of many bedding plants cultivated in several countries of the Mediterranean. Inside the greenhouse, daily temperatures were maintained between 20°C and 30°C, light was provided only by natural solar radiation. The bedding plants were grown into pots (diameter 14 cm, height 12 cm) containing 1.5 L of peat. Fertigation was scheduled daily and applied with subirrigation system to ensure that water and nutrients were nonlimiting.

Immediately after cutting, leaves were transported on ice to the laboratory. Leaf length was measured from lamina tip to the point of intersection of the lamina and the petiole, along the midrib of the lamina, while leaf width was measured from end-to-end between the widest lobes of the lamina perpendicular to the lamina mid-rib. L, W, and LA of each leaf were measured and recorded using an area meter (*WinDIAS 2; Delta-T Devices Ltd*., Cambridge, UK) calibrated to 0.01 cm^2.

The relationships were evaluated by fitting regression models with the linear regression procedure of *SPSS* (*SPSS Inc*., Chicago, IL, USA.) and the stepwise elimination option, as reported by Miranda and Royo (2003a). The internal validity of the models was tested by coefficient of determination (R^2), Mean Square Error (MSE), and Predicted Residual Error Sum of Squares (PRESS). Residuals were analyzed to determine the presence of outliers and nonconstant error variance. Outlier is defined as

$$\text{Outlier} = \begin{cases} 0 \text{ if } |r_i| \le k\sigma \\ 1 \text{ otherwise} \end{cases} \quad (1)$$

where, by default k = 3, and scale σ is computed as corrected median of the absolute residuals (Peksen 2007).

LA was the dependent variable and the independent variables were L, W, L^2, W^2, and the product L × W. MSE, PRESS, Error Sum of Squares (SSE), and the values of the coefficients (*b*) and constants (*a*) were also reported (Table 1), and the final model was selected based on the combination of the highest R^2, the lowest MSE, the lowest PRESS, and when the PRESS values are reasonably close to SSE. Individualized models for each cultivar of the different bedding plants have been built. In all individual models involved alone LW parameter, which was the main parameter explaining a big part of total variation for LA. In addition, *Wilkes-Shapiro W* statistic test result revealed that data pooled from all cultivars showed normal distribution. For this reason, data were pooled and a single relationship calculated to develop leaf area prediction model for bedding plants. Finally, using two measurements (*i.e.*, L and W) introduces potential problems of collinearity, resulting in poor precision in the estimates of the corresponding regression coefficients. For detecting collinearity, the variance inflation factor (VIF) (Marquardt 1970) and the tolerance values (T) (Gill 1986) were calculated.

$$\mathrm{VIF} = \frac{1}{(1-r^2)} \quad (2)$$

$$\mathrm{T} = \frac{1}{\mathrm{VIF}} \quad (3)$$

where r is the correlation coefficient. If the VIF value was higher than 10 or if T value was smaller than 0.10, then collinearity may have more than a trivial impact on the estimates of the parameters, and consequently one of them should be excluded from the model.

Validation experiment: In addition, to validate the developed models of bedding plants and to increase practical applicability, a validation experiment was conducted in the summer 2010 on leaf samples of Fiesta Gitana yellow (pot marigold), Anna (dahlia), Diamond purple (sweet William), Adara (geranium), Bravo (petunia) and Power yellow blotch (pansy) grown under greenhouse conditions at the private farm of FlorGuarino (Sicily, Italy). These cultivars were selected as the most representative bedding plants cultivars cultivated in Italy.

To validate the model, about 400 leaves of Fiesta Gitana yellow, Anna, Diamond purple, Adara, Bravo and Power yellow blotch actual leaf area and leaf width and length were determined by the previously described procedures. Two techniques reported by Miranda and Royo (2003a,b; 2004) were used to validate the models for bedding plants: (*1*) the validation data set was used to produce a validation model by re-estimating the model parameters using the Stepwise Regression Option approach to develop the estimation model and the models were compared for consistency; (*2*) regression parameter estimates from the estimation models were used to predict outcomes for observations in the validation data set and then the mean squared prediction error (MSPR) was calculated and compared with the MSE of the regression fit to the model building data set (Neter *et al.* 1996). In order to compare the predicted leaf area (PLA) to the observed leaf area (OLA) for the cultivars of bedding plants during the summer growing season, graphical procedures (Bland and Altman 1986) were used. Scatter plots of values for the PLA against the OLA are presented (Fig. 1). GLM procedure of *SPSS* was used to evaluate the linear relationship for OLA and PLA. Values for PLA were subtracted from OLA for the cultivars Fiesta Gitana yellow, Anna, Diamond purple, Adara, Bravo and Power yellow blotch, and differences were plotted against the OLA for each of them. Lack of agreement was evaluated by calculating the relative bias, estimated by the mean of the differences (d) and the standard deviation (SD) of the differences (Fig. 2). Normality test was carried out to obtain a *Wilkes-Shapiro W* statistic using examines procedure of *SPSS* (Marini 2001).

Results and discussion

Leaf shape: One of the leaf shape traits is the length:width ratio (L:W). For pot marigold, the highest L:W ratio was recorded on 'Fiesta Gitana orange' (2.71), followed by both 'Fiesta Gitana light yellow', and 'Fiesta Gitana yellow' (2.51), whereas the highest L:W ratio of dahlia was observed in both 'Margareth', and 'Tessy' (1.56), followed by 'Blanca', 'Emily', and 'Anna' (1.46). Moreover, for sweet William, cultivars such as 'Coral' and 'Diamond purple' produced the widest leaves (L:W ratio was 3.54 and 3.36, respectively), while cultivars 'Diamond pink', and 'Diamond white' had narrow leaves (L:W ratio was 4.58 and 4.92, respectively). The leaf shape trait was lower than one for geranium and ranged from 0.78 to 0.82 among cultivars. Finally, the L:W ratio for petunia was recorded on 'Bravo' (2.02), followed by 'Duo' (1.74), 'Velvet' (1.64), and 'Hurrah' (1.56), whereas the highest leaf shape trait for pansy was observed on 'Power yellow blotch' (1.61) followed by both 'Power purple blotch', and 'Power ocean blotch' (1.42), and finally on 'Power white blotch' (1.37).

Collinearity test: As a preliminary step to model calibration, the degree of collinearity among W and L was analyzed. The VIF ranged from 3.2 to 5.8 for pot marigold, from 3.0 to 8.8 for dahlia, from 1.4 to 3.1 for sweet William, from 5.2 to 8.6 for geranium, from 3.3 to 4.3 for petunia, and from 1.2 to 2.6 for pansy. Moreover, T values ranged from 0.17 to 0.31 for pot marigolds, from 0.11 to 0.33 for dahlia, from 0.32 to 0.72 for sweet William, from 0.12 to 0.19 for geranium, from 0.23 to 0.30 for petunia, and from 0.38 to 0.81 for pansy depending on the cultivar. In all cultivars, VIF was less than 10 and T was greater than 0.10, showing that the collinearity between L and W can be considered negligible (Gill 1986) and these variables can be both included in the model.

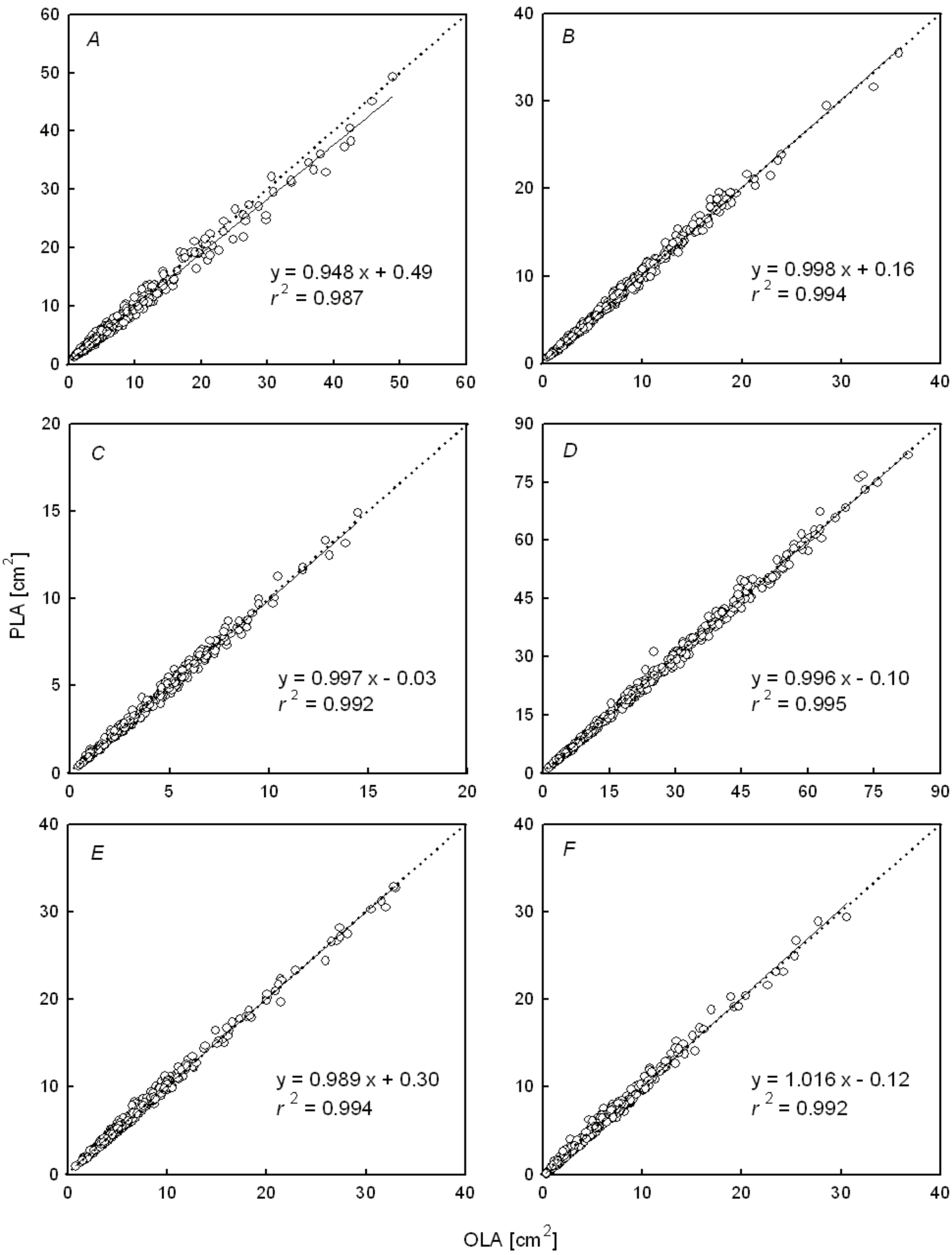

Fig. 1. Plot of predicted leaf area (PLA) using model 3 *vs*. observed values of single leaf areas (OLA) of Fiesta Gitana yellow (pot marigold, *A*), Anna (dahlia, *B*), Diamond purple (sweet William, *C*), Adara (geranium, *D*), Bravo (petunia, *E*) and Power yellow blotch (pansy, *F*), coming from the validation experiment. *Solid line* represents linear regression lines of model 3. *Dotted lines* represent the 1:1 relationship between the predicted and observed values.

Model calibration: Regression analysis demonstrated significant relationships (P<0.001) between LA and midvein length (L), maximum leaf width (W), the product of length and width (LW), the square of length (L^2), and the square of width (W^2) (Table 1). This is in agreement with previous studies (Fallovo *et al.* 2008, Kandiannan *et al.* 2009, Kumar 2009) on nondestructive model development for predicting LA using simple linear measurements. However, suitability of these models varied based on the selection criteria previously described. Except for model 1, all models produced a coefficient of determination (R^2) greater than 0.85 (Table 1). Based on selection criteria previously described (higher R^2, lower MSE, lower PRESS, and when the PRESS

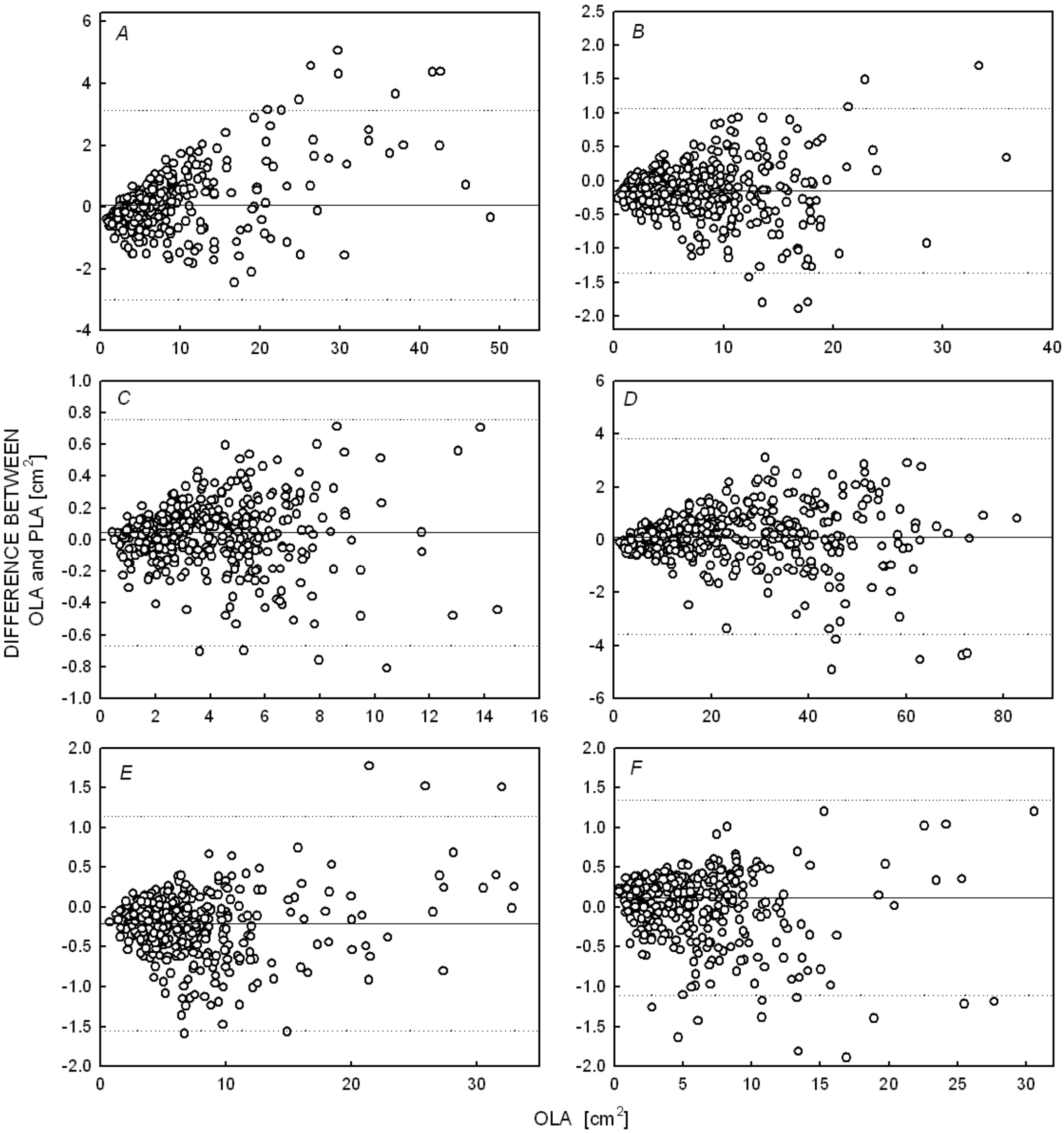

Fig. 2. The difference between predicted leaf areas (PLA) estimated by model 3 from pooled data from nineteen bedding plants cultivars and observed leaf area (OLA) *vs*. the observed leaf area of Fiesta Gitana yellow (pot marigold, *A*), Anna (dahlia, *B*), Diamond purple (sweet William, *C*), Adara (geranium, *D*), Bravo (petunia, *E*) and Power yellow blotch (pansy, *F*), coming from the validation experiment. The *solid line* is the mean of the differences. The *broken lines* are the limits of agreement, calculated as d ± 3 SD; where d is the mean of the differences, and SD is the standard deviation of the differences. If the differences are normally distributed, 97% of the differences in a population will lie between the limits of agreement.

values were reasonably close to SSE), this study demonstrated that models with a single measurement of L (Models 1 and 4, Table 1) were less acceptable for estimating LA of bedding plants as a result of their relatively lowest coefficient of determination (R^2), higher MSE, and higher PRESS values. An improvement was possible for single LA estimation when W^2 (Model 5) was used as the independent variable (Table 1). To find a model to predict single LA accurately for bedding plants of all cultivars, the product of LW was used as the independent variable (Model 3). We preferred this linear model (LA = 0.55 + 0.56 LW for pot marigolds, LA = 0.28 + 0.58 LW for dahlia, LA = –0.06 + 0.69 LW for sweet William, LA = 0.07 + 0.68 LW for geranium, LA = 0.21 + 0.64 LW for petunia, and LA = –0.26 + 0.71 LW for pansy) for its accuracy: highest R^2 (greater than 0.99), smallest MSE, smallest PRESS, and to the reasonably close PRESS value to SSE (Table 1). PRESS criterion and SSE are measures of how well the use of the fitted values for a subset model can predict the observed values of the response value Yi. Some evidence of the internal validity of the fitted model is to compare PRESS and SSE (Miranda and Royo 2003a). PRESS value is always larger than SSE (Table 1) because the regression

Table 1. Fitted coefficient (*b*) and constant (*a*) values of the models used to estimate the bedding plants leaf area (LA in cm^2) of single leaves from length (L) and width (W) measurements. [a]Coefficient of determination (R^2), mean square errors (MSE), predicted residual error sum of squares (PRESS), and error sum of squares (SSE) of the various models are also given. [b]Standard errors in parenthesis; L and W were in cm. All data were derived from the calibration experiment.

Model No.	Form of model tested	Fitted coefficient and constant *a* [cm^2]	*b*	R^{2a}	MSE[a] [cm^2]	PRESS[a] [cm^2]	SSE[a] [cm^2]
		Pot marigold (*Calendula officinalis* L.)					
1	LA = *a* + *b*L	–7.50 (0.29)[b]	2.96 (0.05)	0.913	7.69	3,086	3,022
2	LA = *a* + *b*W	–7.99 (0.22)	8.02 (0.09)	0.950	4.47	1,790	1,755
3	LA = *a* + *b*LW	0.55 (0.06)	0.56 (0.01)	0.991	0.82	331	323
4	LA = *a* + *b*L^2	0.61 (0.14)	0.21 (0.01)	0.945	4.02	1,616	1,580
5	LA = *a* + *b*W^2	0.78 (0.09)	1.42 (0.01)	0.978	1.95	783	767
		Dahlia (*Dahlia pinnata*)					
1	LA = *a* + *b*L	–6.24 (0.14)	3.38 (0.03)	0.926	2.06	1,702	1,684
2	LA = *a* + *b*W	–6.22 (0.13)	5.12 (0.05)	0.939	1.69	1,399	1,384
3	LA = *a* + *b*LW	0.28 (0.02)	0.58 (0.01)	0.994	0.18	150	148
4	LA = *a* + *b*L^2	0.47 (0.06)	0.37 (0.01)	0.957	1.19	981	969
5	LA = *a* + *b*W^2	0.41 (0.04)	0.87 (0.01)	0.980	0.55	453	450
		Sweet William (*Dianthus barbatus* L.)					
1	LA = *a* + *b*L	–2.98 (0.11)	1.45 (0.02)	0.882	0.86	502	497
2	LA = *a* + *b*W	–2.94 (0.12)	6.29 (0.11)	0.858	1.03	603	597
3	LA = *a* + *b*LW	–0.06 (0.02)	0.69 (0.01)	0.992	0.06	33	32
4	LA = *a* + *b*L^2	0.38 (0.06)	0.14 (0.01)	0.893	0.78	455	450
5	LA = *a* + *b*W^2	0.20 (0.06)	2.75 (0.04)	0.907	0.82	481	476
		Geranium (*Pelargonium* × *hortorum*)					
1	LA = *a* + *b*L	–18.92 (0.36)	8.48 (0.07)	0.942	12.29	10,121	10,002
2	LA = *a* + *b*W	–20.26 (0.37)	7.09 (0.06)	0.944	11.98	9,881	9,756
3	LA = *a* + *b*LW	0.07 (0.06)	0.68 (0.00)	0.995	1.03	851	842
4	LA = *a* + *b*L^2	0.90 (0.14)	0.80 (0.01)	0.963	5.73	4,722	4,667
5	LA = *a* + *b*W^2	–0.22 (0.1)	0.56 (0.01)	0.987	2.72	2,240	2,216
		Petunia (*Petunia* × *hybrida*)					
1	LA = *a* + *b*L	–6.72 (0.21)	3.43 (0.04)	0.917	2.35	1,333	1,313
2	LA = *a* + *b*W	–7.19 (0.24)	5.86 (0.08)	0.895	2.97	1,693	1,660
3	LA = *a* + *b*LW	0.21 (0.04)	0.64 (0.00)	0.992	0.22	127	125
4	LA = *a* + *b*L^2	1.20 (0.11)	0.33 (0.01)	0.928	2.05	1,154	1,142
5	LA = *a* + *b*W^2	0.04 (0.09)	1.07 (0.01)	0.954	1.31	748	733
		Pansy (*Viola wittrockiana*)					
1	LA = *a* + *b*L	–6.72 (0.27)	3.79 (0.07)	0.820	5.10	3,034	2,964
2	LA = *a* + *b*W	–5.57 (0.19)	5.02 (0.08)	0.882	3.36	2,003	1,950
3	LA = *a* + *b*LW	–0.26 (0.03)	0.71 (0.00)	0.990	0.28	166	164
4	LA = *a* + *b*L^2	–0.28 (0.11)	0.49 (0.01)	0.898	2.90	1,718	1,685
5	LA = *a* + *b*W^2	0.32 (0.06)	0.90 (0.01)	0.961	1.10	654	639

fit for the i^{th} case, when this case is deleted in fitting, can never be as good as that when the i^{th} case is included. In the current study, PRESS value of all bedding plants was reasonably close to SSE for the LA model 3 (Table 1) and supports the validity of the fitted regression model and of the MSEs as an indication of the predictive capability of this model (Neter *et al.* 1996). Based on these considerations, both L and W measurements were necessary to estimate bedding plants LA accurately.

The shape coefficient (regression coefficient of model 3) can be described by a shape between an ellipse (0.78) and a triangle (0.5) of the same length and maximum width. Our shape coefficients (0.56 for pot marigold, 0.58 for dahlia, 0.69 for sweet William, 0.68 for geranium, 0.64 for petunia, and 0.71 for pansy) agreed closely with those calculated for other crops. Values of 0.63 have been reported for broccoli (Stoppani *et al.* 2003), 0.69 for pepper (De Swart *et al.* 2004), 0.63 for zucchini squash (Rouphael *et al.* 2006), 0.68 for sunflower (Rouphael *et al.* 2007), 0.64 for eggplant (Rivera *et al.* 2007), 0.70 for *Euphorbia* x lomi Rauh Thai hybrids (Fascella *et al.* 2009), 0.50 for watermelon (Rouphael *et al.* 2010b) 0.72 for rose (Rouphael *et al.* 2010c).

Table 2. Statistics and parameter estimates from regression model for leaf area (LA, cm^2) estimation. The estimation models (EM) were developed from nineteen bedding plants cultivars sampled in the spring growing season in 2010. Validation models (VM) were developed from six bedding plants cultivars sampled in the summer growing season in 2010.

Statistic or parameter estimate	Pot marigold		Dahlia		Sweet William		Geranium		Petunia		Pansy	
	EM	VM	EM	VM	EM	VM	EM	VM	EM	VM	EM	VM
Intercept	0.546	0.160	0.275	0.156	-0.058	0.002	0.070	0.284	0.211	–0.056	–0.258	–0.092
Standard error of intercept	0.062	0.066	0.025	0.036	0.017	0.021	0.061	0.100	0.038	0.033	0.034	0.034
Regression coefficient for LW	0.561	0.586	0.585	0.582	0.693	0.689	0.682	0.679	0.636	0.639	0.708	0.691
Standard error of regression coefficient	0.003	0.003	0.002	0.002	0.003	0.003	0.002	0.002	0.002	0.002	0.003	0.003
Prediction sum of squares (PRESS)	331	-	150	-	33	-	851	-	127	-	166	-
Error sum of squares (SSE)	323	336	148	70	32	18	842	513	125	60	164	67
Mean squared prediction error (MSPR)	-	1.105	-	0.200	-	0.047	-	1.327	-	0.206	-	0.186
Mean square error (MSE)	0.820	0.892	0.180	0.176	0.060	0.044	1.030	1.277	0.220	0.162	0.280	0.177
$P>F$	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Coefficient of multiple determination R^2	0.9907	0.9873	0.9935	0.9943	0.9923	0.9920	0.9951	0.9955	0.9921	0.9948	0.9901	0.9921

Model validation: Parameter estimates and statistics obtained from *SPSS* outputs are presented for the LA estimation and validation models (Table 2). The regression coefficients for LW determined in the estimation models were very similar to that of the validation models for all bedding plants and the R^2 values were similar for both models (Table 2), indicating the applicability of the proposed model 3 to data beyond those on which the model is based (Neter *et al.* 1996). Moreover, a means of measuring the actual predictive capability of the models is to use them to predict each case in the validation data set and then to calculate the MSPR. If the MSPR is fairly close to the MSE based on the regression fit to the estimation data set, then the MSE for the selected regression model is not seriously biased and gives an appropriate indication of the predictive ability of the model. In the current study, the MSPR from the validation data set for pot marigold leaf area did not differ greatly from the MSE of the estimation data set (Table 2). The same case was also observed for the dahlia, sweet William, geranium, petunia, and pansy leaf-area models (Table 2). This implies that the MSE based on the estimation data set is a reasonably valid indicator of the predictive ability of the estimation regression model (Neter *et al.* 1996).

Comparisons between OLA versus PLA using model 3 for the validation set derived from summer 2010 experiment showed a close correlation coefficient $r = 0.98$, $P<0.0001$), and the PLA values were very close to the OLA values, giving an underestimation of 5.2%, 0.2%, 0.3%, 0.4%, and 1.1% in the prediction for pot marigold, dahlia, sweet William, geranium, and petunia, respectively, and an overestimation of 1.6% in the prediction for pansy (Fig. 1). However, correlation is an inappropriate analysis to explain relationship between PLA and OLA, and a plot of the differences between PLA and OLA against OLA may be more informative (Bland and Altman 1986, Marini 2001). Plotting differences against OLA value also allows investigation of possible relationships between measurement error and the true values. Lack of agreement between estimation PLA and OLA can be evaluated by calculating the bias, estimated by the mean of the differences (d) and the SD of the differences. In Fig. 2, a solid line represents the mean of the differences. If the differences are normally distributed, 97% of the differences will lie between d ± 3 SD, which is the case in the current study, in which a few plots were out of these lines, whereas the rest of the plots were placed between lines.

Conclusions: To summarize, we developed simple predictive models to accurately estimate the area of leaves for several bedding plants. The length–width model can provide more accurate estimations of bedding plants leaf area across cultivars than those based on single length or width measurement. These models are excellent and non-destructive tools for studying leaf growth and development in bedding plants without the use of any expensive instruments, *e.g*., digital camera with image measurement software or leaf area planimeter.

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