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Modeling individual leaf area of rose (*Rosa hybrida* L.) based on leaf length and width measurement

Y. ROUPHAEL[*,+], A.H. MOUNEIMNE[**], A. ISMAIL[***], E. MENDOZA-DE GYVES[#], C.M. RIVERA[#], and G. COLLA[#]

Department of Crop Production, Faculty of Agricultural Engineering and Veterinary Medicine, Lebanese University, Dekwaneh-Al Maten, Lebanon[*]
Department of Environment and Natural Resources, Faculty of Agricultural Engineering and Veterinary Medicine, Lebanese University, Dekwaneh-Al Maten, Lebanon[**]
Department of Food Technology, Faculty of Agricultural Engineering and Veterinary Medicine, Lebanese University, Dekwaneh-Al Maten, Lebanon[***]
Dipartimento di Geologia e Ingegneria Meccanica, Naturalistica e Idraulica per il Territorio, Università della Tuscia, 01100 Viterbo, Italy[#]

Abstract

Accurate and nondestructive methods to determine individual leaf areas of plants are a useful tool in physiological and agronomic research. Determining the individual leaf area (LA) of rose (*Rosa hybrida* L.) involves measurements of leaf parameters such as length (L) and width (W), or some combinations of these parameters. Two-year investigation was carried out during 2007 (on thirteen cultivars) and 2008 (on one cultivar) under greenhouse conditions, respectively, to test whether a model could be developed to estimate LA of rose across cultivars. Regression analysis of LA *vs*. L and W revealed several models that could be used for estimating the area of individual rose leaves. A linear model having L×W as the independent variable provided the most accurate estimate (highest r^2, smallest MSE, and the smallest PRESS) of LA in rose. Validation of the model having L×W of leaves measured in the 2008 experiment coming from other cultivars of rose showed that the correlation between calculated and measured rose LA was very high. Therefore, this model can estimate accurately and in large quantities the LA of rose plants in many experimental comparisons without the use of any expensive instruments.

Additional key words: individual leaf area; linear measurements; nondestructive methods; *Rosa hybrida* L.; validation.

Introduction

Plant leaf area (LA) is a key variable for most agronomic and physiological studies involving plant growth, light interception, transpiration, photosynthetic efficiency, and responses to fertilizers and irrigation (De Swart *et al*. 2004, Rouphael *et al*. 2006). Therefore, LA strongly influences growth and productivity; estimating LA is a fundamental component of crop growth models (Lizaso *et al*. 2003).

Measuring the surface area of a large number of leaves can be both time-consuming and labour costly. Many methods have been devised to facilitate the measurement of LA. However, these methods, including those of tracing, blueprinting, 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. LA can be also measured quickly, accurately, and nondestructively using a portable scanning planimeter (Daughtry 1990), but it is only suitable for small plants with few leaves (Nyakwende *et al*. 1997) and not feasible for large leaves. An alternative method to measure LA is to use image analysis with image measurement and analysis software. The capture of an image by a digital camera is rapid, and the analysis using proper software is accurate (Bignami and Rossini 1996), but the processing is time-consuming, and the facility is generally expensive and not suitable for

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[+]Corresponding author; fax: +961-(0)1 495786, e-mail: joerouphael@yahoo.com
Abbreviations: GLM – general linear model; L – leaf midvein length; LA – individual leaf area; L × W – product 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; W – maximum leaf width.

nonflat leaves measurement, because pictures taken not exactly perpendicularly can cause erroneous LA evaluation. Therefore, an inexpensive, rapid, reliable, and nondestructive method for measuring LA is required by agronomists. Accurate, nondestructive measurements permit repeated sampling of the same leaves over time and exclude biological variation in destructive methods (De Swart *et al*. 2004). Especially when using unique plants, for example in genetically segregating populations, nondestructive measurements are of a great value. If the mathematical relationships between LA and one or more dimensions of the leaf (length and width) could be clarified, a method using just these models to estimate LA would be more advantageous than many of the methods mentioned above (Beerling and Fry 1990). Various combinations of measurements and various models relating length and width to area have been developed for several fruit trees (Montero *et al.* 2000, Demirsoy *et al*. 2004, Serdar and Demirsoy 2006, Cristofori *et al*. 2007, Mendoza-de Gyves *et al*. 2007, Tsialtas *et al*. 2008) and vegetable crops (Stoppani *et al*. 2003, De Swart *et al*. 2004, Salerno *et al*. 2005, Cho *et al*. 2007, Peksen 2007, Rouphael *et al*. 2006, Rivera *et al*. 2007, Olfati *et al*. 2009, Rouphael *et al*. 2010, in press) while information on the estimation of ornamental plant LA, in particular rose (*Rosa hybrida* L.) is still lacking.

The accuracy of the predictions is dependent on the variation of leaf shape between cultivars. Since leaf shape (length:width ratio) may vary among different genetic proveniences (Stoppani *et al*. 2003), we needed a reliable model of nondestructive LA estimation to use in a physiological study of rose leaves independently of the genetic material.

Therefore, the aims of this study were: (*1*) to develop a model for LA prediction from leaf length and width measurement in rose that would be able to accommodate the effect of changes in leaf shape between cultivars and which could be used for rose plants of all accessions without recalibration and (*2*) to assess the reliability of the selected model on an independent set of data from other cultivar.

Materials and methods

Data collection: Fourteen rose (*Rosa hybrida* L.) cultivars collected from experimental and private farms were used to develop the leaf area (LA) prediction model. Wide varieties of fully expanded leaf samples were used. Area of the different cultivar leaves ranged from 1 to 79 cm^2, length from 1.2 to 12.3 cm and width from 1.0 to 8.5 cm (Table 1). Leaves were selected randomly from different levels of the canopy, during the spring-summer growing season in 2007 and 2008.

Model building: A total of 2002 rose leaves (about 150 leaves per cultivar) were measured for LA, length (L) and width (W) in the preliminary calibration experiment coming from thirteen cultivars: ‘Vivaldi’, ‘Queen Elizabeth’, ‘Virgo’, ‘Velvet Star’, ‘Anna’, ‘New Dawn’, ‘Alba’, ‘Fairy’, ‘Iceberg’, ‘White Success’, ‘Kardinal’, ‘Rockstar’, and ‘Grand Gala’ grown under greenhouse conditions at the private farm ‘Vivai Michellini’ (Latium region, central Italy). These cultivars were selected as a representative sampling of many roses cultivated in the Mediterranean region (Spain, Italy, and France).

Immediately after cutting, leaves were placed in plastic bags and 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 (Fig. 1) by a ruler. Values of L [cm] and W [cm] were rounded to the nearest 0.1 cm. The area of each leaf (LA) was measured using an area meter (*LI-3100; LICOR*, Lincoln, NE, USA) 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), Predicted Residual Error Sum of Squares (PRESS). Residuals (r_i) were analyzed to determine the presence of outliers and nonconstant error variance. Outlier is defined as:

$$\text{Outlier} = 0 \text{ if } |r_i| \leq k\sigma \text{ or } 1 \text{ otherwise} \quad (1)$$

where, by default k = 3, and scale σ is computed as corrected median of the absolute residuals (Cankaya *et al*. 2006, Peksen 2007).

LA was the dependent variable and the independent variables were L, W, L^2, W^2, and the product L × W. Mean square error (MSE), prediction sum of squares (PRESS), Error Sum of Squares (SSE), and the values of the coefficients (*b*) and constants (*a*) were also reported (Table 2), 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 have been built. In all individual models involved alone L×W 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 was calculated to develop LA prediction model for rose. 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

Table 1. The leaf shape (length:width ratio), mean, minimum (min) and maximum (max) values for the leaf length, leaf width, and leaf area of rose cultivars. *Standard errors in parenthesis. **Coefficient of determination (r^2) and mean square errors (MSE in cm^2) of the linear regression between leaf width (W) and leaf length (L).

	Leaf length [cm]			Leaf width [cm]			Leaf area [cm^2]			L:W (±SE)	r^2	MSE**
Cultivars	mean	min	max	mean	min	max	mean	min	max			
New Dawn	3.2	1.8	6.0	2.3	1.0	3.4	5.9	1.8	14.7	1.39 (0.012)*	0.894	0.13
Kardinal	4.8	2.5	7.0	3.4	1.5	5.5	12.9	3.3	26.9	1.40 (0.009)	0.884	0.14
Fairy	3.1	1.6	6.0	2.1	1.0	3.4	5.6	1.1	14.7	1.43(0.015)	0.906	0.17
White Success	5.7	1.8	12.3	3.8	1.0	8.5	19.2	1.8	79.2	1.47 (0.012)	0.902	0.35
Rockstar	5.8	2.3	11.0	3.7	1.1	7.2	17.7	2.2	52.7	1.52(0.012)	0.875	0.33
Virgo	3.9	1.6	8.5	2.4	1.0	5.8	8.3	1.0	35.1	1.55 (0.014)	0.885	0.18
Vivaldi	3.2	1.2	6.0	2.1	1.0	3.7	5.7	1.4	15.4	1.55 (0.016)	0.770	0.18
Queen Elizabeth	4.0	1.8	8.0	2.5	1.0	5.1	8.5	1.8	30.3	1.56 (0.015)	0.858	0.24
Alba	3.5	1.8	7.7	2.2	1.0	5.1	6.7	1.8	6.7	1.57 (0.015)	0.838	0.19
Anna	4.5	1.5	8.5	2.8	1.0	5.7	11.3	1.0	34.0	1.61 (0.014)	0.905	0.25
Velvet Star	5.5	1.5	8.7	3.4	1.0	5.7	15.2	1.1	34.2	1.63 (0.012)	0.892	0.28
Grand Gala	5.3	1.8	10.5	3.1	1.0	7.0	13.4	1.8	52.6	1.66 (0.015)	0.804	0.31
Dallas	5.2	1.8	9.0	3.1	1.0	7.6	12.2	1.8	37.0	1.67 (0.011)	0.810	0.25
Iceberg	3.8	1.8	7.5	2.4	1.3	3.6	6.4	2.1	20.0	1.77 (0.018)	0.773	0.22

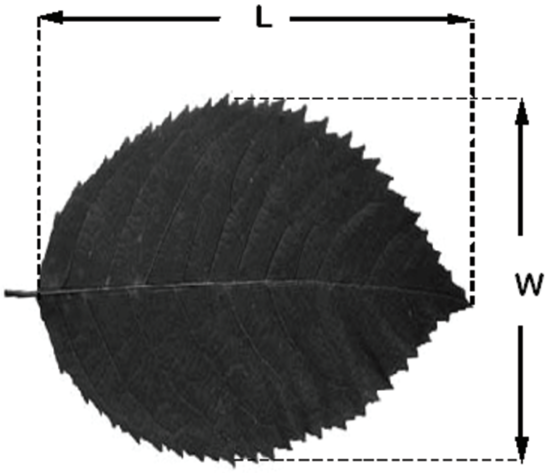

Fig. 1. Rose leaf showing the position of leaf length (L) and width (W) measurement.

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 (Cristofori *et al.* 2007, Fallovo *et al.* 2008).

Validation experiment: In addition to validate the developed model and to increase practical applicability, a validation experiment was conducted in the spring-summer 2008 on leaf samples of 'Dallas' grown in a simplified hydroponic system at the Experimental Farm of Tuscia University, central Italy (42°25′N, 12°08′ E, 310 m a.s.l.). This cultivar was selected as the most representative rose cultivar cultivated in Italy.

To validate the model, 220 leaves of 'Dallas' were used to determine LA and leaf width and length by the previously described procedures. Two techniques reported by Miranda and Royo (2003a,b; 2004) were used to validate the models: (*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 LA (PLA) to the observed LA (OLA) for the cultivar 'Dallas' during 2008 growing season, graphical procedures (Bland and Altman 1986) were used. Plots of values for the PLA against the OLA are presented (Fig. 2). *GLM* (*General Linear Model*) procedure of *SPSS* was used to evaluate the linear relationship for OLA and PLA. Values for PLA were subtracted from OLA for the cultivar 'Dallas' 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. 3). Normality (Gaussian distribution) test was carried out to obtain a *Wilkes-Shapiro W* statistic using examines procedure of *SPSS* (Marini 2001).

Results and discussion

One of the leaf shape traits is the length:width ratio (L:W). In the current experiment, significant differences ($p<0.05$) in L:W ratio were recorded among genotypes (Table 1). Cultivars 'New Dawn', 'Fairy', 'Kardinal', and 'White Success' produced the widest leaves (L:W ratio ranged from 1.39 to 1.47). Moreover, cultivars 'Iceberg', 'Grand Gala', and 'Dallas had narrow leaves (L:W ratio ranged from 1.66 to 1.77), while cultivars 'Vivaldi', 'Queen Elizabeth', 'Virgo', 'Velvet Star', 'Anna', 'Alba', and 'Rockstar' exhibited an intermediate leaf shape value (L:W ratio ranged from 1.52 to 1.63) (Table 1).

As a preliminary step to model calibration, the degree of collinearity among W and L was analyzed. The VIF ranged from 2.7 to 8.3, and T values ranged from 0.12 to 0.36, depending on the cultivar. In all cultivars, VIF was < 10, and T was > 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.

Model calibration: Regression analysis demonstrated strong relationships ($p<0.001$) between leaf area (LA) and midvein length (L), maximum leaf width (W), the product of length and width (L×W), the square of length (L^2), and the square of width (W^2) (Table 2). This is in agreement with previous studies (Tsialtas and Maslaris 2005, Rouphael *et al.* 2006, Cristofori *et al.* 2007, Mendoza-de Gyves *et al.* 2007, Peksen 2007, Rivera *et al.* 2007, Rouphael *et al.* 2007, Antunes *et al.* 2008, Fallovo *et al.* 2008, Tsialtas *et al.* 2008, Kandiannan *et al.* 2009, Kumar 2009, Rouphael *et al.* 2010, in press) 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 0.90 (Table 2). Based on selection criteria previously described (higher r^2, lower MSE, lower PRESS, and when the PRESS values were reasonably close to SSE), this study demonstrated that models with a single measurement of L (models 1 and 4, Table 2) were less acceptable for estimating LA of rose due to their 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 independent variable (Table 2). To find a model to predict single LA accurately for rose plants of all cultivars the product of L×W was used as independent variable (model 3). We preferred this linear model [LA = 0.56 + 0.72 (L×W)] for its accuracy: highest r^2 (> 0.99), smallest MSE, smallest PRESS, and to the reasonably close PRESS value to SSE (Table 2). 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 Y_i. 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 because the regression 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 (1461) of rose was reasonably close to SSE (1453) for the LA model 3 (Table 2), 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 the above considerations, both L and W measurements were necessary to estimate rose LA accurately.

The shape coefficient [regression coefficient (parameter b) 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 (0.72) agreed closely with those calculated for other crops. Values of 0.69 have been reported for pepper (De Swart *et al.* 2004), 0.64 for eggplant (Rivera *et al.* 2007), 0.63 for zucchini squash (Rouphael *et al.* 2006), 0.68 for sunflower (Rouphael *et al.* 2007), 0.69 for persimmon (Cristofori *et al.* 2008), 0.74 for hazelnut (Cristofori *et al.* 2007), 0.59 for *Vitis vinifera* L. (Montero *et al.* 2000), 0.70 for *Euphorbia* × *lomi* Rauh (Fascella *et al.* 2009) and 0.63 for broccoli (Stoppani *et al.* 2003).

Table 2. Fitted constant (*a*) and coefficient (*b*) of the models to estimate the leaf area (LA) from leaf length (L) and leaf width (W) measurements in rose. *Standard errors in parenthesis; L and W were in cm. **Coefficient of determination (r^2), mean square errors (MSE in cm^2), predicted residual error sum of squares (PRESS), and error sum of squares (SSE) of the various models are also given. All data were derived from the calibration experiment in 2007 (n = 2002).

model No.	model tested	Fitted coefficient and constant* *a* (±SE)	*b* (±SE)	r^{2**}	MSE**	PRESS**	SSE**
1	LA = $a + b$L	–10.465 (0.173)	4.85 (0.037)	0.896	8.392	16901	16784
2	LA = $a + b$W	–9.571 (0.138)	7.29 (0.046)	0.927	5.900	11888	11800
3	LA = a + bL×W	0.560 (0.029)	0.717 (0.002)	0.991	0.726	1461	1453
4	LA = $a + bL^2$	0.327 (0.063)	0.467 (0.002)	0.947	3.344	6724	6688
5	LA = $a + bW^2$	1.228 (0.051)	1.034 (0.004)	0.969	2.457	4952	4914

Table 3. Statistics and parameter estimates from regression model for leaf area (LA, cm^2) estimation. The estimation model was developed from thirteen rose genotypes sampled in 2007. Validation model was developed from one rose genotype ('Dallas') sampled in 2008.

Statistic or parameter estimate	Estimation model	Validation model
Intercept	0.560	0.699
Standard error of intercept	0.029	0.117
Regression coefficient for L×W	0.717	0.709
Standard error of regression coefficient	0.002	0.006
Prediction sum of squares (PRESS)	1461	-
Error sum of squares (SSE)	1453	139.96
Mean squared prediction error (MSPR)		0.725
Mean square error (MSE)	0.726	0.719
Coefficient of multiple determination r^2	0.991	0.989

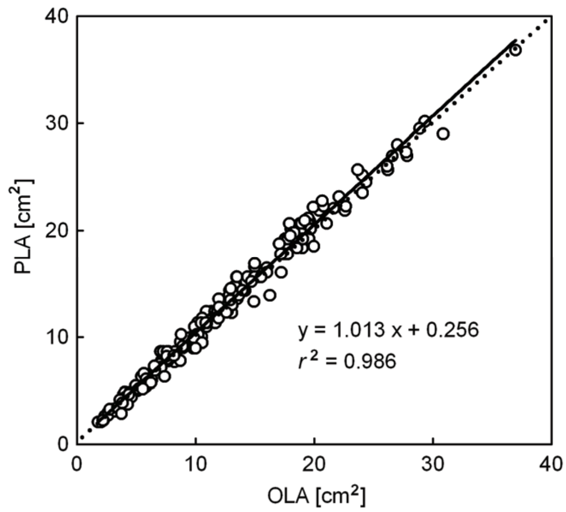

Fig. 2. Plot of predicted leaf area (PLA) using model 3 (LA = 0.56 + 0.72 L×W) versus observed values of single leaf areas (OLA) of cv. 'Dallas', during 2008 (validation experiment). Solid line represents linear regression lines of model 3. Dotted lines represent the 1:1 relationship between the predicted and observed values.

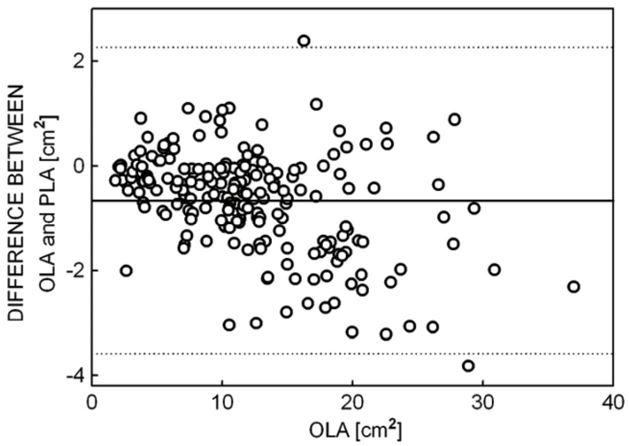

Fig. 3. The difference between predicted leaf areas (PLA) estimated by model 3 from pooled data from thirteen rose genotypes and observed leaf area (OLA) of 'Dallas' genotype versus the observed leaf area of 'Dallas' genotype sampled in 2008 (validation experiment). The *solid line* is the mean of the 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.

Model validation: Parameter estimates and statistics obtained from *SPSS* outputs are presented for the LA estimation and validation models (Table 3). The intercept, the regression coefficient for L×W of the estimation and validation models were not significantly ($p = 0.23$ and $p = 0.48$, respectively) different, and the r^2 values were similar for both models (Table 3), 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 mean of the squared prediction errors, 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 rose LA did not differ greatly from the MSE of the estimation data set (Table 3). 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 observed leaf area (OLA) versus predicted leaf area (PLA) using model 3 for the validation set derived from 2008 experiment, showed a close correlation ($r = 0.99$, $p<0.0001$), and the PLA values were very close to the OLA values, giving an overestimation of 1.3% in the prediction (Fig. 2). However, correlation is insufficient 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 estimated 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. 3, a solid line represents the mean of the differences. If the differences are normally distributed, 97% of the differences lie between d ± 3 SD, which is the case in the current study, where a few plots were out of these lines while the rest of the plots were placed between lines.

Conclusions: To summarize, we can conclude that the length-width model can provide more accurate estimations of rose LA across cultivars than those based on single length or width measurement. Because leaf width and midvein length are dimensions that can be easily measured in the field, greenhouse and pot experiments, use of this model would enable researchers to make non-destructive measurements or repeated measurements on the same leaves. Such models can estimate accurately and in large quantities the LA of rose in many experimental comparisons without the use of any expensive instruments, *e.g.*, a LA planimeter or digital camera with image measurement software.

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