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# A simple and low-cost method for leaf area measurement in *Euphorbia x lomi* Thai hybrids

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**Abstract: Simple, accurate, and low-cost methods to determine individual leaf areas of plants are a useful tool in physiological and agronomic research. Determining the individual leaf area (A) of *Euphorbia x lomi* Rauh Thai hybrids involves measurements of leaf parameters, such as length (L) and width (W), or some combinations of these parameters. Two experiments were carried out in 2008 on five genotypes (calibration experiment) and on one cultivar (validation experiment) under greenhouse conditions to test whether a model could be developed to estimate leaf area across genotypes. Regression analyses of A versus L and W revealed several models could be used for estimating the area of individual *Euphorbia x lomi* leaves. A linear model having LW as the independent variable (A = 0.691 LW – 1.428) provided the most accurate estimate ($R^2$ = 0.981, MSE = 7) of *Euphorbia x lomi* leaf area. Validation of the model having LW of leaves coming from other genotypes showed that the correlation between calculated and measured areas was very high. Using the selected model agronomists and physiologists can estimate accurately and reliably the leaf area of *Euphorbia x lomi* hybrids without destructive measurements or the use of expensive instruments (e.g. a leaf area planimeter or digital camera with image measurement software).**

## 1. Introduction

Since leaf area plays an important role in photosynthesis, light interception, water and nutrient use, and crop growth (Williams, 1987), a simple, rapid, accurate, and low-cost method for the estimation of leaf area may be useful to determine the relationship between leaf area and plant growth rate (Cho *et al*., 2007). The total leaf area of a plant can be obtained by either direct or indirect measurements. The direct method consists of removing all leaves from the plant and measuring them. This method is destructive and requires adequate, potentially expensive equipment. Indirect methods, instead, are non-destructive, less expensive, and can provide accurate leaf area estimates (Norman and Campbell, 1989). Indirect methods are useful when this equipment is not available or non-destructive measurements are required, for example measurements carried out on plants growing in pots of controlled experiments or when using unique plants, as in genetically segregating populations. One of the most frequently used non-destructive and indirect method is estimating leaf area from mathematical equations involving linear measurements such as leaf length or leaf width, or some combination of these variables, which are generally chosen for their simplicity and accuracy (Blanco and Folegatti, 2003).

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 and Demirsoy, 2003; Demirsoy *et al*., 2004; Serdar and Demirsoy, 2006; Cristofori *et al*., 2007; Mendoza-de Gyves *et al*., 2007) and vegetable crops (Blanco and Folegatti, 2003; Stoppani *et al*., 2003; De Swart *et al*., 2004; Rouphael *et al*., 2006; Rivera *et al*., 2007) while information on the estimation of ornamental plant leaf areas, in particular *Euphorbia x lomi* Rauh, an interspecific hybrid between *Euphorbia milii* Des Moulins and *E. lophogona* Lamarck (Spurge family), is still lacking. This is a succulent spiny shrub, with long lanceolate leaves and brightly colored bracts, usually cultivated as a potted flowering plant but also grown as a bedding or garden plant (Rauh, 1979).

The accuracy of the predictions is dependent on the variation of leaf shape between genotypes. Since leaf

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shape (length:width ratio) may vary among different genetic materials (Stoppani *et al.*, 2003), we needed a good non-destructive leaf area estimation model to use in physiological studies of *Euphorbia x lomi* plants, independently of the genetic materials.

Therefore, the aims of this study were to develop a model for leaf area prediction from linear measurements of leaf length and width in *Euphorbia x lomi* Thai hybrids able to accommodate the effect of changes in leaf shape between genotypes and to assess the robustness of the model on an independent set of data from another genotype.

## 2. Materials and Methods

*Data collection*

Six *Euphorbia x lomi* Rauh Thai genotypes (Porn Ying Yai, Duan Tawan, Manee Chintana, Soi Budsanin, Nam Chok, and Sabckaeron Suk) were used to develop a leaf area prediction model. Wide varieties of fully expanded leaf samples were used. Leaves varied in size from large to small for each genotype and were selected randomly from different levels of the canopy during the spring growing season in 2008.

*Model building*

A total of 500 *Euphorbia x lomi* Thai hybrid leaves (about 100 leaves per genotype) were measured for leaf area (A), length (L) and width (W) in the model building experiment coming from five genotypes: Porn Ying Yai, Duan Tawan, Manee Chintana, Soi Budsanin, and Sabckaeron Suk under greenhouse conditions at the Experimental Farm of the Research Unit for Mediterranean Flower Species of Bagheria, near Palermo (Italy) (lat. 38°5'N, long. -13°30'E, altitude 23 m above sea level) during the 2008 growing season. The plants were grown in linear polypropylene containers (2760 L) filled with coconut coir dust and perlite (1:1 v/v) in double rows (row spacing of 0.4 m) giving a plant density of 6.2 plants $m^{-2}$.

Immediately after cutting, leaves were placed in plastic bags and were transported on ice to the laboratory. Leaf L was measured from lamina tip to the point of intersection of the lamina and the petiole, along the midrib of the lamina, while leaf W was measured from end-to-end between the widest lobes of the lamina perpendicular to the lamina mid-rib. Values of L and W were recorded to the nearest 0.1 cm. The area of each leaf (A) was measured using an area meter (LI-3000; LICOR, Lincoln, NE, USA) calibrated to 0.01 $cm^2$.

The dependent variable (A) was regressed with different independent variables, including L, W, $L^2$, $W^2$, and the product L x W. Mean Square Error (MSE) and the values of the coefficients (b) and constants (a) were also reported, and the final model was selected based on the combination of the highest coefficient of determination ($R^2$) and the lowest MSE. These statistics were applied to each individual genotype and to combined data points of all genotypes for each model.

*Model validation*

To validate the developed model and to increase practical applicability, a validation experiment was conducted in spring 2008 on leaf samples of Nam Chok grown at the Experimental Farm of the Research Unit for Mediterranean Flower Species of Bagheria. This genotype was selected as one of the most representative and appreciated *Euphorbia x lomi* Thai hybrids cultivated in Italy and in other countries. The plants were grown in double rows (0.4 m between rows); the final density was 6.2 plants $m^{-2}$.

To validate the model, about 100 leaves of Nam Chok actual leaf area and leaf width and length were determined by the previously described procedures. Leaf area of individual leaves was predicted using the best model from the calibration experiment (model building) and was compared with the actual leaf area. The slope and intercept of the model were tested to see if they were significantly different from the slope and intercept of the 1:1 correspondence line (Dent and Blackie, 1979). Regression analyses were conducted using the SigmaPlot 8.0 package (SigmaPlot, Richmond, California, USA).

## 3. Results and Discussion

One of the leaf shape traits is the length:width ratio (L:W). In the current experiment, significant differences ($P< 0.05$) were recorded on L:W ratio among genotypes (Table 1). Duan Tawan, Nam Chok and Sabckaeron Suk produced the largest leaves (L:W ratio ranged from 2.21 to 2.33); Soi Budsanin had narrow leaves (L:W ratio was 3.32), while Porn Ying Yai and Manee Chintana exhibited an intermediate leaf shape value (L:W ratio ranged from 2.88 to 2.92) (Table 1).

Table 1 - Leaf shape (length:width ratio) values for individual *Euphorbia x lomi* Thai genotypes

| Genotypes | L:W | $R^2$ (y) | MSE (y) |
|---|---|---|---|
| Porn Ying Yai | 2.92 (0.014) (z) | 0.745 | 0.91 |
| Duan Tawan | 2.21 (0.016) | 0.759 | 0.60 |
| Manee Chintana | 2.88 (0.019) | 0.720 | 1.10 |
| Soi Budsanin | 3.32 (0.026) | 0.797 | 0.61 |
| Nam Chok | 2.33 (0.018) | 0.805 | 1.05 |
| Sabckaeron Suk | 2.25 (0.017) | 0.752 | 0.96 |

(z) Standard errors in parenthesis.
(y) 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).

*Model calibration*

Leaf W, leaf L and functions of these dimensions were significantly ($P < 0.001$) correlated with A (Table 2). When A was regressed with L or W, alone (Models 1 and 2), a curvilinear relationship was obtained. A linear relationship was found between A and L x W, and between A and $L^2$ or $W^2$ (Models 3, 4 and 5). Based on selection criteria previously described (higher $R^2$ and lower MSE), this study demonstrated that models with a single measurement of W (Models 2 and 5) (Table 2) were less acceptable for estimating leaf area, due to their lowest coefficients of determination and higher MSE values. An improvement was possible for single LA estimation when L and $L^2$ were used as independent variable (Models 1 and 4). To find a model to predict single leaf area accurately for plants of all genotypes, the product of L x W was used as independent variable (Model 3). We preferred this linear model for its accuracy (smallest MSE and the highest $R^2$) (Table 2). Therefore, both L and W measurements were necessary to estimate *Euphorbia x lomi* leaf area accurately. The shape coefficient (slope 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 coefficient (0.70) agreed closely with those calculated for other crops. Values of 0.69 have been reported for pepper (De Swart *et al.*, 2004), 0.63 for zucchini (Rouphael *et al.*, 2006), 0.59 for *Vitis vinifera* L. (Montero *et al.*, 2000), and 0.74 for hazelnut (Cristofori *et al.*, 2007). Moreover, results of the present study were in accordance with previous studies on establishing reliable equations for predicting leaf area through measuring leaf dimensions. Leaf area estimation models in some crop species (Demirsoy and Demirsoy, 2003; Demirsoy *et al.*, 2004; De Swart *et al.*, 2004) were developed using leaf length and width as performed in our study. These models can also easily be used in physiological and agronomical studies.

Possible genotype differences using the selected model were analyzed. Slopes of the genotypes were slightly different (Table 3). However, when leaf area estimations using an equation derived for a single cultivar vs. the overall model were compared, they were not significantly different. These results suggest that a "universal" leaf area estimation model for *Euphorbia x lomi* Thai hybrids is plausible, unless other genotypes differ greatly in leaf morphology from those used in this experiment.

*Model validation*

Comparisons between measured vs. calculated leaf area using Model 3 (A = 0.691 LW – 1.428) for the validation set derived from the 2008 experiment on hybrid Nam Chok, showed a high degree of correlation and provided quantitative evidence of the validity of the area estimation model (Fig. 1). The regression line of the measured vs. calculated values were not significantly (P = 0.78) different from the 1:1 correspondence. Moreover, the calculated values of A were very close to the measured values, giving an underestimation of 1.3% in the prediction.

To summarize, we can conclude that the length-width model (i.e. Model 3) can provide accurate esti-

Table 2 - Fitted coefficient (b) and constant (a) values of the models used to estimate the *Euphorbia* x *lomi* leaf area (A) of single leaves from length (L) and width (W) measurements (combined data for five *E.* x *lomi* Thai genotypes)

| Model No. | Form of model tested | Fitted coefficient and constant | | $R^{2\,(y)}$ | MSE (y) |
|---|---|---|---|---|---|
| | | a | b | | |
| 1 | A = a + bL | -34.49 (1.41) (z) | 5.98 (0.11) | 0.862 | 41 |
| 2 | A = a + bW | -18.46 (1.26) | 12.42 (0.27) | 0.824 | 50 |
| 3 | A = a + bLW | -1.42 (0.31) | 0.70 (0.005) | 0.981 | 7 |
| 4 | $A = a + bL^2$ | 0.37 (0.66) | 0.24 (0.004) | 0.860 | 42 |
| 5 | $A = a + bW^2$ | 8.02 (0.75) | 1.36 (0.03) | 0.801 | 56 |

(z) Standard errors in parenthesis; L and W were in cm.
(y) Coefficient of determination ($R^2$) and mean square errors (MSE, in $cm^2$) of the various models are also given. All data were derived from the calibration Experiment (n = 500 leaves).

Table 3 - Fitted coefficient (b) and constant (a) values for individual *Euphorbia x lomi* Thai genotypes using the model A = a + bLW, where A is the leaf area of single leaves, and LW is the product of length and width measurements

| Genotypes | Fitted coefficient and constant | | $R^{2\,(y)}$ | MSE (y) |
|---|---|---|---|---|
| | a | b | | |
| Porn Ying Yai | -1.93 (0.58) (z) | 0.71 (0.009) | 0.983 | 3 |
| Duan Tawan | -1.45 (0.43) | 0.69 (0.018) | 0.952 | 9 |
| Manee Chintana | -0.14 (0.70) | 0.70 (0.009) | 0.980 | 5 |
| Soi Budsanin | -0.27 (0.38) | 0.64 (0.011) | 0.975 | 2 |
| Sabckaeron Suk | 0.61 (0.70) | 0.65 (0.013) | 0.971 | 8 |

(z) Standard errors in parenthesis; L and W were in cm.
(y) Coefficient of determination ($R^2$) and mean square errors (MSE, in $cm^2$). All data were derived from the calibration Experiment.

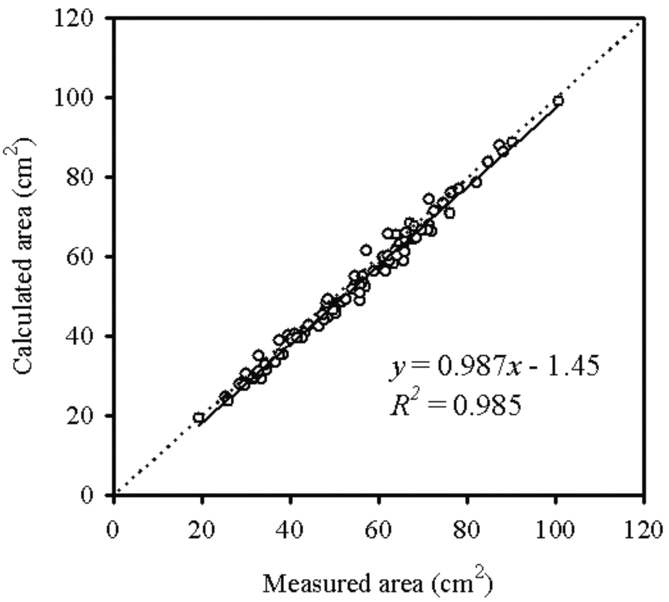


Fig.1 - Measured vs. calculated values of single leaf areas of hybrid Nam Chok during the validation experiment using Model 3 [A = 0.696 LW – 1.428], where A is individual leaf area ($cm^2$) and LW is the product of leaf length (cm) × leaf width (cm). Solid line represents linear regression line of Model 3. Dotted line represents the 1:1 relationship between the measured and calculated values.

mations of Thai *Euphorbia x lomi* leaf area across genotypes, allowing agronomists and physiologists to estimate, accurately and in large quantities, the leaf area of these Spurge family hybrids with no destructive measurements.

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