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A Simple and Accurate Equation for Estimating Individual Leaf Area of Eggplant from Linear Measurements

C. M. Rivera, Y. Rouphael, M. Cardarelli and G. Colla
(Dipartimento di Geologia e Ingegneria Meccanica, Naturalistica e Idraulica per il Territorio, Università della Tuscia, Viterbo, Italy)

Summary

Simple, accurate and non-destructive equations determining leaf area of plants are important for many experimental comparisons. Determining the individual leaf area (LA) of eggplant (*Solanum melongena* L.) involves measurements of leaf parameters [length (L) and width (W)]. Two year investigation were carried out during 2005 (on five cultivars) and 2006 (on one cultivar) under open field and greenhouse conditions, respectively to test whether a equation could be developed to estimate leaf area across cultivars. Regression analyses of LA versus L and W revealed several equations that could be used for estimating the area of individual eggplant leaves. A linear equation having LW as the independent variable provided the most accurate estimate (R^2 = 0.97, MSE = 89) of eggplant LA, but double the time required for leaf measurement. The quadratic equation (LA = 0.763 L + 0.340 L^2) exhibited a high accuracy and precision (R^2 = 0.92, MSE = 220) in estimating individual eggplant LA. We preferred this equation because of its simplicity and convenience, as it only involves one variable. Validation of the quadratic equation having L of leaves measured in the 2006 experiment under greenhouse conditions showed that the correlation between calculated and measured areas was very high.

Key words. leaf area – leaf parameters – linear measurements – non-destructive methods – *Solanum melongena* L.

Introduction

Accurate and simple measurements of leaf surface areas are of special concern to plant scientists as well as to process engineers handling these materials. Measurement of leaf area is of value in studies of plant nutrition, plant competition, plant soil-water relations, plant protection measures, crop ecosystems, respiration rate, light reflectance, and heat transfer in heating and cooling processes (Mohsenin 1986).

Leaf area can be measured either by destructive or nondestructive measurements. Accurate, nondestructive measurements permit repeated sampling of the same plants over time and have the advantage that biological variation could be avoided (De Swart et al. 2004). A common approach for nondestructive leaf area estimation is to develop ratios and regression estimators by using easily measured leaf parameters such as length and width (Schwarz and Kläring 2001). Various combinations of measurements and various equations relating length and width to area have been developed for several vegetable crops such as muskmelon (Panta and NeSmith 1995), tomato (Schwarz and Kläring 2001), cucumber (Blanco and Folegatti 2003), broccoli (Stoppani et al. 2003), pepper (De Swart et al. 2004), and zucchini squash (Rouphael et al. 2006), while information on the estimation of eggplant (*Solanum melongena* L.) leaf areas is still lacking. The accuracy of the predictions however, is dependent on the variation of leaf shape between genotypes. Since leaf shape (length:width ratio) may vary among different genetic materials (Stoppani et al. 2003), we needed a good equation for non-destructive leaf area estimation for use in physiological and agronomical studies of eggplant independently of the genetic materials. Therefore, the aims of this study were (1) to compare existing predictive leaf area equations for eggplant leaves using non-destructive measurements; and (2) to assess the accuracy of the optimum equation selected using an independent dataset.

Material and Methods

A total of 2523 eggplant leaves were measured for leaf area (LA), length (L) and width (W) in the preliminary calibration experiment (2005), and 477 leaves were measured for the validation experiment in 2006.

The five eggplant (*Solanum melongena* L.) cultivars 'Beatrice', 'Lucina', 'Melana', 'Miss Bell' and 'Galina' were grown in summer 2005 (15 June–23 September) under field conditions at the Experimental Farm of Tuscia University, central Italy (latitude 42 °25 ' N; longitude 12 °08 ' E; altitude 310 m). These cultivars were selected as a representative sampling of many eggplants cultivated in Italy. Plants were grown in a randomised completely block design with 4 replications. The size of each plot was 4.8×10 m (four rows per plot). Row spacing was 1.20 m and the distance between plants in the row was

0.40 m providing a plant density of 2.1 plants m^{-2}. All plots received the same amount of total N (150 kg ha^{-1}), P (100 kg ha^{-1}), and K (200 kg ha^{-1}) fertilizers. All of the P and 40 % of the N and K fertilizers was applied prior to planting and thoroughly mixed to the soil. The remaining 60 % of N and K was added equally at weekly intervals through the drip irrigation system, starting 2 weeks after transplanting until the second harvest. Irrigation was scheduled twice weekly and applied with drip-irrigation system to ensure that water was non-limiting. Weeds were controlled by hand as needed and there was no problem related to diseases and insects.

About 500 leaves of different sizes and ages representing the whole field were randomly collected for each cultivar 75 days after transplanting. Immediately after cutting, leaves were placed in plastic bags and cooled on ice for transport 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. In addition, 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 dependent variable (LA) was regressed with different independent variables, including L, W, and LW. Mean Square Error (MSE) and the values of the coefficients (a,b) were also reported (Table 1) Equation selection required a balance between predictive qualities of the equation (highest coefficient of determination (R^2) and the lowest MSE) and the economy of including the least number of variables necessary to predict cucumber leaf area accurately. These statistics were applied to each individual cultivar and to combined data points of all cultivars for each equation.

In addition to validate the developed equation and to increase practical applicability in different environment conditions, a validation experiment was conducted in spring-summer 2006 (15 April–30 July) on leaf samples obtained from 'Galina' eggplant grown under greenhouse conditions at the Experimental Farm of Tuscia University, central Italy. Plants were grown in single rows (1.2×0.4 m) at a plant density of 2.1 plants m^{-2}. Pre-planting fertiliser $(NH_4)_2HPO_4$; K_2SO_4 was spread and disked at the following rates (kg ha^{-1}): 100 N, 52 P and 83 K. Plants were side-dressed with 50 kg ha^{-1} N and 62 kg ha^{-1} K using $Ca(NO_3)_2$ and KNO_3 twice at 3 week intervals, starting 4 weeks after transplanting. Irrigation was applied by drip-irrigation and was scheduled using tensiometres to ensure that water was non-limiting (the high and low tension set points were −30 and −1 kPa, respectively). Plants were kept free from weeds, insects, and diseases using standard greenhouse management.

About 500 leaves of 'Galina' 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 equation (s) from the calibration experiment 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).

Results and Discussion

Model calibration

Regression analysis demonstrated significant ($P<0.001$) relationships between LA and W, L, and the product of W and L (Table 1). Several researches have been carried out to establish reliable relationships between the leaf area and the leaf dimensions of different vegetable crops like muskmelon (Panta and NeSmith 1995), tomato (Schwarz and Kläring 2001), cucumber (Blanco and Folegatti 2003), broccoli (Stoppani et al. 2003), pepper (De Swart et al. 2004), and zucchini squash (Rouphael et al. 2006). In the current experiment, leaf area of single leaves could be predicted on the basis of either length or width alone but predictions improved when quadratic terms were added, especially equations with a single measurement of L (Table 1). Further improvement was possible when the products of length and width [LW or $(LW)^2$] were used. Based on selection criteria previously described (least number of variables, higher R^2 and lower MSE), this study demonstrated that equations with a single measurement of W (equations 3 and 4, Table 1) were less acceptable for estimating leaf area, due to their lowest coefficient of determination and higher MSE values. Equations 5 and 6 (Table 1) that required two measurements per leaf could estimate leaf areas of open field eggplant

Table 1. Fitted coefficients (a, b) of the equations used to estimate the eggplant leaf area (LA) of single leaves from length (L) and width (W) measurements (combined data for five eggplant cultivars).

Equation No.	Form of equation tested	a	b	R^2†	MSE†
1	$LA = a\,L$	0.614 (0.046)*	–	0.730	725
2	$LA = a\,L + bL^2$	0.763 (0.083)	0.340 (0.005)	0.918	220
3	$LA = a\,W$	9.917 (0.076)	–	0.721	752
4	$LA = a\,W + bW^2$	2.251 (0.165)	0.772 (0.016)	0.872	346
5	$LA = a\,LW$	0.641 (0.002)	–	0.967	89
6	$LA = a\,LW + bLW^2$	0.660 (0.004)	−0.00008 (0.0001)	0.967	88

*Standard errors in parenthesis; L and W were in cm.
†Coefficient of determination (R^2) and mean square errors (MSE in cm^2) of the various equations are also given. All data were derived from the calibration experiment 2005 (n=2523 leaves).

accurately, but doubled the time required for leaf measurement. The shape coefficient (slope of equation 5) 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 (0.64) 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 broccoli (Stoppani et al. 2003), 0.62 for blueberries (NeSmith 1991), and 0.59 for *Vitis vinifera* L. (Montero et al. 2000). The quadratic equation having L as independent variable (equation 2) exhibited a high accuracy and precision (one of the smallest MSE values and one of the highest R^2 values) in estimating individual eggplant leaf areas (Table 1). We preferred this equation because of its simplicity and convenience, as it only involves one variable. As stated by Robbins and Pharr (1987), model selection requires a balance between predictive qualities of the model and the economy of including the least number of variables necessary to predict leaf area.

Possible cultivar differences using the equation 2 were analysed. Regression coefficients (a, b) of the eggplant cultivars were not significantly different. Moreover, when leaf area estimations using an equation derived for a single cultivar versus the overall equation were compared, they were not significantly different. Therefore the equation: $LA = 0.763\ L + 0.340\ L^2$ can provide accurate estimations of individual eggplant leaf area across cultivars.

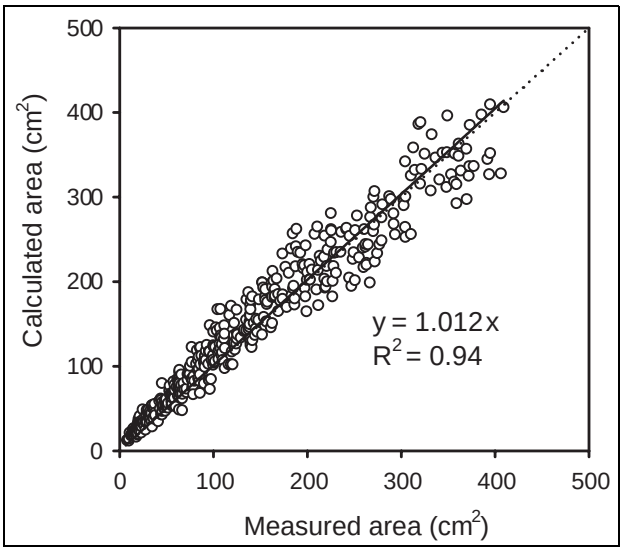


Fig. 1. Measured versus calculated values of single leaf areas of eggplant under greenhouse conditions, during 2006 using equation 2 [$LA = 0.3398\ L^2 + 0.7627\ L$, where LA is individual leaf area (cm^2) and L is the leaf length (cm)]. Solid line represents linear regression line of equation 2. Dotted line represents the 1:1 relationship between the measured and calculated values.

Model validation

Comparisons between measured *vs*. calculated leaf area using equation 2 ($LA = 0.763\ L + 0.340\ L^2$) for the validation set derived from the greenhouse experiment, showed a high degree of correlation and provided quantitative evidence of the validity of the area estimation equation (Fig. 1). The regression lines of the measured *vs*. calculated values were not significantly (P=0.47) different from the 1:1 correspondence. Moreover, the calculated values of LA were very close to the measured values, giving an overestimation of 1.2 % in the prediction. Finally, the equation would likely be useful under a open-field and greenhouse conditions.

In summary, equations that required two measurements per leaf exhibited the highest accuracy and prediction, but double the time required for leaf measurement. Regression equation with the single measurement of leaf length (equation 2) can accurately predict leaf areas for greenhouse and field-grown eggplant cultivars. With this equation, agronomists and physiologists can estimate accurately and in large quantities the leaf area of eggplant plants without the use of any expensive instruments, e.g., leaf area planimeter or digital camera with image measurement software.

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Addresses of authors: C. M. Rivera, Y. Rouphael, M. Cardarelli and G. Colla(corresponding author), Dipartimento di Geologia e Ingegneria Meccanica, Naturalistica e Idraulica per il Territorio, Università della Tuscia, 01100 Viterbo, Italy,
e-mail: giucolla@unitus.it.