International Journal of Plant Production 5 (4), October 2011
ISSN: 1735-6814 (Print), 1735-8043 (Online)
www.ijpp.info

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Modeling individual leaf area of basil (*Ocimum basilicum*) using different methods

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Received 5 March 2011; Accepted after revision 30 April 2011; Published online 15 September 2011

Abstract

Leaf area (LA) is a valuable key for plant physiological studies, therefore accurate and simple models for LA determination are important for many experimental comparisons. A greenhouse experiment was conducted from October 2009 to February 2010 in two basil cultivars (Purple Ruffles and Genovese) to estimate LA, leaf dry weight (DW), leaf fresh weight (DW) and leaf dimensions (width-W and length-L). The aim of the work was to establish a non-destructive model of leaf are estimation. Regression analyses of LA versus FW, DW, L and W revealed several models that could be used for estimating the area of individual basil leaves. Among the models, one based on the sum of dimension squares was the most accurate for Genovese [LA = 0.209 (L^2 + W^2) + 0.25, R^2 = 0.895, RMSE = 0.794 and while the product of dimension squares was the most suitable for Purple Ruffles [LA=0.013 (L^2W^2) +4.963 R^2= 0.817, RMSE = 1.170].

Keywords: *Ocimum basilicum*; Dry weight; Fresh weight; Leaf length; Leaf width; Individual leaf area estimation.

Introduction

Basil (*Ocimum basilicum* L.) is a herbaceous annual species of Lamiaceae family, which has about 3500 species distributed among 210 genera (Blank et al., 2004). In Iran, it is grown mainly for its fresh or dried leaves which are used as fresh consumption, flavorants or condiments.

The leaves are the commercially important plant parts, and by estimating leaf area (LA), the production could be predicted. Also, LA is a key factor for physiological studies involving plant growth, light interception, photosynthetic efficiency, evaporation and also responses to fertilizers and irrigation (Blanco and Folegatti, 2005). Thus, LA strongly influences growth and productivity and its estimation can be a fundamental component of crop growth models (Lizaso et al., 2003; Rouphael et al., 2010a). Both direct and indirect methods can be used for total LA estimation (Kandiannan et al., 2009). Different direct methods are available to measure LA for any single leaf e.g. laser planimeters, scanner methods (Caldas et al., 1992), gravimetric methods (Ross et al., 2000), projected area (Westoby and Wright, 2003), integrating sphere (Serrano et al., 1997) and a fix camera with image analysis software (Granier et al., 2002). However, measurement of LA with direct methods for a whole plant or part of the plant is time-consuming and involves a large amount of labour (Leroy et al., 2007).

Many methods have been devised to facilitate the measurement of LA but these methods, including those of tracing, blueprinting, photographing, or using a conventional planimeter, require the excision of leaves from the plants. Plant canopy is also damaged, which might cause problems to other measurements. (Cristofori et al., 2007) Leaf area can be measured quickly and non-destructively using a portable scanning planimeter (Daughtry, 1990), but it is suitable for small plants with few leaves (Nyakwende et al., 1997).

Many equations have been built in order to estimate LA non-destructively. Montgomery (1911) first suggested that we can estimate area of single leaves from linear measurements such as leaf length (L) and width (W) of each lamina (Leroy et al., 2007). This non-destructive models for LA determination have been built for many species such as bean (Bhatt and Chanda, 2003), white clover (Gamper, 2005), sugar beet (Tsialtas and Maslaris, 2005; Tsialtas and Maslaris, 2008), taro (Lu et al., 2004), radish (Salerno et al., 2005), strawberry (Demirsoy et al., 2005) and zucchini (Rouphael et al., 2006) , kiwi (Mendoza-de Gyves et al., 2007), chestnut (Serdar and Demirsoy, 2006), hazelnut (Cristofori et al., 2007), eggplant (Rivera et al., 2006), hazelnut (Cristofori et al., 2007), Persimon (Cristofori et al., 2008), medlar (Mendoza-De Gyves et al., 2008), small fruits (Fallovo et al., 2008), roses (Rouphael et al., 2010b), watermelon (Rouphael et al., 2010c); sunflower (Rouphael et al., 2007), and Euphorbia × lomi (Fascella et al., 2009).

An indirect, destructive method for estimating LA is a function of dry weight of plant parts or total above the weight of dry ground (Jonckheere et al., 2004). It has been reported that leaf and/or total dry weight have a close relationship to LA in soybean (Lieth et al., 1986), wheat (Aase, 1978), alfalfa (Sharrett and Baker, 1985), barley (Romas et al., 1983) and cotton (Ghaderi and Soltani, 2007).

The objective of this study was to develop functions between plant LA and plant vegetative characteristics in basil cultivars grown under greenhouse conditions. Leaf dimensions (*L* and *W*) or combinations of both, the leaf dry weight (*DW*) and the leaf fresh weight were measured. Although several prediction models are available to estimate leaf area for numerous crops, there is no information about estimation of leaf area for basil. In this paper an attempt has been made to establish a best method for estimation of leaf area of basil crop.

Material and Methods

An experiment was conducted in a research greenhouse at Ferdowsi University of Mashhad (36°17′ N, 59°36′ E) in Iran from October 2009 to February 2010. Seeds of two cultivars of *Ocimum basilicum* (Purple Ruffles and Genovese) were sown in two seed plot that were filled with peat moss. Two weeks after germination, when the second true leaf appeared, seedlings were transferred to a irrigated field (6×3 m^2) with a planting density of 15×15 cm. The growing conditions were optimum (max. and min. temperature 35 ºC and 11 ºC, respectively and RH: 60%). In sixth leaf stage, a fertilization was applied including NPK (20:20:20).

Sampling was done 54 days after sowing (DAS) in first harvest and 150 leaves per cultivar harvested randomly from the upper 1/3 of the plants. Immediately after cutting, leaves were placed in plastic bags and were transferred on ice to the laboratory of Ferdowsi University of Mashhad. Leaf length (*L*) was measured from lamina tip to the point of intersection of the lamina and stem and width (*W*) were measured from tip to tip between the widest lamina with a simple ruler. Values of *L* and *W* were recorded to the nearest 0.1 cm. The LA each leaf was measured using an area meter (Licow) calibrated to 0.001 cm^2. The *FW* of leaves also measured, and then for the determination of the *DW*, leaves were placed in oven at 60 ºC for 48 h. The *FW* and *DW* were measured to the nearest 0.001 g.

The dependent variable (LA) was regressed with different independent variables, including *FW*, *DW*, *L*, *W*, L^2, W^2, the products of these dimension ($L+W$, $L\times W$, L/W, $L^2\times W^2$, L^2+W^2) and also with fresh and dry weight of leaves to identify appropriate functions for use in models estimating leaf area of *Ocimum basilicum*. Root mean square error (RMSE) and the values of the coefficients (*b*) and constants (*a*) were also reported. The estimated LA was determined by fitting the equation and the final model was selected based on the combination of the highest coefficient of determination (R^2) and the lowest RMSE. 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, Equal 1) (Marquardt, 1970) and the tolerance values (*T*, Equal 2) (Gill, 1986) were also calculated.

Equal 1. $$VIF = \frac{1}{1-r^2}$$

Equal 2. $$T = \frac{1}{VIF}$$

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.

Results

Linear measurements

As a preliminary step to model calibration, the degree of collinearity among *W* and *L* was analyzed. The VIF (1.36 and 1.46) and T value (0.68 and 0.73) were acceptable for Genovese and Purple Ruffles respectively, because in two genotypes, 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.

Leaf *W*, leaf *L* and functions of these dimensions were found for both cultivars (Tables 1 and 2).

It was seen significantly ($P<0.001$) correlation with LA and different kind of dimension (Tables 1 and 2). Based on selection criteria previously described (higher R^2 and lower RMSE) we select the best model for estimation leaf area of Basil. Except for Models 2 and 3, all models produced a coefficient of determination (R^2) equal to or greater than 0.84 in Genoves cultivar (Tables 1). Also in Purple Ruffles cultivar it has been shown that except for

Models 1, 2, 3 and 4, other models produced a coefficient of determination (R^2) equal to or greater than 0.71, that this part of research showed that models with only measurement of *W* in Genovese cultivar and that models with only measurement of one variable *L* or *W* in Purple Ruffles were not more acceptable for estimating leaf area, due to their lower coefficient of determination (R^2) and highest RMSE values and as seen at tables 1 and 2, the best model for Genovese and Purple Ruffles cultivars are 7 and 8 model respectively. Therefore, both *L* and *W* measurements were necessary to estimate Basil leaf area accurately.

Table 1. Fitted coefficient (b) and constant (a) values of the models used to estimate the basil (genoves cultivar) leaf area (LA) of single leaves from length (*L*) and width (*W*) measurements.

Model number	Form of model tested	Fitted coefficient and constant		R^2	RMSE
		a	b		
1	LA=a+bL	-7.528	2.931	0.840	0.984
2	LA=a+bW	-6.323	5.088	0.670	1.408
3	LA=a+bW^2	1.580	0.805	0.686	1.377
4	LA=a+bL^2	0.992	0.247	0.848	0.957
5	LA=a+bLW	-0.020	0.520	0.892	0.807
6	LA=a+b (L+W)	-9.539	2.134	0.893	0.802
7	LA=a+b (L^2+W^2)	0.25	0.209	0.895	0.794
8	LA=a+b (L^2 W^2)	4.987	0.012	0.876	0.912

Table 2. Fitted coefficient (b) and constant (a) values of the models used to estimate the basil (Purple Ruffles cultivar) leaf area (LA) of single leaves from length (*L*) and width (*W*) measurements.

Model number	Form of model tested	Fitted coefficient and constant		R^2	RMSE
		a	b		
1	LA=a+bL	-5.654	2.533	0.601	1.711
2	LA=a+bW	-3.012	4.272	0.605	1.702
3	LA=a+bW^2	3.072	0.028	0.621	1.667
4	LA=a+bL^2	2.011	0.205	0.598	1.717
5	LA=a+bLW	0.516	0.504	0.799	1.331
6	LA=a+b (L+W)	-8.347	2.000	0.758	1.331
7	LA=a+b (L^2+W^2)	0.866	0.190	0.717	1.441
8	LA=a+b (L^2 W^2)	4.963	0.013	0.816	1.170

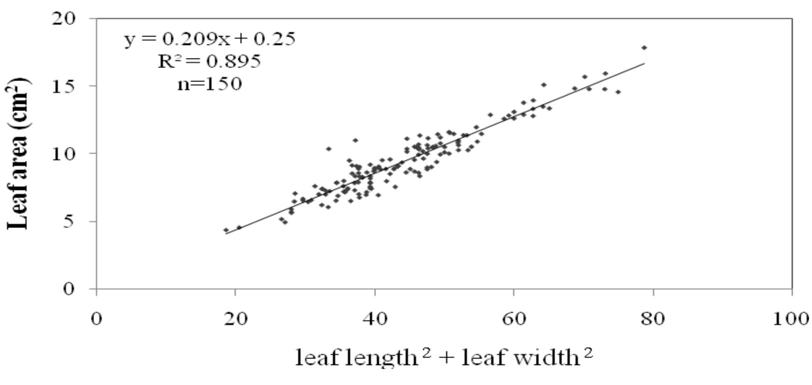

Figure 1. Relationship between leaf area (LA) and leaf length $(L)^2$ + leaf width $(W)^2$ of Single leaves from Genoves cultivar. The equation for the regression line is [LA = 0.209 ($L^2 + W^2$) + 0.25], (n=150).

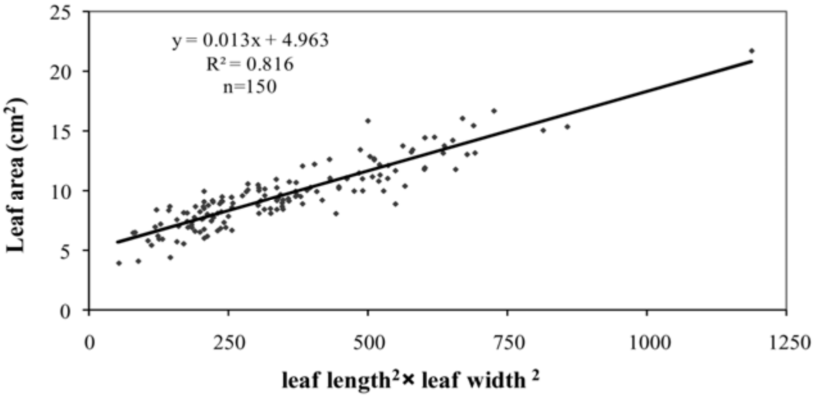

Figure 2. Relationship between leaf area (LA) and leaf length $(L)^2$ × leaf width $(W)^2$ of Single leaves from Purple Ruffles cultivar. The equation for the regression line is [LA=0.013 (L^2W^2) +4.963], (n=150).

Relationship of leaf area to leaf dry and fresh weight

Leaf area ranged from 7.1 to 15.9 cm^2 per plant in Genovese and Purple Ruffles cultivars, respectively, corresponding to 0.23 to 0.46 g the fresh weight of per plant leaf at 54DAS. Also dry weight was varied between 0.013 to 0.045 and in Genovese and Purple Ruffles cultivars, respectively. The linear function fitted well between leaf area and leaf fresh weight in Genovese cultivar but there was not in Purple Ruffles cultivar (Table 3; Figures 3 and 4), Although between three methods for estimating single leaf area, the best methods for estimating was linear measurement (Table 3) in two cultivars, more over its simplicity, it is a non-destructive methods. However that best methods for estimating single leaf area in basil is linear measurement, but there is a good acceptance for estimating based on fresh weight in Genovese cultivar and we can also use this methods if we have not much time for many measurements.

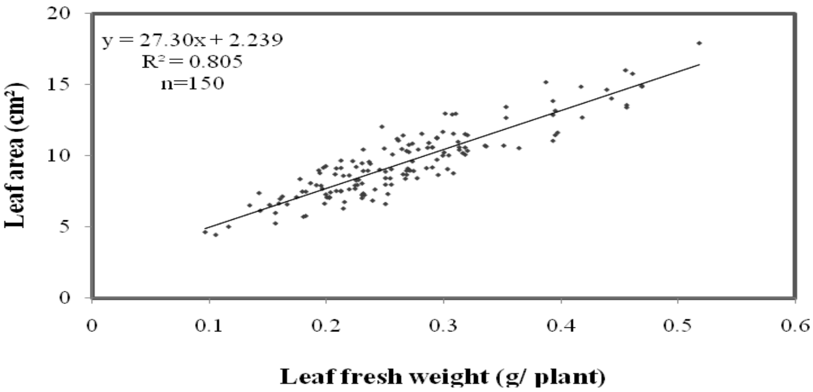

Figure 3. Relationship of leaf area to fresh weight of leaf described by the linear function in Genovese cultivar (n=150).

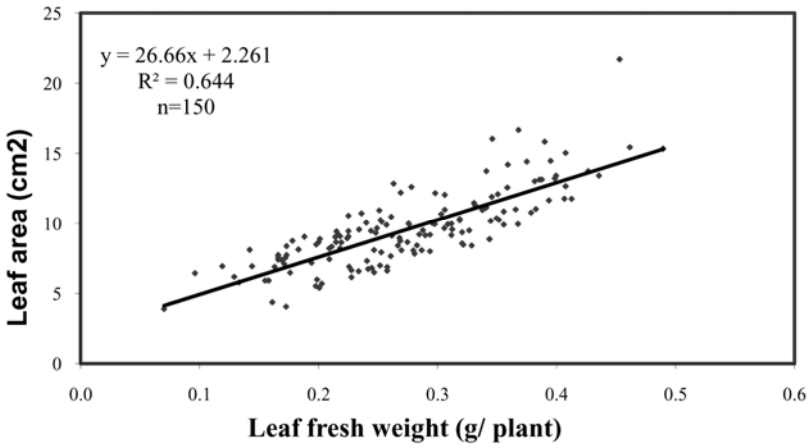

Figure 4. Relationship of leaf area to fresh weight of leaf described by the linear function in Purple Ruffles cultivar (n=150).

Table 3. Evolution of three methods for estimating leaf area for two cultivar of basil (Genovese and Purple Ruffles).

	Genoves				Purple Ruffles			
	R^2	RMSE	Mean±SD	Var	R^2	RMSE	Mean±SD	Var
Estimated Leaf Area (Fresh weight)	0.805	1.362999	9.496±2.215	4.905	0.644	1.615	9.578±2.17	4.769
Estimated Leaf Area (Dry weight)	0.549	2.509667	9.496±1.829	3.344	0.566	1.783	9.577±2.04	4.193
Estimated Leaf Area (Liner measurment)	0.895	0.794379	9.477±2.331	5.433	0.816	1.170	9.451±2.38	5.712

Model validation

To validate the model, about 50 leaves of sweet basil plant per cultivar were taken from different experiment during February, 2010. Actual leaf areas, leaf length, leaf width, dry and fresh weight were determined by the previously described procedures. Leaf area of individual leaves was predicted using the best model from the calibration experiment and was compared with the actual leaf area. Regression analyses were conducted. Comparisons were made between measured versus calculated leaf area of leaves collected from different experiment during 2010 by using Equal (7) [LA=$0.209(L^2+W^2)+0.25$] and Equal (8) [LA=$0.013(L^2W^2)+4.963$] for Genovese and Purple Ruffles cultivars respectively, where LA is individual leaf area (cm^2), L is the leaf length (cm) and W is leaf width (cm). The leaf area was estimated by the model strongly agreed with the measured value of leaf area of the leaves it is evident from higher value of R^2=0.887, R^2=0.84 and lower RMSE=1.0462, RMSE=0.825 for Genovese and Purple Ruffles cultivars respectively (Figures 5 and 6).The validation of the model showed that sweet basil leaf area could be measured quickly, accurately, and non-destructively by using the developed model.

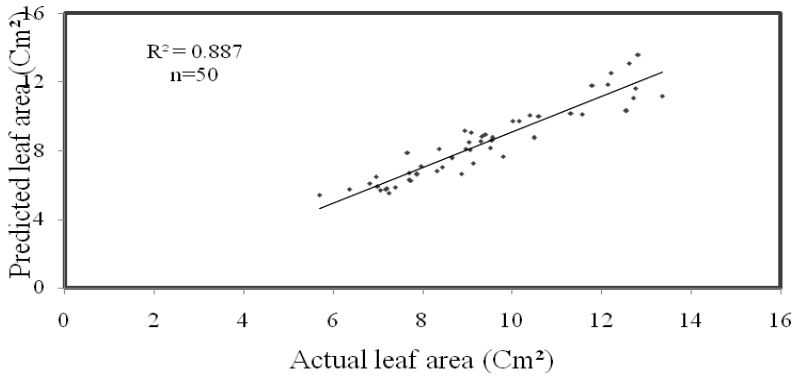

Figure 5. Comparison of actual and predicted leaf area in Genovese cultivar (n=50).

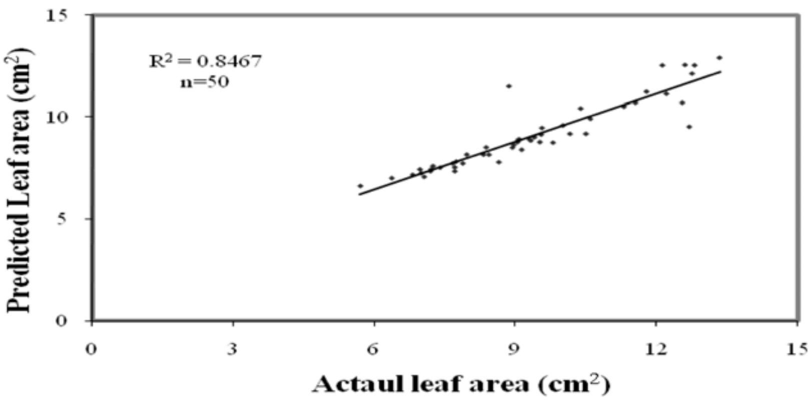

Figure 6. Comparison of actual and predicted leaf area in Purple Ruffles cultivar (n=50).

Discussion

Leaf area is one of the important growth factor for plants especially in vegetables (greens) and lack of accurate model is a limitation for calculating LA (Kandiannan et al., 2009). Leaf area estimated using different methods (Robbins and Pharr, 1987; Montero et al., 2000; Williams Iii and Martinson, 2003). Result of current study showed that area of basil leaves is well correlated to the product of its length and width with high R^2 values. The close relationship between measured leaf area and calculated leaf area based on combination of leaf length and width found in this study is in agreement with those reported by Cristofori (2007) in hazelnut, Kandiannan (2009) in ginger, Potdar (1991) in banana and Karimi (2009) in pisthashio. Leaf length or width solely did not provide a good variable to calculation leaf area, and it is in contrast with Williams Iii and Martinson (2003) and Cho et al. (2007) that stated a single variable of either leaf length or leaf width has a good correlation with leaf area. There are many reports which show estimate of leaf area from leaf dry and fresh weight data (Sharrett and Baker, 1985; Ma et al., 1992). In the present study a nearly good relationship found between fresh weight of leaves in Genovese cultivar and leaf area and we can also use this method if we have not much time for many measurements. Finally linear function models described this relation is better than the other equation types. The validation of the model showed a strong agreement between predicted and measured data and could be estimated quickly, accurately, and non-destructively by using the developed model.

Acknowledgement

This research was supported by the Ferdowsi University of Mashhad and authors would like to thank for financial support of this university.

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