BRIEF COMMUNICATION

Allometric model for nondestructive leaf area estimation in clary sage (*Salvia sclarea* L.)

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Abstract

Leaf area estimation is an important biometrical observation recorded for evaluating plant growth in field and pot experiments. In this study, conducted in 2009, a leaf area estimation model was developed for aromatic crop clary sage (*Salvia sclarea* L.), using linear measurements of leaf length (L) and maximum width (W). Leaves from four genotypes of clary sage, collected at different stages, were used to develop the model. The actual leaf area (LA) and leaf dimensions were measured with a Laser Area meter. Different combinations of prediction equations were obtained from L, W, product of LW and dry mass of leaves (DM) to create linear ($y = a + bx$), quadratic ($y = a + bx + cx^2$), exponential ($y = ae^{bx}$), logarithmic ($y = a + b\text{Ln}x$), and power models ($y = ax^b$) for each genotype. Data for all four genotypes were pooled and compared with earlier models by graphical procedures and statistical measures *viz.* Mean Square Error (MSE) and Prediction Sum of Squares (PRESS). A linear model having LW as the independent variables ($y = -3.4444 + 0.729$ LW) provided the most accurate estimate ($R^2 = 0.99$, MSE = 50.05, PRESS = 12.51) of clary sage leaf area. Validation of the regression model using the data from another experiment showed that the correlation between measured and predicted values was very high ($R^2 = 0.98$) with low MSE (107.74) and PRESS (26.96).

Additional keywords: clary sage, nondestructive, regression model, *Salvia sclarea*, validation.

Clary sage (*Salvia sclarea* L., family Lamiaceae) is a xerophytic biennial or perennial plant native to southern Europe. It is cultivated in England, France, Bulgaria, Southern Russia, and USA for perfume industry. The plant inflorescence possesses very strong aroma, characterized by a fresh floral and herbaceous odour. Thus it has an economic value for the flavour and fragrance industries. Leaf area (LA) is an essential component to estimate plant growth through its incidence on crop physiology mechanisms (Ray and Singh 1989, Portela 1999, Bhatt and Chanda 2003). Thus, accurate assessment of leaf area is essential for evaluation of plant performance at the individual, community and even ecosystem level, as leaf area affects biomass production and nutrient cycling in the ecosystem (Meier and Leuschner 2008). Determination of leaf area of leaves having complex shapes is difficult, time consuming and subject to larger errors. Destructive method of leaf area estimation has no scope for successive measurement of the same leaf, moreover it also damages plant canopy, which adversely affects other measurements of plant growth. Several models have been used to estimate leaf area (Williams and Martinson 2003, Lu *et al.* 2004, Rouphael *et al.* 2006, Hyojin and Park 2009). Estimation of leaf area from mathematical models involving linear measurements of leaves is relatively accurate and nondestructive. A mathematical model can be derived by correlating L, W, or LW with the actual leaf area (LA) of a leaf using regression analysis. The nondestructive methods based on linear measurements are quicker and easier to be executed and offer precision and accuracy as demonstrated for several field crops *viz.* eggplant (Rivera *et al.* 2007), sunflower (Rouphael *et al.* 2007), faba bean (Peksen 2007), stevia (Ramesh *et al.* 2007), persimmon (Cristofori *et al.* 2008), medlar (Mendoza De Gyves *et al.* 2008), small fruits (Fallovo *et al.* 2008), euphorbia

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Abbreviations: CV – coefficient of variation; DM – dry mass of leaves; L – leaf length; L/W – length width ratio; LA – leaf area; LW – product of length and width of the leaf; MSE – mean square error; PRESS – prediction sum of squares; W – leaf width.

(Fascella *et al*. 2009), saffron (Kumar 2009), ginger (Kandiannan *et al.* 2009), roses (Rouphael *et al*. 2010a), and watermelon (Rouphael *et al*. 2010b). Such information on estimation of leaf area in clary sage is not available. The procedure used for developing the model has been used earlier by Tsialtas and Maslaris (2005); Tsialtas *et al*. (2008) and Fallavo *et al*. (2008). The aim of this study was, therefore, to establish a LA prediction model based on L and W measurements and to test the accuracy of the proposed model for LA estimation, and to characterize variability within different accessions of clary sage.

Four genotypes of clary sage (V1, V2, V3, and V4) were planted in the experimental farm of the Institute of Himalayan Bioresource Technology, Palampur (32°06′ 05″ N, 76°34′10″ E, at 1325 m a.s.l.) in December 2008. Fifty representative leaves of three genotypes (V1, V2, and V3) and 10 leaves of one genotype (V4) were selected at random, at early and at maximum vegetative stages of clary sage. The leaves have a woolly-texture leaves and were 7.4–28.7 cm long and 2.4–15.4 cm broad. Thus total 320 leaves were carefully detached for preliminary calibration. LA, L, W, and L/W of all leaves were measured with a Laser Area meter (*CI 203 CID, Inc*., USA). The leaf samples were then oven dried at 70ºC till constant mass. The relationship between leaf area as a dependent variable and L, W, LW, L/W, $L^{0.5}$, L^2, $W^{0.5}$, W^2, L^2W^2, and leaf dry mass (DM) as independent variables was determined using regression analysis on data of individual genotype and together in combination of all the genotypes. Coefficients of determination (R^2) were calculated and the equation that presented the highest R^2 was used in the estimations. The linear (y = a + bx), quadratic (y = a + bx + cx^2), power (y = ax^b), exponential (y = ae^{bx}), and logarithmic (y = a + bLnx) model equations and multiple regression analyses were developed for each genotype using *Microsoft Excel 7.0* software. In the above linear and quadratic model equations, y is the measured leaf area, a is the intercept, b is the slope, c is the constant, and x is the independent variable. The estimated leaf area was determined by fitting the equation. Then estimated and measured leaf areas were compared by testing the significance of regression equation and R^2 between them. The final model was selected based on the combination of the highest R^2 and lowest mean square error (MSE) and prediction sum of squares (PRESS). PRESS, a statistics based on the leave-one-out technique proposed by Allen (1974), was also used to compare different models.

For validating the model, 345 leaves of clary sage were taken from different experiments in August 2009. Actual leaf area, leaf length and width were determined by the previously described procedures. Leaf area of individual leaf was estimated using the best model 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. Additionally, the coefficient of variation (CV) was also used to validate the models.

The equations based on dry mass (DM) had the lowest R^2 value as compared to other equations, and hence were not included in model development. These results conform to those of Kumar (2009) for saffron. There are many reports which suggest calculation of leaf area from leaf dry mass data (Ma *et al.* 1992). However, in our study the R^2 value of the model involving dry mass was not as high as of those involving L, W, and LW. In order to establish an accurate LA prediction model, L^2, W^2, and L^2W^2 were used as suggested by other researchers (Salerno *et al.* 2005, Serdar and Demirsoy 2006).

Table 1. Prediction equation between length and width product and leaf area [cm^2] of clary sage. MSE – mean square error; PRESS – prediction sum of squares; CV – coefficient of variation.

Equation	Genotype	Constants a	b	c	R^2	MSE	PRESS	CV
y = a + bx	V1	0.2845	0.6792		0.9913	4.51	1.13	5.13
	V2	–3.7961	0.7223		0.9898	96.43	24.15	11.87
	V3	1.9546	0.6693		0.9860	9.36	2.34	4.43
	V4	–6.7015	0.7666		0.9910	111.51	27.90	6.10
	combined	–3.4444	0.7290		0.9904	50.05	12.51	9.80
y = a + bx + cx^2	V1	0.5970	0.6683	7.00×10^{-5}	0.9913	4.49	1.12	5.12
	V2	–2.4755	0.7033	3.00×10^{-5}	0.9900	94.87	23.74	11.77
	V3	2.5916	0.6561	6.00×10^{-5}	0.9860	9.36	2.34	4.43
	V4	6.2214	0.6230	3.00×10^{-4}	0.9925	792.42	198.05	16.25
	combined	–2.1529	0.7096	4.00×10^{-5}	0.9906	49.10	12.27	9.71
y = ax^b	V1	0.7117	0.9901		0.9836	4.54	1.13	5.15
	V2	0.6438	1.0129		0.9919	102.41	25.61	12.23
	V3	0.8095	0.9653		0.988	9.49	2.38	4.46
	V4	0.6993	1.0081		0.9889	130.19	32.53	6.59
	combined	0.6596	1.0098		0.9912	55.89	13.97	10.36

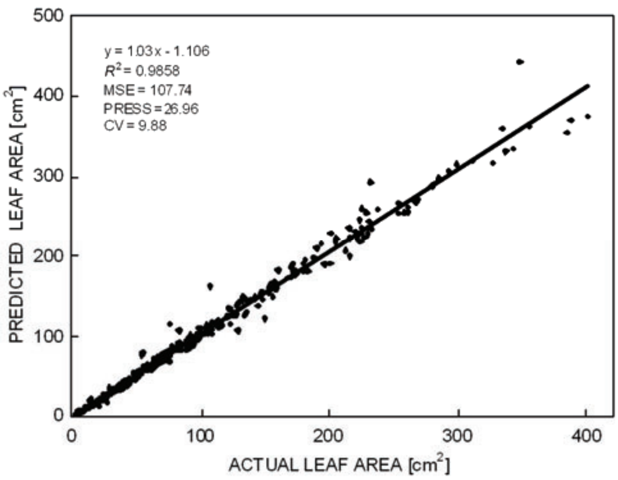

Fig. 1. Validation of the actual *vs*. predicted values of single leaf areas using equation y = –3.4444 + 0.729 LW, where y is individual predicted leaf area, LW is the product of length and breadth of the leaf. *Solid line* represents linear regression line of the model.

Leaf area prediction equations, when leaf length and width were considered together, are presented in Table 1. The equations considering product of leaf length and width as independent variable for leaf area estimation shows 99% association with actual leaf area, as the R^2 ranged from 0.9836 to 0.9913 for different genotypes. It indicates that this model is highly reliable across a range of cultivars. MSE ranged from 4.49 to 792.42 and CV from 4.43 to 16.25. PRESS values of different models ranged from 1.12 to 198.05. Also, the quadratic model having LW as an independent variables showed high R^2 and thus it could be an accurate LA prediction model for clary sage.

For each group, the fitted models were ranked according to their R^2 values. The model with the highest R^2 across all groups was regarded as the best model. Thus, based on R^2 value it was found that use of both length and width of the leaf was imperative for estimation of the leaf area of clary sage. Though quadratic model [$y = -2.1529 + 0.7096\ LW + 4.00 \times 10^{-5}\ (LW)^2$] had the lowest values of MSE (48.72), PRESS (12.27) and CV (9.68) than the other equations, it did not differ much from the linear model (y = –3.4444 + 0.729 LW). Also, the R^2 of these two equations (0.9904 *vs*. 0.9906) are almost similar. Thus, the linear model is better to adopt owing to convenience in calculation. Low MSE, PRESS, and CV show that the calculated LA is close to the actual LA, thus MSE, PRESS, and CV should be the main criterion for selecting an LA depiction model when a precise estimation of the LA is necessary. The coefficient of regression was reported to be a good measure of predictive ability of a model. All regressions were significant, and the entire coefficient of determination exceeded 0.99. Thus, considering both criteria (high R^2 and low MSE, PRESS, and CV) the linear model (y = –3.4444 + 0.729 LW, R^2 = 0.9904, MSE = 50.05, PRESS = 12.51, CV = 9.80, n = 320) can be proposed for LA estimation in clary sage, irrespective of the genotype. Models based on L×W have already been established for other species such as zucchini (Rouphael *et al.* 2006), kiwi (Mendoza-de Gyves *et al.* 2007), and hazelnut (Cristofori *et al.* 2007). The model based on single variable measurement offers the advantage of more efficient data correction, less complex calculation (NeSmith 1992) and requires less time for leaf measurement. But in accordance with the suggestion of Ramesh *et al.* (2007) based on *Stevia* crop, LA measurement involving both length and width is more precise than estimates based on one dimension. The equations with lower R^2 and high CV were eliminated at the beginning of this study. According to Lu *et al.* (2004), simple, linear relationships between leaf dimensions and LA would be preferable. In our work, the best-fitted curves between LA and leaf dimensions (L and W) were those of quadratic functions, however leaf area prediction based on linear dimensions agreed well with that with the quadratic functions, at different stages of the plant. Since no model has been developed so far for LA estimation in clary sage, this work could be valuable information. Comparisons were made between measured and calculated LA of leaves collected from different experiments (y = –3.4444 + 0.729 LW). For validating the model, 345 random samples of clary sage leaves were taken during June 2009. Their LA was measured with leaf area meter and also estimated by adopting the linear equation. The leaf area estimated by the model strongly agreed with the measured value of leaf area, as evident from higher value of R^2 (0.9858), and lower values of MSE (107.74), PRESS (26.96), and CV (9.88). Also, the linear regression for the relationship between measured and estimated values was not significantly different from the 1:1 line (Fig. 1). It suggested that linear model can well be used to predict clary sage leaf area.

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