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Nondestructive, simple, and accurate model for estimation of the individual leaf area of som (*Persea bombycina*)

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Abstract

Nondestructive approach of modeling leaf area could be useful for plant growth estimation especially when number of available plants is limited and/or experiment demands repeated estimation of leaf area over a time scale. A total of 1,280 leaves were selected randomly from eight different morphotypes of som (*Persea bombycina*) established at randomized complete block design under recommended cultural regimes in field. Maximum leaf laminar width (B), length (L) and their squares B^2, L^2; leaf area (LA), and lamina length × width (L×B) were determined over two successive seasons. Leaf parameters were significantly affected by morphotypes; but seasons had nonsignificant impacts on tested features. Therefore, pooled seasonal morphotype means of each parameter were used to establish relationship with LA. L and its square L^2 did not provide accurate models for LA predictions. Considerably better models were obtained by using B (y = 2.984 + 7.9664 x, R^2 = 0.615, $P \geq 0.001$, n = 119) and B^2 (y = 12.784+ 0.9604 x, R^2 = 0.605, $P \geq 0.001$, n = 119) as independent variables. However, maximum accuracy of prediction of LA could be achieved through a simple linear relationship of L×B (y = 8.2203 + 0.4224 x, R^2 = 0.843, $P \geq 0.0001$, n = 119). The model (LA:L×B) was validated with randomly selected leaf samples (n = 360) of som morphotypes and highly significant ($P \leq 0.001$) linear function was found between actual and predicted LAs. Therefore, the last model may consider adequate to predict leaf area of all cultivars of som with sufficient fidelity.

Additional key words: leaf area prediction; leaf length; leaf width; nondestructive methods; regression model; validation.

Introduction

Som (*Persea bombycina,* King ex Hook. f., Lauraceae) is a perennial evergreen tree cultivated extensively in the north-eastern states of India for its foliage as a feed to muga silkworm (*Antherea assamensis*). At present eight morphotypes of som are utilized for the production of golden colour muga silk (Choudhuri 1981, Bhau *et al.* 2009). Variants of som determined the quality of cocoon and silk production abilities (Tazima and Choudhury 2005).

Estimation of leaf area is an essential component of plant growth analysis and studies related to solar radiation interception, photosynthesis, biomass accumulation, transpiration, and energy transfer by plant canopies (Jonckhere *et al*. 2004). Besides, leaf shapes are often useful for plant cultivar classification and species identifications (Neto *et al.* 2006, Du *et al.* 2007). Indeed, som morphotypes are mainly classified on the basis of leaf shape and other associated morphological features (Singh *et al.* 2000). Besides, muga silkworms are commercially reared 3 to 4 times per year in a som garden; therefore, leaf number and area are considered as important determinants of quantity assessment prior to rearing of silkworm (Seth 2000). Leaf area measurement in field is time-consuming, laborious, expensive and often needs sophisticated equipment (Cirak *et al.* 2005, Kumar and Sharma 2010). Up till, nondestructive models for LA prediction have been developed for many trees such as chestnut (Serdar and Demirsoy 2006), hazelnut (Cristoferi *et al.* 2007), cherry (Demirsoy and Demirsoy 2003), peach (Demirsoy *et al.* 2004), sago palm (Nakamura *et al.* 2005), and different other horticultural plants (Uzun and Celik 1999). These estimates are based

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Abbreviations: B – leaf lamina width; B^2 – square of leaf width; L – leaf lamina length; L^2 – square of leaf lamina length; LA – leaf area; LxB – leaf lamina length x width; LSD – least significant difference; R^2 – coefficient of determination.
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on various foliar parameters, which are used to establish linear, quadratic and/or exponential functions derived from best fitted regression equations. Of which, linear models are preferable due to their simplicity in field application (Montgomery and Peck 1992). Though, a report is available to correlate leaf shape features of different variants of som using regression study (Yadav and Goswami 1992), still LA prediction model for som has not reported yet. Therefore, the aim of the study was to determine the relationships among leaf dimensions and LA in eight morphotypes, to develop common prediction models of LA determination applicable to all som variants, and to validate the most suitable LA prediction model with actual values.

Materials and methods

Eight som morphotypes (presently available) were evaluated at the field of Regional Muga Research Station, Boko (92 m a. s. l.; 25°5’31”N, 91°24′00”E), India in two consecutive seasons coincided with ‘Kotia’ (November 2009) and ‘Chotua’ (March 2010) commercial muga silkworm rearing of Assam, India. Morphotypes were maintained in randomized complete block design with 3 m × 3 m spacing. Number of test plants was 20 with four replications per morphotype. The experiment was conducted between 100th and 120 th day after drastic pruning at 2.8 m height in each season, as the developmental period is most suitable for silkworm rearing. The soil was alluvial (entisol) having sandy clay loam in texture, pH: 7.6, with organic carbon 0.72%, available N: 230 kg ha^{-1}, available P: 30.2 kg ha^{-1} and available K: 210.7 kg ha^{-1} at the time of initiation of the experiment. The recommended doses of inorganic N as urea (87 g plant^{-1}), inorganic phosphorus as P_2O_5 (125 g plant^{-1}) and inorganic potassium as K_2O (33 g plant^{-1}) in two splits after each commercial harvest (June and November) and a solitary dose of farm yard manure 10 kg plant^{-1} during May were applied (Chakravorti *et al*. 2005). From each replication subplot, fully expanded leaves were randomly collected (8 to 15 position from the top) from the primary branch. A total of 1,280 leaves were measured for all the morphotypes. Average of five measurements comprised the value of each replication. LA was determined using leaf area meter (*Delta T Devices*, Cambridge, UK). L and B were measured from the lamina tip to the point of petiole intersection along the midrib and from a point halfway across the lamina length, respectively.

Data were subjected to analysis of variance with morphotype and season as major factors. When *F* values were significant ($P<0.05$), *Fisher*’s least significant differences (LSD) were calculated (Gomez and Gomez 1984). Multiple regression analysis of the data was performed with pooled seasonal data of various subsets of independent variables (L, B, and L×B) using *Statistica 8.0* software (*Statsoft Inc*., Tulsa, USA). Relationships of LA with foliar features were generated with predicted intervals for the mean data in simple regression lines using the same software. The use of two measurements (L and B) often associated with problem of collinearity and resulted into poor precision in the estimates of the corresponding regression coefficients (Cristofori *et al.* 2007). Therefore, collinearity was measured using variance inflation factor (VIF, Marquardt 1970) and tolerance values (T, Gill 1986) as follows:

$$\mathrm{VIF} = 1/(1 - r^2) \quad (1)$$

$$\mathrm{T} = 1/\mathrm{VIF} \quad (2)$$

where *r* is the correlation coefficient. If the VIF value is >10 and T value < 0.10, then it seems the collinearity may have more than a trivial impact on the estimates of parameters, and consequently, one of them needs to be excluded from the model (Fallovo *et al.* 2008).

To validate the best fitted equation, replicated predicted values, obtained from the leaves of randomly selected som morphotypes ($n = 360$), were plotted against actual leaf areas using *Excel* software version *8.0*.

Results and discussion

We used L, B, their squares, and L×B to assess the LA prediction model of som morphotypes. These parameters are widely used for development of LA prediction model for different plants (Uzun and Celik 1999, Gamper 2005, Tsialtas and Maslaris 2008). As the first step of model calibration, we have judged the degree of collinearity among all L and B. Morphotype variations of VIF and T values ranged from 3.08 to 9.09 and 0.11 and 0.32, respectively. In all morphotypes, VIF and T values were < 10 and > 0.1, respectively. It indicated that collinearity between L and B was negligible (Gill 1986) and therefore, both variables may be included in the model. Our result on collinearity measurement is in agreement with previous reports on persimmon (Cristofori *et al.* 2008), rose (Rouphael *et al.* 2010) and hazelnut (Cristofori *et al.* 2007). The leaf shape feature (Fig. 1) is measured as leaf lamina length: width ratio (L/B) in the present study and significant differences ($P<0.05$) were observed among morphotypes (Table 1). The M-1 to M-3 and M-7 possessed grater L/B ratios (mean ranges from 3.3 to 3.3). But, M-4, M-5 and M-6 exhibited lesser leaf shape values (mean range: 1.7–2.8). Significant morphotypes variance was also observed for all foliar parameters measured (Table 2). Morphotype variances were not significant by seasons of data collection. Morphotype × season effect was also nonsignificant for all parameters.

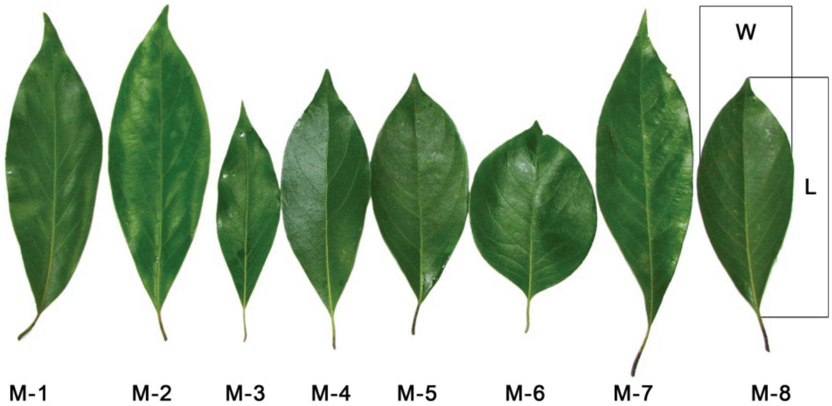

Fig. 1. Leaf shape of eight morphotypes of som (*Persea bombycina*) showing the position of leaf lamina length (L) and width (B) measurement. M-1 to M-8 denote eight morphotypes of som.

Table 1. The leaf shape (length:width ratio; L:B) mean, minimum (min) and maximum (max) values for eight som (*Persia bombycina*) morphotypes. *Standard error in parenthesis. Coefficient of determination (R^2) and mean square error (MSE) of the linear regression between leaf lamina length (L) and width (W).

Morphotype	L:B ratio mean	min	max	R^2	MSE [cm^2]
M-1	3.5 (0.051)*	3.2	4.0	0.75	0.36
M-2	3.4 (0.041)	3.1	3.7	0.84	0.42
M-3	3.8 (0.036)	3.4	3.6	0.92	0.11
M-4	2.7 (0.040)	2.5	2.9	0.69	0.24
M-5	2.8 (0.033)	2.5	3.0	0.81	0.36
M-6	1.7 (0.032)	1.5	1.9	0.79	0.18
M-7	3.3 (0.038)	3.0	3.5	0.82	0.34
M-8	2.4 (0.045)	2.2	2.8	0.78	0.42

Moreover, variance component estimates for morphotypes were greater than estimates for the season and morphotype × season interactions in all parameters. The morphotype M-2 possessed highest LA, L and L×B (Table 3). The mean LA of tested som cultivars was 31.72. The variation of L×B was maximum (3.3 fold) across the morphotypes, than other parameters. Moreover, the trend of grossly identical mean values of studied leaf morphological features of eight morphotypes across seasons (data not shown) and estimated mean sum of squares of seasonal effect (measured by nonsignificant *ANOVA*) indicating that we had obtained consistent results. Besides, the results also indicate that there was a strong genetic, rather than environmental influence in leaf shape features determination in som morphotypes, at least at the time of measurement of data coincided with the stage suitable for silkworm rearing. Our results are in congruence with the findings of Tsialtas *et al.* (2008), where leaf morphology was not affected by the growing season in grapevine leaves.

Table 2. Analysis of variance for leaf lamina length (L), lamina width (B) lamina length × width (L×B), and leaf area (LA) of eight som (*Persia bombycina*) morphotypes at field environment of Boko, Assam, India. Data are pooled values based on 15 observations each of two consecutive seasons (November 2009 and March 2010). cv % – coefficient of variation; df – degree of freedom; ^{ns}, *, ** and *** indicate nonsignificant, significant at $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

Source	Mean squares df	L [cm]	B [cm]	L×B [cm^2]	LA [cm^2]
Morphotype	7	$1,777.1^{***}$	154.8^{**}	$6,224.5^{**}$	$13,808.3^{***}$
Season	1	0.104^{ns}	0.13^{ns}	116.1^{ns}	24.4^{ns}
Season × morphotype	7	0.356^{ns}	0.08^{ns}	82.1^{ns}	22.6^{ns}
cv%		11.8	8.4	14.7	12.8

Like many of the previous nondestructive leaf area prediction model development reports (Esner and Jubb 1988, Demirsoy *et al*. 2005, Tsialtas and Maslaris 2007), we have utilized multiple regression analysis for the development of leaf area prediction model for som. As simple linear relationships between LA and other foliar dimensions are preferable for easy use and rapid measurement (Lu *et al*. 2004), we have emphasized more on the establishment of linear model, rather than on the generation of extra accuracy with complex functions. Moreover, our priority was the development of a generalized model suitable for all available morphotypes of som for wider acceptance with reasonably high accuracy. Further, leaf dimension squares (like L^2 and B^2) often used to increase accuracy of linear models for LA predictions (Smith and Kliewer 1984, Montero *et al*. 2000). Therefore, we have included these two features in the study.

The regression analysis showed that (Table 4) most of the variation in LA values could be explained by L, B, their squares (L^2 and B^2), or product (L×B). LA was significantly related with L, L^2, B, B^2, and L×B. However,

Table 3. Mean values of leaf lamina length (L), lamina width (B), lamina length × width (L×B) and leaf area (LA) of eight som (*Persea bombycina*) morphotypes at field environment of Boko, Assam, India. Data are pooled values based on 15 observations each of two consecutive seasons (November 2009 and March 2010). LSD – least significant difference.

Morphotype	L [cm]	B [cm]	L×B [cm^2]	LA [cm^2]
M-1	15.70	4.38	60.03	35.23
M-2	17.01	4.96	84.88	43.35
M-3	9.86	2.58	25.61	18.66
M-4	11.88	4.37	52.18	27.06
M-5	10.80	3.85	41.65	26.79
M-6	9.43	5.53	52.29	37.24
M-7	15.10	4.58	68.78	38.30
M-8	11.04	4.59	50.72	27.27
Mean	12.59	4.36	55.64	31.72
LSD $_{(0.05)}$	1.27	0.41	10.4	6.27

relationship of LA with L and L^2 showed low R^2 values (range: 0.492 to 0.525) and higher standard error of estimates (range: 18.06 to20.27). Therefore, generated data using L and L^2 could not be used for reliable LA prediction model development. B and its square B^2 were relatively good predictor of LA (Table 3) than L dimensions, as the former features showed better linear relationship with LA (y = 2.984 + 7.966 x, R^2 = 0.612, $P \leq 0.001$, n = 119; y = 12.784 + 0.960 x, R^2 = 0.645, $P \leq 0.001$, n = 119, respectively). However, maximum accuracy of LA prediction was achieved through a simple linear relation with L×B (y = 8.220 + 0.422 x , R^2 = 0.843, $P \leq 0.0001$, n = 119; Fig. 2). The strong relationship between LA with LxB found in the study is in agreement with those reported in Zucchini (Rouphael *et al.* 2006), hazelnut (Cristofori *et al.* 2007) and beet (Tsialtas and Maslaris 2008). However, the accuracy of prediction models of LA was much higher ($R^2 \geq 0.91$, $P \leq 0.00001$),

Table 4. Regression components and leaf area prediction models of som (*Persea bombycina*) developed by using various leaf dimension parameters. LA – leaf area, L – leaf lamina length, L^2 – square of L, B – leaf lamina width, B^2 – square of B and L×B – lamina length × width. df for all estimations was 119.

Variable Dependent	Independent	Linear relationship y = a + bx	Standard error of estimation	R^2	$P \leq F$
LA [cm^2]	L [cm]	y = 4.597 + 2.1542 x	20.27	0.492	0.05
LA [cm^2]	L^2 [cm^2]	y = 17.839 + 0.083 x	18.06	0.525	0.001
LA [cm^2]	B [cm]	y = 2.984 + 7.966 x	6.46	0.612	0.001
LA [cm^2]	B^2 [cm^2]	y = 12.784 + 0.96 x	5.53	0.645	0.001
LA [cm^2]	L×B [cm^2]	y = 8.220 + 0.422 x	3.85	0.843	0.0001

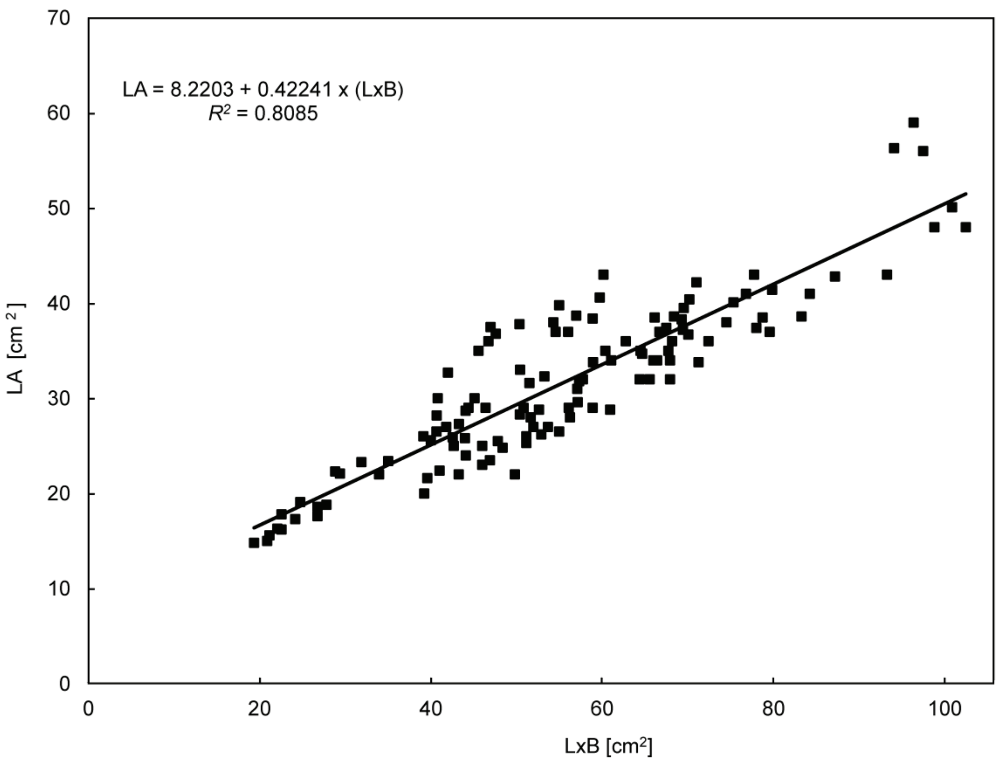

Fig. 2. Best fitted linear regression lines showing the relationship of leaf area (LA) with lamina length × width (L×B) of pooled values of eight som (*Persea bombycina*) morphotypes at field environment of Boko, Assam, India.

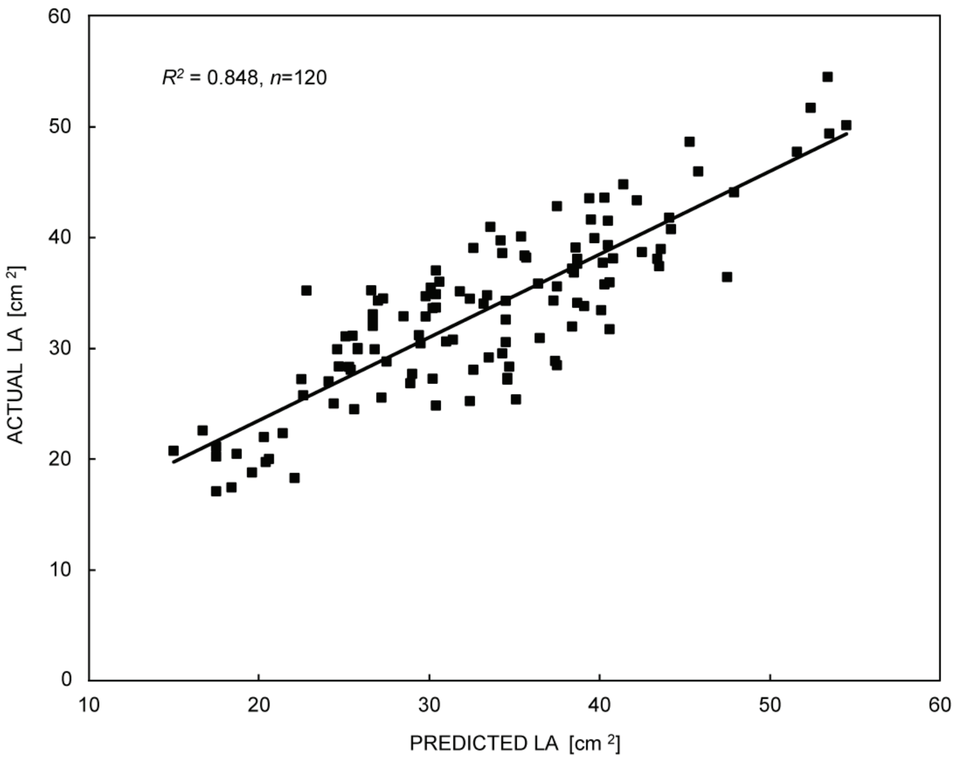

Fig. 3. The relationship between actual leaf area (LA) and predicted LA of pooled values of eight som (*Persea bombycina*) morphotypes.

when the linear relationship was calculated on the individual morphotype based values of L×B or the quadratic function equation deduced from the pooled values of L×B of all morphotypes (data not shown).

Though plethora of leaf area prediction models are available in different plants, still information on the validation of developed model is limited (Cemek *et al* 2011, Serdar and Demirsoy 2006, Tsialtas *et al.* 2008). We have validated our model by comparing the relation of actual and predicted LA values according to Celik and Uzun (2002) and Demirsoy *et al.* (2005). A comparison was carried out between the actual leaf areas, randomly selected from all eight morphotypes by using leaf area meter and predicted areas of those leaves deduced from the developed best fitted simple linear model using L×B values (Fig. 3). The relationship showed high value of coefficient of determination (R^2 = 0.848, $P \leq 0.001$).

Conclusions: Our findings, for the first time, showed LA prediction model based on the linear relationship of L×B that could be used in the comparison of som morphotypes for various filed applications. Moreover, as the leaf lamina length and width are the dimensions that can be easily measurable nondestructively, the developed model could be used for rapid, accurate and repeated estimation of LA in large quantities of som trees without using any expensive instruments.

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