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A simple linear model for leaf area estimation in Persian walnut (*Juglans regia* L.)

I. Keramatlou[a,1], M. Sharifani[b,*], H. Sabouri[c,2], M. Alizadeh[a,1], B. Kamkar[d,3]

[a] Department of Horticultural Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan 49189-43464, Iran
[b] Department of Horticultural Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Basij SQ., Gorgan 49138-43464, Golestan, Iran
[c] Department of Plant Production, Gonbad Kavous University, Gonbad-e Kavus 49717-99151, Iran
[d] Department of Agronomy Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Basij SQ., Gorgan 49138-43464, Iran

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ABSTRACT

Accurate non-destructive leaf area estimation is a useful subject of study for the fields of applied plant science, physiology and plant genetic engineering. The relationship between leaf area and fruit is very important in fruit crops. It determines the nut size and nut filling potential. The aim of this paper is to produce a statistical model based on linear measurements such as leaflet length and width in combination with other simple parameters. Measurements of leaf length, width and surface area of the leaflets were achieved using digital photography. Digital images of Persian walnut (*Juglans regia* L.) leaves were prepared in August 2011 for 14 genotypes and in 2012 for one genotype under open field conditions. Results of regression analysis indicated several models are suitable to estimate leaf area of Persian walnut. The linear model with LW as an independent variable (LA = 1.11 + 0.69LW) with accurate estimation (maximum R^2 = 0.99 and lowest MSE = 10.09) was the best model. To determine the leaf area, the earliest measurements to take would be the length and width of leaflets. Total area of the leaflets is then reported as leaf area. In conclusion, in walnut, number of leaflets and leaflet area should be involved in the estimation of leaf area.

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1. Introduction

Leaf area (LA) is a measurable trait indicating status of growth and development in plants. In addition, this variable reveals some physiological characters of fruit trees. These include light interception, photosynthetic efficiency, respiration, evaporation, transpiration, fruit set, water balance and response to fertilizers (Syvertsen et al., 2003). Thus, in turn LA determination could enable researchers to clarify attributes of fruit yield and quality. Studies in several fruit crops indicated the close relationship of leaf surface area with fruit and nut quality (Marquard, 1987; Santesteban and Royo, 2006; Torri et al., 2009) fruit maturity (Usenik et al., 2008) fruit weight in chestnut and kiwifruit (Famiani et al., 2000). Further, the prediction of leaf area is extended to different crops such as mango (Ghoreishi et al., 2012) and pomegranate (Meshram et al., 2012). The relationship between LA and fruit set stated as determiner tool for the elucidating nut size and filling in pecans, (Torri et al., 2009). In the cultivar 'Mohawk', it has been observed that a leaf:fruit ratio of 4, equivalent to a LA of 1150 cm^2, produced better quality nuts than a leaf:fruit ratio of 2. Furthermore, in the cultivar 'Western', a leaf:fruit ratio of 2, equivalent to an LA of 575 cm^2, was needed for nut filling (Marquard, 1987).

(Lampinen et al., 2011) showed that previous year spur leaf area was strongly related to spur viability and its flowering; specifically, greater leaf area in one year led to the higher probability of spurs survival into the next year. Therefore, higher leaf area caused higher probability for the spurs to bear more flowers. Ratio of LA to fruit mass ratio determined the time of veraison in Sauvignon Blanc and Pinot Noir grapevines (Parker et al., 2014). Finally, LA is essential to evaluate vegetative growth and to estimate crop production potential.

Leaf area can be measured by destructive as well as non-destructive techniques. Many methods have been devised to facilitate the measurement of leaf area. These methods included drawing, blueprinting, photographing, image analysis and use

* Corresponding author. Tel.: +98 1732423303.
E-mail addresses: iskaramatlou@gmail.com (I. Keramatlou), mmsharif2@gmail.com (M. Sharifani), saboriho@yahoo.com (H. Sabouri), behnamkamkar@yahoo.com (B. Kamkar).
[1] Tel.: +98 9112740812.
[2] Tel.: +98 172 2237508.
[3] Tel.: +98 1732427060.

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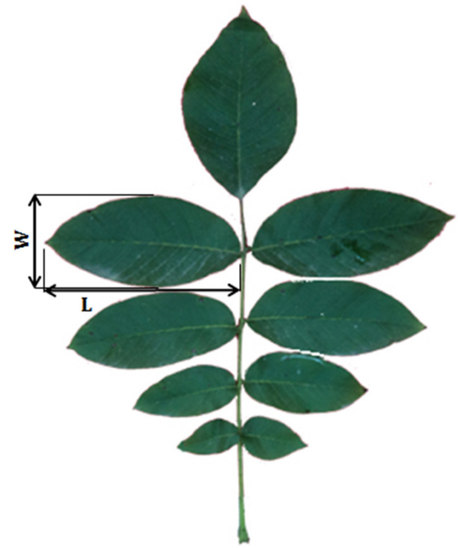

Fig. 1. Indicating measurements of Persian walnut leaflet length (L) and width (W).

of a conventional planimeter or an electronic leaf area meter. However, these methods mostly require the excision of leaves from the plant. In addition, the processing is time-consuming and the equipment required to perform such measurements is generally expensive. Therefore, another simple, inexpensive, rapid, reliable and non-destructive method for estimating leaf area is required for experiments and researchers.

A common approach for leaf area estimation is to develop ratios and regression estimators by using easily measured leaf parameters such as leaf length and width (Schwarz and Kläring, 2001). Various combinations of measurements and various models relating length and width to area have been developed for many horticultural crops such as grape (Williams and Martinson, 2003) cherry (Demirsoy and Demirsoy, 2003), peach (Demirsoy et al., 2004), strawberry (Demirsoy et al., 2005), hazelnut (Cristofori et al., 2007), small fruits (Fallovo et al., 2008), pecan (Torri et al., 2009), Iranian table grapes (Eftekhari et al., 2011) and rose (Gao et al., 2012), while a suitable method to quickly estimate leaf area is still lacking for Persian walnut (*Juglans regia* L.), which has compound leaves, normally consisting of opposite leaflets on a central rib (rachis) and one terminal leaflet resulting in an odd number of leaflets per leaf (Fig. 1).

The accuracy of the predictions depends on the variation of leaflet shape among genotypes. Since leaflet shape (length:width ratio) may vary among different genetic provenances, a reliable model of non-destructive estimation of leaf area is required. Such a model may be utilized as a tool in physiological studies of Persian walnut leaves independently of the genetic materials. Therefore, the aims of this research were to: (1) design and evaluate a number of regression models to estimate walnut LA by non-destructive methods; and (2) to assess the reliability of the model on an independent set of data from multiple genotypes grown under different environmental conditions.

2. Materials and methods

The present study was performed in a Persian walnut orchard located at Golestan province, Minoudasht region, Iran (latitude 37°04′N; longitude 55°32′E; altitude 1060 m) in a silt loam soil type. The annual mean temperature was 16.3 °C, with annual mean rainfall of 690 mm.

2.1. Data collection

Fourteen Persian walnut (*J. regia* L.) accessions propagated through seed were selected and used to develop a leaf area prediction model. Genotypes were coded as G1 to G14. These accessions were selected as a representative sampling of the many walnut genotypes available throughout the Northeastern Iran. Sampling was performed in early August when the leaves were fully developed. The walnut trees used within the experiment had ages of 20 years old. Peripheral leaves from the middle portion of shoots that developed during the same growing season were harvested. Leaves were selected randomly from different levels of the tree canopy ranging from 2 to 3 m from the soil level and all around the crown. Ten leaves were sampled from each accession, each with 7 to 11 leaflets broad elliptic form with entire margin. According to Torri et al., 2009, leaves were kept refrigerated in plastic bags at 4 °C for 24 h until the determinations were made. An image was captured where a sample was placed on a fixed white background along with a ruler, using a digital camera (Sony Cyber-Shot DSC-W290 with a resolution of 12.1 megapixel; Sony, Tokyo). Then the width (W) was measured in the medium part of the leaflet and the length (L) was determined from the base to the apex of the leaflet lamina (Fig. 1) using a Digimizer image analysis software (version 4). The area of each leaflet (LA in cm^2) was measured using the same software. In total, 1124 walnut leaflets from 14 accessions were measured for leaflet L and W and LA parameters.

2.2. Statistical methodology

The dependent variable (LA) was regressed with different independent variables, including L, W, L^2, W^2, $(L+W)^2$ and the product $L \times W$. Root mean squared error (RMSE) and the values of the constants (a) and coefficients (b) were also reported, and the final model was selected based on the combination of the highest coefficient of determination (R^2) and the lowest RMSE. Moreover, using two measurements (i.e., length and width) introduced potential problems of co-linearity, resulting in poor precision in the estimates of the corresponding regression coefficients. For detecting co-linearity, the variance inflation factor (VIF) (Marquaridt, 1970) and the tolerance values (T), were calculated (Gill, 1986).

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

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

where r is the correlation coefficient between length and width of leaflet. If the VIF value was higher than 10 or if T value was smaller than 0.10 then co-linearity may have more than a trivial impact on the estimates of the parameters, and consequently one of those should be excluded from the model (Gill, 1986).

2.3. Model validation

In order to validate the developed model and to increase practical applicability in different environmental conditions, a validation experiment was conducted in the summer 2012 on leaflet samples of G_{15} genotype grown at the Experimental Farm of Minoudasht Agriculture Office (latitude 37°13′N, longitude 55°22′E, altitude 211 m). To validate the model, 136 leaflets of the G15 genotype were used to determine leaf width, length as well as leaf area by the previously described procedures. Leaf area of individual leaflets was predicted using the best model from the calibration experiment and was compared with the actual leaf area. Moreover, to compare the predicted leaf area (PLA) to the observed leaf area (OLA) for the genotype G15 during 2012 growing season, graphical procedures (Martin Bland and Altman, 1986) were used. Scatter

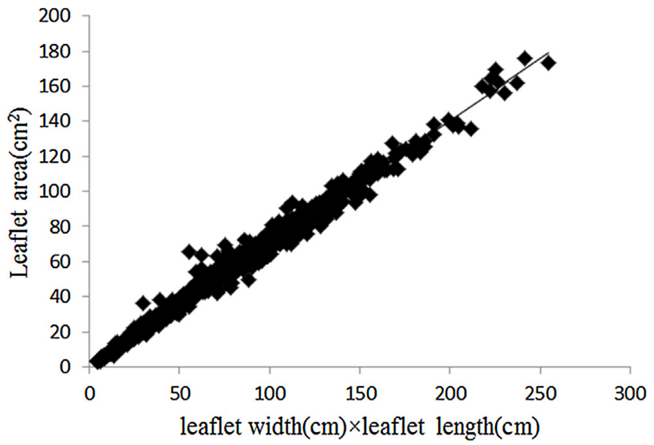

Fig. 2. Relation between leaflet area and leaflet length × leaflet width of single leaflets from 14 Persian walnut genotypes measured in the calibration experiment held by 2011. The equation for the regression line is LA = 1.11 + 0.69LW.

plots of values for the PLA against the OLA are presented in Fig. 2. SPSS was used to evaluate the linear relationship for OLA and PLA of G15.

3. Results and discussion

As a preliminary step to model calibration, the degree of co-linearity among leaf width and length was analyzed. The VIF ranged from 5.2 to 7.4 and *T* values ranged from 0.13 to 0.21, depending on genotypes. In all genotypes, VIF was <10 and *T* was >0.10, showing that the co-linearity between length and width can be considered negligible and these variables can be both included in the model (Gill, 1986).

3.1. Model parameterization

Regression analysis demonstrated a strong relationship ($P < 0.001$) between leaf area (LA) and mid vein length (L), leaf width (W), the product of length and width (LW), the square of the sum of length and width $(L + W)^2$, the square of length (L^2) and the square of width (W^2) (Table 1). This is in agreement with previous studies (Cristofori et al., 2007; Mendoza-de Gyves et al., 2007; Peksen, 2007; Rouphael et al., 2007); on non-destructive models for predicting leaf area using measurement of leaf length and width. However, suitability of these models varied based on the selection criteria previously described. Except for model 1, all models produced a coefficient of determination (R^2) equal to or greater than 0.90 (Table 1). Based on selection criteria previously described (higher R^2, lower MSE), this study demonstrated that models with a sole measurement of leaf length (models 1) were less acceptable for estimating leaf area, due to their lowest coefficient of determination (R^2), higher MSE values. To find a model to predict leaf area accurately for plants of all genotypes the product of leaf length × leaf width was used as an independent variable (model 3). We preferred this model (LA = 1.11 + 0.69LW) for its accuracy: highest R^2 (0.99), smallest MSE (10.1).

Based on the above considerations, both leaflet length and width measurements were necessary to estimate Persian walnut leaf area accurately. The results reported in the present research were in accordance with previous studies on some species of fruit trees such as hazelnut (Cristofori et al., 2007), apple (Palmer, 1987), and kiwifruit (Mendoza-de Gyves et al., 2007), where the leaf area estimation models were developed using the multiplicative equation (length × width).

The shape coefficient (regression coefficient 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 (0.69) agreed closely with those calculated for other crops. Values of 0.74 have been reported for hazelnut (Cristofori et al., 2007) and 0.59 for grapevine (Montero et al., 2000). The applied model analyzed the possible genotype differences among the samples. Regression coefficients of the genotypes were slightly different (data not shown). However, when an equation derived for a single cultivar versus the overall model was compared, no significant differences were found. These results suggest that a universal leaf area estimation model for Persian walnut is plausible, unless other genotypes differ greatly in leaf morphology from those used in this experiment.

3.2. Model evaluation

Comparisons between observed leaflet area (OLA) versus predicted leaflet area (PLA) using model 3 (LA = 1.11 + 0.69LW) for the validation set were derived from 2012 experiment on G15 genotype. The results indicated a close correlation ($r = 0.99$, $P < 0.0001$), between OLA and PLA. Furthermore, the PLA data were very close to the OLA values (Fig. 3.).

Finally, it may be concluded that the length–width model can provide more accurate estimations of Persian walnut leaf area across genotypes and environments than those based on single length or width measurement. Leaf width and mid vein length are easy access parameters in field, greenhouse and pot experiments. Therefore use of this equation would enable researchers to make non-destructive or repeated measurements on the same leaves. Such a model may be accurately utilized to estimate the leaf area of walnut trees without the necessity of application of any expensive instruments, e.g., a leaf area planimeter or digital camera or image measurement software.

Further determination of total leaf area in different periods of Persian walnut growth possibly can reveal the status of water balance, potential of fruit set, fruit maturity, kernel quality and rate of photosynthesis. "In addition, there is a need to develop an instrument with equipped with software that can provide projections of

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

Model no.	Form of model tested	Fitted coefficient and constant		R^2 [b]	RMSE
		a	b		
1	LA = a + b (L)	−29.5(0.87) [a]	6.93 (0.07)	0.88	115
2	LA = a + b (W)	−34.5 (0.60)	15.6 (0.10)	0.94	51.9
3	LA = a + b (LW)	1.11 (0.16)	0.69 (0.002)	0.99	10.1
4	LA = a + b $(L + W)^2$	0.72 (0.22)	0.15 (0.0006)	0.98	18.0
5	LA = a + b (L^2)	1.99 (0.45)	0.32 (0.002)	0.92	75.1
6	LA = a + b (W^2)	5.87 (0.42)	1.30 (0.01)	0.92	72.0

[a] Standard errors in parenthesis; L and W were in cm.
[b] Coefficient of determination (R^2), mean square errors (MSE in cm^2) of the various models are also given. All data were derived from the calibration experiment held by 2011 (n = 1124 leaves).

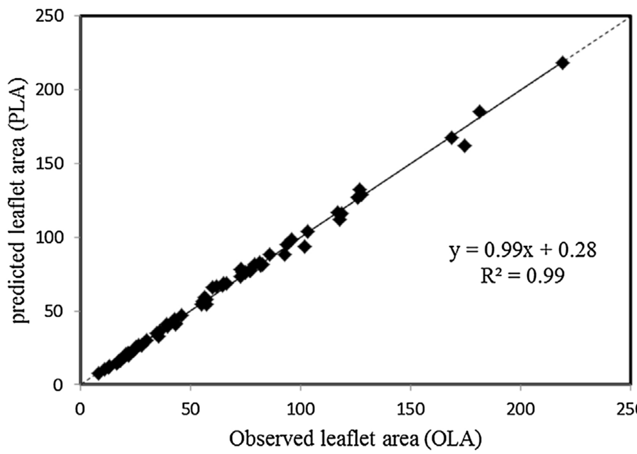

Fig. 3. Observed leaflet area (OLA) versus predicted leaflet area (PLA), during 2012 (validation experiment) using model 3, LA = 1.11 + 0.69LW, LA is individual leaf area (cm^2) and LW is product of leaflet length (cm) × leaflet width (cm). Solid line represents linear regression lines of Model 3. Dotted lines represent the 1:1 relationship between the OLA and PLA.

expected future trends of fruit development based on calculated leaf areas."

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