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Estimation of Leaf Growth on the Basis of Measurements of Leaf Lengths and Widths, Choosing Pistachio Seedlings as Model

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Abstract: Measurement or estimation of leaf growth indices is essential for understanding crop responses to experimental treatments. The objective of this study was to develop regression models for estimating leaf area (LA), fresh weight (FW), and dry weight (DW) of potted-grown pistachio (*Pistacia vera* L. 'Badami') from measurements of leaf length (L) and width (W). Leaf length and width were measured at three month after bud break. Measured leaf length and width ranged from 9.4 to 11.3 and 2.3 to 6 cm respectively. Linear regression equations were fitted and evaluated using alternatively the length, the width and their product as independent variables. Regression using L×W variable fitted the data better. It found that the linear functions give the best fit in terms of R^2 and root mean square of error (RMSE). Leaf length × width provided good estimation of leaf area, fresh weight, and dry weight of leaves of pistachio seedlings. However, leaf length and width solely were not good estimation of leaf growth indices. It was concluded that leaf area, fresh weight, and dry weight of leaves of pistachio seedlings can be estimated or simulated as a linear function of L×W with reasonable accuracy. Also we found a reasonable relationship between leaf dry weight and leaf area.

Key words: Leaf area estimation, Leaf growth estimation, Modeling, Non-destructive methods, *Pistacia vera* L.

INTRODUCTION

Pistachio (*Pistacia vera* L.) is one of the most important commercial trees grown in Iran, Turkey, and recently in the USA. There are many cultivars of pistachio in Iran and some of theme use for rootstock. Pistachio 'Badami' is one of the most common cultivars that uses as pistachio rootstock in Iran. Rootstocks have important roles on growth and performance of trees; so they are the subject of many physiological studies. Leaf is an important plant organ, and is associated with photosynthesis and evapotranspiration; therefore, leaf growth measurements are required in most physiological and agronomic studies involving plant growth (Guo and Sun, 2001). Growth rate of pistachio seedlings is slow and number of leaves produced by seedlings is limited; therefore these characteristics act as restricting factors in measurement of leaf growth indices (leaf area, fresh weight, and dry weight) that often requires in physiological studies. Moreover, measurement of these factors is potentially time-consuming and costly processes.

Montgomery (1911) first suggested that leaf area of a plant can be calculated from linear measurement of leaves using a general relationship A=b×L×W where b is a coefficient. Such a mathematical equation for estimating leaf area reduces sampling effort and cost, and may increase precision where sample of leaf size are difficult to handle. The non-destructive methods based on linear measurements are quicker and easier to be executed and present good precision for the study of plant growth in several crops (Manivel and Weaver, 1974; Sepaskhah, 1977; Strik and Proctor, 1985; Pedro Júnior *et al.,* 1986; Robbins and Pharr, 1987; Silva *et al.,* 1998; Gutiérrez and Lavín, 2000; Astegiano *et al.,* 2001; Guo and Sun, 2001); however, there are no prediction equations for *Pistacia vera* (L.) for estimation of leaf area and other leaf growth indices through nondestructive method.

The objective of this study was to develop a non-destructive methodology for estimating the leaf area, fresh weight, and dry weight for pistachio seedlings, based on linear measurement models.

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MATERIALS AND METHODS

The experiment was conducted at Shiraz, Fars, Iran in spring 2008. Shiraz is situated at 29°37 N 52°32 E. The ecoclimate of Shiraz is subtropical. Seeds of *Pistacia vera* (L.) 'Badami' were brought from Rafsanjan and planted in 25×50 plastic bags containing 16 kg fertile soil. Sampling was done at 90 days after beginning of second season of growth and 300 leaves harvested and brought to the laboratory of Department of Horticultural Science of Shiraz University.

The maximum length and width of all leaves was measured by a ruler. Leaf area measured by portable leaf-areameter model (Area Meter AM200 – ADC Bioscientific). The length (without petiole) and width of leaves were measured to the nearest 1 mm (Figure 1) and the area to the nearest 1.0 mm^2. Fresh weight of leaves also measured, and then for measuring the dry weight, leaves placed in 70°C oven for 48 hour. The fresh and dry weight of leaves was measured to the nearest 0.001 g.

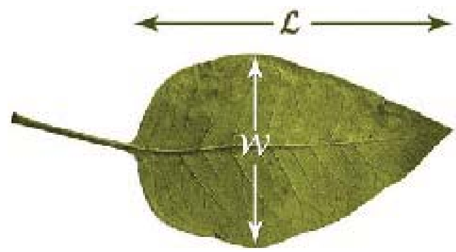

Fig. 1: The picture presents how leaf length (L) and width (W) is measured.

Different regression models were examined for describing (L), (W), and (L×W) relationships to leaf area (LA), dry weight (DW), and fresh weight (FW) of leaves. Two-thirds of the data (200 cases) were allocated by random sampling to the modeling set and one-third (100 cases) to the validation set. The modeling set was employed to examine the linear and non-linear relationships between length and width of leaves and their growth indices (LA, DW, and FW) using correlation analysis, and linear and non-linear regression analysis. The best model was selected based on coefficient of determination (R^2) and root mean square of error (RMSE). The estimation errors were studied by the validation set. RMSE of estimation was calculated as:

$$RMSE = [\Sigma(P-O)^2/(n-1)]^{0.5}$$

Where *P* is the predicted leaf area, *O* is the measured leaf area and n is the number of observation. Regression analysis was carried out using SPSS 16.0 software for windows and Microsoft Excel 2003.

RESULTS AND DISCUSSION

Relationship of Leaf Length and Width to Leaf Area:

Leaf width and length ranged from 2.3 to 6.0 and 3.4 to 11.8 cm respectively, and leaf area extend between 730 to 5165 mm^2. There found no good correlation between W and LA or L and LA (Figure 2); but the linear function described well relationship between L×W and leaf area (Table 1; Figure 3A). Minimum RMSE values obtained were 124.04mm^2 for linear equation and 123.94mm^2 for cubic equation of its corresponding means of leaf area (Table 1). R^2 values for the equation was high (0.98). Therefore, two general equation ($LA = 76.97LW + 35.985$; $R^2 = 0.9816$; RMSE = 124.04mm^2 ~ 5.9% of mean of data) or ($LA = -0.0017LW^3 + 0.1746LW^2 + 71.786LW + 79.966$; $R^2 = 0.9816$; RMSE = 123.94mm^2 ~ 5.9% of mean of data) can be suggested.

Relationship of Leaf Length and Width to Fresh and Dry Weight of Leaves:

Leaf fresh and dry weight varied from 0.37 to 1.36 and 0.13 to 0.59 g respectively. There were not good relationship between W and LA or L and these factors; but the linear functions described well relationship between L×W and FW and DW of leaves. Figure 3B and C shows leaf fresh and dry weight plotted as a function of L×W. R^2 values for fresh weight were greater than 0.94, while they were more than 0.97 for dry weight. The lowest RMSE values obtained for fresh weight, were about 0.05 g (9.4% of the mean), indicating that the relationships are appropriate (Table 2). Based on the R^2 and RMSE values, the linear equation (FW

= 0.0186LW - 0.0037, R^2 = 0.945; RMSE = 0.05g ~ 9.4% of mean of data) and the power equation (FW = $0.0166LW^{1.0287}$, R^2 = 0.936; RMSE = 0.05g ~ 9.4% of mean of data) are suggested as more accurate correlations between L×W and fresh weight (FW).

The lowest RMSE value obtained for dry weight was about 0.010 g (8.01% of the mean), in both linear and polynomial order 2 equations (Table 3). R^2 and RMSE values for these equations are very close.

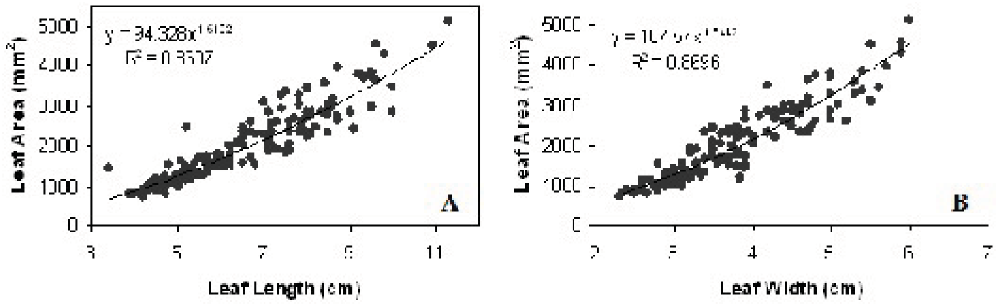

Fig. 2: There found no reasonable correspondence between leaf length (A) and/or leaf width (B) and leaf area.

Table 1: Correlation between L×W and leaf area.

Equation type	Equation	R^2	RMSE
Power	LA = $83.143LW^{0.9818}$	0.981	124.13
Order 4	LA = $-0.0004LW^4 + 0.0604LW^3 - 2.8973LW^2 + 132.44LW - 318.22$	0.981	147.86
Order 3	LA = $-0.0017LW^3 + 0.1746LW^2 + 71.786LW + 79.966$	0.981	123.94
Order 2	LA = $-0.0085LW^2 + 77.503LW + 28.988$	0.981	124.12
Linear	LA = 76.97LW + 35.985	0.981	124.04

Table 1. Order 3 and linear function explain the best connection between LA and L×W. Coefficient of determination (R^2) of these functions are high (98.16%) and root mean square of error (RMSE) are low (5.43%) of mean of leaf area data.

Table 2: Correlation between L×W and leaf fresh weight.

Equation type	Equation	R^2	RMSE
Power	FW = $0.0166LW^{1.0287}$	0.936	0.05
Order 5	FW = $5E\text{-}10LW^5 - 1E\text{-}08LW^4 - 2E\text{-}06LW^3 + 6E\text{-}05LW^2 + 0.02LW - 0.0318$	0.941	0.06
Order 4	FW = $3E\text{-}06LW^3 - 0.0003LW^2 + 0.0289LW - 0.094$	0.941	0.06
Order 3	FW = $8E\text{-}08\ LW^4 - 9E\text{-}06\ LW^3 + 0.0003\ LW^2 + 0.017\ LW - 0.0158$	0.941	0.06
Linear	FW = 0.0186LW - 0.0037	0.945	0.05

Table 2. The best correlation between L×W and leaf fresh weight, obtained by the linear and power equations. Coefficient of determination (R^2) of these functions are high (~94%) and root mean square of errors (RMSE) are low (9.4%) of mean of leaf fresh weight data.

Table 3: Correlation between L×W and leaf dry weight.

Equation type	Equation	R^2	RMSE
Power	DW = $0.0058LW^{1.0653}$	0.962	0.015
Order 4	DW = $-4E\text{-}08LW^4 + 5E\text{-}06LW^3 - 0.0002LW^2 + 0.0118LW - 0.0344$	0.969	0.030
Order 3	DW = $-2E\text{-}07LW^3 + 3E\text{-}05LW^2 + 0.0067LW - 0.0013$	0.961	0.016
Order 2	DW = $2E\text{-}06\ LW^2 + 0.0075LW - 0.0082$	0.970	0.010
Linear	DW = 0.0076LW - 0.0095	0.971	0.010

Table 3. The best linkage between leaf dry weight and L×W found by linear and polynomial order 2 functions. Coefficient of determination (R^2) of these functions are high (97.10%) and root mean square of errors (RMSE) are low (8.01%) of mean of leaf dry weight data.

Relationship of Leaf Dry Weight to Leaf Area:

Equations using dry weight (DW) had strong relationships with LA, manifested in high coefficients of determination (R^2) of the equations and low standard error of estimates (Table 4). There found a good correlation between the dry weight of leaves and leaf area. Linear function described the best relationship between the two factors (LA =9620.3DW + 96.8; R^2 = 0.984; RMSE = 140.50 mm^2 ~ 6.4% of mean) (Figure 3D). This equation had the highest R^2 and the lowest RMSE values among the other equations.

Model Evaluation:

In order to validate the suggested equations, data of 100 leaves that were not involved in modeling set were analyzed (validation set). For independent data, measured leaf area varied between 679 and 3632 mm^2 with a mean of 1540.33 mm^2. Dry weight of validation set ranged between 0.069 and 0.387 g and mean of the data was 0.152 g. Fresh weight of this set varied from 0.14 to 0.92 g and the mean was 0.36 g.

Precision of elected equations evaluated using validation set data to recommend the best model to predict the leaf growth indexes. Predicted values of different leaf growth indexes by the general regression models are presented in Figure 4 (as red line) versus the measured data from leaves. Results showed that linear equations provided the best estimates of leaf area, fresh weight, dry weight and leaf area respectively (Figure 4). RMSE of estimations were very low and reasonable (Table 5), therefore, the relationships appear to be valid and exact.

Table 4: Correlation between leaf dry weight and leaf area.

Equation type	Equation	R^2	RMSE
Power	$LA = 9357.2DW^{0.9072}$	0.97	145.60
Order 4	$LA = -98405\ DW^4 + 96111\ DW^3 - 30721\ DW^2 + 13701\ DW + 24.235$	0.97	141.16
Order 3	$LA = -9129.9\ DW^3 + 7399.3\ DW^2 + 8243.8\ DW + 279.82$	0.97	141.94
Order 2	$LA = 257.46\ DW^2 + 9867.1\ DW + 176.67$	0.97	142.44
Linear	$LA = 9620.3\ DW + 96.8$	0.98	140.50

Table 4. Linear function explains the best relationship between leaf dry weight (g) and leaf area (mm^2). Coefficient of determination (R^2) of this function is high (98.45%) and root mean square of error (RMSE) is low (6.47%) of mean of leaf area data.

Table 5: RMSE and RMSE:Mean% values for predicted data.

Relationship	Regression Model	RMSE	RMSE:Mean %
Leaf Area - LW	LA = 76.97LW + 35.985	138.46 mm^2	8.93
Fresh Weight - LW	FW = 0.0186LW - 0.0037	0.042 g	11.59
Dry Weight - LW	DW = 0.0076LW - 0.0095	0.019 g	12.64
Leaf Area - Dry Weight	LA = 9620.3DW + 96.8	143.99 mm^2	9.43

Table 5. The best equations selected as model to predict leaf growth indexes of pistachio. RMSE and RMSE/MEAN% values for predicted data calculated based on data obtained from linear models and directly measured data. The table shows the accuracy and reliability of models.

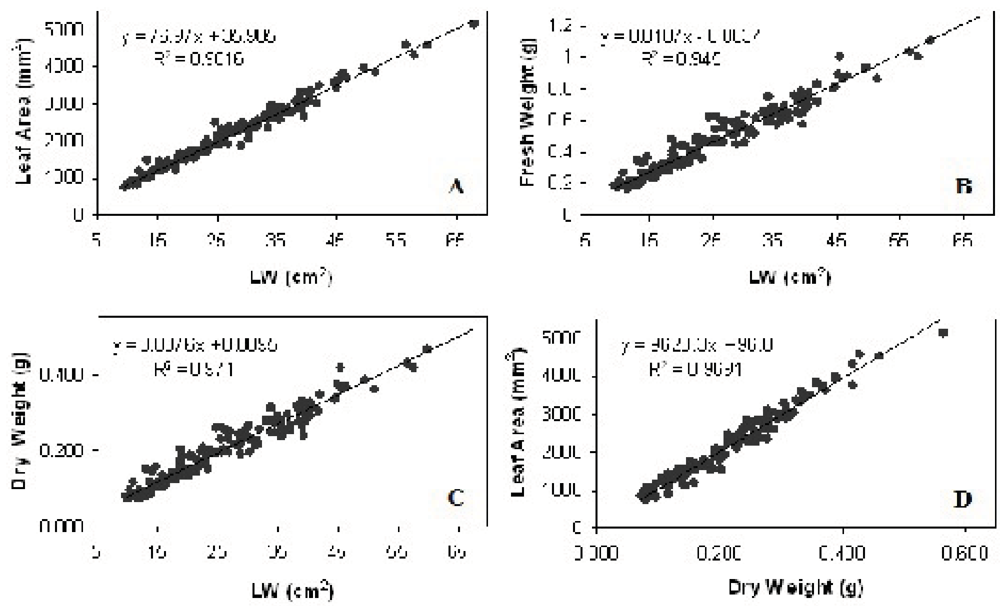

Fig. 3: Relationship of (A) leaf area (mm2), (B) fresh weight (g), and (C) dry weight (g) to L×W (cm2), and (D) Linkage between leaf area (mm2) and leaf dry weight (g); described by the linear function.

Discussion:

Many researches have been carried out to estimate leaf area using leaf length and width (Robbins and Pharr, 1987; Gamiely *et al.,* 1991; Montero, *et al.,* 2000; Williams Iii and Martinson, 2003). In the present study regression equations were fitted using the length, and the width of leaves and the product of them as independent variables to estimate leaf area and the other leaf growth indices. Leaf length or width solely did not provide a good variable to estimate leaf growth indices, 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 and or leaf weight.

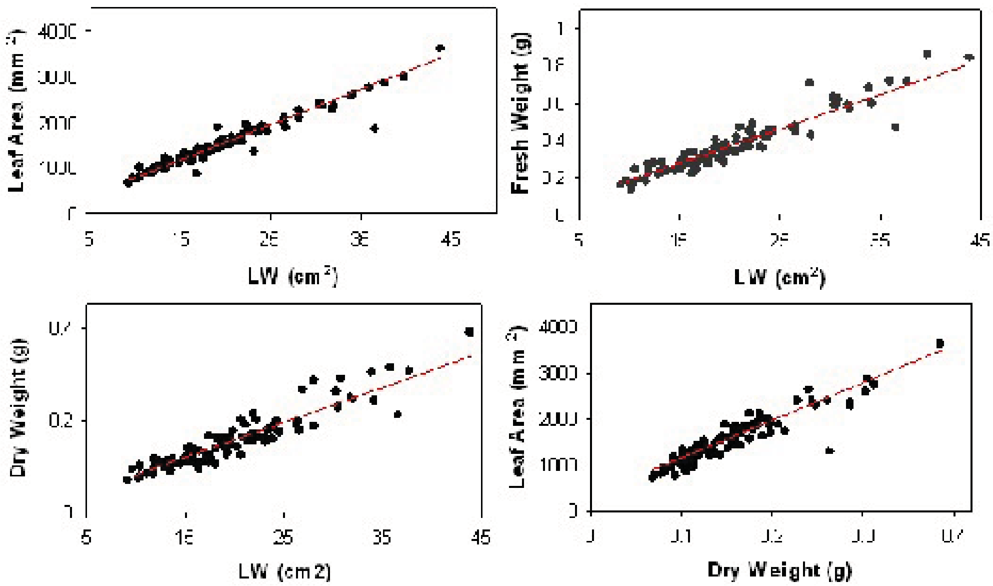

Fig. 4: Predicted factors using suggested linear regression models (the red line) versus the measured data of leaves.

Results of the current study showed that the product of these factors is a good variable to determine leaf growth indexes using regression models. The variable with the highest explanatory capability was used to develop a general equation to predict LA, FW, and DW. We found that area of pistachio leaves is well correlated to the product of its length and width with high R^2 values. R^2 values for fresh weight (94%) and dry weight (97%) of leaves were less than the R2 value for leaf area (98%), and this is likely due to differences in specific leaf area (Cho *et al.,* 2007). Cho *et al.* (2007) stated that SPAD data are useful to determine leaf yield; however in this study, involvement of SPAD data in equations, decreased R^2 and increased RMSE values of fresh weight and dry weight data (data not shown).

There are many reports which suggest calculation of leaf area from leaf dry weight data (Sharrett and Baker, 1985; Ma *et al.,* 1992). In the present study a good relationship found between leaf dry weight and leaf area. linear function described this relation better than the other equation types. Romas *et al.* (1983) in barley and Ratta *et al.* (2000) in grasses also found that a simple, linear regression model between leaf area and vegetative dry weight is adequate to estimate leaf area. However, Sharret and Baker (1985) in alfalfa, Payne *et al.* (1991) in pearl millet, and Akram-ghaderi and Soltani (2007) in cotton reported that power equation best described the relationship between leaf area and leaf dry weight. Marshall (1968) suggested that such relationship changes during plant growth and along with changes in environmental conditions. However, for the models used in this study the seasonal changes of temperature did not adapted.

The best equations selected as model to predict leaf growth indexes of pistachio. Evaluating the suggested models by validation set data, showed a strong agreement between predicted and measured data. The results of the current study showed that pistachio leaf area, fresh weight, and dry weight may be estimated by regression models including the product of leaf length and leaf width values. Accuracy of the general equations fitted in the present work to predict LA, FW, and DW were relatively high. These equations provide a simple, accurate, non-destructive and time saving tool to predict growth of pistachio leaves *in situ*. Some sacrifice in accuracy is inevitable, but using larger populations in the experiments may reduce the deviations. For more precise modeling, environmental factors and computer systems as well as growth factors should be included in the models. The applicability of the suggested equations to other cultivars and/or environment conditions should be tested.

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