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# Quick Estimation of Apple (Red Delicious and Golden Delicious) Leaf Area and Chlorophyll Content

**P. AHMADI MOGHADDAM[1**]**

Department of Biosystem Engineering, Agricultural Faculty, College of Agriculture, Urmia University, Urmia, I. R. Iran

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**ABSTRACT-** The evaluation of leaf area and leaf nutritional value is important for crop growth modeling and estimations of its performance. The purpose of this study was to use image processing techniques to develop an economical method to ease the assessment of nutrient status and leaf area (LA) of plants and to compare the outcomes of this method with linear models. Leaf area and leaf chlorophyll ($LC_{hl}$) estimation models were developed for apples (Red delicious and Golden delicious) using a leaf area meter and a chlorophyll meter (SPAD 502), respectively. Linear models were developed by measuring the length (L) and width (W) of the leaves. Two models (LA= 1.01 + 0.82LW and LA= 1.23 + 0.87LW) were found to provide the most accurate estimations ($R^2$ = 0.94, MSE = 4.6 and $R^2$ = 0.94, MSE = 4.7) of leaf area for Red Delicious and Golden Delicious apples, respectively. Results also showed that the image processing technique was able to estimate leaf area with good accuracy ($R^2$ = 0.98, MSE = 4.6). The calculation of leaf area in this technique is easier and more accurate than linear models. The best model for leaf chlorophyll estimation using the image processing technique was ($LC_{hl}$= - 0.55 red - 0.45green +0.32 blue + 92) which was highly accurate ($R^2$ = 0.95, MSE = 7.4).

**Keywords: Apple, Image processing, Leaf area, Leaf chlorophyll, Linear measurement**

## INTRODUCTION

Apples are one of the most valuable food and commerce crops. More than 65 million tons of apples were produced around the world in 2010. Iran produced 10 million tons of apples in 2010, ranking fifth in the world (6). Proper nutrition of apple trees is one of the most important factors contributing to the improvement of fruit quality and quantity.

*Assistant Professor
** Corresponding Author

Detection of nutrient deficiencies and proper use of fertilizers are essential to the safety and profitability of agricultural crops. Sustainability of the leaves affect crop growth and bio-productivity. Hence, the investigation of leaf status gains great significance in plant growth studies.

Leaf area (LA) is a key variable for most agronomic and physiological studies involving plant growth, nutrition, competition, soil-water relations, respiration rate, light reflectance, evaporation, and responses to fertilizers and irrigation (1, 11). Leaf area also plays an important role in determining proper application rates of insecticides and fungicides. Leaf color can also indicate the amount and proportion of leaf chlorophyll ($LC_{hl}$) which is, in turn, closely related to tree nutrient status. Nitrogen (N) is an essential nutrient in the growth of agricultural crops. The first sign of nitrogen deficiency is leaf pallor. Hence, leaf color as a function of chlorophyll content, can be used as an index to detect nutrient status (7). Since LA and $LC_{hl}$ strongly influence crop growth and productivity, estimating LA and $LC_{hl}$ are fundamental components of crop growth models (9).

There are several methods for measuring leaf area, the most important of which being the graphical method, length and width correlation, special leaf, and the use of leaf area meters for LA measurement (11). Despite the fact that leaf area meters are fast and accurate, they are usually tedious to use and expensive (8). In addition, three convenient techniques are used to measure leaf chlorophyll content including plant analysis, leaf chlorophyll measurement, and remote sensing systems for leaf nitrogen measurement (13). Campbell et al. (2) found high correlations between leaf chlorophyll content and chlorophyll index (SPAD) measured by a chlorophyll meter.

Measuring leaf area and leaf chlorophyll contents of large numbers of leaves is often costly, time consuming and destructive. The total LA and $LC_{hl}$ of a tree can be calculated by either direct or indirect methods. The former consists of separating and measuring all the leaves of the plant. This method is destructive and requires expensive equipment. Indirect, non-destructive methods, however, are user-friendly, less expensive, and can provide accurate LA and $LC_{hl}$ estimations.

Remote sensing has a high potential to obtain large amounts of plant data cheaply and rapidly. The introduction of computer based image-processing systems has further boosted the applications of machine vision techniques for the assessment of plant characteristics such as leaf area and chlorophyll content (3, 14, 19). Generally speaking, computer based systems include a scanner or digital camera to capture the image of the leaf and specifically developed software to analyze the images. Liangliang et al. (8) and Pagola et al. (15) successfully developed digital images to assess the chlorophyll content of winter wheat and barley.

Although LA and $LC_{hl}$ are important parameters in growth studies, little research has been conducted on the simultaneous evaluation of both, and the need is felt for the development of inexpensive, precise and quick methods of leaf area and leaf chlorophyll measurements. In the present study, one such method is proposed based on image processing techniques.

## MATERIALS AND METHODS

### Data collection

Apple leaves from different trees of two cultivars (Red Delicious and Golden Delicious) were randomly harvested from trees of the faculty of agriculture, Urmia University, Iran, every 20 days during the growing season of spring and summer 2012, A total of 210 leaves, 105 from each cultivar, were measured in order to develop the best fitting model for predicting the LA and $LC_{hl}$ of apple leaves. The leaves varied in size from small to large and in chlorophyll content from poor to strong. Immediately after cutting, leaves were placed in plastic bags and transported to the laboratory.

Images were taken from each leaf by a scanner (SCX- 4300 Samsung model) while their chlorophyll content was being simultaneously measured using a chlorophyll meter (SPAD-502, Minolta Co, Japan). In order to increase the accuracy of leaf chlorophyll measurement, sampling was repeated 10 times and an average was calculated. Leaf area was also simultaneously measured using a leaf area meter (AM200). Maximum width (W) (at the widest point perpendicular to the midrib) and maximum length (L) (from lamina tip to the point of petiole intersection along the midrib) were measured using a digital micrometer with an accuracy of 0.1mm (Table 1).

**Table 1. Means ± standard deviations, minimum (Min) and maximum (Max) values for the length (L) and width (W), leaf area and leaf chlorophyll of Red Delicious and Golden Delicious apples**

| Cultivar | L | | | W | | | Leaf Area | | | Leaf Chlorophyll | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean ± SD | Max | Min | Mean ± SD | Max | Min | Mean ± SD | Max | Min | Mean ± SD | Max | Min |
| Red Delicious | 8.17 ± 2.3 | 13.1 | 3.5 | 3.78 ± 1.1 | 6.2 | 1.6 | 25.5 ± 5.1 | 48.1 | 0.61 | 28.13 ± 5.3 | 52 | 0.7 |
| Golden Delicious | 8.53 ± 2.2 | 14.1 | 3.6 | 3.82 ± 1 | 6.3 | 1.6 | 25.9 ± 4.7 | 49.5 | 0.73 | 29.25 ± 5.5 | 49 | 3.5 |

### Model Building

### LA linear regression model

The dependent variable (LA) was regressed with independent variables including L, and W. In such models, linear and nonlinear equations with arbitrary coefficients are defined to estimate leaf area. In the next step, the values estimated by the model are compared with actual values and the error value is calculated. Total error is minimized by changing model coefficients. Finally, the best model according to the highest value of $R^2$and the lowest value of MSE is chosen. Five models (Models I–V) were used for LA estimation (Table 2).

### LA estimation by image processing model

In this method, the image processing technique is used to estimate leaf area. Due to their irregular leaf shape, it is difficult to determine the exact leaf length and width in some crops. In such cases, an image processing technique is recommended to estimate leaf area. In this method, a typical scanner would scan the leaves. Depending on the size of leaf, 10 leaves can be scanned at each given time. The value of the leaf area is then calculated using image processing techniques. In the present study, MATLAB software (Version 7.6, 2011, Math works Company) was used for image processing.

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

| Variety and model number | Model Form | Fitted coefficient and constant | | $R^2$ | MSE |
|---|---|---|---|---|---|
| | | a | b | | |
| Red delicious | LA = a + bWL | 1.01 | 0.82 | 0.94 | 4.6 |
| 1 | LA = aW + bL | 12.53 | 25.1 | 0.75 | 18.1 |
| 2 | LA = $aW^2$ + bL | 0.85 | 15.2 | 0.92 | 5.56 |
| 3 | LA = aW + $bL^2$ | 42.48 | 0.13 | 0.91 | 8.16 |
| 4 | LA = $aW^2$ + $bL^2$ | 0.95 | 0.17 | 0.94 | 4.9 |
| 5 | | | | | |
| Golden delicious | LA = a + bWL | 1.23 | 0.87 | 0.94 | 4.7 |
| 1 | LA = aW + bL | 9.56 | 23.2 | 0.81 | 14.3 |
| 2 | LA = $aW^2$ + bL | 0.89 | 14.3 | 0.91 | 8.2 |
| 3 | LA = aW + $bL^2$ | 39.8 | 0.17 | 0.92 | 5.9 |
| 4 | LA = $aW^2$ + $bL^2$ | 0.91 | 0.14 | 0.95 | 3.5 |
| 5 | | | | | |

Data obtained from such model have to be calibrated. Therefore, 10 cards with standard dimensions were scanned and their areas calculated by the model. Calibration coefficient was calculated according to the actual size of the cards and this coefficient was used to correct leaf area calculations.

## $LC_{hl}$ estimation by image processing models

In order to model leaf chlorophyll, image preprocessing was used first. Digital color images consist of three red, green, and blue components which were all extracted in RGB* space after removing the background. The average of each image was then calculated and different models were submitted to estimate leaf chlorophyll content (Table 3), whereupon the best model was selected. The results of this selection are presented in the next section.

**Table 3. Different models for leaf chlorophyll estimation of the red and golden varieties**

| Varity and model number | | Model Form | $R^2$ | MSE |
|---|---|---|---|---|
| Red delicious | 1 | LChl = - 0.61 R + 74 | 88 | 10.7 |
| | 2 | LChl = - 0.69 G + 93 | 74 | 14.3 |
| | 3 | LChl = - 0.74 B + 102 | 66 | 24.4 |
| | 4 | LChl = - 0.55 R - 0.45 G +0.32 B + 92 | 93 | 7.1 |
| Golden delicious | 1 | LChl = - 0.72 R + 83 | 87 | 11.4 |
| | 2 | LChl = - 0.75 G + 91 | 77 | 12.3 |
| | 3 | LChl = - 0.78 B + 112 | 69 | 21.6 |
| | 4 | LChl = - 0.58 R - 0.48 G +0.41 B + 93 | 94 | 6.5 |

*Red, Green, Blue

## Model performance evaluation

To validate the selected models, 40 new leaves were selected from each cultivar. The LA and $LC_{hl}$ of each individual leaf were predicted using the best model and compared with the actual LA and $LC_{hl}$. In this study, the mean square error of prediction (MSE), minimum prediction accuracy (MPA) and the coefficient of determination ($R^2$) were considered for the evaluation of the accuracy of the models.

To detecting co-linearity, the variance inflation factor (VIF) (9) was calculated. VIF was calculated for the parameter estimation to detect inter-correlations between variables.

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

where, r is the correlation coefficient. If the VIF value exceeds 10 and the co-linearity has a larger rather than a trivial effect on the estimation of the parameter, one of them should be excluded from the model.

## RESULTS

The first step in LA modeling is investigating the degree of co-linearity between W and L. The VIF was 1.34 and 1.55 for Red Delicious and Golden Delicious, respectively. In the two varieties, VIF was smaller than10, indicating that the co-linearity between L and W can be considered negligible.

Regression analysis demonstrated strong relationships among LA, maximum leaf width, and maximum leaf length for the two varieties. Linear and quadratic models were evaluated to estimate leaf area. The results showed that linear model number 1 and non-linear model number 5 had the highest accuracy (Table 2). Since there was no significant difference between the accuracy of these two models, the linear model (LA= 1.01+0.82WL) was selected as the best. Fig. 1 shows correlations between the data estimated by this model and the actual data.

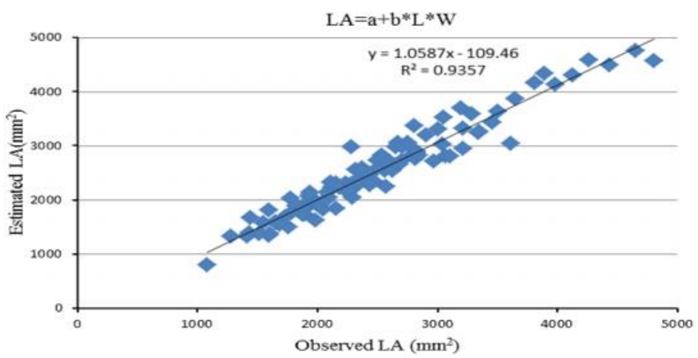


**Fig. 1. Relationship between observed and estimated values of single leaf areas using model 1 [LA = 1.01 + 0.82LW]**

In the second method of leaf area estimation, image processing techniques were used. After removing the background and segmenting the leaves, leaf area was calculated according to the number of pixels. Fig. 2 shows the results of the image processing steps. Fig. 3 shows correlations between data obtained from the model and the actual data. Results showed that the image processing method was more accurate than the linear model used. Due to the fact that linear models apply one equation to all shapes of leaves disregarding their minor differences, this equation causes errors. Conversely, the image processing method extracted the leaf area more accurately.

**a) b) c)**

**Fig. 2. Various stages of leaf processing a) original image b) Separation of leaves c) Converted to binary image**

In order to find a reasonable model to estimate the chlorophyll content (SPAD values) of the leaves, relationships between principle components (R, G and B) and $LC_{hl}$ were considered. The results of this analysis are shown in Table 2.

The results of leaf chlorophyll content modeling using the image processing technique showed that there was a significant relationship between the main components of the image and leaf chlorophyll in RGB color space. Regression analysis showed that the red component of the image had the highest correlation. Using optimization, the best model was developed for chlorophyll content estimation (Table 3). Calculating the difference between red and blue components can help estimate leaf chlorophyll content (model 4).

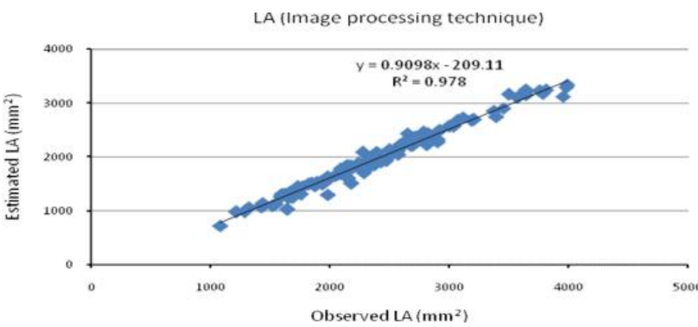


**Fig. 3. Relationship between observed values and estimated values by image processing technique**

## Model validation

Before using the developed models in the present study, it is necessary to validate them with a new data set. For this purpose, L, W, LA and $LC_{hl}$ of 45 new samples were measured. Using the best selected models, chlorophyll content and leaf area were then estimated. The results confirmed the accuracy of the developed models (Fig.4 and 5). Evaluation of the models showed that leaf area and chlorophyll content could be estimated with good accuracy (Table 4).

**Table 4.Validation of developed models for LA and LChl (golden variety)**

| Model | $R^2$ | MSE | MPA% |
|---|---|---|---|
| **LA (linear regression)** | **0.95** | **5.3** | **83.8** |
| **LA (image processing)** | **0.98** | **4.6** | **89.3** |
| **LChl** | **0.95** | **7.4** | **78.2** |

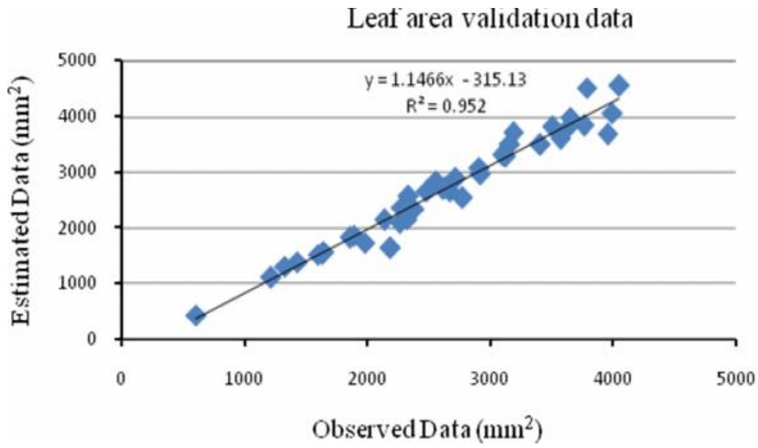


**Fig. 4. Relationship between predicted leaf area by linear models and observed leaf area in the validation data set**

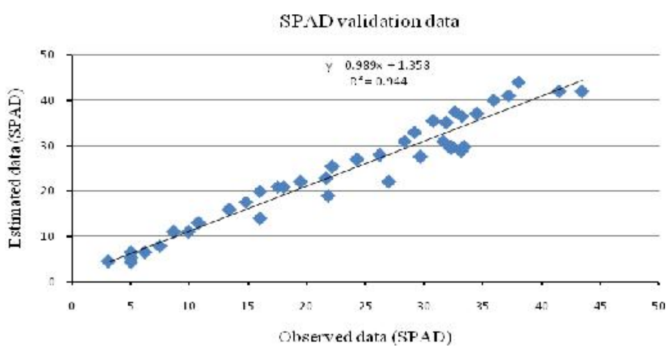


**Fig. 5. Relationship between predicted leaf chlorophyll by linear models and observed leaf chlorophyll in validation data set (red and golden)**

# DISCUSSION

The results show that VIF is smaller than 10, indicating that the co-linearity between L and W can be considered negligible and the variables can both be included in the model of two varieties. Values of 0.69 and 0.63 have been reported for pepper (5), and zucchini squash (17), respectively. In this study, the linear model (LA=1.01 + 0.82WL) was found to be the best. Cristofori et al. (4) showed that the best model for estimating a single hazelnut leaf area is the linear model (LA= 2.59 + 0.74WL), which has a $R^2$=0.98. Peksen (16) also presented a linear model (LA=0.91 + 0.68WL) that had a high accuracy ($R^2$=0.97) in estimating faba bean leaf areas.

Leaf length and width measurement accuracy is very important in linear modeling methods. Reduced measurement accuracy in W and L decreases the accuracy of the model. In addition, when the number of samples is large, measuring W and L can be tedious, complicated and time consuming. Image processing techniques are more efficient for irregular leaves and have higher accuracy rates than linear regression models. Another advantage of image processing techniques is that they can measure the areas of several leaves simultaneously.

The best linear model proposed by Williams and Timothy (20) in a grape leaf area estimation has a $R^2$=0.90 that is not very accurate. Since grape leaves are irregularly shaped, linear modeling is not suitable for them. In this case, using image processing techniques is useful. Investigations showed that the main color component of the leaf decreases with the increase of leaf chlorophyll content, and the red component decreases faster as compared to the blue and green components. Namrata et al (12) reported that the reflectance of the red component was the best among other bands used to assess nitrogen stress in potatos.

## CONCLUSIONS

Plant yield and quality are affected by photosynthesis and transpiration rate, which are closely related to total leaf area and leaf chlorophyll. Since leaf growth is dependent on nutritional value and chlorophyll content, simultaneous measurements of chlorophyll content and leaf area are important. Although many studies have been carried out using length and width measurements, little attention has been given to the simultaneous measurement of leaf L and W and chlorophyll status. In the present study both were measured, and the validation of the developed models showed that leaf area and leaf chlorophyll of apple trees could be measured more quickly and accurately using image processing techniques. Such techniques are recommended for application to other trees with minor changes.

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# تخمین سریع مساحت و مقدار کلروفیل برگ سیب (رقم زرد و قرمز لبنانی)

**پرویز احمدی مقدّم[1]**[**]

[1]گروه مهندسی مکانیک بیوسیستم- دانشکده کشاورزی- دانشگاه ارومیه – ج. ا. ایران

**چکیده-** اندازه‌گیری ارزش غذایی و مساحت برگ برای توسعه مدل‌های رشد و همچنین تخمین عملکرد محصول اهمیت زیادی دارد. هدف از این تحقیق توسعه یک روش اقتصادی و در عین حال سریع برای اندازه‌گیری وضعیت غذایی و مساحت برگ گیاهان با استفاده از تکنیک پردازش تصویر و همچنین مقایسه آن با مدل های خطی می‌باشد. مدلهای تخمین مساحت و مقدار کلروفیل برای برگ سیب (رقم قرمز و زرد لبنانی) صورت گرفت. مساحت واقعی برگ با دستگاه مساحت سنج اندازه‌گیری شد. مقدار کلروفیل برگ نیز توسط دستگاه کلروفیل متر (SPAD) اندازه‌گیری شد. دو مدل خطی (LA = 1.01 + 0.82 LW and LA = 1.23 + 0.87 LW) با دقت ($R^2 = 0.94$, MSE=4.6 and $R^2 = 0.94$, MSE = 4.7) به ترتیب برای تخمین مساحت برگ در ارقام سیب درختی قرمز و زرد لبنانی بدست آمد. همچنین نتایج نشان داد که تکنیک پردازش تصویر قادر به تخمین مساحت برگ با دقت بالاتری نسبت به مدل‌های خطیمی‌باشد ($R^2 = 0.98$, MSE = 4.6). علاوه بر این محاسبه مساحت برگ در این روش، آسانتر و سریعتر از مدل‌های خطی می‌باشد. بهترین مدل برای تخمین کلروفیل برگ با تکینک پردازش تصویر، مدل LC= -0.55 red- 0.45 green+0.32 blue + 92 بوده که دارای دقت مناسب می‌باشد ($R^2$=0.95, MSE=7.4).

**واژه های کلیدی: اندازه‌گیری خطی، پردازش تصویر سیب. کلروفیل برگ، مساحت برگ**

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**استادیار

**مکاتبه کننده