Individual Leaf Area Modelling of Chili Pepper (*Capsicum frutescens*) Using Leaf Dimensions and Weight

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**ABSTRACT**

Leaf area information is required in various horticultural and physiological studies. Leaf area measurements require easy, quick and possibly non-destructive methods. The objective of this study was to establish equations to estimate leaf area (LA) using leaf length (L), width (W), fresh weight (FW) and dry weight (DW), “length × length” (L²), “width × width” (W²), “length × width” (L×W), “length + width” (L+W), “length – width” (L-W) of pepper (*Capsicum frutescens*) as a vegetable and spice plant. A soil-cultured experiment was carried out in 2010 under greenhouse conditions to study relationship between leaf dimension and weight with leaf area of this plant.Observed leaf area was obtained by an automatic measuring device and leaf dimensions were measured by a ruler. Regression analyses of LA versus L, W, FW, DW, L², W², “L×W”, “L+W” and “L-W” was done and led several models that could be used for estimating the area of individual chili pepper leaves. A linear model employing “L×W” as an independent variables [LA=0.498 (L×W) + 0.054] resulted in the most accurate estimation (R² = 0.855, RMSE = 1.28) of pepper leaf area. Validation of the regression models showed that the correlation between measured and simulated values using this equations were quite acceptable (R² = 0.855, d = 1.00 and T = 0.14).

**Keywords:** Fresh weight, leaf length, leaf width, linear model, non-destructive methods, regression models.

**Abbreviations:**

A: Leaf Area; d: Index of Agreement; DW: Dry Weight; E: Efficiency; FW: Fresh Weight; L: Length; L²: Length Square; PAR: Photosynthetically Active Radiation; R²: Coefficient of Determination; RMSE: Root Mean Square Error; T: Tolerance Value; VIF: Variance Inflation Factor; W: Width; W²: Width Square.

**INTRODUCTION**

Chili pepper is rich in phosphorus, iron, calcium and vitamins A and C. The carotenoid capsanthin is the most important pigment of *Capsicum*. The pungent principle capsacin is present on the placenta of the fruits and is said to retain its pungency at dilutions of one part per million (Purseglove et al., 1981). Chili pepper has been employed in a variety of folk remedies to treat asthma, inflamed gums, lumbago, neuralgia, pneumonia, rheumatism, sores, cancers, and tumors (Duke, 2001). In China, the leaves are used to treat toothache, while the fruit is considered useful for stomach disorder, rheumatism, and for increasing circulation of the blood (Small, 1999).

Green leaves play a critical role in crop growth and development. Leaves receive the photosynthetically active radiation (PAR) and ultimately utilize it to produce dry matter. Moreover, they are the main path for transpiration and carbon harvesting, and are a key variable to study the plant in response to fertilizer, irrigation, pruning and other physiological aspects (Smith and Kliwer, 1984). An accurate leaf area measurement plays a key role in understanding crop growth and its environment (Kumar, 2009). Leaf area (A) measurements, especially under field conditions, are often destructive and time consuming (Tsialtas and Maslaris, 2005). However, leaves may have complex shapes making leaf area determination more difficult and subject to larger errors. Furthermore, it is not possible to make successive measurement of the same leaf, and plant canopy would be damaged which can cause problems to other measurements of the experiment (Tsialtas and Maslaris, 2005).

A large number of methods, either destructive or non-destructive, have been developed to measure leaf area. The leaf area can be determined by using some expensive instruments and developed prediction models (Robbins and Pharr, 1987). Recently, new instruments, tools and
machines such as hand scanners and laser optic apparatuses have been developed for some leaf area measurements. However, these are very expensive and complex devices for basic and simple studies and not available everywhere. Despite various methods used to estimate leaf area such as Lu et al. (2004), the most common approach is to develop ratios and regression estimators by using easily measured leaf parameters such as length (L) and width (W) (Kvet and Marshall, 1971), dry matter and leaf specific area (Lieth and Pasian, 1991; Lee and Heuvelink, 2003). These methods usually save time and are non-destructive.

Non-destructive methods allow measurements to be repeated during the plant’s growth period, and reduce the variability associated with destructive sampling procedures (Nesmith, 1992). Thus, prediction model which can estimate leaf area without harming the plant can provide researchers with many advantages in horticultural experiments as follow: 1) the models enable researchers to measure leaf area on the same plants during the plant growth period and may reduce variability in experiments (Gamiely et al., 1991; Nesmith, 1991, 1992); 2) reliable models eliminate the need for expensive instruments and labour; 3) measurement will be easy, quick and thus saving time if a reliable equation be resulted or chosen; 4) using reliable equation, consistent results will be obtained; 5) modelling equation cost nothing.

The non-destructive methods based on linear measurements are fast and easy to be executed and resulted in good precision and high accuracy as demonstrated for several crops like lettuce (Guo and Sun, 2001), cucumbers (Blanco and Folegatti, 2005; Cho et al., 2007), zucchini squash (Rouphael et al., 2006), eggplant (Rivera et al., 2007), sunflower (Rouphael et al., 2007), hazelnut (Cristofori et al., 2007), faba bean (Peksen, 2007), kiwifruit (Mendoza-De Gyves et al., 2007), stevia (Ramesh et al., 2007), persimmon (Cristofori et al., 2008), medlar (Mendoza-De Gyves et al., 2008), and small fruits (Fallovo et al., 2008). The main aim of this study was to find the best model and allometric correlation based on estimate leaf area for chili pepper plants.

MATERIALS AND METHODS

Plant Preparation:

Soil cultured pepper plants were grown under greenhouse conditions in College of Agriculture located in University of Birjand from January to June 2010. Air temperature and relative humidity ranged between 38°C (day) - 17°C (night) and 60%-70%, respectively. Light intensity was about 40.5 (molm⁻²s⁻¹). Irrigation and nutrition were performed based on conventional practices. About 45 days after germination, 100 seedlings were chosen and planted in the space of 50×80 cm on and between rows.

Sample Taking and Measurement:

One fully-expanded leaf sample was randomly prepared from each plant. Each sample was separately taken into plastic bags and transported to the laboratory for destructive measurement of leaf area using leaf area meter (Delta T-Devices Ltd., Burwell, and Cambridge, England). Consequently, leaf fresh weight, length and width of each sample were measured. The maximum length and width of all leaves was measured by a ruler. Width was evaluated from the widest area to the nearest 1 mm, and length was calculated from the top to the end of the blade without petiole to the nearest 1 mm. Then, samples were taken into oven under 80°C for 24 h and dry weight of each was measured. The fresh and dry weights of leaves were measured to the nearest 0.001 g.

Model Validation:

Multiple regression analysis was performed on the samples. A search for the best model to predict leaf area (LA) was conducted with various subsets of the independent variables namely, length (L), width (W), dry weight (DW), fresh weight (FW), length square (L²), width square (W²), “length × width” (L×W), “length + width” (L+W) and “length – width” (L-W). The best model was selected based on coefficient of determination (R²), root mean square error (RMSE), efficiency (E), index of agreement (d), variance inflation factor (VIF) and tolerance value (T). RMSE of estimation was calculated as (Janssen and Heuberger, 1995):

$$RMSE = (\sum (P_i - O_i)^2/N)^{0.5}$$ (1)

Where \(P\) is predicted leaf area, \(O\) is measured leaf area, \(N\) is number of observation, and \(i=1...N\).

Comparison between the best two models (higher \(R^2\) and lower \(MSE\)) was addressed by calculating the statistic \(E\), i.e., the accuracy of model 1 relative to model 2 (Allen and Raktoe, 1981):

$$E_{12} = MSE_1/MSE_2$$ (2)

Where \(MSE_1\) and MSE₂ are the mean square error of the predictions with model 1 and 2, respectively:

$$MSE_1 = \sum (P_{1i} - O_i)^2$$ (3)

$$MSE_2 = \sum (P_{2i} - O_i)^2$$ (4)

The statistic \(E\) is dimensionless and varies from 0 to infinity. A value of \(E\) between 0 and 1 implies that model 1 is superior to model 2. If \(E\) is greater than 1 then model 2 is better.

The index of agreement (d) measures the degree to which the predictions of a model are
error free, and is dimensionless (Willmott, 1981). The d values range from 0, for complete disagreement, to 1, for perfect agreement between the observed and predicted values. The index d was calculated as:

$$d = 1 - \frac{\sum (P_i - O_i)^2}{\sum (|P_i - \bar{O}| + |O_i - \bar{O}|)^2}$$  \hspace{1cm} (5)

where \( \bar{O} \) is the average of the observed values.

For detecting collinearity, the variance inflation factor (VIF) (Marquardt, 1970) and the tolerance values (T) (Gill, 1986) were calculated:

$$VIF = \frac{1}{1 - r^2}$$  \hspace{1cm} (6)

$$T = \frac{1}{VIF}$$  \hspace{1cm} (7)

where \( r \) is the correlation coefficient. If the VIF value was higher than 10 or if T value was smaller than 0.10, then collinearity may have more than a trivial impact on the estimates of the parameters, and consequently one of them should be excluded from the model.

RESULTS AND DISCUSSION

Various mathematical models for indirect estimation of leaf area of different plant species have been presented (Guo and Sun, 2001; Rouphael et al., 2006; Cristofori et al., 2008; Fallovo et al., 2008; Spann and Heerema, 2010). Although no model has been developed to predict chili pepper leaf area, however, the present study results were in agreement with some of the previously mentioned investigations on non-destructive model development for predicting leaf area using simple linear leaf measurements. Minimum and maximum data for considering independent variables are shown in Table 1. Each of these variables were used to evaluate their relationship with actual leaf area, and power, linear and exponential relationships were studied. But, because of higher \( R^2 \), only linear relationship was shown in this study (Fig. 1).

The present results indicated that among tested equations, the seventh equation considering Length × Width (LA=0.498 (L×W) + 0.054) has the highest \( R^2 \) (0.855) and lowest RMSE (1.28). Therefore it is a good equation for non-destructive measurement of leaf area compared with others. This equation indicated that Length×Width for chili pepper is strongly related with actual leaf area (as seen in Table 2). Regarding to date presented in Table 4, it is clear that the highest and lowest SE are resulted from seventh, eighth and forth equations, respectively.

Table 1. Mean ± standard deviations, minimum (Min) and maximum (Max) values for the leaf length (L), width (W), fresh weight (FW), dry weight (DW), "length × width" (L×W), Length2 (L²), Width2 (W²), “length × width” (L×W), “length + width” (L+W) and “length – width” (L-W) used for chili pepper plants observed data, model building and testing.

<table>
<thead>
<tr>
<th>Plant parameters</th>
<th>Sample N.</th>
<th>Mean ± SD</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (cm)</td>
<td>100</td>
<td>6.70±2.84</td>
<td>4.40</td>
<td>9.00</td>
</tr>
<tr>
<td>Width (cm)</td>
<td>100</td>
<td>3.39±.95</td>
<td>2.50</td>
<td>4.70</td>
</tr>
<tr>
<td>Fresh weight (g)</td>
<td>100</td>
<td>37.72±3.03</td>
<td>14.00</td>
<td>70.00</td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>100</td>
<td>0.11±0.66</td>
<td>0.04</td>
<td>0.18</td>
</tr>
<tr>
<td>Length² (cm)</td>
<td>100</td>
<td>45.24±2.86</td>
<td>19.36</td>
<td>81.00</td>
</tr>
<tr>
<td>Width² (cm)</td>
<td>100</td>
<td>11.75±2.94</td>
<td>6.25</td>
<td>22.09</td>
</tr>
<tr>
<td>Length × Width (cm²)</td>
<td>100</td>
<td>22.94±3.11</td>
<td>11.00</td>
<td>42.30</td>
</tr>
<tr>
<td>Length + Width (cm)</td>
<td>100</td>
<td>10.05±3.06</td>
<td>6.90</td>
<td>13.70</td>
</tr>
<tr>
<td>Length – Width (cm)</td>
<td>100</td>
<td>3.26±1.89</td>
<td>1.60</td>
<td>5.10</td>
</tr>
</tbody>
</table>

Table 2. Regression models, \( R^2 \) and RMSE used for leaf area estimation of chili pepper.

<table>
<thead>
<tr>
<th>Plant parameters</th>
<th>Equation N.</th>
<th>Equation</th>
<th>( R^2 )</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (cm)</td>
<td>1</td>
<td>LA=2.920 (L) - 7.951</td>
<td>0.709</td>
<td>1.81</td>
</tr>
<tr>
<td>Width (cm)</td>
<td>2</td>
<td>LA=5.977 (W) - 8.796</td>
<td>0.764</td>
<td>1.63</td>
</tr>
<tr>
<td>Fresh weight (g)</td>
<td>3</td>
<td>LA=0.269 (Fresh W) + 1.336</td>
<td>0.807</td>
<td>1.47</td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>4</td>
<td>LA=18.96 (Dry W) + 9.412</td>
<td>0.938</td>
<td>3.29</td>
</tr>
<tr>
<td>Length² (cm)</td>
<td>5</td>
<td>LA=0.219 (L²) + 1.555</td>
<td>0.721</td>
<td>17.70</td>
</tr>
<tr>
<td>Width² (cm)</td>
<td>6</td>
<td>LA=0.847 (W²) + 1.530</td>
<td>0.760</td>
<td>1.64</td>
</tr>
<tr>
<td>Length × Width (cm²)</td>
<td>7</td>
<td>LA=0.498 (L×W) + 0.054</td>
<td>0.855</td>
<td>1.28</td>
</tr>
<tr>
<td>Length + Width (cm)</td>
<td>8</td>
<td>LA=2.219 (L+W) - 10.82</td>
<td>0.823</td>
<td>1.41</td>
</tr>
<tr>
<td>Length – Width (cm)</td>
<td>9</td>
<td>LA=2.731 (L-W) + 2.573</td>
<td>0.314</td>
<td>2.78</td>
</tr>
</tbody>
</table>

The highest d value was shown for equations 1, 7 and 8 (Table 3). The lowest MSE and T value, and the highest VIF are obtained for equation 7 (Table 3), which confirmed the goodness of seventh model to estimate leaf area. Data showed the low difference between RMSE and MSE related to equations 7 and 8 (Tables 2 and 3). Statistic E was used to compare these equations and
models, and results indicated that model 7 was better than model 8 (Table 4). Leaf area is one of the important growth parameters and one must record it for effective monitoring of the growth and development of plant in the experiment. Lack of accurate model is a limitation for calculating LA. Non-destructive method of the estimation of LA has several advantages without compromising on accuracy (Peksen, 2007; Antunes et al., 2008; Kandiansan et al., 2009). Many studies have been carried out to estimate leaf area by measuring leaf dimensions. In general, the combination of leaf length (L) and maximum width (W) has been used as the parameters of leaf area models (Peksen, 2007; Antunes et al., 2008). Various mathematical models for indirect estimation of leaf area of different plant species have been described (Peksen, 2007; Antunes et al., 2008). Present study results are in agreement with some of the previous studies mentioned above on model development for predicting leaf area using simple linear measurements (Falvo et al., 2008; Cristofori et al., 2008).

Fig. 1. Plot of predicted leaf area, estimated by model versus the observed leaf area using independent variables (A-I).
predicted leaf area using the proposed model. A close relationships were found between actual leaf area and predicted leaf area area estimation to compare models for chili pepper.

<table>
<thead>
<tr>
<th>Plant parameters</th>
<th>Equation N.</th>
<th>SE</th>
<th>MSE</th>
<th>d</th>
<th>VIF</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (cm)</td>
<td>1</td>
<td>0.28</td>
<td>3.26</td>
<td>1.00</td>
<td>3.44</td>
<td>0.29</td>
</tr>
<tr>
<td>Width (cm)</td>
<td>2</td>
<td>0.29</td>
<td>2.65</td>
<td>0.99</td>
<td>4.24</td>
<td>0.23</td>
</tr>
<tr>
<td>Fresh weight (g)</td>
<td>3</td>
<td>0.30</td>
<td>2.17</td>
<td>0.99</td>
<td>5.18</td>
<td>0.19</td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>4</td>
<td>0.07</td>
<td>10.81</td>
<td>0.77</td>
<td>1.04</td>
<td>0.96</td>
</tr>
<tr>
<td>Length² (cm)</td>
<td>5</td>
<td>0.29</td>
<td>313.18</td>
<td>0.99</td>
<td>3.58</td>
<td>0.28</td>
</tr>
<tr>
<td>Width² (cm)</td>
<td>6</td>
<td>0.29</td>
<td>2.69</td>
<td>0.99</td>
<td>4.17</td>
<td>0.24</td>
</tr>
<tr>
<td>Length × Width (cm²)</td>
<td>7</td>
<td>0.31</td>
<td>1.63</td>
<td>1.00</td>
<td>6.90</td>
<td>0.14</td>
</tr>
<tr>
<td>Length + Width (cm)</td>
<td>8</td>
<td>0.31</td>
<td>1.98</td>
<td>1.00</td>
<td>5.65</td>
<td>0.18</td>
</tr>
<tr>
<td>Length - Width (cm)</td>
<td>9</td>
<td>0.19</td>
<td>7.71</td>
<td>0.98</td>
<td>1.46</td>
<td>0.68</td>
</tr>
</tbody>
</table>

CONCLUSION
In this study, very close relationships were found between actual leaf area and predicted leaf area area estimation to compare models for chili pepper.

RESULTS
Results showed that chili pepper leaf area could be monitored quickly, accurately, and non-destructively by using the "leaf length × leaf width" model.

REFERENCES


