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Full Length Research Paper

Modeling of the individual leaf area and dry weight of oregano *(Origanum onites* L.) leaf using leaf length and width, and SPAD value

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The study was aimed to modeling of the individual leaf area and dry weight of oregano *(origanum onites* L.) using leaf length, leaf width and SPAD value. Non-destructive approach of modeling can be very useful for plant growth estimation. To predict individual leaf area and dry weight of oregano, models were developed using leaf length, leaf width, SPAD value, and different combinations of these variables. Eight regression equations, commonly used for developing growth models, were compared for accuracy and adaptability. The two nonlinear models developed were as follows: individual leaf area LA = $0.104 + 0.363W + 0.380L^2 – 0.028W^2$ (R^2 = 0.973) and dry weight DW = $0.0018 + 0.00102WS + 3.45E^{-4}$ LWS (R^2 = 0.928), where *L* is the leaf length, *W* the leaf width, *S* the SPAD value, and *LWS* = *L x W x* S. For validation of the model, estimated values for individual leaf area and dry weight showed strong agreement with the measured values, respectively. Leaf dry weight, especially, was estimated with a higher degree of accuracy through the use of a SPAD value, as well as leaf length and width. Therefore, it is concluded that models presented herein may be useful for the estimation of the individual leaf area and dry weight of oregano with a high degree of accuracy.

Key words: Oregano, modeling, SPAD value, leaf length, leaf width.

INTRODUCTION

The genus *Ocimum basilicum* L. (Lamiacae) comprises more than 150 species and is considered as one of the largest genera of the lamiaceae family. *O. basilicum* L. (sweet basil) is an annual herb which grows in several regions all over the world (Sajjadi, 2006; Simon et al., 1990; Grayer et al., 1996). Oregano grows in the cool moist and tropical rain forest zones in annual temperatures between 6 - 24°C and receiving 500 - 8000 mm annual precipitation. Although, sweet basil is cultivated in different climatic and ecological conditions, the most favorable conditions are found in countries with a warm climate. Oregano develops best under long days in sunny conditions. *Ocimum* spp. contain a wide range of essential oils rich in phenolic compounds and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins (Phippen et al., 1998). The leaves and flowering tops of sweet basil are used as carminative, galactogogue, stomachic and antispasmodic medicinal plant in folk medicine (Duke, 1989). Antiviral, antimicrobial and anti-inflammatory activities of this plant have also been reported (Ozbek et al., 2007; Baratta et al., 1998; Caliskan et al., 2009).

Leaf area has been measured in experiments concerning some physiological phenomenon such as light, photosynthesis, respiration, plant water consumption and transpiration In addition, leaf number and area of a plant have an important role in some cultural practices such as training, pruning, irrigation, fertilisation, etc (Odabas et al., 2009; Odabas et al., 2007; Odabas et al., 2005). The

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leaf area estimation models aiming to predict leaf area non-destructively can provide researches with many advanages in agricultural experiments. Leaf area plays an important role in photosynthesis, light interception, water and nutrient use, crop growth and development. Non-destructive method for the estimation of leaf area may be useful to determine the relationship between leaf area and plant growth rate. Simple regression models, related to leaf area and crop growth rate, were applied to estimate crop yields. Since leaf development has a strong relationship with crop growth, knowing the change in leaf area may be useful for estimating crop growth. Considering that leaf area and crop growth are both affected by nutritional conditions, more reliable results may be obtained through the addition of nutritional factors to the models (Monterio et al., 2000; Cho et al., 2007). A chlorophyll meter is also useful for the prediction of crop production.

SPAD values are indirectly related to nitrogen concentration. For simple, rapid, and accurate estimation of leaf dry weight, various non-destructive tests, measurable with ease, should be added (Cho et al., 2007; Le Bail et al., 2005).

Common measurements for prediction equations in some models carried out previously have included leaf width, leaf length, petiole length, main and/or lateral vein length, and different combination of these variables. Some researchers have tried using new equipment and tools such as hand scanner or laser optic apparatuses for predicting plant growth non-destructively, but these are very expensive investments for basic and simple research (Cirak et al., 2005). The objective of this study was to develop models of estimating leaf area and dry weight of oregano using leaf length, leaf width and SPAD values.

MATERIAL AND METHOD

Plant material and experimental conditions

This research was conducted in outdoor and greenhouse at Ondokuzmayis University, Vocational School of Bafra in Turkey from April 1 to September 1, 2008.

Dimension of the greenhouse length; width and height were separately 15, 8 and 3 m. Seeds were sown in viols. After germinated, seedlings were transferred to (40 × 30 × 15 cm) pods. In the present study *Origanum onites* L. seeds were used and set up in 100 pots in greenhouse and outdoor. Greenhouse and outdoor were separated shaded and un-shaded parts, however, 50% transparent polyethylene cover was used for shading. Leaf samples were collected at the same time in greenhouse and outdoor. The peat used in the research and had a pH value of 7.

Model construction

Leaf samples were randomly taken from all plants from April to September 2008 at a five time intervals. In this period, 10 leaves were collected for each plant within the first three day of April, May, June, July and August to catch the different phases of leaf morphogenesis. Thus, 50 leaf samples for each plant and a total of 5000 leaves for all plants were processed at the same day as they were collected in the following manner. First, they were placed on the photocopier desktop by holding flat and secure and copied on A3 sheet (at 1:1 ratio). Second, a Placom Digital Planimeter (Sokkisha Planimeter Inc., Model KP-90) was used to measure actual leaf area of the copy. Selection of leaf dimensions for measurement was governed by variation in leaf characteristics (e.g., size, shape, and symmetry) and practical constraints (e.g., ease and accuracy of measurements under field conditions). Given these concerns, we chose maximum leaf width (W) and leaf length (L) to correlate with leaf area. Leaf width (cm) was measured from tip to tip at the widest part of the lamina and leaf length (cm) was measured from lamina tip to the point of petiole intersection along the midrib. The leaf positions were selected with regard to points that could be easily identified and used to facilitate the measurement of leaf length and width. Dry weights were determined after drying for 72 h 70°C. The SPAD readings were taken with a chlorophyll meter (Apogee Model CCM200) and recorded as a mean of 10 measurements for each individual leaf.

The most common regression equations used to develop plant growth models were evaluated for accuracy and adaptability. All equations were composed of various subsets of independent variables, such as leaf length (L), leaf width (W), SPAD values (S), L^2, W^2, LW, *LS,* and *LWS*. The eight models determined to be the most suitable for estimating total leaf area (LA), fresh weight (FW) and dry weight (DW) of cucumber were selected. All variables in the models below were significant at P = 0.05 level.

$$F = a + bLW \quad (1)$$

$$F = a + bW + cLW \quad (2)$$

$$F = a + bL + cW + dLW \quad (3)$$

$$F = a + bL + cW^2 + dLW \quad (4)$$

$$F = a + bW + cL^2 + dW^2 \quad (5)$$

$$F = a + bL + cL^2 + dW^2 \quad (6)$$

$$F = a + bLW + cLWS \quad (7)$$

$$F = a + bLS + cLWS \quad (8)$$

Where *F* is the leaf area, fresh or dry weights; *L* the leaf length, *W* the leaf width, LW = L × W, LS = L × SPAD, and LWS = L × W × SPAD; a, b, c and d are the constant. All data was analyzed using the SAS (Statistical Analysis Software) Program. Slopes, intercepts and regression coefficients of the models were compared using the SAS REG procedure. Correlation coefficients were calculated between measured and estimated data (Cho et al., 2007).

RESULTS

Of the all models, four consisting of leaf length (L) and leaf width (W) were selected for estimation of the leaf area (LA) of oregano (Table 1). Equation 5 had a higher R^2 value with a lower root mean square error (RMSE) than other equations tested.

Figure 1 shows that both leaf length and leaf width were highly related to leaf area of oregano. Equations with P > 0.05 and lower R^2 values were eliminated at the beginning of this study. To estimate leaf fresh weight (FW) of cucumber, 3 models using *L* and W, and one model using L, W, and SPAD were selected (Table 1). Of

Table 1. Regression models for the estimation of leaf areas (LA) of oregano.

Regression model	Equation	R^2	RMSE	Pr > *F*
LA = 1.466 + 0.041LW	1	0.954	42.33	<0.0001
LA = 1.608 - 0.723W + 0.400LW	2	0.972	41.64	<0.0001
LA =-0.625 + 1.301L - 0.042W + 0.029LW	3	0.970	41.26	<0.0001
LA = 0.104 + 0.363W + $0.380L^2 – 0.028W^2$	5	0.973	41.11	<0.0001

All variables in the models above are significant at P = 0.05. LA is leaf area; L is leaf length; W is leaf width; LW is L × W.

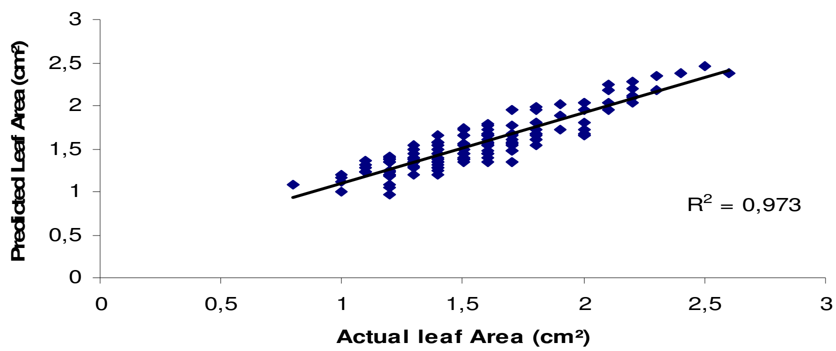

Figure 1. Relationship between actual and predicted leaf areas (cm²) of oregano.

Table 2. Regression models for the estimation of dry weight (DW) of oregano.

Regression model	Equation	R^2	RMSE	Pr > *F*
DW = 0.0012 + 0.0024LW	1	0.893	0.233	<0.0001
DW = 0.0025 + 0.0011L + 0.0043W + 0.0031LW	3	0.915	0.226	<0.0001
DW = 0.0018 + 0.00102WS + $3.45E^{-4}$ LWS	7	0.928	0.199	<0.0001

All variables in the models above are significant at *P* = 0.05. DW is leaf dry weight; *L is* leaf length; W is leaf width; *LW* = *L* × W; *LS*= *L* × SPAD value; *LWS*= *L* × *W* × SPAD value.

the three models, Equation 5 showed the highest R^2 with a lower RMSE. By adding the SPAD value, accuracy of the model was slightly increased but not significantly.

Using the same method as in DW, 3 models for estimating leaf dry weight (DW) were selected (Table 2). Equation 7 showed the highest R^2 value with the lowest RMSE among the models using *L* and W. However, the addition of SPAD values resulted in an increase in the R^2 of the model from 0.893 - 0.928, a significant difference.

According to these results, leaf length and width contribute to accurately determine leaf area of the plant, but not dry weight. However, SPAD values enabled us to increase the accuracy of the model for estimation of leaf dry weight.

DISCUSSION

Leaf area is routinely measured in experiments interesting crops where some physiological phenomenon such as light, photosynthesis, respiration, plant water consumption and transpiration is being studied (Centritto et al., 2000). In addition, leaf number and area of a plant are important in terms of cultural practices such as training, pruning, irrigation, fertilization etc. The leaf area estimation models that aim to predict leaf area non-destructively can provide researches with many advantages in agricultural experiments. Moreover, these kinds of models enable researchers to carry out leaf area measurements on the same plants over the course of a study, resulting in reduced experimental variability (NeSmith, 1992). Leaf area can be determined by using expensive instruments and/or predictive models. Recently, new instruments, tools and machines such as hand scanners and laser optic apparatuses have been developed for leaf and fruit measurements. These are very expensive and complex devices for both basic and simple studies. Furthermore, non-destructive estimation of leaf area saves time as compared with geometric measurements (Robbins and Pharr, 1987). For this reason, several leaf area prediction models were produced for some plant species in previous studies but, to the authors' knowledge, there are no published reports related to leaf area prediction model for any medicinal plant.

In this study, the individual leaf area was well correlated with leaf length and leaf width, with high R^2 values (Tables 1), whereas leaf dry weight had a relatively low R^2 value, likely due to differences in specific leaf area (SLA) (Table 2). Although the shape of a leaf was not a significant factor in the estimation of leaf area and leaf fresh weight, an additional factor, SPAD, was required to estimate leaf dry weight more accurately. Non-destructive and rapid estimation of leaf area, many methods have been applied. This method was rapid and relatively accurate but it required trained operators. In our experiment, rulers were used directly to measure the leaf length and leaf width.

According to the results of the current study, cucumber leaf area and dry weight may be estimated by nonlinear regression models including leaf length, leaf width, and SPAD values. For more precise modeling, environmental factors and computer systems as well as growth factors should be included in the models.

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