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A new, simplified and non-destructive estimate of leaf area, leaf fresh and dry weight of greenhouse cucumber *Cucumis sativus* (Cucurbitaceae) by linear measurements

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Abstract

The estimation of leaf area and leaf fresh and dry weight are used in agronomy to follow the development of crops, and to predict light interception and productivity. In small plant populations using non-destructive methods of leaf area measurements could be useful. In this study because of equipment limitations, the transparent paper and simple ruler were used. A mathematical model can be assessed by correlation between leaf length (L), width (W) or length x width (LW) to the actual leaf area of a sample leaves using regression analysis. SPAD values (S) also used for high accuracy estimation of leaf fresh and dry weight. Regression models including highest R^2 value and lowest RMSE selected. Y=-4.043-1.415LW+1.050 L^2 +1.063 W^2 ($R^2 = 0.984, RMSE = 12.09$) Y=-0.125+0.00741 LW+0.000208 LWS (R^2=0.945, RMSE= 0.58), Y=-0.0435+0.0000467 LWS+0.00134 LW (R^2=0.887, RMSE= 0.17) were the best predicted models for estimation of leaf area, leaf fresh weight and leaf dry weight, respectively. To evaluate the accuracy of the model, estimated values of individual leaves were plotted against measured values. The SPAD value parameter using in fresh and dry weight equation with higher accuracy, showed that SPAD meter is a relatively inexpensive, fast, easy, and non-destructive machinery for recognition of crop N nutrition that has simplified research in plant development. Therefore, it is concluded that high accuracy models can be used resulting in time and effort saving for estimation of leaf area, leaf fresh and dry weight in order to trace crops growth.

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Introduction

Leaf growth is one of the most important feature of plants which affects plant productivity under different environments, environmental stresses and agronomic management significantly alter the leaf growth in different plant species (Hooker 1907, de Jesus, do Vale *et al.* 2001, Blanco and Folegatti 2005, Córcoles, Domínguez *et al.* 2015). Plant productivity depends on their ability to intercept and capture the solar radiation, plants modify their leaf area and canopy structures to gain the desirable amount of solar energy and biomass (Gifford, Thorne *et al.* 1984, Mikias Yeshitila 2016). In plant growth analysis, the leaf area index (LAI) which is described as the total one side area of leaf tissue per ground unit surface area (Watson 1947) and specific leaf area (SLA) which is described as the ratio of leaf area to leaf weight (Stoppani, Wolf *et al.* 2003) are also calculated from the leaf area. Therefore, correct measurements of leaf area are necessary for comprehending the plant growth response under different environmental conditions (de Jesus, do Vale *et al.*, 2001).

Researchers have been using many various methods for estimating leaf area for several plant species (Küßner and Mosandl 2000, Jonckheere, Fleck *et al.*, 2004). (Marshall 1968) has been categorized methods to leaf area measurements as direct and indirect. A direct method requires measuring the foliage of plant canopy sample by leaf area meter, removing all leaves of plants and measuring them (Sadik, Al-Taweel *et al.*, 2011). All direct methods that are not mentioned here which is named destructive are very expensive and not available in developing countries, moreover, this approach is not used in low plant density at laboratory researches or in field conditions, the whole plant removal is not possible because of the limited-number (Norman and Campbell 1989, Mendoza-de Gyves, Cristofori *et al.* 2008, Córcoles, Domínguez *et al.*, 2015, Shabani and Sepaskhah 2017).

Due to all methods, the predicted models derived from mathematical equations consisting linear measurements of leaves (Beerling and Fry 1990, Coombs, Hall *et al.*, 2014) are comparatively accurate. It can be used without damaging and injuring plants (Kvet and Marshall 1971). (Montgomery 1911) for the first time suggested that the linear measurement of leaves can be used to calculate leaf area, using a general relationship A=b*I*W where b is a coefficient (Montgomery 1911). Several measurement methods related to leaf length and leaf width and also their combination have been used for different kinds of plant species, which of generally chosen for their simplicity and accuracy (Montero, De Juan *et al.* 2000, Williams III and Martinson 2003, Blanco and Folegatti 2005), such as onion (Córcoles, Domínguez *et al.* 2015), *Helicteres isora* L. (Kumbhani, Kuvad *et al.* 2017), hydroponically cucumber (Cho, Oh *et al.* 2007). Also, there are many reports available for using leaf dry and fresh weight to assess the leaf area of plant with high accuracy (Sharratt and Baker 1986, Cho, Oh *et al.* 2007). Leaf area has been found concerning linear leaf dimensions, which are used in various plants to assess leaf area (Uzun and Çelik 1999, Lu, Lu *et al.*, 2004, Kandiannan, Parthasarathy *et al.*, 2009). Since leaf expansion has a strict correlation with plant growth, understanding a variation in leaf area may be beneficial for evaluating plant growth (Cho, Oh *et al.*, 2007). Therefor to follow continues alterations in leaf area and the subsequent growth, a modeling procedure is required.

The object of this study is to make a new, simple mathematical models to predict the leaf area in a soil greenhouse cucumber using non-destructive leaf area measurement. This model should be applied for various plant growth stages with simplest equipment such as ruler that is find everywhere. In this paper a millimeter graph paper method is described.

Material and methods

Plant material and experimental conditions

This study was carried out from May to October 2016 in greenhouse of Pouyesh high school of Qazvin, (Qazvin Province, Iran). Total available area of the greenhouse was 100m². Seeds of the cucumber (cultivar Sina 189, Seminis, Royal Sluis Seed Company) were selected based on their extended use in the country.

Seeds were soaked for 24 hours, followed by 72 hours keeping within wet tissue papers for germination. Germinated seeds were sown in nursery beds prepared from standard growing mixers of sands in 2cm depth of pots. Seedling with the appearance of the second or third true leaves was transplanted into a culture bed into the greenhouse. Plants were spaced rows with a planting density of 2.5 plants per m^2. All agronomic practices like rate and frequency of fertilizer applications, disease and pest management were applied according to standard recommendations.

Model construction and validation

Different leaves sizes from large (fully expanded leaves) to smallest for each plant were chosen from various heights of the plant canopy between 0.5 and 4 meters from the soil surface during the growing period. A total number of 180 leaves (about 120 plants) from the individual plant were measured for leaf area, leaf length and leaf width. Leaf length was measured from lamina tip to the interception of the lamina and petiole along midrib of the lamina, while leaf width was measured from end to end between the widest lobes of the lamina. Leaf area was measured using millimeter graph paper. Each leaf was spread over millimeter graph paper, and the outline of leaves was drawn. Using the paper blade, the area of the millimeter graph paper lies around the outline was cut and weight. One cm^2 of the same millimeter graph paper was also cut and weighed. For the accuracy of the experiment leaves of the same size were measured more than once. The equation, Leaf area (cm^2)= x/y, where x is the weight of the graph paper lied over the leaf outline (g) and y is the weight (g), of the cm^2 area of the graph paper, was used to calculate the leaf area nondestructively. In terms of not having the necessary facilities (leaf area meter) to determine leaf area in order to validate the model, also a transparent millimeter paper was used in different ways. In this approach other isolated leaves in the same size were drawn over the transparent paper, and count the cells covered by the leaf area, therefore the accurate area of leaves was attached. In order to measure fresh and dry weights of leaves, the cutting leaves of the plants were kept in plastic bags in order to save humidity and transferred in the laboratory to determine their fresh weight. Dry weights were measured after drying for 72 hours at 72°C. The various subsets of independent variable were used to make equations such as leaf length (L), leaf width (W), and parameters that are derived from them such as L^2, W^2, LW, Ln L, Ln W, L/W. The most appropriate models with highest R^2 value and lowest RMSE were chosen for estimating the leaf area, fresh weight and dry weight. Finally the two sets of estimates were linked according to y=a + bx, where y is a leaf area estimated by using transparent millimeter paper as a dependent variable and x is a leaf area estimated by using millimeter graph paper as independent variable. The regression equation computed by using IBM SPSS Statistics 24.

Results

In this study, we used the non-destructive leaf area, fresh and dry weight of leaf prediction models for greenhouse cucumber (*Cucumis sativus*). There are several models which have been reported in the linear estimating leaf area for many crops as mentioned above. It is concluded that there is a close relationship between leaf length, leaf width and other parameters derived from and leaf area, leaf dry and fresh weight. In this study there are several models which are used for estimating leaf area, among these models the best models with the highest R^2 value and lowest RMSE were selected (Table 1).

Table 1. Selected models for leaf area estimation in cucumber with coefficients.

Equation	Regression model	R^2	RMSE	Pr>F
1	Y=-1.520+0.699LW	0.982	12.98	<0.000
2	Y=-2.571+0.353 L^2+0.345W^2	0.983	12.49	<0.000
3	Y=-35.661+4.754L+0.517L^2	0.941	23.29	<0.000
4	Y=-4.043-1.415LW+1.050L^2 + 1.063W^2	0.984	12.09	<0.000
5	Y=-10.655-11.064L+12.843W+0.63L^2	0.981	13.31	<0.000

(Fig. 1) Shows that leaf area of cucumber are high correlation with leaf length and leaf width. Therefore, these two parameters could be considered as the most relevant parameters involved in the leaf area estimation. To estimate leaf, fresh weight (FW) of cucumber, best models with higher R^2 value, lowest RMSE were selected (Table 2). According to the above methods, 5 models used also for estimating leaf dry weight (DW) (Table 3). Results show that there are close relationships between leaves length and leaves width with leaves areas and also leaves fresh weight, but are less confidence with leaves dry weight. Therefore, SPAD value could be used as a more reliable index to predict the model for estimation of the leaves dry weight. Fig. 2, shows the relationship between SPAD value and leaf dry weight.

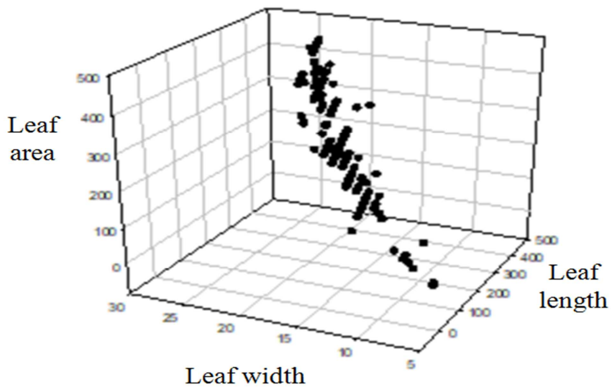

Fig. 1. Relationship among leaf length, leaf width, and leaf area of cucumbers (n= 179).

Table 2. Selected models for leaf fresh weight estimation in cucumber with coefficients.

Equation	Regression model	R^2	RMSE	Pr>F
1	Y=0.0175LW-0.4395	0.934	0.6	<0.000
2	Y=-0.125+0.00741LW+0.000208LWS	0.945	0.58	<0.000
3	$Y=-0.483+0.0101L^2+0.00730W^2$	0.943	0.58	<0.000
4	$Y=1.516-0.385L+0.00503WS+0.0203L^2$	0.94	0.57	<0.000

Table 3. Selected models for leaf dry weight estimation in cucumber with coefficients.

Equation	Regression model	R^2	RMSE	Pr>F
1	Y=0.0036LW-0.0271	0.881	0.18	<0.000
2	Y=-0.0435+0.0000467LWS+0.00134LW	0.887	0.17	<0.000
3	Y=0.0638+0.0000712LWS-0.0000845LS	0.835	0.18	<0.000
4	Y=0.197-0.000327WS+0.0000837LWS	0.886	0.18	<0.000
5	Y=0.174-0.00663L-0.0174W+0.00428LW	0.882	0.18	<0.000

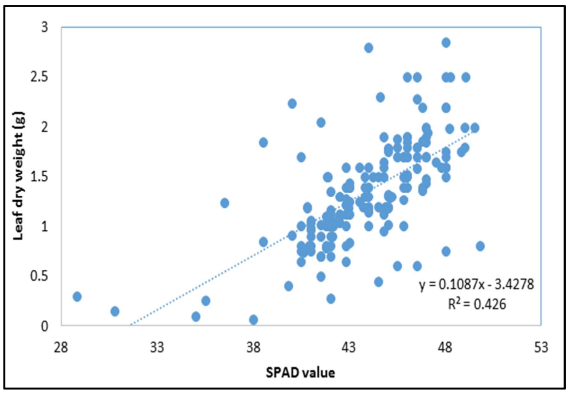

Fig. 2. Relationship between SPAD value and leaf dry weight of cucumbers (n=179).

Model validation

Estimated data plotted against measured data to prove the validity of models for the evaluation of the individual leaf area, fresh and dry weights. Eq. (4) were used to estimate the leaf area (LA), and were closely in agreement with the measured value, with R^2 =0.996 (Fig. 3). Eq. (2) from table (2) and Eq. (2) from table (3) were used to estimate the leaf fresh weight (FW) and leaf dry weight (DW) were also strongly agreed with the measured value, with R^2 =0.974 and R^2 =0.95, respectively (Fig. 4, Fig. 5).

In comparison, between all three parameters, there was moderately lower agreement for dry weight (DW) with the measured value.

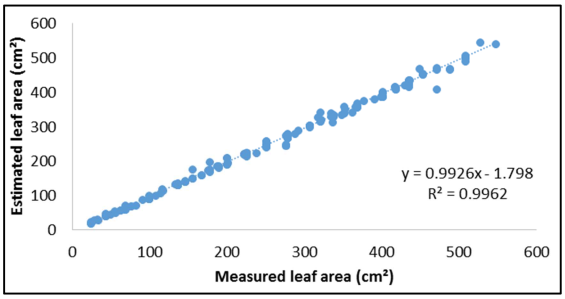

Fig. 3. Comparison of measured and estimated leaf areas of cucumbers (n=179).

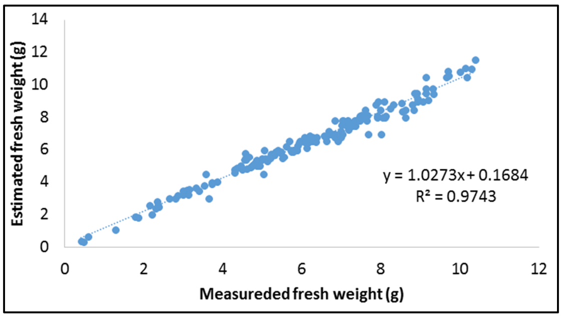

Fig. 4. Comparison of measured and estimated leaf fresh weights of cucumbers (n=179).

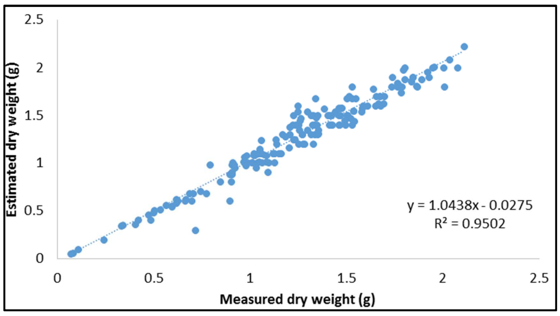

Fig. 5. Comparison of measured and estimated leaf dry weights of cucumbers (n=179).

Discussion

Many researchers have been implemented leaf dimensions to assess leaf area. As a rule, leaf area was considered the dependent variable and leaf length and leaf width as independent variables (Robbins and Pharr 1987, Montero, De Juan *et al.*, 2000, Williams III and Martinson 2003). (Cittadini and Peri 2006) estimated leaf area using a leaf length and width for sweet cherry. (Olfati, Peyvast *et al.*, 2009) using this method to predict leaf area of red cabbage. We used either single variable models or their combinations. In order to avoid exposure to the problems of collinearity between leaf length and leaf width, single variable models were preferred (Williams III and Martinson 2003), but in previous research mentioned above and also our models demonstrated that although single variable models showed relatively high correlations with high R^2 value with leaf area, but showed lower correlations with fresh and dry weight. (Cho, Oh *et al.*, 2007) emphasized these results in their experiment.

In this study, there are high correlations between individual's leaf length and leaf width and leaf area and leaf fresh weight, but because of specific leaf area (SLA) differences, there is a relatively lower correlation in comparison with leaf area and leaf fresh weight, between individual's leaf length and leaf width with leaf dry weight. There is very low probability that the shape of leaves plays an influential role in the model construction. There is another parameter SPAD value, which is shows nutritional conditions in cucumber plants, can be used for higher accuracy of the model specially leaf fresh and dry weight. Therefore, it is a reliable parameter which can be used for model construction.

SPAD value have a higher effect on dry weigh model construction, relative lower effect on fresh weight construction, and approximately have no effect on leaf area model construction. Yield can be predicted with SPAD value, if it has a close relationship between plant fresh and dry weight (Le Bail, Jeuffroy *et al.*, 2005). To validate the accuracy of the model, the estimated growth data and measured data showed good agreement with each other. Leaf area ratio to plant dry weight considered as specific leaf area (SLA) changed with plant growth stages, therefore, its changes may result in the lower accuracy of leaf dry weight prediction models (Cho, Oh *et al.*, 2007).

Therefore, leaf length and width were conducive to precisely leaf area determination of the plant, but not dry weight. Nevertheless, SPAD values increment the accuracy of the model to estimate the leaf dry weight (Caliskan, Odabas *et al.* 2009).

Some researchers have suggested new equipment application such as scanner or laser optic apparatuses to predict plant growth non-destructively, but these methods involve high costs and also may be inaccessible in developing country (Odabas and Ayan 2005). (Williams III and Martinson 2003)used a series of photographic standards of different ages and sizes of tomato leaves. These methods are rapidly and with high relative accuracy, but it required developed equipment and camera and also trained operators. In our method and similar cases, the only thing which is needed is ruler that is available for everyone everywhere to measure leaf length and width. The only limitation to use this method is that it is not too fast, but it is accurate, simple and non-destructive. Furthermore, these types of models enable researchers to implement leaf area measurements on the same plants during the growth period, resulting in reduced experimental variability (NeSmith 1992). It is obvious, more practical computer-based image processing systems will be essential for more rapid and accurate plant characteristic measurements which is not available for all growers.

There are other factors such as temperature and integral radiation that has affected leaf area and dry matter production, respectively (NeSmith 1992). However, in this study, because of greenhouse condition and available equipment limitation, it was very difficult to adapt seasonal variation of temperature and integral radiation. Therefore, according to this study, it is accessible to predict leaf area, leaf fresh and dry weights by using leaf length, leaf width and also SPAD value.

It is predicted that the model suggested in this paper can be used in other similar condition that there is no restriction for water or nutritional supplies, but in conditions that the plants suffer from diseases, pests or weeds it is not recommended. In the beginning, however it could be time-consuming, but when the model was predicted, it can be beneficial for managers and growers in agriculture and can be considered as a decision support system tool since it can be beneficial for monitoring crop growth while growing season progressed, and provide practical information about all the nutritional and sources demand crop need for biomass production. Therefore, the leaf area estimation models that intend to forecast leaf area non-destructively can have numerous benefits for the growers in agricultural experiments.

Conclusions

In general leaf area measurement is a key factor in experiments where some physiological events such as light, photosynthesis, respiration, plant water supplies and transpiration is being investigated (Centritto, Loreto *et al.* 2000). Plus, leaf number and leaf area of the plant are influential in terms of cultural practices such as training, pruning, irrigation, fertilization etc (Caliskan, Odabas *et al.*, 2009). As a general result, leaf length and leaf width have a strong relationship with leaf area and also leaf fresh weight, but an influential factor SPAD value were needed to predict leaf dry weight in line with leaf length and leaf width. With using these models for leaf area estimation, sampling efforts and cost could be decreased, and may increase accuracy where samples of leaf size are out of reach. Moreover, these three models with high R^2 value and low RMSE could be used by growers in the cucumber greenhouse in the site of experiment which have carried out, because of environmental and physiological similarity for cucumber plant growth period. There are other studies for leaf area estimation in cucumber but in this study using most available staffs according to our country facilities, it could be more functional. For more precise modelling, environmental factors and computer systems as well as growth factors should be considered.

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