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Full Length Article

Effect of Deficit Irrigation on the Estimation of Cucumber Leaf Area under Greenhouse Conditions

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Abstract

Simple, accurate, and nondestructive methods of quantifying leaf area are important for many experimental comparisons. The aims of this study were to establish models to estimate the leaf area of cucumber plants grown under greenhouse conditions and evaluate the effects of different irrigation water levels on this estimation. Six irrigation levels based on evaporation from a Class A Pan (I_{20}:20%, I_{40}:40%, I_{60}:60%, I_{80}:80%, I_{100}:100% and I_{120}:120% of) were utilized. A total of about 690 leaves from different irrigation levels were collected and relationships among leaf width (W), length (L) and leaf area (LA) were investigated. Estimation models were derived using Multiple Linear Regression (MLR) method and Full Cross Validation was used to validate the models. The Root Mean Square Error of Prediction (RMSEP) and R^2 values were used to compare the models. It was found that mean leaf area values were affected by different irrigation amounts. Mean leaf areas in I_{20} and I_{40} were significantly lower than the other irrigation treatments. A strong non-linear relationship was found between cucumber W, L and LA ($R^2 \geq 0.94$). Three LA prediction models with W (Model 1), L (Model 2) and W and L (Model 3) were developed for each irrigation levels and for the combined data. RMSEP and R^2 values were 16.3 cm^2 and 0.99 for the model of combined data for all leaves from all different irrigation treatments. In conclusion, for various physiological and morphological studies, model developed in this study can be used as non-destructive technique to estimate cucumber leaf area from leaf size for each irrigation levels and for the combined data in a shorter time with less labor and budget. © 2018 Friends Science Publishers

Keywords: Cucumber; Deficit irrigation; Leaf area estimation; Water stress

Introduction

Cucumber (*Cucumis sativus*) is an important vegetable, which is the second most important source of vegetable cultivated in open fields and greenhouses in Turkey with a production amount of about 1.8 million tons (TURKSTAT, 2017). Turkey ranks fourth in cucumber production in the world after China, Russia, and Iran with a total yearly production of 1,780,472 tons (FAOSTAT, 2014). Although cucumbers have a water content of about 95%, they are rich in vitamins A and C. In addition, since fruit juice is alkaline, it is also used in cosmetic products (Kaygisiz, 2000). Due to these characteristics, many scientific studies have been established on cucumber.

Leaves are the most important organs in which light energy is absorbed and used in the production of the metabolites necessary for plant growth through photosynthesis. In an environment where other environmental conditions are optimal, plant production is determined by the amount of light energy the plant can capture (Kanemasu *et al.*, 1985). Accordingly, leaf area is the most important factor promoting plant growth and productivity (Kandiannan *et al.*, 2002). The amount of leaf area is the main factor in plant development due to its effect on the amount of absorbed photosynthetic radiation (Lawlor, 1995). Leaf is an important plant organ for the photosynthesis as well as evapotranspiration (ET). For this reason, the measurement of leaf growth is a crucial application in evaluating most agronomic and physiological studies including plant development (Guo and Sun, 2001).

Usually grown in the greenhouse, cucumber (*Cucumis sativus* L.) plants need much water during their life time, so drought stress is a limiting factor for their growth and development (Wang *et al.*, 2012). Total leaf area depends on the number and size of leaves which are negatively affected by water stress and nutrient deficiency (Longnecker, 1994). Reduced intake of water and nutrients in plants leads to a decrease in the absorption of photosynthetic radiation and therefore slowing of photosynthesis (Koc and Barutcular, 2000). As the leaf area is related to photosynthetic activity, it also affects carbohydrate metabolism, dry matter production, yield and quality (Centritto *et al.*, 2000).

Classical methods for leaf area estimation deal with pruning or detaching the leaves and measuring the leaf area. This method is tedious and time consuming. Leaf detaching methods are generally not preferred, especially when working with small plots or a small number of plants. In addition, some other methods require a high level of

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technical knowledge, operation and maintenance of expensive equipment. Researchers are often unable to provide these conditions. For this reason, it is important to develop a simple, inexpensive and non-destructive method for estimating leaf area.

Non-destructive leaf area measurement without detaching the leaves is more preferred due to the ability of the investigator to provide studying on the same plant and leaf thus to reduce the high variation that can emerge in the trials. In addition, the ability to determine the leaf area with simple linear measurements cancels out the need for expensive and complex leaf area measurement devices. For this reason, prediction with the aid of mathematical models using leaf size data obtained by linear leaf measurements is very useful in plant studies (Camas *et al.*, 2005).

There have been some studies to determine the leaf area for different plants including grapevines (Manivel, 1974), strawberry (Strik and Proctor, 1985), maize (Stewart and Dwyer, 1993), lettuce (Guo and Sun, 2001), safflowers (Camas *et al.*, 2005), chestnut (Serdar and Demirsoy, 2006), bean (Peksen, 2007), hazelnut (Cristofori *et al.*, 2007), oregano (Caliskan *et al.*, 2010), citrus (Femat-Diaz *et al.*, 2011), and tomato (Kucukonder *et al.*, 2016). There have also been several studies on cucumber plants under different conditions such as Blanco and Folegatti (2005) suggested a prediction equation for leaf area in the conditions of different salinity and grafting for greenhouse cucumbers while Cho *et al.* (2007) suggests a prediction equation in the conditions of using leaf length, width, and SPAD values for hydroponically grown cucumbers. However, to the best of our knowledge, there is no study on the estimation of leaf area of crops under water stress caused by deficit irrigation application. The answer to the question whether or not a separate model is necessary for plant leaves under different irrigation treatments was not studied before.

For this reason, the aim of this study was to use the leaf size (width and height) to non-destructively predict the leaf area of cucumber plants subjected to different levels of irrigation in greenhouse conditions and to investigate the effect of the limited watering on the leaf area estimation. In this way, it could be possible to determine the leaf area in shorter time, with less labor and less errors thanks to a simple equation that can easily be used on spreadsheet software. The model developed from this study will be useful for researchers in the calculation of leaf area used in physiological, morphological and other studies on cucumber.

Materials and Methods

Experimental Infrastructure

The cucumber plants bought from a private company (Cukurova Tarim, Mersin, Turkey) in seedling stage were grown in a plastic covered greenhouse located near Samandag city (Latitude: 36°06' N; Longitude: 35°57' E) of Hatay province, Turkey. The cucumber variety was "Ural-F1" which is one of the common varieties used in the study region. The region where the study area is located has a typical Mediterranean climate with warm and rainy winters and hot and dry summers. The study area had six parcels, one parcel for each irrigation treatment (Fig. 1). Each study block consisting of two plant rows was 12.0 m x 1.5 m with a total area of 18 m^2. Cucumber seedlings were planted in double rows with 50 cm plant spacings and 50 cm row spacings while 100 cm space was available between the blocks (Fig. 1). The study was arranged in randomized multiple block design with three replications.

Six irrigation levels based on evaporation from a Class A Pan (I_{20}:20%, I_{40}:40%, I_{60}:60%, I_{80}:80%, I_{100}:100% and I_{120}:120%) were utilized. The amounts of irrigation water applied along with the plant water consumptions are given in Table 1.

Leaf Measurements

Leaf width and length dimensions (in cm) were measured and used to define the leaf shape (Fig. 2) (Stewart and Dwyer, 1993). The widest part of the foliage was taken as leaf width (W) as leaf length (L) was defined as the distance between the two furthest points (from lamina tip to the point of petiole intersection along the midrib) of the foliage.

Approximately 115 leaf samples from each irrigation level making a total of about 690 leaf samples were collected. Leaf widths (W) and lengths (L) of the leaf samples were measured with a digital caliper (Asimeto IP67 model, Asimeto Co, Weißbach, Germany). Leaf area measurements (in cm^2) were taken using an electronic planimeter (X-Plan 300C+, Ushikata Mfg. Co. Ltd., Tokyo, Japan).

Statistical Analysis

The leaf area means of different irrigation levels (I_{20} through I_{120}) were compared and the effect of water stress on the average leaf area was examined. In addition, the relationship between leaf width, leaf length, and leaf area were used to develop estimation models to predict leaf areas from leaf width and length. Linearization procedure was used since a non-linear relationship existed between the leaf area and the leaf width and length parameters (Gomez and Gomez, 1984).

In mathematical modeling, UnScrambler software (Version 9.7, Camo Sofware, Oslo, Norway) was used. Full Cross Validation method was utilized to test the models. Root Mean Square Error of Prediction (RMSEP) and R^2 values were used to compare the performances of the different models. RMSEP values were calculated using the formula given below (Esbensen, 2009):

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{N}\left(y_i - y_{i,ref}\right)^2}{n}}$$

Where; y_i: predicted leaf area (cm^2), $y_{i,ref}$: measured

Table 1: Applied irrigation water (I) and plant water consumption (total evapotranspiration, ET)

Irrigation treatment	I (mm)	ET (mm)
I_{20}	121	294
I_{40}	202	349
I_{60}	283	400
I_{80}	364	451
I_{100}	445	495
I_{120}	526	539

Table 2: Effect of different irrigation water amounts on the mean leaf area

	I_{20}	I_{40}	I_{60}	I_{80}	I_{100}	I_{120}
Number of leaves (n)	100	141	97	93	132	131
Mean leaf area (cm^2)	183.8	198.8	270.0	280.1	253.0	292.1
Standard deviation	114.6	155.0	172.1	187.0	156.5	188.5
Standard error	11.5	13.1	17.5	19.4	13.6	16.5

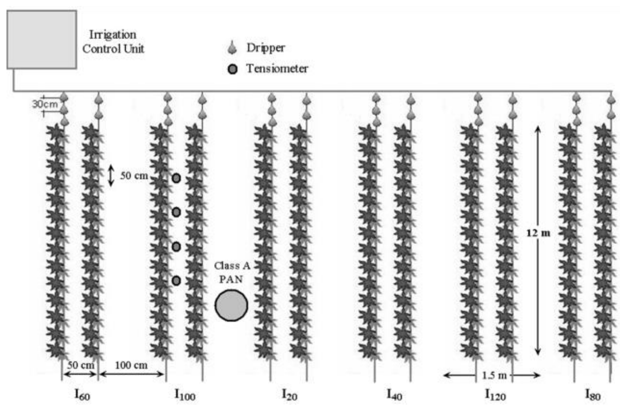

Fig. 1: Study design depicting the surface drip irrigation and plant locations

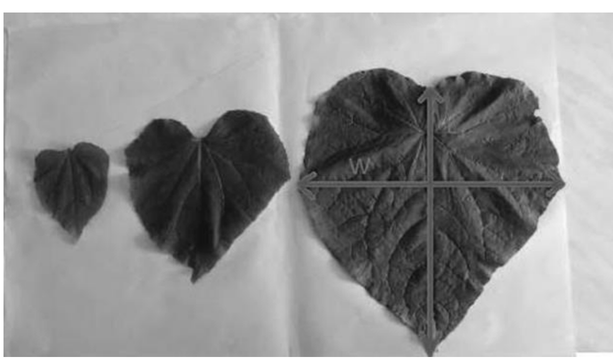

Fig. 2: Cucumber leaf length (L) and width (W)

leaf area (cm^2), and *n*: number of leaf samples.

Results

The effect of different irrigation levels on the mean leaf area was examined (Table 2). Mean leaf area values of plants grown at I_{20} and I_{40} irrigation levels (183.8 cm^2 and 198.8 cm^2) were found to be significantly lower than those of other irrigation levels (≥253.0 cm^2) (Table 2). The highest mean leaf area (292.1 cm^2) was obtained from the I_{120} irrigation level. As shown in Table 2, the mean leaf area values increased as the amount of applied irrigation water increased. It was seen that water applications encouraged plant vegetative growth and increased the leaf area.

In the study, the relationships between leaf width, leaf length and leaf area of cucumber plants grown at different irrigation levels (I_{20}, I_{40}, I_{60}, I_{80}, I_{100} and I_{120}) were also investigated and illustrated in Fig. 3. It was found that there existed a high correlation ($R^2 \geq 0.94$) and a non-linear relationship between leaf width and leaf area as well as leaf length and leaf area (Fig. 3). R^2 values on the figures represent the result of the model fit between the leaf area and the squared leaf width and squared leaf length.

In order to understand whether different models are required for each irrigation level, the relationship between leaf width, leaf length and leaf area is examined on a single graph by combining data for all irrigation levels (Fig. 4). In this inquiry, it was seen that there was no significant dispersion or variation in the data. As different irrigation levels did not affect the relation between leaf width, leaf length and leaf area significantly, it was stipulated that one single model could be used to predict leaf area from leaf width or leaf length for all irrigation levels (Fig. 4). For this reason, the measured data were combined for all irrigation levels during the modeling phase. In addition, as indicated by the graphs obtained from the combined data, leaf width and leaf area relationship ($R^2 = 0.98$) showed a slightly more homogeneous data distribution than leaf length and leaf area relationship ($R^2 = 0.96$). The R^2 values given on the graphs are from the leaf area estimation model using the squared leaf width and squared leaf length.

Since there was a non-linear relationship between leaf width and leaf area and leaf length and leaf area (Fig. 3 and 4), data transformation was performed by taking the square of the leaf width and leaf length to make the data linear. The data before and after the linearization were given in Fig. 5. It can be seen that the linearization by squaring worked well for the current data.

Three different models were computed for each of the six irrigation levels (I_{20} through I_{120}) as well as combined data and studied in the modeling phase of the data analysis to find the best estimation model predicting the leaf area (LA) from the leaf width (W) and leaf length (L):

Model 1: Predicting LA from only squared W ($LA=f(W^2)$)
Model 2: Predicting LA from only squared L ($LA=f(L^2)$)
Model 3: Predicting LA from both squared W and squared L ($LA=f(W^2, L^2)$).

Model equations and corresponding RMSEP and R^2 values for each irrigation level (I_{20} through I_{120}) as well as combined data are presented in Table 3.

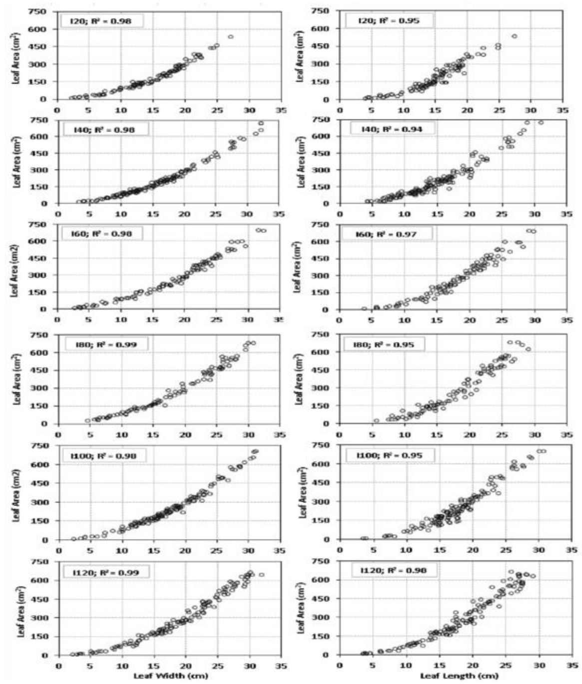

Fig. 3: Relation of leaf area to leaf width and leaf length for different irrigation levels (I_{20}, I_{40}, I_{60}, $I_{80,}$ $I_{100,}$ I_{120})

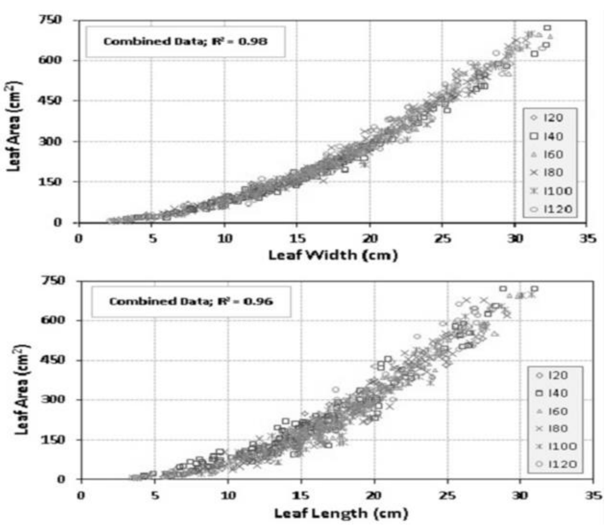

Fig. 4: Relation of leaf area to leaf length and leaf width for combined data

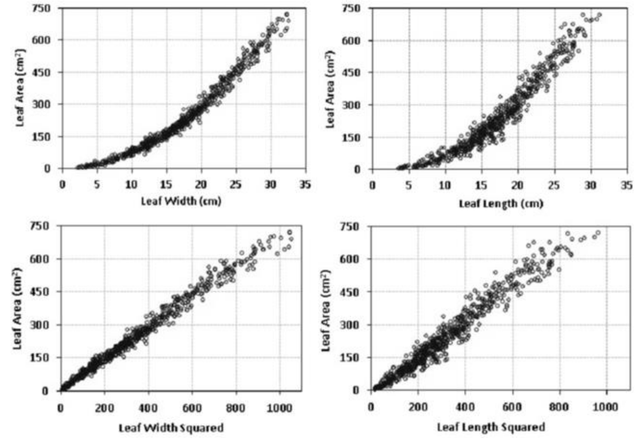

Fig. 5: Relation of leaf area to leaf length and leaf width for combined data before linearization (top) and after linearization (bottom)

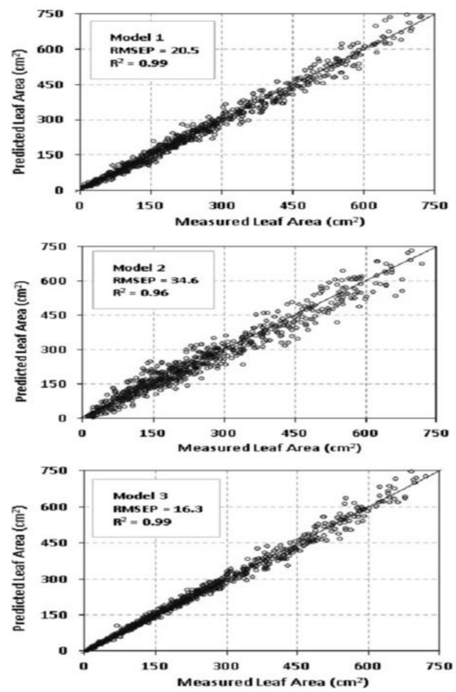

Fig. 6: Measured vs. predicted leaf areas for three different models for the combined data

In addition, graphs showing the relationship between the measured leaf area and the estimated leaf area are given for combined data for all irrigation levels in Fig. 6.

Discussion

Water stress is a limiting factor for crop growth and development (Wang *et al*., 2012). Leaf area depends on the size of leaves which are negatively affected by water stress and nutrient deficiency (Longnecker, 1994). Significant numbers of studies were carried out to predict leaf area from leaf size for different plants including strawberry, maize, lettuce, safflowers, chestnut, bean, hazelnut, oregano, citrus, tomato and grapevines (Strik and Proctor, 1985; Stewart and Dwyer, 1993; Guo and Sun, 2001; Camas *et al.*, 2005; Serdar and Demirsoy, 2006; Cristofori *et al.*, 2007; Peksen, 2007; Caliskan *et al.*, 2010; Femat-Diaz *et al.*, 2011; Kucukonder *et al.*, 2016).

Table 3: Leaf area prediction models and their RMSEP and R^2 values

Irrigation Level	Model No	Model Equation	RMSEP (cm^2)	R^2
	Model 1	$LA = 6.1833 + 0.7241 * (W)^2$	14.5	0.98
I_{20}	Model 2	$LA = -14.3952 + 0.8146 * (L)^2$	31.8	0.92
	Model 3	$LA = -1.8631 + 0.5731 * (W)^2 + 0.1872*(L)^2$	12.2	0.99
	Model 1	$LA = 8.4439 + 0.6672 * (W)^2$	14.6	0.99
I_{40}	Model 2	$LA = -4.8366 + 0.8049 * (L)^2$	31.1	0.96
	Model 3	$LA = 3.4703+0.5198 * (W)^2 + 0.1859*(L)^2$	12.1	0.99
	Model 1	$LA = 12.5957 + 0.6927 * (W)^2$	21.9	0.98
I_{60}	Model 2	$LA = -23.9969 + 0.8374 * (L)^2$	33.6	0.96
	Model 3	$LA = -2.0919 + 0.4826 * (W)^2 + 0.2642*(L)^2$	18.0	0.99
	Model 1	$LA = 8.5587 + 0.7140 * (W)^2$	22.1	0.99
I_{80}	Model 2	$LA = -33.3219 + 0.8771 * (L)^2$	37.8	0.96
	Model 3	$LA = -7.2621+ 0.5126 * (W)^2 + 0.2586*(L)^2$	17.8	0.99
	Model 1	$LA = 10.3602 + 0.7001 * (W)^2$	17.5	0.99
I_{100}	Model 2	$LA = -28.5522 + 0.8301 * (L)^2$	33.9	0.95
	Model 3	$LA = -3.6852+ 0.5202 * (W)^2 + 0.2252*(L)^2$	13.7	0.99
	Model 1	$LA = 5.7216 + 0.7131 * (W)^2$	25.8	0.98
I_{120}	Model 2	$LA = -15.0768 + 0.8339 * (L)^2$	37.8	0.96
	Model 3	$LA = -5.7699+ 0.4775 * (W)^2 + 0.2881*(L)^2$	20.0	0.99
Combined	Model 1	$LA = 8.3319 + 0.7015 * (W)^2$	20.5	0.99
Data	Model 2	$LA = -18.3528 + 0.8308 * (L)^2$	34.6	0.96
	Model 3	$LA = -2.8849+ 0.5023 * (W)^2 + 0.2474*(L)^2$	16.3	0.99

In the current study, it was observed that different irrigation levels had a significant effect on mean leaf area. Mean leaf area values of plants grown at I_{20} and I_{40} irrigation levels (183.8 cm^2 and 198.8 cm^2) were found to be significantly lower (Table 2). Leaf area reductions due to deficit irrigation were reported by Lanari *et al.* (2015) for grapevine. They reported that the reduction in water availability of 40% of daily evapotranspiration limited shoot and leaf growth affecting leaf number, shoot elongation, and leaf area. It seemed that the plants slowed down the development especially on the leaves to adapt to the water stress. Guendouz *et al.* (2016) also declared that the differences between leaf areas of stressed and non-stressed conditions equaled to 17.2% for durum wheat cultivars. In the present study, improvement of leaf growth was observed as the water restriction decreased and the plant water need was met. The highest mean leaf area (292.1 cm^2) was obtained from the I_{120} irrigation level (Table 2). The mean leaf area values increased as the amount of applied irrigation water increased. It was seen that water applications encouraged plant vegetative growth and increased the leaf area.

In the present study, a high and non-linear relationship was found between leaf area and leaf length and width (Fig. 3); so, taking square of leaf width and leaf length gave satisfactory results for the estimation of the leaf area from the squared leaf width and squared leaf length. Therefore, a high correlation between squared leaf width, squared leaf length and leaf area were found ($R^2 \geq 0.94$) (Fig. 5). Similar non-linear relationships between leaf area and leaf length and width were also reported by other researchers for various crops (Blanco and Folegatti, 2005; Caliskan *et al.*, 2010; Eftekhari *et al.*, 2011; Kucukonder *et al.*, 2016). Squaring the leaf width and leaf length was one of the mostly used transformation method for leaf area studies (Blanco and Folegatti, 2005; Camas *et al.*, 2005; Serdar and Demirsoy, 2006; Cristofori *et al.*, 2007; Caliskan *et al.*, 2010; Eftekhari *et al.*, 2011; Kucukonder *et al.*, 2016).

The use of leaf width to predict the leaf area compared to leaf length provided slightly better accuracy for all irrigation levels (I_{20} through I_{120}) and the combined data (Table 3). Also, it was found out that RMSEP values for I_{20} and I_{40} irrigation levels for the Model 3 (12.2 and 12.1 cm^2 respectively) were significantly lower than the RMSEP values for other irrigation levels (I_{60} through I_{120}) (between 13.7 and 20.0 cm^2) (Table 3). This could be explained based on the finding that the mean leaf sizes and areas were significantly lower for I_{20} and I_{40} irrigation levels compared to other irrigation treatments (I_{60} through I_{120}) (Table 2). In addition, the use of leaf width and leaf length together in the prediction of leaf area provided lower RMSEP values for all irrigation levels and combined data (Table 3). Concerning the combined data, for the model estimating leaf area from squared leaf width (Model 1), RMSEP value was 20.5 cm^2 and R^2 value was 0.99 (Table 3); whereas, the RMSEP and R^2 values were 34.6 cm^2 and 0.96, respectively for the model estimating the leaf area from the squared leaf length (Model 2). The use of leaf width to predict the leaf area compared to leaf length provided slightly better accuracy. The model estimating leaf area from both leaf width and leaf length together (Model 3) had a significantly better performance than that of Model 1 and Model 2 (RMSEP of 16.3 cm^2 and R^2 of 0.99). This shows that Model 3 can predict the leaf area with higher accuracy than the other two models for combined data for all irrigation levels (I_{20} through I_{120}). The use of leaf width and leaf length together in the prediction of leaf area provided lower RMSEP values for all irrigation levels and combined data (Table 3). Similar results were reported for grapevine leaves by Eftekhari *et al.* (2011) that the use of a non-linear model in which leaf width and length are used together gave more precise results for leaf area estimation than the other linear models in which only width or length of the leaves were used.

The study results show that leaf area of cucumber plants can be determined non-destructively with high accuracy by using the leaf width and leaf length for all irrigation levels without a need for an expensive leaf area measurement device. In this way, it could be possible to determine the leaf area in shorter time, with less labor and less errors thanks to a simple equation that can easily be used on spreadsheet software. A strong non-linear relationship was found between cucumber leaf width, leaf length and leaf area for all irrigation treatments (I_{20} through I_{120}) ($R^2 \geq 0.94$).

Cucumber leaf area can be determined from either leaf width or leaf length; however, the use of both leaf width and leaf length together provided a higher prediction accuracy. The study showed that the cucumber leaf area could easily be determined non-destructively from either leaf width or leaf length or using both of them without a need for an expensive leaf area measurement device. Using separate models for the plants obtained from the irrigation levels of I_{20} and I_{40} compared to other irrigation treatments provided lower RMSEP values.

Conclusion

It is possible to determine the cucumber leaf areas non-destructively without detaching the leaves from the plant in a shorter time with less labor and budget. The model developed from this study will be useful for researchers in the calculation of leaf area used in physiological, morphological and other studies on cucumber.

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