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Individual modelling of leaf area in cress and radish using leaf dimensions and weight

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ABSTRACT

Purpose: The objective of this study was to establish equations to estimate leaf area (LA) using length (L), width (W), fresh weight (FW) and dry weight (DW), length × width (L×W), width/length (W/L) of cress leaves as a leafy vegetable and radish leaves as a root vegetable. **Research method:** An experiment was carried out under greenhouse conditions to study the relationship between leaf dimension and weight with LA of these two vegetable plants. Observed LA was obtained by an automatic measuring device and leaf dimensions were measured by a ruler. Regression analyses of LA versus L, W, FW, DW, L×W and W/L led several models that could be used for estimating the area of individual cress and radish leaves. **Findings:** A linear model employing FW as an independent variable [LA=0.295 (Fresh W.)+ 1.430] resulted the most accurate estimate (R^2 = 0.912, RMSE = 1.52) of cress LA. For radish, a linear model using W as an independent variable [LA=22.50 (W) + 7.46] showed the most accuracy (R^2 = 0.874, RMSE = 11.26) for estimating LA. Validation of the regression models showed that the correlation between measured and simulated values using these equations was quite acceptable for radish and cress (R^2 = 0.922, 0.876), respectively. **Research limitations:** Evaluation of more leafy vegetables possibly had better results. **Originality/Value:** The results showed that cress and radish LA could be monitored quickly, accurately, and non-destructively by using the leaf FW and leaf W models, respectively.

INTRODUCTION

Cress or garden cress is rich in vitamins A and C, iron, and calcium. It contains isothiocyanates with antibacterial properties (Xue, 2001). Cress is used to increasing sexual power and acts as a diuretic and purgative. It is also used to treat pleurisy, dropsy, asthma, and coughing with nausea, vitamin C deficiency, liver disease, hemorrhoids, and as an abortifacient (Perry, 1980). Radish leaves usually are medium green and lobed and have a rough texture that contains high amounts of vitamin A, B, C and calcium, pectin, phytin, iron, manganese, and copper. It is used to treat asthma, cough, diarrhea, dysentery, and malnutrition (Xue, 2001).

Green leaves play a critical role in crop growth and development. Leaves receive the photosynthetically active radiation (PAR) and ultimately utilize it producing dry matter (Demarty et al., 2007). Factors related to leaf area, such as photosynthesis and transpiration rate, directly affect the plant productivity, which makes the leaf area (LA) a key variable in physiological studies involving plant growth, light interception, photosynthetic efficiency, evapotranspiration, and responses to fertilizers and irrigation (Blanco & Folegatti, 2005). An accurate LA measurement plays a key role in understanding crop growth and its environment (Kumar, 2009). Leaf area measurements especially under field conditions are often destructive and time-consuming (Tsialtas & Maslaris, 2005). However, leaves may have complex shapes making LA determination more difficult and subject to larger errors. Furthermore, it is not possible to make a successive measurement of the same leaf, and plant canopy would be damaged which cause problems to other measurements of the experiment (Tsialtas & Maslaris, 2005).

A large number of methods, either destructive or not, have been developed to measure LA. The LA can be determined by using some expensive instruments and developed prediction models (Robbins & Pharr, 1987). Recently, new instruments, tools, and machines such as hand scanners and laser optic apparatuses have been developed for leaf area measurements that these are very expensive and complex devices for both basic and simple studies. Despite various methods used to estimate LA (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 & Marshall, 1971), dry matter and leaf specific area (Lee & Heuvelink, 2003; Lieth & Pasian, 1991). 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 LA without harming the plant can provide researchers with many advantages in horticultural experiments as following: 1) the models enable researchers to measure LA on the same plants during the plant growth period and may reduce variability in experiments (Nesmith, 1992) reliable models eliminate the need for expensive instruments and labor; 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 and 5) modeling 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 cucumbers (Blanco & Folegatti, 2005; Cho et al., 2007), zucchini squash (Rouphael et al., 2006), sunflower (Rouphael et al., 2007), hazelnut (Cristofori et al., 2007), faba bean (Peksen, 2007), stevia (Ramesh et al., 2007), persimon (Cristofori et al., 2008), potato (Busato et al., 2010), rose (Rouphael et al., 2010), gladiolus (Schwab et al., 2014), coneflower (Aminifard et al., 2016) and Crotalaria juncea (De Carvalho et al., 2017). However, based on the literature review, no model has been developed to predict cress and radish LA. Since each species shows characteristic patterns of leaf morphology, it is necessary to generate specific models of leaf area estimation. Therefore, the main aim of this study was to find the best model and allometric correlation based on estimate LA for two garden vegetable plants.

MATERIALS AND METHODS

Soil cultured cress and radish plants were grown under greenhouse conditions in the Faculty of Agriculture located in University of Birjand from January to March 2015. Air temperature and relative humidity ranged between 24 °C (day) and 21 °C (night) and 60-70%, respectively. The light intensity was about 40.5 mol. m^{-2} s^{-1}. Irrigation and nutrition were performed based on conventional practices.

About 50 days after planting, 100 cress plants were selected and one fully-expanded leaf sample was prepared from each plant. While for radish, once their roots grew adequate, which was about 60 days after planting, 180 plants taken out completely. Each sample (plant) was separately taken into plastic bags and transported to the laboratory for destructive measurement of LA using LA meter (Delta T-Devices Ltd., Burwell, and Cambridge, England). Thereafter, leaf fresh weight (FW), length (L) and width (W) of each sample were measured. The maximum L and W of all leaves were measured by a ruler. Width was evaluated from the widest area with a precision of 1 mm, and L was calculated from the top to the end of the blade without petiole with a precision of 1 mm. The samples were dried in oven at 80 °C for 24 h and dry weights were then measured. The fresh and dry weights (DW) of leaves were measured with a digital balance of accuracy of 0.001 g. Mean, maximum and minimum of all samples were calculated.

Multiple regression analysis was performed on the data. A search for the best model to predict LA was conducted with various subsets of the independent variables such as: L, length square (L^2); W, width square (W^2); length × width (L×W); FW, DW, length + width (L+W); and width/length (W/L). The best model was selected based on the coefficient of determination (R^2), root means square of error (RMSE), efficiency (E), index of agreement (D), variance inflation factor (VIF) and tolerance value (T).

The relationship between leaf area as a dependent variable and independent variables was determined using regression analysis on data from 50 leaves. Coefficients of determination (R^2) were calculated and the equation that presented the highest R^2 was used in the estimations. Then estimated and measured leaf areas were compared by testing the significance of the regression equation and degree of goodness of fit (R^2) between estimated and observed values. The final model was selected based on the combination of the highest R^2 and the lowest root mean square error (RMSE). Root mean square error of estimation was calculated based on Janssen and Heuberger (1995):

Equation 1: $RMSE = [\sum (Pi-Oi)^{2}/N]^{0.5}$

Where P = predicted LA, O = measured LA, N = 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 & Raktoe, 1981):

Equation 2: $E_{12} = MSE_1/MSE_2$

Where MSE_1 and MSE_2 are the mean square error of the predictions with model 1 and 2, respectively:

Equation 3: $MSE_1 = \Sigma (P_1i - Oi)^2$, $MSE_2 = \Sigma (P_2i - Oi)^2$

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 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:

Equation 4: $d = 1 - [\Sigma (P_i-O_i)^2] / \Sigma [(|P_i-\bar{O}|) + (|O_i- \bar{O}|)]^2$

Where $\bar{O}$ is the average of the observed values.

For detecting collinearity, the VIF (Marquardt, 1970) and the T (Gill, 1986) were calculated:

Equation 5: $VIF=1/1-r^2$, $T=1/VIF$

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.

To validate the models, about 100 leaves of each cress and radish plants were taken and actual leaf area, leaf fresh weight, and width were determined by the previously described procedures. Leaf area of individual leaves was predicted using the best model from the calibration experiment and was compared with the actual leaf area. The slope and intercept of the model were tested to see if they were significantly different from the slope and intercept of the 1:1 correspondence line (Dent & Blackie, 1979). Regression analyses were then conducted.

RESULTS AND DISCUSSION

Minimum and maximum data for considering independent variables about both plants are shown in Table 1. Each of these variables was used to evaluate its relationship with actual LA, and power, linear and exponential relationships were studied.

The results indicated that among tested equations, the third equation considering leaf FW (LA=0.295 FW +1.430) showed the highest R^2 (0.912) and lowest RMSE (1.52) and the second equation employing leaf W (LA=22.50 W +7.46) with the highest R^2 (0.875) and the lowest RMSE (11.26) for cress and radish plants, respectively, means these are suitable equations for non-destructive measurements of LA compared with others. These equations indicated that leaf FW for cress and leaf W for radish strongly related with actual LA (Table 2, Fig. 1, and Fig. 2).

Regarding Table 3, it is clear that the highest SE and the lowest MSE are obtained for equation 3 for cress and equation 2 for radish, which confirmed the goodness of these models to estimate LA. The highest index of agreement (D) value obtained for the first, fourth and seventh equations for cress (Table 3). There was no difference of d value for different equations related to radish (Table 3). The VIF and T of these data (Table 3) showed no correlation between variables especially leaf dimensions. Data showed the low difference between RMSE and MSE related to equations 3 and 5 for cress and equations 2 and 3 for radish (Tables 2 and 3). Statistic E was used to compare these equations and models, and results indicated that model 3 was better than model 5 for cress and for radish model 2 was better compared to model 3 (Table 4). To validate the developed models for the estimation of individual leaf area, measured and predicted data were compared. The leaf areas, estimated by equations 3 and 2, strongly agreed with the measured value, with R^2 = 0.922 and R^2 = 0.876 for cress and radish, respectively (Fig. 3).

Table 1. Mean, minimum and maximum values for measured independent variables of cress and radish leaves

Plant parameters	Sample No.	Mean ± SDᵻ	Min.	Max.
		Cress		
Length (cm)	100	6.44±4.23	4.00	11.50
Width (cm)	100	3.20±4.21	2.00	5.30
Fresh weight (g)	100	35±4.97	12.00	92.00
Dry weight (g)	100	0.31±0.47	0.12	0.83
Length×Width (cm^2)	100	21.88±4.48	8.20	58.65
$Width^2$ (cm)	100	10.96±4.16	4.00	28.09
Length+Width (cm)	100	9.70±3.88	6.40	14.20
		Radish		
Length (cm)	180	17.40±2.38	9.00	25.50
Width (cm)	180	3.19±2.99	0.82	7.19
Fresh weight (g)	180	8.02±2.88	3.50	14.10
Length×Width (cm^2)	180	58.93±28.86	7.65	178.31
$Length^2$ (cm)	180	313.32±23.63	81.00	650.25
$Width^2$ (cm)	180	12.00±0.16	1.00	52.00
Length+Width (cm^2)	180	20.59±2.68	9.85	31.99
Width/Length (cm)	180	0.18±2.66	0.07	0.42

ᵻ Standard deviations (SD), minimum (Min) and maximum (Max), length (L), width (W), fresh weight (FW), dry weight (DW), $length^2$ (L^2), $width^2$ (W^2), length× width (L × W), length + width (L + W), fresh $weight^2$ (FW^2) and dry $weight^2$ (DW^2).

Table 2. Regression models, R^2 and RMSE used for leaf area estimation

Plant parameters	Equation No.	Equation	R^2	RMSE
		Cress		
Length (cm)	1	LA=2..969 (L)-8.023	0.855	1.72
Width (cm)	2	LA=5.691(W)-7.060	0.810	2.02
Fresh weight (g)	3	LA=0.295 (FW)+1.430	0.912	1.52
Dry weight (g)	4	LA=33.56 (Dry W.)+1.721	0.861	1.88
Length×Width (cm^2)	5	LA=0.467(L×W)+0.907	0.890	1.56
$Width^2$ (cm)	6	LA=0.847 (W^2)+1.968	0.808	2.01
Length+Width (cm)	7	LA=2.403 (L+W)-12.18	0.719	2.40
		Radish		
Length (cm)	1	LA=7.370 (L)-48.97	0.557	21.15
Width (cm)	2	LA=22.50 (W)+7.46	0.874	11.26
Fresh weight (g)	3	LA=15.00 (FW)-40.83	0.816	13.58
Length×Width (cm^2)	4	LA=0.847 (L×W)+29.39	0.814	13.70
$Length^2$ (cm)	5	LA=0.203 (L^2)+15.62	0.547	21.40
$Width^2$ (cm)	6	LA=2.784 (W^2)+46.05	0.793	14.44
Length+Width (cm)	7	LA=6.142 (L+W)-47.20	0.703	17.32
Width/Length (cm)	8	LA=494.6 (W/L)-9.118	0.692	17.64

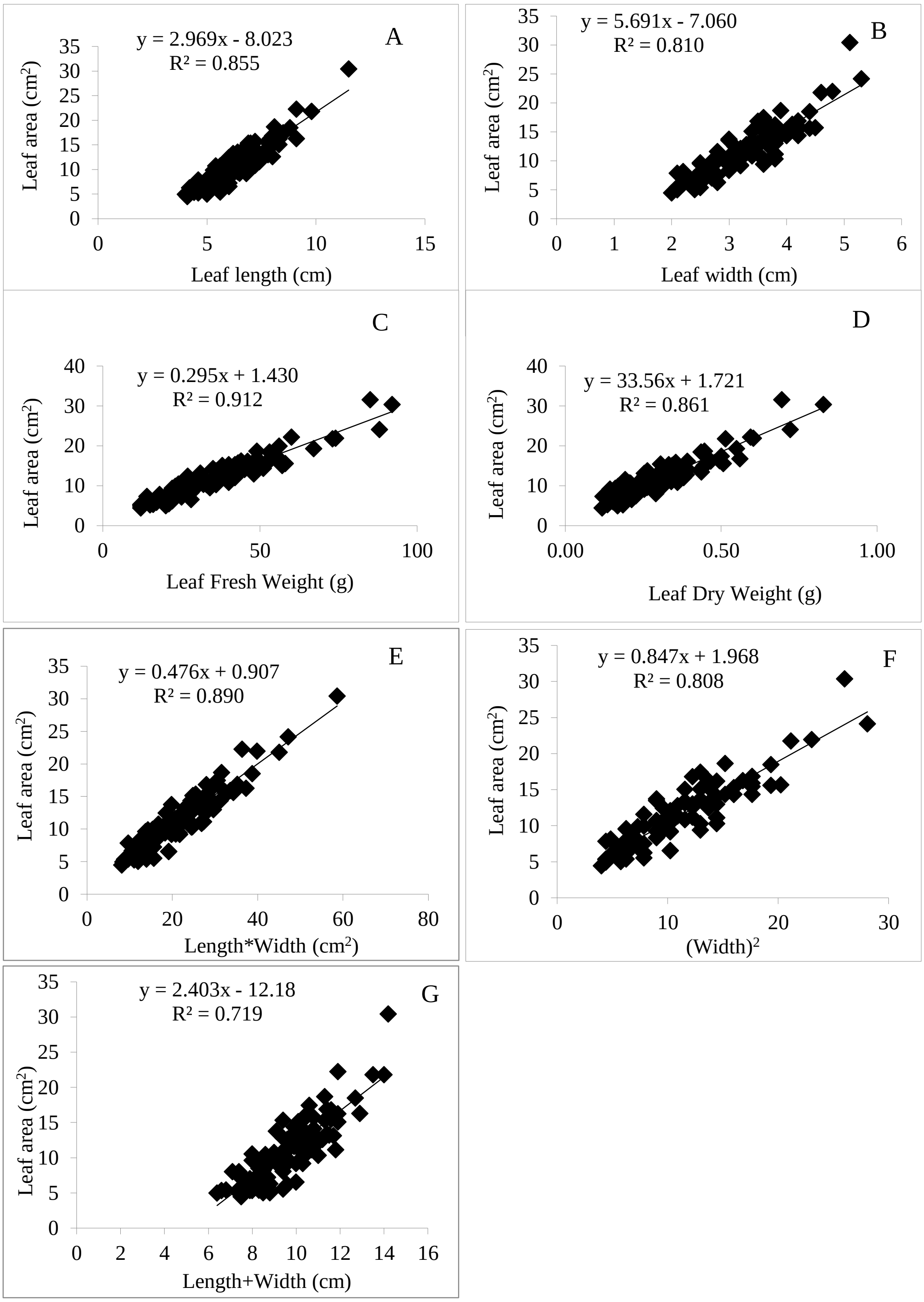

Fig 1. Plot of predicted leaf area, estimated by model vs. the observed leaf area using independent variables for cress plant (A-G)

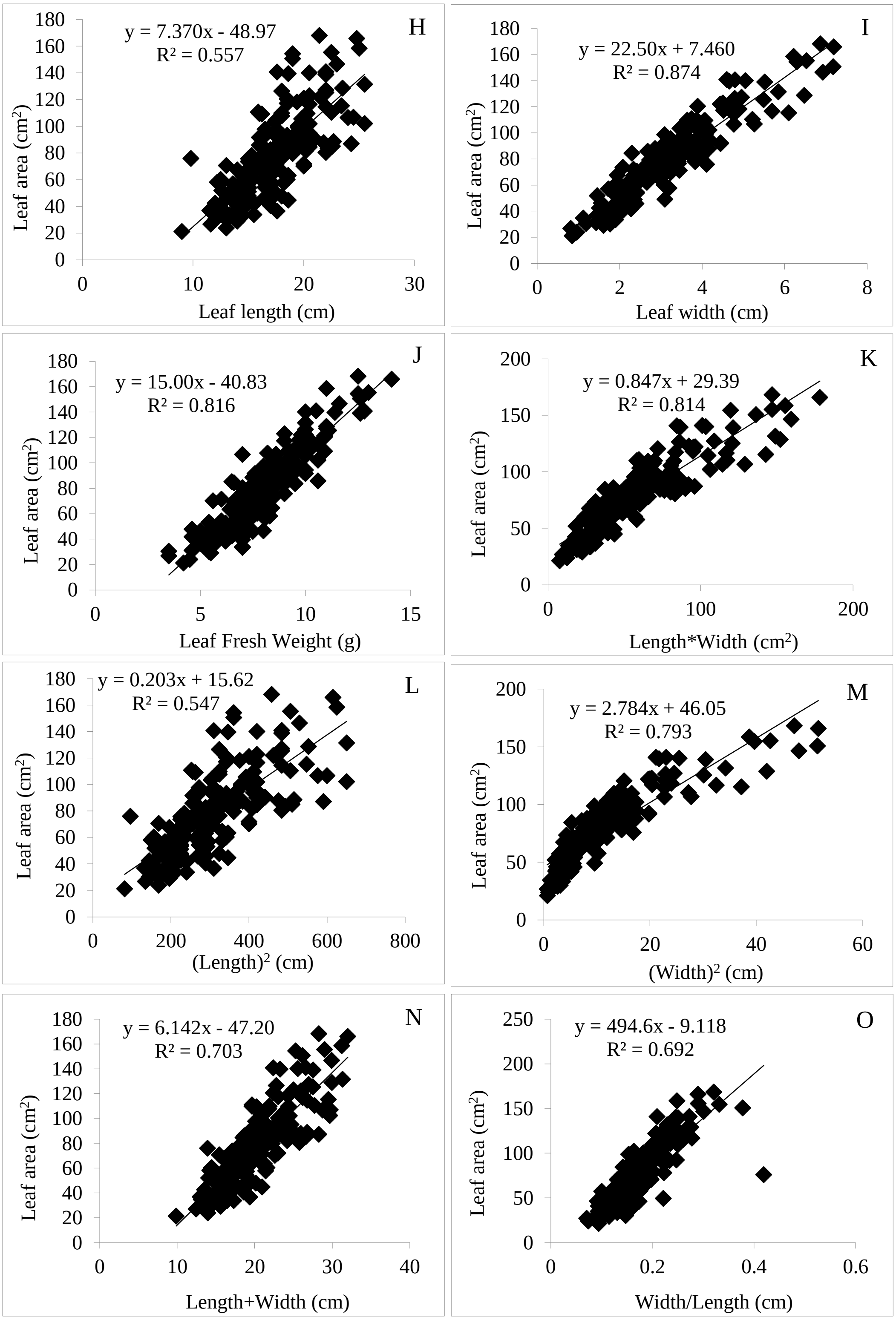

Fig 2. Plot of predicted leaf area, estimated by model vs. the observed leaf area using independent variables for radish plant (H-O).

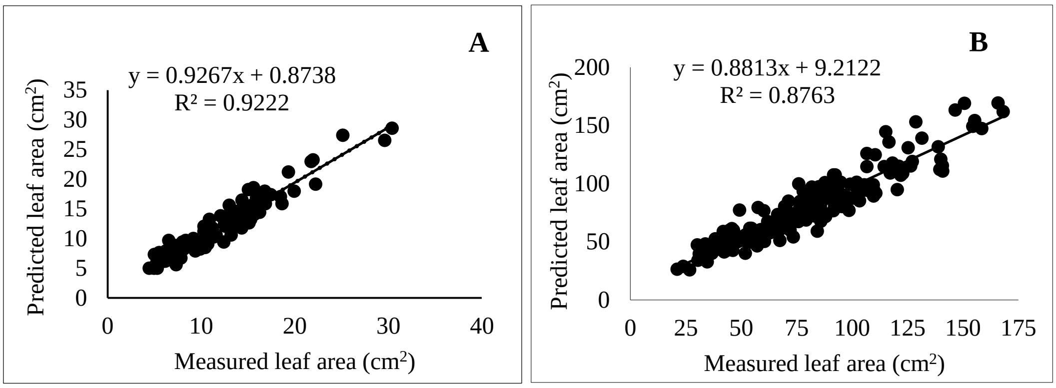

Fig. 3. Comparison of measured and predicted leaf areas of cress (A) and radish (B)

Leaf area is one of the most important growth parameters and for effective monitoring of the growth and plant development, it should be recorded several times accurately. Lack of an accurate model is a limitation for calculating LA. Non-destructive method of the estimation of LA has several advantages without compromising on accuracy (Antunes et al., 2008; Kandiannan et al., 2009; Peksen, 2007). Many studies have been carried out to estimate the leaf area by measuring leaf dimensions. In general, the combination of leaf L and maximum W has been used as the parameters of LA models (Antunes et al., 2008; Peksen, 2007). The results of present study were in agreement with the previous studies on model development for predicting LA using simple linear measurements (Cristofori et al., 2008; Busato et al., 2010; Rouphael et al., 2010; Schawb et al., 2014; Aminifard et al., 2016; De Carvalho et al., 2017). In this study, very close relationships were found between actual leaf area and predicted leaf area using the proposed model.

Table 3. Statistics and parameters yielded from regression models for LA estimation to compare models

Plant parameters	Equation No.	SE[Ɨ]	MSE	d	VIF	T
		Cress				
Length (cm)	1	0.42	2.97	0.99	6.89	0.14
Width (cm)	2	0.42	4.07	0.78	5.26	0.19
Fresh weight (g)	3	0.50	2.32	0.81	11.36	0.09
Dry weight (g)	4	0.47	3.52	0.99	7.19	0.14
Length×Width (cm^2)	5	0.45	2.42	0.97	9.09	0.11
$Width^2$ (cm)	6	0.42	4.03	0.50	5.21	0.19
Length+Width (cm)	7	0.39	5.77	0.98	3.56	0.28
		Radish				
Length (cm)	1	1.78	447.33	0.98	2.26	0.44
Width (cm)	2	2.22	126.80	0.99	7.94	0.13
Fresh weight (g)	3	2.14	184.41	0.99	5.43	0.18
Length×Width (cm^2)	4	2.15	187.69	0.99	5.38	0.19
$Length^2$ (cm)	5	1.76	458.07	0.98	2.21	0.45
$Width^2$ (cm)	6	0.01	208.56	0.98	4.83	0.21
Length+Width (cm)	7	1.99	299.94	0.98	3.37	0.30
Width/Length (cm)	8	1.98	311.08	0.96	3.25	0.31

Ɨ Standard error (SE), Mean square errors (MSE), index of agreement (d), variance inflation factor (VIF) and tolerance value (T).

Table 4. Calculation of statistic E to find the best equation

Equations	MSE	E12
	Cress	
Equation 3	2.32	(MSE3/MSE5)=0.958
Equation 5	2.42	(MSE5/MSE3)=1.043
Radish		
Equation 2	126.80	(MSE2/MSE3)=0.687
Equation 3	184.41	(MSE3/MSE2)=1.454

CONCLUSION

In conclusion, results showed that cress and radish LA could be monitored quickly, accurately, and non-destructively by using the leaf FW and leaf W models, respectively. With these models, agronomists and physiologists can estimate the leaf area of cress and radish plants accurately and in large quantities.

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مدل‌سازی سطح برگ شاهی و تربچه با استفاده از ابعاد و وزن برگ

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چکیده:

اطلاعات مربوط به سطح برگ در مطالعات مختلف باغبانی و فیزیولوژیکی مورد نیاز است. اندازه‌گیری سطح برگ نیاز به روش‌های آسان، سریع و در حد امکان غیر مخرب دارد. هدف از این مطالعه تعیین معادلات برای برآورد سطح برگ با استفاده از طول (L)، عرض(W) ، وزن‌تر (FW) و وزن خشک (DW) ، طول × عرض، طول + عرض، عرض/ طول، طول × طول و عرض × عرض برگ های شاهی (*Lepidium sativum* L.) به عنوان سبزی برگی و تربچه (*Raphanus sativus* L.) به عنوان سبزی ریشه ای بود. آزمایش حاضر در سال ۲۰۱۵ تحت شرایط گلخانه برای مطالعه رابطه بین اندازه برگ و وزن با سطح برگ در این دو سبزی انجام شد. سطح برگ مشاهده شده با استفاده از یک دستگاه اندازه‌گیری اتوماتیک و ابعاد برگ توسط خط‌کش اندازه‌گیری شد. تجزیه و تحلیل رگرسیون سطح برگ نسبت به‌L ، W، FW، DW، W × L، W + L، W / L، L^2 وW^2 ، چندین مدل را برای برآورد سطح برگ گیاهان شاهی و تربچه تعیین کرد. مدلی خطی با استفاده از FW به عنوان یک متغیر مستقل [LA=0.295 (Fresh W.)+ 1.430] دقیق ترین برآورد (R^2 = 0.912, RMSE = 1.52) را از سطح برگ شاهی نشان داد. برای تربچه، مدلی خطی با استفاده از W به عنوان یک متغیر مستقل + [LA = 22.50 (W) 7.46] دقت بیشتری (R2 = 0.874, RMSE = 11.26) در برآورد سطح برگ داشت. تایید مدل های رگرسیون نشان داد که همبستگی بین مقادیر اندازه گیری شده و شبیه سازی شده با استفاده از این معادلات به ترتیب برای تربچه و شاهی (R2 = 0.922, 0.876) قابل قبول بود.

کلمات کلیدی: متغیرهای مستقل، شاهی، روش غیر تخریبی، تربچه، مدل‌های رگرسیونی