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Modelling individual leaf area of cress and radish 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 length (L), width (W), fresh weight (FW) and dry weight (DW), length×width (L×W), length + width (L+W), width/length (W/L), length^2 (L^2) and width^2 (W^2) of cress (*Lepidium sativum* L.) leaves as a leafy vegetable and radish (*Raphanus sativus* L.) as a root vegetable. A soil-cultured experiment was carried out in 2010 under greenhouse conditions to study 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, L+W, W/L, L^2 and W^2 led several models that could be used for estimating the area of individual cress and radish leaves. A linear model employing FW as an independent variables [LA=0.295 (Fresh W.)+ 1.430] resulted in 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) estimating LA. Validation of the regression models showed that the correlation between measured and simulated values using these equations were quite acceptable (R^2 = 0.922, 0.876).

Key words: Independent variables, leaf area simulation, *Lepidium sativum, Raphanus sativus*

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 increase 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 contain 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 for producing dry matter (Demarty *et al.*, 2007). Factors related to leaf area, such as photosynthesis and transpiration rate, directly affect plant productivity, which makes leaf area (LA) a key variable in physiological studies involving plant growth, light interception, photosynthetic efficiency, evapotranspiration and answers to fertilizers and irrigation (Smith and Kliewer, 1984; Blanco and 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 and 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 successive measurement of the same leaf and plant canopy would be damaged which cause problems to other measurements of the experiment (Tsialtas and Maslaris, 2005).

A large number of methods, either destructive or not, have been developed to measure LA. The LA could 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 leaf area measurements, however these were 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 has been 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 (Lee and Heuvelink, 2003; Lieth and Pasian, 1991). Such methods usually saved time,were non-destructive and allowed measurements to be repeated during the plant's growth period and reduced variability associated with destructive sampling procedures (Nesmith, 1992). Thus, prediction model which could estimate LA without harming the plant could provide researchers with many advantages in horticultural experiments, which are as follow: 1) the models enabled researchers to measure LA on the same plants during the plant growth period and reduced variability in experiments (Gamiely *et al.,* 1991; Nesmith, 1991, 1992); 2) reliable models eliminate the need for expensive instruments and labor; 3) measurement will be easy, quick and thus time-saving if a reliable equation emerged or was chosen; 4) use of reliable equations ensured consistency of results; 5) modeling equation cost nothing. The non-destructive methods based on linear measurements were 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), persimon (Cristofori *et al.,* 2008), medlar (Mendoza-De Gyves *et al.,* 2008), small fruits (Fallovo *et al.,* 2008), potato (Busato *et al.,* 2010), rose (Rouphael *et al.*, 2010), gladiolus (Schawb *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 estimation of LA for two garden vegetable plants.

Materials and methods

Soil cultured cress and radish plants were grown under greenhouse conditions at Faculty of Agriculture,University of Birjand, from January to March 2015. Air temperature and relative humidity ranged between 24 ºC (day) and 21 ºC (night), 60-70 %, respectively. Light was about 40.5 mol/m^2/s. Irrigation and nutrition were provided as per conventional practices. For cress, about 50 days after planting, 100 plants were chosen and one fully-expanded leaf sample was prepared from each. For radish, after development of their roots for about 60 days after planting, 180 plants were taken out completely. Each 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). Consequently, leaf fresh weight (FW), L and 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 to the nearest 1 mm and L was calculated from the top to the end of the blade without petiole to the nearest 1 mm. Then, samples were oven-dried at 80°C for 24 h and dry weight of each was measured. The fresh and dry weights (DW) of leaves were measured to the nearest 0.001 g. The mean, maximum and minimum of all samples were calculated.

Multiple regression analysis was performed on the samples. A search for the best model to predict LA was conducted with various subsets of the independent variables namely, 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 coefficient of determination (R^2), root mean 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 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): $RMSE = [\sum (Pi\text{-}Oi)^2/N]^{0.5}$

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 and Raktoe, 1981): $E_{12} = MSE_1/MSE_2$

Where MSE_1 and MSE_2 are the mean square error of the predictions with model 1 and 2, respectively: $MSE_1 = \Sigma (P_1i - Oi)^2$, $MSE_2 = \Sigma (P_2i - Oi)^2$

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:

$d = 1 - [\Sigma (P_i\text{-}O_i)^2]/ \Sigma [(|P_i\text{-}\bar{O}|) + (|O_i - \bar{O}|)]^2$ Where $\bar{O}$ is the average of the observed values.

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

$VIF=1/1-r^2$, $T=1/VIF$

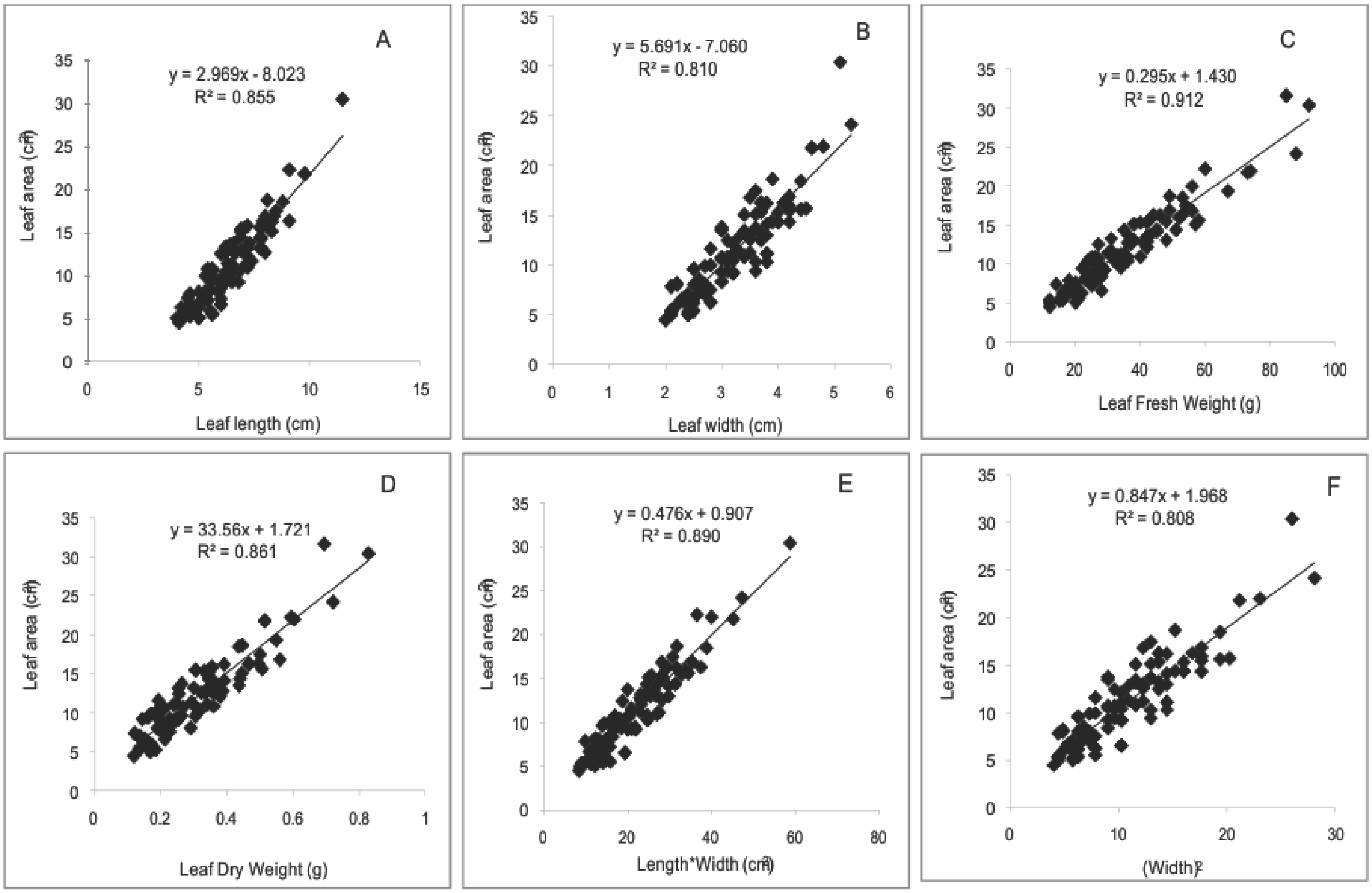
Where, r is the correlation coefficient. If the VIF value was higher than 10 or if T value was smaller than 0.10, then collinearity may have more than a trivial impact on the estimates of the parameters and consequently one of them should be excluded from the model.

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 and Blackie, 1979). Regression analyses were 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 are good means for non-destructive measurement 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 and Fig. 1 and Fig. 2). As per Table 3, it is clear that the highest SE and the lowest MSE were obtained for equation 3 for cress and equation 2 for radish, which confirmed the goodness of these models to estimate LA. The d



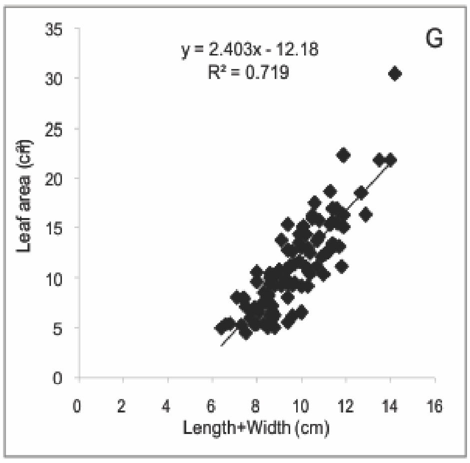


Fig 1. Plot of predicted leaf area, estimated by model vs. the observed leaf area using independent variables for cress plant (A-G).

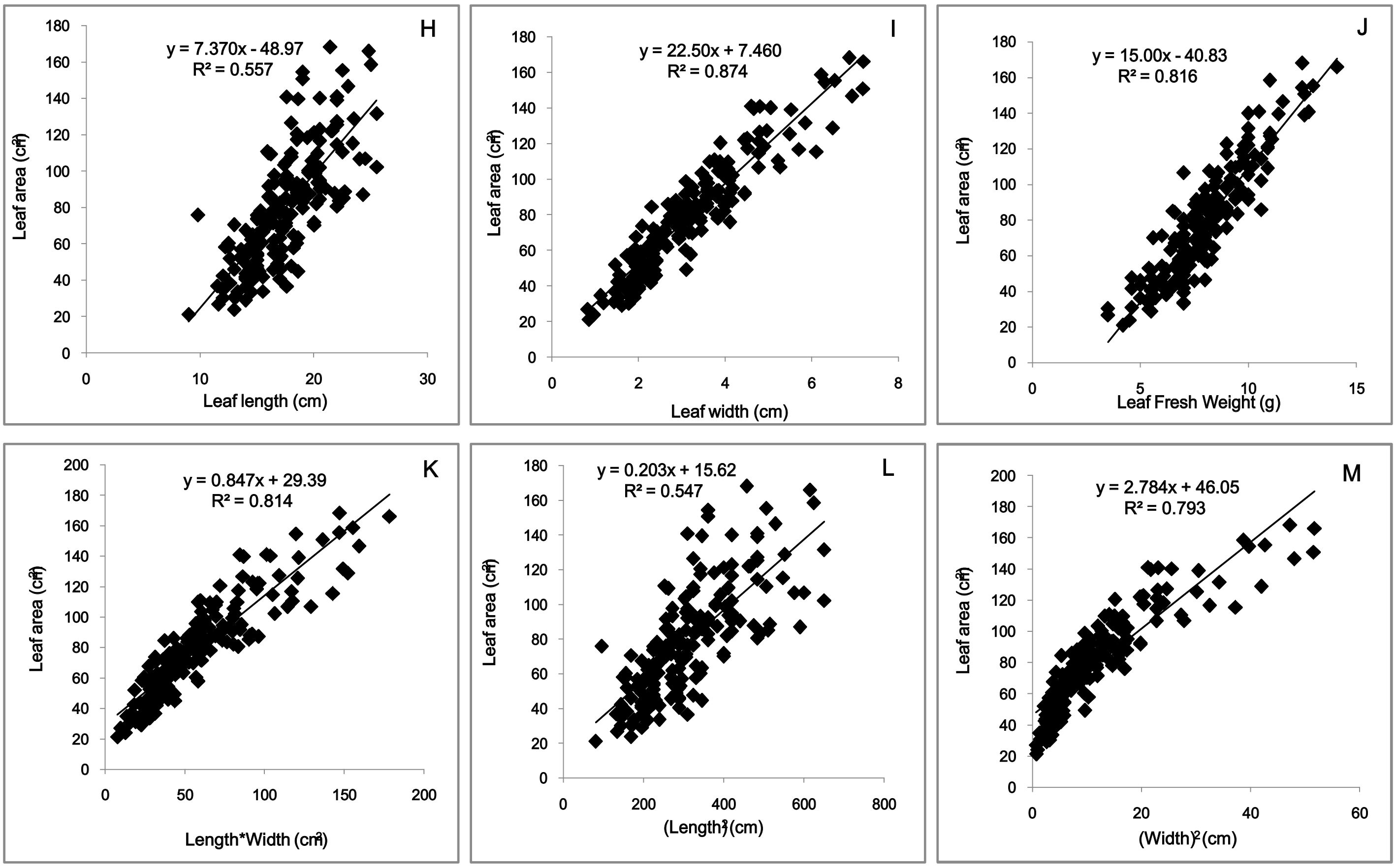
Table 1. Mean, minimum and maximum values for measured independent variables of cress and radish leaves.

Plant parameters	N	Mean±SDƗ	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

Ɨ 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).

value was highest 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).

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 methods of estimating LA have several advantages without compromising on accuracy (Antunes *et al.,* 2008; Kandiannan *et al.,* 2009; Peksen, 2007). Many studies have been carried out to estimate leaf area by measuring leaf dimensions. In general, the combination of leaf L



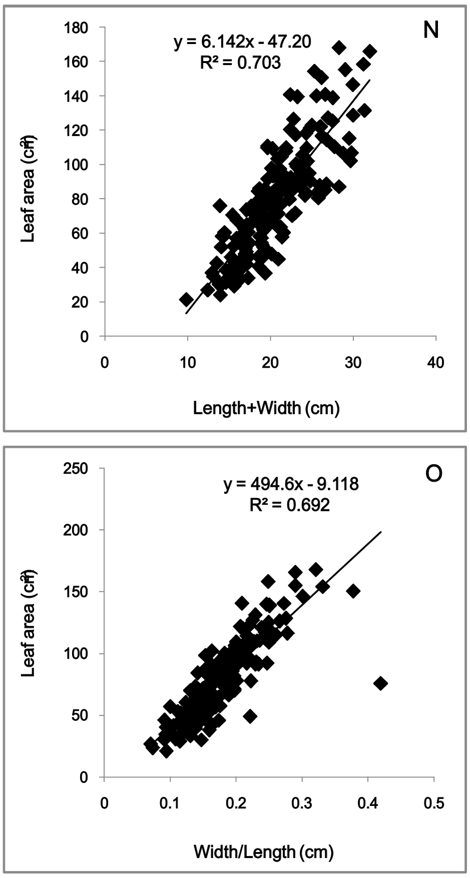


Fig 2. Plot of predicted leaf area, estimated by model vs. the observed leaf area using independent variables for radish plant (H-O).

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

Plant parameters	Eq. 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

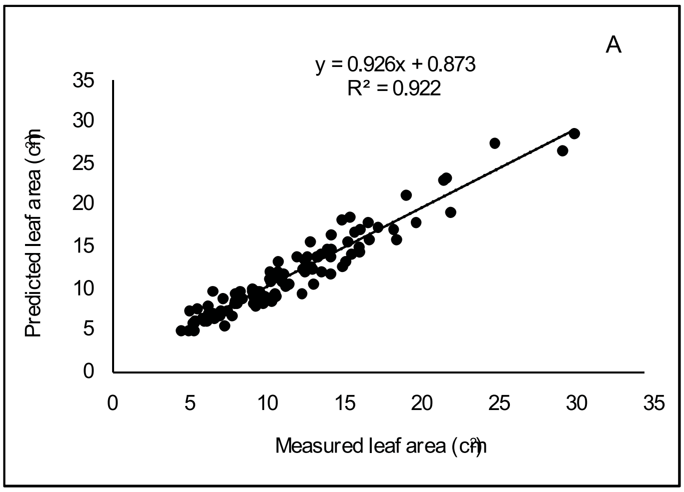
and maximum W has been used as the parameters of LA models (Antunes *et al.,* 2008; Peksen, 2007). Present study results were in agreement with the previous studies on model development for predicting LA using simple linear measurements (Antunes *et al.,* 2008; Cristofori *et al.,* 2008; Fallovo *et al.,* 2008; Guo and Sun, 2001; Peksen, 2007; Rouphael *et al.,* 2006; Busato *et al.,* 2010; Rouphael *et al.,* 2010; Schawb *et al.,* 2014; Aminifard *et al.,* 2016; De Carvalho *et al.,* 2017).

To conclude, very close relationships were found between actual LA and predicted LA using the proposed model. 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 accurately

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

Plant parameters	Eq.	SE[ƚ]	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

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



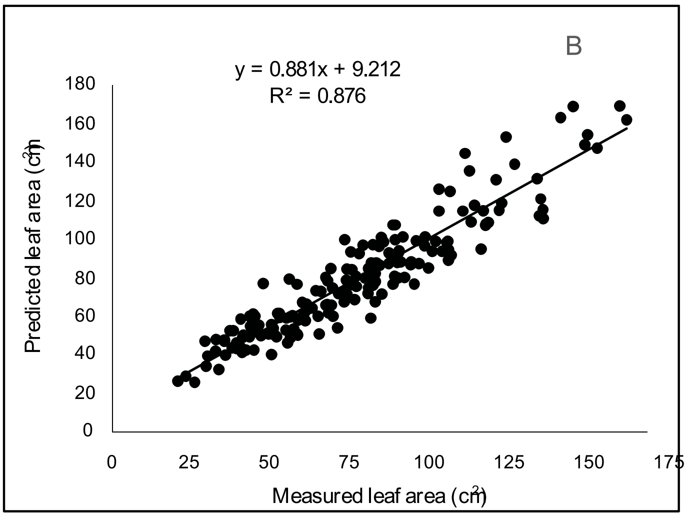


Fig. 3. Comparison of measured and predicted leaf areas of cress (A) and radish (B).

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

and in large quantities the leaf area of cress and radish plants.

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