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Non-destructive model to estimate leaf area in *Epilobium* species

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Abstract

Leaf area (LA) is one of valuable parameters in plant physiological studies. Therefore, the use of a non-destructive, accurate and simple model to estimate LA is very important. This research aimed to develop a non-destructive model to estimate LA accurately in *Epilobium* species. To estimate LA, leaf length (L) and leaf width (W) of five *Epilobium* species were determined. Moreover, the actual leaf area, using leaf area meter was measured. Regression analysis of LA versus L, W and LW revealed several models to predict LA in *Epilobium* species. Out of the models, the best fitted and validated model which is recommended to estimate LA accurately in each species, was quadratic model based on two dimensions (L and W), including the *E. algidum* (LA = 0.1264 + 0.6562 (L×W) + 0.0366 $(L×W)^2$), *E. parviflorum* (LA = – 3.144 + 1.323 (L×W) – 0.030 $(L×W)^2$), *E.* sp. (LA = 0.4236 + 0.3033 (L×W) + 0.1368 $(L×W)^2$), *E. hirsutum* (LA= 2.2417 + 0.2202 (L×W) + 0.0029 $(L×W)^2$), and *E. frigidum* (LA = 0.2119 + 0.4162 (L×W) + 0.1191 $(L×W)^2$), all with $R^2 \geq 0.80$.

Keywords: *Epilobium*; Leaf area; Leaf length; Leaf width; Non-destructive; Quadratic regression model

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Introduction

The *Epilobium* genus of perennial herbaceous plant which belongs to Onagraceae family consists of about 200 species with worldwide distribution. It has been widely used in traditional medicine and its fresh leaves used in tea and salad (Raven 1976; Vitalone *et al.* 2001). The extracts of aerial parts of *Epilobium* taxa have many therapeutic properties, including anti-proliferative, antioxidant, antitumor and also anti-inflammatory activity (Vitalone *et al*. 2001; Stolarczyk *et al*. 2013). Today, human societies have shown a high tendency to use herbs and their derivatives, hence increasing their production and yield is very important. The leaves have many different responsibilities in the plant such as photosynthesis, assimilation, and represent the main surface for physiologically active exchange and likewise biologically active components such as, flavonoids (e.g., kaempferol, mirycetin and quercetin) (Hiermann 1983; Averett and Raven 1984), steroids (Hiermann and Mayr 1985) and tannins (Stolarczyk *et al*. 2013) that in some of the species of *Epilobium* such as *E. hirsutum* and *E. parviflorum* has been found to be concentrated in the leaves. Therefore, leaf traits such as leaf area (LA) can have a significant effect on the estimation and the determination of growth, development and leaf structure. LA is an important characteristic for studying of the parameters such as leaf area ratio and specific leaf area which has been related to crop management (Reich *et al*. 1992; Borsato *et al*. 2008; Schmildt *et al*. 2014). LA is largely evaluated in physiological studies and also has a key role on transpiration, net assimilation rate, plant evapotranspiration (ET), light interception, plant growth, photosynthetic efficiency, plant productivity and survival (Chen *et al*. 1997; Wright *et al*. 2005). LA could be evaluated through many destructive (direct methods) and non-destructive (indirect methods) (Kubner and Mosandl 2000). However, most of the direct methods used to estimate LA, require the excision of leaves from the plants and scanning the leaves in the lab, so because of leaf destruction, plant canopy is damaged, which may alter other characters by interfering on physiological and phenological responses due to the reduction in canopy. Furthermore, direct methods are time-consuming and need advanced and expensive equipment, such as portable scanners, area integrators and laser optic apparatuses, (Mousavi Bazaz *et al*. 2012; de Souza *et al*. 2015). Whereas, the indirect (non-destructive) methods are based on mathematical equations and linear measurements of plant leaves (including leaf length (L) and leaf width (W)) and also digital images which are relatively more accurate and require less time and can be used without any destruction at different phenological stages of the plant (Cho *et al*. 2007; Ramesh *et al*. 2007; Pezzini *et al*. 2018; Schwab *et al*. 2014). Several equations have been developed to estimate LA by non-destructive methods. Montgomery (1911) first suggested that LA can be estimated by using linear measurements such as leaf length and width. Non-destructive models have been developed for many plant species such as *Bergenia purpurascens* (Zhang and Liu 2010), *Helianthus* (Maldaner *et al*. 2009), *Zizyphus joazeiro* (Maracajá *et al*., 2008), *Crotalaria juncea* (Cardozo *et al*. 2011), *corylus* (Cristofori *et al*. 2007), *castanea* (Serdar and Demirsoy 2006), *Actinidia deliciosa* (Mendoza-de Gyves *et al*. 2008), and *Phaseolus vulgaris* (Bhatt and Chanda 2003). Therefore, the objective of this study was to develop and validate the best fitted models to estimate the leaf area based on leaf dimensions (L and W), in some *Epilobium* species for the first time.

Material and Methods

Plant material

The seeds and the whole plant (Herbarium sample) of five *Epilobium* species (Onagraceae family) were collected from natural habitats of Iran at the seed full ripening stage in June and July 2014 (Table 1). The identification of plant species was conducted at Iranian Biological Resource Center (IBRC). The species consisted of *Epilobium algidum*, *E. parviflorum*, *E. sp.*, *E. hirsutum* and *E. frigidum*. The experiment was conducted in the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. The seeds were cultured *in vitro* on MS culture medium (Murashige and Skoog 1962) and incubated at 25 ºC with a 16-h photoperiod under 16/8-h light/dark. After three weeks, plantlets were transferred for acclimatization to pots, containing autoclaved soil within *ex vitro* condition and kept in a growth chamber. This procedure was described by Turker *et al.* (2008) with some modifications. For leaf area (dependent variable) estimation a total of 250 leaves were randomly collected for each species. The actual leaf area of each leaf was measured with an area meter (Delta-T Ltd., Cambridge, UK) calibrated to 0.01 cm^2. Then, the largest leaf length (L) and width (W) in cm were measured, using a millimeter ruler that is illustrated in Figure 1, and also the product of length with width was obtained for all leaf samples.

Model development

The dependent variable (leaf area, LA) for each sampled leaf was regressed to build the model with independent variables, including L, W and L×W. The descriptive statistics such as minimum, maximum, mean, standard error, variance (Var.) and Kurtosis of L, W, as well as LA in each species are presented in Table 2. Thereafter, regression models, linear ($Y = a + bx$), power ($Y = ax^b$) and quadratic ($Y = a + bx + cx^2$) were examined between the dependent variable (LA) and independent variables, including L, W and L×W. Also, the existence of collinearity between these variables was verified by the variance inflation factor (VIF) (Marquaridt 1970), using SPSS software. If VIF for a variable was >10, the variable should be removed from the model.

For each model, we obtained the coefficient of determination (R^2), the root mean square error (RMSE), mean square error (MSE) and Akaike information criteria (AIC) (Table 3). The criteria to select the best fitted model was based on the combination of the highest R^2 and lowest MSE, RMSE and AIC for each species (Floriano *et al*., 2006; Souza and Amaral 2015). The formula for AIC is as follows:

$$AIC = n * Ln\,(SSE/n) + 2k$$

n= number of observations

k = number of parameters

Table 1. Geographical information of collected Iranian endemic *Epilobium* species.

Species	Sample ID	Province	Locality	Altitude (m)	Latitude (E)	Longitude (N)
E. algidum	P1012150	Tehran	Havir	2221	35°41´	52°24´
E. parviflorum	P1012148	East Azerbaijan	Marand	1747	45°46´	38°25´
E. sp.	P1012144	Chahar Mahal Bakhtiari	Kohrang	2569	50°03´	32°27´
E. hirsutum	P1012152	Mazandaran	Chalus	80	36°38´	51°24´
E. frigidum	P1012139	Tehran	Firoozkooh	2591	35°54´	52°43´

Figure 1. *Epilobium* leaf indicating the position of leaf length (L) and width (W) linear measurement.

Table 2. Minimum (Min), maximum (Max), mean, standard error (SE), variance (Var.) and kurtosis for leaf length, leaf width, length × width and leaf area of *Epilobium* species.

Species	Width (cm)					Length (cm)				
	Min	Max	Mean ± SE	Var.	Kurtosis	Min	Max	Mean ± SE	Var.	Kurtosis
E. algidum	0.50	1.20	0.87 ± 0.02	0.03	- 0.64	2.00	4.00	2.97 ± 0.08	0.33	- 0.65
E. parviflorum	1.40	2.20	1.60 ± 0.02	0.03	2.03	2.80	4.00	3.45 ± 0.04	0.08	- 0.09
E. sp.	0.50	1.40	0.80 ± 0.02	0.03	0.16	1.60	3.00	2.15 ± 0.04	0.08	0.24
E. hirsutum	1.30	3.30	1.97 ± 0.06	1.05	1.40	3.00	7.80	4.84 ± 0.17	1.50	- 0.56
E. frigidum	0.50	1.00	0.87 ± 0.02	0.03	- 0.76	2	3.50	2.58 ± 0.06	0.19	0.17

Table 2 (continued)

Species	Length × Width (cm^2)					Leaf area (cm^2)				
	Min	Max	Mean ± SE	Var.	Kurtosis	Min	Max	Mean ± SE	Var.	Kurtosis
E. algidum	1.15	4.80	2.66 ±0.14	0.96	- 0.53	1.00	4.00	2.16 ± 0.13	0.91	- 0.73
E. parviflorum	4.20	8.00	5.58 ± 0.13	0.91	1.40	2.00	5.00	3.04 ± 0.12	0.73	0.72
E. sp.	1.00	4.20	1.76 ± 0.09	0.39	2.88	1.00	4.00	1.44 ± 0.05	0.37	4.38
E. hirsutum	4.42	25.74	4.78 ± 0.21	2.21	1.10	3.00	9.00	9.97 ± 0.64	20.96	2.35
E. frigidum	1.15	3.35	2.28 ± 0.10	0.50	- 0.86	1.00	3.00	1.84 ± 0.10	0.58	- 1.21

Validation of models

To validate the best-fitted model (with highest R^2, and lowest MSE, RMSE, and AIC), an experiment was performed in the summer-autumn 2018 on leaf samples of five *Epilobium* species grown at the same condition (described previously) in the growth chamber. In this regard about 50 leaves from different plants of each species were used to measure independent variables (L and W) and dependent variable (LA), using the same procedures described above to obtain observed LA (OLA). Leaf area (PLA) was predicted for each species using the best fitted model. Then, a PLA and OLA of each *Epilobium* species were regressed. Regression analyses were conducted using the SigmaPlot 12.5 package and Excel (version. 2016). Scatter plot of OLA against PLA is presented in Figure 2 (Mousavi Bazaz *et al.* 2012).

Software and tools

Statistical analyses were performed using the Microsoft Office Excel® 2013 and SPSS (Version 22, SPSS Inc, Chicago, IL, USA). Regression equations were fitted using SigmaPlot (ver.12.5, Richmond, California, USA).

Results

This study used a simple non-destructive model to predict leaf area of *Epilobium* species by measuring of length, width and the number of leaves. All the data for each five species (including L, W, L×W and also the actual leaf area) are documented in Table 2. Leaf length, Leaf width and observed leaf area for all species varied from 1.60 to 7.80 cm, 0.5 to 3.30 cm and 1 to 9 cm^2, respectively (Table 2). Among five *Epilobium* species examined in the present study, *E. hirsutum* and *E.* sp. had the highest and the lowest LA, respectively, and likewise the species differed in leaf dimensions (Table 2). The VIF varied between 1.25 and 2.78, depending on the species and leaf traits. Because VIF was < 10 in all *Epilobium* species, collinearity among independent variables (L, W, L × W) can be inconsiderable, hence all variables were included in the model (Gill 1986). The three models (linear, power, quadratic) and their statistical criteria such

Table 3. Parameter and statistic estimates from regression models to estimate the leaf area (LA) from leaf length (L) and leaf width (W). Statistic estimates are coefficient of determination (R^2), root mean square error (RMSE) mean square error (MSE), and Akaike information criteria (AIC).

Species	Type	Equation	R^2	RMSE	MSE	AIC
E. algidum	Linear	LA = – 0.1513+ 0.8711 L×W	0.80	0.41	0.17	-82.30
E. algidum	Linear	LA = – 2.3254 +1.5092 L	0.84	0.37	0.13	-92.63
E. algidum	Linear	LA = – 1.0344 + 3.6717 W	0.49	0.67	0.45	-33.83
E. algidum	Quadratic	$LA = 0.1264 + 0.6562 (L\times W) + 0.0366 (L\times W)^2$	0.84	0.36	0.13	-93.49
E. algidum	Quadratic	$LA = -0.9383 + 0.5896 L +0.1469 (L)^2$	0.81	0.41	0.17	-82.68
E. algidum	Quadratic	$LA = 1.6951 + -3.0715 W + 3.9720 (W)^2$	0.51	0.65	0.42	-36.24
E. algidum	Power	$LA = 0.7493 L\times W^{1.0783}$	0.80	0.41	0.17	-82.51
E. algidum	Power	$LA = 0.2308 L^{2.0195}$	0.84	0.37	0.13	-93.08
E. algidum	Power	$LA = 2.6372 W^{1.6085}$	0.50	0.66	0.43	-35.07
E. parviflorum	Linear	LA = – 1.758 + 0.859 L×W	0.92	0.24	0.05	-137.24
E. parviflorum	Linear	LA = – 4.7194 + 2.2452 L	0.56	0.56	0.31	-51.79
E. parviflorum	Linear	LA = – 3.6743 + 4.1755 W	0.76	0.41	0.16	-83.02
E. parviflorum	Quadratic	$LA = -3.1441 + 1.323 (L\times W) - 0.030 (L\times W)^2$	0.92	0.23	0.05	-138.77
E. parviflorum	Quadratic	$LA = -5.4099 + 2.6462 L - 0.0578 (L)^2$	0.56	0.56	0.31	-51.80
E. parviflorum	Quadratic	$LA = -3.4803 + 3.9502 W + 0.0643 (W)^2$	0.76	0.41	0.16	-83.02
E. parviflorum	Power	$LA = 0.2312 L\times W^{1.4930}$	0.91	0.24	0.05	-133.19
E. parviflorum	Power	$LA = 0.1334 L^{2.5113}$	0.55	0.56	0.31	-51.23
E. parviflorum	Power	$LA = 1.1446 W^{2.0304}$	0.76	0.41	0.16	-82.57
E. sp.	Linear	LA = – 1186 + 0.8819 L×W	0.82	0.25	0.06	-104.52
E. sp.	Linear	LA = – 2.0031 + 1.5970 L	0.60	0.38	0.14	-72.49
E. sp.	Linear	LA = – 0.5977 + 2.5344 W	0.65	0.35	0.12	-59.61
E. sp.	Quadratic	$LA = 0.4236 + 0.3033 L\times W + 0.1368 (L\times W)^2$	0.84	0.23	0.05	-106.29
E. sp.	Quadratic	$LA = 5.0646 - 4.8927 L + 1.4621 (L)^2$	0.69	0.33	0.11	-72.54
E. sp.	Quadratic	$LA = 2.0082 - 4.0630 W + 3.9475 (W)^2$	0.76	0.29	0.08	-63.34
E. sp.	Power	$LA = 0.7455 L\times W^{1.1299}$	0.83	0.24	0.06	-105.85
E. sp.	Power	$LA = 0.1922 L^{2.5650}$	0.65	0.35	0.12	-72.28
E. sp.	Power	$LA = 1.9680 W^{1.6084}$	0.69	0.33	0.11	-60.99
E. hirsutum	Linear	LA = 1.8576 + 0.2932 L×W	0.81	0.64	0.41	-39.46
E. hirsutum	Linear	LA= – 0.2501 + 1.0384 L	0.73	0.76	0.58	-21.50
E. hirsutum	Linear	LA= – 0.6834 + 2.7705 W	0.63	0.89	0.80	-5.98
E. hirsutum	Quadratic	$LA= 2.2417 + 0.2202 (L\times W) + 0.0029 (L\times W)^2$	0.84	0.59	0.35	-46.91
E. hirsutum	Quadratic	$LA = 6.1553 - 1.7230 L + 0.2795 (L)^2$	0.81	0.63	0.40	-40.56
E. hirsutum	Quadratic	$LA = 3.3555 - 1.1250 W + 0.8953 (W)^2$	0.66	0.86	0.74	-9.82
E. hirsutum	Power	$LA = 1.1173 L\times W^{0.6404}$	0.78	0.69	0.47	-31.83
E. hirsutum	Power	$LA = 0.7912 L^{1.1341}$	0.74	0.75	0.57	-22.93
E. hirsutum	Power	$LA = 2.1406 W^{1.1736}$	0.64	0.88	0.78	-6.80
E. frigidum	Linear	LA = – 0.3678 + 0.9680 L×W	0.80	0.33	0.10	-130.90
E. frigidum	Linear	LA = – 1.7276 + 1.3817 L	0.63	0.45	0.20	-91.00
E. frigidum	Linear	LA = – 0.9487 + 3.2054 W	0.83	0.51	0.26	-97.00
E. frigidum	Quadratic	$LA = 0.2119 + 0.4162 (L\times W) + 0.1191 (L\times W)^2$	0.81	0.32	0.10	-138.47
E. frigidum	Quadratic	$LA = -1.2678+ 1.5377 L + 0.0625 (L)^2$	0.63	0.45	0.20	-103.20
E. frigidum	Quadratic	$LA = 2.293 - 4.607 W + 4.243 (W)^2$	0.56	0.49	0.24	-116.63
E. frigidum	Power	$LA = 2.2784 L\times W^{1.7367}$	0.81	0.35	0.12	-133.13
E. frigidum	Power	$LA = 0.323 L^{1.8477}$	0.63	0.45	0.20	-98.08
E. frigidum	Power	$LA = 1.9047 W^{1.2043}$	0.54	0.51	0.26	-103.32

as MSE, RMSE and AIC, are shown in Table 3. Among the models, we selected the best model based on several selection criteria (higher R^2 and lower MSE, RMSE, AIC). The coefficient of determination (R^2) was more than 0.65 for the power and quadratic models of *E. hirsutum* and *E.* sp. In this regard the quadratic models based on the L×W with the highest coefficient of determination ($R^2 \geq 0.80$) and the lowest values of MSE and RMSE compared with other models were selected as the best models for five *Epilobium* species (Table 3). Thus, both L and W variables were needed to estimate *Epilobium* LA accurately.

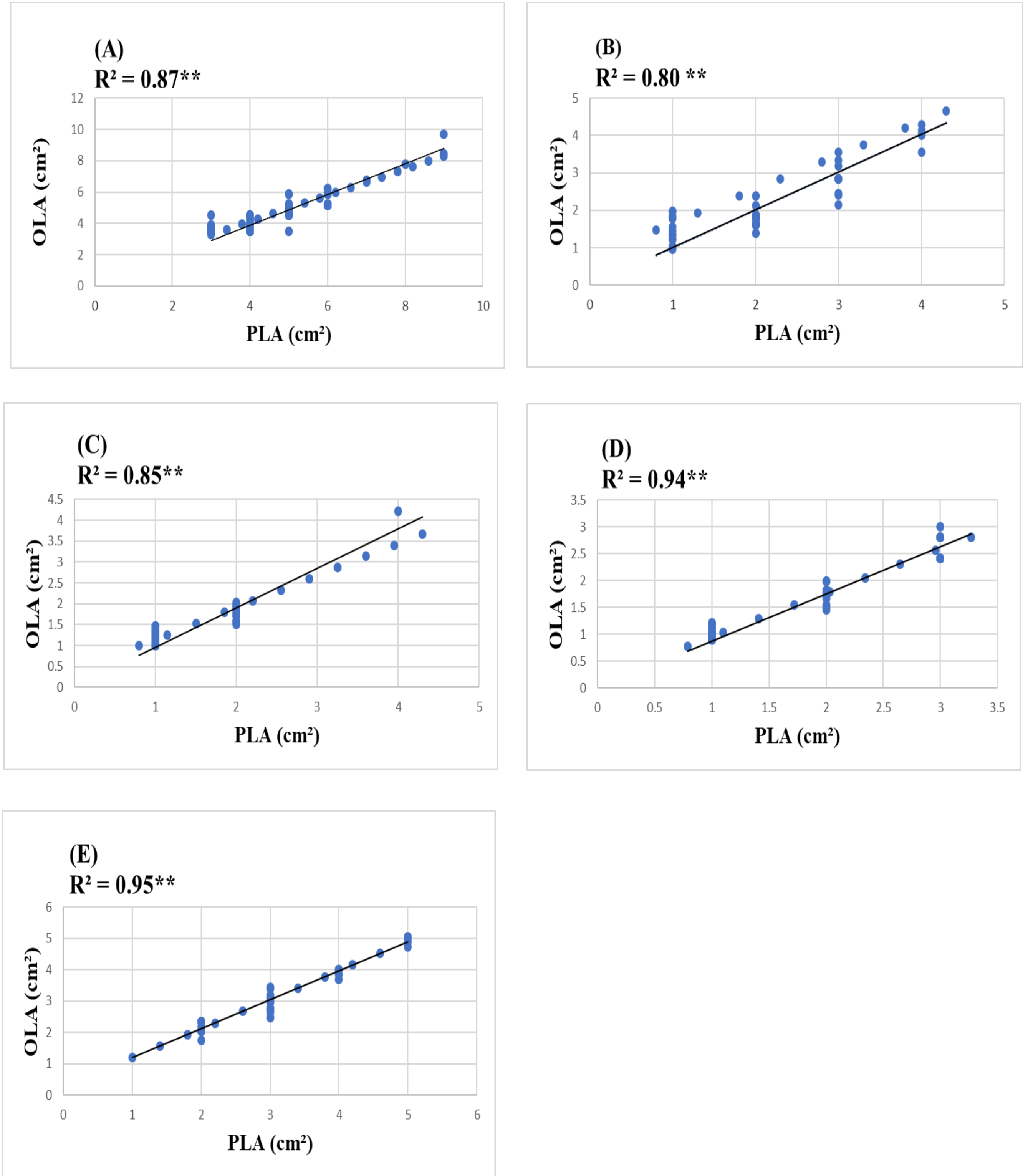

Figure 2. Plot of predicted leaf area (PLA) vs. the observed leaf area (OLA) for *Epilobium hirsutum* (a), *E. algidum* (b), *Epilobium* sp. (c), *E. frigidum* (d), and *E. parviflorum* (e) by using the model LA = a + b (L×W).
**Significant at $p \leq 0.01$.

The regression between PLA and OLA showed strong relationship ($R^2 \geq 80$) by using a non-destructive model based on L×W for five *Epilobium* species. According to these results, the quadratic model based on L×W showed higher coefficient of determination ($R^2 \geq 0.80$) and lower MSE as compared to linear and power equations for the five species of *Epilobium* that are shown as follows:

E. algidum (LA = 0.1264 + 0.6562 (L×W) + 0.0366 $(L\times W)^2$), *E. parviflorum* (LA = – 3.144 + 1.323 (L×W) – 0.030 $(L\times W)^2$), *E.* sp. (LA = 0.4236 + 0.3033 (L×W) + 0.1368 $(L\times W)^2$), *E. hirsutum* (LA= 2.2417 + 0.2202 (L×W) + 0.0029 $(L\times W)^2$), and *E. frigidum* (LA = 0.2119 + 0.4162 (L×W) + 0.1191 $(L\times W)^2$), $R^2 \geq 0.80$.

Discussion

LA is one of the important physiological growth parameters for plants and measuring it accurately without damaging the plant is necessary in some researches. In this regards several studies have been conducted to estimate LA, by using linear dimensions (L, W, L^2, W^2, L×W, $L^2 \times W^2$), indirect (non-destructive) methods, and mathematical models of linear, power and quadratic types (Cho *et al*. 2007; Rouphael *et al*. 2010; de Carvalho *et al*. 2017). In the present study, we evaluated the non-destructive estimation of LA in *Epilobium* species from linear dimensions to select the best model. Result of the current study indicated that area of *Epilobium* leaves is well related to L×W with high R^2 values ($R^2 \geq 0.80$), so presenting the best-fitted parameters for the quadratic model. These R^2 values (Table 3) agree with other previous reports to select the best fitted model for LA estimation in plants, i.e. (durian (*Durio zibethinus*, LA = 0.888 (LW) – 4.961, $R^2 = 0.91$), (cerrado species: *Styrax pohlii*, LA = 0.582 + 0.683 LW, $R^2 = 0.98$; *Styrax ferrugineu*, LA = –0.666 + 0.704 LW, $R^2 = 0.97$; *V. ferruginea*; LA = 0.463 + 0.676 LW, $R^2 = 0.96$) (Souza and Habermann 2014; Souza and Amaral 2015; Kumar *et al*. 2017).

To determine the best fitted model, low values of MSE, RMSE, and AIC are also important. Therefore, the models we selected to estimate LA for *Epilobium* species not only had the highest R^2 ($R^2 \geq 0.90$) but also had lower value of AIC, MSE, and RMSE than those presented by the other models in our study (Table 3). We found strong relationship between PLA and OLA in *Epilobium* species based on the product of leaf length and width in the quadratic model (Y = a + b (L×W) + c $(L\times W)^2$) with high coefficient of determination R^2 ($R^2 \approx 0.81$-0.92; $p < 0.001$) (Figure 2). In this regard we propose the best fitted models for *Epilobium* species as: *E. algidum* (LA = 0.1264 + 0.6562 (L×W) + 0.0366 $(L\times W)^2$), *E. parviflorum* (LA = –3.144 + 1.323 (L×W) – 0.030 $(L\times W)^2$), *E.* sp. (LA = 0.4236 + 0.3033 L×W + 0.1368 $(L\times W)^2$), *E. hirsutum* (LA= 2.2417 + 0.2202 (L×W) + 0.0029 $(L\times W)^2$), and *E. frigidum* (LA = 0.2119 + 0.4162 (L×W) + 0.1191 $(L\times W)^2$) (Table 3). All the best fitted models for *E. parviflorum, E.* sp and *E. frigidum* were derived from the two dimension (LW) regression showed the highest R^2 value (0.92, 0.84 and 0.81, respectively) while other models based on a single dimension of L or W exhibited the lowest R^2 ($R^2 \approx$

0.55-0.69 for L, $R^2 \approx$ 0.54-0.76 for W) (Table 3).These results are in agreement with previous reports in which the model for LA estimation was based on the combination of leaf length and width and is derived from the two-dimension regression (L and W), showing high R^2 values (Cristofori *et al*. 2007; Karimi *et al*. 2009; de Souza *et al*. 2015). Souza and Amaral (2015) in the study on *Vernonia ferruginea* reported low R^2 for the models derived from the single dimension regressions [$R^2 \approx$ 0.40 (for L) and $R^2 \approx$ 0.66 (for W and W^2)]. They also indicated high R^2 for the models derived from the two-dimension regressions [$R^2 \approx$ 0.96 (for LW) and 0.95 (for L^2W^2)]. de Souza *et al*. (2015) in a study on the multiple Vochysiaceae species, reported the lowest R^2 (0.79) for the model that derived from a single-dimension regression (L) and the highest R^2 (0.99) for the models derived from the two-dimension regressions (LW). However, Oliveira *et al*. (2019) developed a model to estimate LA by using linear measurements (L and W) on *Schinus terebinthifolius* Raddi and mentioned the best fitted model based on only leaf dimension W (LA = -2.6646 + 2.2124 (W) + 1.3953 $(W)^2$) due to its high precision in the estimation of LA.

In case of *E. algidum* and E. *hirsutum* speices, we observed the highest R^2 (0.84), respectively for the best fitted model from two-dimension regressions and the model derived from single-dimension (L) also showed high R^2 (R^2 ≈0.81-0.84 and (R^2 ≈0.73-0.81 in *E. frigium* and *E. hirsutum* speices, respectively, so the R^2 range observed in *E. algidum* and E. *hirsutum* by single (L)- and two-dimension (LW)-derived models was very narrow. Narrow R^2 range has been observed when developing the best model for estimating LA in *vitis* (Williams III and Martinson 2003), *castanea* plants (Serdar and Demirsoy 2006) and *Vernonia ferruginea* (Souza and Amaral 2015). The reason for observing narrow R^2 range in the models of *E. algidum* and *E. hirsutum* speices might be due to the ratio between L and W, so it can be considered an exception for these species. de Souza *et al*. (2015) reported that in the cases of narrow R^2 range in dwas is considered in our study. Moreover, Nakanwagi *et al*. (2018) reported in *Salvia hispanica* L. and *Solanum aethiopicum* that the models with L and combination of both L and W provided high accuracy and good LA estimation, which can be considered in *E. algidum* and *E. hirsutum* speices to estimate LA

In overall, we showed that the quadratic model ($Y = a + b\,(L\times W) + c\,(L\times W)^2$) based on the combination of L and W for each five *Epilobium* species, estimates LA better than the other models. In agreement with our results, Mousavi Bazaz *et al*. (2012) in their study on two cultivars of *Ocimum basilicum* (Purple Ruffles, Genovese) to estimate LA by using non-destructive method, established the best fitted model, containing L and W for each cultivar separately (Mousavi Bazaz *et al*. 2012). Hence, the quadratic model derived from the two dimensions (L and W) that we proposed, could be used to estimate LA of *E. algidum*, *E. parviflorum*, *E.* sp, *E. hirsutum* and *E. frigidum* with high accuracy. In the present study, the validation tests also determined the quadratic models with the highest R^2 ($R^2 \geq$ 0.90) and the lowest MSE, RMSE, and AIC for each species separately, and these models can be used to estimate LA for these species without any destructive method, just by using a ruler and a calculator.

Conclusions

The acquisition of nondestructive methods to estimate LA accurately as an important growth parameter in plants are useful. In this regard to accurately estimate LA, validation tests are very critical to determine the best fitted model derived from two-dimension regression of leaf length (L) and width (W), with the highest R^2 and the lowest MSE, RMSE, and AIC. Accordingly, we highlighted in the present study, the quadratic model based on the two-dimension regression (L and W) which is recommended for the leaf area prediction of five *Epilobium* species with high accuracy and without any destructive effect for the first time.

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Conflict of Interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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مدل غیر تخریبی تخمین سطح برگ در گونه‌های اپیلوبیوم

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چکیده

سطح برگ (LA) یکی از پارامترهای مهم در مطالعات فیزیولوژی گیاهی است. بنابراین، استفاده از مدل غیر تخربی دقیق و ساده برای تخمین سطح برگ بسیار مهم است. هدف از این تحقیق ایجاد مدل غیرتخریبی برای تخمین سطح برگ به صورت دقیق در گونه‌های اپیلوبیوم بود. به منظور تخمین سطح برگ، طول برگ (L) و عرض برگ (W) پنج گونه اپیلوبیوم اندازه‌گیری شد. علاوه براین، سطح برگ واقعی با استفاده از دستگاه سنجش سطح برگ اندازه‌گیری شد. تجزیه رگرسیون سطح برگ با L، W و LW چندین مدل را به منظور پیش بینی سطح برگ در گونه‌های اپیلوبیوم فراهم نمود. از بین مدل‌ها بهترین و معتبرترین مدل که برای تخمین دقیق سطح برگ در هر گونه پیشنهاد شد، مدل کوادراتیک بر پایه دو بعد L و W، همه با $R^2 \geq ۰/۹۰$ به شرح بود:

E. algidum (LA = 0.1264 + 0.6562 (L×W) + 0.0366 $(L\times W)^2$), *E. parviflorum* (LA = – 3.144 + 1.323 (L×W) – 0.030 $(L\times W)^2$, *E*. sp. (LA = 0.4236 + 0.3033 (L×W) + 0.1368 $(L\times W)^2$), *E. hirsutum* (LA = 2.2417 + 0.2202 (L×W) + 0.0029 $(L\times W)^2$), and *E. frigidum* (LA = 0.2119 + 0.4162 (L×W) + 0.1191 $(L\times W)^2$).

واژه‌های کلیدی: اپیلوبیوم؛ سطح برگ؛ عرض برگ؛ غیر تخریبی؛ طول برگ؛ مدل رگرسیونی کوادراتیک