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NONDESTRUCTIVE LEAF AREA AND FRESH WEIGHT ESTIMATION FOR *Taraxacum kok-saghyz* Rodin AND THEIR SAMPLING NUMBER

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Abstract

Kok-saghyz (*Taraxacum kok-saghyz* Rodin), Russian dandelion, is a perennial plant widely recognized as one of the most promising sources of natural rubber. The works to utilize natural rubber are underway in the United States, China and Western Europe (Germany, Spain, Czech Republic and the Netherlands). The aim of this study was to determine nondestructive models for estimating leaf area and fresh weight of Russian dandelion plants. Regression analyses were performed between leaf area, fresh weight, leaf length, and leaf width in two hundred and fifty leaf samples collected during different growth stages of Russian dandelion plants. Data from another fifty leaves were used for validating the proposed models. Regression analyses were performed among ten data groups with different numbers of data randomly selected from the total three hundred leaves data set to determine the smallest sampling number for applying the final models correctly. The model for estimating leaf area (LA) is: $LA = 6226.424 + 26.31L + 545.334W - 313.993L^{0.5} - 3138.047W^{0.5} - 0.009L^2 - 3.86W^2 + 0.057LW$, with R^2 and RMSE values of 0.818 and 168.29, respectively. The model for estimating leaf fresh weight (FW) is: $FW = 1125.572 - 24.857L + 233.070W + 0.055LW + 276.956L^{0.5} - 1264.466W^{0.5} + 0.067L^2 - 1.964W^2$, with R^2 and RMSE values of 0.735 and 87.84, respectively. At least ten leaf samples are required when applying the two models. Determining transformed forms of leaf dimensions that are linearly related to leaf area and fresh weight, and integrating all of them into one equation maybe a better solution for establishing models to estimate leaf area and fresh weight of plant species, particularly those with higher variation among individual leaves.

Keywords: *Taraxacum kok-saghyz* Rodin, leaf length, leaf width, estimation model, regression analysis

Russian dandelion (*Taraxacum kok-saghyz* Rodin) is a perennial herbaceous plant,_rare and endemic Eastern Tien Shan species, growing on depleted and saline soils, The plants were studied as a local rubber plant in 1930-1940 and is now widely recognized as one of the most promising sources of natural raw materials for rubber production [1, 2]. The plant may contain up to more than two hundred leaves, which form a basal rosette above the root. Leaf shape is narrow obovate or oblanceolate with entire or undulate margins, and without a petiole [3]. It is well known that leaf area plays an important role in plant growth analysis. Leaf area and leaf weight measurements are necessary to estimate leaf area index [4-6], photosynthesis rates, light interception, water and nutrient use and crop growth [7-9]. Due to these leaf characteristics, it is difficult to monitor the aboveground growth status of Russian dandelion plants directly; therefore, it is necessary to develop indirect methods to estimate the leaf area and fresh weight of the plant.

Among various methodological approaches for estimating leaf area and fresh weight, indirect and nondestructive estimating methods have been widely applied for their inexpensive, rapid and simple features [6, 10]. Additionally, indirect methods enable researchers to measure leaf area and fresh weight on the same plants during the plant growth period. This may reduce variability in experiments [11-14]. In nondestructive methods, leaf area and weight are usually estimated by traits such as leaf length, leaf width, growing degree days, and petiole length. The proposed models for estimating leaf area and weight are dependent on the growth traits and leaf shape of the plant. Amongst these investigations, those which correlated leaf length and width with leaf area and fresh weight are most common [6, 15-18].

Although Russian dandelion has been studied for decades, leaf area and weight prediction models have not been developed for this plant until this paper.

Therefore, the objective of this study was to develop a reliable model for leaf area and fresh weight estimates of Russian dandelion, based on leaf dimensions, which can be applied in studies of the plant.

Techniques. Russian dandelion plants were grown in Harbin City, China in June, 2014. The experimental site is situated at N45°34'59.9", E126°34'18.8". The climate is temperate continental monsoon with a mean annual temperature of 4.2 °C and a mean annual precipitation of 532 mm. Standard crop management practices were followed in the experiment.

Russian dandelion leaf morphology varied among different plants. Two months after the seedlings were planted, fifty representative and integrated leaves were randomly selected at 14-day intervals from the plot. Each leaf sample was measured for fresh weight on an electronic balance, and the length and maximum width of the leaves were measured with a simple ruler. Length was measured from the lamina tip to the point of intersection of the lamina and stem. Width of the leaves was measured from tip to tip between the widest lamina. Leaf fresh weight was measured to ten percent of a milligram. Leaf length and width was measured to the nearest millimeter on a linear scale. Thereafter, all leaves were arrayed and marked with a serial number on white paper with a ruler as a standard measurement, covered with a white plastic film to flatten them. Photographs were taken and presented in JPG format. The leaf samples were then dried at 80°C till constant weight and measured for dry weight. Photographs were viewed using ImageJ software (version 1.48) and leaf area for each leaf was calculated using this software. Detailed procedures were followed according to the method described by H.M. Easlon and A.J. Bloom [19]. A total of two hundred and fifty leaves were measured for leaf area, fresh weight, leaf width, and leaf length in the preliminary calibration experiment.

The relationships between leaf area and fresh weight as dependent variables, and leaf length and leaf width as independent variables were determined using regression analysis on data from two hundred and fifty leaves. Independent variables were transformed into other forms (square, square root, exponent, etc.) to establish linear relationships with leaf area and fresh weight. Therefore, scatter-plot matrixes were established using OriginPro software (version 9.0) (OriginLab Corporation, USA) to find linear relationships between leaf area, fresh weight and transformed leaf length and width forms. The model equations were then developed based on scatter-plot matrixes using SPSS (Statistical Product and Service Solutions, version 19.0) software (IBM, USA). Equations with the highest coefficients of determination (R^2) were used in the estimations. Both estimated (*Sim.Yi*) and measured (*Obs.Yi*) leaf areas and fresh weights were compared by testing the significance of the regression equation and the degree of goodness of fit (R^2) between them. The final model was selected based on the combination of the highest R^2 and the lowest root mean square error (RMSE) [4]. Calculation of RMSE as follows (n for number of measurements, n = 250):

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(Sim.Yi - Obs.Yi)^2}{n}}.$$

To validate selected models, fifty leaves of Russian dandelion were selected randomly from the plot in October, 2015. Observed values of leaf length, width, area and fresh weight were determined as described as above. Simulated values of leaf area and fresh weight were obtained and compared with the observed values through correlation analysis. The slope and intercept of the model were tested to determine whether they were different from the slope and intercept of the 1:1 correspondence line at 0.05 level [20]. Regression analyses were conducted using SPSS (version 19.0) software. To determine the smallest sampling number of the proposed model, a hypothesis was proposed. Ten groups, with same number (sampling number) of leaf data selected randomly from tree hundreds leaves data set, were established. Estimated values of leaf area and fresh weight were calculated through the proposed model using the ten groups of leaf data. Linear regressions were performed between estimated values and measured values. If all the significant values of linear regression equations were smaller than 0.05, the sampling number was appropriate. Ten groups of fifty individual leaves were tested initially and if all significant values of the linear regression equations were smaller than 0.05, then ten groups of twenty five leaves data were tested next. The process could continue until the smallest sampling number was found.

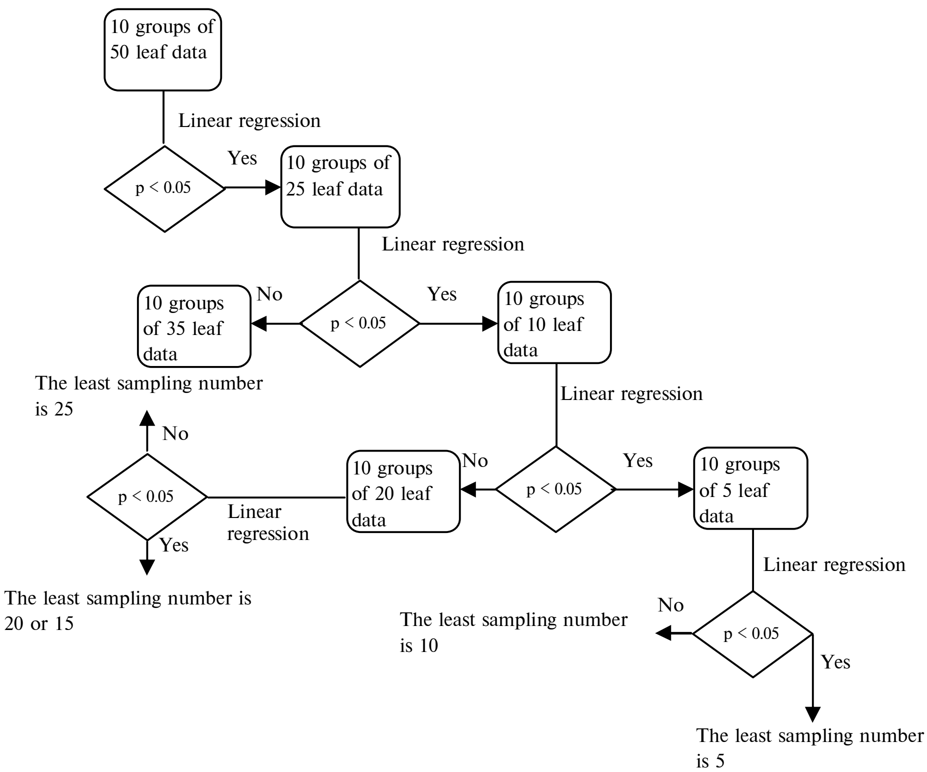
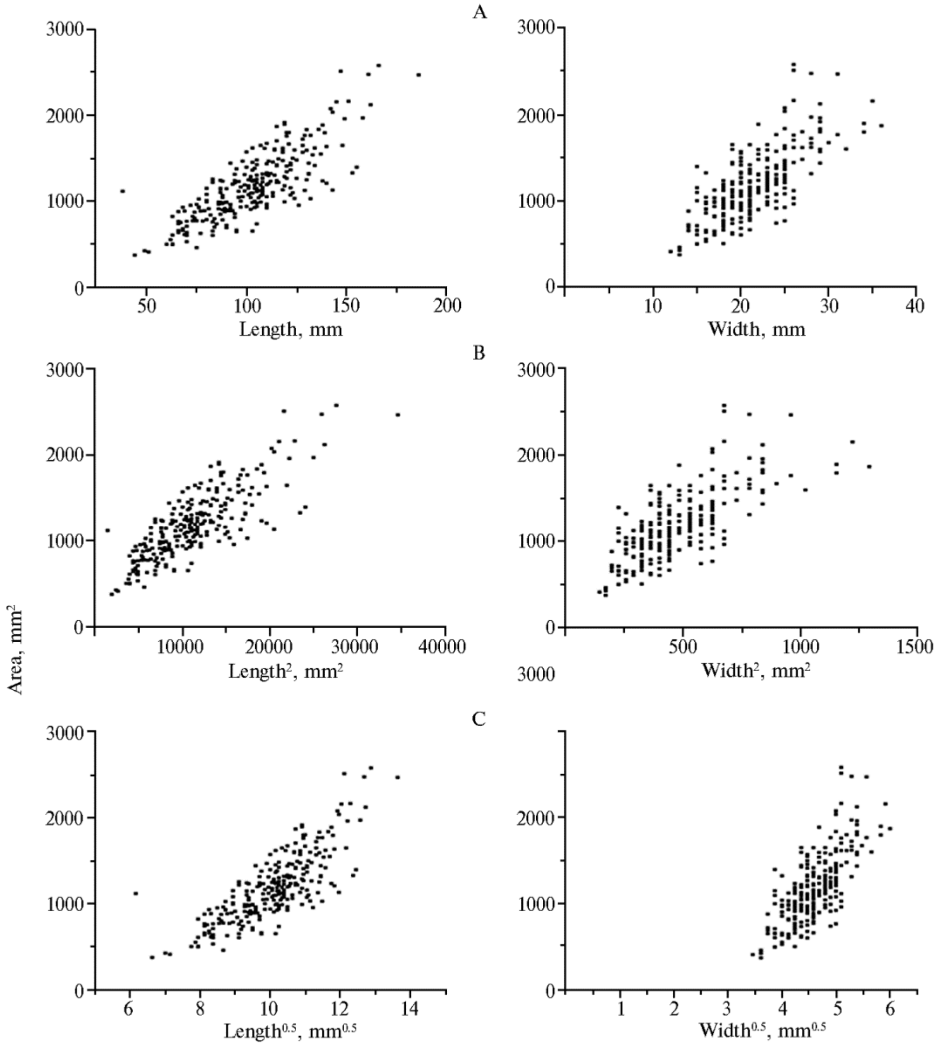


Fig. 1. Road map of determining sampling number of the proposed model for Russian dandelion (*Taraxacum kok-saghyz* Rodin) leaf nondestructive biometry.

Results. Evaluation of leaf morphology [21-24] and the development of adequate models [25] are relevant for many plants. The procedure we used to estimate the sampling number of Russian dandelion was as shown (Fig. 1).

L e a f a r e a m o d e l s e s t a b l i s h e d a n d v a l i d a t e d. Variability of the leaf



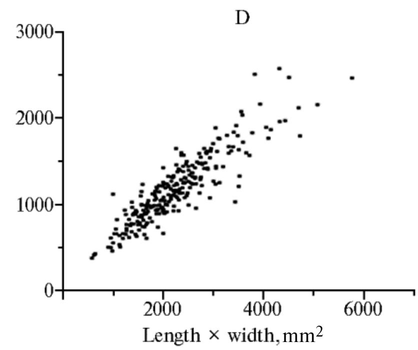


Fig. 2. Scatter-plot matrixes made between leaf area and leaf length and width (A), **square of leaf length and width** (B), **square root of leaf length and width** (C), **multiply leaf length by width** (D) **for Russian dandelion (*Taraxacum kok-saghyz* Rodin) as per suggested linear regressions.**

morphology among Russian dandelion plants is well-known [3], which increases relevance of developing a model for a non-structural assessment of biometric indicators of the leaves.

Leaf area was linearly related to leaf length and leaf width (A), square root of leaf length and width (B), square root of leaf and width (C) and value of leaf length multiplied by width (D). Other transformed forms of leaf length and width were not linearly related to leaf area. Relative equations Leaf area was linearly related to leaf length and leaf width (A), square root of leaf length and width (B), square root of leaf and width (C) and value of leaf length multiplied by width (D). Other transformed forms of leaf length and width were not linearly related to leaf area. Relative equations ($LA = a + bL + cW$; $LA = a + bL^2 + cW^2$; $LA = a + bL^{0.5} + cW^{0.5}$; $LA = a + bLW$) were established one by one (Table 1). It was found that when two different transformed forms of leaf length and width which are linearly related to leaf area, were included in one equation, the R^2 value increased and RMSE value would decreased, except in equation 8. When three or four different variables (equation 9 and 10, see Table 1) were included, R^2 values were the highest, namely 0.818. However, equation 10 had a lower RMSE value than equation 9.

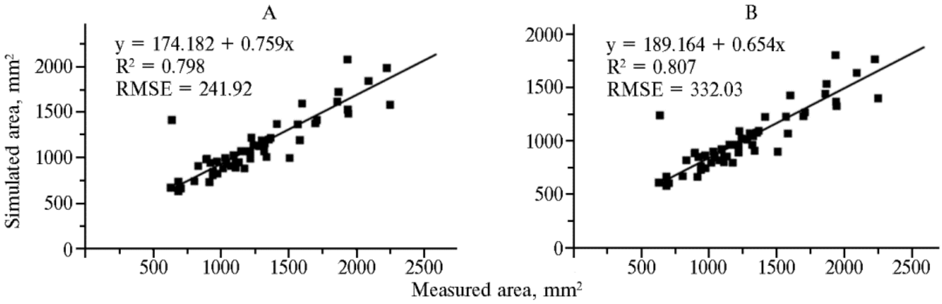


Fig. 3. Validation of the measured vs. estimated values of Russian dandelion (*Taraxacum kok-saghyz* Rodin) single leaf using model 9 (A) **and model 10** (B). Solid line represents linear regression line.

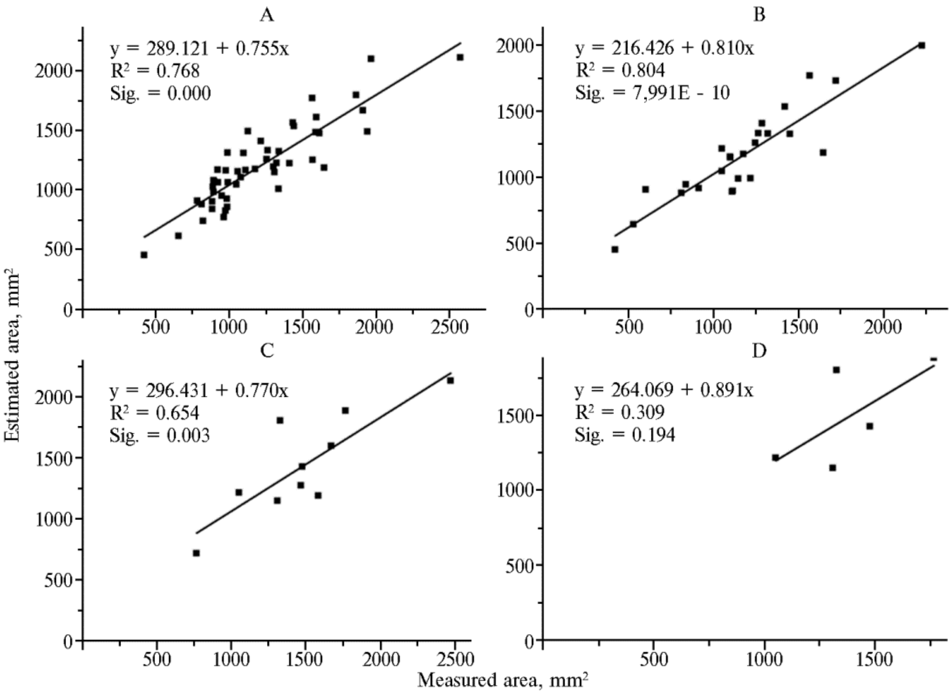


Fig. 4. Sampling number validation of proposed leaf area model for Russian dandelion (*Taraxacum kok-saghyz* Rodin) (one example): fifty leaves (A), twenty five leaves (B), ten leaves (C) and five leaves (D) validation, Sig. stands for significance level.

Validation experiments demonstrated that both leaf areas estimated using model 9 and model 10 were very similar to the measured value of leaf area. The linear regression for the relationship between both measured and estimated values were the same to the 1:1 line at 0.05 level (Fig. 2 and Fig. 3). Model 10 had

1. Estimated models for leaf area of Russian dandelion (*Taraxacum kok-saghyz* Rodin)

No	Variables	Regression models	Constant								R^2	RMSE
			a	b	c	d	e	f	g	h		
(1)	L, W	LA = a + bL + cW	–744.204	9.962	42.043						0.810	171.96
(2)	LW	LA = a + bLW	225.999	0.424							0.805	174.30
(3)	L^2, W^2	LA = a + bL^2 + cW^2	222.184	0.047	0.907						0.810	171.87
(4)	$L^{0,5}$, $W^{0,5}$	LA = a + b$L^{0.5}$ + c$W^{0.5}$	–2609.279	195.109	396.179						0.795	178.54
(5)	L, W, LW	LA = a + bL + cW + dLW	–356.135	6.274	23.674	0.171					0.813	170.74
(6)	L, W, L^2, W^2	LA = a + bL + cW + dL^2 + eW^2	–392.679	2.062	46.133	0.037	0.287				0.816	169.20
(7)	L, W, $L^{0,5}$, $W^{0,5}$	LA = a + bL + cW + d$L^{0.5}$ +e $W^{0.5}$	711.166	25.248	38.795	–306.976	29.071				0.816	169.00
(8)	$L^{0,5}$, $W^{0,5}$, LW	LA = a + b($L^{0.5}W^{0.5}$) + cLW	–220.583	18.881	0.231						0.808	172.92
(9)	L, W, L^2, W^2, $L^{0,5}$, $W^{0,5}$	LA = a + bL + cW + d$L^{0.5}$ + e$W^{0.5}$ + fL^2 + gW^2	6321.276	21.902	579.001	–248.841	–3346.881	0.002	–3.984		0.818	168.34
(10)	L, W, L^2, W^2, $L^{0,5}$, $W^{0,5}$, LW	LA= a + bL + cW + d$L^{0.5}$ + e$W^{0.5}$ + fL^2 + gW^2 + hLW	6226.424	26.310	545.334	–313.993	–3138.047	–0.009	–3.866	0.057	0.818	168.29

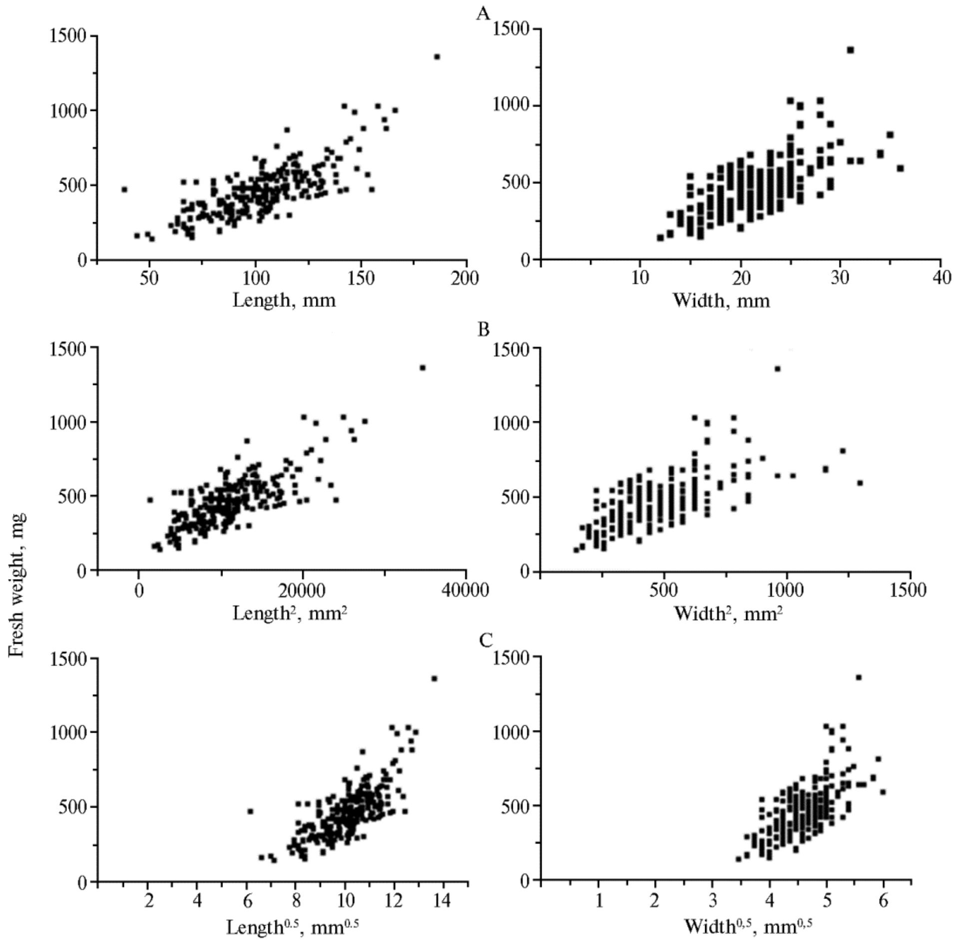
N o t e. L is leaf length, W is leaf width, LA is leaf area.

2. Estimated models for leaf fresh weight of Russian dandelion (*Taraxacum kok-saghyz* Rodin)

No	Variables	Regression models	Constant								R^2	RMSE
			a	b	c	d	e	f	g	h		
(1)	L, W	FW = a + bL + cW	–304.693	4.243	15.378						0.699	93.69
(2)	LW	FW = a + bLW	74.630	0.172							0.703	93.00
(3)	L^2, W^2	FW = a + bL^2 + cW^2	76.556	0.021	0.319						0.717	90.83
(4)	$L^{0.5}$, $W^{0.5}$	FW = a + b$L^{0.5}$ + c$W^{0.5}$	–1041.233	81.679	147.543						0.678	96.85
(5)	L, W, LW	FW = a + bL + cW + dLW	29.784	1.064	–0.455	0.147					0.709	92.03
(6)	LW, L^2, W^2	FW = a + bLW + cL^2 + dW^2	77.149	0.048	0.016	0.205					0.718	90.74
(7)	L, W, L^2, W^2, $L^{0.5}$, $W^{0.5}$	FW = a + bL + cW + dLW + eL^2 + fW^2	–45.050	–4.630	32.631	0.086	0.033	–0.587			0.734	88.11
(9)	L, W, L^2, W^2, $L^{0.5}$, $W^{0.5}$, LW	FW = a + bL + cW + dLW + e$L^{0.5}$ + f$W^{0.5}$ + gL^2 + hW^2	1125.572	–24.857	233.070	0.055	276.956	–1264.466	0.067	–1.964	0.735	87.84

N o t e. L is leaf length, W is leaf width, FW is leaf fresh weight.

a higher R^2 value than that of model 9 and was selected as the final model.



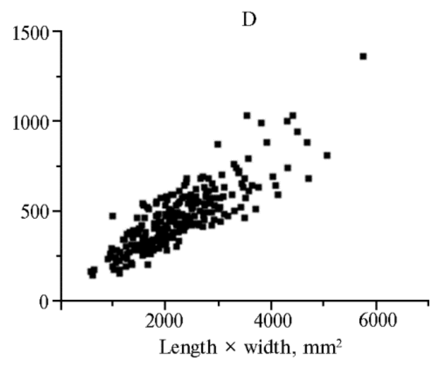


Рис. 5. Scatter-plot matrixes made between fresh weight and leaf length and width (A), **square of leaf length and width** (B), **square root of leaf length and width** (C), **multiply leaf length by width** (D) **for Russian dandelion (*Taraxacum kok-saghyz* Rodin) as per suggested linear regressions.**

To determine the smallest sample number of the proposed model (10), ten groups were established from the three hundred leaves total data set. The groups comprised fifty, twenty five, ten and five leaves data. Linear regression analyses were performed between estimated values and measured values. The results indicated that all significant values of linear regression equations established from ten groups of fifty, twenty five and ten leaves data were smaller than 0.05. However, some significant values of linear regression equations established from ten groups of five leaves data were bigger than 0.05 (Fig. 4). This suggests that the smallest sampling number required is ten when the proposed model (10) is applied.

Leaf fresh weight model established and validated. Scatter-plot matrix figures (Fig. 5) indicated that leaf length, width and other three transformed forms, namely (length, width), ($length^2$, $width^2$), ($length^{0.5}$, $width^{0.5}$), length multiplied by width, were linearly related to leaf fresh weight. When all the four forms were included in one equation, the final regression equation with the highest R^2 and the lowest RMSE values was established (Table 2). The final simulated equation is: $FW = 1125.572 - 24.857L + 233.070W + 0.055LW + 276.956L^{0.5} - 1264.466W^{0.5} + 0.067L^2 - 1.964W^2$.

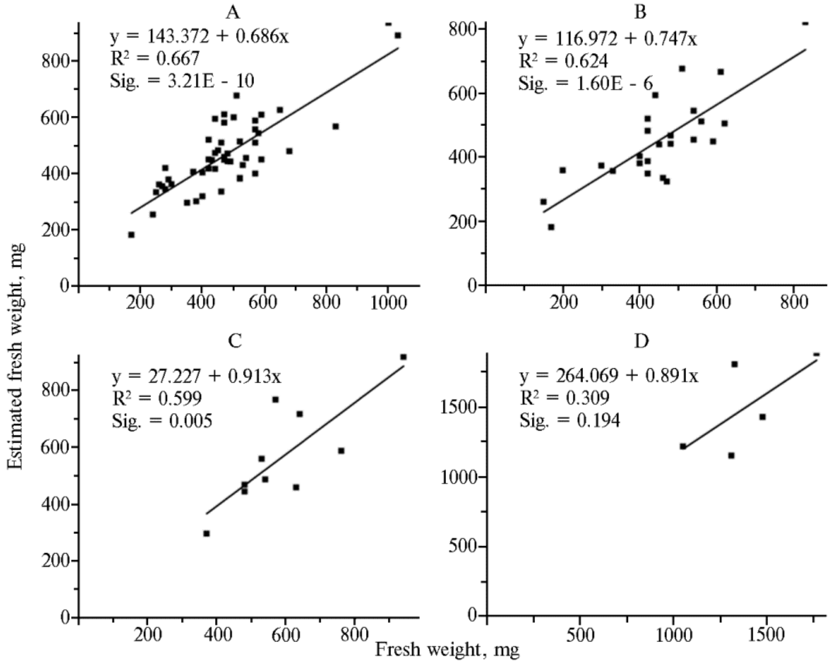


Fig. 6. Sampling number validation of proposed fresh weight model for Russian dandelion (*Taraxacum kok-saghyz* Rodin) (one example): fifty leaves(A), twenty five leaves(B), ten leaves(C) and five leaves (D) validation. Sig. stands for significance level.

Regression analysis to determine the minimum sampling (Fig. 6) showed results similar to the leaf area (at least 10 leaves).

Thus, four transformed forms of leaf length and width, i.e. (length, width), (length^2, width^2), (length$^{0.5}$, width$^{0.5}$), (length × width), were found to be approximately linearly related to leaf area and fresh weight. When the four transformed forms were integrated in one equation, regression models were established for the estimation of leaf area and fresh weight of Russian dandelion, without the destruction of leaves. The equation for estimating leaf area is: $LA = 6226.424 + 26.31L + 545.334W - 313.993L^{0.5} - 3138.047W^{0.5} - 0.009L^2 - 3.86W^2 + 0.057LW$, R^2 and RMSE values for the model are 0.818 and 168.29 respectively. At least ten leaves are required when the model is applied. The equation for estimating leaf fresh weight is: $FW = 1125.572 - 24.857L + 233.070W + 0.055LW + 276.956L^{0.5} - 1264.466W^{0.5} + 0.067L^2 - 1.964W^2$, R^2 and RMSE value for the model are 0.735 and 87.84 respectively. At least ten leaves are required when this model is applied. The proposed models need to be validated in other varieties of Russian dandelion in the future. For plants species with higher variation among individual leaves, determining transformed forms of leaf dimensions that are linearly related to leaf area and fresh weight and integrating all of them into one equation maybe a better solution.

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