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Estimation of leaf area of stem lettuce (*Lactuca sativa* var *angustana*) from linear measurements

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Stem lettuce (*Lactuca sativa* L. var. *angustana*) is a very popular garden crop in China. Its stem is used as a vegetable. There are many different cultivars of stem lettuce grown in various regions of the country, although based on leaf shape, these can be categorized into long and oval leaf types.

Leaf is an important organ of plants, which is closely related to photosynthesis and evapo-transpiration. Therefore leaf-area measurements are required in most physiological and agronomic studies involving plant growth. Leaf area is also involved in the derivation of many indices of plant growth, which are frequently used in the plant growth analysis (Potdar and Pawar 1991).

Many methods of leaf-area measurements have been developed (Robson and Sheehy 1981). Direct methods for determining leaf area are restricted to the use of an automatic area-integrating meter. However, for a researcher who doesn't have an electronic area meter, non-destructive methods of measuring leaf area are limited to using leaf geometric shapes, dot counting, and light interception. One of the most frequently used non-destructive and indirect methods is estimating leaf area from mathematical formulae involving linear measurements of leaf or leaflet. The accuracy of linear measurements of leaf area was reviewed by Wiersman and Bailey (1975).

In recent years, prediction of leaf area by mathematical models has become a common approach (Potdar and Pawar 1991, Thornley and Johnson 1990). This procedure involves measuring the length, width, leaf weight and area of a set of leaf samples, and calculating several coefficients on leaf parameters for predicting areas of subsequent samples. However, proper selection of the independent variable(s) and time of sampling are cardinal in modeling prediction equations for plant growth systems by regression analysis.

Previous investigations concentrated on generating a single regression equation for all growth stages of plant (Sepaskhah 1977). This method presents a number of shortcomings such as overlooking changes in leaf morphology with growth stages and growth systems (Robbins and Pharr 1987). In the present study, separate equations for leaf-area estimation were developed for different genotypes of stem lettuces at different growth stages. The study was conducted to (*i*) develop separate prediction models for estimating individual leaf area of different genotypes of stem lettuce plants at different growth stages; (*ii*) demonstrate the reliability of making leaf-area estimations at different stages of plant growth using different methods; and (*iii*) identify the most suitable parameters to be used in estimating leaf area of the 2 stem lettuce cultivars at different stages of plant growth using different methods.

Field and laboratory procedures

The field experiment was conducted during 1997–98 to 1998–99 at the farm, College of Agriculture and Biotechnology, Hangzhou. The cultivars were sown on 11 October, and transplanted after 1 month when plants had 3–4 true leaves, and then grown under normal field conditions with standard cultural practices in randomized block design with 3 replications and at 2 plant spacings (30 cm × 30 cm and 30 cm × 40 cm). The plot size was 3 m × 25 m.

Leaf samples used in this experiment were obtained from 2 cultivars ('Erbaipi', long leaf type and 'Yuanye', oval leaf type). A total of 300 measurable leaves of different sizes, each from 30 randomly selected plants of both cultivars were taken at different growth stages (8th, 16th, 20th and 24th week). The maximum length and width of each leaf was measured to 0.1 cm. The individual leaf area measurements were made with a LI-188b leaf-area meter calibrated to 0.01 cm^2.

Regression model calculation

Regression analyses were made between leaf area and suggested parameters using a general model of the following order :

$$Y = a + bX$$

where a and b are model constants.

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Table 1 Means standard errors, Sb^, and ranges of lengths, width, and areas of leaf from 2 cultivars at different developmental stages

Time	CV		No.	Mean	Minimum value	Maximum Value
8 week	Long	Area	300	24.18	5.6	39.1
		L	300	12.02	5.2	15.5
		W	300	2.73	1.7	3.8
	Oval	Area	300	27.84	8.2	54.15
		L	300	11.38	4.5	17.0
		W	300	3.53	2.0	5.1
16 week	Long	Area	300	36.21	12.8	62.3
		L	300	12.22	6.8	15.6
		W	300	4.38	2.5	6.6
	oval	Area	300	58.40	15.4	116.5
		L	300	13.63	6.0	19.1
		W	300	5.61	3.1	10.2
20 week	Long	Area	300	123.11	78.3	155.7
		L	300	22.42	16.6	26.0
		W	300	7.07	5.5	8.1
	oval	Area	300	200.30	99.6	280.9
		L	300	25.05	17.4	29.6
		W	300	10.74	7.0	13.5
24 week	Long	Area	300	151.03	116.9	188.2
		L	300	27.94	26.3	32.2
		W	300	7.11	5.8	8.1
	oval	Area	300	212.63	159.8	284.0
		L	300	26.64	24.0	31.7
		W	300	11.53	9.2	13.9

The estimation equation for the measured leaf area (LA) was determined using the stated parameters as independent variables: length (L), width (W), and length × width (L × W). For each cultivar, 4 regression models at 4 different leaf development stages were developed.

Individual leaves and cultivars at a certain or different stage differed significantly in length, width and leaf area, indicating a wide diversity in the materials (Table 1). The size of leaves of both cultivars also greatly changed with plant growth, the mean length and width of leaves of long leaf cultivar 'Erbaipi' were 12.02 cm and 2.73 cm at the 8th week, respectively, which increased to 27.94 cm and 7.11 cm at the 24th week (Table 1). Whilst the mean leaf length and width of oval leaf cultivar 'Yuanye' increased from 11.38 cm and 3.53 cm at the 8th week to 26.6 cm and 11.53 cm at the 24th week respectively.

It was observed (Table 2) that in prediction equations involving L as variable for 2 cultivars at different growth stages, the values of regression coefficient, b, and the intercept, a, were different among cultivars and/or different growth stages, and the statistical results (Se, Sb^, R^2) were also different. Meanwhile, similar tendency of changes in various values were also observed in regression analyses when leaf width was used as variable (Table 2).

Regression equations were also calculated using the L × W as variable (Table 2). It is obvious that the use of L × W as opposed to only L or W for leaf area increased the coefficients of determination (R^2), and decreased the Se and Sb^, so the use of L × W was satisfactory in predicting area. Although the slopes (b) of the regression equations calculated for leaf areas for 2 cultivars at different stages did not differ too much, they were still different, also the value changed a lot with the growth stage. Therefore, predicting leaf area by L × W using a fixed regression model at different growth stage was questioned.

In the present study, R^2 reduced with the growth of plants, irrespective of independent variable used.

The b constants found in this study when L × W was used as the independent variable in 2 cultivars was closely identical to those calculated for other crops (Hughes and Proctor 1981, Ray and Singh 1989). Therefore, the separate leaf-area estimation equation should be determined for higher accuracy for each cultivar during the various vegetative stage of growth to get a satisfactory estimate of leaf area at a specific stage.

Leaf morphology is even related to cultural systems in cucumber, larger leaves were observed in plants grown in hydroponic sand culture (Robbins and Pharr 1987), suggesting a need of separate equation to have a precise prediction of leaf area even for the same variety grown under different cultural system. In the present experiment, development of separate regression equation for estimation of leaf area for 2 distinct leaf morphology cultivar well met the requirement.

Prediction models for the 2 cultivars at different stages of

Table 2 Regression analysis using L, W and L×W variables for predicting the area of individual levels of 2 cultivars at different developmental stages

Time	CV	Actual area	Predicted area	Sb<	Se	R^2	a	b
Week 8	Long	24.18	24.46	0.3429	3.4394	0.9519	−13.2912	3.1202
	Oval	27.84	27.80	0.3842	5.1228	0.9444	−8.4443	3.1884
Week 16	Long	36.21	35.32	0.4548	4.6065	0.9835	−29.2414	5.6312
	Oval	58.40	57.89.	0.8362	12.8835	0.9649	−52.6931	8.1591
Week 20	Long	123.11	120.17	2.7091	24.4539	0.9256	−71.5246	8.9242
	Oval	200.30	190.43	2.1371	23.9315	0.9350	−166.7451	14.6791
Week 24	Long	151.09	145.22	3.0514	25.7448	0.9267	−141.9900	10.4986
	Oval	212.68	195.95	3.4733	25.0133	0.9298	−94.7631	12.0270
				W variable				
Week 8	Long	24.18	24.17	1.5466	3.4934	0.9588	−13.5408	13.8310
	Oval	27.84	27.55	1.2283	3.3966	0.9792	−28.6249	15.9275
Week 16	Long	36.21	35.38	1.5572	7.6918	0.9764	−11.3501	10.8527
	Oval	58.40	59.14	1.2155	8.1135	0.9968	−50.5352	19.4204
Week 20	Long	123.11	120.72	4.3717	21.6272	0.9381	−51.6650	25.5628
	Oval	200.30	203.53	4.3739	24.5816	0.9470	−112.4223	29.1114
Week 24	Long	151.09	148.10	4.6244	18.7982	0.9136	−20.5453	24.1751
	Oval	212.68	205.76	3.4369	15.3014	0.9302	−41.4319	23.1628
				L×W variable				
Week 8	Long	24.18	24.30	0.03753	1.8778	0.9708	1.8203	0.6484
	Oval	27.84	27.83	0.03578	2.6559	0.9689	1.7974	0.5995
Week 16	Long	36.21	35.76	0.03110	3.0143	0.9934	3.6512	0.5993
	Oval	58.40	57.92	0.03101	6.2414	0.9868	3.5885	0.6491
Week 20	Long	123.11	121.01	0.08745	10.0982	0.9476	−12.5000	0.8856
	Oval	200.30	196.84	0.07442	17.9862	0.9681	7.0590	0.7048
Week 24	Long	151.09	152.33	0.08875	14.7932	0.9242	19.3696	0.6618
	Oval	212.68	207.95	0.08168	15.0561	0.9250	52.0496	0.5610

All R^2 values significant at the 5% level

plant growth showed that the values of model parameters, b, and the intercept, a, were different for each of the character studied, although the statistical result (R^2) did not differ much, especially at corresponding leaf-development stages. The b constant in a regression equation involving a measurement of leaf length and width was not significantly different even in various crops, with values of 0.5~0.8 (Bhan and Pande 1966, Hughes and Proctor 1981, Ray and Singh 1989). In our study, b value was relatively stable when L × W was used as the independent variable, akin to others (Hughes and Proctor 1981, Ray and Singh 1989), but it changed a lot when L or W was used as the independent variable (Table 2).

Sampling at the 16th week of growth was best stage for estimation of leaf area using regression equation for both the cultivars compared to other stage, for any of the parameters may offer a reliable leaf-area prediction. It was easy to infer the influence of leaf shape on leaf-area determination in the present study.

Growth habit of plant affected its leaf development. However, the lack of consistence in coefficients of regression (Table 2) at the 4 stages of leaf development for both cultivars indicates that analysis based on a single sampling data may not provide a more accurate result especially when leaf architecture changes with leaf development.

Our research on predicting leaf area of 2 cultivars at different growth stages differed from most published materials. This detailed study is helpful in searching a suitable model for a specific stage. An important consideration in formulating prediction equations by regression is the choice of the independent variable (s). Therefore, 4 prediction equations, involving various independent variables (the leaf length, the leaf width, and the product of leaf length × leaf width), were formulated for estimating leaf area by use of the linear regression equation, $y = a + bx$. Results of these analyses (Tables 2) showed that LA was closely related to all parameters (L, W, and L × W). However, the models that incorporated both L and W (L × W), gave the highest coefficients of regression (R^2) predicting ability of the regression model, for both cultivars at all stages of leaf development. But coefficient of regression (R^2) are smaller when only length or width of the leaf is measured. Hence leaf-area estimations were most reliable with the models where the product of L and W (L × W) was used (Tables 2). Our present result confirms the hypothesis that product of leaf length and leaf width could be a reliable parameter in estimating leaf area.

This study has shown that leaf-area estimations based on linear measurements *in situ* are reliable. It has also been revealed that consideration of differences in leaf shape varying with cultivars and growth stages is also crucial in estimating leaf area. The approach used here is rapid, inexpensive, simple and precise for leaf-area determination in crops especially with non-lobed leaves. Hence regression models based on linear measurements can provide a rapid, and convenient approach for the determination of leaf area in stem lettuce.

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SUMMARY

A study was carried out during 1997–98 and 1998–99 to estimate leaf area of stem lettuce (*Lactuca sativa* L. *angustana*) from linear measurement. Sampling at the 16th week of growth was found best for estimation of leaf area using regression equation for 'Erbaipt' and 'Yuange' cultivars compared with the sampling done at other stage. The leaf-area estimations based on linear measurements *in situ* were found reliable. Regression models based on linear measurements can provide a rapid and convenient approach for the determination of leaf area in stem lettuce.

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