

LEAF AREA ESTIMATION IN JOJOBA (*SIMMONDSIA CHINENSIS* (LINK.) C.E. SCHNEIDER) SEEDLINGS

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ABSTRACT

The leaves are variously shaped in Jojoba seedlings and may be broadly categorized into two types. The leaves produced on the lower node (s) are generally smaller with obtuse apex in contrast to the commonly occurring leaves of upper nodes which are comparatively larger with acute apex. Of 115 leaves studied from 12 seedlings there were 85 acute apex and 30 were obtuse apex leaves (ratio: 2.83: 1). The graphically measured one-sided leaf area (LAM) of 115 individual leaves of *Simmondsia chinensis* (Link.) C.E. Schneider varied from 0.13 – 11.57 cm² (mean = 4.11 ± 0.237 cm²; CV = 56.68%). The leaf area (LAM) was found to correlate significantly with multiplicative parameter of leaf length and breadth (LL x LB) as simple linear model ($R^2 = 0.963$, $F = 2982.04$ ($p < 0.0001$)) as well as the power model ($R^2 = 0.982$; $F = 6246.31$ ($p < 0.0001$)). Leaf area also correlated significantly with LL and LB linearly combined in a multiple linear correlation / regression model ($R^2 = 0.958$; $F = 136.0$ ($p < 0.0001$)). The coefficient k estimated arithmetically as $k = \text{Area}_{\text{measured}} / (\text{LL} \times \text{LB})$ averaged to 0.64713 ± 0.00823 (CV = 13.64%). On statistical comparison, the estimated leaf areas on the basis of above models were not found to vary significantly from the measured areas of the leaves (LAM) and therefore, may be used for leaf area estimation in Jojoba. However, in view of simplicity and convenience and accuracy of estimates, the method of using average k factor may be recommended for the estimation of leaf area in Jojoba. The magnitude of k averaged to 0.65879 ± 0.00717 in case of acute apex leaves, 0.61425 ± 0.02342 for obtuse apex leaves and 0.64713 ± 0.008229 for the pooled sample of the two types of the leaves.

Key Words: *Jojoba* (*Simmondsia chinensis* (Link.) Schneider, Arithmetic and Allometric estimation of leaf area.

INTRODUCTION

The leaf area is directly related to light interception, photosynthesis, transpiration and carbon gain and storage. It is considered to be the most important single determinant of plant productivity (Linder, 1985; Kathirvelan and Kalaiselvan, 2007). The estimation of leaf area is, however, a time-consuming and laborious task. The applicability of allometric methods in leaf area estimation was shown by Huxley (1924) first time in some grasses. Pearsall (1927) used allometric relationships in carrot and turnip to predict root storage through shoot growth estimation. Leaf area estimation in several species has been undertaken by many workers for various reasons (Kemp, 1960; Jain and Misra, 1966; Williams *et al.*, 1973; Aase *et al.*, 1978; Hatfield *et al.*, 1976; Elasner and Jubb, 1988; Chinamuthu *et al.*, 1989; O'Neal *et al.*, 2002; Williams III and Martinson, 2003; Kathirvelan and Kalaiselvan, 2007; Cristofori *et al.*, 2007; Khan, 2008, 2009, Ahmed and Khan, 2011).

Jojoba (*Simmondsia chinensis* (Link.) C.E. Schneider) is an economically important plant which yields very costly oil for several industrial uses (NRC, 2004). In this paper, allometric relationship of leaf area of this species with such linear measurements as length and breadth of the leaf is investigated besides generally employed arithmetic procedure for determining leaf area through calculation of mean multiplication coefficient (K), as employed by several workers (Kathirvelan and Kalaiselvan, 2007; Khan, 2008, 2009; Ahmed and Khan, 2011, Khan *et al.*, 2015a). This study has been undertaken to develop a simple but efficient way of ascertaining leaf area in Jojoba so that photosynthetic area of its juveniles may be determined in our experiments related to Root Rot - Root Knot disease complex in this species.

MATERIALS AND METHODS

One hundred and fifteen (115) leaves of various sizes from 12 healthy seedlings of Jojoba grown in pots under normal agricultural practices in experimental field of department of Botany, University of Karachi, were collected and their linear measurements were recorded for the length of leaf (lamina length lying between the point of midrib insertion and the apex of the leaf (LL) and lamina breadth (LB) at the broadest points. To determine true leaf area, the leaf outline was carefully drawn on graph paper and area determined with all possible precision and accuracy. The multiplication factor (K) was calculated by employing the formula, $K = \text{LEAF Area}_{\text{measured}} / (\text{LL} \times \text{LB})$. Employing average value of the multiplication factor k, leaf areas were calculated as $\text{Leaf Area}_{\text{computed}} = K (\text{length} \times \text{breadth})$ for comparison with the observed areas of the leaves. Leaf aspect ratio was calculated as LB/LL (Lu *et al.* (2012). The location and dispersion parameters of the data were calculated (Zar, 1994). The skewness and kurtosis (g_1 and g_2 , respectively) were calculated as $g_1 = K_3 / (K_2')^{3/2}$ and $g_2 = K_4 / (K_2')^2$, respectively - K_s , are moments around mean (see Shaukat and Khan, 1979). The standard errors of skewness and kurtosis (Sg_1 and Sg_2 , respectively) were given as:

$$Sg_1 = \sqrt{6N(N-1) / (N-2)(N+1)(N+3)} \text{ and}$$

$$Sg_2 = \sqrt{24N(N-1)2 / (N-3)(N-2)(N+3)(N+5)}.$$

Table 1A. Location and dispersion parameters of length, breadth, aspect ratio, leaf area, multiplicative parameter and Coefficient K of acute apex leaves (N = 85) of Jojoba seedlings.

Parameter	LL (cm)	LB (cm)	Aspect ratio	LAM (cm ²)	LL x LB	K	LAK	LAPOW	LAMR
Mean	3.8635	1.9647	0.526093	5.15741	7.813	0.65879	5.15741	5.14203	5.15761
SE	0.0968	.04434	0.0137	0.2224	0.3099	0.00717	0.22239	0.200259	0.21138
Q2	4.0000	2.000	0.5000	5.110	7.980	0.64778	5.110	5.25668	5.28970
CV (%)	23.09	20.81	24.02	39.75	36.57	10.02	39.75	36.80	37.79
g1	-0.810	0.059	1.978	0.597	0.009	1.209	0.597	0.015	-0.478
Sg1	0.261	0.261	.261	0.261	0.261	0.261	0.261	0.261	0.261
g2	0.198	1.720	6.127	1.000	-0.366	1.570	1.00	-0.369	1.008
Sg2	0.517	0.517	0.517	0.517	0.517	0.517	0.517	0.517	0.517
Min.	1.00	0.70	0.294	.4300	0.70	0.558	0.430	0.4545	0.1625
Max.	5.00	3.30	1.053	11.57	14.52	0.887	11.57	9.5988	9.990
KS-z	1.374	0.914	1.175	0.603	0.498	1.381	0.603	0.498	0.515
p	0.046	0.374	0.126	0.861	0.915	0.044	0.861	0.965	0.954

Table 1B. Location and dispersion parameters of length, breadth, aspect ratio, leaf area, multiplicative parameter and Coefficient K of obtuse apex leaves (N = 30) of Jojoba seedlings.

Parameter	LL (cm)	LB (cm)	Aspect ratio	LAM (cm ²)	LL x LB	K	LAK	LAPOW	LAMR
Mean	1.3950	1.0877	0.77231	1.09333	1.6943	0.61425	1.09333	1.073664	1.09331
SE	0.0816	0.09715	0.04084	0.17916	0.24298	0.02342	0.17916	0.169510	0.170893
Q2	1.3250	1.0000	0.73864	0.82000	1.3750	0.60062	0.8200	0.83217	1.04140
CV (%)	32.04	48.92	29.97	89.76	78.55	20.88	89.75	86.47	85.71
g1	0.353	1.134	0.356	1.876	1.641	0.653	1.876	1.792	1.010
Sg1	0.427	0.427	0.427	0.427	.427	0.427	0.427	0.427	0.427
g2	-0.157	0.718	-0.465	3.956	2.998	0.263	3.956	0.638	0.571
Sg2	0.833	0.833	0.833	0.833	0.833	0.833	0.833	0.033	0.833
Min.	0.50	0.50	0.3846	0.1300	0.25	0.3833	0.130	1.288	0.170
Max.	2.50	2.40	1.2687	4.4600	6.00	0.9298	4.440	4.1738	3.499
KS-z	0.796	0.914	0.512	1.026	0.866	0.632	1.026	0.919	0.768
p	0.550	0.374	0.956	0.243	0.442	0.819	0.243	0.367	0.598

Table 1C. Location and dispersion parameters of length, breadth, aspect ratio, leaf area, multiplicative parameter and Coefficient K of all types of leaves (N =115) of Jojoba seedlings.

Parameter	LL (cm)	LB (cm)	Aspect ratio	LAM (cm ²)	LL x LB	K	LAK	LAPOW	LAMR
Mean	3.2370	1.7390	0.587280	4.11017	6.2381	.64713	4.11018	4.10097	4.10906
SE	0.1243	0.05433	0.01786	0.23735	0.34343	.008229	0.23731	0.23325	0.227368
Q2	3.5000	1.9000	0.53333	4.45000	6.4600	0.64087	4.450	4.21073	4.74810
CV (%)	41.21	33.51	32.61	59.68	59.04	13.64	61.93	61.01	59.27
g1	-.407	-0.365	1.462	0.346	0.057	0.398	0.346	0.308	-0.525
Sg1	0.226	0.226	0.226	0.226	0.226	0.226	0.226	0.226	0.226
g2	-1.179	-0.166	1.875	-0.150	-0.969	1.462	- 0.150	-0.946	-0.609
Sg2	0.447	0.447	0.447	0.447	0.447	0.447	0.447	0.447	0.447
Min.	0.50	0.50	0.2941	0.1300	0.25	0.3833	0.1300	0.1385	1.2755
Max.	5.00	3.30	1.2687	11.570	14.52	0.9298	11.570	8.555	8.7171
KS-z	1.621	1.248	1.907	0.732	0.840	1.372	0.732	0.871	1.155
p	0.010	0.089	0.001	0.658	0.480	0.048	0.658	0.434	0.139

Acronyms: LL, leaf length; LB; leaf breadth; LAM, Leaf area measured; K, coefficient; LAK, leaf area estimated via K; LAPOW, leaf area estimated via power equations (Table 2); LAMR, leaf area estimated via equations of multiple regression (see Fig. 2). Aspect ratio = LB / LL (after Lu *et al.* (2012).

Linear and power relationships of leaf area with multiplicative parameter of $LL \times LB$ were determined. In addition to it, the regression coefficients were also determined by employing multiple regression method fitting in the allometric model, $Y = a + b_1LL + b_2LB \pm SE$ (Zar, 1994). The arithmetic and allometric methods were compared for their precision and suitability. The data was analyzed using SPSS version 12.

RESULTS AND DISCUSSION

Jojoba seedlings produce variously shaped leaves (Khan *et al.*, 2015b) which may broadly be categorized into two groups.

1. The leaves produced at lower nodes - generally smaller, obtusely-apexed and retuse occasionally.
2. The leaves produced on upper nodes are generally larger and acutely-apexed, elliptic, lanceolate to oblanceolate and ovate.

Amongst 115 leaves collected from 12 healthy Jojoba seedlings, 30 leaves were obtusely-apexed and 85 were acutely-apexed leaves i.e. acutely-apexed and obtusely-apexed leaves were in a ratio of 2.83:1. Various location and dispersion parameters pertaining to these leaves and their pooled sample are outlined in Table 1.

Leaf length

Leaf length in case of the pooled sample of leaves ($N = 115$) varied from 0.50 to 5.0 cm (mean: 3.24 ± 0.124 ; CV: 41.21%). Leaf length of acute apex leaves averaged to 3.86 ± 0.097 cm (1.0-5.0 cm). These leaves varied by 23.09% in length. The obtuse apex leaves were smaller and averaged to 1.40 ± 0.082 cm in length (0.5 to 2.5 cm) varying 32.04% in size (Table 1).

Leaf breadth

Leaf breadth in case of the pooled sample of leaves ($N = 115$) varied from 0.50 to 3.3 cm (mean: 1.739 ± 0.543 ; CV: 33.51%). Leaf breadth of acute apex leaves averaged to 1.96 ± 0.44 cm (0.70 – 3.3 cm) – varying by 20.81%. The obtuse apex leaves were smaller and their breadth 1.088 ± 0.097 cm (0.5 to 2.4 cm) varying 48.92% in size (Table 1).

Leaf Shape Consistency

Length / breadth ratio may give some indication about consistency of leaf shape with size (Verwijst and wen, 1996). In present studies, breadth / length ratio (aspect ratio after Lu *et al.* (2012), a converse of Length / breadth ratio, was calculated in the two types of leaves. Aspect ratio in apex acute leaves averaged to 0.5261 ± 0.0137 (varying from 0.294 to 1.053; CV = 24.02%). Median (0.50) was located near the mean. In obtuse apex leaves, aspect ratio averaged to 0.7723 ± 0.0408 and varied from 0.385 to 1.269 (CV: 28.97%)(Table 1; Fig. 1). Aspect ratio varied somewhat more in obtuse apex leaves as compared to that in acute apex leaves. Aspect ratio in both types of leaves distributed symmetrically (Table 1).

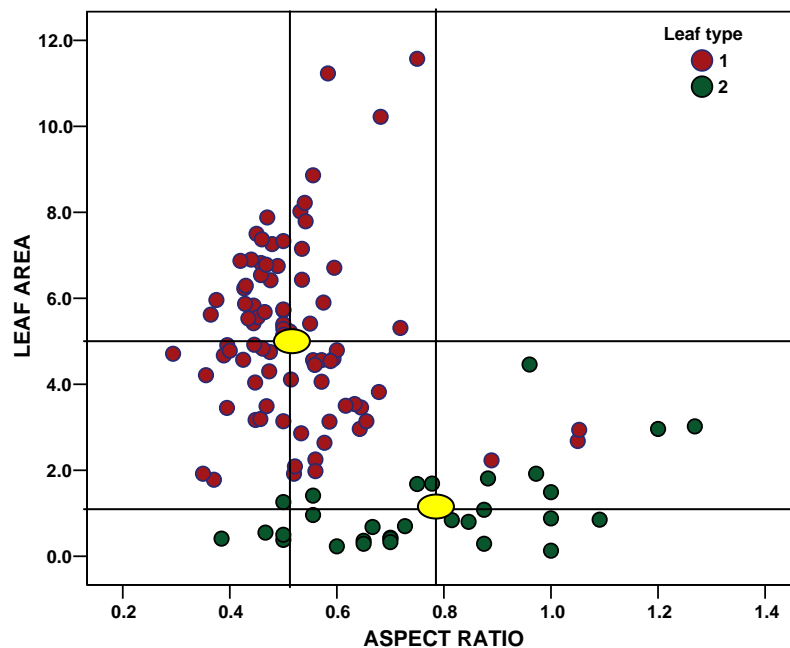


Fig. 1. Leaf area (cm^2) and aspect ratio (Leaf breadth / Leaf length) relationship in two types of leaves of Jojoba. Type 1, acute apex leaves ($N = 85$) and Type 2, leaves with obtuse apex ($N = 30$).

It followed from the data that acute apex leaves were larger in size with low aspect ratio whereas obtuse apex leaves were smaller but with larger aspect ratio.

Leaf Area

The measured one-sided single leaf area (LAM) of acute apex leaves varied from 0.43 to 11.57 cm² (mean = 5.157 ± 0.2224 cm²; CV = 39.75%). The mean leaf area of obtuse apex leaves quite low (mean = 1.0933 ± 0.1792 cm² (varying from 0.136 to 4.460 cm²; CV= 89.76%). In both types of leaves, LAM distributed normally (Table 1). The leaf area was found to correlate with LL and LB significantly but the values of r^2 were low. LB was, however, more closely related with LAM than LL with LAM (Table 2).

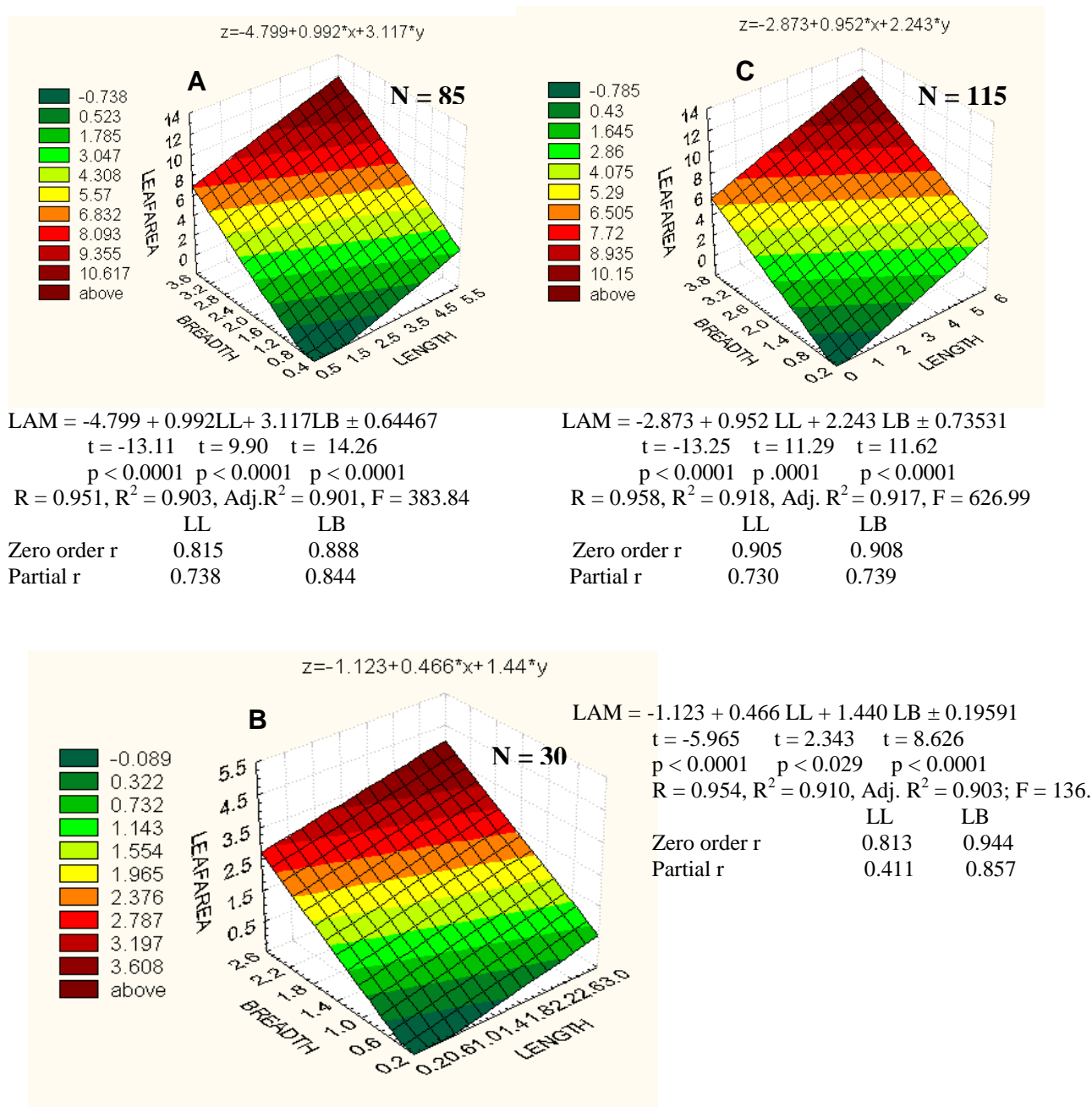


Fig. 2. Relationship of leaf area measured (LAM) with LL and LB as given by multiple linear regression

Table 2. Linear correlation and regression between leaf area and linear dimensions of leaves.

Leaf area	Length				Breadth			
	r ²	a	b	F	r ²	a	b	F
Leaf area -Acute apex leaves, N=85	0.664	-2.080	1.873	164.15 (p < 0.0001)	0.788	-3.590	4.452	164.15 (p < 0.0001)
Obtuse apex leaves, N=30	0.662	-1.398	1.786	54.73 (p < 0.0001)	0.892	-0.851	1.741	230.21 (p < 0.0001)
Pooled sample, N=115	0.819	-1.480	1.727	511.96 (p < 0.0001)	0.825	-2.788	3.967	531.39 (p < 0.0001)

Linear simple and multiple regression models obtained by regression of LAM with LL and LB separately or in combination yielded significant equations (Fig. 2) The zero order or partial correlations in multiple linear correlation and regression analyses for either type of leaves or the pooled sample of leaves were relatively higher with LB than that with LL i.e. LAM depended somewhat more on LB than LL.

The leaf area LAM was found also to relate with multiplicative parameter of LL x LB significantly as simple linear model.

LA M (cm²) = - 0.24646 + 0.69167 (LL x LB) ± 0.5490 Acute apex leaves (N = 85)
R² = 0.929; F = 1088.54 (p < 0.0001)

LA M (cm²) = -0.12000 + 0.71614 (LL x LB) ± 0.2378 Obtuse apex leaves (N = 30)
R² = 0.929; F = 1088.54 (p < 0.0001)

LA M (cm²) = -0.12163 + 0.67839 (LL x LB) ± 0.048849 Pooled sample of leaves (N = 115)
R² = 0.929; F = 1088.54 (p < 0.0001)

Table 3. Relationship of leaf area with multiplicative parameter of leaf length and width (Power model).

Leaf types	Best fit Power equations
Leaves with Acute apex (N = 85)	$\text{LAM (cm)}^2 = 0.650668. (\text{LL} \times \text{LB})^{1.00593} \pm 0.098446$ $t = 21.93 \quad t = 44.55$ $p < 0.00001 \quad p < 0.00001$ $R = 0.9797; R^2 = 0.9599, \text{Adj. } R^2 = 0.9595; F = 1984.46 (p < 0.00001)$
Leaves with obtuse apex (N = 30)	$\text{LAM (cm)}^2 = 0.587212. (\text{LL} \times \text{LB})^{1.094567} \pm 0.19591$ $t = 26.37 \quad t = 22.54$ $p < 0.00001 \quad p < 0.00001$ $R = 0.9735; R^2 = 0.9478, \text{Adj. } R^2 = 0.9459; F = 508.22 (p < 0.00001)$
Leaf types (pooled) (N = 115)	$\text{LAM (cm)}^2 = 0.593706. (\text{LL} \times \text{LB})^{1.050052} \pm 0.13016$ $t = 42.11 \quad t = 76.03$ $p < 0.00001 \quad p < 0.00001$ $R = 0.9911; R^2 = 0.9822, \text{Adj. } R^2 = 0.9822; F = 6246.31 (p < 0.00001)$

The power model was, however, the best fit model to relate LAM with multiplicative parameter, LL x LB in case of acute apex and obtuse apex leaves or pooled leaf sample as given in Table 3. All these equations were not only highly significant but had very high explanatory power to account for variation in LAM by LL x LB in two types of leaves and their pooled sample.

In present studies, the coefficient k was arithmetically estimated as $k = \text{Area}_{\text{measured}} / (\text{LL} \times \text{LB})$. This parameter averaged to 0.65879 ± 0.00717 in acute apex leaves. In case of obtuse apex leaves K averaged to 0.61425 ± 0.02342 and in the pooled sample, mean magnitude of K was 0.64713 ± 0.008229 .

To check validity of various predictive models, leaf areas were estimated - a) on the basis of equations of multiple regression model (LAMR), and b) on the basis of power model equations (LAPOW). A new parameter of leaf area (LAK) was also generated arithmetically on the basis of average values of coefficient k ($\text{Area estimated} = k (\text{LL} \times \text{LB})$) for acute apex and obtuse apex leaves and their pooled sample. The statistical descriptive properties of these parameters are presented in Table 1.

Many workers have undertaken leaf area estimation allometrically as well as mathematically and have obtained significant results with many plant species e.g., *Fragaria* spp. (Demirsoy *et al.* (2005); *Xanthosoma* spp. (Goenaga and Chew (1991); *Arachis hypogaea* (Kathirvelan and Kalaiselvan, 2007); hazel nut (Cristofori *et al.* (2007); millet (Persaud *et al.* (1993); *Prunus avium* (Citadani and Peri, 2006); in 15 fruit spp. (Uzun and Celik, 1999); sunflower (Bange *et al.* (2000), cotton (Akram-Ghaderi and Sultani, 2007), *Nicotiana plumbaginifolia* (Khan, 2008), improved genotypes of *Coffea arabica* and *C. canephora* (Brinate *et al.*, 2015) and *Ficus religiosa* (Khan, 2009). The fitness of power model to estimate leaf blade area has been reported in several species e.g., in *Coffea arabica* and *C. canephora* with high precision ($R^2 = 0.998$) and accuracy irrespective of cultivar and leaf size and shape (Atunes *et al.*, 2008), in 'Niagara' ($R^2 = 0.992$) and 'DeChunac' ($R^2 = 0.963$) grapevines (Williams III and Martinson, 2003); groundnut (Kathirvelan and Kalaiselvan, 2007), *Nicotiana plumbaginifolia* (Khan, 2008), *Jatropha curcas* (Ahmed and Khan, 2011), *Capparis cartilaginea* (Khan *et al.*, 2015a), *Hibiscus sabdariffa* (Nnebue *et al.* 2015), *Vicia faba* (Erdoğan, 2012) and cassava (*Manihot esculenta*, morphotype Phillipine) (Karim *et al.*, 2010).

The variously estimated leaf areas (LAK, LAPOW and LAMR) were compared with the measured leaf area (LAM). The linear correlation coefficients between measured leaf area (LAM) and LAK, LAMR and LAPOW, separately indicated that these parameters were highly significantly correlated with LAM (Table 4). In apex acute and obtuse acute leaves and the pooled sample of the two leaf types, the estimation of leaf area with respective k factors gave the best results. All of the methods employed above appeared to be more or less equally suitable to estimate leaf area in *Jojoba* through such simple measurements as length and breadth of the leaves intact with the plant. Owing to the simplicity and convenience and the accuracy of estimation, using mean k coefficient may be recommended for estimation of leaf area in *Jojoba*. The magnitude of k averaged to 0.65879 ± 0.00717 in case of acute apex leaves, 0.61425 ± 0.02342 for obtuse apex leaves and 0.64713 ± 0.008229 for the pooled sample of the two types of the leaves.

Table 4. Relationship between LAM and variously estimated leaf areas as given by Pearson product moment correlation coefficient.

Leaf types	Pearson Product Moment Correlation Coefficient (r)		
	LAM vs. LAK	LAM vs. LAPOW	LAM vs. LAMR
Acute apex leaves (N = 85)	1.0	0.964	0.951
Obtuse apex leaves (N = 35)	1.0	0.972	0.954
Pooled leaf sample (N = 115)	1.0	0.982	0.958

LAM, leaf area measured; LAK, leaf area estimated through K ; LAPOW, leaf area estimated through power equations (Table 3) and LAMR, leaf area estimated through multiple regression equations on the basis of LL and LB (Fig. 2).

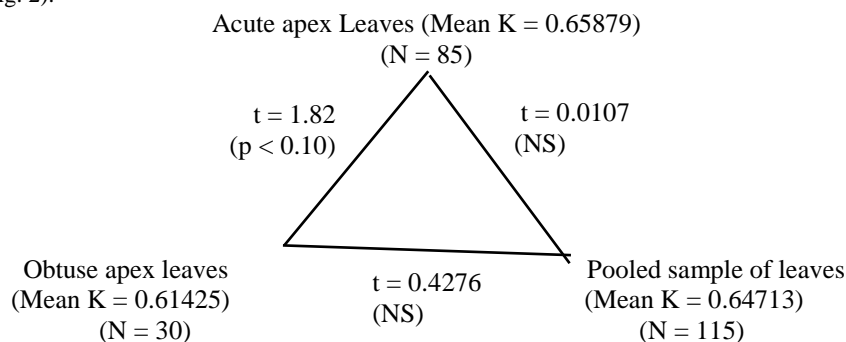


Fig. 3. Comparison of average values of k for acute apex and obtuse apex leaves and their pooled sample in terms of t-test

The mean k value of 0.64713 for pooled sample of leaves was not significantly different from mean k value of 0.65879 for acute apex leaves ($t = 0.0107$, NS) and mean value of 0.61425 for obtuse apex leaves ($t = 0.4276$, NS) (Fig. 3). Mean k value of acute apex leaves was also not significantly different from the mean k value for obtuse apex leaves ($p < 0.10$). It signifies the fact that mean k value of 0.64713 may generally be applicable to estimate leaf area irrespective of the leaf types.

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