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LEAF ARCHITECTURE AND NON- DESTRUCTIVE LAMINA AREA ESTIMATION IN *BAUHINIA RACEMOSA* LAMK.

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Abstract

The architecture and non-destructive lamina area estimation is described in *Bauhinia racemosa* Lamk. The graphically measured one-sided leaf area (LAM) of 50 individual leaves of *Bauhinia racemosa* Lamk. varied from 0.55 to 18.53cm^2 (mean = 11.4179 ± 0.5586 cm^2; CV = 34.60%). The overall leaf shape, as given by aspect ratios, appeared to be maintained with age. Based on the criterion of LAWG (1999), apex shape was, however, found to be lobed in 62% of the leaves, emarginate in 36 % of the leaves and retuse in 2% leaves (young ones). Apical and basal angles were wide obtuse but apical angle was substantially larger than basal angle. The Leaf length LL was determined as LL = LM + La + Lb where La was the apical leaf extension length and Lb the basal leaf extension length. Allometric methods such as simple linear, power model and mutiple correlation and regression analyses were employed using midrib length (LM), leaf breadth (LB) or leaf length (LL) or their multiplicative parameters (LM x LB or LL x LB) as independent variables against LAM in addition to the arithmatic methods to determine mulplication coefficients (leaf form factors) k and k′ on the basis of k = Leaf area $_{\text{measured}}$ / (LM x LB) and k′ = Leaf area $_{\text{measured}}$ / (LL x LB) were determined to arrive at simple and useful models to estimate lamina area. The power model was, the good fit model relating LAM with LM x LB or LL x LB. Amongst the two, LAM was obviously better correlated with LM x LB ($R = 0.984$; $F = 1496.64$, $p < 0.0001$) than LL x LB ($R = 0.968$; $F = 712.92$, $p < 0.0001$). k averaged to 1.2727 ± 0.0176 with variability around 9.78% only and k ′ averaged to 0.787014 ± 0.01453 with variability around 13%. Comparison of various models indicated that power based models and arithmetic factors k and k′ were the best fit to estimate leaf area in *B. racemosa*. The use of k in leaf area estimation appears to be more suitable as k′ involves determination of leaf length, LL = LM + La + Lb, a cumbersome way to work with leaves attached with the plants.

Introduction

The development of predictive models for leaf area estimation is important and a useful tool in studies related to the plant growth and development. 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 investigated 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, Khan *et al*, 2015 a and c). Such simple and accurate methods eliminate the need of expensive leaf area meters (Gamiely *et al.*, 1991). In this paper leaf architecture and lamina area estimation in *Bauhinia racemosa* Lamk., a useful leguminous arid land tree species, have been undertaken.

Materials and Methods

Fifty leaves of various sizes from a mature tree of *Bauhinia racemosa* growing in the campus of University of Karachi were collected and their linear measurements were recorded for the length of midrib (distance from the proximal most to the distal most point of the mid-vein (LM) and lamina breadth (LB) at the broadest points on the margin i.e. perpendicular to LM. 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 = Leaf area $_{\text{measured}}$ / (LM x LB). Employing average value of the multiplication factor k, leaf areas were calculated as Leaf Area $_{\text{computed}}$ = k (LM x LB) for comparison with the measured areas of the leaves. Leaf architectural parameters (apical leaf extension (La) and basal leaf extension (Lb) lengths and apex and base angles) were determined according to LAWG (1999). Since *B. racemosa* leaves are provided with La and Lb, the leaf length (LL) was determined as LL = LM +La +Lb (LAWG, 1999). Leaf aspect ratios were calculated as LB / LM and also as LL / LB (Lu *et al.* (2012). The multiplication factor calculated as Leaf area $_{\text{measured}}$ / (LL x LB) was designated as k′. The location and dispersion parameters of the data were calculated (Zar, 1994). The skewness and kurtosis (g1 and g2, respectively) were calculated as $g_1 = K_3 / (K_2')^{3/2}$ and $g_2 = K_4 / (K_2')^{2}$, respectively - Ks, are moments around mean (see Shaukat and Khan, 1979). The standard errors of skewness and kurtosis (Sg1 and Sg2, respectively) were given as: Sg1=√ 6N (N-1) / (N-2) (N+1) (N+3) and Sg2= √ 24N (N-1)2 / (N-3) (N-2) (N+3) (N+5).

Linear and power law relationships of leaf area with multiplicative parameters of LM x LB and LL x LB were determined. In addition to it, the regression coefficients were also calculated by employing multiple regression method fitting in the allometric model, $Y = a + b_1LM + b_2LB \pm SE$ and also as $Y = a + b_1LL + b_2LB \pm SE$ (Zar, 1994). The arithmetic and allometric methods were compared for their precision and suitability. The data was analyzed using SPSS version 12.

Table 1. Location and dispersion of leaf architectural and lamina area parameters of *B. racemosa* leaves (N=50). All the quantitative parameters presented appeared to be symmetrical in distribution.

Statistical Parameters	Petiole (cm)	LB (cm)	LM (cm)	LM x LB	k	LAM (cm^2)	Aspect ratio*	La Lobe I (mm)	La lobe II (mm)	Lb lobe I (mm)	Lb lobe II mm)	Apex angle (°)	Base angle (°)
Mean	1.4200	4.4718	1.9440	8.9993	1.2727	11.4179	2.3494	7.2900	7.1700	4.3100	4.8500	246.18	229.24
SE	0.02900	0.12597	0.0602	0.44128	0.01756	0.55864	0.04942	0.29798	0.30826	0.25511	0.24085	1.318	1.417
Median	1.4000	4.6000	2.0000	8.9700	1.2650	11.4300	2.2950	8.00	7.000	4.000	5.000	248.00	230.00
CV (%)	14.44	21.11	21.88	34.67	9.78	34.60	14.87	28.90	30.40	41.85	35.12	3.79	4.37
Skewness	-0.163	-1.465	-0.822	-0.378	0.426	-0.302	0.237	-0.929	-0.334	0.425	-0.161	-0.718	-0.462
Kurtosis	-0.474	4.234	0.978	-0.087	0.752	-0.045	0.057	1.715	0.533	0.056	-0.690	1.316	-0.218
Minimum	1.00	0.90	0.60	0.54	1.02	0.55	1.50	1.00	1.00	1.00	1.00	220	208
Maximum	1.80	5.90	2.60	14.79	1.65	18.53	3.14	12.00	12.00	9.00	8.00	265	250
KS-z	0.919	0.796	0.901	0.506	0.509	0.519	0.963	1.203	0.790	0.927	0.927	1.113	1.066
P	0.367	0.350	0.391	0.960	0.958	0.951	0.312	0.110	0.561	0.357	0.356	0.1638	0.206

Statistical Parameters	LAK (cm^2)	LAMR (cm^2)	LAPOW (cm^2)	LL	LL x LB	Aspect ratio**	k′	LAK′ (cm^2)	LAMR′ (cm^2)	LAPOW′ (cm^2)	La (Leaf) (mm)	Lb (Leaf) (mm)
Mean	11.4534	11.5444	11.4269	3.1250	14.3799	1.4356	0.787014	11.4179	11.4112	11.4047	7.230	4.58
SE	0.56161	0.47690	0.56609	0.0808	0.67453	0.0311	0.01453	0.55854	0.53827	0.60968	0.27291	0.21541
Median	11.4161	12.0096	11.379	3.150	14.30	1.4359	0.789838	11.430	11.3474	11.1996	7.50	4.500
CV (%)	34.67	29.21	35.03	18.28	32.66	15.29	13.05	34.61	33.35	37.80	26.69	33.26
Skewness	-0.378	-0.661	-0.363	-0.437	-0.358	-0.850	-1.109	-0.302	-0.358	-0.187	-1.159	-0.009
Kurtosis	- 0.087	0.047	0.118	-0.419	-0.189	9.3333	11.045	-0.0450	0.189	-0.457	1.814	-0.431
Minimum	0.69	3.14	0.660	1.83	1.76	0.46	0.3134	0.55	1.340	0.950	1.0	1.0
Maximum	18.83	17.09	18.89	4.10	22.79	2.12	1.1472	18.53	18.060	19.29	10.50	7.50
KS-z	0.506	0.496	0.530	0.782	0.505	1.171	1.181	0.501	0.505	0.510	0.959	0.572
p	0.960	0.967	0.962	0.562	0.961	0.129	0.123	0.951	0.961	0.957	0.316	0.899

LM, midrib length; LB, leaf breadth; LM x LB (midrib length x Leaf breadth multiplicative parameter; k, multiplication factor (ML x LB based); LAM, Leaf area measured; aspect ratio*, LM/LB; aspect ratio**, LB/LL; La, apical leaf extension length; Lb, basal leaf extension length; LAK, k-based area estimated; LAMR, leaf area estimated on the basis of multiple regression with LM and LB; LAPOW, leaf area based on power equation (with LM x LB as independent variable; LL, leaf length (LM+ La +Lb); LL x LB, leaf length x leaf breadth multiplicative parameters; Aspect ratio** (LB/LL ratio); k′; muliplicative parameter (LL x LB based) LAK′, k′-based area estimated; LAMR′, leaf area estimated on the basis of multuple regression with LL and LB; LAPOW′, leaf area based on power equation with a multiplicative independent variable, LL x LB.

La, apical leaf extension length; Lb, basal leaf extension length. SE of skewness (Sg1) = 0.337 and SE of kurtosis (Sg2) = 0.662.

Results and Discussion

Leaf architecture

The quantitative architectural parameters of leaves are presented in Table 1. Leaf of *B. racemosa* is simple, petiolate, alternate with distichous phyllotaxy. Lamina placed at around right or higher angle to the petiole (Fig. 1). Leaf lamina bilobed. Lamina width (LB) is larger than midrib length (LM) i.e. midrib length from umbo to apical notch. The aspect ratio was, therefore, 2.349 ± 0.04942 (Table 1). Midrib extension ($\leq$ 1mm) present in the apical notch (Fig. 1). Such extension of midrib has also been shown in some species of *Bauhinia* (*B. tomentosa, B. malabarica, B, purpurea, B. variegata, B. rufescens* etc,) by Das and Paria (1999). Midrib extension beyond leaf lamina (around 1mm long) is also reported in *Bauhinia monandra* (Australian Tropical Rianforest Plants; keys.trin.org.au/key-server/data ….html). Umbo is embayed in sinus and lamina base cordate (i.e. leaf base sinus with straight or convex sides and Lb > 0 (see LAWG, 1999). Leaf is hairy on both surfaces but densely hairy on ventral side. Midrib is more hairy than rest of the lamina. Hairs uni- to multicellular greatly varying in length. Few hairs are very large. Leaf stipulate (stipule green, hairy, 1 mm or lesser in size). Younger leaves are yellowish green in colour (low chlorophyll content). They slowly darken in green colour.

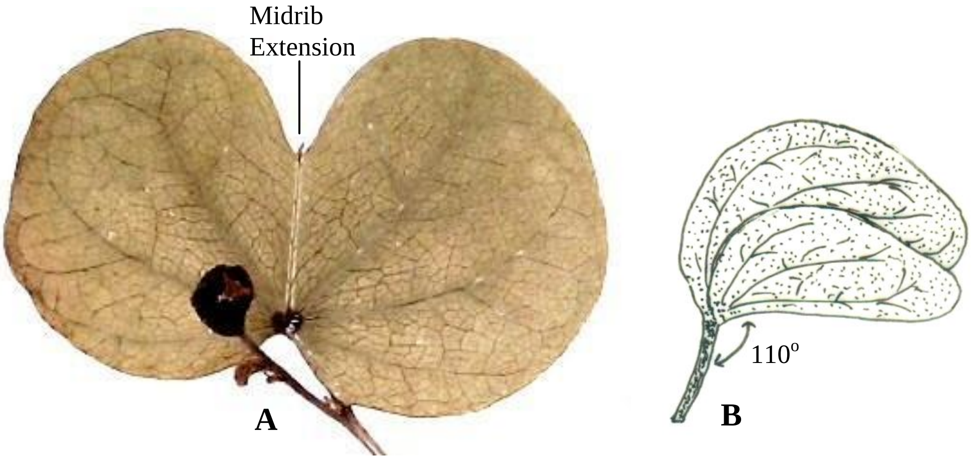


Fig. 1. Dorsal surface view of dry *B. racemosa* leaf (A) and lateral view (B).

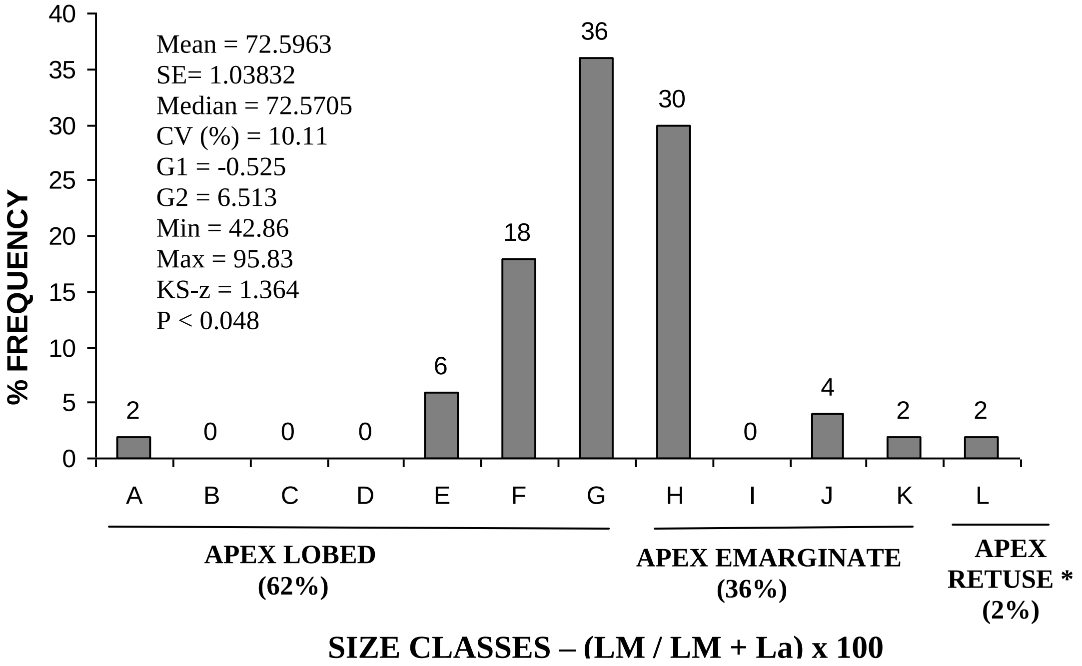


Fig. 2. Frequency distribution of midrib proportion [(LM / LM + La) x 100]. LM, Midrib length; La, Apical leaf extension length. Acronyms to the size classes: A, < 45; B, 45-50; C, 50-55; D, 55-60; E, 60-65; F, 65-70; G, 70-75; H, 75-80; I, 80-85; J, 85-90; K, 90-95; L >95. *,Very young leaves.

Petiole length (PL): The petiole length ranged from 1.0 to 1.8 cm averaging to 1.42 ± 0.029 cm.
Midrib length (LM) : Midrib length varied from 0.6 to 2.60 cm (mean: 1.944 ± 0.06.2; CV: 21.88%) (Table 1).
Leaf breadth (LB): Leaf breadth (N =50) varied from 0.90 to 5.9 cm (mean: 4.4718 ± 0.1560; CV: 21.11%).

Leaf Shape Consistency: Leaf shape is very intricate and cannot be modeled with high accuracy with simple geometrical figures. Length / breadth ratio may, however, give some indication about consistency of leaf shape with size (Verwijst and wen, 1996). In present studies, breadth / midrib length ratio (aspect ratio after Lu *et al.* (2012), a converse of Length / breadth ratio, was calculated. Aspect ratio in leaves averaged to 2.3494 ± 0.0494 (varying from 1.50 to 3.14 with as low variation as 14.87%. The magnitude of median (2.295) was located near the mean (Table 1). The aspect ratio followed symmetrical distribution pattern amongst leaves. Aspect ratio, calculated as Leaf breadth / Leaf length, averaged to 1.4356 ± 0.0311 and varied from 0.46 to 2.12 (CV = 15.29%). Aspect ratios calculated as above showed non-significant correlations with LAM (r = 0.138 and 0.218, respectively). It appears that overall leaf shape, as given by aspect ratios, is maintained with age.

Apical and Basal leaf extension lengths (La and Lb): Apical leaf extension length (La; distance on a perpendicular from the distal most point of mid-vein to the distal most extension of the leaf tissue) averaged to 7.29 ± 0.298 mm in case of one leaf lobe of *Bauhinia* leaf and 7.17 ± 0.308 mm in the other lobe of the leaf. The magnitude of La in the two lobes varied by a quantum of 28.9 and 30.4% (Table 1). La was not significantly different in the two lobes of the leaves (t = 0.455, p < 0.651, NS). According to LAWG (1999) in leaves with La > 0, there may be three categories of apex shape on the basis of magnitude of LM / LM + La i.e. if LM is 95-99% of LM + La leaf apex is retuse, if the length LM is 75-90% of LM + La, the leaf apex is emarginate and when LM is < 75% of LM + La, the leaf apex is lobed. On the basis of this criterion, 62% of the leaves exhibited lobed apex, 36% emarginate and only 2% of the leaves were retuse (Fig. 2) i.e. there exists variation in leaf apex type in *B. racemosa*. It was observed that younger smaller leaves were retuse but as they mature the apical notch deepens and leaves become emarginate to lobed on maturity. This is explicit with the following relationship of LM / (LM + La) with LAM. The variation in LAM accounted for c 34% variation in LM / (LM + La).

$LM / (LM + La) = 55.1382.\ LAM^{0.115275} \pm 0.08929;\ R = 0.5816,\ R^2 = 0.3383;\ F = 24.54\ (p < 0.0001)$ …. Eq. 1

The magnitude of Lb (basal leaf extension length (distance on a perpendicular from the proximal most point of the mid-vein to the proximal most extension of the leaf tissue) was considerably lesser than La and averaged to 4.31 ± 0.255 and 4.81 ± 0.241 mm in the two lobes, respectively. The mean Lb magnitude of the two lobes was significantly different from each other (t = 2.194, p < 0.033) i.e. Lb of one lobe was larger than the other lobe of the leaf.

Apex and basal angles: The apex and base angles averaged to 248.16 ± 1.318 and 229.24 ± 1.417^o (wide obtuse in both cases). The apex and basal angles varied very little in magnitude (3.79 and 4.37%, respectively (Table 1). Apex angle was significantly larger than the basal angle (t = 7.87, p < 0.0001).

Leaf length (LL): The leaf length (LL = LM + La + Lb) averaged to 3.125 ± 0.081 cm varying from 1.83 to 4.10 cm (CV =18.28).

Leaf Area: The measured one-sided single leaf area (LAM) varied from 0.55 to 18.53 cm^2 (mean = 11.418 ± 0.5586cm^2; CV = 34.6%). LAM distributed normally (Table 1). The leaf area was found to correlate with LM, LL and LB significantly but the values of r^2 were somewhat low. LB was, however, more closely related with LAM than LM or LL (Table 2).

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

Y	Midrib Length (LM) ….X_1				Leaf Length (LL) ….X_2				Leaf Breadth (LB) ….X_3			
LAM	R^2	a	b	F	R^2	a	b	F	R^2	a	b	F
	0.765 ***	-4.653	8.282	186.34 ***	0.800 ***	-7.913	6.186	192.06 ***	0.870 ***	-7.084	4.318	322.41 ***

***, p < 0.0001

Linear simple and multiple regression models (Table 2; Fig. 3) obtained by regression of LAM with LM, LL and LB separately or in combination yielded significant equations (Table 2, Fig. 3 and 4). The zero order or partial correlations in multiple linear correlation and regression analyses were relatively higher with LB than that with LM i.e. LAM depended somewhat more on LB than LM. The leaf area LAM was found also to relate with multiplicative parameter of LM x LB and LL x LB significantly as simple linear model as given below. Equation # 2 based on LM x LB had around 39% more explanatory power to define LAM than equation. # 3 based on LL x LB.

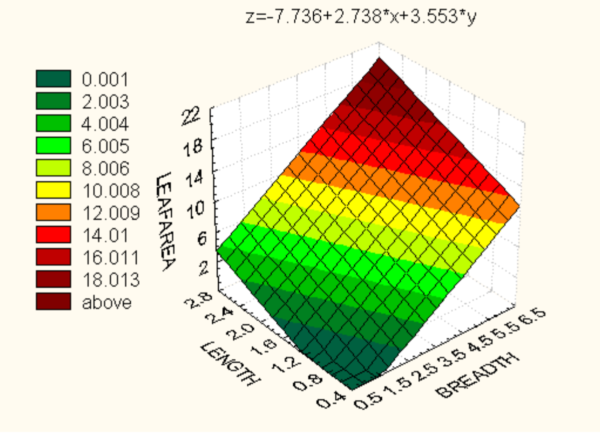
LA M (cm^2) = 0.384+ 1.2260 (LM x LB) ± 0.99328; $R^2 = 0.969$; F = 726.99 ($p < 0.0001$) ….. Eq. 2.

LA M (cm^2) = -0.064+ 0.798 (LL x LB) ± 1.05931; $R^2 = 0.930$; F = 633.38 ($p < 0.0001$) …... Eq. 3.

The power model was, however, the good fit model relating LAM with LM x LB (Fig. 5) or LL x LB (Fig. 6). Amongst the two, LAM was obviously better correlated with LM x LB (R = 0.984; F = 1496.64, p < 0.0001) than LL x LB (R = 0.968; F = 712.92, p < 0.0001) (Fig.4). 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), cassava (*Manihot esculenta*, morphotype Phillipine) (Karim *et al*., 2010) and Simmondsia chinensis (Khan *et al*., 2015c).

In present studies, the coefficient k was arithmetically estimated as k = Area $_{\text{measured}}$ / (LM x LB). This parameter averaged to 1.2727 ± 0.0176 with variability around 9.78% only (Table 1). In 88% of the cases k value ranged between 1.2 and 1.6. k ′ (estimated as k′ = Area $_{\text{measured}}$ / (LL x LB), averaged to 0.787014 ± 0.01453 and also in 88% of the cases k′ magnitude fell between 0.7 and 0.8. There was, however, more variation associated with k′ (13.05%) than k (9.7%). By this virtue k appeared to be relatively more suitable for leaf area estimation in *B. racemosa* than k′.

To check validity of various predictive models, leaf areas were estimated - a) Leaf areas estimated on the basis of equations of multiple regression models (Fig. 3 and Fig. 4) were designated as LAMR and LAMR′), b) leaf areas estimated on the basis of power model equations (Fig. 5 and 6) were designated as LAPOW and LAPOW′ and c) the leaf areas estimated on the basis of average values of k (leaf area, LAK = 1.2727 (LM x LB)) and k′ (leaf area LAK′ = 0.787014 = k′ (LL x LB). The statistical descriptive properties of these parameters are presented in Table 1.

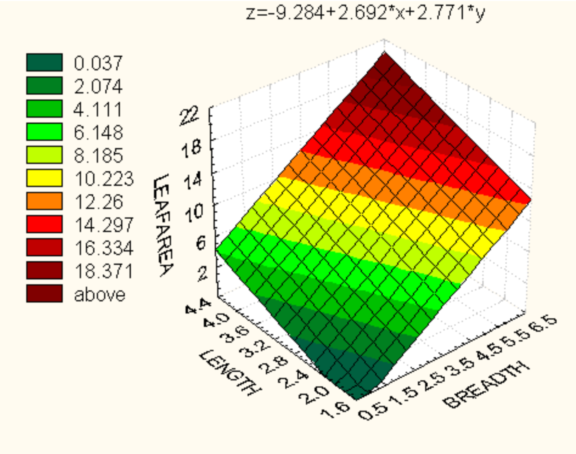


Multiple Correlation & Regression

C= -7.7360 (t = -9.23, $p < 0.0001$)
b1 = 2.7389 (t = 8.33, $P < 0.0001$)
b2 = 3.5530 (t = 5.16, $p < 0.0001$)
SE = 1.16511
R = 0.958
$R^2 = 0.917$
Adj $R^2 = 0.914$
F = 260.56 ($p < 0.0001$)

	ML	LB
Zero order r:	0.892	0.933
Partial r:	0.601	0.772

Fig. 3. Surface plot of Leaf area (LAM) as function of midrib length (LM) and leaf breadth (LB). Multiple correlation and regression statistics is given in inset.



Multiple Correlation & Regression

C= -9.281(t = -10.40, $p < 0.0001$)
b1 = 2.692 (t = 8.83, $P < 0.0001$)
b2 = 2.771 (t = 5.83, $p < 0.0001$)
SE = 1.1064
R = 0.962
$R^2 = 0.925$ Adj $R^2 = 0.0.922$
F = 288.80 ($p < 0.0001$)

	LL	LB
Zero order r:	0.894	0.933
Partial r:	0.648	0.790

Fig. 4. Surface plot of Leaf area (LAM) as function of leaf length (LL) and leaf breadth (LB). Multiple correlation and regression statistics is given in inset.

The variously estimated leaf areas (LAK, LAK′, LAMR, LAMR′ LAPOW and LAPOW′) were compared with the measured leaf area (LAM). The linear correlation coefficients between measured leaf area (LAM) and estimated areas separately were highly significant (Table 4). The average value of LAM was not found to vary significantly from average value of LAK (t = - 0.252, p < 0.802), LAK′ (t = 0.035, p < 0.986), LAMR (t = - 0.840, p < 0.405), LAMR′ (0.045, p< 0.964), LAPOW (t = - 0.064, p < 0.949) and LAPOW′ (t= 0.07, p < 0.938). All the methods employed appeared to be more or less equally suitable to estimate leaf area in *B. racemosa* through such simple measurements as midrib length, leaf length and leaf breadth of the leaves intact with the plant.

Many workers have undertaken leaf area estimation allometrically as well as mathematically and have obtained useful 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).

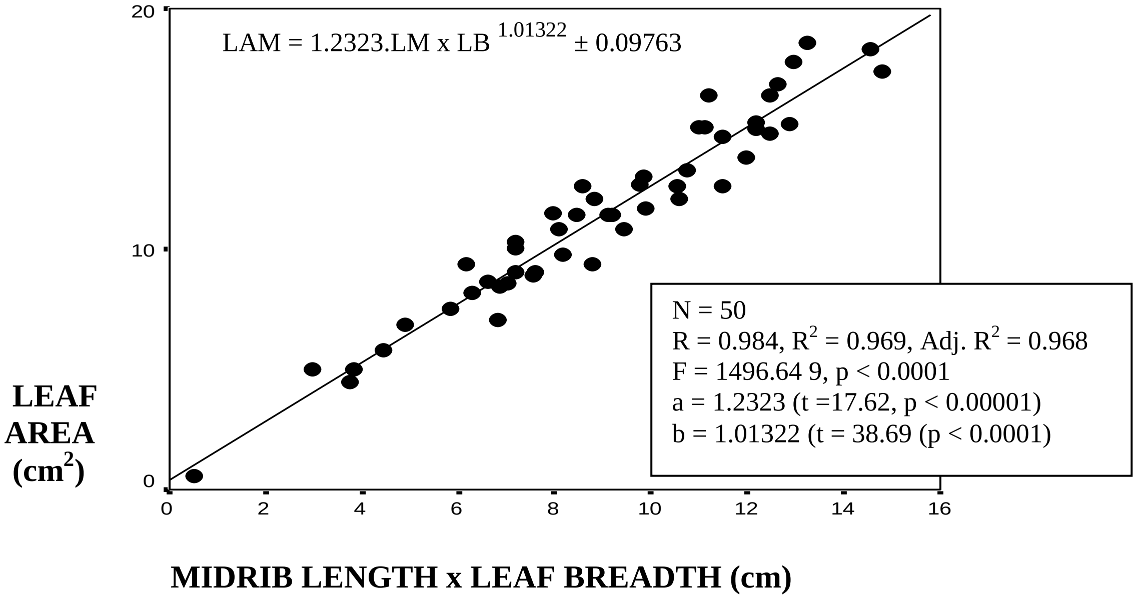


Fig. 5. Power law relationship of leaf area (LAM) with multiplicative variable of midrib Length (LM) x leaf breadth (LB) .

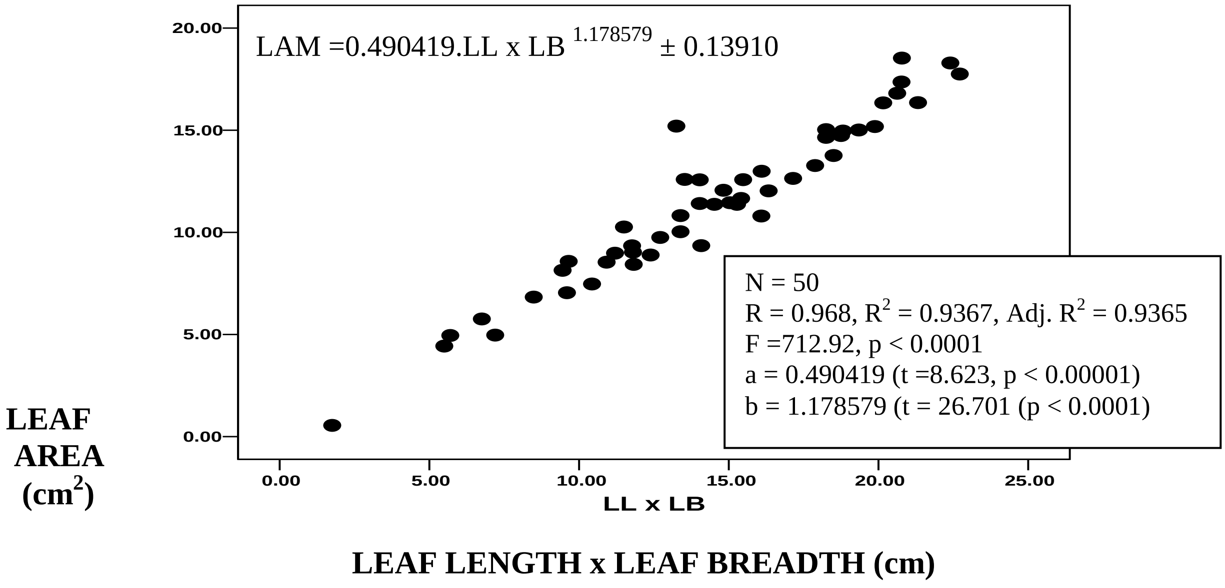


Fig. 6. Power law relationship of leaf area (LAM) with multiplicative variable of leaf Length (LL) x leaf breadth (LB).

However, owing to the simplicity and convenience and the accuracy of estimation, using mean k coefficient (1.2727) while measuring midrib length and leaf breadth may be recommended for estimation of leaf area in this species. The estimation of leaf area using average value of k′ appears to be equally effective as k, but using k′ shall necessitate to determine leaf length (LL = LM + La + Lb) – a cumbersome approach and tedious measurement activity with leaves attached to the plant.

Table 4. Relationship between LAM and variously estimated leaf areas as given by linear correlation.

Statistical Parameters	LAM vs. LAK	LAM vs. LAMR	LAM vs. LAPOW	LAM vs. LAK′	LAM vs. LAMR ′	LAM vs. LAPOW ′
r	0.969	0.968	0.970	0.999	0.964	0.963
F	726.09	763.83	725.88	-	633.38	606.69
p	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

See text for explanation of acronyms.

It may be mentioned here that environmental interactions may influence any such model in plants (Robbins and Pharr, 1987) as suggested above in *B. racemosa.*

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