

PLANT-SIZE DATA AND ESTIMATION OF SOME VITAL LEAF CHARACTERISTICS IN NATURALLY GROWING *NICOTIANA PLUMBAGINIFOLIA* VIV. (SOLANACEAE) IN KARACHI

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ABSTRACT

Nicotiana plumbaginifolia Viv. (Tex-Mex tobacco) is recorded from Karachi as a rare winter weed in flower beds and growing as road-side plant near plant-nurseries and along sewerage lines. Data on its plant size and the leaf characteristics, based on sample size of 120 leaves, are presented here. Measured Leaf area of radical leaves varied from 24.44 to 126.03 cm² (mean 80.8 ± 5.86 cm²; CV = 34.04%) and for cauline leaves from 0.61 to 124.36 cm² (mean: 22.29 cm²; CV = 126.38%). The regression coefficients were computed from multiple regression to fit in the allometric model – $Y = a + b_1X_1 + b_2X_2$, where Y is the true leaf area (LA) and X₁ and X₂ were leaf length and leaf breadth, respectively. The predictive equation was as follows:

$$\begin{aligned} \text{Log}_e \text{LA} &= -0.38295 + 0.88962 \text{Log}_e L + 1.06053 \text{Log}_e B \pm 0.098195 \\ t &= -5.72 \quad t = 17.97 \quad t = 29.04 \\ p &< 0.00001 \quad p < 0.00001 \quad p < 0.00001 \\ R^2 &= 0.9953; \text{Adj. } R^2 = 0.9952; F = 12241.7; r = 0.9976 \\ \text{OR} \\ \text{LA} &= \text{antilog} [-0.38295.L^{0.88962}.B^{1.06053}] \end{aligned}$$

Also, the value of the multiplication factor K for each leaf was found by employing the formula, $K = \text{LA} / (L \times W)$ and employing average K value, 0.58206, the leaf area for each leaf was computed as $\text{Leaf area}_{\text{computed}} = K \times L \times W$. By comparing the estimated leaf area with measured leaf area it was found that allometric estimation was little better than k factor analysis in the present case.

Leaf Dry matter of individual leaves (LDM) behaved more or less in the same manner as LA. Average multiplication factor K_{LDM} was arrived at 0.00267619. The estimated LDM using formula, $\text{LDM} = K_{\text{LDM}} \times L \times B$, was not statistically different from the measured LDM ($X^2 = 4.40$, df = 118; NS). The Estimated LDM related closely with measured LDM ($r = 0.9312$; df = 118; $p < 0.00001$).

Specific Leaf Area (SLA) was expressed as one-sided graphically determined leaf area of a fresh leaf divided by its oven-dry mass (cm²/g⁻¹) and the inverse of SLA was referred to as Specific leaf mass (SLM). SLA varied substantially from 63.5 to 576.83 (CV= 36.58%) amongst the leaves investigated. The curvilinear or logarithmic relationship of SLA with LA appeared to be little better than simple linear relationship between them. In short, smaller are the leaves, lower the SLA. Larger are the leaves, larger the SLA. SLA of cauline leaves was consistently lower than that of radical leaves in all individual plants examined. Considering overall variation in SLA in the two types of leaves, SLA was comparatively of higher order in rosette leaves (354.09 ± 21.39 ; N = 22, CV= 28.3%) than cauline leaves (231.59 ± 7.58 cm²/g⁻¹; N = 98, CV= 32.4%). High SLA of rosette leaves may be a significant factor enabling early ground cover by basal leaves for light interception in this species.

The Leaf to leaf analysis of SLA in leaves on the main stem and the lateral branches in two individual plants showed almost a regular trend of increase of SLA from apex to the base of the plants. Apical leaves had lower SLA than subsequent basal leaves. Exposure of axial leaves to comparatively more irradiance may probably be the reason for low SLA of axial leaves. SLA related with ratio of Leaf Dry Mass contents (LDMC) negatively.

Specific Leaf Mass (SLM), an inverse of SLA, varied more in cauline (42.8%) than in radical leaves (25.8%). The values of SLM for cauline leaves were, however, generally higher (mean = 0.004901 ± 0.0002 ; range: 0.00241 to 0.01574) than for radical leaves (mean = 0.00303 ± 0.00166 ; range: 0.00173 to 0.00432). Since there appears no significant difference in hydrature between radical and cauline leaves, differences in SLM in them appears to be attributable to environmental differences. Possibly exposure of axial leaves to more irradiance and consequently their more active photosynthetic role is the reason for their high SLM.

Key Words: *Nicotiana plumbaginifolia* Viv., Leaf Area, Leaf dry matter (LDM), Specific Leaf Area (SLA), Specific Leaf Mass (SLM), Leaf Dry Mass Contents ratio (LDMC), Allometric analysis.

INTRODUCTION

Nicotiana plumbaginifolia Viv. (Tex-Mex Tobacco or sweet scented tobacco or leadwort-leaved tobacco or smaller tobacco or Neeshnam Other or wild tobacco) is said to be a weed native to Mexico and the West Indies. It is widely reported from Rawalpindi (called Geedar tobacco in Punjab), Peshawar and Lahore districts of Pakistan (Nasir, 1985). It has not been previously reported from Karachi but for the last few years we observed this species colonizing derelict areas, flowerbeds and lawns in the campus of Govt. National College Karachi, after winter rains (Fig.1), growing as one of the component of roadside vegetation near Islamia College, Karachi (April 11, 2008) and also along the sewerage line in Abbasi Shaheed Hospital, Karachi (June, 2008).

It is said to be a useful rapidly growing plant. According to Powers (1874) its leaves are sun-dried, finely cut and smoked. It has widely been studied and employed in physiological, biochemical and genetic and molecular research. This paper attempts to estimate some vital leaf characteristics of *Nicotiana plumbaginifolia* viv. (Solanaceae) from its population in Karachi.

Phytography:

Erect pubescent herb up to 1m tall, stem hollow at maturity, leaves radical and cauline, sessile, variable in size, pubescent, elliptic-oblong or oblanceolate, entire or margin wavy, cuneate to decurrent. Panicle lax. Padicel c 3 mm, pubescent glandular. Calyx around 10 mm pubescent nervose, lobes linear lanceolate. Corolla tube 3-4 cm long limb 1 to 1.5 cm broad, lobes five glaucous. Mature corolla white above but pink on the underside (Fig. 2). Anthers small, ovary bicarpellary, syncarpous, superior, axile placentation, style c 2 cm long, stigma roughly bifid green. Capsule ovoid included in persistent calyx. Seeds small (much less than 1mm) sub-globose to angular, minutely rugose-reticulate, brown.

MATERIALS AND METHODS

Ten healthy *N. plumbaginifolia* plants were harvested randomly and carefully with their root system, after heavy irrigation of these plants with water to loosen soil, in April 2007 from its population composed of not more than 25 plants, emerging after late December (2006) rainfall and abounding with sandy derelict area in the campus of Govt. National College, Karachi. Then again, during May 11 to 16, 2008, five 40 - 45 days old *N. plumbaginifolia* plants from the same locality and one plant from amongst the roadside plants near Islamia College, Karachi were similarly harvested for morphometric studies. The harvested plants were kept in an ice box and brought to the laboratory immediately and processed for determination of various morphological attributes.

From six plants harvested in 2008 (plant # 11-16; Table 1), One hundred and twenty expanded leaves were collected and immediately floated on water for some time in dark for rehydration (Garnier *et al.*, 2001b; Li *et al.*, 2005). After blotting the surplus water, the leaves were weighed while turgid. Then their linear measurements were recorded for leaf length and leaf breadth 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 - though wavy margins in some cases imposed some difficulty. For dry weight determination, leaves were kept continuously at 70 °C for two days and then weighed. Specific Leaf Area (SLA) was expressed as the ratio of one-sided leaf area (after Westoby *et al.*, 2000) to dry leaf mass ($\text{cm}^2.\text{g}^{-1}$). Leaf dry matter content ratio (LDMC) was calculated as the ratio between leaf dry mass and saturated fresh mass ($\text{g}.\text{g}^{-1}$). Specific leaf mass (SLM) was equal to SLA^{-1} .

The regression coefficients were computed from multiple regression to fit in the allometric model – $Y = a + b_1X_1 + b_2X_2$, where Y is the true leaf area (LA) and X_1 and X_2 were leaf length and leaf breadth, respectively. Also, average ratio or the multiplication K factor was found by employing the formula, $K = \text{LA} / (\text{L}*\text{W})$. Employing average K value, the leaf area was computed as $\text{Leaf area}_{\text{computed}} = K*\text{L}*\text{W}$. The two methods were compared and discussed. Similar statistical analyses were employed for dry matter estimation of leaf based on linear measurements of leaf length and breadth.

OBSERVATIONS, RESULTS AND DISCUSSION

Morphometry

The morphometric parameters of 16 randomly selected reproductive plants of *N. plumbaginifolia* collected over a period of two growing seasons is depicted in Table 1. These plants emerged at different dates in derelict area of college campus as a result of intermittent showers of winter rains in late December of 2006 and those emerging after ploughing and irrigation of flower beds in spring 2008- one of them was collected from near Islamia College, Karachi. The plants collected were, therefore, differentially-aged but all in flowering and fruiting stage. The height of the plants varied substantially. The tallest plant had shoot length of 101 cm. The maximum dry phytomass attained by any plant was 39.3g. The Leaf Area per plant could only be measured in harvest of 2008 and depending upon plant size, it ranged from 4.34 to 12.79 $\text{dm}^2.\text{Plant}^{-1}$. Being an annual the plant behaved as a surface feeder and roots mainly feed by ramifying profusely in the upper 20 - 25 cm soil profile. The lateral roots were larger than the main tap root (Fig. 3). On dry phytomass basis, root component of plant occupied a *per cent* proportion of 6.76 ± 0.51 (CV = 30.6%) (Fig.4).



Fig. 1. *Nicotiana plumbaginifolia* growing in lawn and the derelict areas of Govt. National College, Karachi after winter rains (2006). A, Vegetative Growth (irrigated flower bed). B, Reproductive phase and C, Plant under *Guaicum officinale* shade in dry grassy lawn and suffering from relatively more moisture deficit. D, Flowers and fruits.



Fig 2. Close up view of flowers (A & B) of *Nicotiana plumbaginifolia* (Note the pinkish underside); C, Young plants showing rosette of radical leaves in contact of soil and semi-amplexicaul cauline leaves above.

The major proportion of dry mass was allocated to leaves ($56.18 \pm 2.92\%$; $CV = 20.08\%$) followed by that of stem ($30.30 \pm 1.93\%$; $CV = 25.5\%$). The reproductive component was much lesser than 10% except in case of plant # 12, with large number of leaves and capsules, allocated slightly larger than 20% of the total plant dry mass to the reproductive component. Experimental determination of allocation pattern of phytomass more accurately is, however, necessitated in view of early death and partially or wholly decay of radical leaves in contact of moist soil. Larger allocation of biomass to leafy component may be due basal rosette habit of the plant with low height and tendency of spreading leaves on the ground to capture more light for survival in woodland as the case reported for *Sonchus asper* (Mehrotra *et al.*, 2004).

The rosette forming leaves of *N. plumbaginifolia* are more or less oblanceolate to spatulate type forming mosaic with fine lateral adjustment with adjacent leaves without overlapping. Deflowering and removal of fruits are known to delay senescence in this species and inhibit axis growth and promote formation of leaves, laminar area and leaf dry weight (Gupta and Chatterjee, 1971).



Fig. 3. Root system of *N. plumbaginifolia* young reproductive plant growing in a flowerbed fertilized with cow dung compost. Secondary roots are larger than the tap root.

Estimation of various vital leaf characteristics

Determination / estimation of leaf characteristics such as leaf area, (LA), leaf dry mass (LDM), specific leaf area (SLA), Specific leaf mass (SLM) and Leaf Dry mass Contents ratio (LDMC) were undertaken with plants harvested in 2008. Location and dispersion parameters of these characteristics are given in Table 2. Various leaf parameters may be described as follows.

Leaf Length (L) and breadth (B)

Average length of radical leaves was 17.3 ± 0.81 and average breadth was 8.00 ± 0.32 cm. L varied slightly more than the B. Cauline leaves were generally smaller (Table 2). Variation in B in cauline leaves was substantially larger (75.8%) as compared to L (53.4%). L and B related with each other positively ($r = 0.9575$; $p < 0.001$) (Table 3).

Leaf Moisture

The moisture content of radical leaves (91.97 ± 1.48 , $CV: 3.95\%$) was not significantly different from that of cauline leaves ($90.15 \pm 0.296\%$, $CV = 2.05\%$).

LDMC: The cauline or axial leaves had higher LDMC (0.09847 ± 0.002954 , $CV = 18.73\%$) than that of radical leaves (0.06801 ± 0.003105 , $CV = 14.4\%$) ($F = 25.2$; $p < 0.001$). LDMC variation was somewhat larger in cauline leaves than radical leaves (Table 2). LDMC related with SLA negatively ($r = 0.5149$; $p < 0.001$).

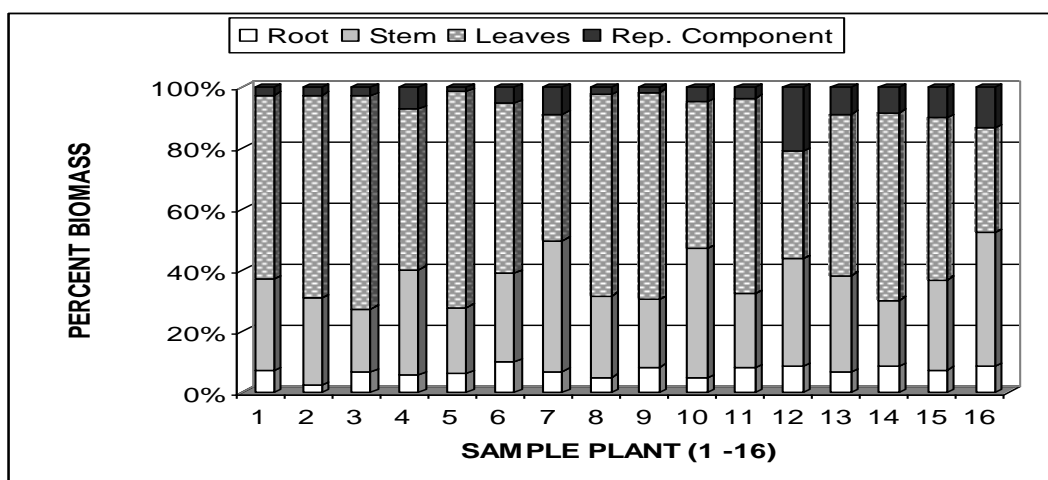


Fig. 4. Percent proportion of biomass in vegetative and reproductive components of *N. plumbaginifolia*. Samples 1-10 harvested in April, 2007 and 11 – 16 in April-May, 2008.

Leaf Shape Constancy

L/B ratio gives some indication about consistency of leaf shape with size (Verwijst and Wen, 1996). Overall L/B variation in radical and cauline leaf samples (N=120) was substantial (34.7%) and of the same order as for cauline leaves (35.02%). The variation of L/B ratio of radical leaves was comparatively low (c 16%) (Table 4). L/B ratio exhibited no correlation with leaf size in case of radical leaves ($r = -0.0071$) but related significantly negatively in case of cauline leaves ($r = -0.5522$; $p < 0.001$) - smaller are the leaves, larger is the L/B ratio. This behaviour of radical and cauline leaves may probably be attributed to their inherent differences in shape and quantum of the changes in their shape with increase in size. Radical leaves are oblanceolate or rarely spatulate in shape and cauline leaves are elliptic-oblong to lanceolate. The radical leaves are less variable in size (34.04%) and axial leaves are much variable in size (126.4%) (Table 2). Shape constancy is more rigid in radical leaves. The changes of shape in axial leaves, from base of the plant to apex, bring relatively larger decrease in their breadths than in their lengths. Thus the leaves in the floral region are smaller and lanceolate to linear in shape.

Table 1. Morphometric parameters of 16 randomly chosen naturally growing plants of *Nicotiana Plumbaginifolia*.

Plant Number	Tap Root Length (cm)	Shoot Length (cm)	Number of Leaves*	Number of Flowers & buds	Number of capsules	Total Leaf Area per plant (dm ²)**	Dry Weight per Plant (g)
HARVEST FOR THE YEAR 2007							
1	15	64	62	16	22	-	11.3
2	11.5	77	53	13	22	-	11.2
3	22.5	69	58	20	28	-	13.5
4	19	86	96	33	154	-	32.0
5	17.6	38	27	9	8	-	3.7
6	8	48	24	5	6	-	3.4
7	17	101	128	19	119	-	26.0
8	16.2	40	41	9	17	-	7.9
9	18	53	58	7	16	-	14.5
10	15	98	206	22	87	-	39.3
Mean	15.98	67.40	75.3	15.30	47.90	-	16.28
SE	1.27	7.24	17.54	2.68	16.63		3.84
CV (%)	26.26	33.96	73.60	55.75	109.8		74.57
HARVEST FOR THE YEAR 2008							
11	15.2	26.2	67	14	4	4.34	14.37
12	12	53.5	138	-	157	9.01	2.72
13	11	59.6	38	14	14	8.52	3.03
14***	12.5	60	56	18	8	9.16	12.05
15	20	47.5	34	13	12	5.92	4.82
16	7.5	51.5	141	12	122	12.79	4.79
Mean	13.03	47.72	79.00	11.83	52.83	8.29	6.96
SE	1.73	5.09	19.76	2.51	27.81	1.20	2.02
CV (%)	32.44	26.15	61.24	51.94	128.9	35.29	71.41

*, Including small leaves at the base of inflorescence axes; **, One-sided leaf area; ***, roadside plant from Islamia College, vicinity.

Table 2. Location and dispersion statistics for some measurements in radical and cauline leaves of *N. plumbaginifolia*.

RADICAL LEAVES							
Parameter	L (22)**	B (22)	LA* (22)	LDM (22)	SLA (22)	SLM (22)	LDMC ratio (10)
Mean	17.93	8.00	80.80	0.2418	354.09	0.00303	0.06801
SE	0.81	0.32	5.86	0.0192	21.39	0.00166	0.00311
Minimum	11.0	4.00	24.44	0.0439	231.55	0.00173	0.05908
Maximum	24.0	9.8	126.03	0.4082	576.83	0.00432	0.09109
CV (%)	21.42	19.00	34.04	37.27	28.33	25.84	14.43
CAULINE LEAVES							
Parameter	L (98)	B (98)	LA* (98)	LDM (98)	SLA (98)	SLM (98)	LDMC ratio (39)
Mean	8.067	3.351	22.294	0.08291	231.59	0.004901	0.09847
SE	0.435	0.257	2.845	0.00945	7.583	0.00021	0.00295
Minimum	2.0	0.50	0.61	0.00720	63.542	0.002407	0.0715
Maximum	19.80	10.00	124.36	0.44540	415.44	0.01574	0.1355
CV (%)	53.44	75.83	126.38	112.86	32.42	42.48	18.73
RADICAL + CAULINE LEAVES POOL							
Parameter	L (120)	B (120)	LA* (120)	LDM (120)	SLA (120)	SLM (120)	LDMC ratio (49)
Mean	9.88	4.203	33.012	0.1120	254.05	0.00456	0.09225
SE	0.520	0.274	3.289	0.0102	8.488	0.00019	0.003001
Minimum	2.00	0.50	0.61	0.0072	63.50	0.001734	0.05908
Maximum	24.00	10.00	126.00	0.4450	576.83	0.01574	0.1355
CV (%)	57.68	71.33	109.09	99.32	36.58	44.80	22.77

L, Leaf Length (cm); B, Leaf Breadth (cm); LA, True Leaf Area (*, One-sided – cm²); LDM, Leaf Dry Mass (g); SLA, Specific Leaf Area; SLM, Specific Leaf Mass; LDMC, Leaf dry matter content ratio. **, Sample size (N).

Table 3. Linear correlation matrix among the leaf attributes (N= 120).

L	L						
B	0.9575	B					
LA	0.9575	0.9699	LA				
LDM	0.8978	0.9486	0.9522	LDM			
SLA	0.7096	0.6503	0.6178	- 0.4446	SLA		
SLM	- 0.6293	- 0.5993	- 0.7323	0.4478	- 0.9389	SLM	

Table 4. Variation of L/B ratio in radical and cauline leaves.

Leaf type	Mean	SE	Minimum	Maximum	CV (%)
Radical (N=22)	2.2559	0.0773	1.802	3.200	15.93
Cauline (N=98)	2.929	0.104	1.532	7.413	35.02
Radical and Cauline (N = 120)	2.806	0.256	1.5319	7.413	34.70

Leaf Area (LA)

Plant 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). It is

required to be estimated in many experimental designs. In present studies, true leaf area (LA) for radical (22) and cauline (98) leaves (120 *in toto*) of *N. plumbaginifolia* was measured graphically and also estimated by two commonly employed methods – k factor analysis and allometric way of estimation. LA of radical leaves varied from 24.44 to 126.03 cm² (mean 80.8 ± 5.86 cm²; CV = 34.04%) and for cauline leaves from 0.61 to 124.36 cm² (mean: 22.29 cm²; CV = 126.38%). The size variation in cauline leaves was comparatively much higher than that in radical leaves mainly due to the fact that size of axial leaves markedly declined from basal to apical region.

Leaf Area Estimation

Allometrics

The leaf length (L) and breadth (B) correlated with LA highly significantly ($R^2 = 0.9575$ and 0.9699 , respectively). L and B collectively accounted for around 95% variation in LA. Logarithmic transformation of L and B improved their relationships with logarithmically transformed LA and accounted for 96.06 and 98.19% variation in leaf area, respectively. Therefore, $\text{Log}_e B$ was two percent better estimator of $\text{Log}_e LA$ than $\text{Log}_e L$.

$$\begin{aligned}\text{Log}_e LA &= -2.0845 + 2.26222 \text{Log}_e L \pm 0.28009 \\ t &= -22.57 \quad t = 53.90 \\ p &< 0.00001 \quad p < 0.00001 \\ R^2 &= 0.9609; \text{Adj. } R^2 = 0.9606; F = 2905.7; r = 0.9803\end{aligned}$$

$$\begin{aligned}\text{Log}_e LA &= 0.78864 + 1.68796 \text{Log}_e B \pm 0.18959 \\ t &= 26.82 \quad t = 80.51 \\ p &< 0.00001 \quad p < 0.00001 \\ R^2 &= 0.9821 \text{Adj. } R^2 = 0.9819; F = 6481.5; r = 0.9910\end{aligned}$$

When the allometric relationship was derived from the multiple linear regression of logarithmically transformed true leaf area (LA) and logarithmically transformed length (L) and width (B) of the leaves the regression coefficients were highly significant. The allometric model for leaf area estimation based on 120 leaves samples during flowering stage of plant was:

$$\begin{aligned}\text{Log}_e LA &= -0.38295 + 0.88962 \text{Log}_e L + 1.06053 \text{Log}_e B \pm 0.098195 \\ t &= -5.72 \quad t = 17.97 \quad t = 29.04 \\ p &< 0.00001 \quad p < 0.00001 \quad p < 0.00001 \\ R^2 &= 0.9953; \text{Adj. } R^2 = 0.9952; F = 12241.7; r = 0.9976\end{aligned}$$

OR

$$LA = \text{antilog} [-0.38295.L^{0.88962}.B^{1.06053}]$$

Here LA was measured in cm² and L and B in cm. The above allometric model was tested for its validity and estimated area of leaves was not found to be significantly different from the true leaf area (Chi square = 30.337; df = 119; NS). The estimated area correlated highly significantly with true leaf area ($r = 0.9920$) (Figure 5). The better fitness of power model than the linear models as suggested here have also been reported in leaf area estimation of *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 and Martinson, 2003) and groundnut (Kathirvelan and Kalaiselvan, 2007).

K factor Analysis

Using the extreme linear measurements (L and B) of the leaves and true leaf area measured (LA), the average “K” factor was arrived at 0.58206 (Range: 0.40063 – 0.79028; SD: 0.05729; CV=9.86%). The leaf area thus estimated for 120 leaves using average “K” factor was tested for its validity and was not found to be significantly different from the measured area (Chi Square = 37.76; df = 119; NS). The estimated area correlated highly significantly with true leaf area ($r = 0.9934$) (Figure 5).

The results of this study indicate that the leaf area of *N. plumbaginifolia* can easily be predicted by simply measuring the length (L) and width (B) of the leaves and employing the allometric model proposed above or using equation $A = K * L * B$ with average “K” factor magnitude of 0.58206.

By comparing the two methods, it is obvious that allometric estimation was better than k factor analysis in the present case. Many workers have undertaken leaf area estimation allometrically and mathematically and have arrived at significant results with many 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), etc. Schurr (1997) while employed k factor analysis in *Nicotiana tabaccum* had arrived at k value of 0.75 which is substantially high as compared to 0.58206 found in case of *N. plumbaginifolia*.

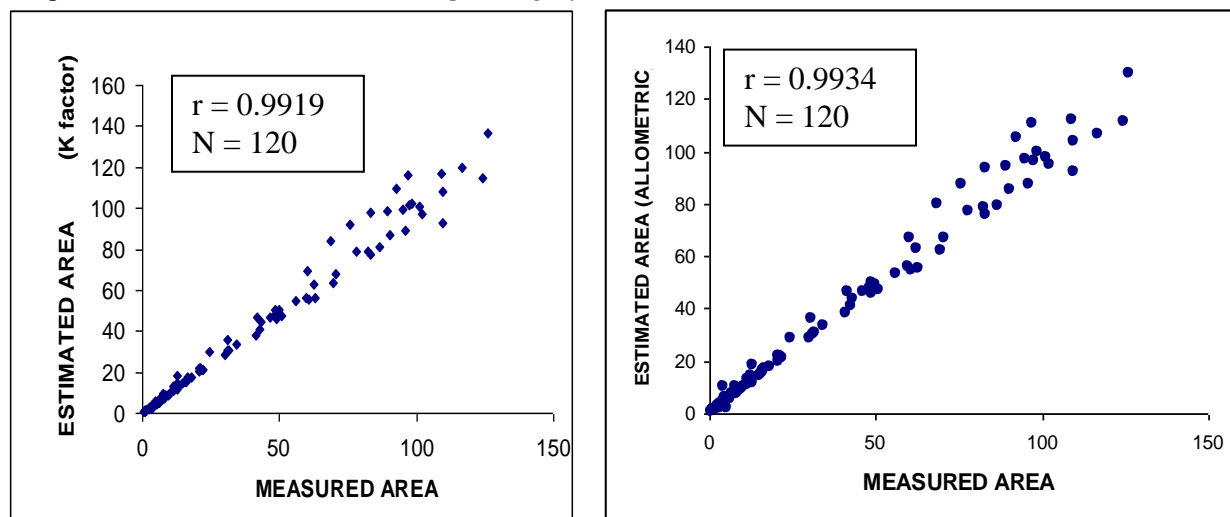


Fig. 5. Relationship between areas of leaf measured and estimated through K factor calculation and allometrically for 120 *N. plumbaginifolia* leaves.

Table 5. SLA as a function of the position of leaf on the plant (base to apex).

Leaf No.	MS	B1	B2	B3	B4	B5	B6	B7
1.	a) Dead R * b) 262.63 R	285.92 204.90	202.63 199.32	232.02 179.18	489.30 187.92	191.30 143.86	161.61 143.55	- 93.65
2.	Dead R * 252.24 R	252.63 107.07	285.18 196.31	209.87 193.66	208.45 158.92	159.83 152.30	194.10 119.51	- 109.73
3 .	257.77 R 301.24 R	258.82 -	155.00 150.00	242.73 137.78	220.94 -	175.93 124.35		
4.	250.22 C 255.55 R		306.96 137.79	208.30 145.98	226.24 -	194.74 82.44		
5.	237.16 C 231.55 R			246.85 117.89	165.63 -	- 63.54		
6.	245.51 C 252.70 C	a) Plant No. 12 b) Plant No. 11 (see Table 1) R, Radical Leaves; C, cauline leaves; *, The radical leaves in contact with soil were partially or wholly dead or dying; B1, Branch 1; B2, Branch 2.....B3, Branch 3and so on; MS, Main stem						
7.	229.99 C 219.78 C							
8.	217.78 C 209.27 C							
9.	211.01 C 184.99 C							
10.	212.60 C 180.64 C							
11.	192.31 C 152.22 C							
12.	240.83 C 128.00 C							

The K factor value is reported to vary with species to species and even variety to variety of a species (Lazarov, 1965). Bozhinova (2006) has reported k factor value to be 0.71 and 0.69 for Burley 1000 and Burley 21 varieties of

tobacco, respectively. In Burley 1317 tobacco cultivar k value is reported to vary with position of leaves on the plant – 0.64 for lower zone leaves, 0.66 for the middle zone leaves and 0.68 for the upper zone leaves (Bozhinova, 2006). Somewhat lower value of 0.58206 in case of *N. plumbaginifolia* may probably be due to comparatively smaller leaf size in this species compared to *N. tabaccum*.

Leaf Dry Matter (LDM)

LDM behaved in similar fashion as leaf area (Table 2). The two parameters related closely positively ($r = 0.9522$) as also reported by Akram-Ghadevi and Sultani (2007) in cotton. LDM also related linearly with leaf length ($r = 0.8978$) and leaf breadth ($r = 0.9486$) positively. LDM relationship with SLA was weak (Table 3).

$$\begin{aligned} \text{LDM (g)} &= -0.061192 + 0.01754 L \pm 0.049204 \\ t &= -6.78 \quad t = 22.15 \\ p &< 0.001 \quad p < 0.001 \\ R^2 &= 0.8061; \text{Adj. } R^2 = 0.8045; F = 490.4 \end{aligned}$$

$$\begin{aligned} \text{LDM (g)} &= 0.364101 + 0.03532 B \pm 0.03537 \\ t &= 6.51 \quad t = 32.55 \\ p &< 0.001 \quad p < 0.001 \\ R^2 &= 0.8998 \text{ Adj. } R^2 = 0.8989; F = 1059.6 \end{aligned}$$

$$\begin{aligned} \text{LDM (g)} &= 0.014901 + 0.0029415 \text{ LA} \pm 0.003413 \\ R^2 &= 0.9067; \text{Adj. } R^2 = 0.9059 F = 1147.2 \end{aligned}$$

Allometrically estimated LDM using above equation for LDM-LA relationship when compared with the measured LDM, the values of the estimated LDM were not significantly different from the measured one ($X^2 = 0.8249$, $df = 118$, NS).

K factor for LDM determination was calculated through formula $K_{\text{LDM}} = \text{LDM} / L * B$. The K_{LDM} values varied from 0.0008574 to 0.00960 (average: 0.0026619). The values of leaf dry matter estimated through formula, $\text{LDM}_K = K_{\text{LDM}} * L * B$, were found not to be significantly different from measured values of LDM ($X^2 = 4.409$, $df = 118$, NS). Measured LDM related positively significantly with LDM_K ($r = 0.9312$, $p < 0.00001$). It follows from the results that LDM of *N. plumbaginifolia* leaves can be easily estimated by simply measuring length and breadth of leaves and using equation, leaf dry matter = $K_{\text{LDM}} * L * B$ with average K_{LDM} 0.0026619.

Specific Leaf Area (SLA):

SLA was expressed here as one-sided graphically determined leaf area of a fresh leaf divided by its oven-dry mass ($\text{cm}^2/\text{g}^{-1}$) and the inverse of SLA was referred to as Specific leaf mass (SLM). SLA of a species is generally regarded as good correlate of potential relative growth rate or mass-based maximum photosynthetic rate (Carnelissen *et al.*, 2003). In our studies, SLA varied substantially from 63.5 to 576.83 (CV: 36.58%) amongst the leaves investigated. Westoby *et al.* (2000) has reported 10-fold variation in SLA among species interspersed in the same habitat. Across 17 species investigated from evergreen Oak forest at 2200m altitude in Kumayoun, Nainital, India, maximum SLA was represented in *Artemisia* ($697.3 \text{ cm}^2/\text{g}^{-1}$) and minimum for *Ainslaea* ($8.09 \text{ cm}^2/\text{g}^{-1}$) and *Calamina* ($8.29 \text{ cm}^2/\text{g}^{-1}$) (Mehrotra *et al.*, 2004).

SLA related positively with both L and B but somewhat more closely with L ($r = 0.7096$, $p < 0.0010$) than B ($r = 0.6503$, $p < 0.001$) or leaf area LA ($r = 0.6178$, $p < 0.001$) (Table 3). It related somewhat weakly with LDM ($r = -0.4446$, $p < 0.001$). The curvilinear or logarithmic relationship of SLA with LA appeared to be little better than simple linear relationship between them. In short, smaller are the leaves, lower the SLA. Larger are the leaves, larger the SLA.

$$\begin{aligned} \text{SLA} &= 201.419 + 1.5939 \text{ LA} \pm 73.39 \\ t &= 22.12 \quad t = 8.53 \\ p &< 0.001 \quad p < 0.001 \\ R^2 &= 0.3817; \text{Adj. } R^2 = 0.3764; F = 72.84; r = 0.6259 \end{aligned}$$

$$SLA = 184.006 + 3.61808 LA - 0.017234 (LA)^2 \pm 71.41$$

$$t = 16.92 \quad t = 5.05 \quad t = -2.76$$

$$p < 0.001 \quad p < 0.001 \quad p < 0.001$$

$$R^2 = 0.4195; \text{Adj. } R^2 = 0.4096; F = 42.274; r = 0.6477$$

$$SLA = 129.0994 + 46.27805 \log_e LA \pm 66.364$$

$$t = 9.84 \quad t = 10.74$$

$$p < 0.001 \quad p < 0.001$$

$$R^2 = 0.4943; \text{Adj. } R^2 = 0.4900; F = 115.345; r = 0.7030$$

$$\log_e SLA = 4.898828 + 0.209346 \log_e LA \pm 0.25372$$

$$t = 97.7 \quad t = 12.7$$

$$p < 0.001 \quad p < 0.001$$

$$R^2 = 0.5778; \text{Adj. } R^2 = 0.5742; F = 161.48; r = 0.7601$$

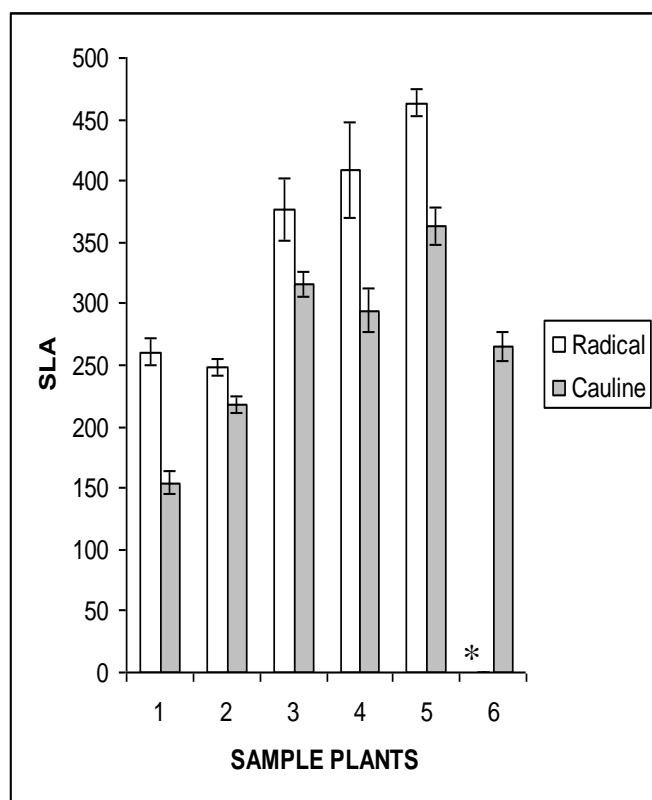


Fig. 6. SLA values ($\text{cm}^2.\text{g}^{-1}$) for radical and cauline leaves for six plants sampled in 2008. *, No radical leaves in good state were available in sample # 6. They have died and decayed wholly or partially.

When SLA of radical and cauline leaves was viewed on the basis of individual plants studied (Fig. 6), SLA of cauline leaves was consistently lower than that of radical leaves.

Considering overall variation in SLA in the two types of leaves, SLA was comparatively of higher order in rosette leaves (354.09 ± 21.39 ; $N = 22$) than cauline leaves ($231.59 \pm 7.58 \text{ cm}^2.\text{g}^{-1}$; $N = 98$) (Table 2). The SLA in the two types of leaves varied more or less equally (28.3% in rosette leaves and 32.4 % in axial leaves). The overall mean value of SLA was moderate - $254.05 \pm 8.49 \text{ cm}^2.\text{g}^{-1}$.

SLA associated with LDMC negatively ($r = -0.5149$, $p < 0.0001$; F , 16.95). Our results are in agreement with Garnier *et al.* (2001b) and Li *et al.* (2005) for species of mobile, semi-fixed and fixed dunes examined collectively. It has, however, been asserted by Garnier *et al.* (2001b) that low SLA values are relatively independent of changes in LDMC except when SLA values are higher than $100 - 150 \text{ cm}^2.\text{g}^{-1}$.

The Leaf to leaf analysis of SLA in leaves on main stem and lateral branches in two individual plants (Table 5) showed almost a regular trend of increase of SLA from apex to the base of the plant. Apical leaves had lower SLA than subsequent basal leaves. Dependence of SLA on a number of attributes such as leaf thickness and leaf tissue density (Witkowski and Lamont, 1991; Westoby, 1998; Wilson *et al.*, 1999), anatomical features of the leaves (Garnier *et al.*, 2001a) temperature to which leaf is exposed (Blackman, 1937; Acock *et al.*, 1979; Acock, 1980), growth stage and leaf maturity (Jonckheeri, *et al.*, 2004), solar radiation (Blackman, 1937; Reddy *et al.*, 1989), carbon dioxide concentration (Lieth *et al.*, 1986), etc. has been suggested among various species. In *N. plumbaginifolia*, high SLA of rosette leaves may be a significant factor enabling early ground cover by basal leaves (cf. Dingkuhan *et al.*, 2001) for light interception. Exposure of axial leaves to comparatively more irradiance may probably the reason for low SLA of axial leaves. High irradiance decreases SLA (Simioni *et al.*, 2004; Akram-Ghaderi and Sultani, 2007). Reddy *et al.* (1989) while studying SLA variation in cotton concluded that 93% of the variation in SLA of cotton leaves could be accounted for by the light flux density within the cotton canopy. High

SLA was found at the base of the canopy and low SLA was found at the top of the canopy. In open canopy situations, SLA gradient may be reverse e.g., in *Salix viminalis* where SLA was found to associate with time-related factors such as leaf maturity or growth stage and happened to be high in upper leaves (Verwijst and Wen. (1996). Variation of SLA with position of leaf on the plant has also been demonstrated in some species such as palm (Awal *et. al* , 2004), barley (Gunn *et. al.*, 1999). Chun-Wang (*et. al.* (2006) have reported increase in SLA from top to bottom of the crown in Scots pine. SLA was higher in the interior of the crown as compared to the crown edge.

Specific Leaf Mass (SLM)

SLM varied more in cauline (42.8%) than radical leaves (25.8%). The values of SLM for cauline leaves were, however, generally higher (mean = 0.004901 ± 0.0002 ; range: 0.00241 to 0.01574) than for radical leaves (mean = 0.00303 ± 0.00166 ; range: 0.00173 to 0.00432) (Table 2). SLM, being inverse of SLA, related negatively with SLA ($r = -0.9389$, $p < 0.001$). SLM was higher for smaller cauline leaves. It related with LA negatively ($r = -0.7323$; $p < 0.001$) (Table 3).

$$\begin{aligned} \text{SLM} &= 0.0074178 - 0.0010593 \text{ LA} \pm 0.001396 \\ t &= 26.88 \quad t = -11.68 \\ p &< 0.001 \quad p < 0.001 \\ R^2 &= 0.5363 \text{ Adj. } R^2 = 0.5324; F = 136.5 \\ r &= -0.7323 \end{aligned}$$

Across 11 lamiaceous species adapted to shade and sunshine environment, Castrillo *et. al.* (2005) have reported lower values of SLM in shade plants and higher values in sun plants. Shade plants had SLM – 0.003 to 0.006 g /cm² and sun plants – 0.009 – 0.016g /cm². Plants under sun have relatively higher values of sugar contents in leaves, low FW:DW ratio and high SLM (Castrillo *et. al.* (2005). The sun leaves of *Heteromeles arbutifolia* have been reported to have higher leaf mass per unit area than shade leaves (Valladares and Pearcy, 1998. In *Claytonia virginica* shade reduced SLM (Anderson and Eickmeier, 1998). SLM was 15% higher in coffee plants in full sunlight than shaded areas (Fahl *et al.*, 1994). Since there appears no significant difference in hydrature between radical and cauline leaves, differences in SLM in them appears to be attributable to environmental differences. Apical leaves for their more active physiological role under direct sun in *N. plumbaginifolia* may be thought to have greater metabolites contents than radical leaves remaining in shade due to upper part of the plant or neighbouring individuals.

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