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SOME MORPHOLOGICAL OBSERVATIONS ON LEAVES OF *CARISSA MACROCARPA* (ECKL.) A. DC. (FAMILY APOCYNACEAE)

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ABSTRACT

The leaves of *Carissa macrocarpa* (Eckl.) A. DC., collected from plants grown as hedge at Oud Metha, Dubai, were studied for their phenotypic traits, morphometric characteristics, leaf area estimation and surface micromorphological structure. Short phytography of the plant has been described. Leaves were variable in shape - ovate, oblong, orbicular or almost round. Leaf length (measured from umbo region to the apex) averaged to 4.18 ± 0.12 cm with a moderate degree of variation (1.4 -7.5 cm, CV: 28.73%). The leaf width at the widest points on the margins averaged to 3.12 ± 0.098 cm varying around 31.33% (1.0 - 6.2 cm). Around 92% of the leaves showed leaf length ranging between 2.1 and 6.0 cm. Aspect ratio (Length / breadth) of leaves in *C. macrocarpa*, averaged to 1.373 ± 0.0268 (varying from 0.87 to 2.36 with moderate variation of 19.21%. Apex angle averaged to 97.04 ± 1.387^o varying around 14.3 %. It was lesser than 90^o in 25% of the leaves, 90^o in 11% of the leaves and greater than 90^o in 64% of the leaves (91 – 148^o). Leaf base angle averaged to 109.44 ± 1.095^o. It was ≤ 90^o in 5% leaves and larger than 90^o (91 – 143^o) in 95% of the leaves i.e., in most of the cases leaf base was obtuse. Base angle related with apex angle as, Base Angle = 55.234 + 0.559 Apex angle ± 7.780. Leaf base in umbo area embayed in sinus in some cases and gave rise to cordate type of leaf base otherwise cuneate. 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 1.33 ± 0.118 (N = 9, 1.0-1.5mm) as found in only in nine leaves. La was zero (non-existent) in 91% of the leaves. Basal leaf extension length (Lb) was observed in 14 leaves only which had cordate leaf base and their umbo region embayed in sinus. Lb was zero (non-existent) in 86% of leaves. Lb averaged to 1.714 ± 0.276mm (N = 14, 0.5 – 4mm). Spines were bifurcate oppositely produced on node and quite variable in size - small to giant (c 10 cm). The value of mean arithmetic coefficient (k) for leaf area estimation was found to be 0.70980 ± 0.008128 varying from 0.51 to 1.0529 (CV: 11.45%) and concentrated around the mean value. Leaf area (LAM, cm^2) was found to relate to the multiplicative parameter, LL (cm) x LB (cm), as given by the equation: (LAM) = 0.702.(LL x LB) $^{1.002}$ ± 0.115, R^2 = 0.968).

Leaf area per leaf, in a sample of 100 leaves, averaged to 9.884 ± 0.5427 cm^2 varying from 1.02 to 27.98 cm^2 (CV: 55.93. It was positively skewed and leptokurtic – asymmetrically distributed. In 85% of the leaves LAM varied from 12.6 to 15.0 cm^2. Under the given growth conditions, Leaf dry matter, in comparison to SLA, appeared to be a better parameter in *C. macrocarpa*.

The leaves of *C. macrocarpa* were hypostomatic. Lower epidermis had stomata generally of anomocytic type but also anisocytic type of arrangement of subsidiaries was seen. Stomata were round or oval or wide elliptical measuring stomatal diameter c 33.5 -35.0 µm and outer stomatal ledge aperture c. 15 µm. They were variable in size and orientation. Stomata with common subsidiary (ies) were present. Anticlinal walls of pavement epidermal cells were straight to curvy. Leaves were trichomatous when young. In a sample of 60 microscopic frames of vision, the stomtal density per mm^2 on the ventral surface of leaves averaged to 283.8 ± 5.41 stomata varying from 190.35 to 432.62, CV: 14.77%. Leaf cuticle appeared to be in form of sheet. There were, however, epicuticular crystalloids, probably waxy, in form of granules and platelets which appeared to fuse to form lumps of various shapes.

Key words: *Carissa macrocarpa* (Eckl.) A. DC., Leaf traits, leaf surface micromorphology, trichomes, Stomata types, Epicuticular waxy crystalloids.

INTRODUCTION

Carissa macrocarpa [(Eckl.) A. DC.] syn. *C. grandiflora* (E. Mey) A. DC., commonly called Natal Plum, belongs to Dogbane family (Apocynaceae). *C. macrocarpa* is found amongst the coastal bushes and often on sand dunes in Southern Africa, occurring up to 1350m (Allam *et al.,* 2016) and flowering round the year (Leeuwenberg and Dilst, 2001). It has glossy foliage and fragrant, starry white jasmine like flowers (Kaunda and Zhang, 2017). It contains latex abundantly (Gabr *et al.,* 2015). The college of Agriculture and Environmental Sciences of University of California, Davis rates the plant as mildly toxic. The ripe fruit is, however, edible. It is an element of Southeastern and Southern African vegetation (White, 1983; Leeuwenberg and Dilst, 2001) and is distributed from Kenya to South Africa (Congo, Kenya, Zambia, Zimbabwe, Mozambique, South Africa) and cultivated in tropical and subtropical areas all over the world including UAE (Abu Dhabi; Leeuwenberg and Dilst, 2001). We found it in Dubai grown in form of hedges in Oud Metha, near Pakistan Education Academy, Dubai, UAE. Allam *et al.* (2016) have studied *Carissa macrocarpa* cultivated in Egypt, for botanical characterization of its leaf, stem and root (anato-histologically). It is an economically important plant in which a number of secondary metabolites (triterpenoids, steroids, alkaloids, flavonoids, saponins, anthraquinones, tannins, etc.) have been reported (Moodley, 2012; Khalil *et al.,* 2015; Allam *et al.,* 2016). It is a source of antimicrobial compounds (Souilem *et al.,* 2018).

In the present paper, the leaves of this species are studied for their phenotypic traits, morphometric characteristics, leaf area estimation and surface micromorphological structure under urban environment of Dubai and frequent pruning stress. The micromorphological characteristics of epidermal surface is important and quite pertinent to be studied in details (Barthlott *et al.,* 1998) since it is the outer most boundary of the plant in contact with the environment. Presently, there is paucity of foliar morphometric data on this species.

Climatic features of Dubai: UAE is located in Middle East, situated on Arabia Peninsula between Oman and Saudi Arabia bordering the Gulf of Oman and the Persian Gulf. It covers an area of 83,600 Sq. km. Its largest city is Dubai, landscape of which is sandy – extreme hot. Days are sunny all the year around. Humidity is discomfortingly high in

coastal region. According to Köppen classification, its climate is of Bwh type (Tropical desert climate) (Köppen and Geiger, 1954) and bioclimate as given by Holdridge (1947) falls into the category of Tropical Desert Bush formation. Brief description of climate of Dubai is given in Khan and Ismail (2019).

MATERIALS AND METHODS

The leaves of *C. macrocarpa* were collected in November 2018 from the plant grown as hedge along the roadside near Pakistan Education Academy, at Oud Metha, Dubai (Fig. 1). The hedges were grown by drip irrigation under the roadside plantation of *Millingtonia hortensis*. These trees casted shade and litter (mainly abscised corolla, fruits and leaves) on the hedge underneath. These hedges were invaded by some plants such as saplings of *M. hortensis*, *Ficus infectoria*, *F. bengalensis* and *Ziziphus* sp. and some herbs like *Launaea* sp. and grasses. The hedges were pruned and maintained regularly but at some places it was dying due to some random reason. Due to carelessness of people, these hedges, at some places, were containing trash and debris, seldom in substantial amount.

Protocols of Hickey (1973) and LWG (1999) were followed for morphological description of leaf. The leaves were kept in an ice box and were detached from the branches while underwater. After drying for surplus water, the linear measurements of leaves were recorded for leaf length (LL) and lamina breadth (LB) at the broadest points. To determine leaf area (LAM), the leaf outlines of fresh leaves were carefully drawn on graph paper and area determined with all possible precision and accuracy. For leaf dry matter (LDM) determination, leaves were kept continuously at 70 ^{o}C for two days and then weighed along with the very small petiole. Specific Leaf Area (SLA) was expressed as the ratio of two-sided leaf area to dry leaf mass ($cm^2.g^{-1}$) following Westoby *et al.* (2000). Specific leaf mass (SLM) was equal to SLA^{-1}. The average ratio or the multiplication factor (K) was also calculated for the leaf by employing the formula, K = LAM / (length x breadth). Employing average values of the multiplication factor we computed the Area computed = K (length x breadth). Bivariate linear and power relationships between the specific leaf characteristics were computed and the regression coefficients were determined by multiple regression method to fit in the allometric model, Y = a + b1 X1 + b2 X2 ± SE in order to relate measured leaf area with linear measurements recorded. The arithmetic and allometric methods were compared for their precision and suitability. The data was analyzed statistically on SPSS ver. 17. 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:

$Sg1=\sqrt{6N (N-1) / (N-2) (N+1) (N+3)}$ and

$Sg2= \sqrt{24N (N-1)^2 / (N-3) (N-2) (N+3) (N+5)}$.

The data were analyzed following Zar (2010) and the normality of various variables was tested on the basis of Kolmogorov-Smirnoff test corrected for Lilliefors significance correction and Shapiro-Wilk test.

Epidermal impressions from fresh leaves were made with clear nail polish (Wang *et al.,* 2006). Stomatal nomenclature suggested by Prabhakar (2004) being simple and based upon structure of stomata and not their ontogenetic pathways was adopted to ascertain stomatal types. For scanning electron microscopy (SEM), air-dried leaf material was mounted on brass stubs and coated with a 250 ^{o}A gold layer with JFC-1500 gold coater. Scanning micrographs were made at 15kV with JEOL JSM-6380A electron microscope at various magnifications. The images were saved digitally on computer. Epicuticular wax crystalloids were identified after Barthlott *et al.* (1998).

Fig. 1. Close up view of the *C. macrocarpa* hedge. Note that the new growth is more vigorous with larger leaves as compared to the leaves of lower canopy.

RESULTS AND DISCUSSION

Phytography

The phytography presented here is based on the plant grown under drip irrigation as road-side hedge in Oud Metha, Dubai (Fig.1). Obviously, being a hedge, the plants have been under intermittant stress of pruning. The brief phytography of the plant is described below (Fig 2- 4).

The plant is densely bushy, latex bearing and profusely branched. Bark greyish smooth longitudinally fissured. Stem woody. When young it is green and hair-bearing sparsely. Spines slender and small, sometimes robust in new growth after pruning – furcate or mostly bifurcate. Leaves exstipulate, opposite. Petiole is short, pubescent when young. It is dorsally-centrally grooved. Spines are produced in the axil of bract on either side of the node. Leaf phyllotaxy is opposite decussate. Leaf blade shiny green dorsally and dull yellow green ventrally coriaceous and brittle when dry, ovate, orbicular or wide elliptical or almost round, acute and mucronate apex, obtuse or rounded sub-cordate and sometimes embayed in sinus at the base. Sometimes the lamina on two sides of midrib is unequal in width. Mature leaves appear to be glabrous shiny above but young leaves exhibit delicate hairs all over lamina dorsally and ventrally both, margins revolute particularly in mature and / or dried leaves. Several [(4) 5-8)] pairs of secondary veins were present, arcuate towards the margin. Sometime up to 11 pairs of secondary veins have been reported (Leeuwenberg and Dilst, 2001). Tertiary venation is reticulate, less apparent. Inflorescence terminal. Pedicel c. 3.0 mm, pubescent when young. Flowers white, pentamerous, opens during day. They are pleasantly fragrant. Sepals green or pale green, unequal in size generally and connate at the base. At the base of sepals, thalamus bears five thick bulbous gland-like growths. Sepals narrowly ovate or narrowly oblong, acute at apex. Corolla white, glabrous (bear hairs on inner side and margins when young), lobes broadly ovate or orbicular, obtuse or rounded at the apex, and thickened in the middle. Stamens five, 1.0 – 1.8 mm, inserted below mouth of corolla tube. Corolla tube (1.0-1.6 cm in length) is densely hairy inside in the region of staminal insertion. Filaments short and anthers c. 2mm in length, dithecous and acutely-apexed. Pistil 2.7-5.8 mm long, Ovary cylindrical or sub-globose. Green in colour but sometimes red, style green, 2 – 3 mm, stigma capitate green, laterally compressed. Ovary is bicarpellary with axile placentation. Stigma bearing tuft of delicate whitish hairs on the top. Fruit ellipsoidal or oblong berry 20-30 mm in length and up to 15 mm in width, green when young and red when ripe. Seeds were not seen. Leeuwenberg and Dilst (2001) described seeds to be plano-convex, obliquely elliptical or ovate. Young fruits are green. Ripe fruits are said to be edible.

The taxonomic characters led to the identification of the plant as *Carissa macrocarpa* on the basis of description presented by Leeuwenberg and Dilst (2001).

Table 2 presents data on architectural morphometry and some vital traits of the leaves. The leaves *C. macrocarpa* are glossy, dark green above and dull paler green below. The plant is characteristically evergreen, twiggy, and branching in a repeated forked manner. Spine rarely single otherwise forked and at times robust particularly in the new growth. The leaves of *C. macrocarpa* exhibited variation in leaf shape (Fig. 3) – ovate, wide elliptic, oblong, orbicular or almost round. They are generally mucronate, acute at the apex and cordate (embayed in sinus) to cuneate at the base.

Leaf length and width

Leaf length (measured from umbo region to the apex) averaged to 4.18 ± 0.12 cm with a moderate degree of variation (1.4 -7.5 cm, CV: 28.73%) (Table 2). The leaf width at the widest points on the margins averaged to 3.12 ± 0.098 cm varying around 31.33% (1.0 - 6.2 cm). Around 92% of the leaves showed leaf length ranging between 2.1 to 6.0 cm and some 85% of the leaves had width ranging from 1 to 5 cm. Leaf venation brochidodromous (Fig. 2C). Leaf base cuneate to cordate. Our data on leaf length and width agree with that of Leeuwenberg and Dilst (2001) collected from Africa – Length: 1.3-7.2 cm and width 0.9-5.3 cm. Allam *et al.* (2016) reports leaf much shorter (1-3cm) and narrower (1-2 cm) from El-Orman Botanical Garden, Giza, Egypt. The data on leaf length (1-5 cm) and width (1-2.5) for plant grown in Garden of King Faisal University, Al-Ahsa region, KSA (Khalil *et al.*, 2015) was also comparatively lesser than that collected from Dubai (in hand).

Aspect ratio (Length / Breadth ratio; LL / LB):

Leaf shape is very intricate matter. It is difficult to be modeled with high accuracy with simple geometrical figures. Length / breadth ratio may, however, give some indications about consistency of leaf shape with size (Verwijst and wen, 1996). Aspect ratio (Length / breadth) of leaves in *C. macrocarpa*, averaged to 1.373 ± 0.0268 (varying from 0.8696 to 2.3636 with moderate variation of 19.21% (Table 2). The aspect ratio (LL / LB) was $\leq$ 1.0 in 9% of the leaves and tended to be $\geq$ 1.0 in 91% of the leaves (> 1.0 to 2.3636) being > 2.0 in 3% of leaves. It followed from the data that leaves were generally larger in length than width but in some cases converse was true. The aspect ratio followed asymmetrical distribution pattern (Table 3; Fig. 5). Aspect ratio showed non-significant

correlation with LAM (r = 0.180, F = 3.294, p < 0.073). It appears that overall leaf shape, as given by aspect ratio, is generally maintained with leaf size in majority of the leaves.

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 1.33 ± 0.118 (N = 9, 1.0-1.5mm) as found in only in nine leaves. La was zero (non-existent) in 91% of the leaves. According to LWG (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, 98% of the leaves exhibited retuse apex, 1% emarginate and 1% retuse intergrading to emarginate type of apex.

Basal leaf extension length (Lb) was observed in 14 leaves only which had cordate leaf base and their umbo region embayed in sinus. Lb was zero (non-existent) in 86% of leaves. Lb averaged to 1.714 ± 0.276 (N = 14, 0.5 – 4mm). When the plants were re-visited in October 2019, a leaf was observed with obliquely inserted lamina at umbo. The leaf had length along midrib, L = 2.7 and 2.9 (for the two sides of midrib, respectively) and breadth, B = 3.8 cm. L / B ratio was 0.763. La measured 1.5 mm on each side midrib and Lb = 2.0 and 3.0 on either side of midrib.

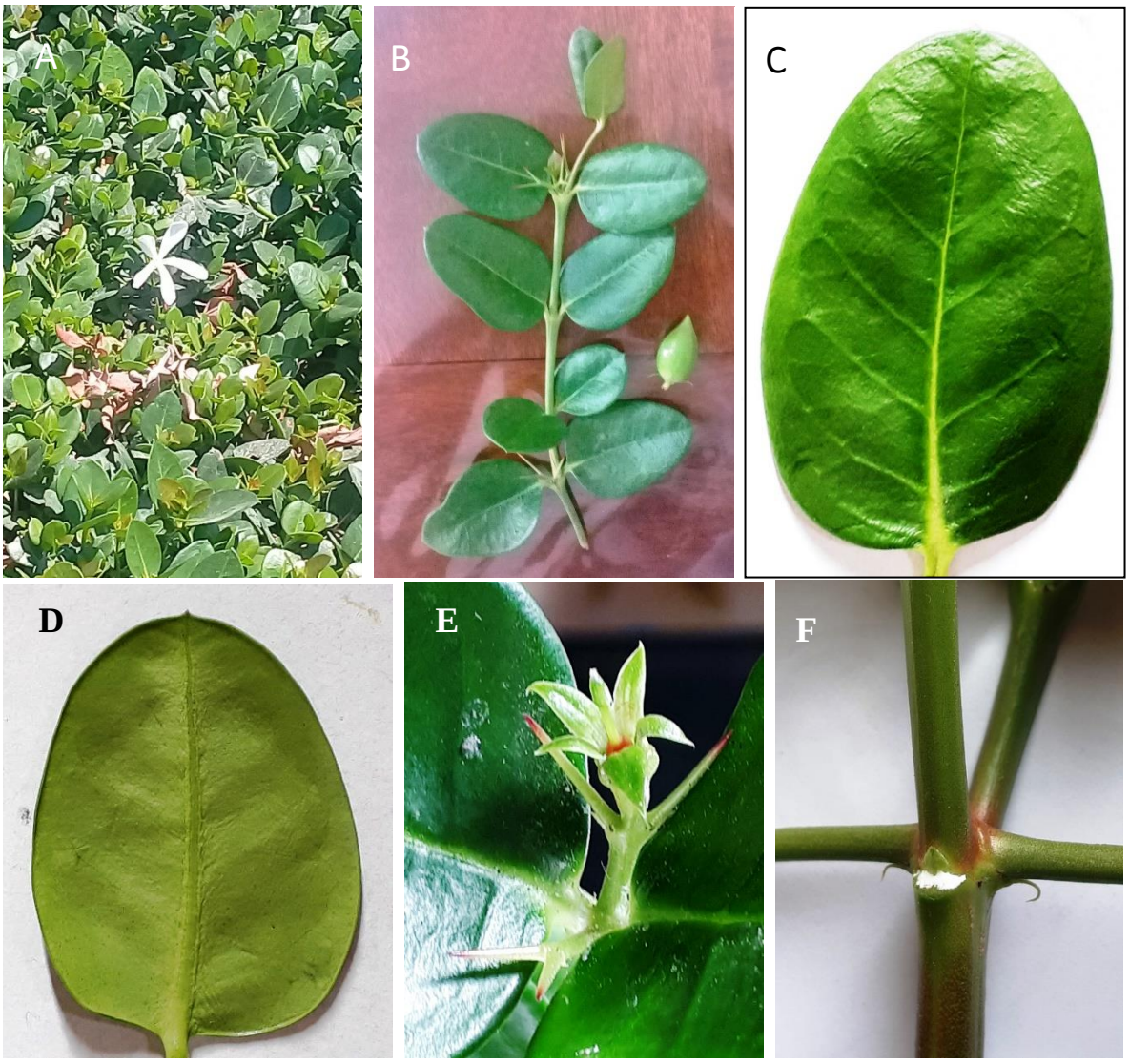


Fig. 2. *Carissa bispinosa* – A, a hedge at Oud Metha, Dubai- showing flower (highly fragrant); B, a twig and a subglobose / ellipsoidal fruit; C, a leaf showing glaucous dorsal surface and brachidodromous type of venation – 7 pairs of sec. veins; D, dull green ventral surface of leaf (mucronate); E, A flower (corolla removed) showing gynocium - red ovary and capitate stigma protected by red-tipped spines. On maturity the spines are stiff and hard, woody and piercing; F, showing bracts on the node – white occlusion is the latex accumulated on the removal of leaf.

Leaf apex angle (A^o)

Apex angle averaged to 97.04 ± 1.387^o varying around 14.3 %. It was lesser than 90^o in 25% of the leaves, 90^o in 11% of the leaves and greater than 90^o in 64% of the leaves (91 – 148^o) i.e. apex angle was acute in 25% leaves and obtuse in 75% of the leaves (as per criterion of LWG, 1999) (Fig. 6, Table 2 and 3).

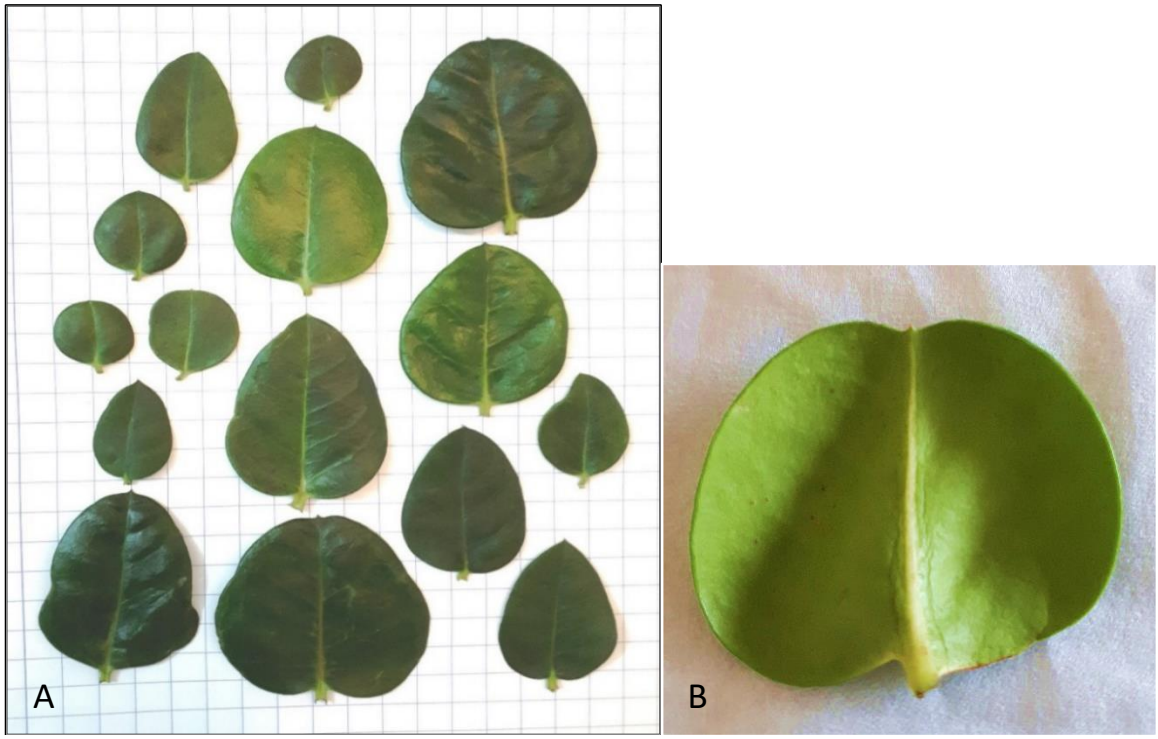


Fig. 3. Leaves of *C. macrocarpa* showing variability in form. Each box is 100 mm^2 in size (A); B, a leaf with obliquely inserted lamina; C, the dorsal and ventral surfaces of very young leaves showing delicate small and whitish hairs on lamina and petiole. The tender green stem also has hairs. These hairs are short-lived and whither soon. They may sometimes be observed as dry powder scattered on the leaf surface in middle-aged leaves.

Fig. 4. A) Dorsal surface of leaf showing whitish dense crust of wilted, dead hairs; B) Corolla tube bearing hairs on inner surface of corolla tube in the region of staminal insertion; C) Bifurcate tuft of delicate hairs on the apex of stigma. Sepals on inner side and margins are often hairy paricularly in very young flowers.

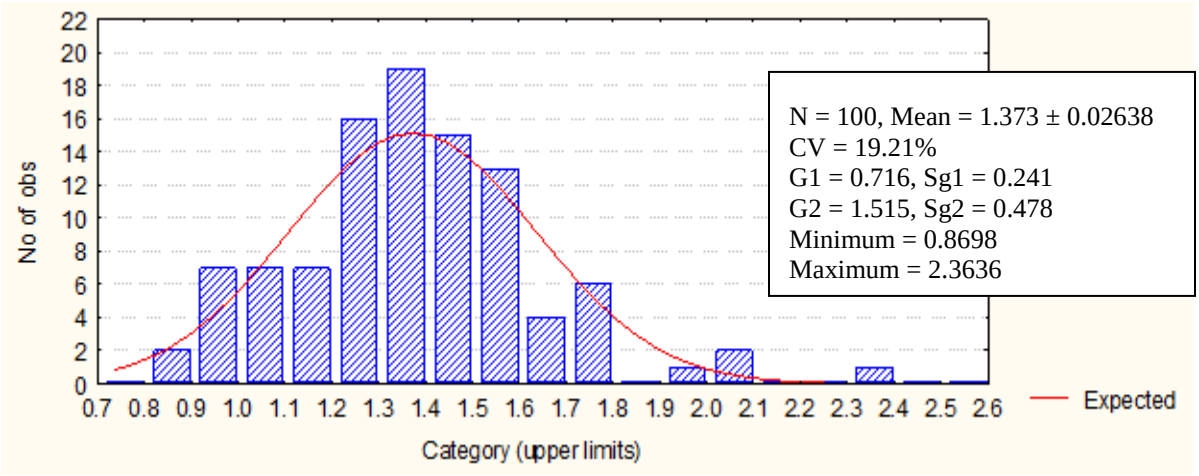


Fig. 5. Frequency distribution of LL / LB ratio (Aspect ratio).

Leaf Base angle (B^o)

Leaf base angle averaged to 109.44 ± 1.095^o. It was ≤ 90^o in 5% leaves and larger than 90^o (91 – 143^o) in 95% of the leaves i.e., in most of the cases it was obtuse. Leaf base in umbo area embayed in sinus in some cases and gave rise to be cordate type of leaf base otherwise cuneate. There was relatively lesser variation in magnitude of basal angle (CV: 10.0%) (Fig. 7, Table 2 and 3).

Relationship between apex and base angles

Apex angle of leaves exhibited significantly positive correlation with base angle (r = 0.707, F = 98.21, p < 0.001) i.e., as the apex angle increased so the base angle and this relationship was best represented by the following best fit equation:

Base Angle = 55.234 + 0.559 Apex angle ± 7.780
t = 9.98 t = 9.91
p < 0.0001 p < 0.0001, R = 0.707, F = 98.21 (p < 0.0001)

Fig. 8 presents the bivariate plot of apex and base angles. The values of apex and base angles concentrated in the mid region of the plot. There appeared four interesting combinational proportionalities between the angles A^o and B^o.

1. Both angle A and B were acute – 3% of leaves.
2. Both angle A and B were obtuse – 61 % of the leaves.
3. Apex acute and base obtuse – 35% of the leaves.
4. Apex obtuse and base acute – very rare, only 1% of the leaves.

It was obvious that the large proportion of leaves (61%) had both apex and base angles obtuse. In 35 % of the leaves, apex angle was acute coupled with the base angle which also happened to be obtuse. The base angle was predominantly obtuse in 96% of the leaves.

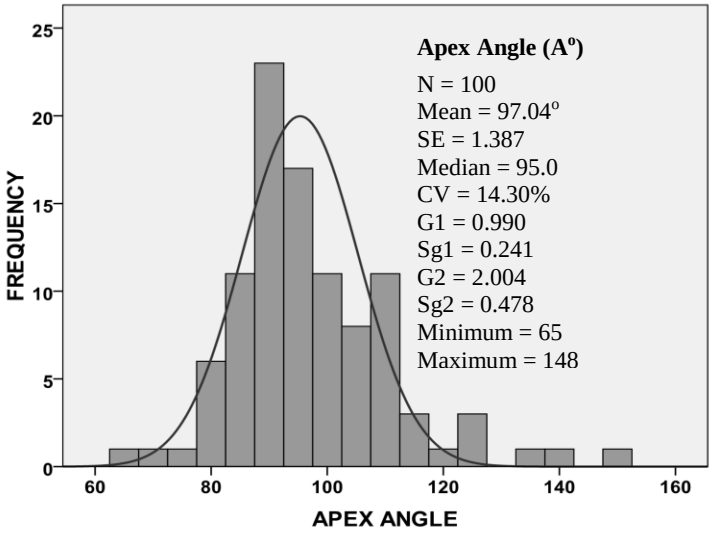


Fig. 6. Frequency distribution of apex angles (^{o}).

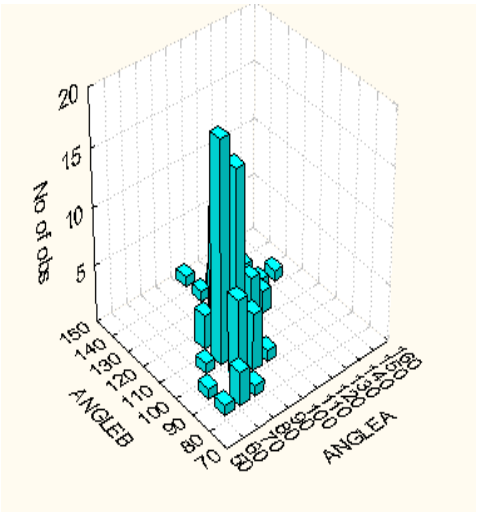
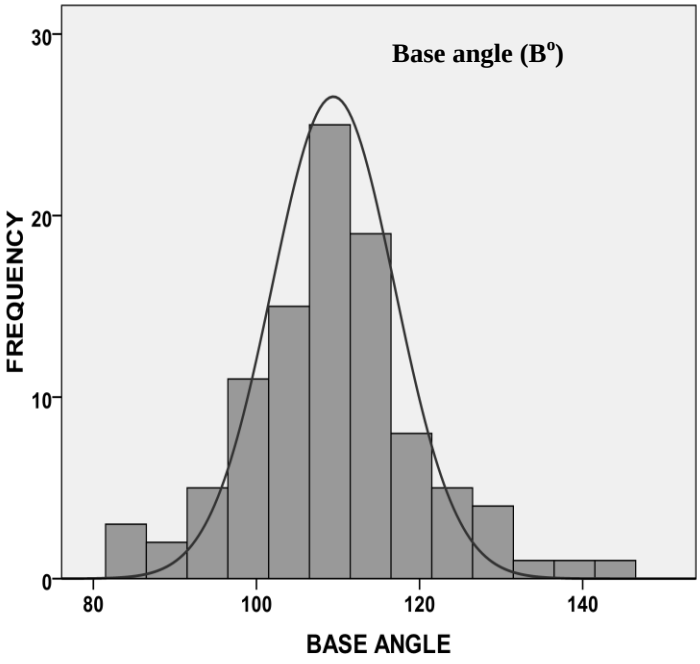


Fig. 8. Bivariate distribution of A^o and B^o.



Base Angle (B^o)
N = 100
Mean = 109.44^o
SE = 1.095^o
Median = 109.0
CV = 10.00%
G1 = 0.276
Sg1 = 0.241
G2 = 0.478
Sg2 = 0.478
Minimum = 84^o
Maximum = 143^o

Fig. 7. Frequency distribution of base angles (^{o}).

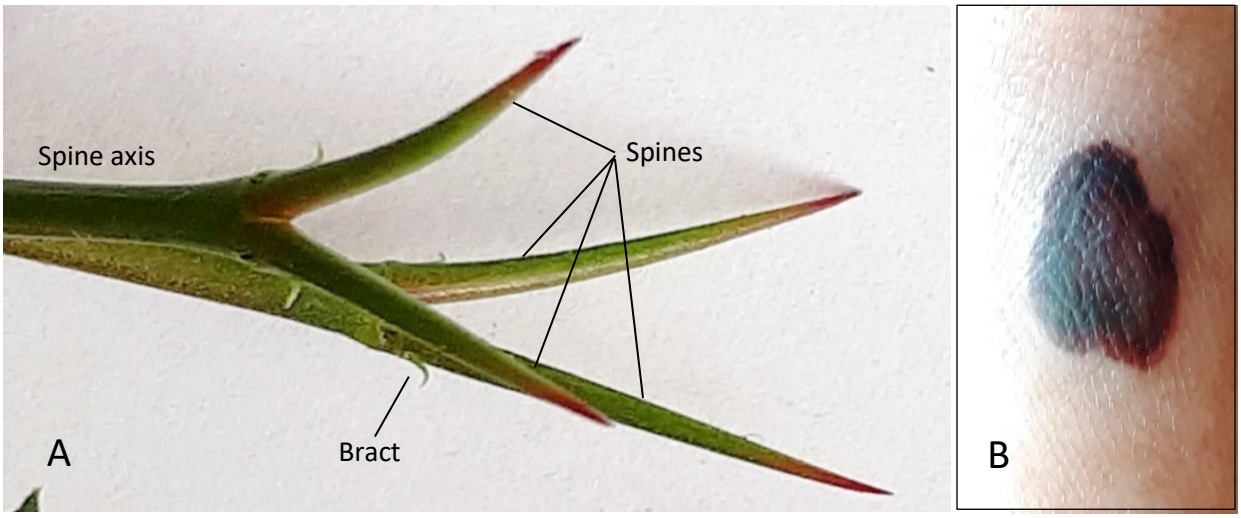


Fig. 9. A, *C. macrocarpa* bifurcate spine - Each produced in the axil of small curved bract. The node generally bears two spines oppositely on either side of the stem axis. B, The local skin reaction on prick by small spines on the thumb.

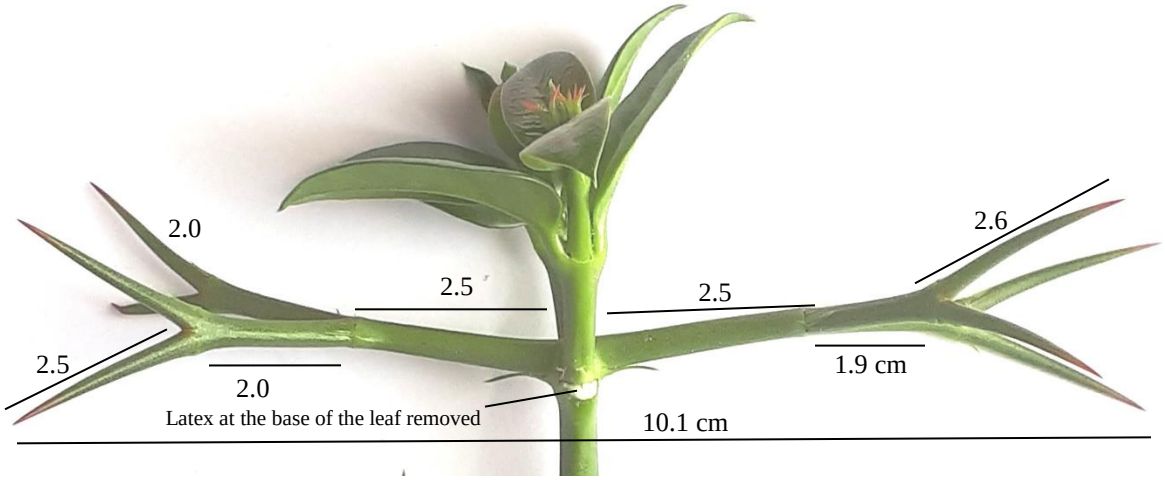


Fig. 10. Giant spines of *C. macrocarpa* (new growth after prunning, January, 2019). The spines were quite symmetrical in shape and size on the either side of the axis. The leaf base is semilunar.

Spines

Not all nodes but some of them bear a pair of spines produced oppositely on either side of a node – sometimes furcate, generally bifurcate. Each spine is produced in the axil of a curved bract. The spines are produced on a node in plane at right angle to the plane of the adjacent pair of leaves. They appear possibly to be the modification of a branch. They are soft when young but hard when mature (Fig. 9 and 10). They occur in various sizes – small, large and giant ones (seen only with new growth). The spines are quite symmetrical in structure and may measure up to 10.1 cm in span cumulatively on either side of the node (Fig. 10). The spines are divided dichotomously, and their measurements of giant spine pair are given in Fig. 10. The angle of divergence in the spine fork appeared to vary between 40 to 60^{o}. Since plant is mildly toxic pricking by spine may cause local skin reaction (Fig. 9 B) which takes quite some time to heal and may need antibiotic therapy.

Measured leaf area (LAM) and estimated Leaf areas (LAK and LAR)

LAM

LAM, in a sample of 100 leaves, averaged to 9.884 ± 0.5427 cm^2 varying from 1.02 to 27.98 cm^2 (CV: 55.93. It was positively skewed and leptokurtic – asymmetrically distributed (Table 2 and 3 amd Fig. 11). In 85% of the leaves LAM varied from 12.6 to 15.0 cm^2 .

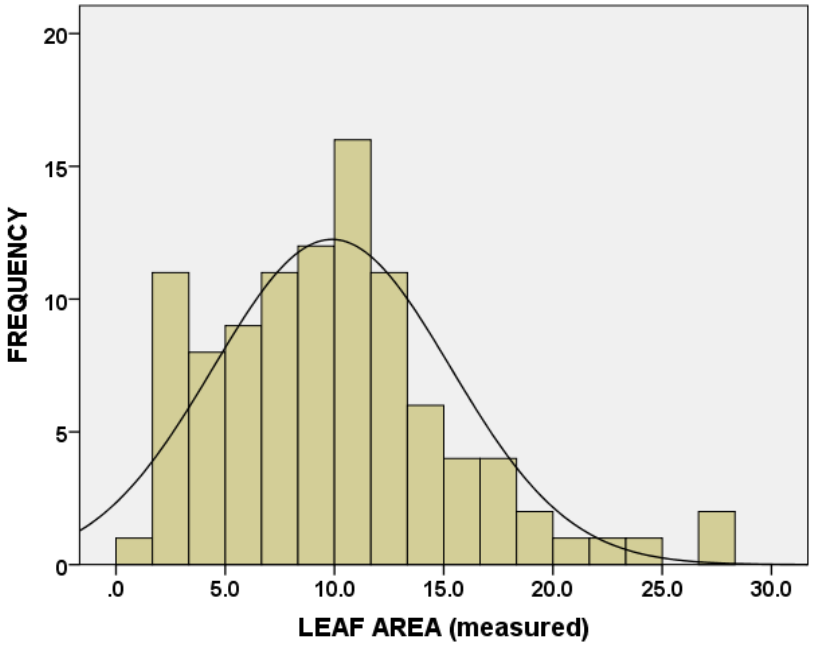


Fig. 11. Frequency distribution of leaf area measured graphically in cm^2.

Table 2. Morphometrics and vital leaf traits data of *C. macrocarpa* leaves.

Statistics	Leaf morphometric parameters												
	LL	LB	LL x LB	LAM	LL/LB ratio	K	A^o	B^o	LDM	SLA*	SLM*	LAK	LAR
N	100	100	100	100	100	100	100	100	100	100	100	100	100
Mean	4.178	3.118	14.00	9.883	1.373	0.7098	7.04	109.44	0.1119	185.86	0.0058	9.937	9.883
SE	0.1201	0.098	0.776	0.5428	0.0264	0.0081	1.387	1.095	0.0071	5.153	0.00017	0.5508	0.5488
Q2	4.450	3.100	13.79	9.670	1.373	0.7081	95.00	109.0	0.0980	183.0	0.0055	9.7847	9.728
CV (%)	28.74	31.33	55.43	55.93	19.21	11.45	14.30	10.00	64.04	27.73	29.08	55.43	55.49
G1	- 0.034	0.474	0.867	0.932	0.716	0.508	0.990	0.276	0.972	1.171	1.516	0.867	0.869
Sg1	0.241	0.241	0.241	0.241	0.241	0.241	0.241	0.241	0.241	0.241	0.241	0.654	0.241
G2	-0.489	0.642	0.654	1.285	1.515	2.527	2.004	0.478	7.078	3.525	4.263	0.654	0.659
Sg2	0.478	0.478	0.478	0.478	0.478	0.478	0.478	0.478	0.478	0.478	0.478	0.478	0.478
Min.	1.40	1.0	2.0	1.02	0.8696	0.510	65.0	84	0.021	78.46	0.0024	1.4196	1.41
Max.	7.5	6.2	37.0	27.98	2.3636	1.053	148.0	143	0.478	410.6	0.0127	26.405	26.30

LL, Leaf length (cm); LB, Leaf breadth (cm); LL x L B, multiplicative parameter of length and breadth; LAM (cm^2); A^o, Apex angle; B^o, Base angle; LDM, Leaf dry mass (g); SLA, specific leaf area (cm^2 /g) and SLM, specific leaf mass (inverse of SLA, g $.cm^{-2}$). Q2, median; CV, Coefficient of variability (%); G1, skewness; Sg1, SE of skewness; G2, kurtosis, Sg2, SE of kurtosis; *, Based on two-sided leaf area; LAK, Leaf area estimated via mean K = 0.709808. LAR, leaf area estimated by regression equation based on a multiplicative parameter (LL x LB) relating to LAM through a power model of regression.

Arithmetic coefficient K and estimation of LAK

To facilitate estimation of leaf area arithmetically, coefficient of leaf area estimation, k, was calculated as k = LAM / (LL x LB) and mean value of k was employed to estimate the area of individual leaves as LAK = (LL x LB) x mean k. The value of mean k in the present studies was found to be 0.709808 ± 0.008128 varying from 0.51 to 1.0529 (CV: 11.45%) and concentrated around the mean value (Table 2; Fig. 12). In 85% of the leaves k value varied from 0.61 to 0.80. It distributed asymmetrically – leptokurtic and somewhat positively-skewed (Table 3).

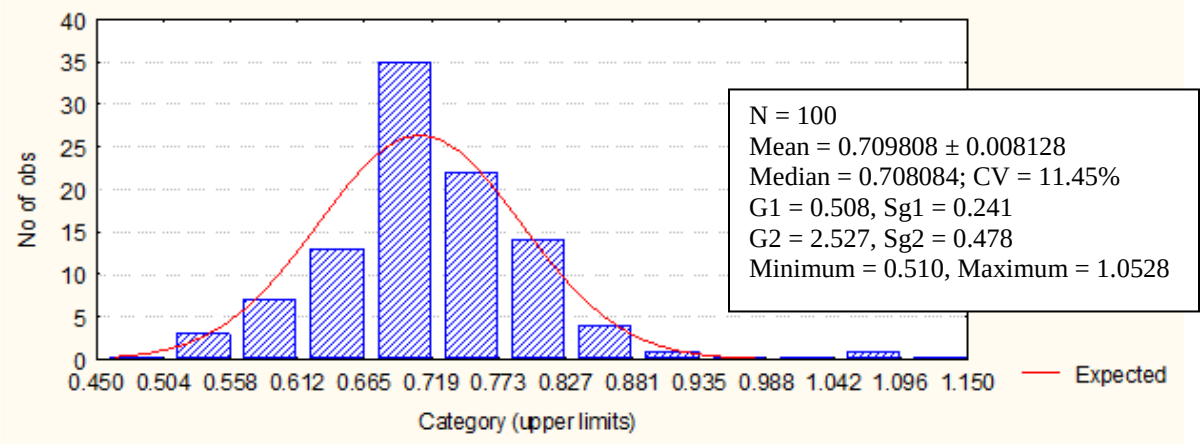


Fig. 12. Frequency distribution of K factor (K = LAM / LL x LB).

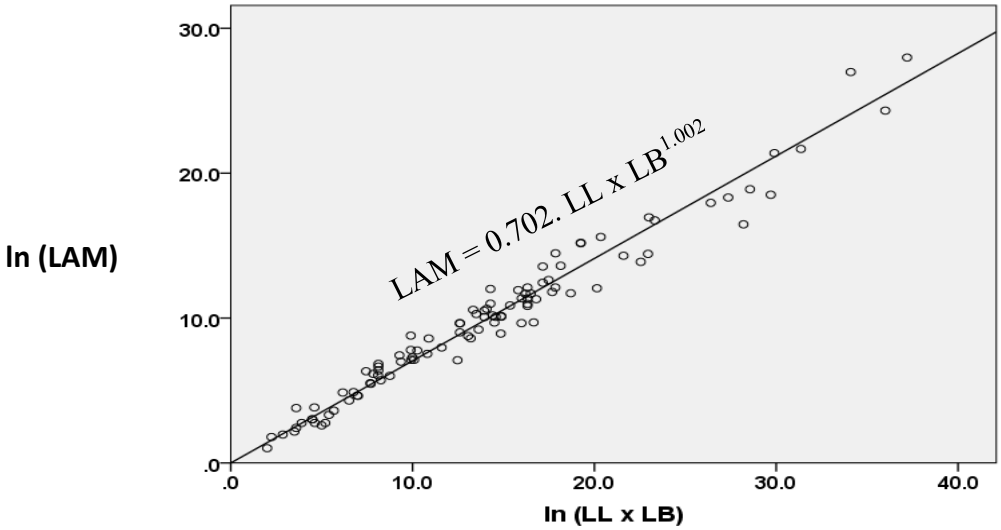


Fig. 13. Relationship of Leaf area measured (LAM) with the multiplicative parameter of LL X LB as given by a power model (see equation I).

Table 3. Test of normality of morphometric parameters.

Leaf Parameters	Kolmogorov-Smirnoff Test**	Shapiro-Wilks Test	Curve
LL	0.106, p < 0.008	0.978, p < 0.091	AS
LB	0.120, p < 0.001	0.973, p < 0.039	AS
LL x L B	0.099, p < 0.016	0.941, p < 0.0001	AS
LAM	0.111, p < 0.004	0.945, p < 0.0001	AS
Aspect ratio = LL/ LB	0.089, p < 0.049	0.963, p < 0.006	AS
K	0.096, p < 0.024	0.965, p < 0.009	AS
A^o	0.130, p < 0.0001	0.0941, p < 0.006	AS
B^o	0.090, p < 0.046	0.979, p < 0.108	AS /S
LDM	0.127, p < 0.0001	0.853, p < 0.0001	AS
SLA*	0.095, p < 0.026	0.935, p < 0.0001	AS
SLM*	0.130, p < 0.0001	0.899, p < 0.0001	AS
LAK	0.099, p < 0.016	0.941, p < 0.0001	AS
LAR	0.1000, p < 0.016	0.941, p < 0.0001	AS

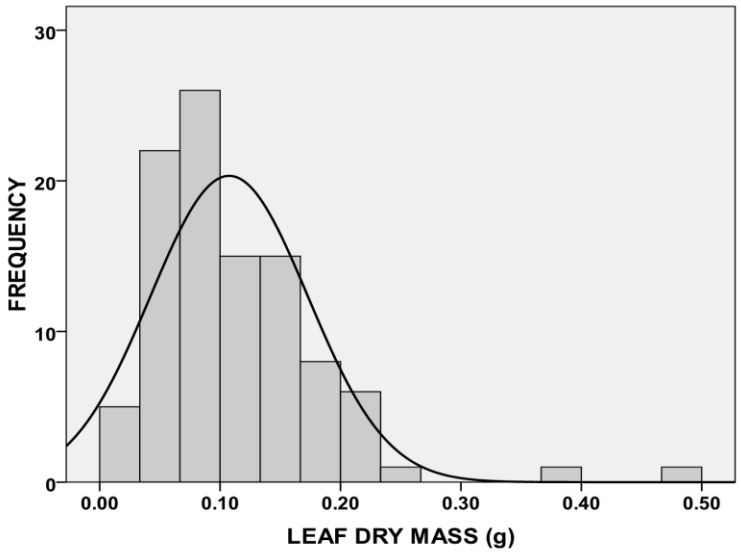
*, based on two-sided leaf area. **, With Lilliefors significance correction. As, Asymmetrical, S, Symmetrical.
The leaf area estimated via K (LAK) averaged to 9.937 ± 5.5082 varying from 1.4196 to 26.4049, CV: 55.43% (Table 2). Like LAM, LAK also distributed asymmetrically (Table 3).

Estimation of leaf area through regression (LAR)

Leaf area for 100 individual leaves was also estimated through following regression equation (best fit power model equation (Fig. 13) between LAM (Y – axis) and a multiplicative parameter of LL x LB (X - axis).

$$LAM\ (cm^2) = 0.702.\ (LL \times LB)^{1.002} + 0.115$$
$$t = 21.28 \qquad t = 54.29$$
$$p < 0.0001 \quad p < 0.0001$$
$$R = 0.984,\ R^2 = 0.968,\ Adj.\ R^2 = 0.967,\ F = 2947.08\ (p < 0.0001)$$

The estimated leaf area through this equation was designated as LAR which in the sample of 100 leaves averaged to 9.883 ± 0.5488 varying from 1.41 to 26.30 cm^2 (CV: 55.49%) (Table 2). Like LAM, LAR in 85% of the leaves varied between 12.6 and 15.0 cm^2. This variable also distributed asymmetrically (Table 3).



LDM (g)
N = 100
Mean = 0.1119g
SE = 0.007166
Median = 0.001750
CV = 64.04%
G1 = 0.972
Sg1 = 0.241
G2 = 7.078
Sg2 = 0.478
Minimum = 0.021
Maximum = 0.478

Fig.14. Frequency distribution of leaf dry mass (LDM, g).

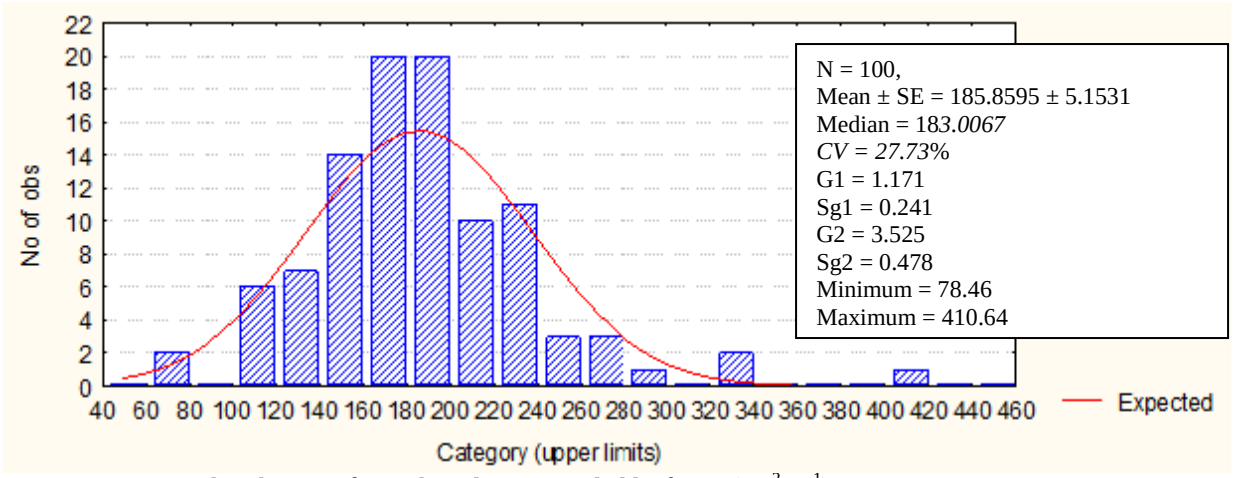


Fig. 15. Frequency distribution of SLA based on two-sided leaf area ($cm^2.g^{-1}$).

The three leaf-size-variables, LAM, LAK and LAR were closely related to each other. LAM, LAK and LAR averaged to 9.8893 ± 0.5428, 9.9377 ± 0.5508 and 9.8833 ± 0.5488 cm^2, respectively, for 100 leaves data set of each. The variables, LAK and LAR, were highly correlated with LAM (r = 0.983, p < 0.0001, in each case). The paired t-test comparisons of the means showed that these mean values were not significantly different from each other (Table 4). The three variables were asymmetrical in distribution and showed variation to be almost comparable. Moreover, they were similar in distribution. From the above results it followed that leaf size of *C. macrocarpa* may be estimated more or less equally well arithmetically by employing mean K value of 0.709808 or

through power model regression equation – LAR = 0.702. (LL x LB)$^{1.002}$. Since by Czekanowski (1913) index of similarity, LAM and LAK were 92% similar to each other and LAM and LAR were 98% similar to each other on the basis of leaf-size composition (Table 5), it appears that the regression method have relatively little edge over the arithmetic method which is although more practical, easier and convenient. These procedures may be useful in determining leaf size in eco-physiological short-term experiments. It may, however, be mentioned here that environmental interactions may influence any such relationships in plants (Robbins and Pharr, 1987).

Table 4. Paired t-test between the pairs LAM- LAK and LAM- LAR.

Pair	Paired difference		t	df	p
	Mean	SE			
LAM – LAK	-0.54395	0.102471	-0.5310	99	0.597
LAM – LAR	-0.000044	0.102165	0 .00001	99	0.999

Leaf dry mass (LDM)

LDM, in a sample of 100 leaves, averaged to 0.1119 ± 0.00717g, varying from 0.021 to 0.478g (CV: 64.04%) and distributed asymmetrically, positively skewed and leptokurtic (Fig. 14, Table 2 and 3). LDM concentrated between 0.051 and 0.25g in 98% of the leaves. In two largest leaves LDM was ≥ 0.35g.

LDM (g) related to Leaf area (LAM, cm^2) significantly positively ($p < 0.0001$) as given by the following equation:

$$LDM\ (g) = 0.014.\ LAM\ (cm^2)^{0.897} \pm 0.265$$

$t = 10.79 \qquad t = 21.40$

$p < 0.0001 \qquad p < 0.0001$

$R = 0.908,\ F = 458.08\ (p < 0.0001)$

Table 5. Percent Frequency distribution of Leaf area measured (LAM) and estimated (LAK & LAR) in various size classes.

Size classes of leaf area (cm^2)	LAM	LAK	LAR
< 2.5	05	04	04
2.6 – 5.0	15	16	16
5.1-7.5	16	18	18
7.6-10.0	16	15	15
10.1 – 12.5	26	23	24
12.6 – 15.0	07	9	08
15.1- 17.5	06	05	05
17.6 – 20.0	04	02	03
20.1 – 22.5	02	05	04
22.6– 25.0	01	01	01
> 25.0	02	02	02

Key to the acronyms:
LAM, Leaf area measured graphically expressed in cm^2;
LAK, leaf area estimated via mean K (K = 0.709808);
LAP, leaf area estimated with the help of power model regression equation (see Fig.13).

As per Czekanowski index of similarity, LAM and LAK were 92% similar and LAM and LAR were 98% similar in their composition based on the size classes of leaves.

Specific Leaf area (SLA)

As calculated following Westoby *et al.* (2000), SLA (based on double-sided LAM) averaged to 185.8595 ± 5.1531 $cm^2.g^{-1}$ and varied from 78.46 to 410.64 $cm^2.g^{-1}$ (CV: 27.3%). In 54% of the leaves, SLA varied between 140 and 200 ($cm^2.g^{-1}$). The leaves of *C. macrocarpa* are provided with palisade on the dorsal side only (Allam *et al.*, 2015), the SLA thus expressed on one-sided leaf area should be 92.923 ± 2.5765 $cm^2.g^{-1}$ which appears quite low as compared to the plants with thin leaves, probably owing to their tough texturedness. SLA distributed asymmetrically (Fig. 15, Table 2 and 3). Simple linear correlations among leaf traits are presented in Table 6. SLA was found to be non-significantly correlated linearly with L, B and LAM but significantly correlated with leaf dry matter content (LDM) and SLM, negatively. LDM related positively significantly with L, B, LAM and SLM. SLA, however, showed significant power model relationship with LAM (given below). Curvilinear or logarithmic relationship of SLA with leaf area has also been reported to be better than simple linear relationship between them in *Nicotiana plumbaginifolia* (Khan, 2008).

$$SLA\ (double\text{-}sided) = 89.627.\ LAM\ (cm^2)^{0.103} \pm 0.265$$

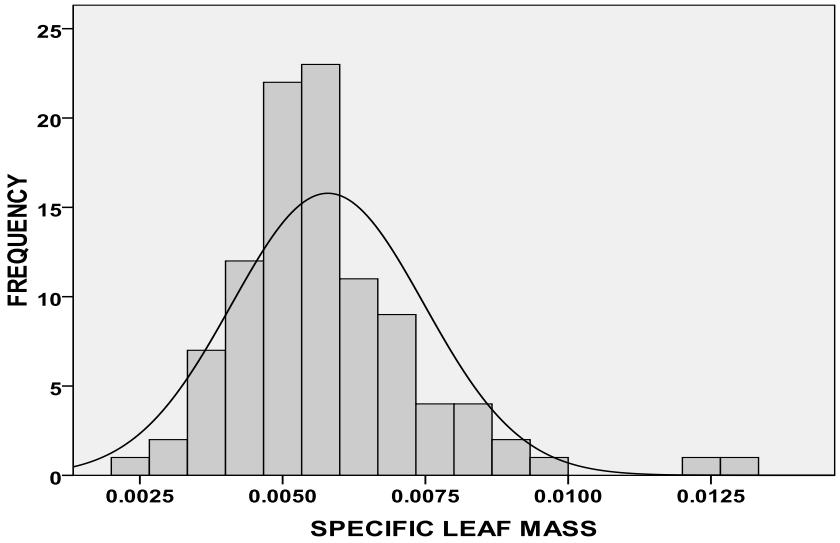
$t = 3.53 \qquad t = 2.46$

$p < 0.001 \qquad p < 0.016,\ R = 0.241,\ F = 6.034\ (p < 0.016)$

Table 6. Correlation (linear, r) matrix among the leaf traits.

L	L				
B	0.838	B			
LAM	0.909	0.946	LAM		
LDM	0.830	0.767	0.859	LDM	
SLA	*0.096*	*0.173*	*0.114*	-0.282	SLA
SLM	0.118	-0.225	*-0.143*	0.264	-0.891

All values of r were significant at least at $p < 0.05$ except those written in italics such as SLA v LAM, SLM v SLA, SLA v L and SLA v B. The relations viz. SLA v LAM and SLM v LAM were better given in power model of linear regression as



SLM
N = 100
Mean = 0.005792
SE = 0.0001684
Median = 0.005464
CV = 29.08%
G1 = 1.516
Sg1 = 0.241
G2 = 4.263
Sg2 = 0.478
Minimum = 0.00240
Maximum = 0.0127

Fig. 16. Frequency distribution of specific leaf mass (inverse of two-sided SLA).

Specific leaf mass (SLM)

Based on as inverse of the double-sided SLA, SLM averaged to 0.005792 ± 0.0001684g.cm^{-2} varying from 0.0024 to 0.0127 (CV: 29.08%) (Table 2, Fig. 16). It was asymmetrical in distribution (Table 3) – positively-skewed and leptokutic. SLM related to LAM by following equation:

$$SLM = 0.014 \,.\, LAM\ (cm^2)^{-0.103} \pm 0.265$$
$$t = 10.79 \quad t = 2.46$$
$$p < 0.001 \quad p < 0.016,\ R = 0.241,\ F = 6.039\ (p < 0.016)$$

Great deal of discussion has been made regarding SLA. It has been reported to vary with position of the leaf on the plants of *Nicotiana plumbaginifolia* (Khan, 2008). Among the four types of leaves recognizable in *Ficus religiosa* (red tender leaves, reddish green developing leaves, yellow green maturing leaves and dark green mature leaves) SLA, SLM, LDM, succulence and moisture content of leaf were found to be the plastic traits. Leaf area, leaf dry matter, LDM and SLM increased significantly with the growth and maturity of the leaves. Pink tender leaves had higher SLA and conversely low LDM and SLM. SLA was low in dark green mature leaves as compared to immature leaves. The tender leaves were more succulent than maturing and mature leaves (Khan, 2009). Liu *et al.* (2016) reported SLA to increase under shade. SLA is also reported to decrease with solar-irradiance in three sites both within and among species comparisons in tropical cloud forest of Bawangling Nature Reserve, South China (Long *et al*., 2011). Ackerly *et al.* (2002) reported that at community level in Chaparral woody plants, the leaf size and SLA both declined with increasing insolation. However, leaf size and SLA were not correlated significantly across species suggesting that these two traits are decoupled and associated with different aspect of performance along the environmental gradient. Li *et al.* (2005) have reported variations in specific leaf area (SLA) and leaf dry matter content (LDM) of 20 species (10 annuals and 10 perennials) that showed different distributional patterns in the Kerqin Sandy Land in Northern China.

In our studies, SLA was found to be non-significantly correlated linearly with L, B and LAM but significantly correlated with leaf dry matter content (LDM) and SLM, negatively. Our results appear to agree with Wilson *et al.* (1999). They reported that SLA is quite variable between replicates and is influenced with leaf thickness. Leaf dry matter, in comparison to SLA, they opined to be a better parameter being independent of leaf thickness.

Regular pruning is considered to be essential to enhance the decorative impact of the plant particulary hedges. The pruning obviously dwarfs plant and leaves of the plant with less food in consequence of tissue removal (Stiles, 1984). Plant growth is thus reduced following pruning. Reduction of leaves and other factors directly or indirectly limit potential growth. Removal of apical bud, however, stimulates buds just below the pruning cut (Reich, 2002). *C. macrocarpa* was pruned by cutting the upper as well as lateral surfaces of the hedge. The new growth was seen to be much vigorous than the lower canopy leaves (smaller generally) lying below the new growth. The new growth was generally vegetative and there was paucity of flowers and fruits in the *C. macrocarpa* hedges in hand. Pruning severity is reported to affect the fruitload and fruit and leaf traits in a variety 'Brigitta' of blueberry (Jorquera-Fontena *et al.,* 2014).

It may be mentioned that *C. macrocarpa* hedge-plants should represent disturbed and unnatural physiological state and this may be the reason that most of the morphometric leaf parameters were generally asymmetric in distribution. The sample leaves being collected from the overall canopy of the plant should obviously be a mixture of old and new growth leaves. One may investigate variation in leaf traits of this plant with respect to the shade and irradiance levels, canopy status i.e., old-growth-canopy (lower canopy leaves) and new-growth-canopy (upper canopy leaves produced after the recent pruning of the plant at known and specified interval) and the varying pruning-intervals. Biochemical status of the leaves should elucidate the eco-physiological responses of the plant under the given stressful conditions in comparison to unpruned individuals.

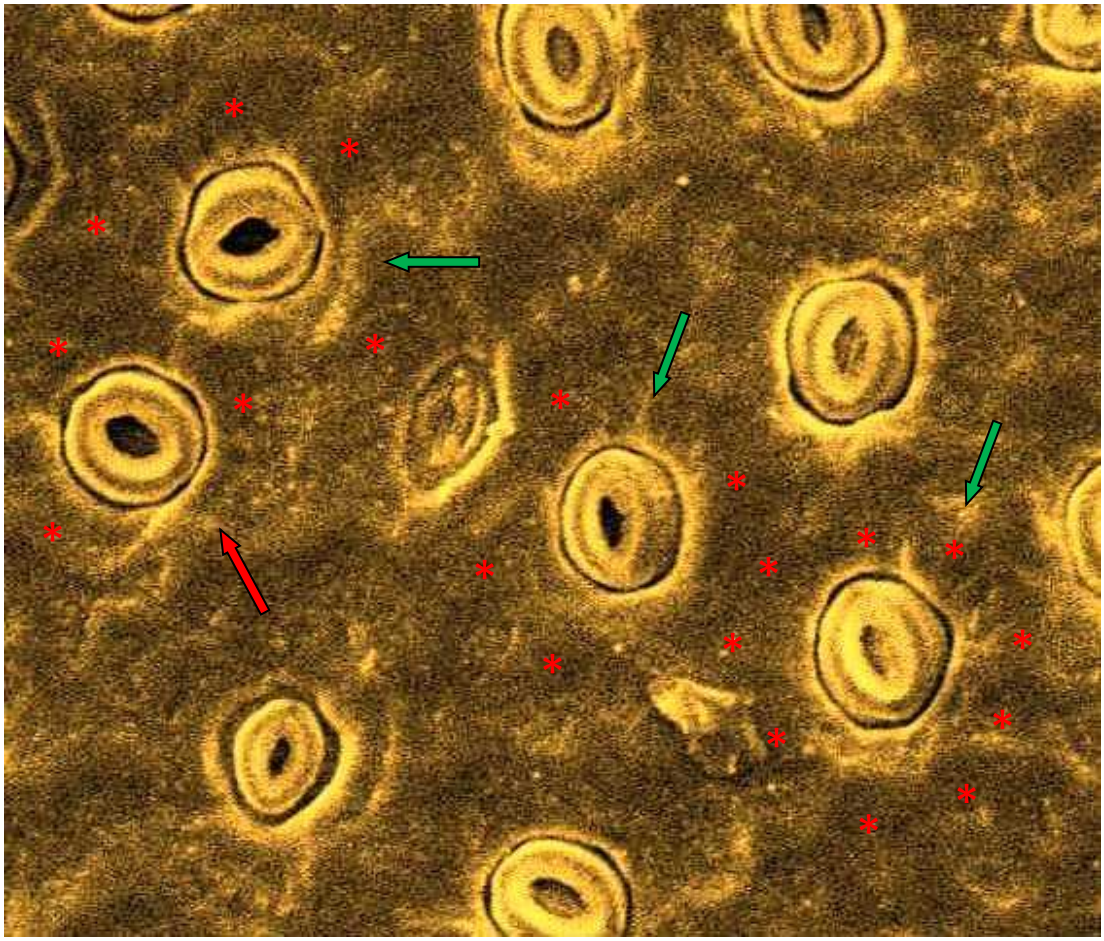


Fig. 17. Surface view of a mature leaf (OM) - In spite of the thick layer of cuticle, impression of the subsidiary arrangement in anomocytic (Green arrows) and anisocytic (Red arrow) forms is quite clear. Subsidiaries are shown by the asterisks. Often there were common subsidiaries between the stomata.

Trichomes

Trichomes are present on younger organs only such as petiole, younger stem, leaves calyx etc. They are non-glandular, conical, whitish, small, delicate structure which are short-lived and whither soon. The trichomes reported from *Mascarenhasia elastica* (Apocynaceae) are also non-glandular simple, long acute with apical cell pointed (Gabr *et al.,* 2015). No glandular trichomes were present in *C. macrocarpa* but they have been reported from other

species of Apocynaceae such as *Carissa spinarum* (trichome with single-celled stalk and bi-celled head) (Gabr *et al.*, 2015). In their same publication, they reported that in *Nerium oleander*, trichomes are non-glandular with short basal cell and long curved apical cell.

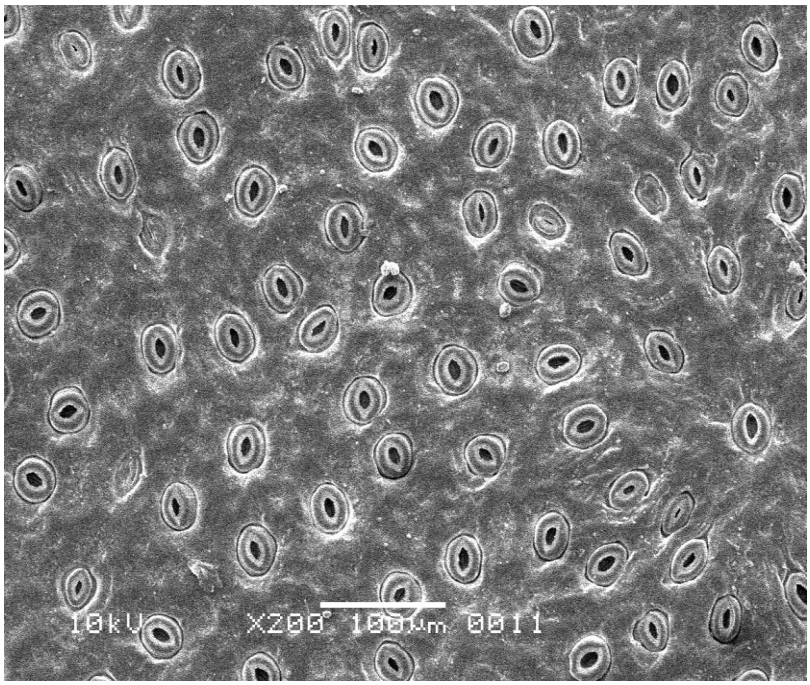


Fig. 18. SEM view of ventral surface of leaf showing densely distributed stomata - round to wide elliptical in shape.

Magnification: 200X.

Thick layer of cuticle has masked the structure of subsidiaries and the ground cells.

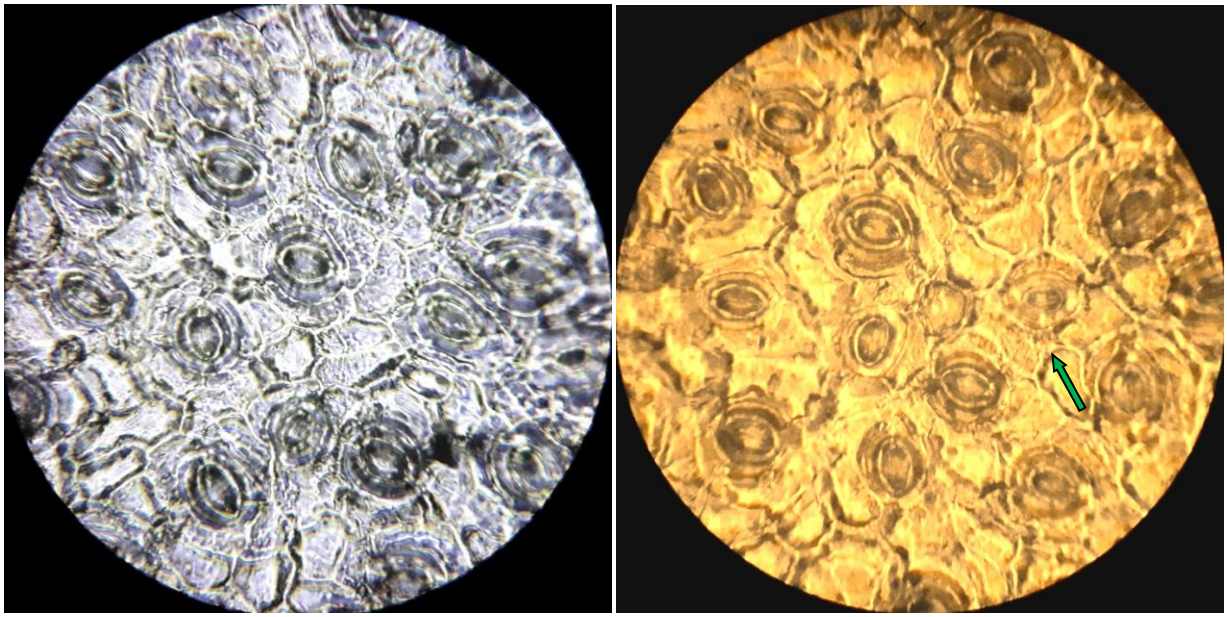


Fig. 19. Two optical microscopic views of the epidermal peel of *C. macrocarpa* showing anomocytic arrangement of subsidiaries for stomata on ventral surface of leaf (green arrow). Magnification: 45 x 15 X.

Stomata

Details of stomata types are presented in Fig. 17-19. The leaves of *C. macrocarpa* are hypostomatic i.e. The upper epidermis is devoid of stomata. In several species of Apocynaceae leaves are hypostomatic except *Catharanthus roseus* which is amphistomatic (Patel, 2003). In *C. macrocarpa*, lower epidermis had stomata generally of anomocytic type but rarely anisocytic type of arrangement of subsidiaries is also present. Across family Apocynaceae, Pant (2003) reported four types of stomata - anomocytic, haplocytic, paracytic and anisocytic. Kanabiran and Ramassamy (1988) also reported tetracytic stomata in 10 Apocynaceae species, beside the above

types but no haplocytic stomata in species studied. Allam *et al.* (2016) have also reported anomocytic stomata from planted Egyptian population of *C. macrocarpa*. Watson and Delwitz (1992) reported that in family Apocynaceae there is a mixture of anomocytic and paracytic and sometimes cyclocytic stomata. In *Mascarenhasia elastica*, an apocynaceous species, stomata are reported to be generally anomocytic with paracytic and actinocytic stomata also (Gabr *et al.*, 2015).

Stomata in *C. macrocarpa* were variable in size and their orientation. Stomata with common subsidiary were frequent (Fig. 19 and 20). *Carissa carandas* has contiguous stomata also (Patel, 2003). Anticlinal walls of epidermal cells were straight to curvy.

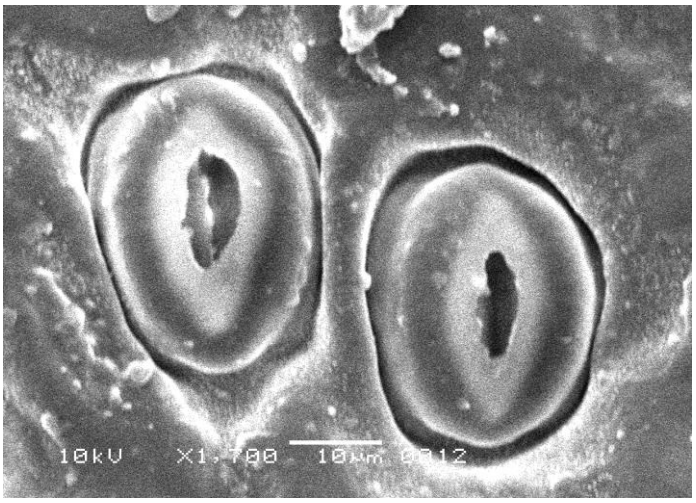


Fig. 20. SEM. Two adjacent stomata with common subsidiary. Epicuticular wax granules are clear. Stomata are round in shape and located in the pits.

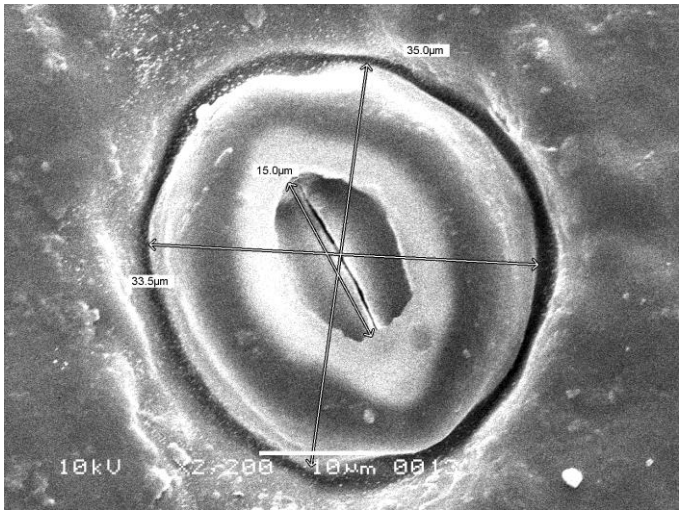


Fig. 21. SEM. A round in shape stoma sitruated in a pit and with well-developed outer ledges – stomatal diameters being 33.5 and 35.0 µm; outer stomatal ledge aperture c 15.0 µm in length

Stomata in *C. macrocarpa* were found situated in circular pits. Metcalfe and Chalk (1979) have reported stomata in pits in *Nerium* (Apocynaceae). Outer ledge of guard cells is well-developed to form incomplete circular dome. The diameters of stomata taken at right angles were more or less comparable, being 35.0 and 33.5 µm, respectively. The stomatal ledge aperture was 15.0 µm in length (Fig. 21).

Stomatal density

In a sample of 60 microscopic frames of vision, the stomatal density per mm^2 on the ventral surface of leaves of *C. macrocarpa* averaged to 283.8 ± 5.41 stomata, varying from 190.35 to 432.62, CV: 14.77% (Fig. 22). The distribution was asymmetrical being positively-skewed and leptokurtic. In around 91.6 % of the observations,

stomatal density was found to fall in the size class of 204-350 stomata per mm^2. Only at few occasions, the density was larger than 350 stomata per mm^2.

Leaf cuticle and epicuticular wax crytalloids

Leaf cuticle appears to be in form of sheet. Allam *et al.* (2016) have described cuticle to be slightly warty. There are, however, epicuticular crystalloids, probably waxy, in form of granules and platelets which appeared to fuse to form lumps of various shapes (Fig. 23).

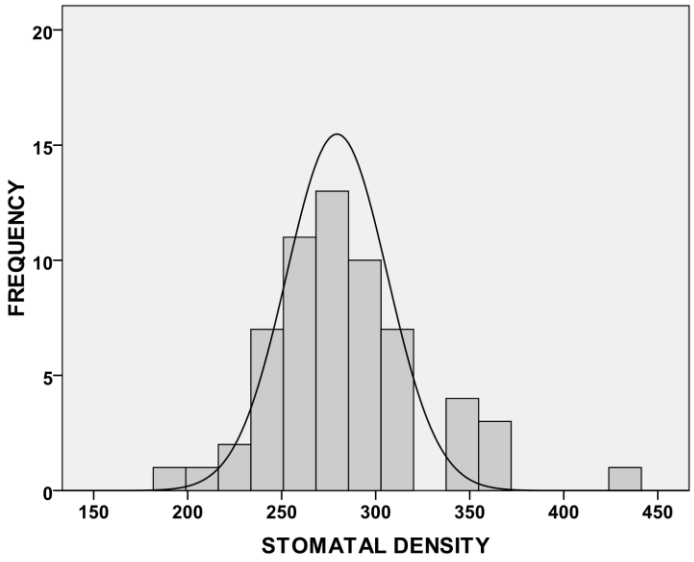


Fig. 22. Frequency distribution of stomatal density per mm^2 of ventral surface of leaf. Dorsal surface is devoid of stomata.

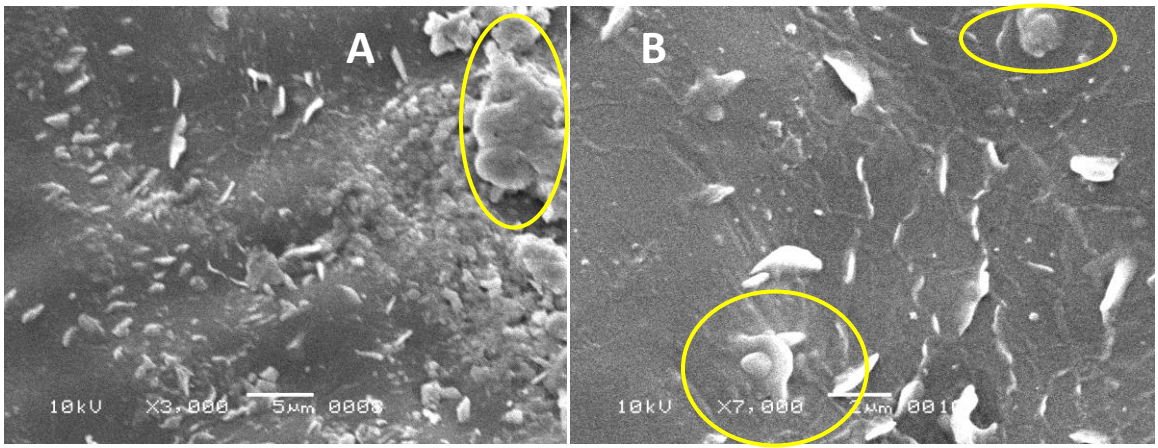


Fig. 23. SEMs of dorsal surface of leaf. Cuticle appears in form of sheet and epicuticular crystalloids in form of granules and platelets. They appear to coalesce to form small irregularly shaped lumps. A, 3000X and B, 7000X.

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