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SOME OBSERVATIONS ON MANGROVE SPECIES, *AEGICERAS CORNICULATUM* (L.) BLANCO OF PAKISTAN WITH REFERENCE TO PROPAGULE, SAPLING AND SAPLING LEAF

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ABSTRACT

Some observations on propagular-size, sapling growth in tidal marsh and macro- and micromorphology of sapling leaves of a true mangrove species, *Aegiceras corniculatum* (L.) Blanco grown in a coastal nursery by planting propagules in tidal marsh of Shah Bunder, Indus delta, are described. Growth included such parameters as net growth (between top of propagule to the end of shoot), number of leaves per sapling, number of internodes in new growth, and photosynthetic area of the sapling. There were crystals containing cells in cortical cells. Spherocrystals and cystoliths also occurred in the cortical cells. In addition to the internal structure of leaves, the morphometric and architectural parameters such as leaf length (LL) and breadth (LB), leaf area measured graphically (LAM), apex and basal angles and aspect ratio (LB / LL) were determined. Lamina area was determined arithmetically using multiplying coefficient K (determined for this species to be 0.6573) for equation, Leaf area = K (LL x LB) and also statistically by developing regression equations for simple (linear and power models) and multiple correlation and regression. Surface micromorphological studies were undertaken with respect to the stomatal types, their density and occurrence of cork warts. On the basis of Dilcher's (1974) stomatal classificatory scheme, paracytic, brachyparacytic, anisocytic and cyclocytic arrangement of subsidiaries was recorded. Subsidiaries distinct and raised in form of ring around the guard cells. Anticlinal walls of epidermal cells were straight to somewhat curvy. Stomatal density averaged to 96.37 ± 1.275 stomata per mm^2 and stomatal size averaged to 38.36 ± 0.873 X 11.94 ± 0.267 µm. The results are discussed with respect to the available literature.

Key Words: *Aegiceras corniculatum* (L.) Blanco. Propagular-size, Sapling growth, Leaf morphometry and architecture and Leaf surface micromorphology.

INTRODUCTION

Historically, one may find eight recorded species of true mangroves from Pakistan coast. Four of them have now disappeared and the existing four species include *Avicennia marina* (Forssk.) Vierh, *Rhizophora mucronata Lam., Aegiceras corniculatum (L.) Blanco and Ceriops tagal (*Pers.) C.B. Rob. This number is much smaller compared to the luxuriant Asian flora of 44 species (Chapman, 1977) which may be explained on the basis of aridity and salinity of Indus delta (Snedaker, 1984) but also due to grazing and cutting and the poor coastal management which caused disappearance of at least four mangrove species once thriving in Indus delta luxuriantly. A summary of knowledge related to mangroves of Pakistan has been published by Snedaker (1984). The management of this ecosystem is very difficult and problematic due to the inaccessibility of most of the areas and the fishing and grazing rights of the local population. From time immemorial the camels have grazed these forests and this have caused serious damages. By far the most important cause of their deterioration in the Indus Delta is the reduction in volume of fluvial discharge due to the diversion of the river Indus into an irrigation system (Qureshi, 2012). The loss of mangroves due to camel grazing is a grave loss of biodiversity in Indus delta.

Aegiceras corniculatum (L.) Blanco. (Syn. Rhizophora corniculata L., Aegiceras majus Gaertn. A. fragrans Koen.) is vernacularly known as black mangrove, River mangroves or Khalsi, Narikandam, Kachang Kachang, Kacang Kacang. It belongs to family Myrsinaceae. In Pakistan, it is distributed in Indus Delta, Karachi (a single tree was seen in Chashma Island in 1988) and Sonmiani (Fig. 1). *A. corniculatum* reaches 6 m or more in height, bears grayish bark. Plant shape is irregular. The plant is highly salt tolerant at germination stage. *A. corniculatum* is poorly known due to its poor occurrence and being not any conspicuous part of mangrove community (Tomlinson, 1986). Saifullah (1999) opined that it only occurs in Indus delta and not in Balochistan. It was collected from Hajamoro Creek (Indus delta) in 1999. It was reported from near Boat Club and Beach luxury hotel, Karachi adjacent to Chinna Creek (Jafri and Saeeda Qaiser, 1975). Saifullah (1999) opined that It is not found anywhere in Kalmat, Miani Hor and Gavater Bay. Tahir M. Qureshi (IUCN) in 2011, however, provided us a picture of an individual of *A. corniculatum* (the largest plant in the area) in Son Miani (Miani Hor) (Fig. 1). It is included in the Red list of IUCN. Salinity appears to be the reason of disappearance of this species besides its cutting for fuel wood and fodder (Saifullah, 1999). Low salinity significantly promotes sapling growth and the optimum growth takes place at 24 $dS.m^{-1}$. (Patel and Pandey, 2009). Higher salinity decreases plant growth. There appears more cold resistance in *A. corniculatum* as compared to *Avicennia marina* (Peng *et al.,* 2015). It attracts several types of moths. It shows analgesic and antidiabetic effects (Roome *et al.,* 2011; Gurudeeban *et al.,* 2012). Among Pakistan's mangroves, unlike *A. marina* and *C. tagal* which are sodiophillic plants, *Aegiceras corniculatum* appears to be relatively a potassiophillic plant (Das and Ghose, 2000) who have reported various elements in this species e.g., N: 1.085, P: 0.076, K: 1.503 and Na: 1.201% (g per100g. D. Wt.). Etymologically, *aik* (Gr.) = goat; keras (Gr.) = horn; *corniculatum* (Latin) = curved like a horn) i.e. referring to the fruits resemblance to the goat's horn. It is a cryptoviviparous species. It bears an endophytic fungus, *Fusarium incarnatum* which yields several cytotoxic alkaloids (Ding *et al.,* 2012) and shows anti-diabetic effects (Gurudeeban *et al.,* 2012). This paper presents brief observations on propagules, saplings and the sapling leaf regarding architecture and surface micromorphology.

Physico-chemical properties of Seawater

Seawater is basic in reaction. pH up to 8.5 but much variable (6.4 to 9.6) in Bakran area probably due to industrial discharge. Mildly basic in Karachi Harbour estuarine ecosystem (7.3-8.1). Temperature is low in winter and high in summer (24.8 -30.0 ^{o}C). Salinity is high. It varies around 40 dS.m^{-1} but has also been recorded as high as 51.4-55.3 dS.m^{-1} in Korangi creek. TDS is low in winter months and high in June. In Indus delta salinity varies from 46.6 to 56.6 dS.m^{-1}. Brief description of physico-chemical properties of Seawater may be seen in Khan *et al.* (2020).

MATERIALS AND METHODS

Propagules of *R. mucronata (*mainly),but also of *A. corniculatum* and *C. tagal* were time to time collected from Sonmiani, Balochistan by Sindh Forest Department (SFD) to establish nurseries for plantation at various places of coastal tidal marsh. One hundred and five propagules from the lot of *A. corniculatum* were studied for their size and were weighed fresh and after drying at 70 ^{o}C for 48h.

Fig. 1. A) An individual of *Aegiceras corniculatum in* Sonmiani Bay, Balochistan, Pakistan (Photo by M.T. Qureshi, then Conservator Coastal Forest). This individual is said to be the largest *A. corniculatum* plant in the area (Pers. Comm. M. T. Qureshi, 2011). B) Sapling of *A. corniculatum* raised in Shah Bunder nursery and later transplanted in drum pot filled with coastal sand and irrigated with Seawater. C) Leaf form of *A. corniculatum*. Ci, dorsal; Cii, Ventral surface. Note that dorsal surface is shining with faintly visible venation. The ventral surface is light green.

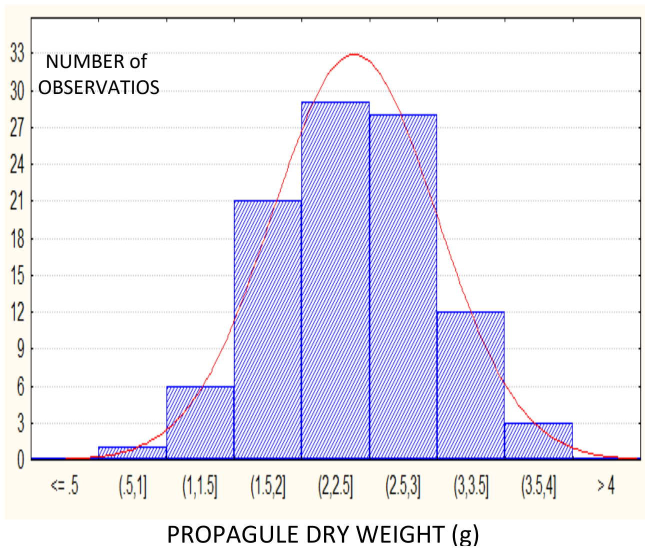
The saplings of this species were collected in 2012 from *A. corniculatum* nursery established in Shah Bunder by SFD. Two-year old Saplings were studied for their growth. Hickey (1979) and LWG (1999) were followed for description of saplings' leaves. To study stomatal types, leaves epidermal impressions were made with clear nail polish (Wang *et al.,* 2006) and the imprints were studied under compound optical microscope for surface micromorphology. 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 Electron (SE) micrographs were made at 5kV with JEOL JSM-6380A electron microscope at various magnifications. The images were saved digitally on computer. Stomatal nomenclature suggested by Dilcher (1974) was adopted to ascertain stomatal types. The diameter of the secretory glands of *A. corniculatum* was measured microscopically. In case of non-circular opening of the gland, diameters on two radii at right angle were measured for an average diameter value. Measurement of stomatal size was made through calibrated ocular scale with slides of the nail polish imprints of the leaf surfaces. The data was analyzed statistically (Zar, 2010).

To determine leaf area, the leaf outline was carefully drawn on graph paper and area (LAM) determined with all possible precision and accuracy. Aspect ratio was determined as leaf breadth (LB) / leaf length (LL). The multiplication factor (K) was calculated by employing the formula, K = leaf area measured / (LL x LB). Employing average value of the multiplication factor K, leaf areas were also calculated as Leaf Area computed (LAK) = K (length x breadth) for comparison with the observed areas (LAM) of the leaves. Bivariate power relationship of leaf area with measured linear dimensions of the leaf were computed and expressed as LAPOW. In addition to it, leaf area (LMULTI) was computed with the help of the regression coefficients determined by employing multiple regression method fitting in the allometric model, $Y = a + b_1LL + b_2 LB \pm SE$.

RESULTS AND DISCUSSION

Propagules:

There is hardly any seed dormancy period after fertilization in *A. corniculatum* (Das and Ghose, 2003). The embryo germinate within fruit (vivipary). Vogel (1980), on the basis of germination, included *A. corniculatum* in *Rhizophora* type. The hypocotyl (propagule) produced is more or less spindle-shaped, slightly curved with narrow tip. Propagule have up to 12 longitudinal ridges on the surface and several warts. *A. corniculatum* propagules collected from Sonmiani Bay, in pot culture under Seawater irrigation at DFO (Karachi) nursery in 1987, showed sprouting in c. 95% propagules within two months of incubation. After c 9 months, the mortality was recorded around 31.3% (Qureshi and Khan, 1988).



N =105
Mean = 2.378g
SE = 0.05841
CV = 24.16%
G1 = - 0.006
Sg1 = 0.236
G2 = - 0.237
Sg2 = 0.467
Min. = 0.6571
Max. = 3.7822
KS-z = 0.586
$p < 0.882$

Fig. 2 A. Frequency distribution of dry weight (g) of *A. corniculatum* propagules. The variable distributed normally. Some 69.5 % of the total propagules fell within a class of 2.0 to 3.5g.

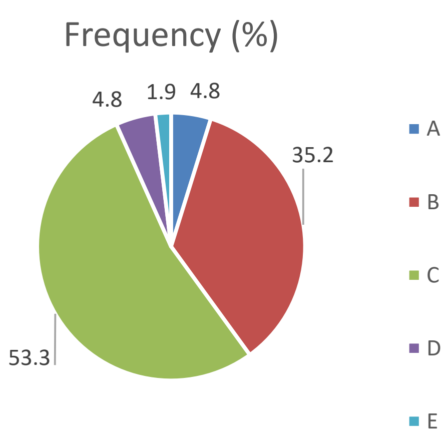


Fig. 2 B. Frequency distribution of moisture contents in propagules.

Class A, ≤ 30.0%;
B, 31-40,
C, 41-50;
D, 51-60 and
E, ≥ 60%
(61-78.8%).

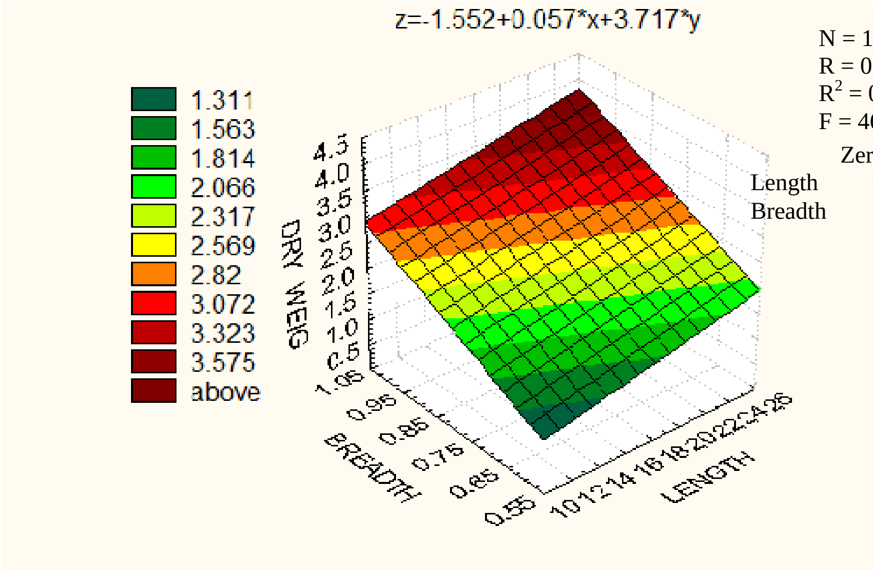


Fig. 3. Relationship of Propagule dry weight (g) with length (cm) and breadth (cm) of the Propagule of *A. corniculatum*.

A sample of randomly selected 105 propagules, out of a lot collected from Balochistan by Sindh Forest department, averaged to 17.16 ± 0.2619 cm (11.2 to 23.3 cm) in length and 0.790 ± 0.0108 cm (0.60 to 1.0 cm) in breadth. The moisture content of the propagules averaged to 41.23 ± 0.6996% varying from 22.60 to 78.80% (Coefficient of variability, CV: 18.65%). Propagular length distributed asymmetrically – showing significantly positive skewness and leptokurtosis. The parameter of moisture content obviously being non-exact as the propagules took time to reach laboratory. The dry weight of propagules after two-day drying at 70 ^{o}C, averaged to 2.378 ± 0.0584g and distributed symmetrically (Fig. 2). The dry weight of propagule appeared to be a function largely of breadth of the propagule (Fig. 3) and related with their lengths and breadths as:

Propagular dry weight (g) = 1.552 + 0.057(Length, cm) + 3.3717 (Breadth, cm).
R = 0.689, R^2 =0.467, F = 46.0 ($p < 0.001$)

Table 1. Morphometric data of two-year old saplings of *A. corniculatum* grown in tidal area of Shah Bunder, Sindh. (N = 4 randomly selected).

Root (cm)	Number of roots	Propagule Length (cm)	Stem height (cm)	Number of internodes	Number of leaves present	Foliar Photosynthetic area (cm^2) per seedling
23.5 ± 2.40	7.5 ± 0.87	19.88 ± 1.05	8.63 ± 0.38	6.00 ± 0.0	6.50 ± 0.86	61.84 ± 6.88
Internodes (Base to apex) length (cm) and Range						
Internode # 1	0.85 ± 0.23 (0.5 -1.5)					
Internode # 2	1.29 ± 0.12 (1.0-1.5)					
Internode # 3	1.40 ± 0.25 (0.70-1.8)					
Internode # 4	1.78 ± 0.11 (0.7 -2.0)					
Internode # 5	1.70 ± 0.08 (1.5-1.9)					
Internode # 6	1.60					

Sapling growth: Morphometric data of 24-months old saplings collected from the tidal marsh nursery of Shah Bunder is presented in Table 1. The sapling growth appeared to be inherently quite slow as sapling in two years could produce at the most six internodes (none of the internode of more than 2 cm in length) above the propagule. The internodal length increased from base to apex significantly. Total net growth was 8.63 ± 0.38 cm. Leaves at lower three nodes have abscised and there were 6-9 leaves associated with the saplings in upper 3-4 nodes (Mean leaf number = 6.50 ± 0.86 per sapling produced from a propagule of 19.88 ± 1.05 cm. The foliar photosynthetic area (FPA) associated with the saplings was 61.84 ± 6.88 cm^2. This quantum of FPA was quite low if compared with equally aged *R. mucronata* sapling at Gwadar tidal nursery which had eight internodes and FPA 144.17 ± 13.96 cm^2

and growth in height 21.30 ± 2.54cm (Khan *et al.*, 2021). This may be attributed to higher growth rate and larger propagular size of *R. mucronata.*

Leaf morphometry and architectural characteristics

Leaves of *A. corniculatum* are ovate-lanceolate, obovate to obovate-oblong, shortly-petiolate, exstipulate, symmetrical, leathery, oval, smooth margined, green above but brownish green below. Leaves are simple, entire-margined, with single prominent midrib ventrally, less apparent pinnate venation and thick cuticle on both surfaces. It is coriaceous and brittle. Leaves are thick, succulent, dorsiventral, and opposite-decussate. They are flat green and thick and leathery. Leaf base is acute and apex generally obtuse but at times acute. The morphometric and architectural parameters of leaves of 24 - month old saplings from Shah Bunder are presented in Table 2.

Petiole: Petiole in *A. corniculatum* saplings was small, cylindrical, and green.

Leaf Length (LL) and breadth (LB): Leaf length *of A. corniculatum* saplings averaged to 5.45 ± 0.14cm (4.2 to 6.7 cm, CV: 13.14%) and breadth to 2.64 ± 0.059 cm (2.10 - 3.2 cm, CV: 11.34%). Around 73.1% of the leaves had length between 4.0 and 6.0cm. Most of the leaves (92.3%) were 2.0 to 3.0cm in breadth and only 7.7% of leaves were larger than 3.0 cm in breadth. The leaves of *A. corniculatum* are fairly consistent in shape as the LL / LB ratio in this species varied only 10.25%. This parameter averaged to 2.10 ± 0.0411 varying from 1.6562 to 2.5417 i.e. lamina was generally twice in length than its width (Table 2). However, Leaf size was comparatively smaller than that in naturally growing trees in Indus delta. Jafri and Saeeda Qaiser (1975) reported its leaf to be 4-8 cm in length and 2-5- 4.0 cm broad.

Leaf shape: In present investigation, the leaf shape consistency was evaluated as Aspect ratio = Breadth / Length of leaves (Table 2). This parameter may give some indication about consistency of leaf shape with size (Verwijst and Wen, 1996). Aspect ratio (LB / LL) averaged to 0.4875 ± 0.0098 (CV = 10.25%) (Table 2; Fig.4). Aspect ratio ranged between 0.41 and 0.50 in 63.3% leaves and between 0.51 and 0.60 in 23.1% leaves. As given below, the multivariate correlation of aspect with LL and LB gave highly significant relationship ($R = 0.992$, $R^2 = 0.985$, $F = 745.7$ ($p < 0.0001$). LL behaved negatively compared to LB in multivariate analysis. On the basis of regression coefficient (b), the aspect ratio was, however, relatively more controlled by leaf breadth than leaf length. The leaves appeared to exhibit considerable consistency of shape.

Aspect = 0.483 - 0.089 LL + 0.185 LB ± 0.00642

t = 40.95, t = -37.2, t = 32.41

p < 0.001, p < 0.0001, p < 0.0001; F = 745.67 (p < 0.0001)

	Zero order r	Partial r
LL	- 0.540	- 0.992
LB	0.269	0.989

The multiplicative factor K: The values of multiplicative factor (K) of the equation K = Area / (LL x LB), tended to be highly leptokurtic in distribution (Table 2) and somewhat positively skewed in *A. corniculatum* leaf. It averaged to 0.6573 ± 0.0182 varying 14.25% only. Around 76.9% of the K values were found to be between 0.6 and 0.7. Being largely concentrated around the mean value it indicated its practical suitability in leaf area estimation.

The area of the leaf blade was found to be 2/3 = 0.6666 of the recorded rectangular area of leaf length x breadth by Cain and Castro (1959) for carefully drawn tracings of leaves of different sizes and shape of eleven rain-forest trees with lanceolate, wide elliptical to somewhat round (obovate) leaf forms with obtuse apex. Carefully analyzed leaf blade area of these leaves averaged to 67.4 % of the length x breadth rectangle varying from 61.9 to 77.7%. It led them to coin a "rule of thumb" that the blade area approximated 2/3 of the length-breadth rectangle in the leaves studied. For ovate-lanceolate, obovate to obovate-oblong leaves of *A. corniculatum,* our results are in agreement with Cain and Castro (1959). K for *Rhizophora mucronata* leaves was found to be 0.6998 ± 0.0062 (Khan *et al.,* 2021). It may, however vary with leaf shape.

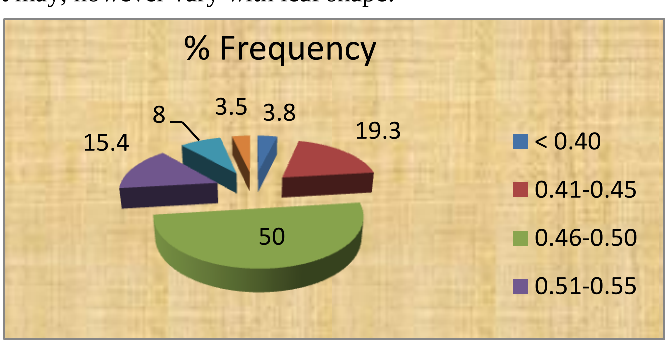


Fig. 4. Aspect (LB / LL ratio) of leaves of two-year old *A. corniculatum* saplings. Some 84.7 % of the aspect values fell between 0.45 and 0.60 indicating fairly high degree of leaf shape consistency.

Apex (AA^o) and Base angles (BA^o): Apex angle averaged to 90.31 ± 1.564^o (generally obtuse) varying between 72^o and 112^o (CV: 8.83%). Apex angle was, however, smaller than 80^o in 3.8% of the leaves. Some 50% of the leaf fell in AA ranging between 81 and 89^o. AA was equal to 90^o in 11.6 % of the leaves and was larger than 90^o in 34.6% of the leaves. Thus besides obtuse-apex leaves, there were apex-acute leaves also in substantial number. The base angle averaged to 60.58 ± 1.542, varying between 39^o and 71^o that is CV: 12.90%. The base angle was lesser than 50^o in 11.5% of leaves, 51-70^o in 88.8% leaves. The base angle larger than or equal to the right angle was observed in none of the leaves. That is all leaves had acute base (Cuneate).

Lamina area: Lamina area was determined graphically and referred to as LAM. The arithmetically determined leaf area via K as multiplication factor (0.6573) using LL x LB of leaves was referred to as LAK. Power model equation (equation 3, Table 3) was used to arrive at Leaf area referred to as LAPOW and Multiple correlation and regression equation (equation 2, Table 3) was employed to estimate leaf area referred to as LMULTI. The location and dispersion parameters of lamina areas determined through various methods are presented in Table 2. LAM, LAK, LAPOW and LMULTI averaged to 9.150 ± 0.460 (CV: 25.63%), 9.437 ± 0.3935 (CV: 21.26%), 9.513 ± 0.4053 (CV: 21.72%) and 9.541 ± 0.4169 (CV: 22.28) cm^2, respectively (Table 2).

Table 2. Morphometric and architectural parameters of leaves (N = 26) of a two-year old sapling of *A. corniculatum.*

Parameters	Mean	SE	CV%	Min	Max
LL (cm)	5.45	0.140	13.14	4.20	6.70
LB (cm)	2.64	0.059	11.34	2.10	3.20
LL / LB ratio	2.0716	0.0411	10.115	1.6562	2.5417
LAM (cm^2)	9.150	0.460	25.63	6.01	13.99
AA (^{o})	90.31	1.564	8.83	72.0	112
BA (^{o})	60.58	1.542	12.90	39.0	71.0
Aspect (LB / LL)	0.4875	0.0098	10.25	0.3934	0.6038
K	0.6573	0.0187	14.54	0.4808	1.0377
LAK (cm^2)	9.437	0.3935	21.26	6.14	13.31
LAPOW (cm^2)	9.513	0.4053	21.72	5.81	13.03
Lmulti (cm^2)	9.541	0.4169	22.28	6.07	13.67

LL, Lamina length (cm); LB, Lamina breadth (cm); LAM, Lamina area measured (cm^2); AA, Apex angle; BA, Base; angle;; Aspect ratio, LB / LL; LAK, Lamina area measured through K; LAPOW, Lamina area estimated through power model equation; Lmulti, Lamina area estimated through multiple linear regression equation. (See Table 3).

Table 3. Correlation and regression analyses for lamina area estimation in *A. corniculatum*.

Simple Linear Correlation and Regression			
Lamina area (cm^2) = 0.277 + 0.636 (LL x LB, cm) ± 1.150, R = 0.877; R^2 = 0.769; Adj. R^2 = 0.769; F = 80.09			
t = 0.26	t = 8.95		
p < 0.795	p < 0.0001 --Eq. # 1.		
Multiple Correlation & Regression			
Lamina area (cm^2) = - 8.161 + 2.237 LL + 2.078 LB ± 0.1.157, R = 0.881; R^2 = 0.776; Adj.R^2 = 0.757, F = 39.84			
t = -3.84	t = 5.20	t = 2.0198	
p < 0.0001	p < 0.0001	p < 0.0001 ----------------------------------Eq. # 2.	
		Zero Order r	Partial r
	LL	0.858	0.735
	LB	0.716	0.388
Power model			
Lamina area (cm2) = 0.736. (LL x LB) $^{0.954}$ ± 0.133, R = 0.853; R^2 = 0.728; Adj.R^2 = 0.717; F = 64.221			
t = 3.158	t = 8.014		
p < 0.004	p 0.0001 --Eq. # 3 (Best fit)		

LL, Lamina length (cm); LB, Lamina breadth (cm); LL x LB, Multiplicative parameter of length and breadth.

Relationships amongst measured and estimated lamina areas: The average values of various leaf area parameters although appeared to be quite comparable to each other, the superiority of estimation methods needed to be tested. When tested through correlation analysis, the estimated leaf areas (LAK, LAPOW and LMULTI) were correlated with LAM highly significantly. LAM related with LAK and LAPOW closely (r = 0.877 and F = 80.09, p < 0.001 and r = 0.877, F= 79.60, p < 0.001, respectively). LAK related with Lmulti highly closely (r = 0.986, F =

851.93, $p < 0.001$). None of the estimated leaf areas differed significantly from measured leaf area ($t_{LAM\text{-}LAK}$, 0.477 (NS); $t_{LAM\text{-}LAPOW}$, 0.6131, NS and $t_{LAM\text{-}LMULTI}$, 0.6262 (NS).

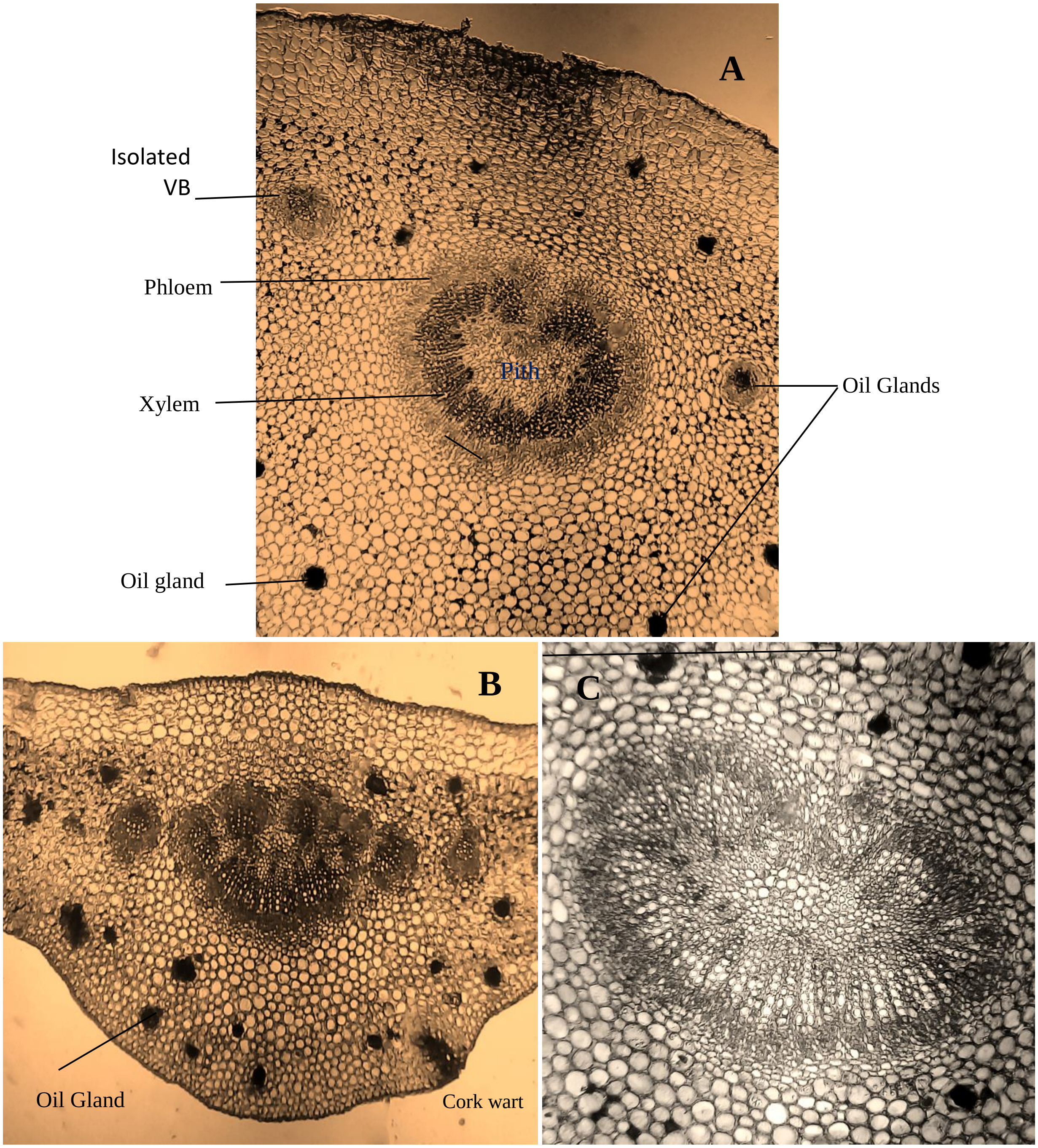
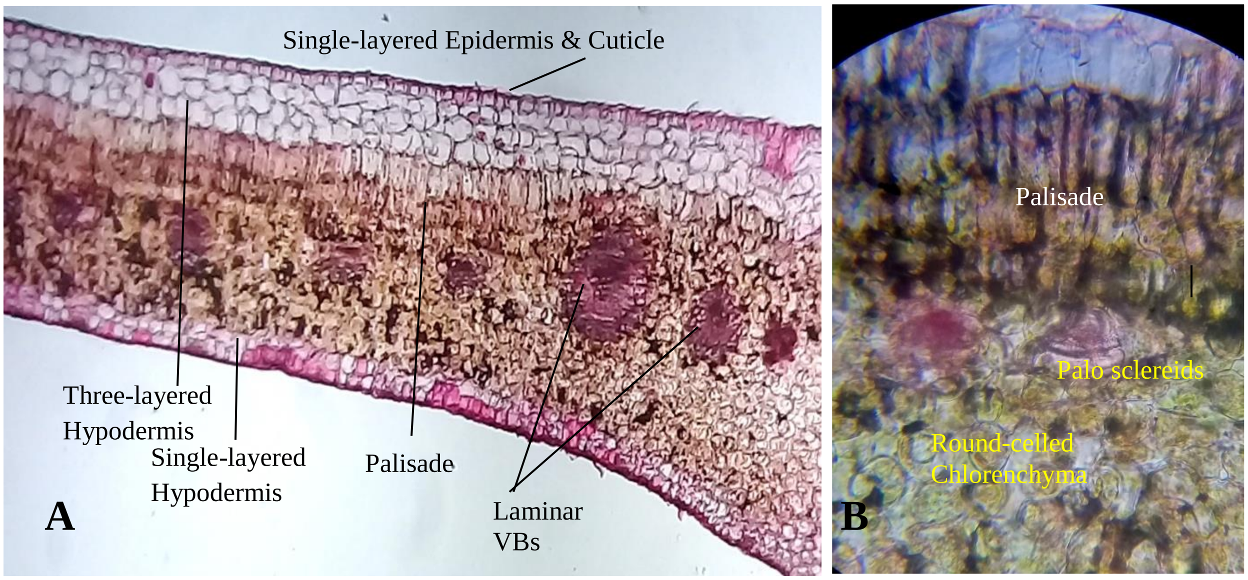


Fig. 5. TS of petiole (A) and TS of leaf through midrib (B & C) of *A. corniculatum*. Cork warts may be seen in midrib region. Endodermis and pericycle are visible in C with little amount of Parenchymatous pith.

It implied that estimation of leaf area on the basis of LL and LB using K as multiplicative factor (0.6573) was as suitable in *A. corniculatum* as through other methods (LMULTI or LAPOW). This contention was further substantiated on the basis of compositional similarity determined via Czekanowski's (1913) index of similarity on the basis of % frequency of occurrence of leaf area values in 20 classes of equal class size interlude. Composition similarity was 76.3% between LAM and LAK, 76.3% between LAM and LAPOW and 84.0% between LAM and LMULTI i.e. maximum composition similarity existed between LAM and LMULTI. The estimation in leaf area on the basis of multiple correlation and regression may, therefore, be recommended for leaf area estimation in *A. corniculatum*. However, owing to its precision, simplicity and convenience using multiplicative coefficient K (= 0.6573) may be more practical way to estimate leaf area in this species. Ahmed and Khan (2011) have also recommended the arithmetic method of using K as multiplication factor to be most accurate in *Jatropha curcas* (with K = 0.858758).



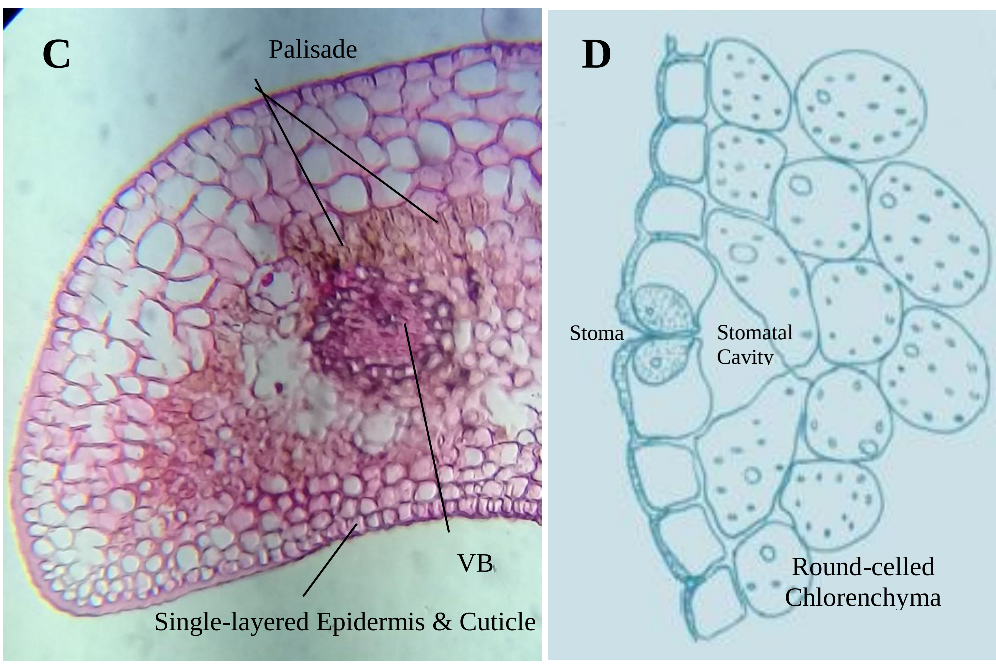


Fig. 6. TS of leaf of *A. corniculatum* near the lateral wing of lamina showing palisade parenchyma near extreme laminar VB. Palisade is composed of elongated cells (B) and round-celled cholorophyllous tissue below palisade. Note cystoliths in water tissue near VB (C), stoma with stomatal cavity in line drawing (D).

Leaf orientation

Leaves in *A. corniculatum* are opposite. A leaf is borne more or less with its flat surface making an angle (c 45^{o}) with horizontal (Fig. 1B). In mangroves (Rhizophoraceae), the optimal leaf temperatures for photosynthesis are very close to the average air temperature of the tropical area (Andrews *et al.*, 1984; Ball *et al.*, 1988). However, the rates of transpiration in all species of mangroves examined are not sufficient to prevent heating of the leaves above ambient air temperature (Ball, 1988). The orientation of leaves on stem in this respect is very important. Ball (1988) has presented an example of *Rhizophora apiculata* when exposed canopy leaves are constrained a horizontal position, the temperature of leaves increased substantially (c. 11 ^{o}C maximally) above ambient air temperature with increased incident radiation. In contrast when leaves were in vertical orientation, they avoided heat load during mid-day when irradiance and air temperatures are greatest. During mid-day, the leaves oriented with 45^o to upright axis received only 20% of the available sun and were approx. 10 ^oC cooler. The leaf angle is, therefore, a compromise between the requirements for illumination and the maintenance of favourable leaf temperature with minimal evaporative cooling effect (Ball *et al*., 1988). Similar observation was made by Andrews and Muller (1985) in *Rhizophora stylosa* stand. Ball (1988) concluded that leaf angle (i.e. inclination to the horizontal) affects the radiant heat loading on the leaf. The role of leaf orientation in *A. corniculatum* needs to be evaluated in detail.

Internal structure of leaf

The anatomical details of leaf are presented in Fig. 5 - 8 and may briefly be described as below.

i) Petiole: Epidermis is composed of smaller squarish or rectangular cells covered with thick **c**uticle (Fig. 5A). Epidermis is one-layered. Hypodermis multi-layered composed of irregular cells. Cortical cells are circular in shape with intercellular spaces amongst them. A number of sclereids are distributed in cortical region – brachyslereids also often present. The major central vascular bundle (VB) is horse-shoe shaped – xylem on the inner side and phloem on outer side. Some isolated small VBs are also present. Xylem vessels are circular. Pith is parenchymatous. Some oil glands may be seen distributed in the cortex.

ii) Lamina: Epidermis is composed of single-layer of small cells covered with cuticle. The number of epidermal cells on adaxial surface has been reported to be 1966.0 ± 55 cells per mm^2 and abaxially 2873 ± 108 cells per mm^2 in this species from India (Seshavatharam and Srivalli, 1989) (Fig. 5 *B and C*). Hypodermis in the midrib region is multi-layered but in laminar region two to three layered dorsally and single layered ventrally (Fig. 6A). Multilayered Hypodermis appears to be characteristic of *A. corniculatum.* Palosclereids were present in round-celled parenchymatous cortex (Fig 6 B). Palosclereids from palisade parenchyma have been reported in *A. corniculatum* quite earlier by Rao (1971). Oil glands were present in petiole and leaf lamina. Palo-sclereids are reported from *Ceriops decandra* and *Bruguiera gymnorrhiza* (Das and Ghose, 1996). They have also reported enlarged terminal tracheids in *A. corniculatum and* also from *C. decandra, Phoenix peludosa,* and *Excoecaria agallocha.* Tannin cells were not found in *A. corniculatum*. The coriaceous nature of leaves may probably be due to sclereids in the mesophyll. Stomata on lower surface only. There appears several oil glands in the mesophyll. Palisade tissue is present below hypodermis. There is single but large horse-shoe shaped VB in the midrib region but there may be several smaller isolated VBs (Fig. 6A) in the lamina presumably owing to reticulate venation. Large intercellular spaces are present in cortical parenchyma near midrib VB (Fig. 6B). There is well developed round-celled cholorophyllous tissue on lower surface near VB and lower side of lamina. There is well-developed aerenchyma which is considered important in mangroves from viewpoint of air flow dynamics. Several crystalliferous cells (spherocrystals) and cystoliths containing cells may be observed in cortex besides water tissue near the central VB (Fig. 7). Cork warts are present on leaf surface. The internal structure of *A. corniculatum* leaf resembles to the anatomical account given by Surya and Hari (2017 a, b) for this species. Hypodermal water tissue on both sides of leaf. Chapman (1975) considered water storage tissue as a characteristic of nearly all mangrove leaves and recognized 4 different categories - (i) Water storage tissue exclusively hypodermal, (ii) Water storage tissue derived from hypodermis plus subsequently modified assimilatory tissue, (iii) Water storage tissue exclusively deep seated and (iv) Leaves without specific water storage tissue. In *A. corniculatum*, water storage tissue appears to be exclusively hypodermal. One may find crystals containing cells in cortical cells. Spherocrystals and cystoliths do occur in the cortical cells (Fig. 7 and 8).

Salt glands

Salt glands appear to be present densely on adaxial epidermis but very rarely on abaxial epidermis of leaves (Fig. 9, 10 and 11).

Salt glands are seen to be the collections of multiple number of cells in *A. corniculatum* (Fig. 11). Das and Ghose (1993) reported 8 radiation cells in a gland of *A. corniculatum* from India. But according to Cardale *et al.* (1971) and Hachings and Saenger (1987) the salt gland of *A. corniculatum consists* of large number of abutting secretory cells (Fig. 9B) and a single large basal cell. The secretory cells and single basal cells are joined with large number of palsmodesmata and the glandular cuticle shows differences between the top and sides of the gland that is said to be due to variation in nature or quality of wax deposition which may be important in secretion process since there is no evidence of pores (Cardale *et al.*, 1971).

Salt is secreted by the cytoplasm of the secretory cells into large vacuole and that secretory cells dry out with the ageing of leaf and salt remains on the surface of leaf as a white powdery layer (Fig. 10) as also reported by Surya and Hari (2017). As measured on the dorsal surface, salt glands density averaged to 903.04 ± 14.5828 glands per cm^2 i.e. 9.03 glands per mm^2. The density, however, was quite variable from 566.17 to 1245.6 per cm^2 (Fig. 12A) with variation: 12.5%. Salt glands distributed asymmetrically with significant leptokurtosis. Diameter of these glands averaged to 48.06 ±1.9144 µm (varying 30.86% i.e. from 19.2 to 80.0 µm). In 65% of the observations the diameter of salt glands fell between 31 – 60 µm (Fig. 12B).

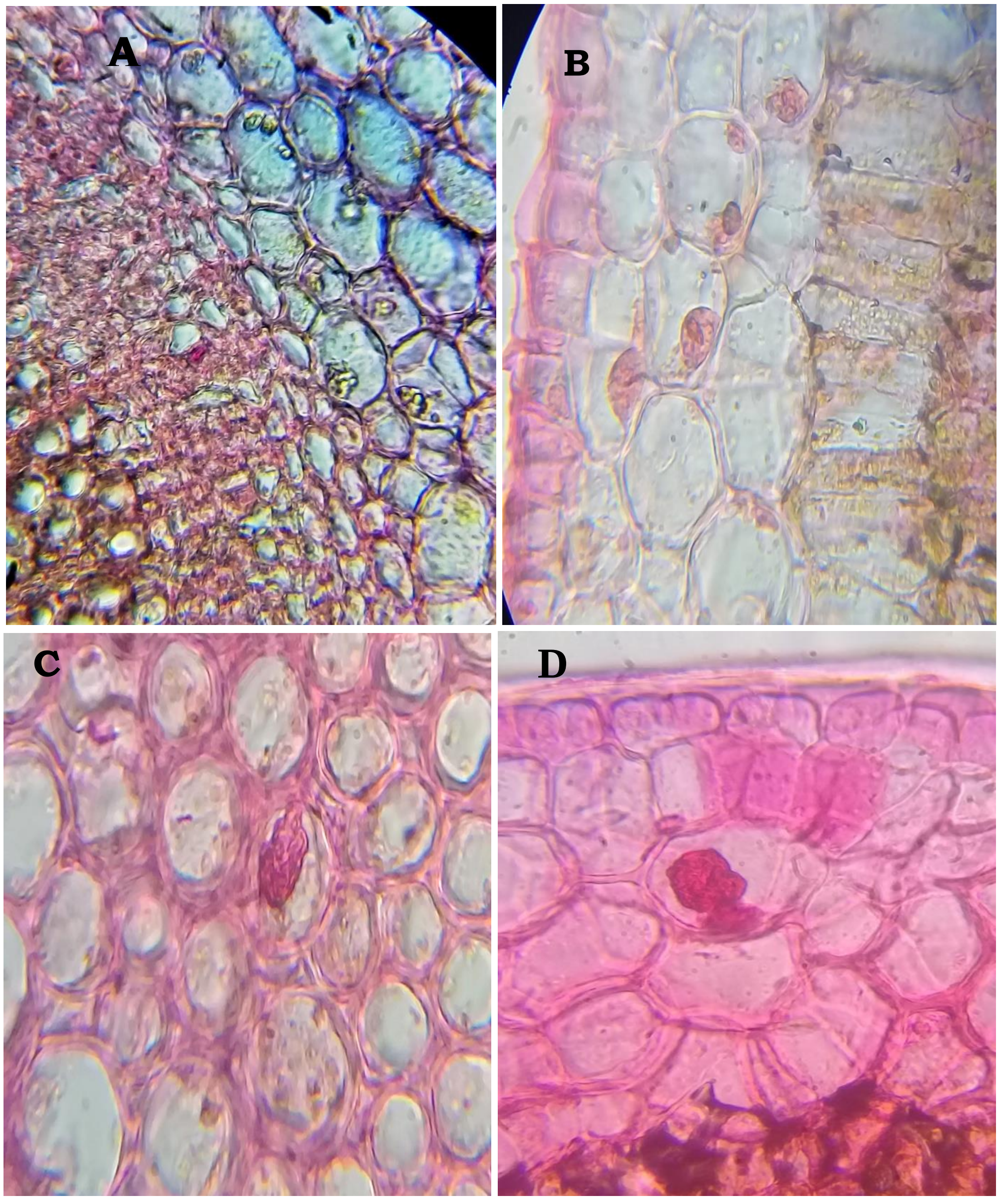


Fig. 7. *A. corniculatum*. Crystals (A), spherocrystals (B) and cystoliths (C and D) present in cortical cells of leaf.

It may be mentioned that xeromorphic characters like thick cuticle, small epidermal cells, water storage tissue, leaf succulence, extensive palisade, presence of the salt glands, tracheids / sclereids, etc. appear to be the eco-anatomical adaptations in *A. corniculatum* in response to high temperature and salinity in coastal environment of Pakistan.

Cork warts

Cork warts are present in *A. corniculatum on* leaf as minute black lesions occurring in different sizes. A degrading wart is shown in (Fig. 13). In *Kandelia (a genus of Family Rhizophoraceae)*, according to Sheue *et al.* (2003) they may occur on both sides of leaf and may be in different sizes. They are said to always originate from stoma or they arise from normal epidermal cells of the adaxial surface in *Kandelia*. Cork warts are thought to play an important role in internal tissue aeration of the mangrove plants due to internal flow (Evans *et al.,* 2009; Evans and Bromberg, 2010). Aerenchyma and cork warts in leaves of mangroves provide internal flow through heated air pressurization to aerate anoxic roots. Warts are reported to have secretory cells in *R. mucronata,* an important laminar characteristic (Replan and Malabrigo, 2017). Warts of *A. corniculatum* and their physiological role need further study.

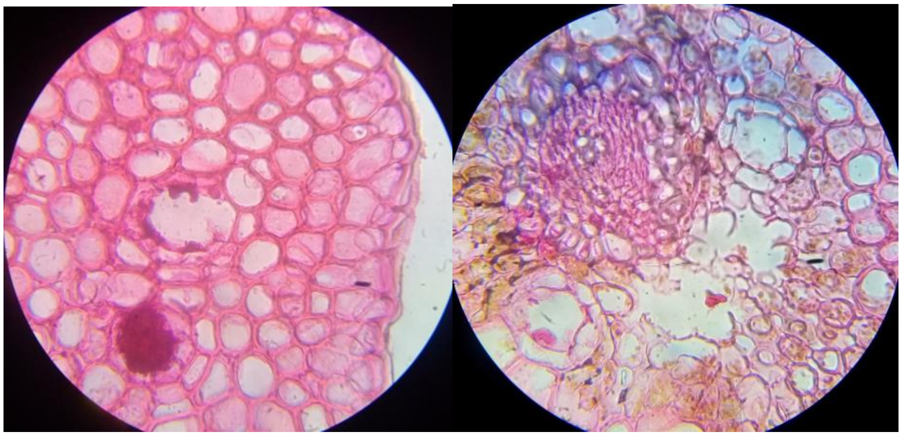


Fig. 8. A crystalliferous cell and an oil gland (A) and VB and water tissue near VB (B).

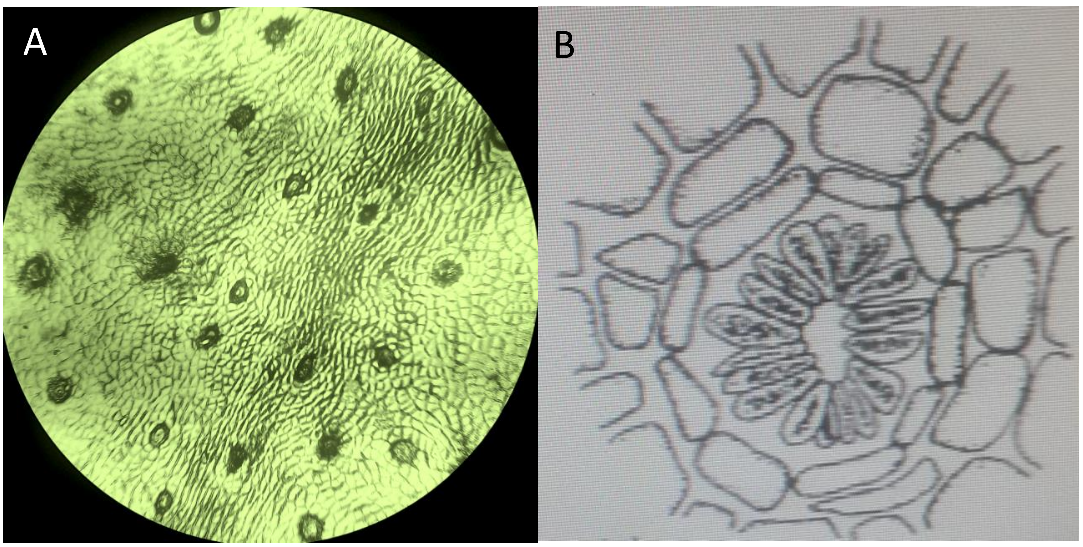


Fig. 9. A) Salt glands on the dorsal surface of *Aegiceras* leaf. B) Salt gland – dorsal view of salt gland of *A. corniculatus*. Magnification: 1,000X. Adopted from Hachings and Saenger (1987).

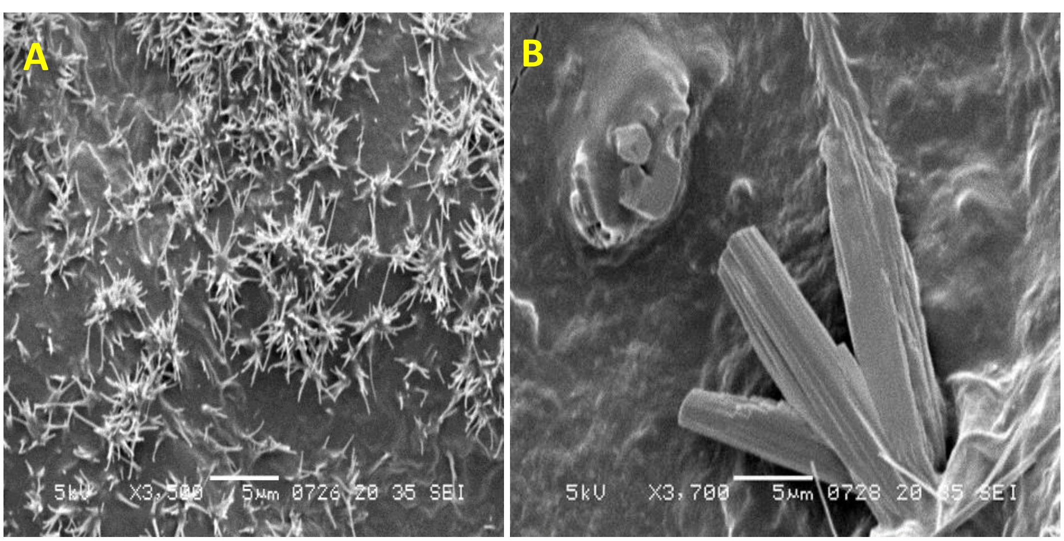


Fig. 10. Dorsal surface of leaf of *A. corniculatum*. A) Cuticle and salt crystals on the surface of leaf**.** B) A wart and a salt gland containing crystals.

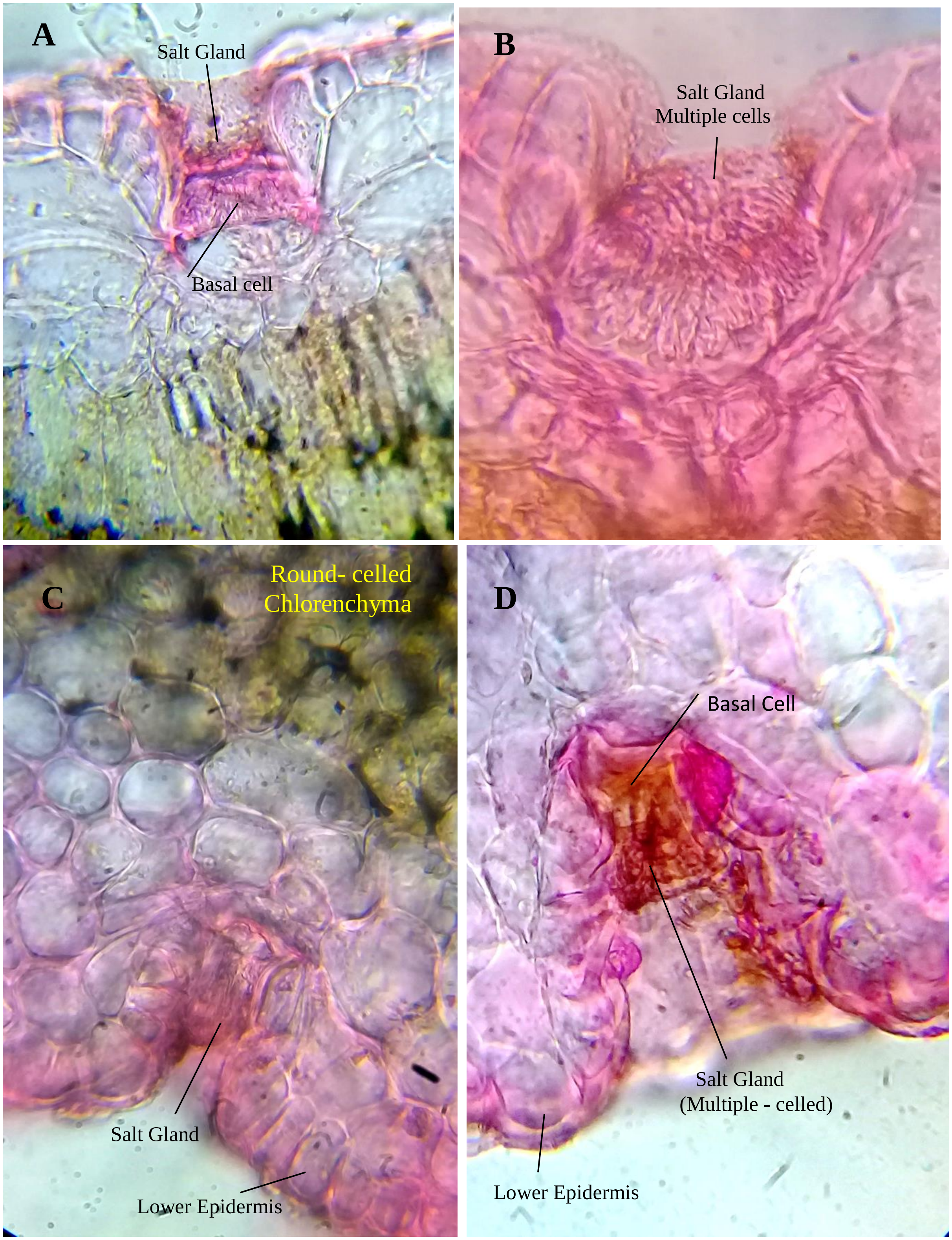


Fig. 11. Salt glands of *A. corniculatum* in upper (A and B) and lower epidermis (C and D) in various developmental stages. There appears some palo sclereids in palisade and round-celled Chlorenchyma (C).

Stomata

Leaves are hypostomatous i.e. dorsal surface is devoid of stomata. Stomata are sunken and localized in the laminar islands between the veins (Fig. 14-17). Stomata were of cyclocytic type (a special type of anomocytic stoma). Stomata are wide-elliptical in shape and oriented in various directions (Fig. 14, 16). Scanning electron microscopy (SEM) indicated five to six distinct subsidiaries arranged around the guard cells in form of raised ring (Fig. 16). In nail polish imprint, however three types of stomata were also observed (paracytic, brachyparacytic and anisocytic (Fig. 17). Das and Ghose (1993) have also reported paracytic stomata in *A. corniculatum.* The guard cells are sunken. The epidermal cells are papillose but remain below the raised ring of the subsidiary cells. Epidermal cells have straight to slightly curved anticlinal walls (Fig. 17). In *A. corniculatum* there are two outer ledges in guard cells as reported by Das and Ghose (1993) (Fig. 15B). The surrounding-cells-arrangement is reported by Metcalfe and Chalk (1979) to be anomocytic and anisocytic in Family Myrsinaceae. Seshavatharam and Srivalli (1989), have reported anomocytic stomata in *A. corniculatum*. Stomata in a sister species *Aegiceras rotundifolia* are reported to be cyclocytic. Diversity of stomata on the same surface of leaf suggests to the confirmation of Stace's (1966) who opined that many genera of *Rhizophoraceae* have basically paracytic subsidiaries but that extra walls usually develop giving the appearance of anomocytic stomata. In a non-mangrove leguminous species, *Cassia fistula* (seedlings), Khan and Zaki (2019) demonstrated transformation of paracytic stoma into anisocytic and anisocytic stoma into anomocytic one. The granules distributed on the leaf surface of *A. corniculatum* were presumably waxy crystalloids (Fig. 16).

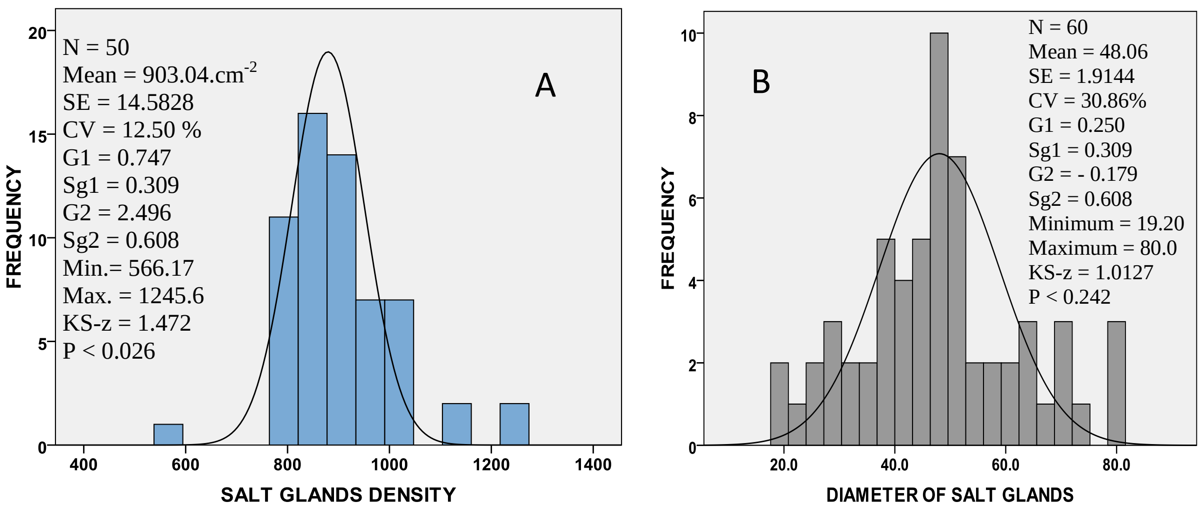


Fig. 12. A) Salt gland density per cm^2 on the dorsal surface of leaf of *A. corniculatum*. Some 80% of the observations fell in the size class of 701-1000 salt glands per cm^2. B) Distribution of salt gland diameter (µm) on the dorsal surface of leaf of *A. corniculatum*.

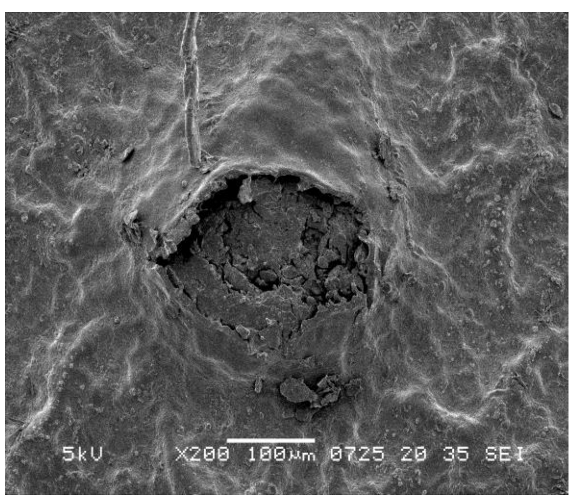


Fig. 13. Degrading cork wart on the dorsal surface of *A. corniculatum*.

In our studies the stomatal density (SD) in *A. corniculatum* averaged to 96.37± 1.27 varying from 49.14 to 137.61 stomata per mm^2 (Fig. 18). On the other hand, Seshavatharam and Srivalli (1989) reported stomatal density in *A. corniculatum* to be 175 ± 28 per mm^2. This variation may presumably be attributed to the seasonal effect or salinity of Sea water or the size of the leaf. Peel *et al.* (2017) have investigated stomatal density in *Rhizophora mangle* collected from River, Lagoon and Beach varying in salinity in Mexican Caribbean and reported average density to be 65.0 ± 12.32 (45.5-87.4), 73.4 ± 13.49 (52.9-97.0) and 74.8 ± 17.32 (54.5-102.5), respectively. In *R. mangle*, they found stomatal density to be negatively correlated with leaf width, leaf length and leaf area, and stem diameter of the plant. Salisbury (1928) also reported stomatal density inversely related to leaf size due to higher cell insertion. Young leaves have large number of stomata but as leaf expands the density declines (Gay and Hurd, 1975). Foliar superficial structures are distantly located in mature leaves as compared to younger leaves in land plants. Since salinity reduces leaf size, it may increase the stomatal density, they opined.

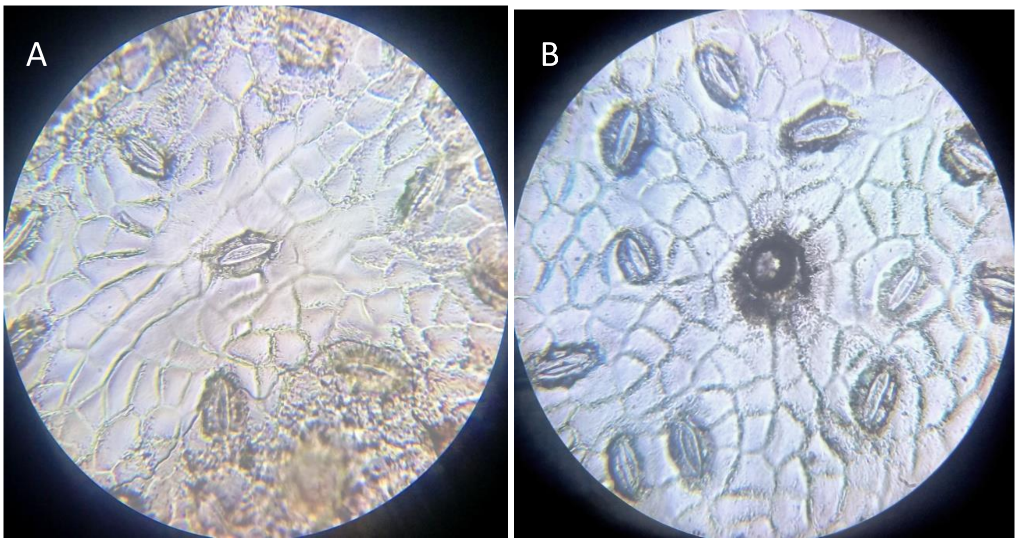


Fig. 14. Stomatal distribution on the ventral surface of leaf of *A. corniculatum*. Less frequently stomata may be seen in vein region (A). Frequently stomata may be seen distributed around a salt gland.

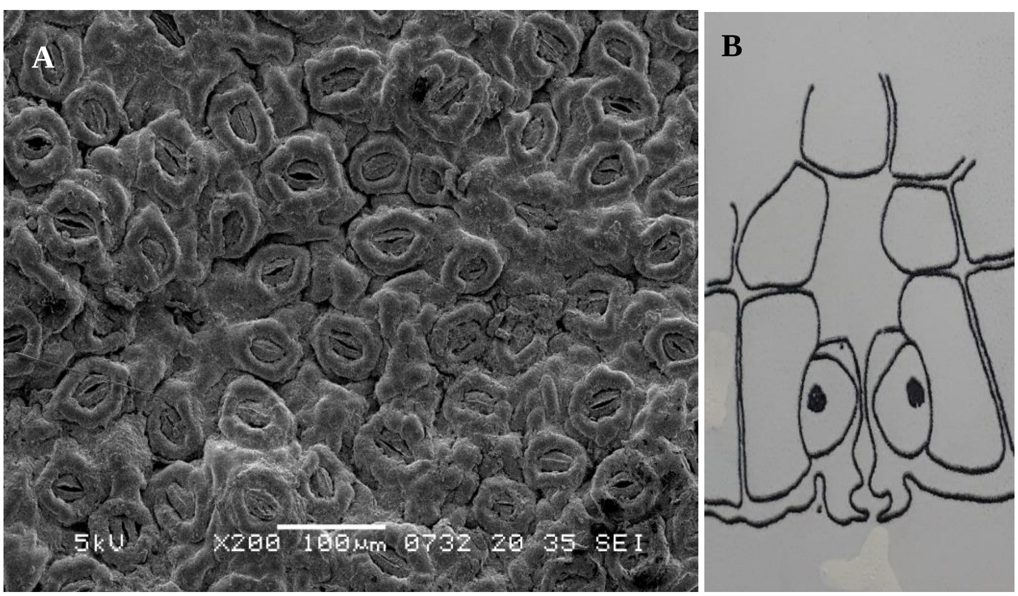


Fig. 15 A) SEM of *A. corniculatum* - ventral surface of young leaf (cuticle removed by hexane treatment). Dense crop of Cyclocytic (special anomocytic) stomata. Subsidiaries raised above the stoma (see Fig. 16 also). B) Line drawing of TS of Stoma with two outer ledges of guard cells. Image adopted from Das and Ghose 1993)

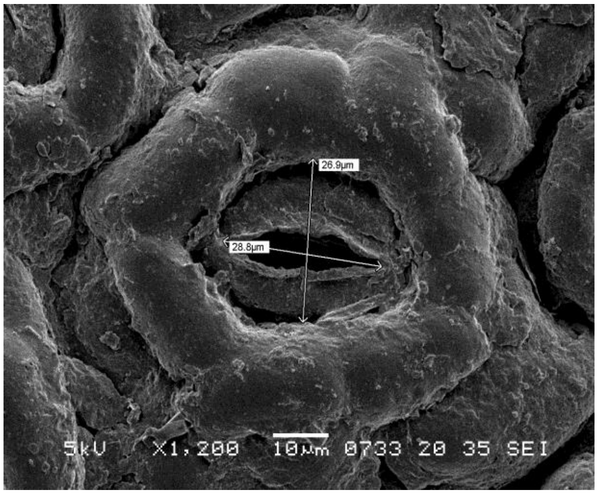


Fig. 16. Enlarged view of a cyclocytic stoma on the young leaf ventral surface of *Aegiceras corniculatum*. Note the raised distinct subsidiaries (six in number) surrounding the guard cells. Stoma is sunken.
Pore length = 28.8 µm. Stomatal width = 26.9 µm. The scattered granular structures may presumably be the waxy crystalloids. Magnification: 1200X

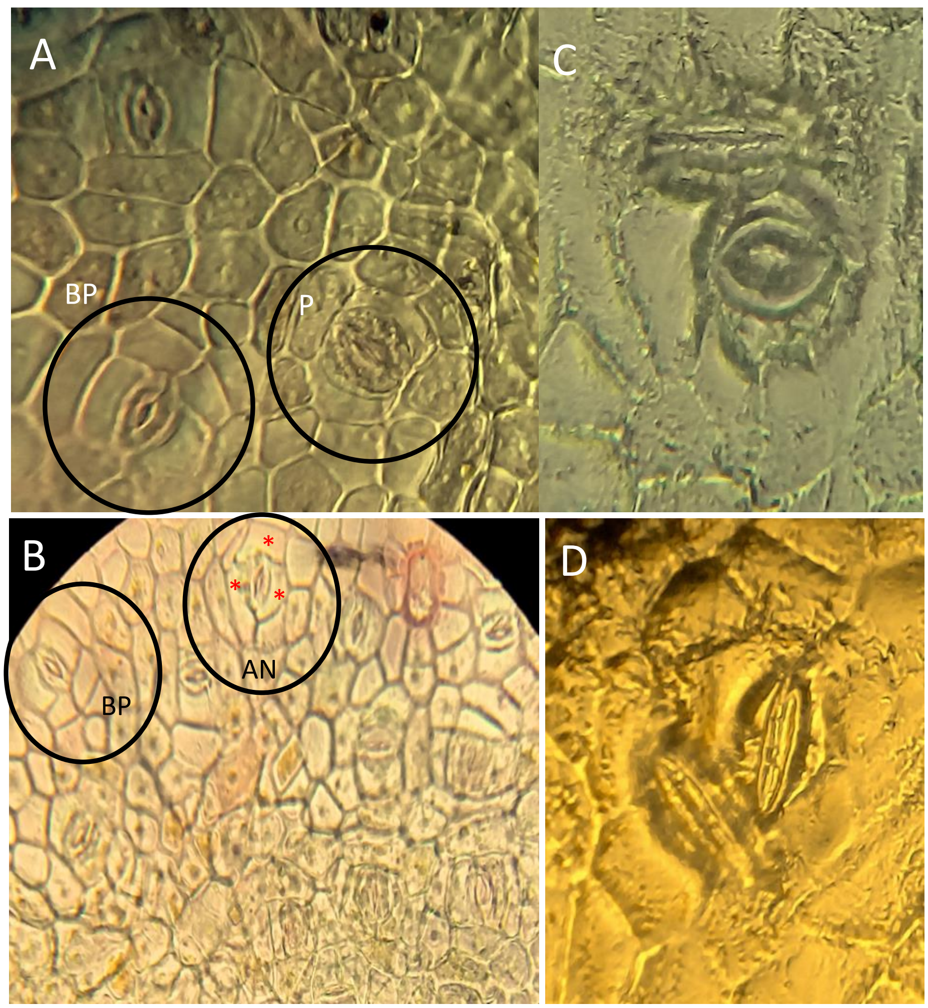


Fig. 17. Stomata of *A. corniculatum* as seen in the epidermal peel of ventral surface of *A. corniculatum* leaf. Paracytic (P, two cells flanking the sides to enclose guard cells completely) and brachyparacytic (BP, two cells flanking the sides but not enclosing completely), and anisocytic (AN, stoma surrounded by three unequal subsidiaries) types of subsidiary arrangement was recorded. Magnification: A, 45 x 10 X (zoom 2 X); B, 45 x 10 X. C and D, Nail polish imprint of stomata lying in close proximity. Such stomata were rare (Magnification: 45 x 10, zoom 4X).

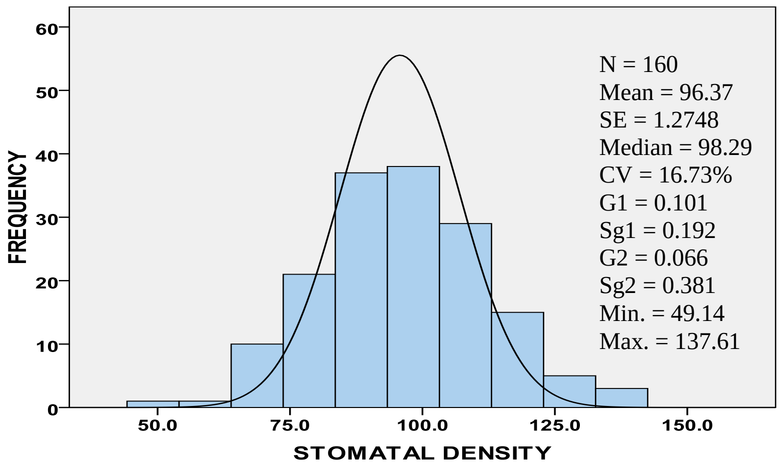


Fig. 18. Distribution of stomatal density per mm^2 on ventral surface of maturing leaf of *A. corniculatum*. Some 87.5% of the observations fell in the size class, 76-125 stomata per mm^2.

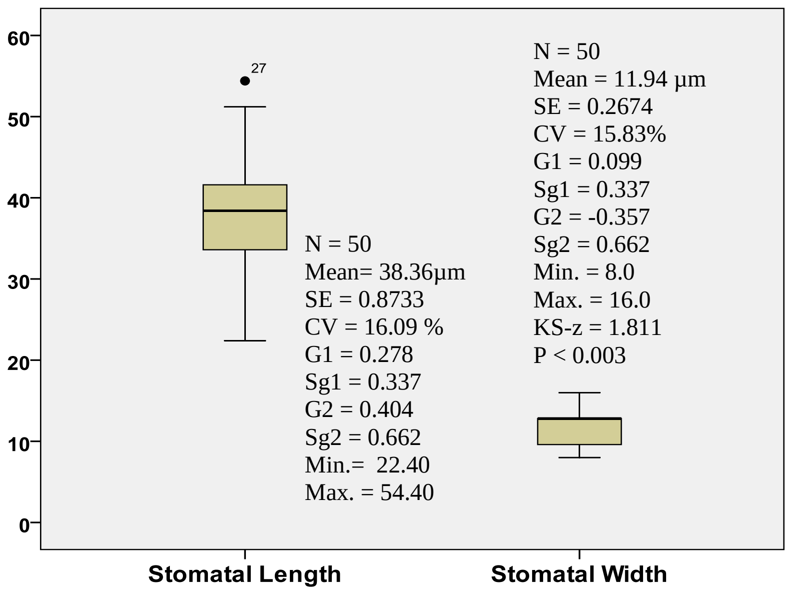


Fig. 19. Box plot representation of stomatal length and width (µm) on ventral surface of leaf of *A. corniculatum.* Length averaged to 38.36 ± 0.8733 µm. There was only one case of very large stomata (54.40 µm in length). Some 64 % of the stomata fell in size class of 31- 40 µm in stomatal length. Stomatal width varied from 8-16 µm (mean: 11.94 ± 0.2674 µm). Some 66% of the stomata had stomatal width of 11-15 µm.

The stomatal size in *A. corniculatum* was found to average 38.36 ± 0.8733 µm in length (ranging from 22.4 to 54.4 µm) and 11.94 ± 0.267 µm (ranging from 8.0 to 16.0 µm) in width (Fig. 19). Length and width varied more or less equally (CV: 16.09 and 15.83 %, respectively. Stomata in *A. corniculatum* are reported to be 47.80 ± 0.26 x 21.35 ± 0.21 µm by Das and Ghose (1993). Seshavatharam and Srivalli (1989), on the other hand, reported stomatal size in this species to be 36.0 ± 2.0 x 20.66 ± 1.8 µm. Our results are more or less in agreement with previous records of foliar stomatal size in *A. corniculatum* from India.

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