BRIEF NOTES ON THE MANGROVE SPECIES *RHIZOPHORA MUCRONATA* LAM. (RHIZOPHORACEAE) OF PAKISTAN WITH SPECIAL REFERENCE TO SAPLING AND LEAF

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ABSTRACT

Growth and leaf characteristics of variously- aged saplings of *Rhizophora mucronata* Lam., a true mangrove species, grown by planting propagules in various nurseries established in tidal areas along the coast of Pakistan or experimental plantations in swampy areas of some islands of Indus delta are described. Growth included such parameters as net growth (from the end of the propagule to the tip of the shoot), number of leaves per sapling, number of internodes in new growth, number of branches and photosynthetic area of the sapling. Internal structure of leaves was also studied. The morphometric and architectural parameters included parameters such as petiole length and weight, leaf length (LL) and breadth (LB), leaf area measured graphically (LAM), apex and base angles, aspect ratio (LB / LL), and Lamina weight. Lamina area was determined arithmetically using multiplying coefficient K for equation, Leaf area = K x LL x LB and also statistically by developing regressive equations for simple (linear and power models) and multiple correlation and regression. Surface Micromorphological studies were undertaken with respect to the stomatal type, their density and occurrence of warts. The results are discussed with respect to the available literature.

Key Words: *Rhizophora mucronata* Lam. saplings growth, leaf morphometry and architecture and leaf surface micromorphology

INTRODUCTION

The coastal forests are found in the Indus delta in Sindh and the coastal areas of Sonmiani, Kalmat and Gavatar Bay in Balochistan. Historically, one finds eight recorded species of true mangroves described from Pakistan coast. Four of them have now disappeared and the existing four species include *Avicennia marina* (Forssk.) Vierh, *Rhizophora mucronata* Lam, *Aegiceras corniculatus* (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 probably be explained on the basis of aridity of Indus delta (Snedaker, 1984) and 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) and recently reviewed by Rafique (2018). Mangroves forests are facing serious stresses which jeopardize their sustainability and their existence in Pakistan. A significant reduction in the fresh water supply and increased marine water pollution from industries as well as over harvesting of mangroves and fishes by the local peoples, sedimentation, population stress and coastal erosion are usually considered to be the immediate causes of mangrove loss in Pakistan. Mangrove deterioration in the Indus Delta is considered to be due to the reduction in volume of fluvial discharge (Qureshi, 2012). If disturbance and shortage of fresh water continue at the present pace, mangrove ecosystem should be completely ruined in the year 2232 (Peracha *et al.*, 2017). Mangrove decline is a global phenomenon.

Seawater in Indus delta 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. It is mildly basic in Karachi Harbour estuarine ecosystem (7.3-8.1). Temperature is low in winter and high in summer (24.8 -30.0 °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 (Ali and Khan, 2012). Batool et al. (2014) has published an overview on this species. It is a C₃ plant (Gillikin and Verheyden, 2005) with potassiophillic nature (Das and Ghose, 2000). R. mucronata is a branched large shrub or moderate-sized tree (up 10 m in height) supported with prop roots and reddish brown bark (Ghafoor, 1984). It is viviparous. It is referred to as loop root mangrove, red mangrove and Asiatic mangrove (Grin, 2006). It helps in coastal stabilization (Naidoo (1985). It is a true mangrove species distributed in N. Australia, South East Asia, and Indo-Pakistan sub-continent, East and South Africa and Micronesia. In Pakistan its major growth is in Son Miani, Balochistan (Fig. 1A) which has been a great source of propagules used in its plantation projects in Indus delta (IUCN, 2012,2013) and also in Abu Dhabi, UAE (Yousif and Sen, 2020). It is phytochemicslly important species. It has multiple uses (Duke, 1983) but lies in red list of IUCN (Duke et al., 2010). There is no Rhizophora stylosa in Miani Hor (Fig. 2). Optimum salinity for growth of the local R. mucronata is reported to be 50% Seawater salinity (Aziz and Khan, 2001). Its leaves changes colour to brown and stem becomes softer under high salinity (Titah et al., 2019). Sewage treated R. mucronata grows but poorly (Nyomora and Njan, 2012) compared to A. marina. High siltation brings negative effects on this species. Under high siltation number of branches are lesser, leaves are smaller and stomatal area is low. Temperature fluctuation, salt stress and high siltation impose water and oxygen stress on R. mucronata ((Du Dauwaerder, 2012; Noor et al., 2015).

R. mucronata leaves may be used as a food supplement as they have plenty of minerals, essential amino acids, fibre, vitamins, protein, unsaturated fatty acids and carbohydrates (Suganthy and Devi, (2016). Honey collected from its flowers is said to be poisonous (CSIR, 1948-1976, seen in Duke, 1983).

Various Projects have been undertaken in the past and in current scenario to conserve the resources of mangrove ecosystems. *Rhizophora mucronata* has been the major species employed in plantation in Indus delta. It may be grown from propagules (seedlings) under frequent Seawater irrigation on costal sand in drum pots (Fig. 1B), but it is not as tolerant as *A. marina* to sewage pollution and salinity. The present paper describes the variously-aged saplings of *R. mucronata* from its nurseries or plantations along the Pakistan coast. Leaf morphometry, architecture, salient features of internal structure and surface micro-morphological characteristics of leaves with reference to stomata and warts have also been described.



Fig. 1. A population of *Rhizophora mucronata* in Son Miani Bay, Baluchistan coast (A; photo by M. Tahir Qureshi) and the sprouted propagules sown in coastal sand and irrigated with Seawater (B) in drum pots at department of Botany, University of Karachi.



valves arch over the stigma in Rhizophora mucronata (A) due to short style and the anther valves do not arch over the stigma due to longer style in Rhizophora stylosa (B). (Tomlinson (1986; page 339). Kogo et al. (1987) have given some data on the germination of Rhizophora stylosa collected from Miani Hor, Balochistan. Since this species has not previously reported from the coast of Pakistan (Ghafoor, 1984) and even from India and Sri Lanka (Rao, 1987; Untawale, 1987), a number of Rhizophora specimens collected from Miani Hor during June, 1988 (flowering period) were studied at department of Botany, University of Karachi, to confirm occurrence of R. stylosa in Pakistan. Our unpublished taxonomic studies indicated that our taxon from Miani Hor belong to the taxon R. mucronata and not to R. stylosa. Our taxon had short style (< 5mm) so the anther valves of dehisced anther could arch over the stigma. In R. stylosa style is longer and valves of the dehisced anther arch over the stigma (cf. Tomlinson, 1986).

Fig. 2. Relationship of style to dehisced anther. The anther

MATERIALS AND METHODS

The saplings of *R. mucronata* of various ages were studied from the tidal marshes of Gwadar in Balochistan and Shah Bunder of Indus delta plantations nurseries frequently inundated with tidal Seawater. Saplings were studied for their growth and the leaves were measured for their length of petiole (PL), lamina length lying between the point of midrib insertion and the apex of the leaf (LL) and lamina breadth (LB) at the broadest points. Hickey (1979) and LWG (1999) were followed for description of leaf. To study stomatal types, leaflet epidermal impressions were made with clear nail polish (Wang *et al.*, 2006) and studied under compound optical microscope. For scanning electron microscopy (SEM), air-dried plant material of leaf was mounted on brass stubs and coated with a 250 °A gold layer with JFC-1500 gold coater. SE micrographs were made at 15kV with JEOL JSM-6380A electron microscope at

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various magnifications. The images were saved digitally on computer. Stomatal nomenclature suggested by Dilcher, 1974) was adopted to ascertain stomatal types. 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 the leaf area, the leaf outline was carefully drawn on graph paper and area (LAM) determined with all possible precision and accuracy. The multiplication factor (K) was calculated by employing the formula, K = 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

The saplings of *R. mucronata* are characterized with overwrapping stipules on the top and scar of the petiole insertion on the young stem. Mature bushes and trees develop stilt roots for support. The prop or stilt root are large and supports a diversity of organisms which is inversely related to water temperature above 34 °C in at least *Rhizophora mangle* (Kolechmainen and Morgan, 1972). *R. mucronata* petiole outline is concave adaxially and convex abaxially (Surya and Hari, 2017b).



Fig. 3. Nursery of *R. mucronata* established in Shah Bunder, Indus delta (A) to raise saplings for transplantation in barren deltaic areas of Indus delta; B ,Jiwani coastal nursery of *R. mucronata*.

Sapling Transplantation and their growth in tidal marsh

The *R. mucronata* populations in Son Miani, Balochistan were the main source of propagules (seedlings) collection. Various nurseries were established by sowing fresh propagules along the coast at several swampy places at different times for uninterrupted supply of saplings (Fig. 3). The propagules of *R. mucronata* (hypocotyls) are elongated rugose structure 25 to 35 cm in length largely varying with size (length and breadth as multiplicative parameter) (Fig. 5). Ghafoor (1984) described hypocotyl to be 20-40 cm long and 1.5-2.0 cm broad. The propagule averaged to $18.48 \pm 0.64g$ in dry weight (CV: 34.65%) (Fig. 4)

The experimental plantation was initiated in 1986 (Qureshi and Khan, 1988) by the Sindh Forest Department (SFD). After the successful preliminary plantations, the Sindh Forest Department later undertook massive plantation in Indus delta. This community participation endeavour broke the earlier Guinness World record of planting *R. mucronata*, the most plants, in 24 h by planting 1.129,294 mangroves saplings on an island near Keti Bunder in Thatta district of Sindh on April 19, 2018. (The Express Tribune, April 19, 2018). Earlier record was there of planting 847, 275 saplings in 24 h in Kharro Chaan, Thatta. (The Express Tribune, 2013). This was a very successful venture. Artificial propagation in certain areas of tropical World has also been successfully achieved – Hawaii (Teas *et al.*, 1975), Florida (Teas, 1977), Andaman Islands (Banerji, 1958) and Pakistan since 1986 (SFD reports) and Abu Dhabi (Yousif *et al.*, 2020). The results of the experimental plantations in Indus delta were presented in Qureshi and Khan (1988) and later discussed by Qureshi (2012). Brief description of sapling growth in coastal plantations is described as below.



Fig. 4. Frequency distribution of propagule dry weight of *R. mucronata* collected from Sonmiani, Balochistan. An average propagule admeasured 29.60 ± 0.364 cm in length and 1.55 ± 0.0226 cm in braedth (1.0 to 2.0 cm).



Fig. 5. Relationship of propagules dry wt. (g) with multiplicative parameter of propagules dimension (Length (cm) x Breadth (cm) in *R. mucronata* – linear and power model.

The Sapling Growth with age at various sites of transplantation along Pakistan coast

The sapling growth of *R. mucronata* for various ages at Sipli, Ratto Kot, Batano, Shah Bunder and Port Muhammad Bin Qasim plantations has been described in Qureshi and Khan (1988).

At 4 months of age at **Ratto Kot**, saplings exhibited net increment in height, 15.24 ± 0.79 cm and 25.63 cm after 10 months.

At Sipli Island, plant after 6 months of sowing had 3-4 internodes (2-8 leaves in all) and net increment of 23.2 \pm 1.27 cm in height. After 9 months the net growth was 27.69 \pm 2.04 cm ranging from 18 to 40.3 cm. After 18 months of sowing, plant showed 47.92 \pm 1.72 cm net increment in height. The frequency of bearing 9-10 internodes was around 33% and that of 11th and 12th internode around 6.6%. The photosynthetic area of the sapling (PAS) in these plantations was determined as PAS = \sum (L x B x 0.666) Where L = Length of the leaf lamina (cm) and B =

breadth (cm) for number of leaves present with the sapling (Cain and Castro, 1959). Total leaf area per sapling in these three plantations was 117.06 ± 15.98 , 204.22 ± 16.83 and 401.89 ± 90.2 cm², respectively i.e. it increased with the age of the plantation.

At Shah Bunder, 10.5 months old sapling showed net increment in height 44.25 ± 2.87 cm with 6.25 ± 0.16 internodes. The plant showed great degree of inhibition in all growth parameters (36 - 58%) at Ratto Kot as compared to that almost equally aged plants at Shah Bunder presumably due to high salinity at Ratto Kot and availability of fertile alluvium at Shah Bunder.

It was learnt from the results of the experimental trial (Qureshi and Khan, 1988) that:

1) *R. mucronata* is the best growth performer in tidal mud flats amongst all the species tested. The 5 years old plantation at **port Muhammad Qasim** had net increment in height 60.08 ± 3.6 cm with total photosynthetic area averaging to 405.77 ± 13.0 cm² per sapling.

2) Larger was the propagule planted, better was the growth of the plant as was indicated by a direct correlation between propagule size and the sapling vigour (Table 1).

	Pearson	Intercept	Slope
Parameter combination	Correlation (r)	'a" [–]	ʻb,
Net growth* (cm) vs. propagule length (cm)**	0.3928 (p < 0.02)	23.40	0.2875
Mean leaf area (cm ²) vs. propagule length (cm)	0.7797 (p < 0.001)	10.95	0.3684
Total leaf area (cm ²) vs. propagule length (cm)	0.8616 (p < 0.001)	66.77	4.6856
Diameter of basal internode (cm) vs. propagule length	0.9376 (p < 0.001)	0.2686	0.0112
(cm)			

Table 1. Correlation and regression between propagule length and seedling vigour (N = 15)

*, This parameter not included propagular length; **, the length of the propagule sown.

3) *R. mucronata* was successfully re-introduced in deltaic islands (sprouting was high (up to 95%). However, the plants under unprotected conditions and exposed to strong winds died substantially whereas in protected muddy swamps they grew well – the protection from winds due to nearby *Avicennia marina* and nutrients richness in mud. This observation has also been confirmed by Yousif and Sen (2020) from plantation in Abu Dhabi from the propagular stock from Pakistan.

4) This species sprouted equally well in sandy and muddy soils but failed to survive in raised sandy areas.

5) To an extent the seedling mortality was attributable to barnacle infestation and the damage caused by the activity of the cambium-eating crab, *Sesarma* in Shah Bunder.

6) The growth is also influenced with salinity - it should be planted in protected swamps with soil-water salinity does not exceed the value of 5.0% salts.

The sapling growth at different ages in nurseries at Gwadar (24 months old, N = 4) and Shah Bunder / Keti Bunder (16 months- and 32 months-old saplings which were studied in July, 2012 is shown in Table 2, 3 and 4. At 24 months of age in Gwadar nursery, saplings showed average net growth of 21.30 ± 2.54 cm with 8 internodes which almost gradually shortened from base to apex. The net growth in length was 84.09 % of the propagule length sown. The sapling had photosynthetic area 144.17 ± 13.96 cm². In Shah Bunder, sapling after 16 months of sowing attained net growth of 37.4 ± 1.36 cm with four internodes quite longer than Gwadar saplings. The photosynthetic area was 145.7 ± 4.20 cm² quite comparable to 24 months old saplings of Gwadar. The net linear growth was 98.06% of the sown propagule length. Better growth at Shah Bunder may be hypothesized to be attributable nutrient rich Seawater of Shah Bunder due to fluvial discharge of River Indus. In Shah Bunder at the age of 32 months the net growth was 43.12 ± 2.57 cm i.e. 152.9 % of the propagule sown. Photosynthetic area associated with saplings averaged to 592.0 ± 88.24 cm² with 35.20 ± 5.3 leaves. The sampling had maximally five branches ranging in length from 12.6 to 17.78 cm. At this age, Dry weight of sapling averaged to 63.70 ± 7.64 (48.07 - 79.43g, CV =26.78%).

The net growth of saplings related positively (r = 0.678, p < 0.001) with age when data of various nurseries at different locations (Ratto Kot, Sipli, Port M. Qasim, Gwadar and Shah Bunder) was analyzed through correlation and regression. With the consideration of zero intercept, net growth related to age as: Net growth (cm) = 1.197. Age in months. The net growth per month was thus around 1.2 cm (Fig. 6).

Average	Number of	Root	Net	Number of					
Propagules	roots	thickness	Stem	internodes	Number of	Photosynthetic			
Height (cm)		(cm)	height		leaves	area (cm ²) per			
			(cm)		present	sapling *			
25.33 ±	8.6 ± 1.28	0.353 ±	21.30 ±	7.33 ±	9.33 ± 0.67	144.17 ±			
1.92		0.0125	2.54	0.20		13.96			
	•	Internodes (Ba	ase to apex) let	ngth (cm) and Ra	ange				
Internode # 1			4.3	$0 \pm 0.76 (2.9 - 5.5)$	5)				
Internode # 2			4.60	$0 \pm 0.70 (4.4 - 5.5)$	9)				
Internode # 3			4.0	$3 \pm 1.36 (1.7 - 6.4)$	4)				
Internode # 4			2.4	$\pm 0.57 (1.8 - 3.5)$	5)				
Internode # 5			1.60	$0 \pm 0.10(1.5 - 1.5)$	8)				
Internode # 6		$1.23 \pm 0.39 \ (0.7 \ \text{-} 2.0)$							
Internode # 7		0.83 ± 0.18 (0.6 – 1.2)							
Internode # 8		0.70							

Table 2. Morphometric data of 24-month-old saplings of *R. mucronata* grown in the tidal area of Gwadar, Balochistan (N = 4, randomly selected).

*, determined on the basis of statistically more valid value of K = 0.6998. Sapling Dry weight $27.11 \pm 4.50g (20.01 - 35.45g, CV = 28.75\%)$

Under salinity of 30 ppt, Basyuni *et al.* (2018) reported net growth (from end of the propagule to the tip the stem) to be 18.90 cm (Seaward plants) after 4 months of sowing in Pulan, Sembilan, N. Sumatra, Indonesia, quite faster at low salinity). At salt concentration of 35ppt the growth reduced to 93%.

After 11 years of plantation at Abu Al Abyad (salinity 43-44 ppt) and Ras Ghanada islands (salinity 45-49 ppt), Yousif and Sen (2020) reported the long term survival of *R. mucronata* to be 81% in Abu Al Abyad and only 10% in in Ras Ghanada (decline due to external factors). The mean plant height after 11 years was $2.06m \pm 0.80$ (SD) at Ras Ghanada and $1.53m \pm 0.36$ (SD). At Abu Abyad 36.4% of plants showed reproduction and 10.3 in Ras Ghanada. Many plants developed stilt roots. The growth of variously- aged plantations made along the Pakistan coast and particularly in Shah Bunder and Keti Bunder need to be comprehensively investigated as effects of salinity on growth and survival of mangrove seedlings is reported to change with age (Kodikora *et al.*, 2018).

Table 3. Morphometric data of 16-month-old saplings of *R. mucronata* raised in the tidal area of Shah Bunder, Sindh. (N = 4, randomly selected).

Average Propagule height (cm)	Number of roots	Root thickness (cm)	Net Stem height (cm)	Number of internodes	Number of leaves present	Photosynth etic area (cm ²) per sapling *			
38.14 ±	8.5 ±	0.313 ±	37.4 ±	3.6 ±	7.2 ±	145.7 ±			
0.86	1.25	0.0125	1.36	0.25	0.49	4.20			
	Internode	s (Base to ape	x) length (cr	n) and Range					
Internode # 1			14.64 ± 1.2	26 (11.6 – 17.8	5)				
Internode # 2		9.50 ± 1.69 (3.8 - 13.7)							
Internode # 3	4.70 ± 0.653 (3.2 - 6.3)								
Internode # 4	$1.48 \pm 0.81 (0.5 - 3.7)$								

*, Determined on the basis of statistically more valid value of K = 0.6998



Fig. 6. The net growth in height of saplings related positively (r = 0.678, p < 0.001) with age when analyzed for the various nurseries irrespective of their locations (Ratto Kot, Sipli, Port M. Qasim, Gwadar and Shah Bunder).

Average Propagule Height	Number of roots	Root thickness (cm)	Stem height (cm)	Number of Branches	Number of leaves present	Photosynthetic area (cm ²) per sapling *				
	0.60 +	0.356 +	<i>43</i> 12 ±	3 20 +	35.20 ±	502.0 + 88.24				
$28.2 \pm$ 0.86	9.00 ± 0.24	0.330 ± 0.0117	43.12 ± 2.57	5.20 ± 0.58	53.20 ± 5.30	392.0 ± 66.24				
0.00	Average length of Branches (cm)									
Branch # 1		1	7.78 ± 0.34	(N = 4; 11.4)	- 22.0)					
Branch # 2		1	6.17 ± 0.60	(N = 3; 15.0)	- 17.0)					
Branch # 3	10.16 ± 3.89 (N = 3; 3.5 - 17.0)									
Branch # 4	14.10 ± 2.72 (N = 5; 7.0 - 19.5)									
Branch # 5		12.6 ± 0.80 (N = 2; 11.8 -13.4)								

Table 4. Morphometric data of 32-month-old saplings of *R. mucronata* grown in the tidal area of Shah Bunder, Sindh. (N = 4, randomly selected).

*, Determined on the basis of statistically more valid value of K = 0.6998. Sapling Dry weight = 63.70 ± 7.64 (48.07 - 79.43g, CV = 26.78%)

Table 5. Morphometric and architectural parameters of leaves (N = 177) of a 32-month-old sapling of *R. mucronata* raised in tidal area of Shah Bunder, Sindh.

Parameters	Mean	SE	CV%	g1	g2	Min	Max	KS-z	р	Curve
LL (cm)	6.658	0.1263	25.24	-0.526	0.329	1.20	11.0	0.792	0.557	S
LB (cm)	3.437	0.07096	27.46	- 0.376	0.451	0.50	6.20	0.957	0.319	S
LAM (cm^2)	16.947	0.58816	46.17	0.350	0.499	0.49	48.06	0.630	0.822	S
$AA(^{o})$	79.31	0.5990	10.05	0.183	1.959	50	105	1.062	0.209	S
$BA(^{o})$	81.42	0.693	11.32	- 0.026	3.418	40	115	0.983	0.289	S
PL (cm)	1.064	0.225	28.11	0.214	0.305	0.3	2.0	1.469	0.027	AS
Petiole wt (g)	0.0247	0.00101	51.38	0.244	-0.733	0.0017	0.0575	0.759	0.611	S
Lamina wt. (g)	0.3802	0.01350	47.26	0.126	- 0.621	0.0121	0.7821	0.744	0.638	S
Aspect ratio	0.5156	0.00465	12.01	0.323	0.964	0.3455	0.7222	1.043	0.227	S
К	0.69975	0.00617	11.72	1.171	10.308	0.4312	1.2147	2.245	0.0001	AS
$LAK (cm^2)$	17.026	0.59324	46.36	0.357	0.377	0.4059	47.72	0.696	0.717	S
LAPOW (cm ²)	16.466	0.57327	46.32	0.356	0.375	0.394	46.113	0.695	0.719	S
Lmulti (cm ²)	16.949	0.5645	44.31	- 0.464	0.476	- 7.25	38.48	0.777	0.582	S

Acronyms: LL, Lamina length (cm); LB, Lamina breadth (cm); LAM, Lamina area measured; AA, Apex angle; BA, Base; angle; PL, petiole length; Aspect ratio, LB / LL; K, multiplication coefficient; LAK, Lamina area measured through K; LAPOW, Lamina area estimated through power model equation; Lmulti, Lamina area estimated through multiple linear equation, SE of skewness = 0.183; SE of kurtosis = 0.363.

Leaf morphometry and architectural characteristics

Leaf is characterized with leaf tip mucro, simple, petiolate with single prominent midrib, less apparent pinnate venation and thick cuticle on both surfaces. The morphometric and architectural parameters of leaves of 32 - month old saplings from Shah Bunder are presented in Table 5. Leaves are thick succulent ovate-lanceolate, dorsiventral, and opposite-decussate. They are flat green, relatively thick and leathery. The leaves are petiolate, entire-margined and obscurely-veined. Midrib is prominently present on ventral surface. Lenticels as brown dots are clearly present on ventral surface (Fig. 7). Leaf base is Cuneate and apex mucronate. Mucro is c 5 mm in length. In mature trees growing naturally mucro may be as large as 9 mm (Ghafoor, 1984). They are succulent and their succulence appears to increase with salinity (Wehe, 1964). Leaves of *Rhizophora* resemble to *Bruguiera* and *Kandelia* in form (Tomlinson, 1986). Leaves of *R. mucronata* decompose relatively rapidly than that of *A. marina*. (Time in days required to decompose 50% leaf mass is 44.3 days in *R. mucronata* and 49.55 days in *A. marina* (Farooqui *et al.,* 2014). Leaf morphometry and architectural details of 32- month old saplings in Shah Bunder is given as below.



Fig. 7. Ventral leaf surface of *R. mucronata* (A) showing lenticels as brown-black dots (B) under high magnification.

Petiole: Petiole in *R. mucronata* saplings was small, cylindrical, and green. The petiolar length (PL) averaged to 1.06 ± 0.225 cm (0.3-2.0 cm) – varying around 28.11%. Petiole length in 91% leaves was between 0.51 and 1.5cm. Petiole weight averaged to 25.7 ± 0.101 g varying from 0.0017 to 0.0574g, CV: 38.64%). In around 79 % of the leaves, petiole weight varied between 2.6 to 7.5mg. Petiole in leaves of mature naturally growing trees is reported to be 1-3 cm long (Ghafoor, 1984).

Leaf Length (LL) and breadth (LB): Leaf length *R. mucronata* saplings averaged to 6.658 ± 0.1263 cm (1.2 to 11.0 cm, CV: 25.24%) and breadth to 3.437 ± 0.271 cm (0.50 - 6.20, CV: 27.46%). Around 81.9% of the leaves had length 6.0 to 9.0 cm. Most of the leaves (88.7%) were 2.0 to 5.0 cm in breadth and only 3% of leaves were larger than 5 cm in breadth. Leaf lamina was comparatively smaller than that in naturally growing trees in Miani Hor. Ghafoor (1984) has reported leaf lamina to be (6 -) 8-15 (-20) cm long and (3.5 -) 4-8 (10) cm broad in naturally growing trees of *R. mucronata* in Miani Hor.

Leaf shape: In present investigation, the leaf shape consistency was evaluated as Aspect ratio = Breadth / Length of leaves (Table 5; Fig.8). This parameter may give some indication about consistency of leaf shape with size (Verwijst and Wen, 1996). Aspect ratio (LB / LL) averaged to 0.5156 ± 0.0047 (CV = 12.01%). Aspect ratio in 86.6 % leaves ranged between 0.41 and 0.6. It was ≤ 0.4 in 4.2% of the leaves. Aspect parameter distributed symmetrically (Fig. 8). It exhibited insignificant correlation with leaf length (r = 0.042, F: 0.313, p < 0.576) but significant correlation with leaf breadth (r = 0.414, F: 36.193, p < 0.001) i.e. Leaf Length and breadth had the explanatory power of aspect ratio variation by 17.1%. Multivariate correlation of aspect with LL and LB gave highly significant relationship (R = 0.946, R² = 0.895, F= 743.0 (p < 0.0001). LL behaved in opposite manner (negatively) compared with LB in multivariate analysis. Aspect ratio was, however, relatively more controlled by leaf breadth than leaf length (Fig. 9). The leaves appeared to exhibit considerable consistency of shape.

The multiplicative factor K: The values of multiplicative factor (K) of the equation $K = \text{Area} / (LL \times LB)$, tended to be highly leptokurtic in distribution (Table 5, Fig. 10) and somewhat positively skewed. It averaged to 0.6998 \pm 0.0062 varying only 11.72% only. Around 87% of the K values were found to be between 0.6 and 0.8. The distribution frequency of K values \leq to 0.5 were only 2.8%. Being largely concentrated around the mean value indicated its practical suitability in leaf area estimation.

Apex (AA°) and Base angles (BA°): Both apex and base angles averaged to be lower than 90°. Mean base angle was, however, slightly larger (81.42° , $40-115^\circ$) than the apex angle (79.31° , $50-105^\circ$) (Table 5). Apex angle was larger than 90° in 6.2% of the leaves only. Apex angle was thus predominantly acute. The base angle was lesser than 50° in 1.1% of leaves, $51-80^\circ$ in 46.9% leaves. It was $81-90^\circ$ in 39.6% of the leaves. The base angle larger than right angle was observed in 12.4% leaves. That is proportionately larger number of leaves had acute base and obtuse in 12.4% of the leaves. It followed that leaves were ovate to elliptical and fairly consistent in form.

Lamina area: Lamina area was determined graphically and referred to as LAM. The arithmetically determined leaf area via K as multiple factor (0.6998) using LL x LB of leaves was referred to as LAK. Power model equation (equation 3, Table 6) was used to arrive at Leaf area referred to as LAPOW and Multiple correlation and regression equation (equation 2, Table 6) 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 5. LAM, LAK, LAPOW and LMULTI averaged to 16.947 ± 0.5882 (CV: 46.077%), 17.026 ± 0.5932 (CV: 46.36%), 16.47 ± 0.5733 (CV: 46.33%) and 16.949 \pm 0.0.5645 (CV: 44.31) cm², respectively. On the basis of Kolmogorov-Smirnoff test, all leaf area parameters tended to be symmetrical (Table 5). Mean foliar area was obviously quite lesser than that of naturally growing trees in Miani Hor.



0



t = 34.66

t = 38.11

Partial correlation r

- 0.935

0.946

p < 0.0001 p < 0.0001

Fi	g 9	 Relationship 	p of as	pect ratio	of leaf	(N = 1)	77) t	o LL	, and L	B in	32	months	old	saplings	of <i>F</i>	R. mucronata
	~					A A										

Table 6 presents the results of correlation and regression analysis for lamina areas estimation in leaves of 32month old saplings of Shah Bunder. All the three equations based on 1) simple correlation and regression leaf area with a multiplicative parameter LL x LB as independent variable, 2) Multiple correlation and regression analysis for leaf area with LL and LB as independent variables and 3) Power model regression for leaf area) with LL x LB as X variable, were statistically significant in terms of r, R, F, and t- values. Amongst these three equations, power model equation was the best fit equation for the estimation of leaf area statistically (Table 6). Many workers have undertaken leaf area estimation allometrically as well as mathematically and have arrived at significant results with many species (Khan, 2008; 2009 for details). The fitness of power model to estimate leaf blade area has been reported in several species e.g., in *Coffea arabica* and *C. canephora* with high precision ($R^2 = 0.998$) and accuracy irrespective of cultivar and leaf size and shape (Atunes *et al.*, 2008), in 'Niagara' ($R^2 = 0.992$) and 'DeChunac' ($R^2 = 0.963$) grapevines (Williams III and Martinson, 2003); groundnut (Kathirvelan and Kalaiselvan, 2007), *Nicotiana plumbaginifolia* (Khan, 2008) and *Salvadora persica* (Khan *et al.*, 2020).

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 more closely (r = 0.973 and F = 3159.95, p < 0.001 and r = 0.973, F= 3156.66, p < 0.001, respectively) than with Lmulti (r = 0.960, F = 2036.48, p < 0.001). It implied that estimation of leaf area on the basis of LL and LB using K as multiplicative factor (0.6998) is the most suitable in *S. persica*. Ahmed and Khan (2011) have also recommended the arithmetic method of using K as multiplication factor to be most accurate in *Jatropha curcas* (with a value of K = 0.858758). Besides being accurate it is simple and convenient also. This contention was further substantiated on the basis of composition 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 90.39% between LAM and LAK, 87% between LAM and LAPOW and 79.4% between LAM and LMULTI i.e. maximum composition existed between LAM and LAK. The estimation in leaf area on the basis of multiplicative factor K (0.6998) may, therefore, be recommended for leaf area estimation owing to its precision, simplicity and convenience.

Lamina dry weight: The lamina weight per leaf averaged to $0.3802 \pm 0.0135g (0.0121 - 0.7821g, CV: 47.26\%)$. It tended to follow normal distribution (Kolmogorov-Smirnoff Test with Lilliefors significance correction) (Table 5). Lamina weight of $\leq 0.250g$ was observed in 22.0% of the leaves. Around 75.7% of the leaves had lamina wt. between 0.251 and 0.750g. Lamina wt. larger than 0.750g was only observed in 2.3% of the leaves. Lamina weight (g) tended to be positively correlated with LAM through a power model as given below: The results signify that the explanatory power of LAM to control Lamina wt. is around 66.5%.

$$\begin{array}{l} Log_e \ Lamina \ weight \ (g) = 0.036 + 0.825 \ (Log_e \ LAM, \ cm^2) \pm 0.0.386 \\ t = 8.192 \quad t = 18.62 \\ P < 0.0001 \quad p < 0.0001; \ R = 0.815, \ R2 = 0.665, \ F = 346.69 \ (p < 0.001) \end{array}$$

Table 6. Correlation and regression analyses for lamina area estimation of leaves (N = 177) in 32-month-old *R*. *mucronata* saplings raised in tidal area nursery of Shah Bunder.

Simple Linear Correlation and Regression						
Leaf area (cm ²) = $0.517 + 0.675$ (LL x LB, cm) ± 1.799 , R = 0.973 ; R ² = 0.947 ; Adi, R ² = 0.947 ; F = 3155.95						
t = 1.61 $t = 56.18$						
p < 0.110 $p < 0.0001$ Eq. # 1. (see Fig. 11)						
Multiple Correlation & Regression						
Leaf area $(cm^2) = -11.697 + 1.481$ LL + 5.466 LB ±2.2138, R = 0.960; R ² = 0.921; Adj. R ² = 0.920, F = 1012.4						
t = -17.16 $t = 5.92$ $t = 12.27$						
p < 0.0001 $p < 0.0001$ $p < 0.0001$ Eq. # 2.						
Zero Order r Partial r						
LL 0.923 0.409						
LB 0.951 0.681						
Power model						
Leaf area (cm ²) = 0.697. (LL x LB) $^{0.999} \pm 0.117$, R = 0.984; R ² = 0.968; Adj.R ² = 0.968; F = 5378.02						
t = 23.16 $t = 73.34$						
p < 0.0001 p 0.0001						

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

Leaf orientation

Leaves in *R. mucronata* are opposite. A leaf is borne more or less with its flat surface making an angle (c 45°) with horizontal (Fig. 12). In 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 °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 received only 20% of the available sun and were approx. 10 °C cooler. The increase in 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. Leaf angle increased with increasing exposure to the sun among the members of Rhizophoraceae. The angle was greater, and hence the proportion of the projected leaf area was smaller, the greater the salinity tolerance of a species. The role of leaf orientation in *R. mucronata* should be evaluated in detail.



Fig.10. Distribution of K





Fig. 11. Relationship of graphically measured lamina area (LAM, cm²) with multiplicative parameter of LL x LB as given by simple linear or power model of regression in 177 leaves of 32-month old saplings of *R. mucronata* (see Table 6).

Fig, 12. Two-year old R. mucronata saplings in drum pot and irrigated with Seawater at Department of Botany, University of Karachi. It was initially grown with propagules in a coastal swamp in pots daily inundated with oceanic currents with tides in Gwadar under Mangrove transplantation Scheme of Coastal Development Authority. Note the alignment of leaves at 45° from the horizontal. The orientation of leaves to sun is verv crucial in mangroves particularly Rhizophora. (See text for explanation).

Leaf temperature is also influenced by leaf size (Ball, 1988). In Rhizophoraceae, leaf size decreases with increasing exposure to radiation (Ball *et al.*, 1988) and is the smaller in the most salt-tolerant species (Ball, 1988). The mangrove leaves are smallest under conditions in which, due to intense radiation and / or limitations to evaporative cooling, they sustain the greatest heat load (Ball *et al.*, 1988).



Fig. 13. TS of petiole of *R. mucronata* - A, Epidermal and cortical region; B, Vascular bundle zone and C, Xylem, phloem and the scattered sclereids in the cortex. A brachyslereid is also visible.

Leaf internal structure

The anatomical details of leaf are presented in Fig. 13 -17 and may be briefly be described as below.

Petiole: Cuticle is thick and appears to be waxy. Epidermis is one-layered and epidermal cells are squarish or rectangular in shape and small in size. Cortical cells are circular to irregular in shape with large intercellular spaces among the cell. A number of sclereids are distributed in cortical region – brachyslereids also often present. Vascular bundle is horse-shoe shaped – xylem on the inner side and phloem on outer side. Xylem vessels are circular (Fig. 13). Pith is parenchymatous

Lamina: *R. mucronata* is often classed as leaf-succulent halophyte. There are, however, no specialized cells of salt glands. Due to the absence of salt glands. Scholander *et al.* (1962) called it a salt excluder. In the leaf epidermis is single-layered and consists of rectangular cells covered with cuticle. Hypodermis in the midrib region is multi-layered but in laminar region two-layered dorsally and single layered ventrally. Sclereids are abundant in cortex. The coriaceous nature of leaves may probably be due to sclereids in the mesophyll. Sclereids are also reported by Tomlinson (1986) in the cortical region of *R. mucronata* for mechanical strength. Stomata on lower surface only-they are sunken and provided with front cavity. Palisade present in both upper and lower region of lamina but somewhat. There is single but large horse-shoe shaped VB in the midrib region but there are several VBs (Fig. 14-16) in the lamina owing to reticulate venation. Large intercellular spaces are present in cortical parenchyma near midrib VB (Fig. 16). This Aerenchyma is considered important in mangroves from air flow dynamics. Several VB (Fig. 17). Tannin and crystalliferous cells are reported in Family Rhizophoraceae. *Rhizophora* has mucilage cells also. Aerenchyma in the leaf is important from the viewpoint of internal air flow. This resembles to the anatomical account given by Surya and Hari (2017 a and b) for *R. mucronata* of Kerala.



Fig. 14. TS of Leaf of *R. mucronata* – Midrib region.



Fig.15. TS of leaf of *R. mucronata* through laminar region (A) and a stoma on the ventral surface of leaf (B).





Fig. 17. R. mucronata Crystals in the cortical cells (A) and a cystoliths (B).



Fig. 18. A) Ventral surface of leaf of two- year old sapling of *R. mucronata* from the central midrib zone. This region of leaf is completely devoid of stomata. The cells polygonal in shape papillose and without intercellular spaces. The anticlinal cell walls are quite straight and cells are somewhat papillose. B) Ventral surface of Leaf of *R. mucronata* sapling showing stomata restricted to the laminar region. The midrib area is devoid of stomata.

Leaf surface micromorphology Stomata

Leaves are hypostomatic. Dorsal surface is devoid of stomata (Fig. 18 A) and they are restricted to ventral surface (Fig. 19B). Stomata are sunken and localized in the non-venous part of the lamina (Fig. 18). Stomata were of cyclocytic type (a special type of anomocytic). Stomata are wide-elliptical in shape and oriented in various directions (Fig. 19, 20, 22A). Five to six subsidiaries are arranged in a ring around the guard cells (Fig. 19). Subsidiaries appear to be located in depression (Fig. 20). The epidermal cells are papillose, more or less round in shape with straight to slightly curvy anticlinal walls (Fig. 21). Rarely stomata are also without guard cells (Fig. 22 B). The guard cells of R. *mucronata* have outer beak-like ledges (Fig. 22 B). Stomata were in close pair sometimes (Fig. 23).

In genus *Poga* of Rhizophoraceae, stomata have been reported to be of anomocytic type and in genera *Anisophyllea* and *Combretocarpus* of this family they have been reported to be paracytic type (Stace, 1966). Metcalfe and Chalk (1950) have reported anomocytic, anisocytic and paracytic stomata on the leaves of genus *Anopyxis* of Family Rhizophoraceae. Stace (1966) opines that many of the genera of *Rhizophoraceae* have basically paracytic subsidiaries but that extra walls usually develop giving the appearance of anomocytic stomata. Das and Ghose (1997) reported cyclocytic stomata in *R. mucronata*. In this species, the surrounding epidermal cells of the guard cells mother cells divide tangentially in a circular way to form a ring of narrow cells of the subsidiary cells. The ontogeny is, therefore, perigenous type and mature stomata, a cyclocytic type. There are no hairs on the

epidermis. Stomata of *R. mucronata* resemble to that of *Kandelia* with 5-6 subsidiaries (Sheue *et al.*, 2003). There are no trichomes in *R. mucronata*.



Fig. 19. Dorsal (A) and Ventral (B) surface of leaf of *R. mucronata*. Dorsal surface has no stomata.



Fig. 20. Magnified view of ventral surface of leaf of *R. mucronata* showing cyclocytic (special type of anomocytic stomata with subsidiaries in depression in ring) surrounded with papillose round to polygonal cells of epidermis.



Fig. 21. A) A stoma of *R. mucronata* in depression under high magnification (1500X). Inner Pore size- 22.8 um in length. B) Stoma position within leaf of *R. mucronata* after Schimper 1891) – Ct, cuticle, ep, epidermis, hp, hypodermis, St, stoma (guard cells).



Fig. 22. Ventral surface of *R. mucronata* leaf (as seen in nail-polish imprint) – A) stomatal distribution and B) A stoma without guard cells.

Stomatal density in freshly collected leaves of *R. mucronata* from the saplings was fairly consistent among the leaves averaging to 143.5 ± 2.23 , 140.64 ± 1.96 , 146.72 ± 2.24 and 144.75 ± 2.11 stomata per mm² respectively in four leaves studied (Table 7). Stomatal density varied but little (CV = 10 to 11%). In the four leaves studied, predominating size class of stomatal density was 120-160 stomata per mm² (occupying a proportion of 79.8, 72.8, 74.5 and 76.4% of the total observations, respectively). The average stomatal density in a mature leaf collected in 2012 from naturally growing plant of *R. mucronata* in Indus delta and preserved in formalin was, however, quite low 95.97 \pm 1.34 and varied between 58.97 and 127.8 stomata per mm² (Fig. 24) which 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 which is lesser than our estimate in *R. mucronata* sapling leaf. In *R. mangle*, Peel *et al.* (2017) 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. Khan *et al.* (2017) reported high glandular density in young smaller leaves than larger mature leaves in *Rhynchosia minima* due to foliar expansion. Since salinity reduces leaf size, it may increase the stomatal density. Davis (1940) reported the influence of salinity changes per se on leaf sizes in *Rhizophora*. It may be mentioned that leaves collected from saplings were smaller than those collected from naturally growing plants.

Stomatal length in *R. mucronata* in present studies averaged to $39.66 \pm 0.626 \mu m$ in leaf I and $37.14 \pm 0.5113 \mu m$ in leaf II (Table 8). Sheue *et al.* (2003) reported guard cells of *Kandelia* to be $33.25 \mu m \times 31 \mu m$ in size. In leaf sample I stomatal size in terms of length in 50 % of the observations belonged to the category of size below 40 μm and 50 % to the category of more than 40 μm and lesser than 50 μm . The length and width of stomata varied almost equally (CV= 14.07 and 15.48 %, respectively). The width of the stomatal apparatus averaged to 29.47 \pm 0.510 in leaf I and 23.20 \pm 0.2733 μm in leaf II. The width size class of 20.1- 40 μm was the dominating class (97.5%) in Leaf I and size class 21-25 μm was predominating in leaf II.

Our results on stomatal size of *R. mucronata* are similar to Samadder and Jayakumar (2015) who have reported stomatal size in *Rhizophora apiculata, R. mucronata* and *R. stylosa* of Andaman and Nicobar to be 41.8 x 22.8, 34.2 x 26.6 and 38 x 26.6 µm, respectively. The stomatal types were reported to be anomocytic in *R. apiculata* and *R. stylosa* and, cyclocytic in *R. mucronata*. Stomatal length in *R. mucronata* is reported to 17.98 µm and width 9.27 µm by Surya and Hari (2017a and b). The depth to which the stomata may be sunken is said to depend on the leaf age (Chapman, 1976).



Fig. 23. Stomatal pairs on ventral surface of leaf of *R. mucronata* preserved in formalin.



Table 7. Stomatal density per mm² in some freshly collected mature leaves of *R. mucronata* from Indus delta.

Parameters	Leaf I	Leaf II	Leaf III	Leaf IV	Pooled data
Ν	55	55	55	55	220
Mean	143.50	140.64	146.72	144.75	143.91
SE	2.2323	1.9571	2.2360	2.11031	1.0716
CV (%)	11.59	10.32	11.30	10.82	11.04
Minimum	108.12	108.12	98.29	108.12	98.29
Maximum	186.75	176.52	176.92	176.92	186.75

Table 8. Stomatal size (pore + guard cells) of *R. mucronata* leaf (ventral surface).

	LEA	FI	Leaf II			
Parameters	Stomatal	Stomatal	Stomatal	Stomatal		
	length (µm)	width (µm)	length (µm)	width (µm)		
N	80	80	70	70		
Mean	39.99	29.47	37.14	23.20		
SE	0.626	0.510	0.51132	0.27334		
CV (%)	14.07	15.48	11.52	9.86		
Minimum	25.60	19.20	28.80	16.0		
Maximum	49.60	41.60	44.80	28.80		

Cork warts

Cork warts are present in *R. mucronata* on ventral side of leaf as minute black lesions occurring in different size (Fig. 25). In *Kandelia (a genus of 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 of the abaxial side but 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 Knudsen internal flow (Evans *et al.*, 2009; Evans and Bromberg, 2010). Aerenchyma and cork warts in leaves of mangroves provide Knudsen internal flow through heated air pressurization to aerate anoxic roots. Attim's model in this connection is crucial and defined by the presence of a trunk with continuous growth, all orthotropic branches with lateral flowers, and continuous or discontinuous branching. This all-purpose generalized model may have considerable plasticity in its expression. Warts are reported to have secretory cells in *R. mucronata*, an important laminar characteristic (Replan and Malabrigo, 2017).



Fig. 25. Three warts on the ventral surface of *R. mucronata* leaf (A). A single wart with diameter 433µm).

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