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SEEDLING STRUCTURE AND SURFACE MICROMORPHOLOGY OF *STERCULIA FOETIDA* L. (MALVACEAE)

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Abstract

The seedling structure and surface micromorphology of *Sterculia foetida* L. (Malvaceae) is described. Seedling of *S. foetida* may be Phanerocotylar-epigeal - Reserve type or Cryptocotylar – hypogeal - Reserve type. When aboveground, cotyledons unfolded, expanded and turned green on exposure to sun, developing dense crop of trichomes. The whole seedling surface was trichomatous. Cotyledonary trichomes were conical and multicellular. Some four kinds of trichomes were observed on leaflets. 1) Long, apically pointed non-glandular unicellular (acicular) trichomes – predominantly abundant. 2) Long, apically curved or hooked, unicellular or uniseriate multicellular trichomes. 3) Long apically club- shaped, Bicellular, uniseriate glandular trichomes. 4) Dark globular (ball-like, peltate) glandular trichomes on the midrib. Epidermal cells in cotyledons were polygonal and straight in anticlinal contour. The adaxial epidermis of leaf was with straight to curvy anticlinal walls and abaxially the epidermal cells were sinuous and irregular in shape. A diversity of stomatal complexes was recorded on cotyledons and leaflets. Stomatal density was lesser on cotyledonary sun-exposed surface than that of leaflets. Leaflets were hypostomatous. Foliar stomatal density varied with the size of leaflets. Stomatal density in the smallest leaflet of 184 mm^2 was 249.14 ± 5.54 (varying from 157.26 to 324.36 stomata per mm^2, CV: 14.05%) and 125.22 ± 3.449 stomata per mm^2 in the largest leaflet (4709 mm^2). Stomatal size inclusive of normal and giant stomata averaged to 21.15 ± 14.86 µm x 14.86 ± 0.176 µm (L x W) in 265 observations. It may be concluded that *S. foetida* is quite rich in trichomal and stomatal diversity.

Key words: *Sterculia foetida* L., Seedling structure, Surface micromorphology, Trichomes and Stomatal complexes.

Introduction

Sterculia foetida L. (Vern. Names: Homrong or Hamrong, Marong, Chammahong, Samrong, Java Olive, Jangli Badam Pinari, Bastard Poon nut, Hazel Sterculia, wild Indian almond, Skunk tree, Banksho Badam, Kalumpang etc.), a multipurpose tropical deciduous tree of ornamental and medicinal value, belongs to family Malvaceae (former Sterculiaceae). The genus *Sterculia* was named after the Latin god Sterculius = god of fertilizer and manure and species name comes from the foul-smelling flowers and leaves of some species of genus *Sterculia.* The genus comprises some 300 species (Thabet *et al.,* 2018). It is distributed in India, Taiwan, Indo-China, Philippines, Hawaii, Indonesia, Australia, and some African countries – now cultivated on small scale in Pakistan (Fig. 1). During flowering phase, it is prominent and presents a brilliant sight due to orange red flowers against leafless state of the plant. The fruits are capsules - red and lobed (Fig. 1B, C). A fruit contains 10-15 seeds (Sudrajat *et al.,* 2018). There are two shells of seed. The outer shell is brown and the inner shell black. The kernel is pale brown. Seed morphology and its taxonomic significance in Family Malvaceae have been described by Abid *et al.* (2016). The Seeds of *S. foetida* are edible but purgative. *Sterculia* seed oil is a high-quality oil of oxidative stability and potentially edible and used in the food industry (Chanyawiwatkul *et al*., 2018). Seed oil, according to Verma *et al*. (1957) contains 71.8% Sterculic acid and minor proportion of oleic and linoleic acids. Empty fruits and seeds are rated as excellent sources of thermal energy (Vaishnavi and Pugazhvadivu, 2017). Seeds extract is reported to be anti-termite (Amuthavalli *et al.,* 2020). It is a species of potential therapeutic value (Thabet *et al.,* 2018). Its leaves can be used as fodder.

It may be planted in areas with low rainfall and air temperature up to 40-45ºC (Nhan *et al.,* 2019). Its wood is easy to cut but not durable. Gum karaya exudates from the stem of the tree. It has some tolerance to salinity and accumulates K in leaves (Lustosa *et al.,* 2017). The environment of 50% shading was found to be most suitable to produce *S. foetida* seedling even under salinity of 5.1 $dS.m^{-1}$ (Lima *et al.,* 2018). In India, a leaf rolling insect (*Sylepta balteata*) is a serious pest (http://www.nparks.gov.sg/florafaunaweb).

The structure and surface micromorphology of seedlings or mature plants are very useful taxonomic tools to characterize plants. Recently, structure and distribution of heteromorphic stomata in a malvaceous species, *Pterygota alata* (Roxb.) R. Br have been studied by Mitra *et al*., (2015). To our knowledge, no such studies have been undertaken in *S. foetida*. The present paper, therefore, describes the seedling structure and surface micromorphology of this species when raised in pots under irrigation.

Materials and Methods

Seeds: The seeds of *Sterculia foetida* were provided by Dr. M. Azeem, department of Botany, University of Karachi. The weight of air-dried seeds selected to sow (N = 30) for germination averaged to 2.785 ± 0.08123g varying from 1.373 to 3.696g per seed (CV= 15.98%). Some 63.4% of seeds belonged to the size class of 2.51 - 3.0g and 23.3% to the size class of 3.1-4g.

Seedling procurement: To procure seedlings, the seeds were sown in plastic pots (five seeds per pot) under shade in, in the first week of April, 2022 at Karachi. Pots contained sandy loam soil with compost manure. Prior to sowing, the seeds were sterilized with 1% sodium hypochlorite solution, rinsed and submerged in water for 2 h. The pots were arranged in completely randomized design under the green shade at 30 ± 2ºC.

Seedling emergence: Seedlings began emerging within a week quite synchronously.

Seedling micromorphology: The seedling type was described according to Garwood (1996). Hickey (1973) and Ash *et al*. (1999) for description of leaf. The impressions of surface of leaflets were made with clear nail polish Wang *et al.* (2006). The nail polish imprints were studied under compound optical microscope for ornamentation and micro-morphological structures. Stomatal nomenclature suggested by Metcalfe and Chalk (1950), Van Cotthem (1970), Dilcher (1974), Wilkinson (1979), Carpenter (2005) and that presented by Mitra *et al.* (2015) for stomatal description in *Pterygota alata* (Roxb.) R. Br. (Family Malvaceae, formerly Sterculiaceae), *Sabo et al.* (2007) for genus *Arum*, Surat un Nisa *et al.* (2019) for *Vincetoxicum arnottianum* (Fam. Apocynaceae), and Den Hartog née Vanter Tholan and Baas (1978) for Celastraceae were followed to ascertain stomatal types. Stomatal size was measured microscopically at 45 x 10 X magnification. The data on stomata and trichomes was analyzed statistically.

Results and Discussion

Seedling: The seedlings were obtained from seeds varying from 1.373 to 3.696g per seed which were more or less comparable to the size of single seed reported from Indonesia (2.89 ± 0.64 to 4.05 ± 0.52g (Sudrajat *et al.*, 2018). The maximum emergence was recorded to be c 60%. Germination in soil was also reported to start in a week reaching to 70% by Rai (2014) in the climate of Meerut District, India. Santos *et al.* (2004) has reported that mechanical scarification and soaking in water for around 24h overcome dormancy. According to the scheme of Garwood (1996), the seedling of *S. foetida* appeared to be Phanerocotylar-epigeal-Reserve type or Cryptocotylar-hypogeal-Reserve type depending upon the seed-sowing-depth. When seeds were sown in superficial soil, cotyledons were sent aboveground by vigorous hypocotylar growth (Fig. 4A, B and C). If sown in deeper layer of soil only epicotyl emerged out lodging cotyledons underground (Fig. 4D). At 20 days of age, the hypocotyl was meaured 4.5cm and epicotyl c 5 cm. The seedling at this age had three leaves (two large and one small) and leaf area per seedling: 9169 mm^2 (Table 1).

Cotyledons: Cotyledons of an imbibed seed were thick (both cotyledons combinedly around 1 cm in thickness), fleshy, heavy, pale green in colour and oval in shape. They were c 2.7 cm in length and 1.5 cm broad in imbibed seeds. Cotyledons were unequal in size. Inner surface was concave and outer surface surface convex. The ridge on the inner surface of smaller cotyledon fitted with the groove of the larger cotyledon (Fig. 2). If sown near soil surface, hypocotyl grows rapidly and brought cotyledons aboveground. They unfolded, expanded and turned green on exposure to sun, developing dense crops of trichomes. After some time, they twisted and got rolled releasing nutrients to developing epicotyl (Fig. 3, 4A, B, C). In seedling bearing one leaf, the two cotyledons, the cotyledons measured 4.3 and 3.3 cm in length.

Hypocotyl: Hypocotyl was c 0.5 cm in diameter and 1cm in length, green and trichomatous in young seedling. In deep-sown seeds, no cotyledons or hypocotyl came aboveground. It was only epicotyl which came above soil (Fig. 4D).

Epicotyl: It grew rapidly into epicotylar stem and palmate shiny leaves. All parts of epicotyl were trichomatous.

Table 1. Leaf morphometry of Primary, secondary and tertiary leaves of 20-day old seedlings of *S. foetida*.

Leaflets	LL	LW	Leaflet area (mm^2)	AA (°)	BA (°)	Total leaf area (mm^2)
Primary leaf (Petiole length: 4.1 cm)						
Leaflet 1	6.9	2.4	960	46	57	
Leaflet 2	7.3	2.2	964	47	48	
Leaflet 3	7.5	3.1	894	44	40	4709
Leaflet 4	8.0	2.3	1102	55	58	
Leaflet 5	6.9	2.0	729	44	52	
Mean leaflet area = 929.8 ± 25.12 mm^2						
Secondary leaf (Petiole length = 3.7 cm)						
Leaflet 1	5.0	1.4	450	50	55	
Leaflet 2	7.4	2.0	857	45	55	
Leaflet 3	7.6	2.2	1138	51	48	4359
Leaflet 4	7..4	1.9	693	36	40	
Leaflet 5	8.0	2.0	1147	52	44	
Mean leaflet area = 857 .0 ± 133.35 mm^2						
Tertiary leaf (Petiole length = 1.2 cm)						
Leaflet 1	10	-	-	-	-	
Leaflet 2	24	-	-	-	-	
Leaflet 3	20	-	-	-	-	101
Leaflet 4	21	-	-	-	-	
Leaflet 5	12	-	-	-	-	
Mean leaf area per seedling = 9169 mm^2						

Acronyms: LL, Leaflet length (cm), LW, leaflet width in cm (widest point), AA, apex angle, BA, base angle,
Leaf area was determined as sum of the areas of the leaflets of a leaf estimated by drawing their accurate outlines on graph paper.

Leaves: Leaf base was swollen with two spinous conical stipules (c 2mm wide in base and 3 mm in length). The leaflets are arranged in umbrella like manner on top of the green petiole (palmate leaf). The leaflets were free but the basal portion was connate. This agreed with Berger (1972). Leaflets were lanceolate in shape in mature plants - acute at base and apex (Table 1) and widest in the mid (Fig. 5). They were revolutely marginate. The leaves were palmate with five leaflets at seedling stage. The mean number of leaflets per leaf averaged 5.47 ± 0.29, N = 15). Berger (1972) reported 5-8 leaflets per leaf which was higher in upper leaves. Palmate leaves are reported to characterize *S. foetida*. Other species of *Sterculia* have been reported to have simple leaves (Hussin & Sani (1998). Leaflets dorsiventral, marginally entire, variable in size and entire (Table 1) covered all over with trichomes. All parts of leaf were trichomatous – quite denser on midrib and the upper foliar surface (Fig. 6).

Epidermal micromorphology

Trichomes: All aerial parts of a seedling were trichomatous (Figs. 3-6). Trichomes heteromorphic of several types (Figs. 7-11). On sun exposed surface of cotyledons trichomes were conical and multicellular. They were long and bluntly narrow at the apex, and densely covering the surface (Figs. 7, 8). The basal part of the trichome was light in colour or colourless. Trichomal density on two cotyledons studied varied (Table 2) significantly ($t = 2.65$, $p<0.001$). Such a difference in trichomal density may be thought to arise as a result of size difference or differential removal of trichomes with time due to random events. Trichomal scars on cotyledons appeared to be circular and their diameters at right angle averaged to 28.32 ± 0.7198 and 26.86 ± 0.6788 µm. The ratio of their magnitudes, 1.081 ± 0.0306, indicated their near- perfect circular shape (Table 3). The scar of broken trichome was also circular on hypocotyl (Fig. 14).

Fig. 1. **A)** *Sterculia foetida* Habit – image from Karachi, Pakistan. **B)** Five-lobed dehiscing fruit. **C)** A fruit lobe split to show black seeds attached on the ventral suture.

Fig. 2. Emergence of seedling from *S. foetida* seeds. **A**, Seed germinating from superficial soil. **B**, Cotyledon from imbibed seed (inner surface view showing an area in the middle depressed in form of a groove in one cotyledon and mid area raised as a ridge in the other cotyledon); **C**, Cotyledon (outer surface view – prominent in convexity) and **D**, Cotyledon of recently emerged seedling showing inner yellow-green and outer brownish cotyledonary surface. Hypocotyl green.

Fig. 3. **A)** Enlarged view of epicotyl represented by a leaf in very early seedling stage, the hypocotyl is hairy. B) Aboveground cotyledons undergone rolling and twisting as they are consumed. C) The inner surface of cotyledons facing sun on emergence. It becomes green and highly trichomatous on exposure to sun. The epicotyl with a single primary leaf emerging in between the cotyledons. Hypocotyl is green, stout and trichomatous.

Fig. 4. Seedlings of *S. foetida*. **A)** Abovground cotyledons rolling and twistimg tightly. **B)** Growth of a primary palmate leaf with several leaflets. **C)** Early seedling with two primary leaves. Aboveground cotyledons and epicotyl are all over trichomatous. **D)** Only epicotyl arising from the soil in case of deep-sown seeds, no cotyledons or hypocotyl aboveground.

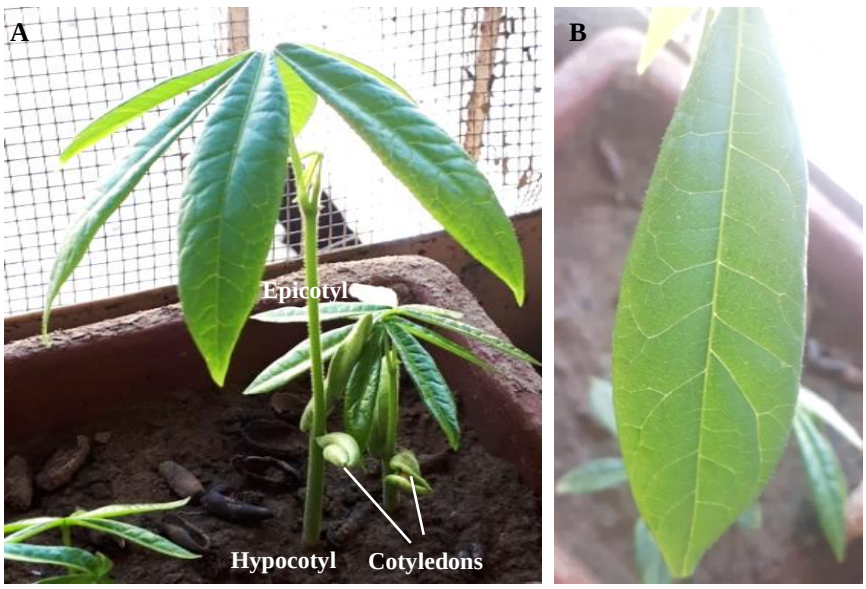


Fig. 5. **(A)** Ten-day old seedlings arising from superficial soil. Smaller seedling arising from smaller seed and larger seedling from the larger (heavier) seed. Note the twisted trichomatous cotyledons and palmate shining leaves with leaflets hanging like an umbrella. The venation is brachidodromous **(B)**.

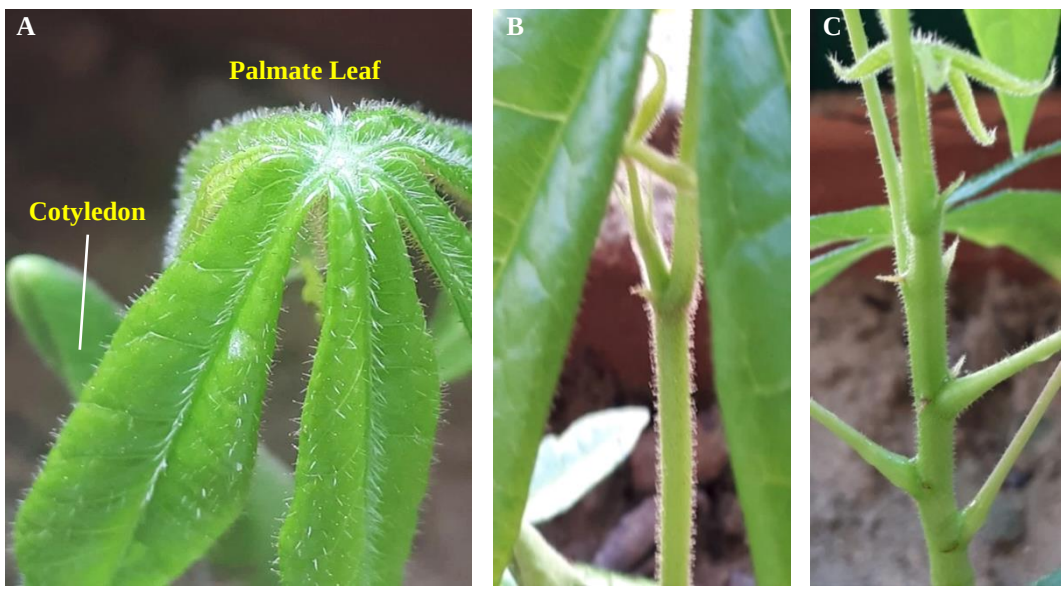


Fig. 6. Trichomes on leaves (**A**) – trichomes are present all over the leaf dorsally and ventrally, but they are denser on dorsal surface i.e., on midrib, and on the top where the leaflets unit (connate). They are present on the margins of leaves also. Trichomes are present on stem and petiole (**B** and **C**). The leaves are stipule-bearing when young (**C**).

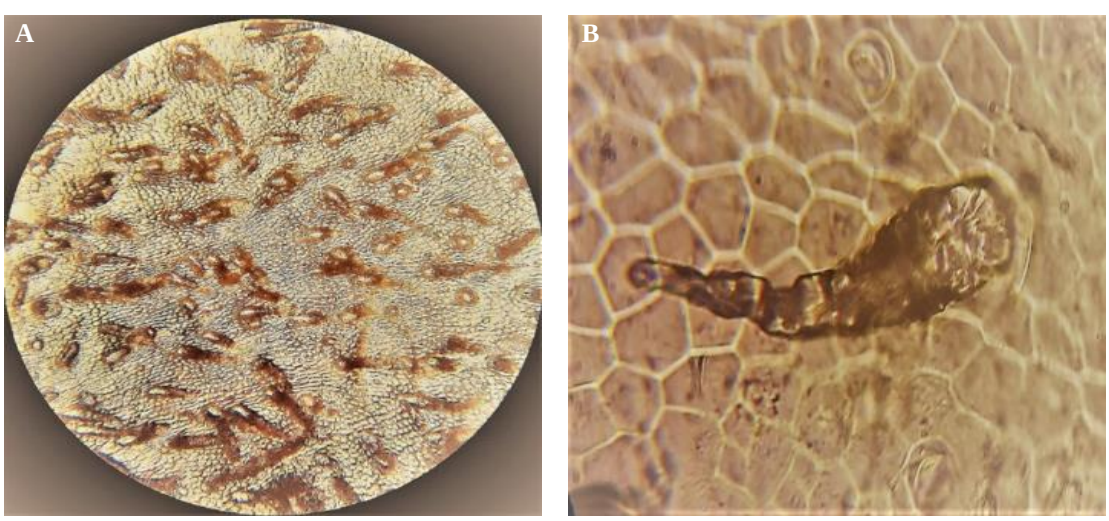


Fig. 7. Cotyledonary surface with multicellular trichomes and stomata interspersed amongst the trichomes (**A**). A single Cotyledonary multicellular conical trichome (**B**). Epidermal cells straight in anticlinal contour. Magnification – **A**) 10 x 10X; **B**) 45 x 10 X.

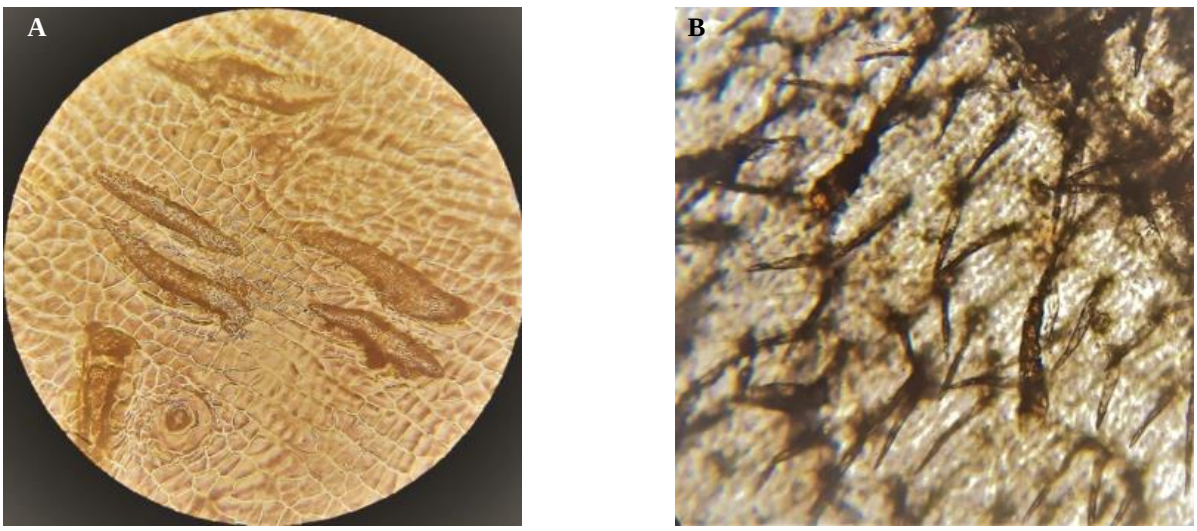


Fig. 8. Multicellular trichomes and a stoma on surface of cotyledon exposed to sun - the anticlinal walls of the epidermal cells are straight (**A**). Trichomes on hypocotyl (**B**) were frequently long, unicellular and apex-pointed. Multicellular trichomes were less-frequent than unicellular trichomes (**B**). Magnification – **A**) 45 x 10X; **B**) 10 x 10X.

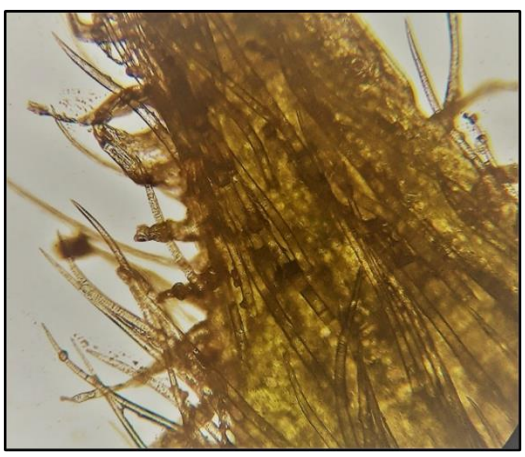


Fig. 9. Dense trichomes on stipules. 10 x 10X.

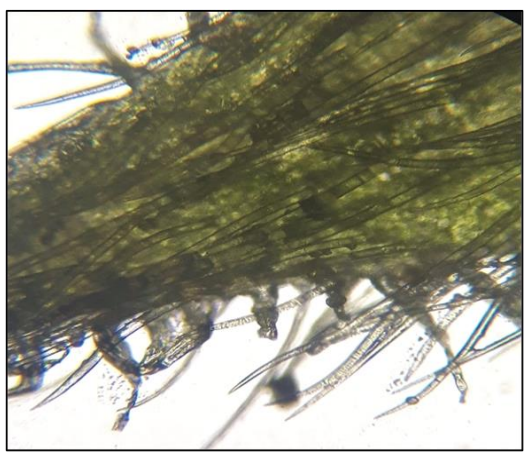


Fig. 10. Surface of young leaf showing trichomes. 10 x 10 X.

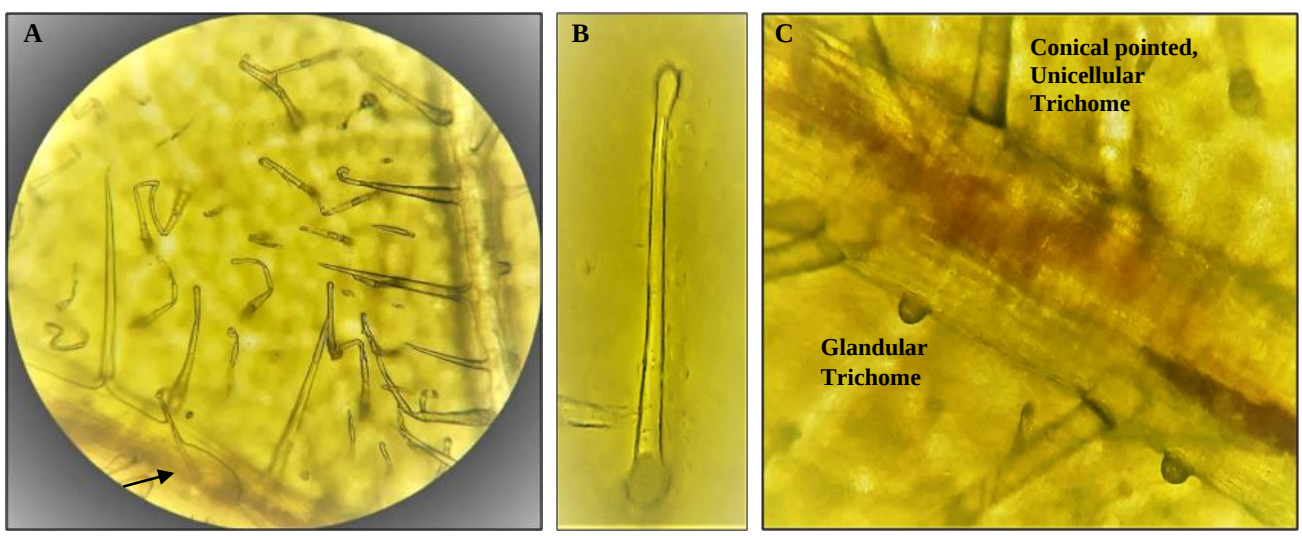


Fig. 11. Ventral surface of leaflet showing;
*, Long conical, apically pointed trichomes arising from the midrib region cells. They are relatively shorter on lamina surface (Fig. 11 A). Magnification: 45 x 10X, zoom 1.6X.
*, Round-ended curved (hooked) or bent type multicellular trichomes arising from lamina and midrib or secondary vein cells (Fig.11 A).
*, A few long apically club-shaped glandular spherical gland (shown by arrow) – Fig. 11A and B. Magnification B: 45 x 10 X; zoom 2.8X.
*, Dark-coloured globular (peltate), glandular trichomes on the midrib amongst the long apically-pointed trichomes (Fig. 11 C). Magnification: 45 x10 X; zoom 2.6X).

The trichomes on hypocotyl were long, frequently unicellular and less frequently multicellular (Fig. 8B). Abscised trichome left circular scar on hypocotyl (Fig. 14). On stipule and young leaf, trichomes were dense, generally long and unicellular (Figs. 9, 10). Trichomes of leaflet are presented in Fig. 11. They generally arose from midrib or veinlets (Fig. 18). They were variable in length, generally shorter on finer vessels or intercostal islands. They may be classified as given below:

1. Long, apically pointed non-glandular unicellular (acicular) trichomes – predominantly abundant.
2. Long, apically curved or hooked, unicellular or uniseriate multicellular trichomes.
3. Long apically club- shaped, Bicellular, uniseriate glandular trichomes.
4. Dark globular (ball-like) glandular trichomes on the midrib.

There were a number of trichomal scars on the leaflets which were circular in outline as on cotyledon and hypocotyl. Their size in terms of diameters measured at right angle (Table 4) in statistically good number of observations (N = 30-50) indicated that they were fairly constant in average size (24.84 ± 1.014 to 29.96 ± 1.357 µm) in four leaflets studied. As regards to the previous publications, trichomes (non-glandular and glandular) were reported along or on veins of the leaf surface in *Buttneria admanensis* of Family Sterculiaceae by Maity (2011). Non-glandular trichomes were acicular, simple or multicellular in this species (Maity, 2011). Hussin & Sani (1998) described a variety of trichomes in the genus *Sterculia* and two types of trichomes in *Sterculia foetida*, 1) Simple cylindrical non-glandular multicellular trichomes and 2) Glandular trichomes. Our observations indicate that *S. foetida* is comparatively richer in trichomal diversity than that reported in earlier studies. Table 5 presents the length of various types of trichomes. Amongst various trichomes, the longest were the acicular unicellular trichomes present on the midrib (613.28 ± 22.07, 225.0 – 1025.0 µm, CV: 33.12%). Distribution of acicular trichome lengths (µm) on a young leaflet of *S. foetida* revealed that some 77.5% of the trichomes belonged to the size class, 251 -750 µm. Shorter than this class were merely 1.3% and larger than this class were 21.20% (Fig. 12). These trichomes appeared to come from a population which tended to be normally-distributed. The smallest trichomes were the dark, glandular globular (39.29 ± 3.26, 25-50 µm, CV: 21.95%.) and the others were of intermediate sizes.

Ground epidermis: Epidermal cells in cotyledons were polygonal and straight in anticlinal contour (Fig. 7B and 8A). The adaxial epidermis of leaf was with straight to curvy anticlinal walls and abaxially the epidermis wall was sinuous and irregular in shape (Fig. 13). Hussin & Sani (1998) have also reported abaxially wavy anticlinal walls in *S. foetida* leaf. The lamina exhibited a well-developed vascular network studded with trichomes (Fig. 18). The costal cells were elongated. Two trichomal scars on vein were also seen with common elongated basal cells having straight anticlinal walls (Fig. 13). Cuticular striation was present on laminar as well as costal cells of the leaflets (Fig. 19).

Table 2. Cotyledonary trichome density per mm^2.

Parameters	Cotyledon I	Cotyledon II
	Trichomal density	Trichomal density
N	30	30
Mean	45.8686	34.074
SE	3.2078	3.08068
Median	49.1449	29.4869
CV (%)	38.29	49.52
G1	0.037	0.740
Sg1	0.427	0.427
G2	-1.121	0.032
Sg2	0.833	0.833
Minimum	19.66	9.63
Maximum	78.63	78.63
KS-T*	0.158 (p<0.055)	0.207 (p<0.002)
Shapiro-Wilk	0.930 (p<0.050)	0.911 (p<0.016)
Symmetry	AS	AS

Acronyms: SE, Standard error of mean; CV, coefficient of variation; G1, Skewness; Sg1, St, error of skewness; G2, kurtosis; Sg2, St. error of kurtosis; KS-T*, Kolmogorov-Smirnoff Test with Lilliefors significance correction; AS, Asymmetrical

Table 3. Size of trichomal scars in terms of scar diameters on the sun-exposed (upper) surface of cotyledon.

Statistical parameters	Trichome Scar size *		
	Diameter I	Diameter II	Ratio
N	60	60	60
Mean	28.32	26.86	1.0811
SE	0.71977	0.67877	0.03061
CV (%)	19.64	19.58	21.93
Minimum	18.72	15.60	0.58
Maximum	40.56	39.00	1.86

*, Diameters I and II measured at right angle

Table 4. Diameters of trichomal scars at right angles (D1 and D2) on ventral surface of variously sized leaflets.

Statistical Parameters	Leaflet A*		Leaflet B*		Leaflet C*		Leaflet D*	
	D1	D2	D1	D2	D1	D2	D1	D2
N	40	40	50	50	30	30	40	40
Mean	29.96	29.52	29.58	26.15	27.72	24.91	24.84	25.82
SE	1.357	1.186	0.649	0.615	1.372	1.404	1.094	1.149
CV (%)	28.65	25.42	15.51	16.64	27.13	30.88	27.84	28.14
Minimum	15.60	18.72	15.60	15.60	17.72	9.36	15.60	15.60
Maximum	53.04	53.04	37.44	37.44	49.92	46.80	49.92	49.92

Leaflet sizes: *, Leaflet A = 358 mm^2, Leaflet B = 842 mm^2; Leaflet C = 1138 mm^2; Leaflet D = 1786 mm^2

Table 5. Comparison of Length (µm) of various types of Trichomes on leaflet.

Trichome type	Length statistics
Long acicular unicellular trichomes	N = 80, Mean: 613.28 ± 22.07, 225.0 – 1025.0, CV: 33.12%.
Long multicellular (on midrib)	N = 20, Mean: 260.63 ± 10.19, 200 – 350, CV: 17.49%
Long multicellular on veinlets in Lamina	N = 25, Mean: 167.0 ± 10.7, 62.5-250, CV: 32.19%
Long apically curved / bent	N = 12, Mean: 290.58 ± 10.70, 250-350, CV: 10.91%
Long Apically gland-dotted	N = 10, Mean: 261.36 ± 15.12, 200-362.5, CV: 19.18%
Peltate spherical glandular trichome on midrib	N = 7, Mean: 39.29 ± 3.26, 25-50, CV: 21.95 %.

Stomatal complexes: The leaf of *S. foetida* was found to be hypostomatous as also reported by Pereira *et al.* (2018). The stomata were round to wide elliptical in shape. There was a variety of stomata oriented in various directions in this species. They may briefly be described as under:

1. **Anomocytic**: Stomata surrounded with few indistinct neighbouring cells (NCs) (Van Cotthem, 1970; Dilcher, 1974). Such stomata were found on hypocotyl [Fig. 14A and 14 (B1), six NCs] and sun-exposed surface of cotyledon (Fig. 14 B2). On cotyledon they were with 3 to 5 NCs (Fig. 16). Also present on leaflets (Figs. 21, 22 A & B, 27 A & B and 31)

2. **Amphiparacytic variate stoma** (Mitra *et al.,* 2015) was found on hypocotyl (Fig. 14 B5) and Fig. 15).

3. **Paracytic**: Two lateral subsidiary cells (SCs) completely flanking stoma (Figs. 20, 21, 24, 28B) on leaflet – Metcalfe and Chalk (1950); Von Cotthem (1970); Dilcher (1974) and Wilkinson (1979). Carpenter (2005) referred to it as holoparacytic.

4. **Actinocytic**: Stomata with five or more radiating subsidiary cells (Van Cotthem, 1970; Wilkinson, 1979 (Fig. 20).

5. **Tetracytic**: Four cells surrounding guard cells in an irregular and variable manner (Dilcher, 1974) – Fig. 21, 28B.

6. **Stephanocytic**: In typical form SCs are arranged radially around the guard cells. The cells are of different shapes and sizes - such stomata are with five subsidiary cells, with two small placed on one side, and one on the other side of the guard cells. One subsidiary cell is present at each pole (Surat un Nisa *et al.* (2019) - (Fig. 21, 28B, 29 D).

7. **Bicyclic Stephanocytic**: Stomata surrounded by two cycles of cells with inner specialized and outer weakly specialized SCs forming more or less defined rosette (Carpenter, 2005) (Fig. 24B).

8. **Laterocytic I**: Two lateral subsidiary cells are on one side and three on the other side of guard cells (sub type L3 of Surat un Nisa *et al.* [2019]). It was originally described by Den Hartog née Vanter Tholan & Baas (1978) for Celastraceae. They suggested it to arise from anisocytic type and to be developmentally related to such complex stomatal types as complex laterocytic, cyclocytic, complex cyclocytic or bi- or tricyclic stomata at least in Celastraceae. They opined that if laterocytic stomata deserve full recognition as a distinctive stomatal type depends on whether they will be found more often outside the Family Celastraceae. Surat un Nisa *et al.* (2019) have described it from *Vincetoxicum arnottianum* (Apocynaceae) and the present study describes it from *Sterculia foetida* (Fam. Malvaceae) – Fig. 28A.

9. **Laterocytic II**: simple laterocytic – see Wilkinson (1979) – Fig. 29 B.

10. **Laterocytic III**. Laterocytic LI type of Surat un Nisa *et al.* (2019) i.e., two lateral subsidiary on one side and one on the other side of guard cells. (Fig. 26A).

11. **Brachyparacytic subtype I:** Stomata are with two lateral SCs one on each side of the guard cells enclosing the completely only at one pole, other pole is with an additional SC (Mitra *et al*., 2015) - Fig. 25.

12. **Staurocytic:** Four SCs more or less equal in size, with the anticlinal walls of the SCs extending at right angles from the poles and the middle of the guards cells (Dilcher, 1974) - Fig. 29A.

13. **Anisocytic:** Stomata with three SCs unequal in size - two larger and one smaller surrounding the guard cells (Metcalf and Chalk, 1950; Dilcher, 1974) – Figs. 27B, 28B, 30).

14. **Hemiparacytic:** One lateral SC flanking the guard cells on one side and on the other side there may be unspecialized cell (Dilcher, 1974; Surat un Nisa *et al.*, 2019; Tomlinson, 1969; Fryns-Claessens & Van Cotthem, 1973) (Figs. 26B, 27A & 30).

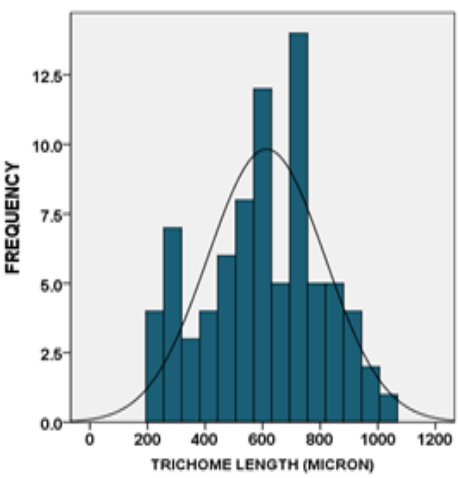
15. B**rachyparahexacytic monopolar variant:** Stomata with five SCs almost resembling Brachyparahexacytic monopolar subtype I of Mitra *et al.* (2015*)* (Fig. 26A).

16**. Contiguous stomata:** Such stomata were present on cotyledons (Fig. 17A & B) and leaflets (Fig. 32). They were of two types – oriented at right angle and juxtaposed type. A unique pair of contiguous stomata was observed on a leaflet situated on top of a flat-topped chimney-like structure formed of single tiers of epidermal cells (Fig. 32). Contiguity of three stomata was evident in Fig. 20.

17. **Giant stomata:** Giant stomata were frequently-occurring in *S. foetida*. These stomata were generally stephanocytic, mono-or polycyclic nature. These stomata

averaged to 26.42 ± 0.416 µm in length (N=45, 23.40 - 40.56 µm, CV: 10.56%) and 18.11 ± 0.479 um (N = 45, 9.56 -28.06 µm, CV: 17.79%) in width. These stomata showed characteristic ornamentation and cuticular striation on subsidiaries. The presence of giant stomata is not uncommon in angiosperms and has been reported from several unrelated families. Boldt & Rank (2010) reported giant stomata (those of > 25 µm in length) in thirty families of dicotyledonous outdoor plants of varied life forms of Germany including Family Malvaceae (*Hibiscus rosa-sinensis*). *Aesculus hippcastanum* (Family Sapindaceae) had largest giant stomata (> 40 µm in length). All giant stomata in their study also invariably showed striation on subsidiary cells (SCs). Mitra *et al.* (2015) in their studies of two varieties of *Pterygota alata* reported that giant stomata were exclusively of amphicyclocytic nature. In *S. foetida*, however, the giant stomata were of stephanocytic or actinocytic or anomocytic types. Rarely, a giant anomocytic stoma was found to have neighbouring cells (NCs) arranged in staurocytic order (two conjoint walls of NCs joining at the poles and two with meeting the poral epidermal wall on lateral sides (Fig. 31).

18. **Persistent Stomatal initials:** They were frequently seen in groups on leaflets of *S. foetida* seedlings (Figs. 20, 21, 23 and 30). Mitra *et al*. (2015) have also reported persistent stomatal initials in leaves of two varieties of *Pterygota alata*, a member of Family Malvaceae from India.



Acicular trichomes
N = 80
Mean = 613.28 µm
SE = 22.700
Median = 612.50
CV % = 33.12
G1 = -0.099
Sg1 = 0.269
G2 = -0.712
Sg2 = 0.532
Minimum = 225.0
Maximum = 1025.0
KST = 0.087
p<0.200
Shapiro-Wilk = 0.975
p<0.116

Fig. 12. Distribution of acicular trichome lengths (µm) on a young leaflet of *S. foetida*. Some 77.5% of the trichomes belonged to the size class, 251 - 750 µm. Shorter than this class were merely 1.3% and larger than this class were 21.20%.

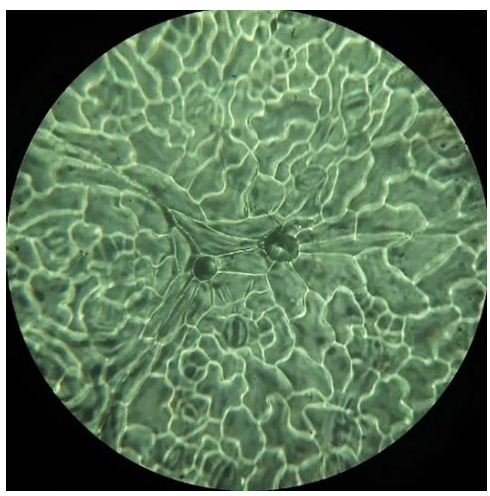
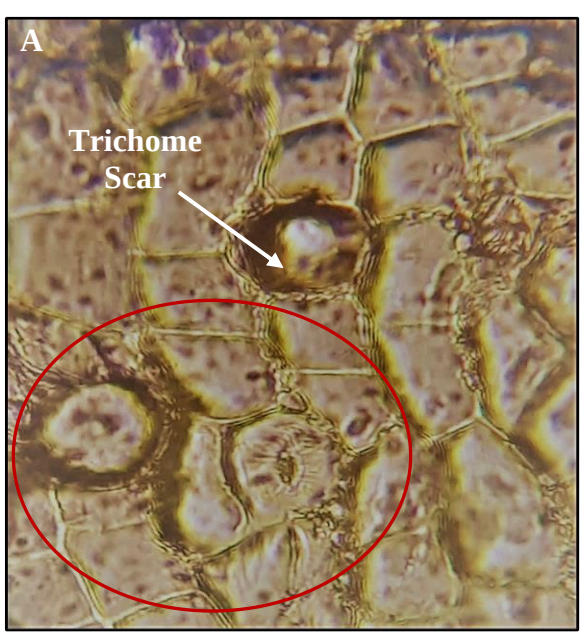


Fig. 13. Scars of two trichomes surrounded with radially elongated basal cells with straight anticlinal walls. Note the common basal cells between them. Magnification: 45 x 10 X.



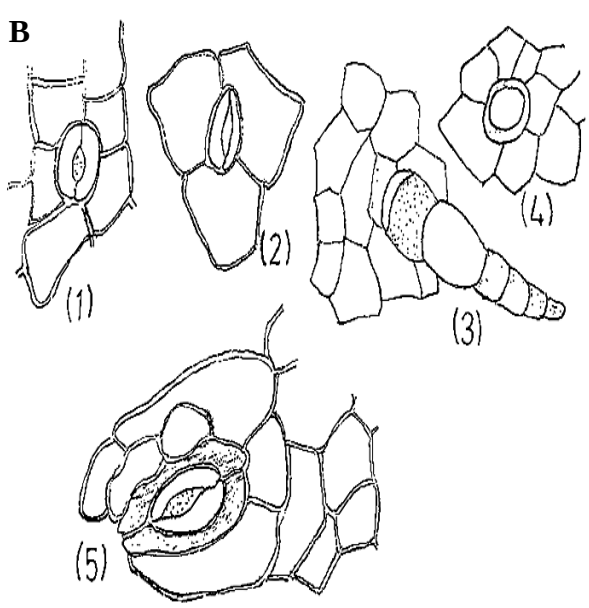


Fig. 14. **A**) Surface of hypocotyl showing two stomata surrounded with 5-6 NCs (enclosed in a circle) and a circular scar of the broken trichome. Note the straightness of the epidermal anticlinal cell walls and trichome base surrounded by six unequal cells Magnification: 45 x 10 X, zoom 1.2 X. **B**) Stoma on hypocotyl - anomocytic (B1), Stoma on cotyledon with 3 NCs (B2), a multicellular conical trichome, common on hypocotyl and cotyledon (B3), A scar of a trichome (B4) and an Amphiparacytic variate stoma (see Mitra *et al.* (2015) on hypocotyl. (Fig. 14 (B5). Stomata not drawn to scale.

Fig. 15. Amphiparacytic variate stoma (see Mitra *et al.*, 2015) on hypocotyl. Magnification: 45 x 10 X, zoom 1.4 X.

Table 6. Cotyledonary stomatal density per mm² on sun-exposed cotyledonary surface.

Parameters	Cotyledon I	Cotyledon II
	Stomatal density	Stomatal density
N	30	30
Mean	28.504	15.39898
SE	3.2428	2.2934
Median	29.4869	14.7435
CV (%)	62.32	81.58
G1	0.234	0.691
Sg1	0.427	0.427
G2	-0.440	0.387
Sg2	0.833	0.833
Minimum	zero	Zero
Maximum	68.80	49.14
KS-T*	0.124 (p<0.2000)	0.171, p<0.025
Shapiro-Wilk	0.961 (p<0.329)	0.903, p<0.010
Symmetry	AS	AS

Acronyms: SE, Standard error of mean; CV, Coefficient of variation; G1, Skewness; Sg1, St, Error of skewness; G2, Kurtosis; Sg2, St. Error of kurtosis; KS-T*, Kolmogorov-Smirnoff Test with Lilliefors significance correction; AS, Asymmetrical

Fig. 16. Stomata on sun-exposed surface of cotyledon – Stoma surrounded with 3 NCs (**A**) and anomocytic stomata with 4- and 5-neighbouring cells (**B** and **C**, respectively). Epidermal cells are straight in anticlinal contour. Magnification: 45 x 10 X, zoom 1.4 X).

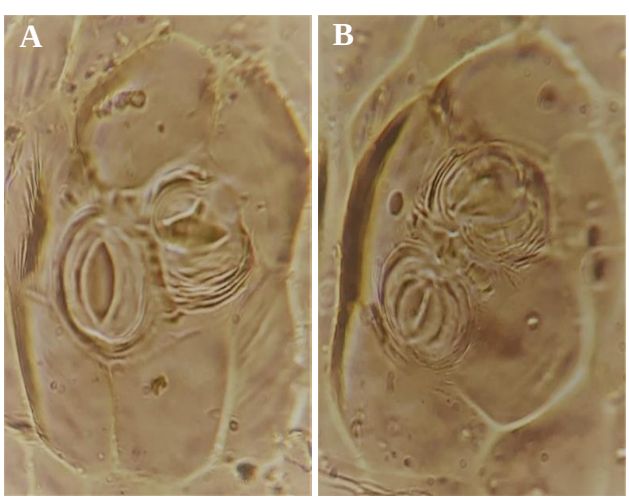


Fig. 17. Cotyledonary surface showing Contiguous stomata (at-right-angle type (**A**) and near juxtaposed type (**B**)' Cotyledonary stomata. Magnification 45 x 10 X, zoom 1. 6 X.

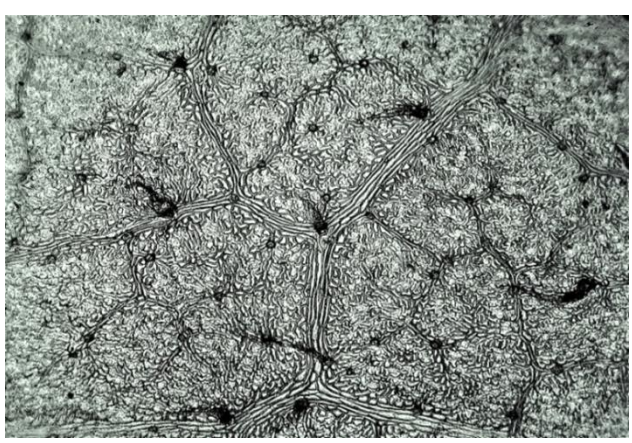


Fig. 18. Surface view of dorsal side of leaflet showing trichomes of different sizes distributed on the veins and the laminar islands. Note the well-developed vascular network. Several trichome have broken leaving behind the scar on the surface. There are no stomata on dorsal surface. Magnification: 10 x 10 X.

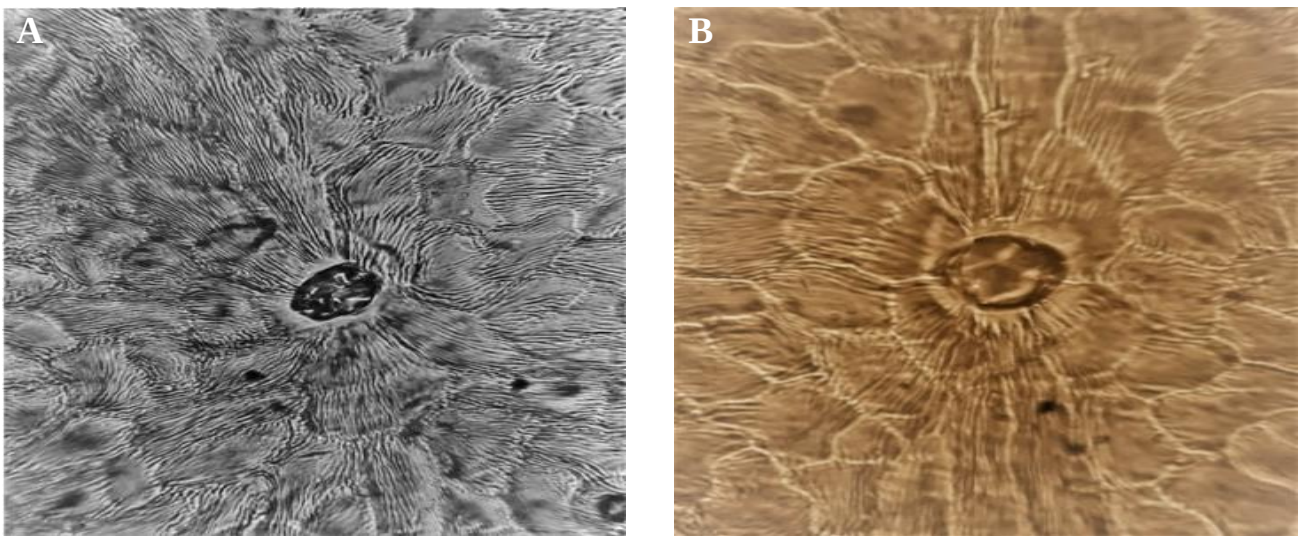


Fig. 19. Dorsal Epidermis cuticular striation of leaflet - Striae are in form of bands running parallel to each other on the periclinal surface of cells and frequently continuing on adjacent cell (s). In the middle of each image is scar of a trichome surrounded with several basement cells. There are no stomata on dorsal side. Magnification: 45 x 10 X, zoom 1.2 X. **A**) Lamina surface; **B**) veinlet surface

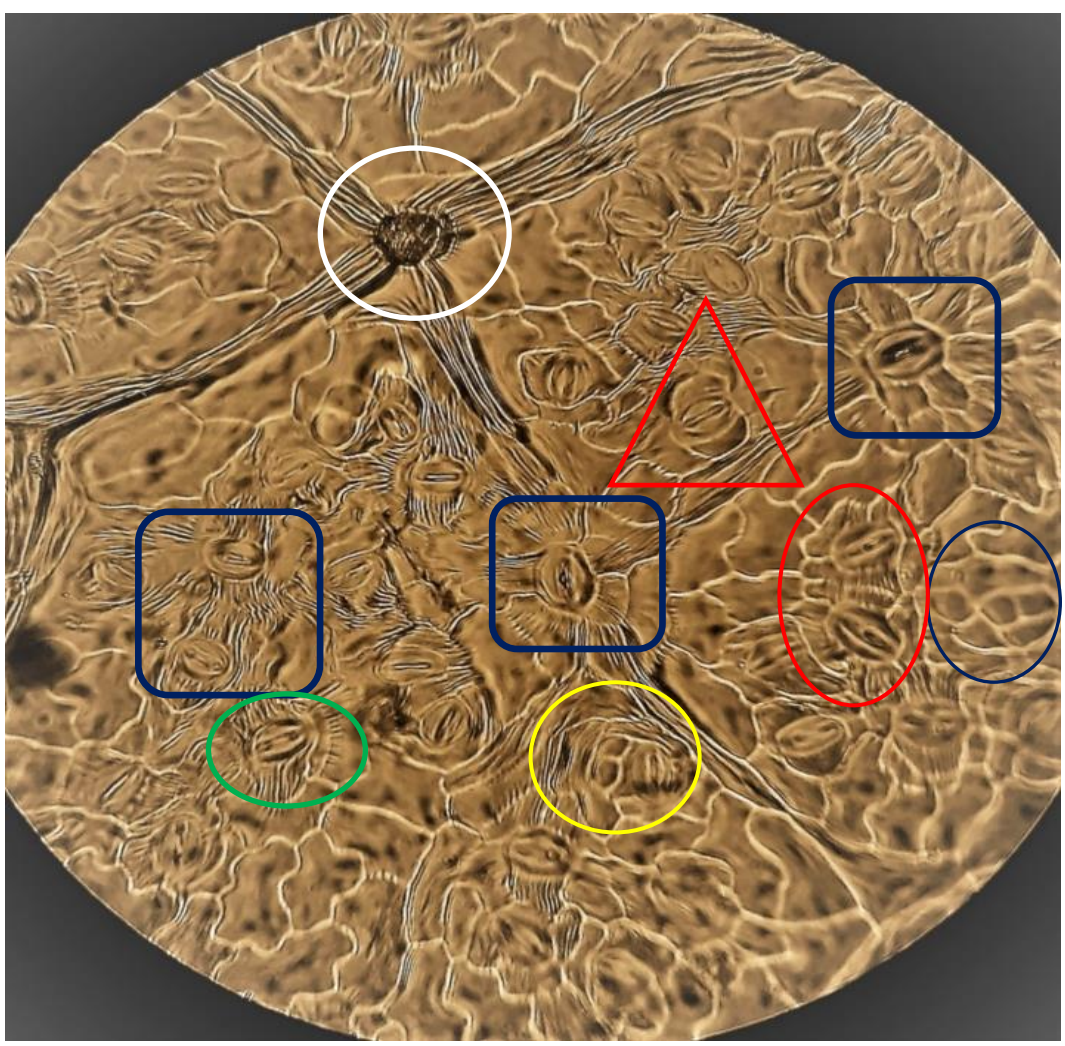


Fig. 20. General view ventral surface of larger leaflet showing vascular region with a scar of a broken trichome (white circle), stomata of two types - brachyparacytic (stoma incompletely surrounded by two subsidiary cells (Red triangle) and actinocytic stoma surrounded by 7-8 cells (Blue squares), and cuticular striation in form of parallel bands on subsidiaries and the costal cells. Paracytic stoma is shown within a green circle. The epidermal anticlinal contour is curvy to sinuous. Two persistent stomatal initials in yellow circle. A cluster of three stomata with no subsidiaries among them (within red circle). Some persistent stomatal initials are also visible (Blue circle). Magnification: 45 x 10 X, zoom 1.4X, enlarged).

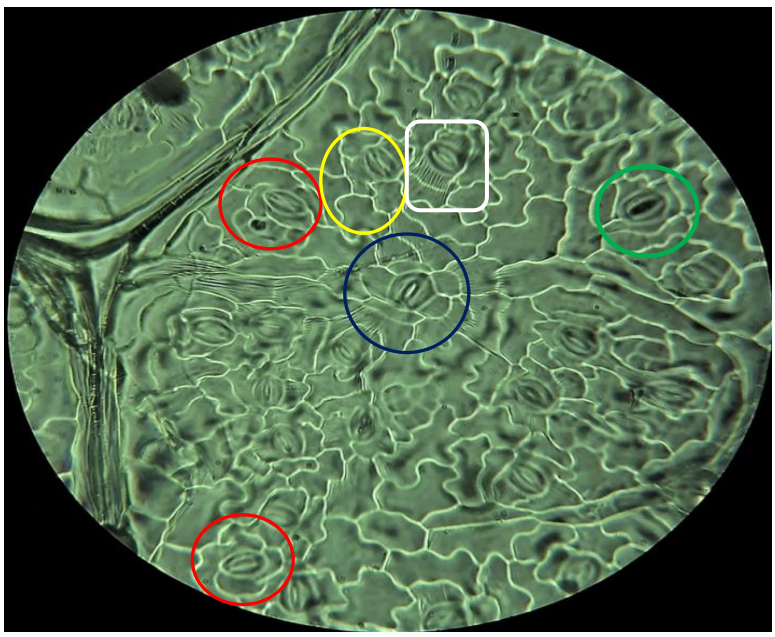


Fig. 21. Ventral surface of leaflet showing paracytic (white square), tetracytic (red circles) and anomocytic stoma (yellow circle). Magnification: 45 x 10, zoom 1.4X). Stephanocytic stoma (five subsidiaries of different shape and size) in blue circle – such stomata are with five subsidiary cells, with two small placed on one side, and one on the other side of the guard cells. One subsidiary cell is present at each pole. Anisocytic in green circle. Magnification: 45 x 10 X.

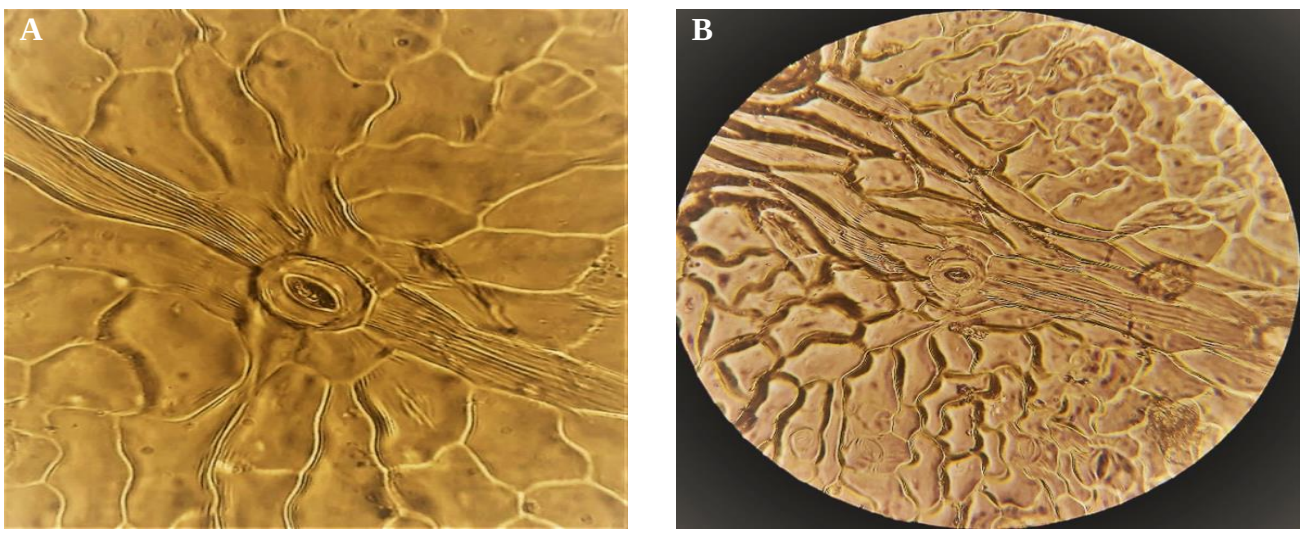


Fig. 22. Anomocytic stoma. (**A**) Surrounded by five elongated cells on the vein of a more or less mature leaflet. Note that the cuticular striation in form of parallel bands. Mag. 45 x 10X, zoom 1.5X. (**B**) Another anomocytic stoma in vascular region where epidermal cells are transversely elongated with striations. The cells of laminar islands are irregular in shape and typically curvy to sinuous. Magnification: 45 x 10X.

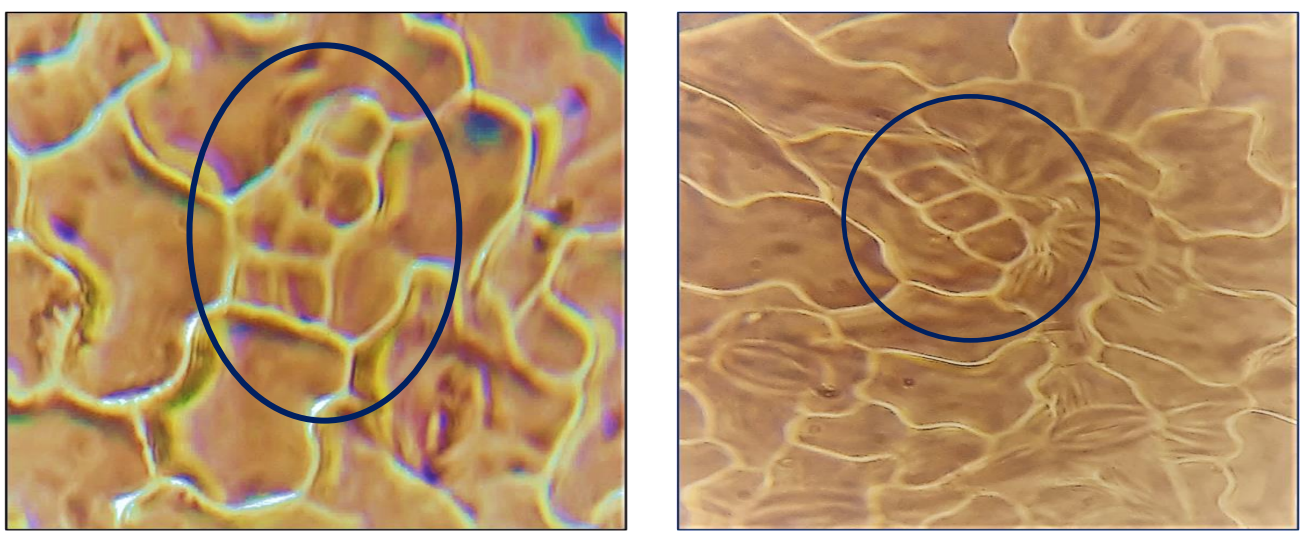


Fig. 23. Persistent stomatal initials in groups. Magnification: 45 x 10 X, zoom 2.0 X).

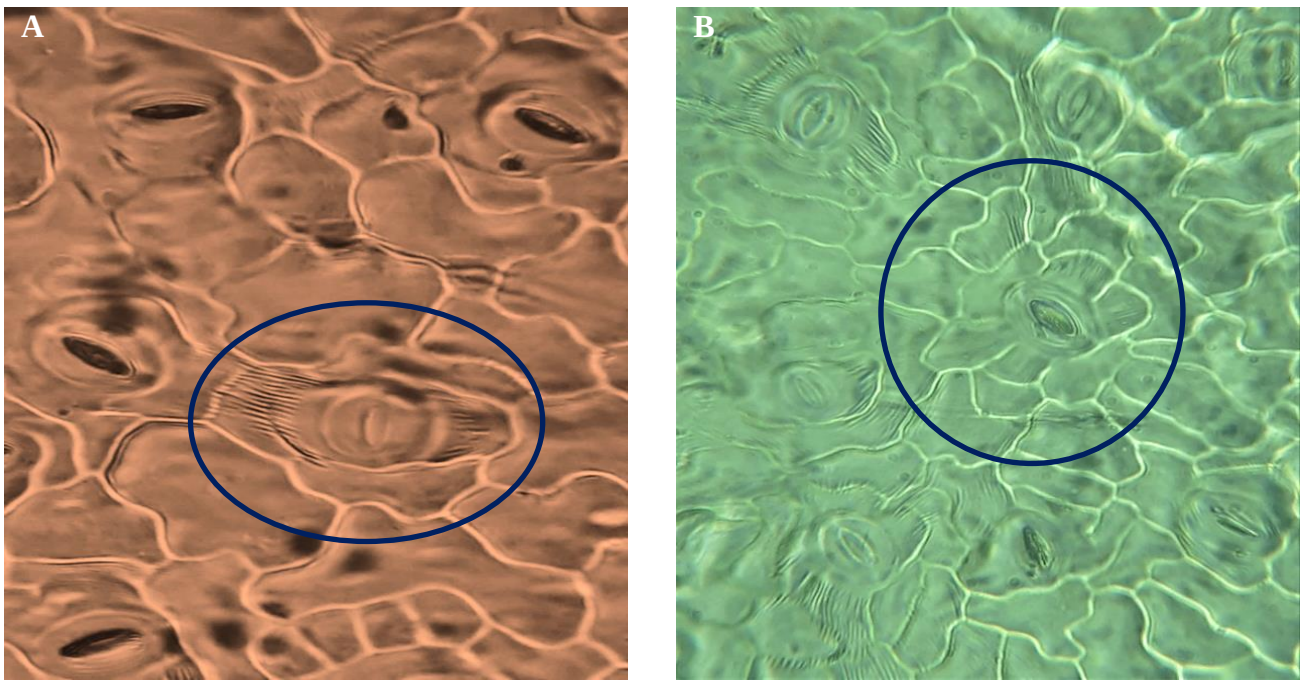


Fig. 24. Paracytic stomata (A) - note the persistent stomatal initials in lower most region of the image. Bicyclic stephanocytic stoma (B). Magnification: 45 x 10 X, zoom 2.0).

Table 7. Length and width (µm) of stomata on sun-exposed (upper) surface of cotyledon.

Statistical parameters	Stomatal size	
	Length	Width
N	60	60
Mean	20.57	18.60
SE	0.6467	0.5859
CV (%)	24.36	24.40
Minimum	9.36	8.58
Maximum	34.32	31.20

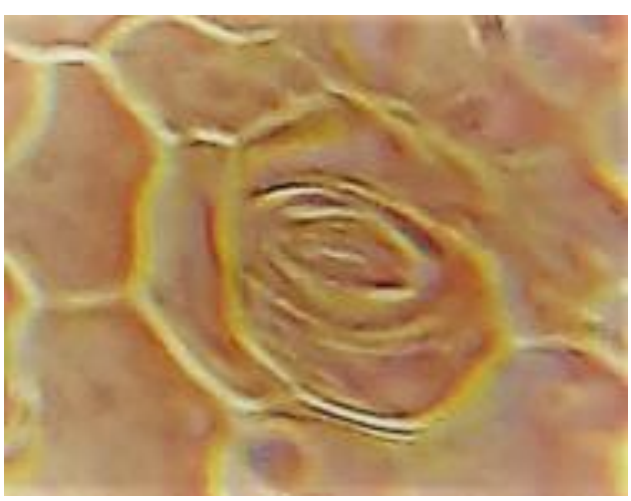


Fig. 25. A stoma - Brachyparacytic subtype I of Mitra *et al.* (2015). Enlarged from an image photographed at 45 x 10X.

Stomatal clustering: Stomata were observed to cluster in the form of an arch on one side of a giant stoma (Fig. 28C) or clustered in groups, separated by a narrow strip of epidermal ground cells (Fig. 30).

Stomata in the family Malvaceae (former Sterculiaceae) were reported to be anomocytic Metcalf & Chalk, 1950). Hussin & Sani (1998) reported anomocytic stomata in *S. foetida*. However, anisocytic, paracytic, cyclocytic Brachyparacytic, amphibrachyparacytic were also found by Maity (2011). Six stomatal types, anisocytic, anomocytic, diacytic, amphipericytic, paracytic and abnormal types were reported in five malvaceous species by Udofia *et al.* (2020). Gangadhara *et al.* (1977) reported anomocytic, paracytic, diacytic, hemidiacytic, single-subsidiary stomatal type and some aberrant types in *Gossypium herbaceum* cotyledon and hypocotyl. Essiett & Iwok (2014) reported laterocytic, brachyparacytic, anisocytic, anomocytic and staurocytic stomata in *Hibiscus* species. Mitra *et al.* (2015) reported stomatal polymorphism from two varieties of *Pterygota alata* viz. *Pterygota alata* (Roxb.) R.Br. var. *alata* and *P. alata* (Roxb.) R.Br. var. *irregularis* (W.W. Sm.) Deb and S.K. Basu (Family Malvaceae) - amphibrachyparacytic (two subtypes), amphicyclocytic (three subtypes), anisocytic, anomocytic, anomotetracytic (one subtype), Brachyparacytic (two subtypes), Brachyparahexacytic (monopolar three subtypes), dipolar (4 subtypes and three intermediate type), Brachyparatetracytic, Cyclocytic (with one subtype), Paracytic, Parahexacytic dipolar, Paratetracytic, Stephanocytic and Giant stomata. Thus, there appears a great diversity of stomatal types in Family Malvaceae.

The presence of stomatal polymorphism (10 types of normal stomata along with 4 subtypes and one intermediate type between stephanocytic and Hemiparacytic and some abnormal stomatal structures both in the vegetative and floral organs of *Canella winterena* (L.) Geartn. (Family Canellaceae) have been reported by Mandal *et al.* (2014). The epidermal cells were polygonal, isodiametric in surface view with straight anticlinal walls. The stomatal complexes included amphibrachyparacytic, anomocytic, anisocytic, brachyparacytic, brachyparatetracytic, cyclocytic, hemiparacytic, laterocytic, holoparacytic, stephanocytic, one

intermediate type between stephanocytic and hemiparacytic, etc. Abnormalities like contiguous stomata, different types of twin stomata and cytoplasmic bridge between adjacent stomata, stomata with single guard cell, pore juxtaposed with normal stomata are also found. Recently, Surat un Nisa *et al*. (2019) have reported stomatal polymorphism in *Vincetoxicum arnottianum* (Fam. Apocynaceae) – demonstrating 10 major stomatal types and 36 stomatal subtypes. Comparing our results with earlier reported studies of Mitra *et al*. (2015) and Surat un Nisa *et al.* (2019), it is obvious that *S. foetida* is rich in stomatal diversity but not as rich as *Pterygota alata* (Fam. Malvaceae) and *Vincetoxicum arnottianum* (Fam. Apocynaceae). Since we dealt with seedlings, stomatal diversity needs to be revisited in mature plants for better elucidation. Surface immaturity often imposes difficulty in stomatal identification.

It may be mentioned that since stomatal polymorphism is reported from unrelated families, it is currently believed that the presence of more than one type of stomata in a species is probably a reflection of precarious balance among the influences of the environment that operate at a level of meristemoids (that form the stomatal mother cells) on one hand and the influences operating at the organ level in deciding the orientation of the cell division at species level on the other hand (Humbert & Guyot, 1969; Mandal *et al.*, 2014) and likely phylogenetic pattern on the basis of polymorphic stomata cannot be drawn (see Mitra *et al*., 52015).

Cotyledonary stomata – density and size: Stomatal density on sun-exposed cotyledonary surface varied with the two cotyledons studied (Table 6). It averaged 28.50 ± 2.24 (varying from zero to 68.8) stomata per mm^2 on one cotyledon and averaged to 15.40 ± 2.29 (varying from zero to 49.14) stomata per mm^2 on the other cotyledon. It is distributed asymmetrically in both cotyledons. The average size of the stomata tended to be 20.57 ± 0.65 x 18.60 ± 0.59 µm (L x W) (Table 7).

Foliar stomata – Density and size: Foliar stomatal density (SD) was studied in ten leaflets of different sizes with 40-50 observations for each leaflet (Table 8). The number of stomata per mm^2 was found to be the function of leaflet size. The density was higher in smaller young leaflets which tended to decline with little irregularity with increase in leaflet size. Stomatal density in the smallest leaflet of 184 mm^2 was 249.14 ± 5.54 (varying from 157.26 to 324.36 stomata per mm^2, CV: 14.05%) and 125.22 ± 3.449 stomata per mm^2 in the largest leaflet (4709 mm^2). Our estimation of stomatal density in *S. foetida* was much higher than that reported by Pereira *et al.* (2018) to be 22.25 ± 0.89 stomata per mm^2 under full-light condition and 20.5 ± 0.82 under half-light condition. It is known from Salisbury (1928) that stomatal density is related to leaf size inversely. Stomatal density on young and small terminal leaflets of *C. fistula* was reported to be quite higher than that on relatively larger leaflets (Khan & Zaki, 2019). Khan & Zaki (2020) also reported leaf-size related inverse association of SD in *Helianthus annuus* Var. US 666. The decrease in SD in larger leaves, as compared to the smaller ones, may be attributed to the foliar epidermal cells' expansion. Young leaves have large number of stomata but as the leaf expands the density declines (Gay & Hurd, 1975).

Foliar stomatal size of normal plus giant stomata in four leaflets studied (N = 265 observations} averaged to 21.15 ± 14.86 µm (L x W). The length and width of the stomata remained more or less unchanged amongst the pooled as well individual leaflets and the coefficient of variability (CV) in any leaflet was < 20% (Table 9).

Table 8. Stomatal density per mm^2 on ventral surface of variously-sized leaflets of a 2-month-old sapling of *S. foetida*.

Leaflet #	Area mm^2	N	Mean	SE	Median	CV (%)	Skewness	Kurtosis	Min.	Max.
I	184	50	249.14	5.5390	255.553	14.05	-0.468	0.147	157.26	324.36
II	237	50	249.46	4.8307	255.553	13.69	-0.510	-0.235	167.09	304.70
II	358	50	252.60	5.0334	245.724	14.09	0.779	0.180	196.58	344.01
IV	842	40	200.60	3.7536	195.090	13.23	-0.289	-0.262	130.56	250.52
V	936	50	214.66	4.4612	216.238	14.70	-0.474	-0.662	137.61	275.21
VI	1102	40	125.22	3.4487	117.950	19.48	0.043	-0.797	78.63	176.92
VII	1390	40	215.45	3.6274	216.238	11.90	0.802	1.067	167.09	294.89
VIII	1786	50	112.44	3.1043	108.12	19.52	-0.032	-0.189	68.80	167.09
IX	2017	50	185.28	4.5282	191.665	15.46	0.891	1.062	98.29	235.90
X	4709	50	125.22	3.4487	117.917	19.48	0.043	-0.797	78.63	176.92

N = Number of microscopic fields of vision observed; SE = St. Error of means; CV = Coefficient of variability (%); St. Error of skewness (0.337 for N = 50, 0.374 for N= 40); St. Error of kurtosis (0.662 for N = 50, 0.733 for N = 40); CV, Coefficient of variability (%); Min. = Minimum; Max. = Maximum

Table 9. Length and width (µm) of stomata on ventral surface of variously sized leaflets (normal + giant stomata both).

Statistical parameters	Leaflet A*		Leaflet B		Leaflet C		Leaflet D		Pooled	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
N	60	60	70	70	75	75	60	60	265	265
Mean	24.34	14.90	19.88	15.49	21.11	14.37	19.08	14.73	21.15	14.86
SE	0.4933	0.3445	0.4429	0.3596	0.3355	0.3318	0.3621	0.3657	0.2334	0.1766
CV (%)	15.70	17.85	18.65	19.42	13.76	19.99	10.90	19.23	17.97	19.35
Minimum	15.60	9.36	9.66	9.36	15.60	9.36	15.60	11.70	9.36	9.36
Maximum	40.56	23.40	28.08	28.08	28.08	24.96	24.96	23.40	40.56	28.08

***,** Leaflet size: A = 358 mm^2, Leaflet B = 842 mm^2; Leaflet C = 1138 mm^2 and Leaflet D = 1786 mm^2

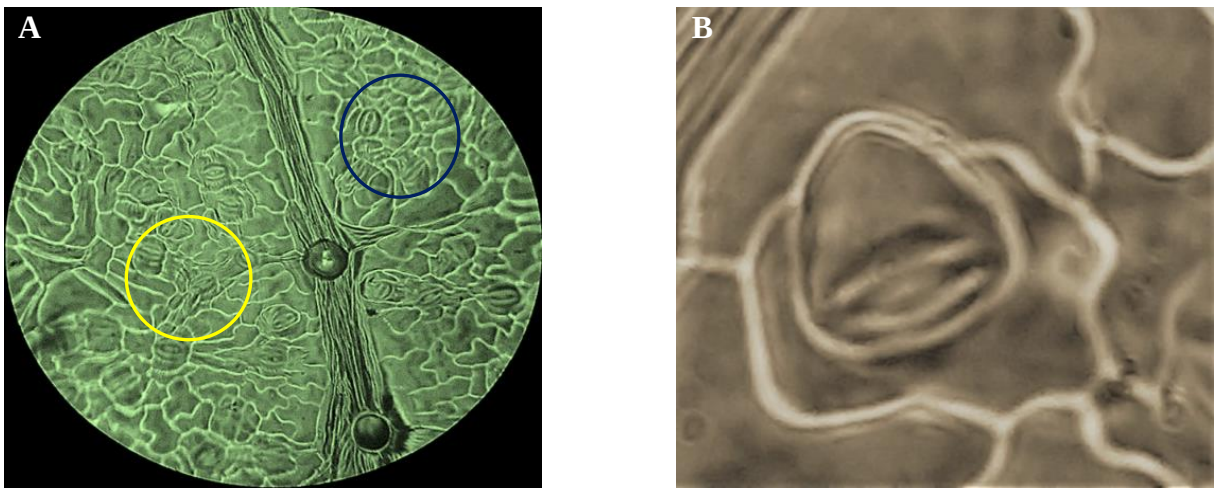


Fig. 26. **A**) Vascular nerve bearing scars of the trichomes and striations parallel to the costal cell's periclinal surface and a laterocytic type stoma (yellow circle) – see Laterocytic LI of Surat un Nisa *et al.* (2019) i.e., two lateral subsidiary on one side and one on the other side of guard cells. Waviness of epidermis cells is apparent. Blue circle indicates Brachyparahexacytic monopolar variant stoma (see Mitra *et al.*, 2015). **B**) Hemiparacytic stoma. Magnification: A, 45 x 10 X and B, Image photographed at 45 x 10 X was enlarged by cropping to show the stoma.

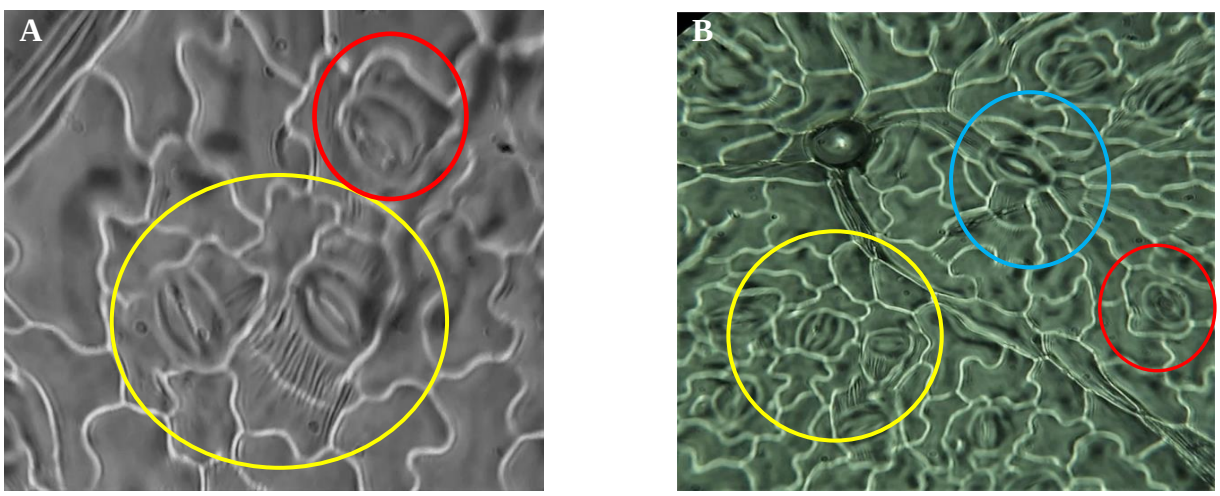


Fig. 27. **A**) Typical paracytic and anomocytic stomata (yellow circles) in close vicinity with a hemiparacytic stoma on ventral surface of leaflet (Red circle). **B**) A brachyparacytic stoma in close association of an anomocytic stoma (Yellow circle), anisocytic stoma (red circle) and a giant stoma connected with a basal cell of a trichome via an elongated curved subsidiary cell (blue circle). Magnification: A, 45 x 10 X, zoom 1.6X; B, 45 x 10 X, zoom 1.2X.

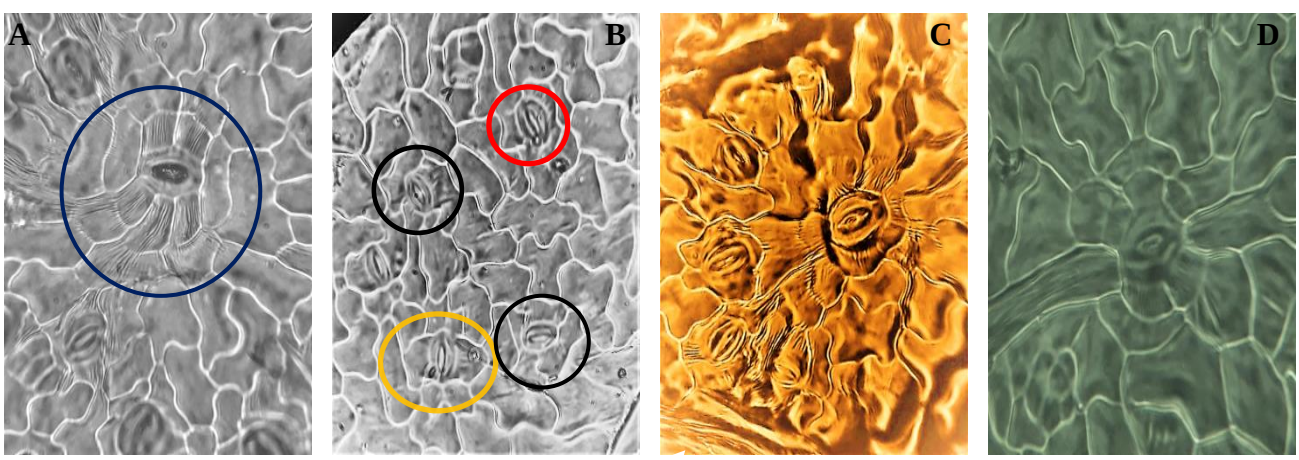


Fig. 28. Ventral surface of leaflet. **A**) Giant laterocytic stoma L3 (Originally described by den Hartog née Vanter Tholan & Baas (1978) for Celastraceae and Surat un Nisa *et al.* (2019) – Fig. 28A). **B**) Various types of stomata – paracytic (arrow), anisocytic (red circle), tetracytic (black circle) and Stephanocytic (Yellow circle). **C**) Cluster of four stomata arranged on one side of a giant amphicyclocytic stoma in form of an arch with prominent striations. Bicyclic stephanocytic stoma on costal region with numerous subsidiary cells, two of them elongated and far-extending (D) SCs connecting with costal cells on either sides. Magnification: A, 45 x 10X, zoom 1.4 x; B, 45 x 10 x, zoom 1.2X; C, 45 x 10X, zoom 1.5X and D, 45 x 10x, zoom 1.4X.

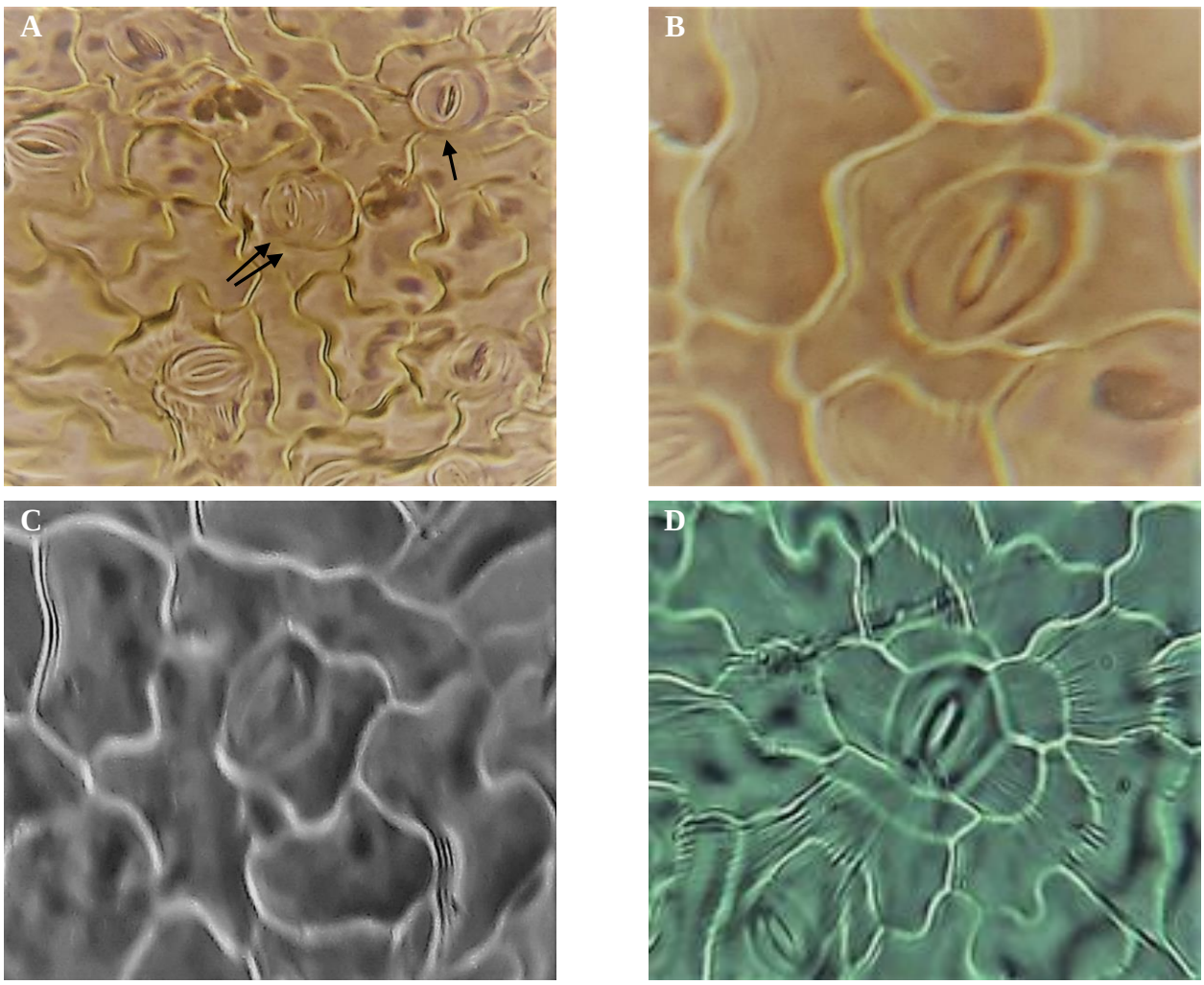


Fig. 29. Ventral surface of leaflet. **A**) Staurocytic (single arrow) and hemiparacytic (double arrow). **B**) Simple Laterocytic stoma. **C**) Hemiparacytic and **D**) Stephanocytic stoma (five subsidiaries of different shape and size) – such stomata are with five subsidiary cells, with two small placed on one side, and one on the other side of the guard cells. One subsidiary cell is present at each pole. Magnification: A, 45 x 10X, zoom 1.2 X; B, 45 x 10, zoom 2.0 X; C, 45 x 10x, zoom 1.6X and D, 45 x 10X, zoom 1.4 X.

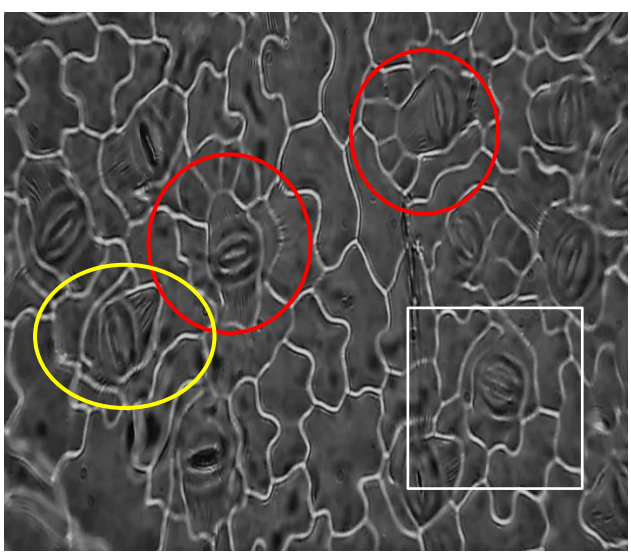


Fig. 30. Ventral surface of young leaflet of *S. foetida*. Stomatal clusters of five and seven stomata separated by a narrow strip of epidermal cells wavy in anticlinal contour. A few persistent stomatal initials are also present in vicinity of stomata (shown inside red circles). Anisocytic stoma in white circle and hemiparacytic in yellow. Magnification: 45 x 10 X, zoom 1.4 X.

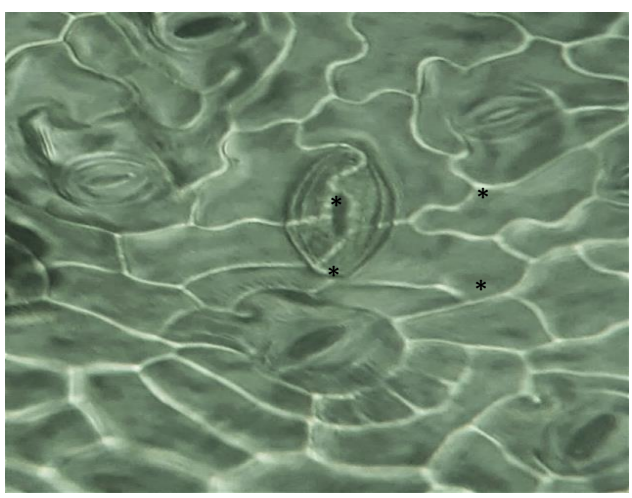
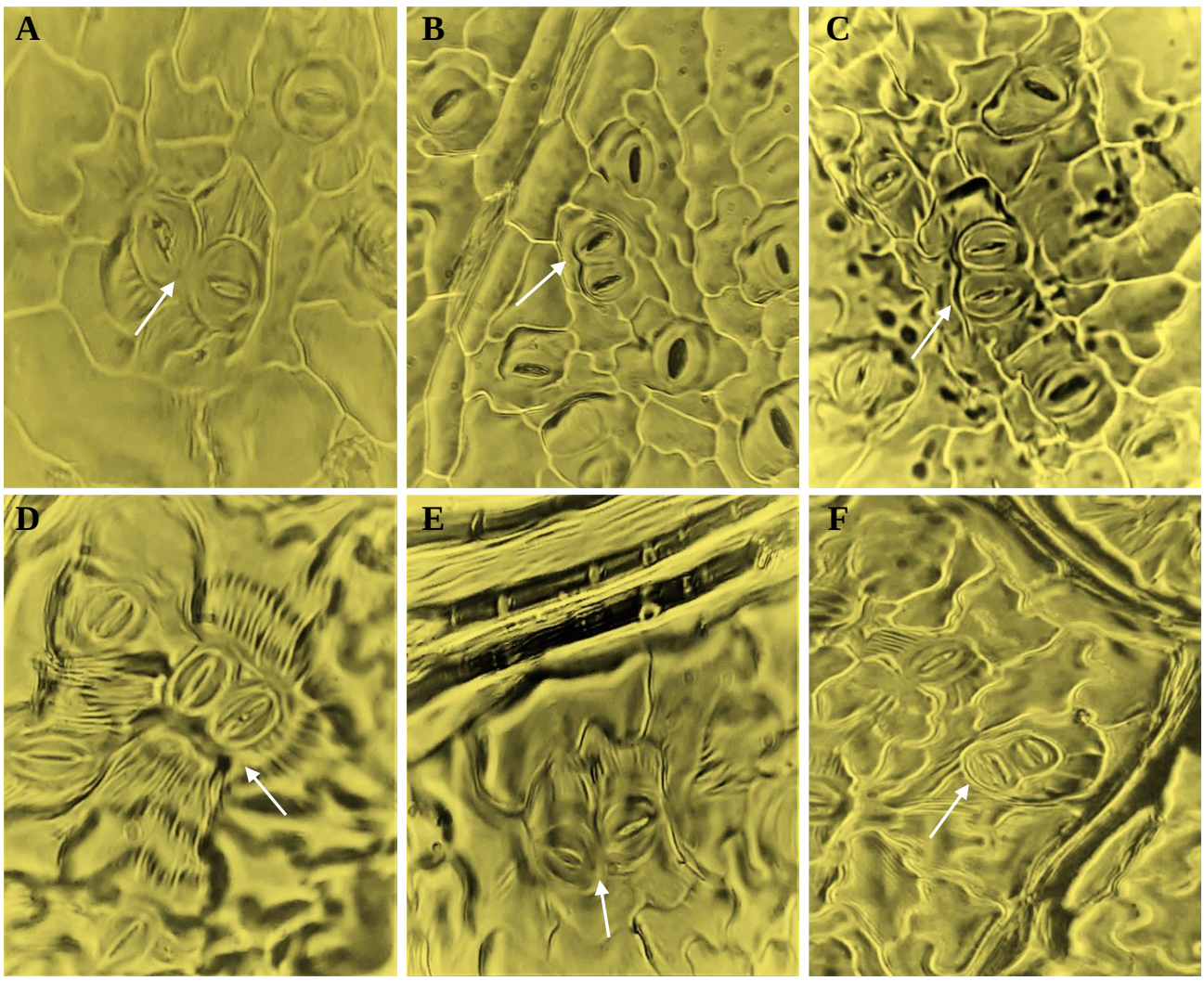


Fig. 31. A stomatal complex in association with four persistent arrested initials in vicinity of a large anomocytic stoma. The two conjoint walls of the indistinct neighbouring cells may be seen joining at the poles of the stoma and the other two conjoint radial tangential walls of the neighbouring cells meeting the poral epidermal wall on lateral side. The large guard cells overlying and partly obscuring neighbouring cells. Image at magnification 45 x 10 X, zoom 1.6 X.



Fig, 32. Contiguous stomata on the ventral surface of leaf. Juxtaposed type (B, C, D and F,) and more or less at-right- angle type (A and E). Note highly striated feather-like subsidiary cells. The contiguous stomata (F) are unique being raised above the ground epidermal cells in form of a flat-topped chimney containing stomata on the top. Magnification: 45 x 10X, zoom 1.4X.

Conclusion

It may be concluded from the results of the present study that *S. foetida* (Family Malvaceae) is quite rich in trichomal and stomatal diversity.

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