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SEEDLING ORNAMENTATION OF *PERISTROPHE PANICULATA* (FORSSK.) BRUMMITT. (ACANTHACEAE)

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ABSTRACT

The ornamentation (trichomes, lithocysts and stomata) of *Peristrophe paniculata* (Forssk.) Brummitt. seedling is described. Seedling appeared to be "Phanerocotylar-epigeal-foliaceous type". *P. paniculata* appeared to consist of lithocysts in all seedling components. Generally, they were of varied shapes and sizes. They are much longer than wider. Each lithocyst contained numerous cystoliths. The lithocyst density on cotyledonary and foliar surfaces was almost comparable -averaging around 13 to 15 lithocysts per mm^2. On cotyledon, lithocysts were predominantly of RR (Both ends round) type. RN (One end obtuse other tapering) or NN (both ends tapering) types were absent from ventral surface of cotyledon. There was, however, merely 6.66 % representation of RN type on dorsal surface of cotyledon. The three types of lithocysts were, however, present on leaf. NN type of lithocysts was 50-58% on ventral and dorsal surfaces. RR and RN types were around 20-25%. There were three types of trichomes 1. Non-glandular uniseriate multicellular larger trichomes, 2. Smaller conical trichomes and 3. Sessile glandular trichomes. Size of normal diacytic stomata was found not to vary on different surfaces of the seedling. Stomatal size was of 25.80 ± 14.85 μm on the dorsal surface of leaf and 27.5 x 14.85 μm on the ventral surface. Foliar stomata had sinuous subsidiaries with wavy anticlinal walls. On leaf surface, besides diacytic stomata, anisocytic and staurocytic stomata were also seen – of course in low number. SEM provided images of the cystoliths and druses. Scanning through EDS attached to the SEM facilitated the elemental composition of the leaf, in the region of cystoliths and the polymorph presumably aragonite, indicated that $CaCO_3$ entered in the composition of the cystolith and the druses.

Key Words: *Peristrophe paniculata*, Acanthaceae, Seedling ornamentation, Trichomes, Lithocysts, Stomata, SEM and EDS Scanning.

INTRODUCTION

Peristrophe paniculata (Forssk.) Brumitt [syn. P. bicalyculata (Retz.) Nees] – (Hindi: Kali Aghedi), also called "The goddess of mercy" (Ogunwande *et al.*, 2010) is a hispid herb reaching up to 1.5m, distributed in Pakistan's provinces of Sindh and Punjab.

P. paniculata is very important medicinal and biologically active herb (Rushmi *et al.*, 2010; Abdulazeez *et al.*, 2009, 2013). It forms populations of variable sizes at several ruderal sites in the campus of University of Karachi, Pakistan, in situations of partial shade of trees and sometimes in open derelict spaces after summer rains as a therophytic colonizer. It also grows among the hedge plants (Khan *et al.*, 2014). They have described structure, composition and pattern in *P. paniculata* dominated vegetation developing in University of Karachi after summer rains.

The present study investigates the surface ornamentation characteristics of *P. paniculata* seedlings with reference to the lithocysts, trichomes and the stomata.

MATERIALS AND METHODS

Nearly one month old seedlings of *Peristrophe paniculata* emerging after summer rains in the derelict field of Botany Department of the University of Karachi were collected for their study. The seedlings were studied for their ornamentation including trichomes, lithocysts and stomata. Seedlings type was described according to Garwood (1996).

Hickey (1973) and LAWG (1999) were followed for description of leaf architecture. The epidermal impressions were made with clear nail polish (Wang *et al.*, 2006).Cotyledons of $1cm^2$ in size were studied for their ornamentation. Foliar trichomes were studies in very young leaves of 0.5 cm^2. Imprints for stomatal studies were taken from leaves of 2 to 5 cm^2 and lithocysts were studied in leaves of 5 and 12 cm^2 in size. Stomatal nomenclature suggested by Prabhakar (2004) being simple and based upon structure of stomata and not their ontogenetic pathways was adopted to ascertain stomatal types. Prabhakar (2004) recognized eleven types of stomata. This nomenclature does not recognize actinocytic and stephanocytic stomata and categorize them as anomocytic type. As a basic criterion, all the cells abutting the guard cells are considered distinct by Prabhakar (2004) from the other epidermal

cells by virtue of their position (i.e. abutting nature to the guard cells) hence he prefers to call them subsidiaries. Length and width of stomatal pores was measured in µm with calibrated micrometer. The data was analyzed statistically (Zar, 2010). For scanning electron microscopy (SEM), unimbibed (air-dried) plant material was mounted on brass stubs and coated with a 250 ^{o}A gold layer with JFC-1500 gold coater. SE micrographs were made at 15kV with JEOL JSM-6380A electron microscope at various magnifications. The images were saved digitally on computer. Elements detector system (EDS) based on Energy dispersive X-ray spectroscopy (EDS) attached to SEM was employed for element detection and quantitative elemental analysis. By this system, single shelled elements are not detected.

RESULTS AND DISCUSSION

Seedling: The seedling of *P. paniculata* is presented in Fig. 1A. Garwood (1996) scheme of seedling types is based on the characters of cotyledonary position (epigeal or hypogeal), exposition (Cryptocotylar or Phanerocotylar) and texture (fleshy or foliaceous) during germination. As per Garwood (1996) scheme, *P. paniculata* seedling appeared to be "Phanerocotylar-epigeal-foliaceous type" (Fig. 1). Such seedlings are also known from *Anogeissus latifolia, Cucumis sativus, Manilkara hexandra* etc (Amritphale and Sharma, 2008).
Root: Tap root system.
Stem: Herbaceous green, 5-6 angled There are ridges and grooves on epicotylar as well as hypocotylar stems in seedling.
Cotyledon: Foliaceous, opposite, petiolate (petiole flat strap –like, c 1.5 cm in length) green, small (1 to 1.5 $cm^{2)}$ in size. Round in shape. Interestingly, new shoot growth may also take place from the axil of the cotyledons (Fig. 1B).
Leaf: Simple, exstipulate, opposite decussate, dorsiventral, apically acute and the base angle obtuse (base cordate). The apex extension length (La) was zero and base extension length (Lb) was = 1-2 (3) mm. Two opposite leaves on a node were more or less equal in size. The first pair of leaves on the stem was smaller in size than the size of the upper pair. Pinnately veined. Veins running in depression and intercostal islands rising above. The leaves especially younger ones were profusely hairy.

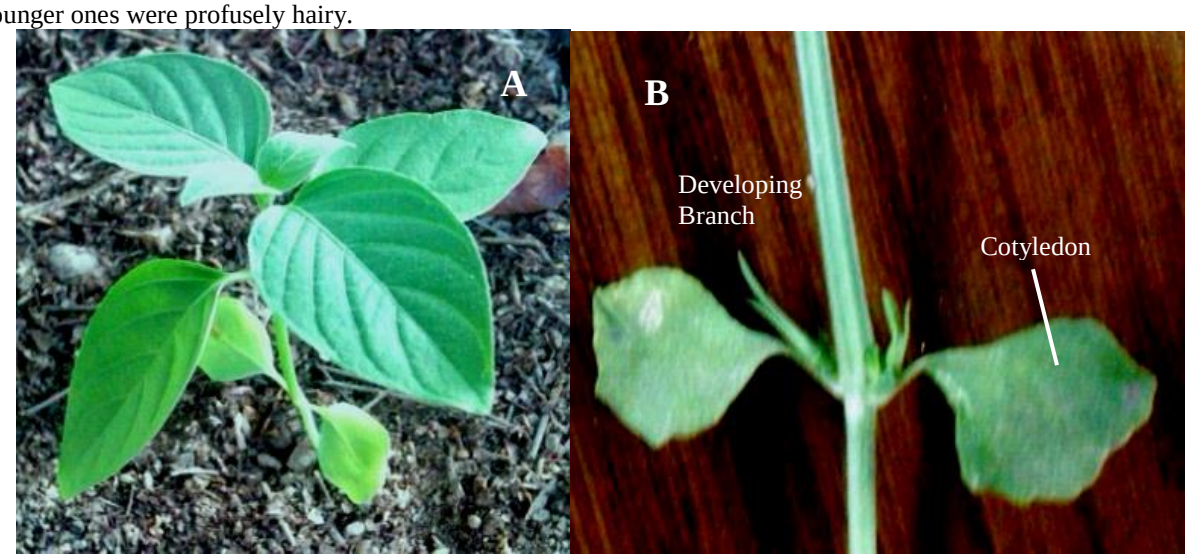


Fig. 1. A, Nearly one month old seedling of *P. paniculata* growing in a derelict site of Karachi University. Cotyledons are still intact. Photo Aug. 2015. The leaves are pinnately veined. The midribs as well as the secondaries run in depression. Fig. 1B exhibits the branch shoots arising from the axil of the cotyledons. Stem is angular with ridges and grooves.

Trichomes

The whole seedling shoot except cotyledon is pubescent. Trichomes are highly variable in size. There were three types of trichomes 1. Non-glandular uniseriate multicellular larger trichomes, 2. Smaller conical trichomes and 3. Sessile glandular trichomes.

Epicotyl is heavily ornamented with trichomes - mostly three-segmented trichomes. The trichomes present on hypocotylar and epicotylar stem were smaller ranging from 72.16 to 213.2 µm. Trichomes on leaf (especially young) were comparatively much larger (2-6 celled), uniseriate, and sharply tapering. They were numerous on veins, margins, apex, and basal part of the leaf including petiole (Fig. 2A, B, C, D and F). The trichomes on the margins arose from a basal collar cell and were directed towards apex. The trichomes present on the laminar intercostal

islands were short, 2-celled, and stout and apically pointed (Fig. 2E). As compared to hypocotyl, trichomes were more numerous on epicotyl but generally restricted on the ridges. These trichomes have swollen basal part but pointed apically. Grooves were generally devoid of trichomes (Fig. 10 A, B and C).

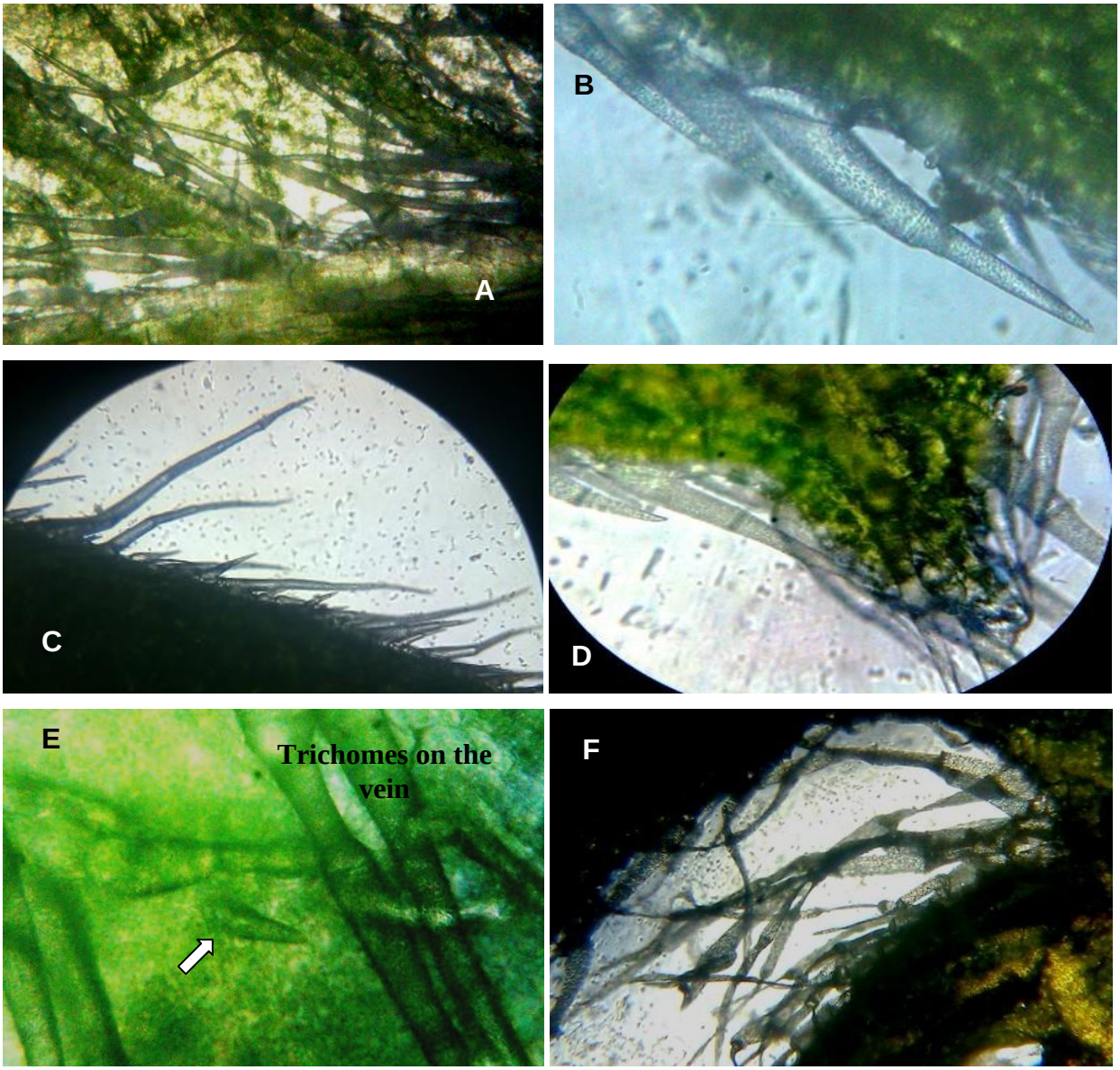


Fig. 2. Multicellular trichomes (2-6 or more celled) of *P. paniculata* leaf as seen under optical microscope. A, midrib and sub-vein region (ventral) B, Lower margin of the leaf; C, Upper margin; D, Apex; E, Laminar island (Arrow indicates the small conical stout trichome); F, Umbo region of the leaf (petiole visible).

Fig. 3 presents the surface structure of epicotyl showing surface ridges and grooves, lithocysts and trichomes. The basal part of epicotylar trichomes was observed to connect with two elongated epidermal cells (Fig. 3) on the ridge portion of the epicotyl. These trichomes were different from those on leaf ventral surface which are upright and provided with bulbous base with a collar at the basement. These trichomes appear to bear druses on their top (Fig. 18A).

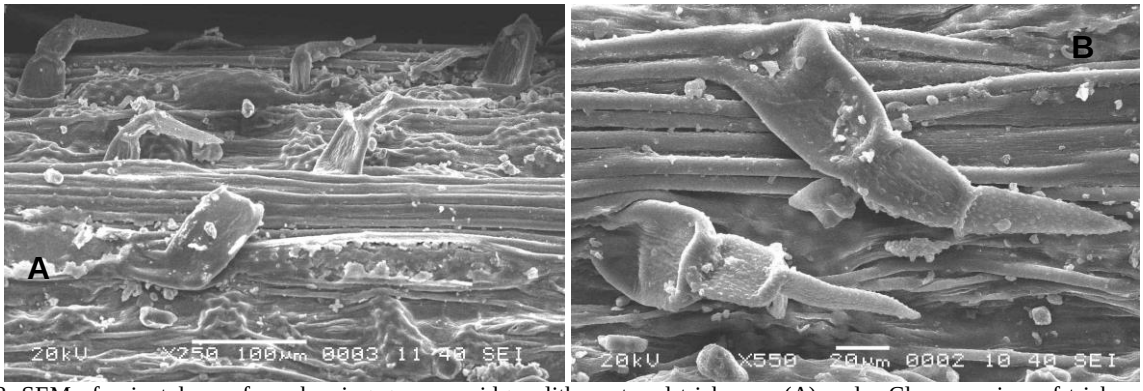


Fig. 3. SEM of epicotylar surface showing grooves, ridges, lithocyst and trichomes (A) and a Close up view of trichomes on epicotyl (B) showing basal connection.

The sessile glandular trichomes were observed only on leaf (Fig. 4). In *P. paniculata*, sessile glandular trichomes (4-celled) were seen on several places of dorsal as well as ventral surface. Such trichomes have also been reported in Acanthaceous *Lepidogathis cristata* by Bhogaonkar and Land (2015). Both, non-glandular and glandular trichomes and elongated lithocysts have also been reported in the leaves of *Justicia acuminatissima*, another Acanthaceous species (Verdam *et al.*, 2012). The grooves of epicotyl contained stomata and a large number of meristemoids. These stomata are generally round and had smooth-walled subsidiaries. On the other hand, lithocysts were distributed on the ridges as well as grooves (Fig. 10A, B and C) along with some crystals (tetrahedrons) presumably of calcium oxalate.

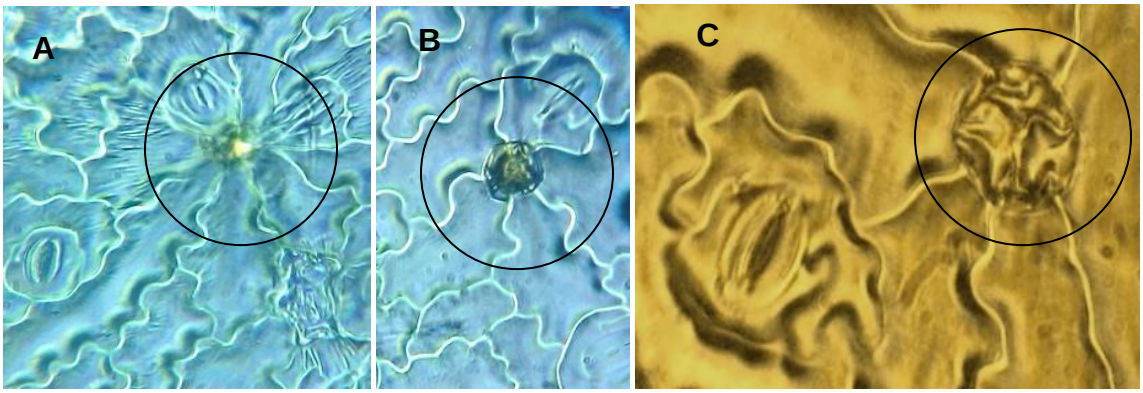


Fig.4. Sessile glandular trichome (SGT) on dorsal (A and B, 45x10X) and ventral surface (C) of leaf.(45x 15 X).

Lithocysts

Cystolith = Gr. "Cavity" and "stone" is a botanical term for the inorganic concretions, usually of calcium carbonate, formed is cellulose matrix in special cells called 'lithocyst' generally in the leaf of plants of certain families. Cystoliths (German – Zystolith, from zyst – cyst - + - lith) are calcium carbonate concretion arising from the cellulose mass of the cells of higher plants. They are well known as intracellular mineralized inclusions which are formed in specialized cells called lithocysts in leaves and other tissues of some angiospermic families. These are the formation of the $CaCO_3$ deposition in higher plants. Cystoliths are internal outgrowth of the cell wall. Lithocysts may occur frequently in several families (Apocynaceae, Moraceae, Urticaceae and Acanthaceae). Lithocyst initial cell have smooth straight wall whereas epidermal pavement cells are sinuous (with wavy anticlinal wall). Lithocysts are cytoplasmically similar to other epidermal cells initially but possess much more active Golgi apparatus and more numerous mitochondria (Watt *et al.*, 1987).

Several authors have used the two terms (lithocyst and cystolith) interchangeably but distinction appears to be essentially maintained since a lithocyst may contain single cystolith or several cystoliths. In *P. paniculata* each lithocyst had several cystoliths. They may number to thousands per cm^2. Setoguchi *et al.* (2013) have indeed reported cystoliths to number 4200/cm^2 in *Celtis sinensis*, 870/cm^2 in *Justicia procumbens and* 3720/cm^2 *in Ficus retusa.* The main body of the cystolith is cellulose extension of the cell wall in which calcium carbonate is deposited in form of granules. With the addition of large amounts of calcium carbonate, the cystolith containing cell

may become an irregular body – nearly filled (Cutter, 1972; Pandey, 2008) In *Ficus elastica* they are reported to have small amount of Silicon, Magnesium and traces of Titanium, Aluminium also (Okazaki *et al.*, 1991). The mineral part of cystoliths in case of *Ficus retusa* is amorphous $CaCO_3$ which transforms to calcite when moistened (Taylor, 1993).

P. paniculata appeared to consist of lithocysts in all seedling components. Generally, they are much longer than wider. On foliar dorsal and ventral surfaces, there was more variation in length (CV= 40.25 and 50.02%, respectively) than their width (CV =17.95 and 28.71%, respectively). Lithocysts are said to be characteristic to the Family Acanthaceae but sometimes they are absent from some Acanthaceous taxa e.g., *Acantha spinosa, Adhatoda beddomei* and *Staurogyne zeylanica* (Patil and Patil, 2011). Lithocysts in *P. paniculata* were of varied shape and sizes (Fig. 5, 6, 7, 8, 9 10, 12). Taken together the whole seedling, they occurred singly, doubly, or forming chains or aggregates of various shapes. They were round, oval, oblong, conical, arc-shaped, bean like, bent sharply like T, Y or V-shaped. The elongated lithocysts may be spindle like or cigar like. These forms of lithocysts have been reported from Family Acanthaceae (Ahmad, 1979; Patil and Patil, 2011). They generally occur singly. The double lithocysts are reported from *Barleria prattensis, Peristrophe montana* and *Sytenosiphonium russellianum* (Patil and Patil, 2011) and Barleria prionitis (Bhogaonkar and Lande, 2012). The lithocysts in *P. paniculata*, on the basis of the endings were classifiable into three types.

1. **Lithocysts with both ends tapering (NN type)** – as also recorded in other Acanthaceous species such as *Strobilanthes anamallaica, Sytenosiphonium russellianum and Pachystachys lutea*. Jani and Rudrappa (2014) recorded such lithocysts from *Peristrophe paniculata*.
2. **Lithocysts with both ends rounded or obtuse (RR type**) – as also recorded in other Acanthaceous species such as *Andrographis alata, A. elongata, Barleria prattensis, B. nemorosa, Stenophonium cordifoliu*m and several other species. Patil and Patil (2011) reported 24 such Acanthaceous species.
3. **Lithocysts with one of the ends either obtuse or tapering (RN type)** – as also recorded in Acanthaceous *Belpperone comosa, Barleriqa nemorosa and B. plumbaginifolia.* Patil and Patil (2011) reported at least 23 such Acanthaceous species. Jani and Rudrappa (2014) recorded RN type of lithocysts from *Peristrophe paniculata*.

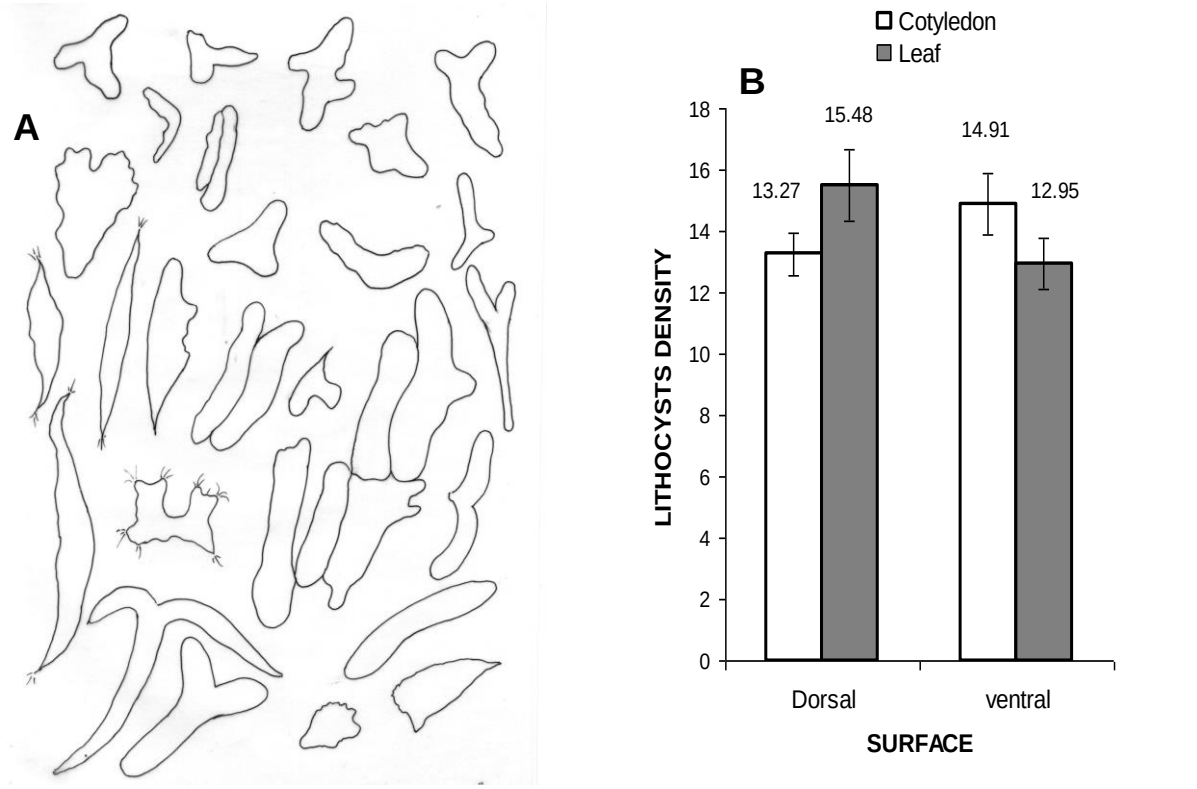


Fig.5. A**)** Free hand illustration of few of the variously shaped lithocysts of *P. paniculata* hypocotyl, cotyledons and leaf. Figures are not drawn to scale and the cystoliths are not shown. **B)** The density.mm^{-2} of lithocysts on the surfaces of seedling cotyledon and the leaf.

The relative abundance of the above-given three types of lithocysts in *P. paniculata* is presented in Table 1. On cotyledon, lithocysts were predominantly Of RR type. RN or NN types were absent from ventral surface of cotyledon. There was, however, merely 6.66 % representation of RN type on dorsal surface of cotyledon. The three types of lithocysts were, however, present on leaf. NN type of lithocysts was dominant type (50-58%) on ventral and dorsal surface. RR and RN types were around 20-25%. It follows from these results that a species may have more than one of the above-said lithocyst types.

The lithocyst density per mm^2 in cotyledon and leaf is presented in Fig. 5B. The lithocyst density in the two components was almost comparable -averaging around 13 to 15 per mm^2 in *P. paniculata.*

Variation in lithocysts has attracted the attention of several taxonomists. Metcalfe and Chalk (1950) gave systematic account of Acanthaceae and categorized seven different groups based on their features. Lithocysts are usually present in Acanthoideae. According to Ahmad (1975), subfamilies Thunbergioideae, Nelsonioideae and Mendoncioideae are characterized by the absence of lithocysts. Exceptionally *Thunbergia laevis* is reported to contain cystoliths (Kumar and Paliwal, 1975). Lithocysts can help in making taxonomic considerations and distinction at species level (Ahmad, 1975; Inamdar *et al.*, 1990).

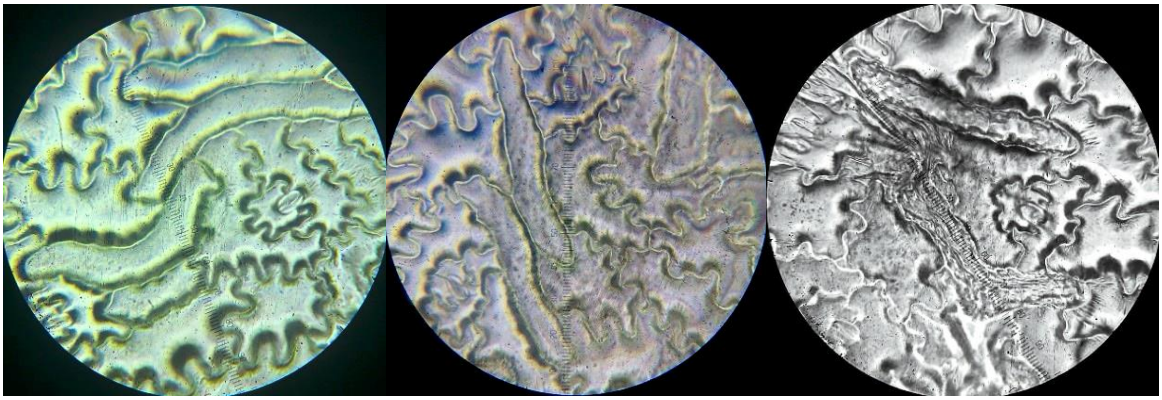


Fig. 6. *P. paniculata* cotyledon dorsal surface. Progressively developing lithocysts.

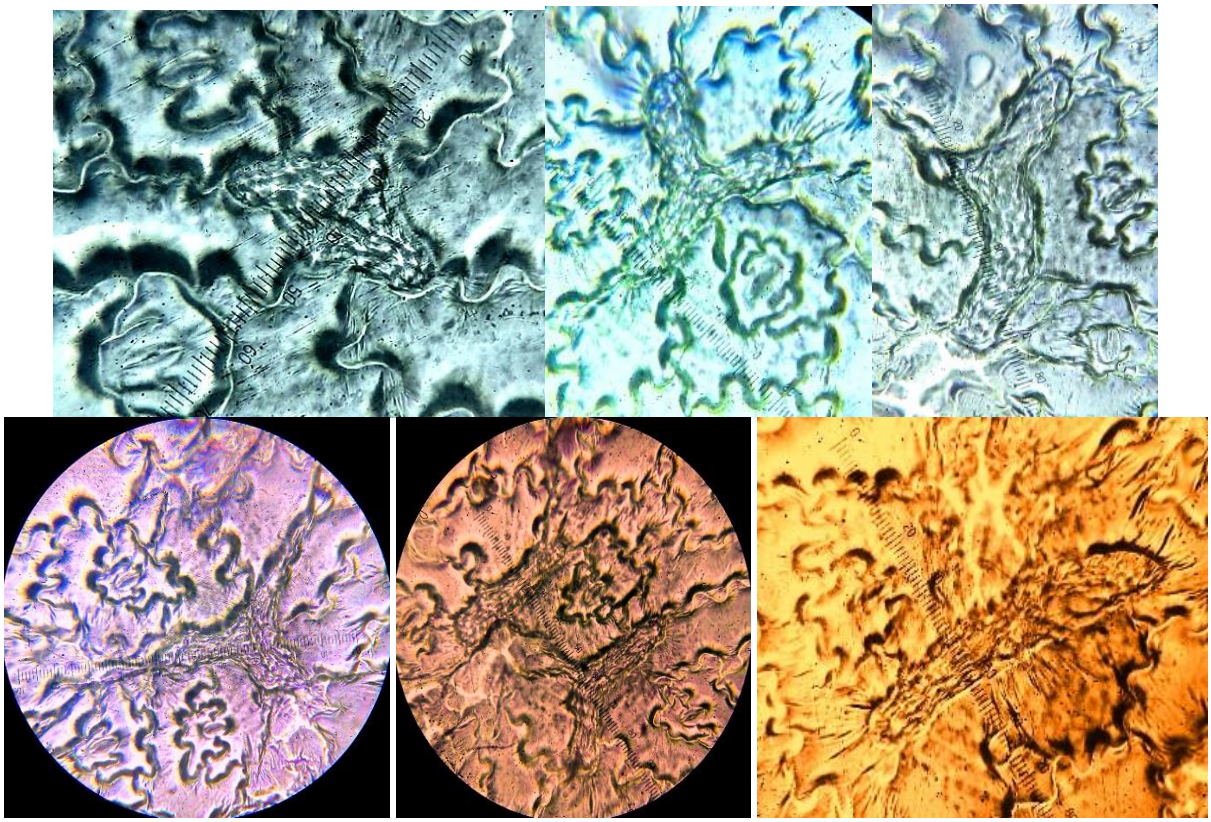


Fig. 7. Variously shaped lithocysts on dorsal surface of *P. paniculata* cotyledon.

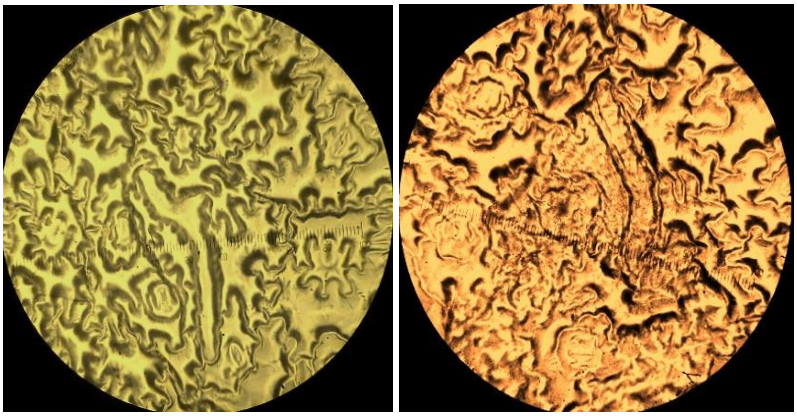


Fig. 8. Lithocysts from ventral surface of a young cotyledon. A, developing lithocyst and B, two fairly developed adjacent lithocyst.

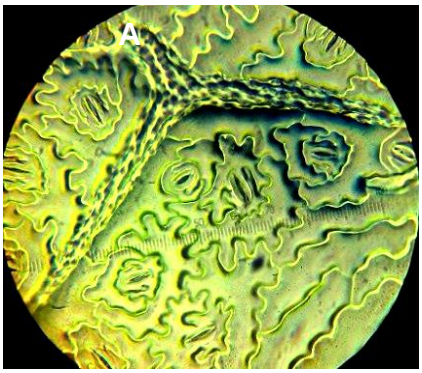


Fig. 9A. T-shaped lithocyst on ventral leaf surface. Fig. 9B. lithocyst separated from a mature leaf (45 x 15 X).

In *P. paniculata* the lithocysts were seen to run abreast the veins and parallel along the veins in the laminar island running parallel to each other. This character is similar to that found in *Lepidogathis cristata* (Bhogaonkar and Lande, 2015).

The location and dispersion parameters of lithocysts size are presented in Table 2. The length and width of the lithocysts varied with respect to the seedling components. The lithocysts were the largest in length on the ventral surface of leaf (326.36 ± 21.07 µm). The lithocysts on the dorsal surface were shorter (167.44 ± 8.70µm). Lithocyst size on dorsal and ventral cotyledonary surfaces was somewhat comparable (199.32 ± 8.33 and 222.55 ±12.09 µm, respectively). The smallest lithocysts were found on epicotylar surface (56.14 ± 4.56 µm). Lithocysts were broader on the ventral cotyledonary surface followed by those of the dorsal cotyledonary surface. They were narrower on leaf and epicotyl. Leaf lithocysts were narrower than that of cotyledons. Bhogaonkar and Lande (2015) have reported lithocyst size in an Acanthaceous taxon, *Lepidogathis cristata*. By comparison, *P. paniculata* lithocysts were larger in length than that in *L. cristata* (dorsal: 183.3 ± 14.19 µm; ventral: 112.90 ± 8.33 µm) but narrower than that in *L. cristata (*dorsal: 51.0 ± 0.033; ventral: 34.50 ± 0.322 µm). Bhogaonkar and Lande (2012) have reported lithocysts size in *Barleria prionitis* to be 85.2 ± 3.16 x 22.3 ± 1.04 µm on dorsal epidermis and 90.5 ± 2.81 x 28.6 ± 1.60 µm on ventral epidermis. *P. paniculata* lithocysts were larger than that of *B. prionitis*.

Table 1. Percent proportion of lithocyst types on dorsal and ventral surfaces of cotyledon and adolescent leaf.

Lithocyst Types *	Cotyledons		Leaf	
	Dorsal	Ventral	Dorsal	Ventral
RR	93.3	100	20.62	25.56
RN	6.66	Zero	20.62	24.44
NN	Zero	Zero	58.7	50.00

*, RR, Ends are round; RN, One end round and other tapered (narrow) and NN, Both ends tapered (narrow).

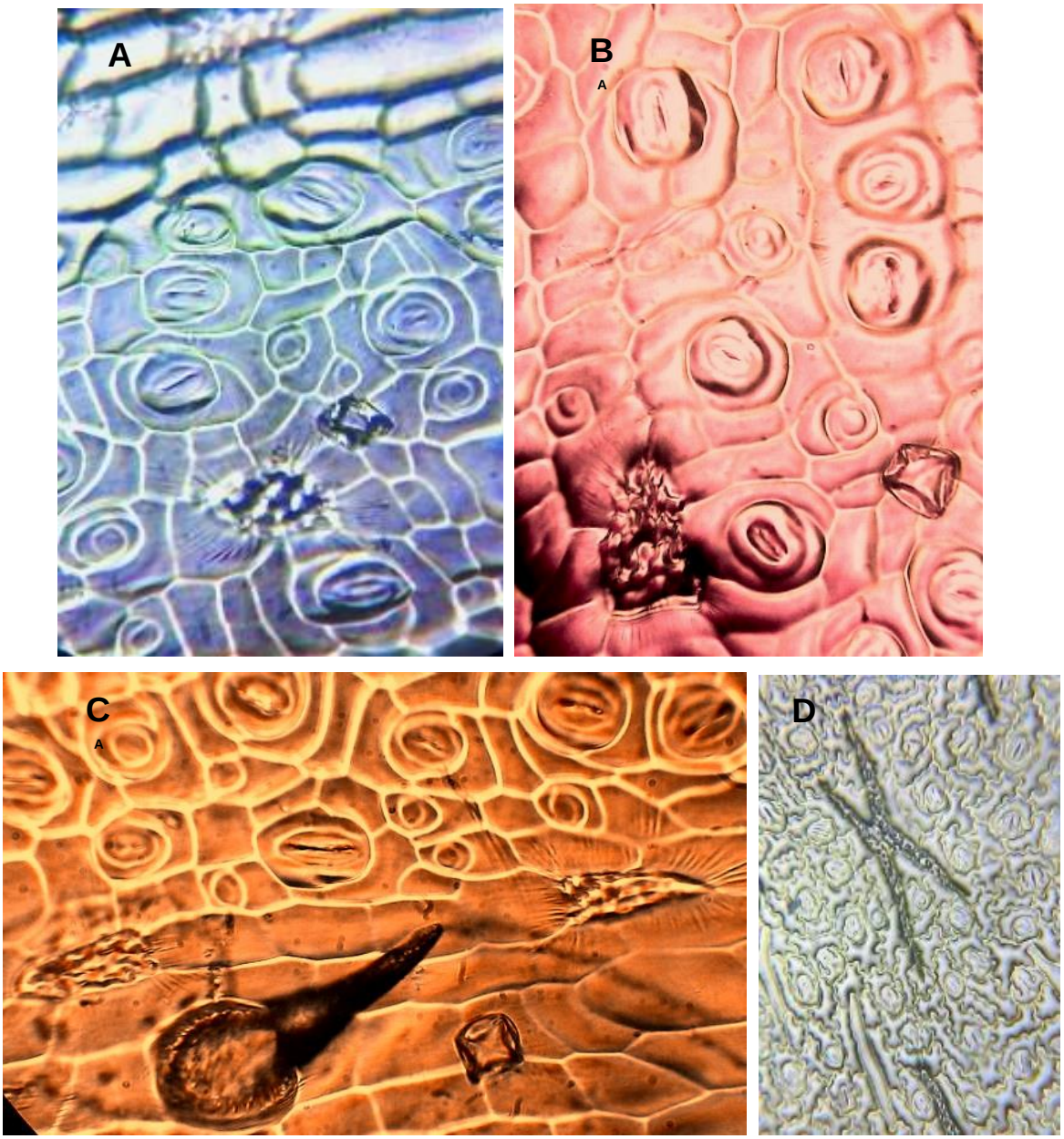


Fig. 10. Surface of epicotyl showing meristemoids, stomata, trichomes and crystals and differentiation of groove and ridge cells. Ridge bears more trichomes (A, B and C). The crystals are presumably of calcium oxalate. Two bent lithocysts are seen to join to form X-shaped structure (D). Images A-C: 45 x 10 X. Image D: 10x10 X.

Calcium oxalate crystals

Some crystals of calcium oxalate were observed on epicotyl (Fig. 10) of *P. paniculata*. These crystals were in form of regular tetrahedrons admeasuring 26 x 26 µm at base. Crystals of Calcium oxalate are known to occur in more than 215 higher plant families (McNair, 1932; Franceschi and Horner; 1980; Lersten and Horner, 2006) including gymnosperms and angiosperms. They may be present in almost all of the parts of the plant (Tütüncü Konyar *et al.*, 2014) and can be located in specific tissues such as epidermis, cortex, phloem, xylem and pith. The occurrence of calcium oxalate crystals in leaf and stem has been reported in *Anabasis articulata*, *Chenopodium album*, *Convolvulus arvensis*, *Datura strumarium*, *Nerium oleander*, *Ricinus communis*, *Rumex nervosus*, *Pergularia tomentosa* and *Withania somnifera* growing naturally in Saudi Arabia (Doaigey (1991). The function of calcium oxalate in plants has been studied by many investigators. They are considered to be participating in Ca-homeostasis, Ca-storage (Franceschi and Horner, 1980), protection against insects and foraging animals. Many

believed that oxalate is an end product of metabolism and that the excess amounts may be toxic to the plant (Franceschi and Horner, 1980). They may be active against foraging animals causing irritation and burning sensation in mouth (Amato, 2006).

Stomata

Three types of stomata were observed on surface of *P. paniculata* seedling (Fig. 10, 11, 12, 13 14 and 15). The characterizing type of stomata to *P. paniculata* is diacytic type (A stoma completely surrounded by only two distinct or indistinctly subsidiaries equal or unequal, parallel, transverse, obliquely oriented to the guard cell but the conjoint wall of the abutting subsidiaries are lateral to the guard cells Subsidiaries may be mono- or polycyclic) which characterizes the family Acanthaceae. The diacytic stomata on hypocotylar and epicotylar surfaces are provided with smooth walled subsidiaries (Fig. 10) whereas in case of leaf the subsidiaries of the diacytic stomata are highly sinuous (Fig.. 13 and 14). Other stomatal types observed on leaf of *P. paniculata* were Anisocytic (A stoma completely surrounded by three subsidiaries, variable in position and shape but one of the subsidiaries distinctly smaller. Subsidiaries mono- to polycyclic.) and Staurocytic types (Stoma completely surrounded by only four subsidiaries variable in shape and size but two of their conjoint walls are polar and two are lateral to the guard cell. Subsidiaries mono- or polycyclic). The subsidiaries of anisocytic and staurocytic stomata were highly wavy in the anticlinal wall. Verdoom *et al.* (2012) have investigated stomatal types in an acanthaceous taxon, *Justicia acuminatissima. Diacyti*c stomatal type in this species was the primary stomatal type which was also associated with anisocytic type of stomata also – of course in small number. This is, therefore, obvious that acanthaceous taxa need to be re-examined with respect to the variability of stomatal types in Family Acanthaceae. Besides diacytic, anisocytic stomata have also been reported in *Avicennia* (Noor-Syaheeru *et al.,* 2015).

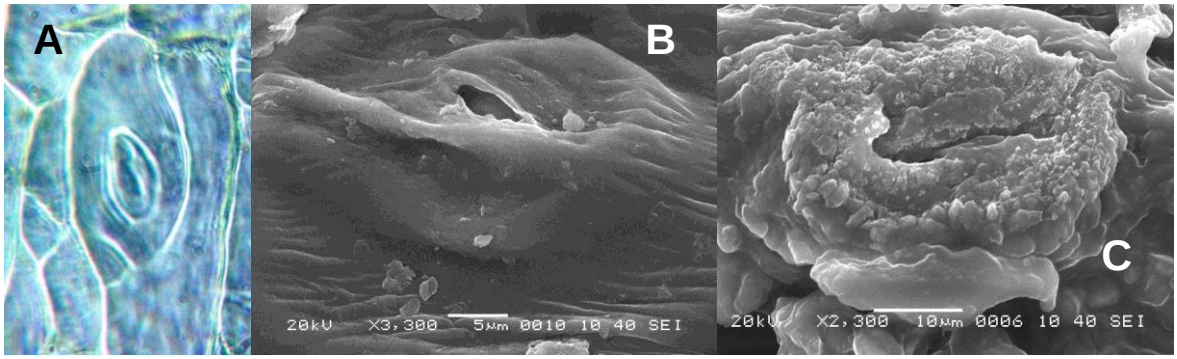


Fig. 11. Three hypocotylar stomata. A, OM image; B and C, SEM images. Thick epicuticular and other deposits apparent particularly in image C –stoma from basal region of hypocotyl. Diacytic nature apparent from image A.

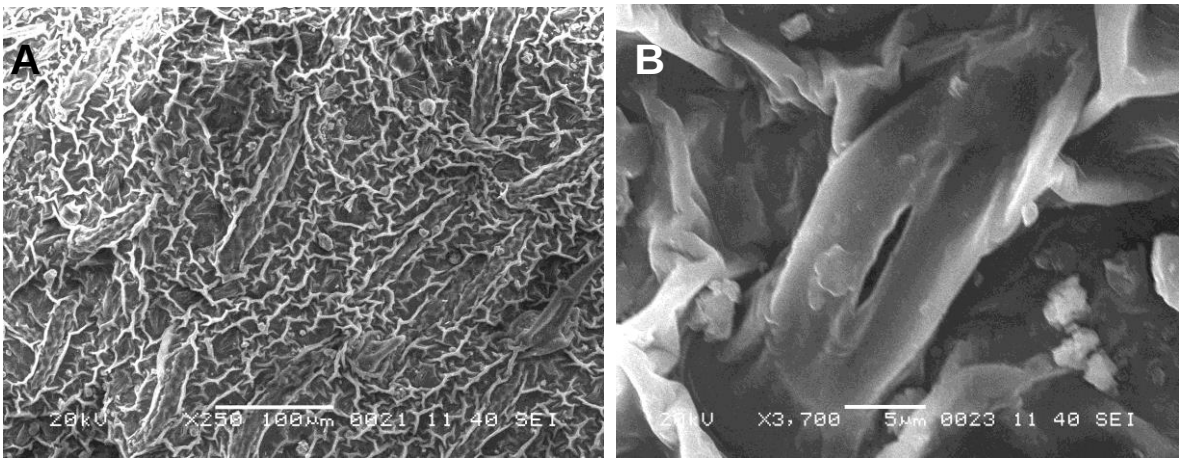
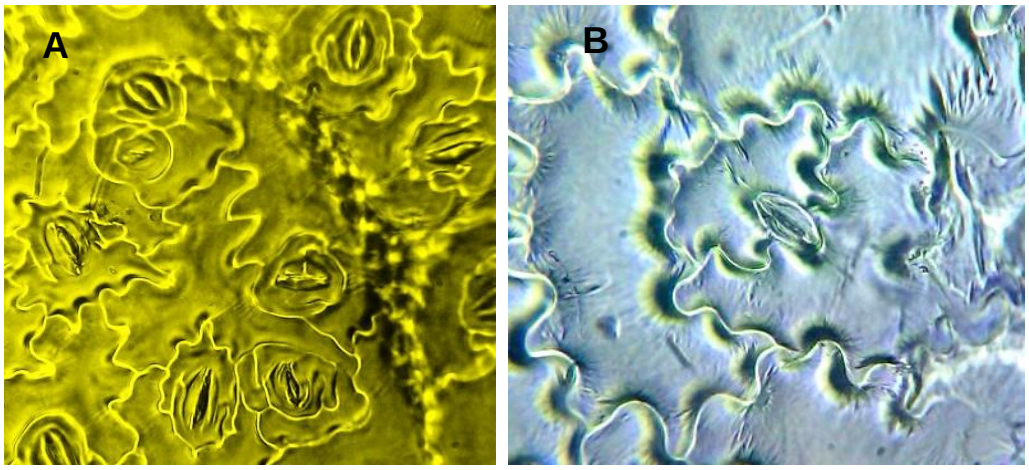


Fig. 12.General view of the dorsal surface showing a crop of lithocysts (A) and a stoma from the dorsal surface of leaf (B).



Fig, 13. The OM view of surface of fresh leaf. A, ventral surface view (45 x 10X). B, The enlarged view of the surface (45 x15 X) to show single stomata and ground epidermal cells. The subsidiaries as well as the pavement epidermal cells are with wavy anticlinal walls.

Stomata on leaf were commonly found to lie in clusters of two stomata. At times they form clusters of three or four stomata (Fig. 16). There were abnormal stomata on the leaf also but they were the variant of diacytic type of stomata. (Fig.17A). Some hemi-bicyclic and bicyclic stomata were also seen but they were kept in broader category of diacytic type (Fig. 17C). There were only diacytic stomata on cotyledons, hypocotyl and epicotyl. Epicotylar stomata had smooth walled subsidiaries and stomatal apparatus generally round (Fig. 10). Foliar stomata had sinuous subsidiaries and elongated stomatal apparatus. (Fig. 13).

Fig, 14. The epidermal cells are burgeoning above the stoma and provided with epicuticular encrustation (45 x 15 X, zoom) - diacytic stomata and a shoe-shaped lithocyst on dorsal surface of leaf. (45 x 15 X).

Stomatal size

Size of diacytic stomata (length and width of stomatal apparatus) on various components of *P. paniculata* seedling is given in Table 2. Size of normal diacytic stomata was found not to vary on different surfaces of the seedling. Stomatal size of *P. paniculata* leaf (Dorsal: 25.80 x 13.51 µm and Ventral: 27.5 x 14.85 µm) was somewhat comparable to an acanthaceous taxon, *Lepidogathis cristata* (Dorsal: 28.50 x 15.0 and Ventral: 34.50 x 21.2 µm) (Bhogaonkar and Lande, 2015) but quite smaller in length than that of *Barleria prionitis* (on dorsal

epidermis 38.7 ± 0.39 x13.0 ± 0.86 µm and on ventral epidermis 34.3 ± 0.32 x 12.5 ± 0.52 µm) (Bhogaonkar and Lande, 2012).

The smaller round diacytic stomata of epicotyl measured 15.07 ± 0.84 µm (varying only by 14.35%) in length and 13.45 ± 0.41 µm (varying by 9.92% only) in width. Foliar anisocytic and staurocytic stomata were larger in size - 33.46 ± 0.66 µm in length and 20.01 ± 0.33 µm in width.

Meristemoids on hypocotylar surface admeasured 19.84 ± 0.71 µm in length and 18.39 ± 0.087 µm in width.

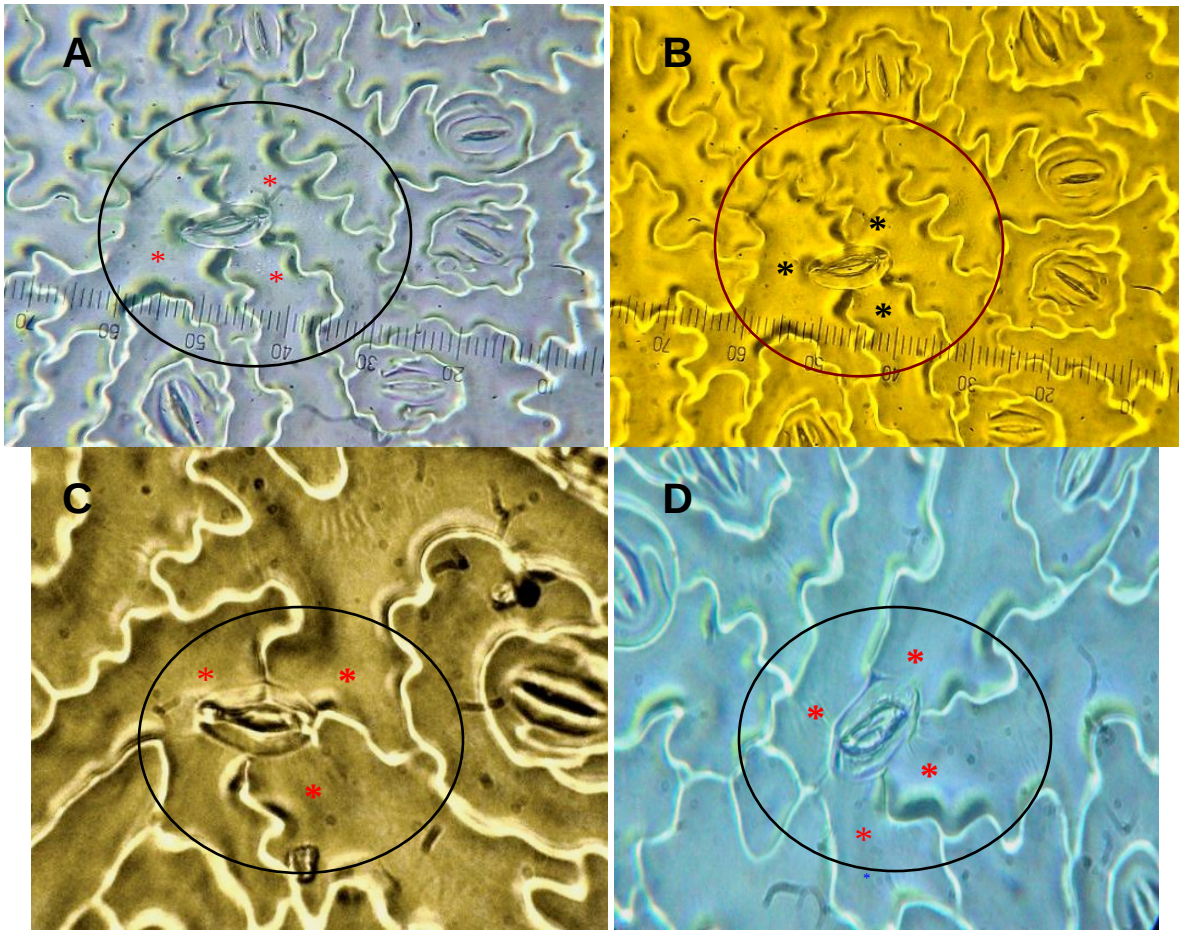


Fig. 15. Anisocytic (A, B and C) and staurocytic type of stoma (D) on the ventral surface of *P. paniculata* leaf. Some ten instances of occurrence of such stomatal types were recorded. Images: 45 x10 X.

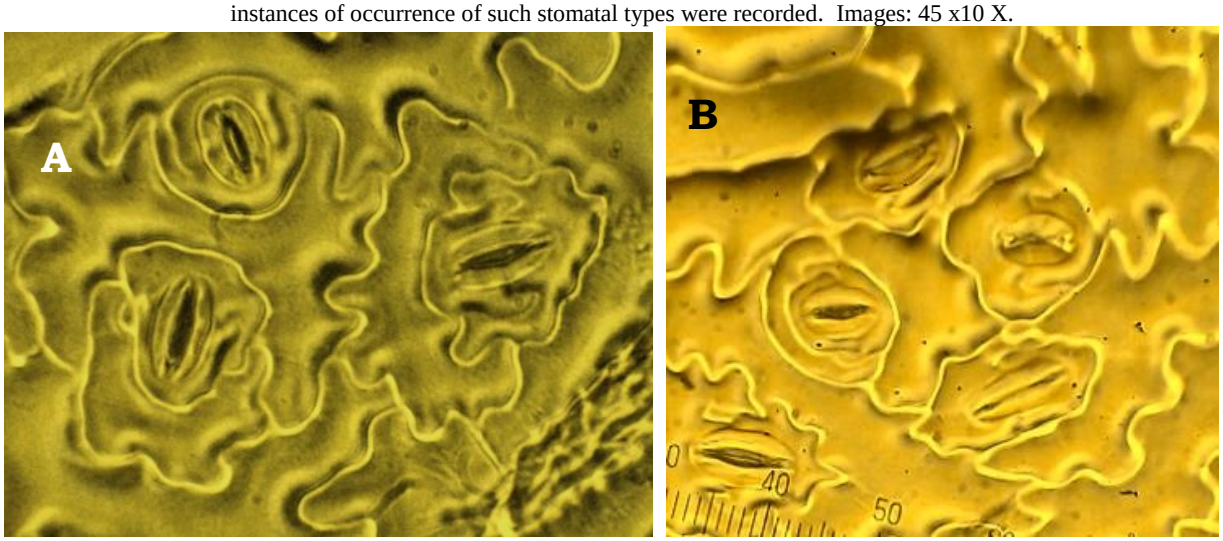


Fig. 16. A cluster of three and four stomata on dorsal surface of leaf. (Photos: 45x10 X).

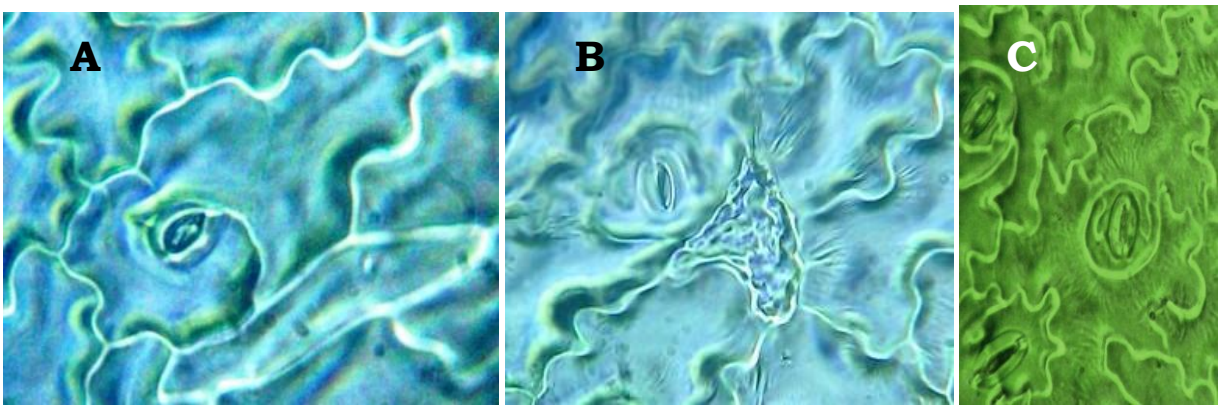


Fig. 17. An abnormal stoma (A) and a lithocyst (B) close to a diacytic stoma on the dorsal surface of leaf. (C) - a smaller foliar stoma surrounded by smooth walled subsidiaries and then a cycle of two normal sinuous subsidiaries. Photos: OM (45x10 X)

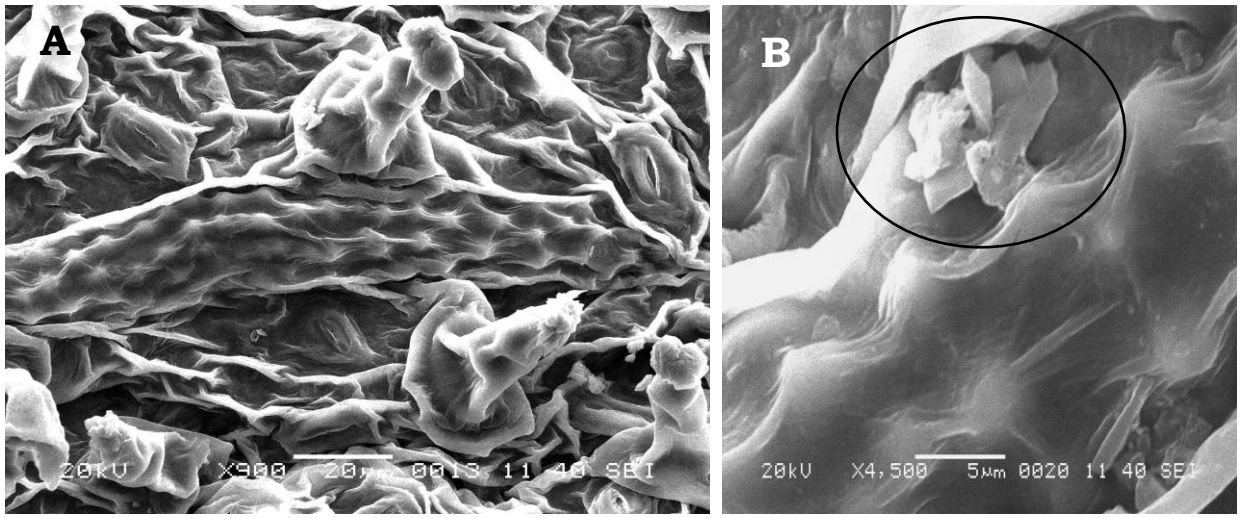


Fig. 18. (A) - Ventral surface of leaf showing trichomes, lithocyst containing cystoliths and stomata. Included inside lithocyst are several cystoliths formed due to the deposition of crystals of calcium salts being wrapped in cellulose. Note the erect trichomes with druses on the top. (B) SEM image of a crystals aggregate (aragonite type) nearby a lithocyst on the ventral surface of leaf. The wrapping of cystoliths inside cellulosic wall is apparent.

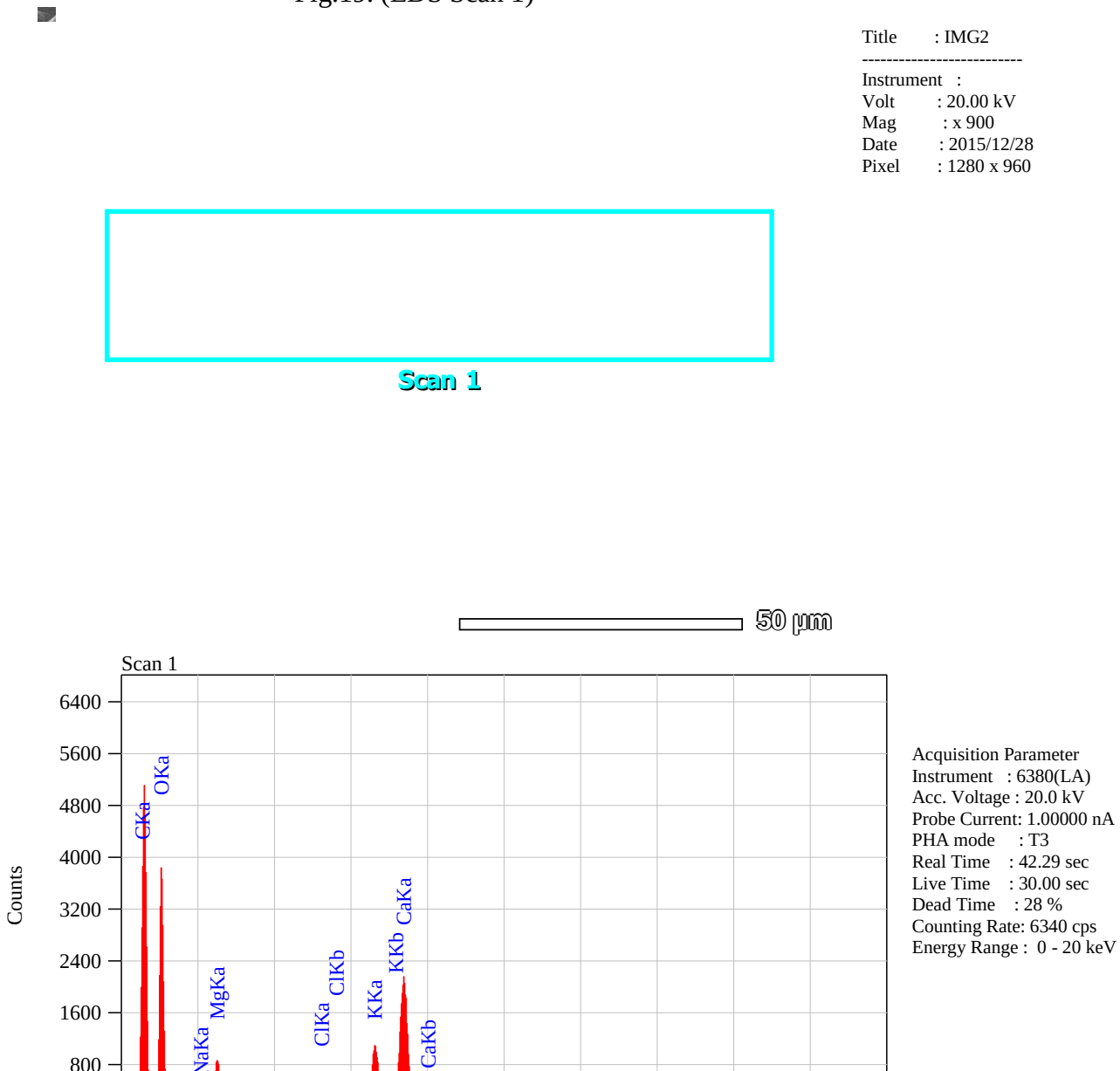
Stomatal density

Stomatal density per mm^2 of various surfaces of *P. paniculata* is presented in Table 3. Stomatal density on dorsal and ventral leaf surfaces distributed normally (KS-z: 0.989, $p < 0.282$ and KS-z: 0.905, $p < 0.386$, respectively) and averaged to be 146.45 ± 2.83 and 168.58 ± 2.70 stomata per mm^2, respectively which was quite higher than That of *L. cristata* (42.2 per mm^2 and 70.2 per mm^2 on dorsal and ventral surface, respectively as reported by Bhogaonkar and Lande (2015). Stomatal density on cotyledons, epicotyl and hypocotyl of *P. paniculata* was lower than that on the leaves. The lowest stomatal density was recorded on dorsal surface of cotyledon (30.67 ± 1.48 per mm^2), which, however, varied by a quantum of 34.04%. On all surfaces, stomatal density distributed normally except dorsal surface of cotyledon deviated from normal distribution (KS-z = 1.449, $p < 0.030$).

EDS determination of Elements

Elements detector system (EDS) based on Energy dispersive X-ray spectroscopy (EDS) attached to SEM was employed for element detection and quantitative elemental analysis. By this system, single shelled elements are not detected. The results of scan 1 and 2 for the foliar region containing several cystoliths in a lithocyst and the area of druses (probably aragonite polymorph) nearby a lithocyst, respectively, are presented in Fig. 19 and 20.

Fig.19. (EDS Scan 1)



ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.4418

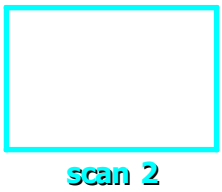
Element	(keV)	mass%	Error%	At%	Compound	mass%	Cation	K
C K	0.277	35.23	0.19	45.38				27.2316
O K	0.525	49.69	0.71	48.05				44.1068
Na K*	1.041	0.27	0.39	0.18				0.2989
Mg K	1.253	2.27	0.29	1.45				2.3593
Cl K	2.621	1.48	0.21	0.65				3.0697
K K	3.312	0.32	0.28	1.31				6.8740
Ca K	3.690	7.74	0.34	2.99				16.0596
Total	100.00	100.00						

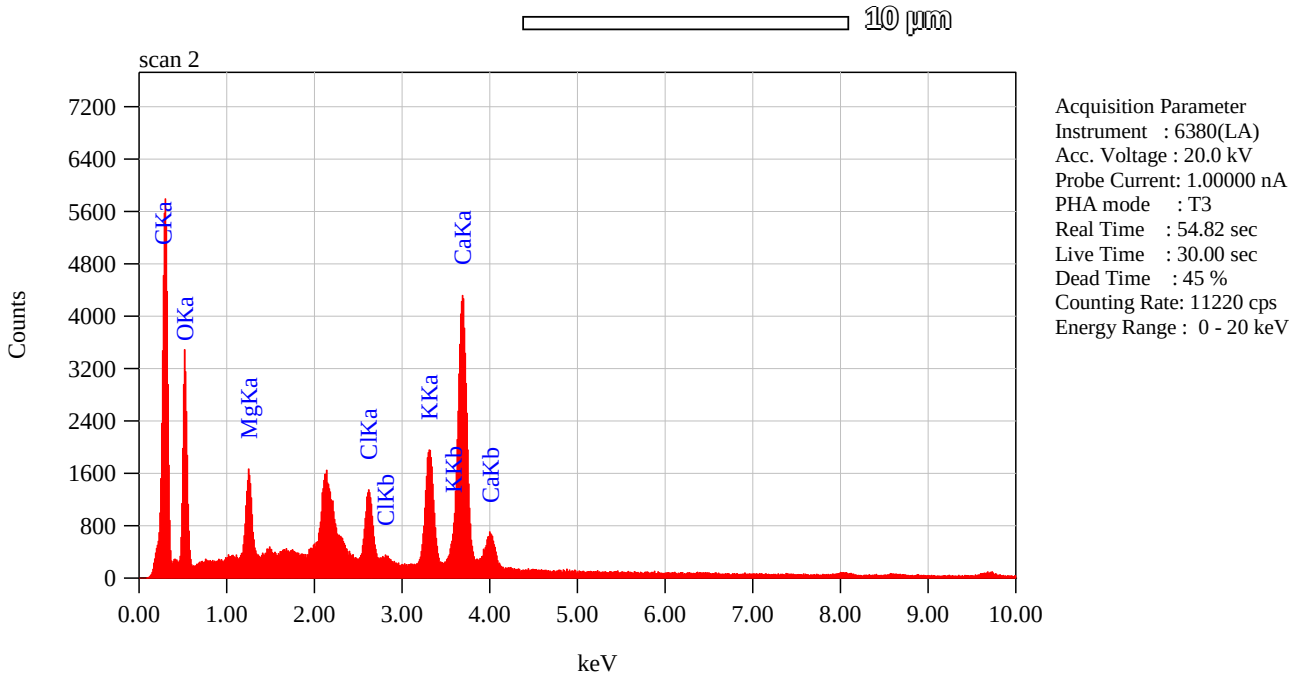
JED-2300 *AnalysisStation* JEOL

Fig. 20 (EDS Scan 2)

Title : IMG2

Instrument :
Volt : 20.00 kV
Mag : x 4,500
Date : 2015/12/28
Pixel : 1280 x 960





Acquisition Parameter
Instrument : 6380(LA)
Acc. Voltage : 20.0 kV
Probe Current: 1.00000 nA
PHA mode : T3
Real Time : 54.82 sec
Live Time : 30.00 sec
Dead Time : 45 %
Counting Rate: 11220 cps
Energy Range : 0 - 20 keV

ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.4101

Element	(keV)	mass%	Error%	At%	Compound	mass%	Cation	K
C K	0.277	35.18	0.19	47.36				24.9453
O K	0.525	41.93	0.78	42.38				30.5517
Mg K	1.253	3.29	0.25	2.19				3.5299
Cl K	2.621	2.34	0.18	1.07				4.9357
K K	3.312	4.70	0.25	1.94				9.9085
Ca K	3.690	12.55	0.30	5.06				26.1289
Total		100.00		100.00				

JED-2300 AnalysisStation JEOL

Table 2. Lithocyst and stomatal size in *P. paniculata* seedling.

Parameters	Length (µm)					Width (µm)				
	N	Mean	SE	CV (%)	Min-Max	N	Mean	SE	CV (%)	Min-Max)
Lithocyst Size (µm)										
Cotyledon (Dorsal)	30	199.315	8.3256	22.88	104.96 -288.64	30	36.135	1.4208	21.54	26.24 -62.32
Cotyledon (Ventral)	30	222.553	12.097	29.77	83.60 - 399.00	30	43.573	1.2555	15.78	26.60 – 57.0
Leaf (Dorsal)	60	167.44	8.7003	40.25	49.20 – 390.32	60	18.040	0.4180	17.95	9.84 – 29.52
Leaf (Ventral)	60	326.36	21.0754	50.02	78.72 – 760.72	60	27.9073	1.0369	28.71	16.40 – 59.40
Epicotyl	35	56.135	4.558	48.03	22.96 - 114.80	35	22.913	1.0426	26.92	9.84 - 36.080
Stomatal Size (µm) - Normal-sized Diacytic type										
Cotyledon (Dorsal)	60	29.547	0.3476	9.11	22.96 - 36.08	60	15.5253	0.2480	12.37	9.84 – 19.84
Cotyledon (Ventral)	60	28.700	0.4641	12.53	19.68 – 37.72	60	16.072	0.2723	13.12	11.48 – 19.68
Leaf (Dorsal)	75	25.803	0.4314	17.83	13.12 – 32.80	75	13.5136	0.2353	15.08	9.84 – 19.84
Leaf Ventral)	75	27.4645	5.3684	16.93	13.12 – 36.08	75	14.8475	0.2045	11.93	11.48 – 19.68
Epicotyl (Groove)	50	25.846	0.5614	15.36	16.40 – 32.80	50	14.6124	0.3277	15.86	9.84 – 19.84
Hypocotyl	50	27.978	0.7430	18.78	16.40 – 41.0	50	16.368	0.4307	18.61	9.84 – 22.96
Meristemoids *	10	19.84	0.711	11.33	-	10	18.37	1.088	18.73	-

*, on hypocotylar and epicotylar surface. .

Table 3. Density per mm^2 of stomata on various organs of *P. paniculata* seedling.

Organ	N	Mean	SE	Median	CV (%)	g1	g2	Sg1	Sg2	Min	Max	KS-z (p) ***
Cotyledon (Dorsal)	50	30.666	1.4764	29.4869	34.04	0.178	-0.617	0.337	0.662	9.83	49.14	1.449 (p < 0.030)
Cotyledon (Ventral)	50	83.153	2.3363	78.6318	19.87	0.166	-1.011	0.337	0.662	58.97	117.95	1.043 (p < 0.227)
Leaf (Dorsal)	100	146.452	2.8270	147.435	19.30	-0.054	-0.436	0.241	0.478	78.63	206.41	0.989 (p <0.282)
Leaf (Ventral)	100	168.583	2.699	167.093	16.01	0.125	0.513	0.241	0.478	88.46	245.72	0.905 (p < 0.386)
Epicotyl (Groove)*	30	126.139	7.302	127.727	31.71	-0.128	-0.462	0.427	0.833	39.32	206.41	0.563 (p < 909)
Epicotyl (Groove)**	30	68.803	4.8975	63.888	38.98	0.567	-0.438	0.427	0.833	29.49	127.78	0.783 (p < 0.572)
Hypocotyl	30	49.80	3.493	49.145	38.42	-0.040	-0.461	0.427	0.833	9.83	88.46	0.623 (p < 0.833)

*, young stomata and meristemoids; **, developed stomata.; ***, Kolmogorov-Smirnoff test.; g1, skewness; g2, kurtosis. Sg1, SE of skewness; Sg2, SE of kurtosis.

In scan 1, the dominating element was Oxygen (49.69%), followed by Carbon (35.23 ± 0.19%), Calcium (7.74 ± 0.34%), Potassium (3.32 ± 0.28%), Magnesium (2.27 ± 0.29%), Chlorine (1.48 ± 0.21 %), and Sodium (0.27 ± 0.39%).

In scan 2, the dominating element was Oxygen (41.93 ± 0.78%), followed by Carbon (35.018 ± 0.19%), Calcium (12.55 ± 0.30%), Potassium (4.70 ± 0.25%) Magnesium (3.29 ± 0.25%) and Chlorine (2.34 ± 0.18%).

On the basis of elemental composition, the two scans showed a similarity of 91.92%, however, it is obvious that Calcium concentration in Scan 2, increased from 7.74 to 12.55% i.e., an increase by a quantum of 62.14% over scan 1. The data indicated that Calcium entered the composition of the druses. The druses in their structure resembled to a polymorph, aragonite of calcium carbonate. It may presumably be thought that the druses and most part of the cystoliths of *Peristrophe paniculata* is composed of calcium carbonate. Tayler (1993) has reported that the mineral part of cystoliths in case of *Ficus retusa* is $CaCO_3$ which transforms to calcite when moistened. Okazaki *et al*. (1991) have isolated cystoliths from *Ficus retusa* and *Celtis sinensis* and analyzed their composition. They reported that in *Ficus elastica* cystoliths besides $CaCO_3$ may also have small amount of Si, Mg and traces of Ti, and Al. $CaCO_3$ content of upper epidermis containing cystoliths in *Justicia procumbens* is reported to be 0.40mg per cm^2. Such parameter for other species viz. *Ficus retusa*, *Ficus elastica* and *Celtis sinensis* is reported to be 1.06, 0.39 and 0.47 mg per cm^2 of their leaves, respectively (Setoguchi *et al.,* 2013).

Lithocysts are formed in the early stages of organ development. Cystoliths inside are formed only when the located tissue is exposed to light. Franceschi and Horner (1980) reported that various physical and chemical parameters (Light, pressure, pH and ion concentration) influence the growth and habit of calcium depositions. Cystoliths are extracellularly formed in the cell wall of the lithocysts. Cystoliths have a stalk and the amorphous calcium carbonate deposited with regular cellulosic fibrils. Plant cell wall is important in $CaCO_3$ nucleation. (Okazaki *et al.,* 1986). In *Justicia procumbens* the main mineral element composition of cystoliths in cotyledon were Ca and P. The Calcium is more deposited in the central part of the cystolith than in the margin area (Lin *et al.,* 2004). The physiological significance of the cystolith is obscure. However, they are suggested as CO_2 and Ca^{++} reservoirs for photosynthesis.

REFERENCES

Abdulazeez M., I.A. Baba, E.Z. Yenusa, S.J. Omanibe and I.H. Oladimeji (2013*).* Antitrypanosomal effect of *Peristrophe bicalyculata* extract on *Trypanosoma brucei* infected rats. *Asian Pacif. J. Trop. Biomed*. 3(7): 523-531.

Abdulazeez, A.M., A.J. Nok, and H. Gwani (2009). *Effect of Peristrophe bicalyculata on lipid profile of P-407 induced hyperlipidemic Wister rats.* Ph. D. thesis. Dept. Biochem. Ahmadu Bello University Zaria, Kaduna, State, Nigeria.

Ahmad, K.J. (1975). Cuticular studies in some species of *Lepidagathis* and *Barleria*. *Bot., Gaz.* 136(1): 129-135.

Ahmad, K.J. (1979). Taxonomic significance of epidermal characters in Acanthaceae. In: *Progress in Plant research.* Today and tomorrow's Printers and Publishers. New Delhi. Vol1. Pp. 135-160.

Amato, P. (2006). The secret life of plant crystals. *Sci. Tech*. 84(6): 26-27.

Amritphale, Dilip and S.K. Sharma (2008). Seedlings of dicots: Form and Function. *Resonance J. Sci. Edu*. 13 (5): 468 -474.

Bhogaonkar, P.Y. and S.K. Lande (2015). Anatomical characterization of *Lepidogathis cristata* Willd.- An ethanomedicinal herb. *J. Global Sci.* 4(5): 2282-2288.

Bhogaonkar, P.Y. and S.K. Lande (2012). Anatomical characterization of *Barleria prionitis* L. A well known medicinal herb. *Biol. Forum – An intern. Journal* 4(1): 1-5.

Cutter, E. (1972). *Plant Anatomy Experiment and Interpretation*. London Edward Ltd.

Doaigey, A.R. (1991). Occurrence, type, and location of calcium oxalate crystals in leaves and stems of 16 species of poisonous plants. *Am. J. Bot*. 78(12): 1608-1616).

Franceschi, V.R. and H.T. Horner (Jr.) (1980). Calcium oxalate crystals in plants. *Bot. Rev.* 45: 361-427.

Garwood, N.C. (1996). Functional morphology of tropical tree seedlings (pp. 59-129). In: *The Ecology of Tropical Forest Tree Seedlings* (Ed. M.D. Swaine), MAB Series, Vol.17, UNESCO, Paris.

Hickey, L.J. (1973). Classification of the architecture of dicotyledonous leaves. *Am. J. Bot.* 60(1): 17-33.

Inamdar, J.A., Chaudhari and R. Rao. (1990). Studies of the cystoliths of Acanthaceae. *Fedds Repert* 101 (7/8): 417-424.

Jani, S. and H.C. Rudrappa (2014). Morphological, structural and micrometric study of cystolith of Family Acanthaceae W.S.R to Kalmagh. *Int. J. Green Pharm*. 8: 13-17.

Khan, D., S.S. Shaukat and M.J. Zaki (2014). Structure, composition and pattern in *Peristrophe paniculata* (Forssk.) Brumitt. Dominated ruderal vegetation emerging after summer rains in Karachi. *Int. J. Biol. Biotech*. 11(2-3): 421-434.

Kumar, S. and G.S. Paliwal (1975).Foliar anatomy of the family Acanthaceae II. The tribe Thunbergieae and Nelsoniaeae. *Acta Bot. Ind*. 3(2): 121-131.

LAWG (Leaf Working Group). (1999). *Manual of Leaf Architecture: Morphological description and Categorization of Dicotyledonous and Net-Veined Monocotyledonous Angiosperms.* Smithsonian Institution, USA. Pp. 65.

Lersten, N.R. and H.T. Horner (2006). Crystal micropattern development in *Prunus serotina* (Rosaceae, Prunoideae) leaves. *Am. J. Bot*. 97: 723-729.

Lin, Miao-Ling, Tsair-Bor Yen, and ling-Long Kuo-Huang (2004). Formation of calcium carbonate deposition in the cotyledons during germination of *Justicia procumbens* L. (Acanthaceae) seeds. *Taiwania* 49 (4): 251-261.

McNair, J.B. (1932). The intersection between substances in plants: essential oils and resins, cyanogens and oxalate. *Am. J. Bot*.19: 255-271.

Metcalfe, C.R. and L. Chalk (1950). *Anatomy of Dicotyledons*. Vol 1- Clerendon Press, Oxford.

Noor-Syaheeru, M.Y. and T. Noraini, R.K. Rashiah and C.A.C. Che-Nurul-Aini (2015). Leaf anatomical characteristics of *Avicennia* L. and some selected taxa in Acanthaceae. *Malaya Nature Journal* 67(1):81-94.

Ogunwande, I.A., T.M. Walker, A. Bansal, W.N. Setzer and E.E.R. Essein (2010). Essential oil constituents and biological activities of *Peristrophe bicalyculata* and *Borreira verticillata. Nat, Prod. Comm*. 5(11): 1815-1818.

Okazaki, M., H. Setoguchi and E. Hisanaga (1991). Inorganic composition of cystoliths isolated from leaves of higher plants. In: Saga *et al*. (1991, Eds.). *Mechanisms and Phylogeny of Mineralization in Biological Systems*. Springer-Verlag, Tokyo.

Okazaki, M., H. Setoguchi, H. Aokia and S. Suga (1986). Application of soft X-ray micro-radiography to observation of cystoliths in the leaves of various higher plants. *Bot. Mag*. 99: 281-287.

Pandey, B.P. (2008). *Plant Anatomy*. 6th Edition, S. Chand & Co., New Delhi. 376 PP.

Patil, A.M. and D.A. Patil (2011). Occurrence and significance of cystoliths in Acanthaceae. *Curr. Bot.* 2(4): 1-5.

Prabhakar, M. (2004). Structure, delimitation, nomenclature and classification of stomata. *Acta Botanica Sinica*, 46 (2): 242-252.

Rushmi, G., P. Jaya, P. Hardik, M. Bhumi and A. Shivani (2010). Peristrophe bicalyculata – A Review. *Pharmacognosy Journal* 2(4): 39-45.

Setoguchi, H., M. Okazaki and S. Suga (2013). Calcification in higher plants with special reference to cystoliths) (pp. 409-418). IN: Crick, R.E. (Ed). *Origin, Evolution and Modern Aspects of Biomineralization in Plants and Animals* (2013). *Springer Science & Business Media* 536 Pp.

Taylor, M.G., K. Simkis, G.N. Greaves, M. Okazaki and S. Maan (1993). An X-ray absorption spectroscopy study of the structure and transformation of amorphous calcium carbonate from plant cystoliths. Proc. Royal Soc. (Biol. Sci.) Vol. 252, issue: 1333. (DOI: 10.1098/rspb.1993.0048).

Tütüncü Konyar, S., N. Öztürk and F. Dane (2014). Occurrence, types and distribution of calcium oxalate crystals in leaves and stems of some species of poisonous plants. *Bot. studies*, 55: 32.

Verdam, M.C.S/, D.T. Ohana, M.C.P. Araujo, F. Guilhon-Simplicio, M.S. de Mensonsa and M.M. Pereira (2012). Morphology and anatomy of *Justicia acuminatissima* leaves. *Brazilean J. Phamacognosy*. Aop 12212.

Wang, Xiu-Mao, Mao Zi-Jun, Choi, Kyung and park, Kwang-Woo (2006). Significance of the leaf epidermis fingerprint for taxonomy of genus *Rhododendron*. *J. Forest Res.*, 17(3): 171-176.

Watt, M.W., C.K. Morrell, D.L. Smith and M.W. Steer (1987). Cystolith development and structure in *Pilea cadieri* (Urticaceae). *Ann. Bot.*, 60: 71-84.

Zar, J.H. (2010). *Biostatistical Analysis*. 5th Ed. Prentice-Hall, Englewood Cliffs, New Jersey, USA.

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