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MICROMORPHOLOGICAL STRUCTURE OF SEEDLINGS OF *RHYNCHOSIA MINIMA* (L.) DC. (PAPILIONACEAE) GROWING IN A DRY RUDERALIZED SITE IN KARACHI

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ABSTRACT

The micromorphological structure of seedlings of *Rhynchosia minima* (L.) DC. is described, from a dry ruderalized site of Karachi, on the basis of optical (OM) and scanning electron microscopy (SEM). The seedling appeared to be "Phanerocotylar-Epigeal-foliaceous" type. Several types of trichomes from cotyledons and leaves and structure of the resin gland (Brown-orange bulky capitate, glandular trichome) are described. Cotyledons are amphitrichomic and amphistomatic with paracytic arrangement of subsidiary cell. *R. minima* leaflets were amphistomatic with much larger number of stomata on ventral surface (283.3 ± 4.39 stomata per mm^2, N = 100, CV = 38.6%) as compared to the dorsal surface (48.06 ± 1.85 stomata per mm^2, N = 100, CV = 38.6%). The diversity of foliar stomata included, paracytic, anisocytic, staurocytic and anomocytic types. The stomatal size on abaxial surface leaf averaged to 18.53 ± 0.36 x 13.17 ± 0.30 µm. The predominating class of stomatal width (pore + guard cells) was 10.1 – 16 µm. Stomata on adaxial surface were comparable in size (19.76 ± 0.22 x 11.19 ± 0.18 µm). Quantitative element analysis based on elements detector system (EDS) of energy dispersive X-ray spectroscopy (EDS) is described.

Key Words: Rhynchosia minima (L.) DC Seedling, micromorphology, trichomes, resin duct, stomata.

INTRODUCTION

Rhynchosia minima (L.) DC. is a pantropical species commonly known as least stout bean, Burn-mouth-vine, and Jumpy bean. It has long synonymy (Basionym: Dolichos minimus L.) and is included in the red list of threatened species (Lopez-Poveda, 2012, IUCN, 2014). It occurs with grasses and shrubs in derelict areas, ruderalized land, and roadside distrurbed areas of Karachi. It is rated as good fodder with no known toxicities (Barnes, 1996; ANG, 2012) but leaves are shed in winter. It appears to be a potential medicinal species also. Jia *et al.* (2015) have reported the occurrence of some novel heteropolysaccharides from *R. minima* roots composed of arabinose, mannose, glucose and galactose of which PRM1 and PRM3 have exhibited strong *in vitro* anti-cancer activity. *R. minima* yields essential oil which has anti-oxidant and antibacterial activities (Gundidza *et al*, 2009) besides anthelmintic potential (Mali and Mahele, 2008). Bhattacharya and Maheshwari (1970) have investigated the extra-floral nectaries in *Leguminales* of Indian flora including *Rhynchosia*, *Vicia* and other genera and Vergas *et al.* (2015) have described the secretory structures and foliar anatomy of *Rhynchosia* spp. from herborized materials of Brazilian collection. *R. minima* appears to exhibit different ecotypes (ANG, 2012), we have undertaken to study the micro-structure of *R. minima* seedlings collected from Karachi University Campus, Karachi, arising after summer precipitation.

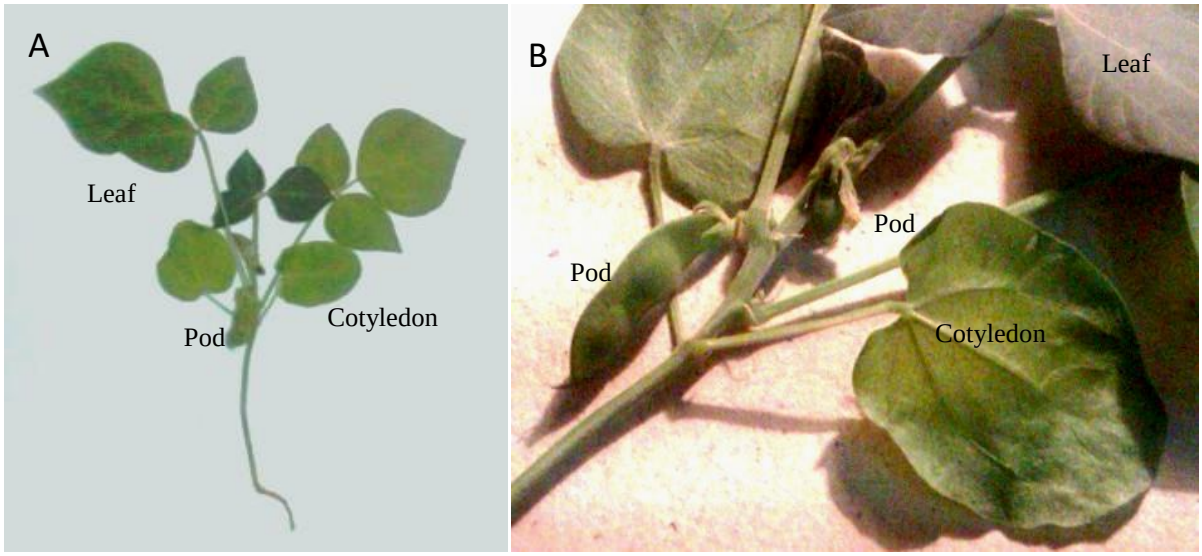


Fig. 1. Seedling of *R. minima* studied. A. Habit; B, Note that seedling under drought has produced two pods while still bearing green photosynthesizing cotyledons.

MATERIALS AND METHODS

Seedlings of *R. minima* were collected from a dry ruderalized site in August 2012 after some 30 days of light rains, from Karachi University Campus. The seedlings were studied for their morphological characters including stomatal types. Seedling type was described according to Vogel (1980) and Garwood (1996). Hickey (1979) and LWG (1999) were followed for description of cotyledon and leaf. To study stomatal types, leaflet epidermal impressions were made with clear nail polish (Wang *et al.*, 2006) and studied under compound optical microscope. 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. The data was analyzed statistically (Zar, 2010). For scanning electron microscopy (SEM), air-dried plant material (cotyledon and leaf) 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. The seedling employed in microscopic studies is shown in Fig. 1.

Fig. 2A. Germination in *R. minima*. It appeared to be "Phanerocotylar-epigeal- foliaceous type" – Cotyledons aboveground due to Hypocotylar elongation, epigeal and leaf like. Testa remained subterranean.

Fig. 2A. Cotyledon of *R. minima* (c. 269 mm^2). Seedling. There arise five vascular nerves in the umbo region embayed in sinus.

RESULTS AND DISCUSSION

R. minima is twining or prostrate ruderal herb attaining height c 30-40cm, stem slender, densely pubescent, striate, and cylindrical. Leaves trifoliate. Inflorescence raceme. Calyx green, the lobes lanceolate, 2-3 mm long; corolla yellow Pods falcate to oblong ovate, flattened, minutely villous, slightly curved with a beak at the apex. Seeds are ovate-reniform, dark brown to almost black, 3- 4 mm long and 1-2 (3) in a pod. The seeds of *R. minima* in soil are exposed to high summer temperature which breaks the seed dormancy under arid field conditions of Karachi and allows seeds to germinate after showers of summer rains (Shaukat and Burhan, 2000).

Seedling: "Seedling" is considered to be the final stage of the regenerative process of a plant from a seed. The use of this term is quite liberal. We have used this term as ecologists employ i.e. stage up to which the cotyledons are attached with the juvenile. According to Léonard's (1957) classification, germination, in *R. minima* is of "type A" germination (cotyledons spreading above soil; no consideration to the presence or absence of the collet). Seedling of *R. minima* appeared to belong to "Type I" seedlings of Klebs (1885) i.e. cotyledons lie above soil due to hypocotylar growth and there is no collet. As per classification by Garwood (1996), it may be rated as "Phanerocotylar-Epigeal-foliaceous" type of seedling (Fig. 2A) which is also seen in *Anageissus latifolia, Cucumis sativus, Manilkara hexandra* and *Terminalia arjuna* (Amritphale, 2004), *Phaseolus vulgaris* (Vogel, 1980) and in *Luffa cylindrica* as well (Khan *et al.*, 2017). However, it appears to be characterized with the fact that seed coat remain subterranean. It may be emphasized that the terms, phanerocotylar and cryptocotylar proposed by Duke (1965) are synonymous to epigeal and hypogeal germination, respectively. However, the use of the two sets of terms appears quite logical (as employed by Garwood, 1996) in the light of a very rare cases of species (*Rollinia saleifolia)* where germination is

epigeal cryptocotylar (Franceschini, 2004). The morphometric characteristics of the seedling studied for surface ornamentation are given in Table 1.

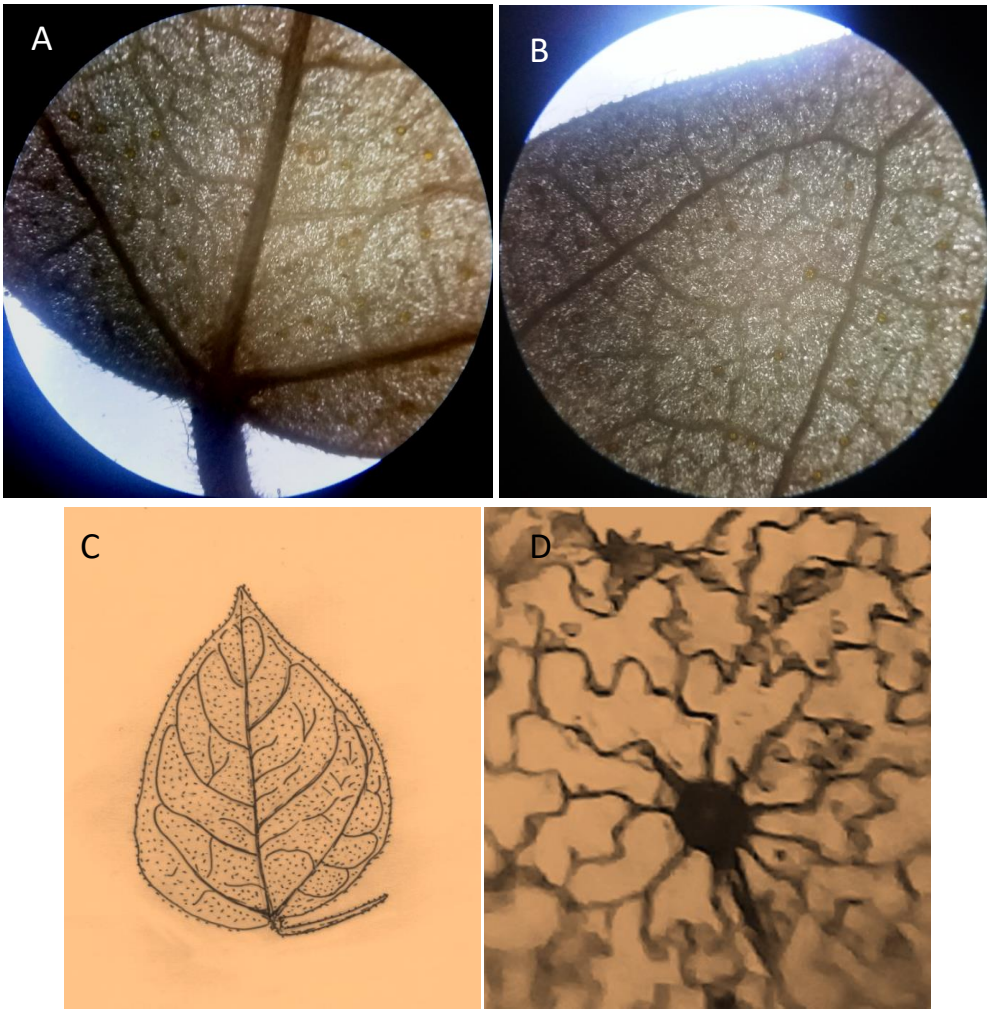


Fig. 3. A, venation in the basal part of a leaflet; B, Venation near the margin; C, Venation of a terminal leaflet drawn (Camptodromous venation with some brachidodromous loops); D, A sharply pointed trichome, the basal part of which is bulbous surrounded by nine sinuous epidermal cells arranged radially around..

Table 1. Morphometric parameters of the seedling studied for surface ornamentation.

Seedling components	Size	Seedling Parameters	Size
Height (cm)	9.5	Lateral leaflet I area (mm^2)	112,229, 300**α
Stem diameter (mm)	1.3	Lateral leaflet II area (mm^2)	79, 417, 280**α
Hypocotyl (cm)	4.2	Terminal leaf area (mm^2)	555, 741, 802**α
Number of leaves	3	Total leaf area (mm^2)	3515 α
Cotyledonary petiole lengths, (cm)	1, 1 *	Photosynthetic area (mm^2)	4041α
Cotyledon I area (mm^2)	269 α	Number of pods	2
Cotyledon II area(mm^2)	257 α	Pod I length (cm)	1.5
Total cotyledonary area (mm^2)	526 α	Pod II length (cm)	0.5
Foliar petiole (cm)	3.1, 4.1, 4.2**	Pod I width (cm)	0.4
Petiolule Length (leaflet I) (mm)	1.5, 2.0, 2.0**	Pod II width (cm)	0.2
Petiolule Length (leaflet II) (mm)	1.4, 1.8, 2.0**	Number of seeds Pod I	2
Petiolule Length (terminal Leaflet) (mm)	7.0, 8.0, 8.0**	Number of seeds Pod II	-

α, One-sided area; *, for two cotyledons respectively; **, in order of lateral leaflet I, lateral leaflet II and terminal leaflet of the three leaves, respectively.

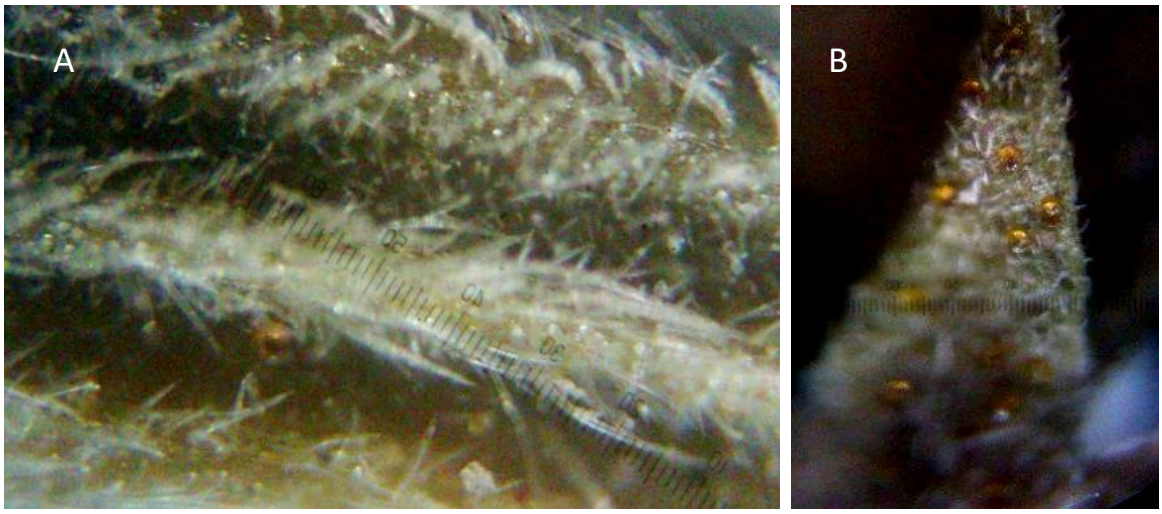


Fig. 4. Surface of stem (A) – very few glands and Outer surface of calyx (B) - showing dense trichomes crop and orange-brown glands (10x10X).

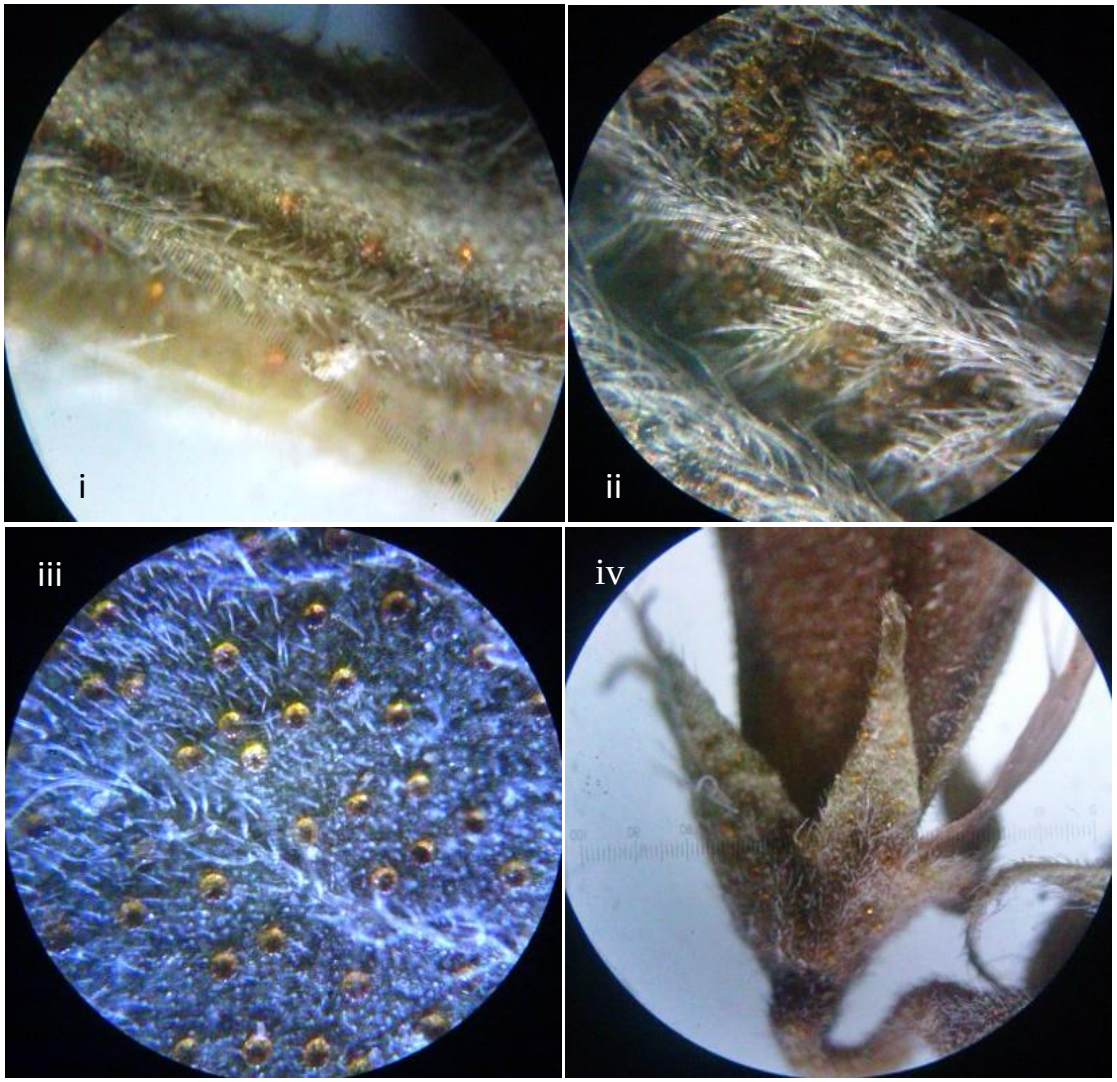


Fig. 5A. Optical microscopic views of surface of various parts of seedling. i, Petiole (10x5 X); ii, Very young leaf (underside, 10x10 X); iii, Mature leaf (underside, 10x10 X); iv, Flower (5 x 10 X). Orange-brown bulky glands may be seen on all parts..

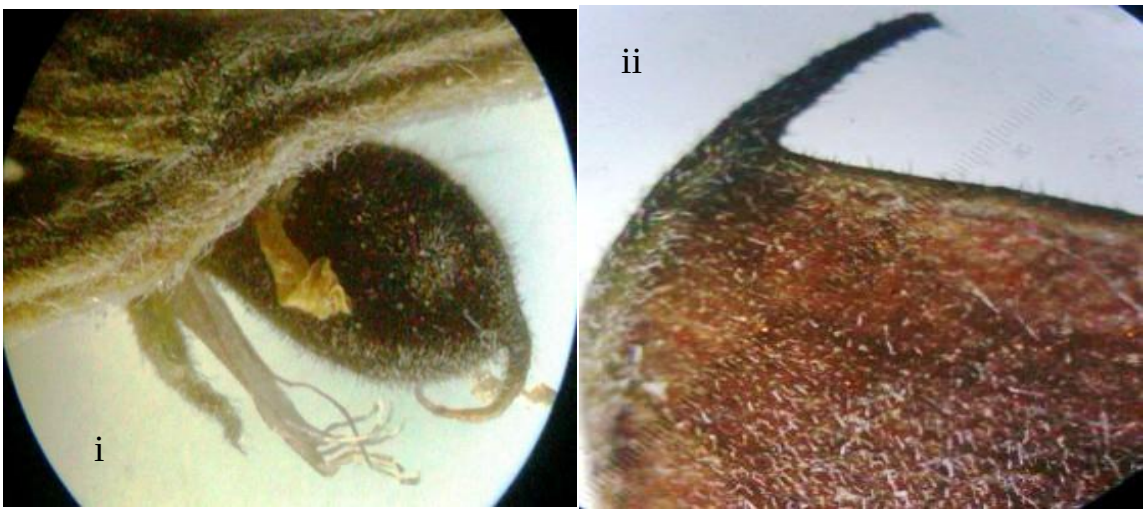


Fig. 5B. Optical microscopic views of surface of Young pod apex (i) and its enlarged view (ii) – showing orange-brown dots of glands (10x5 X).

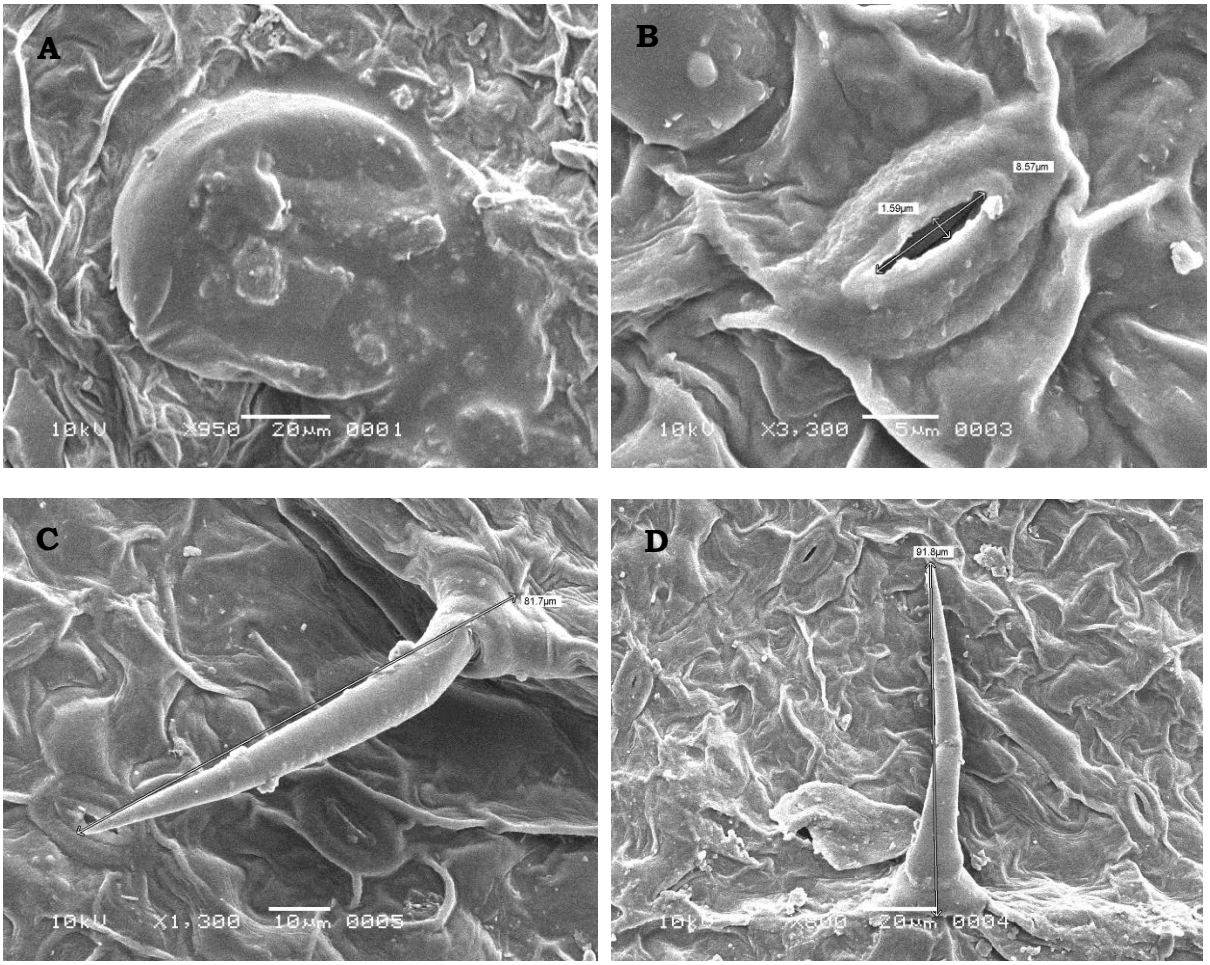


Fig.6. Dorsal surface of Cotyledon: A) A gland in a depression (c 100 µm in diameter), B) stoma (8.57 x 1.59 µm pore size), C and D) eglandular sharply-pointed trichomes (81.7 and 91.8 µm in length, respectively).

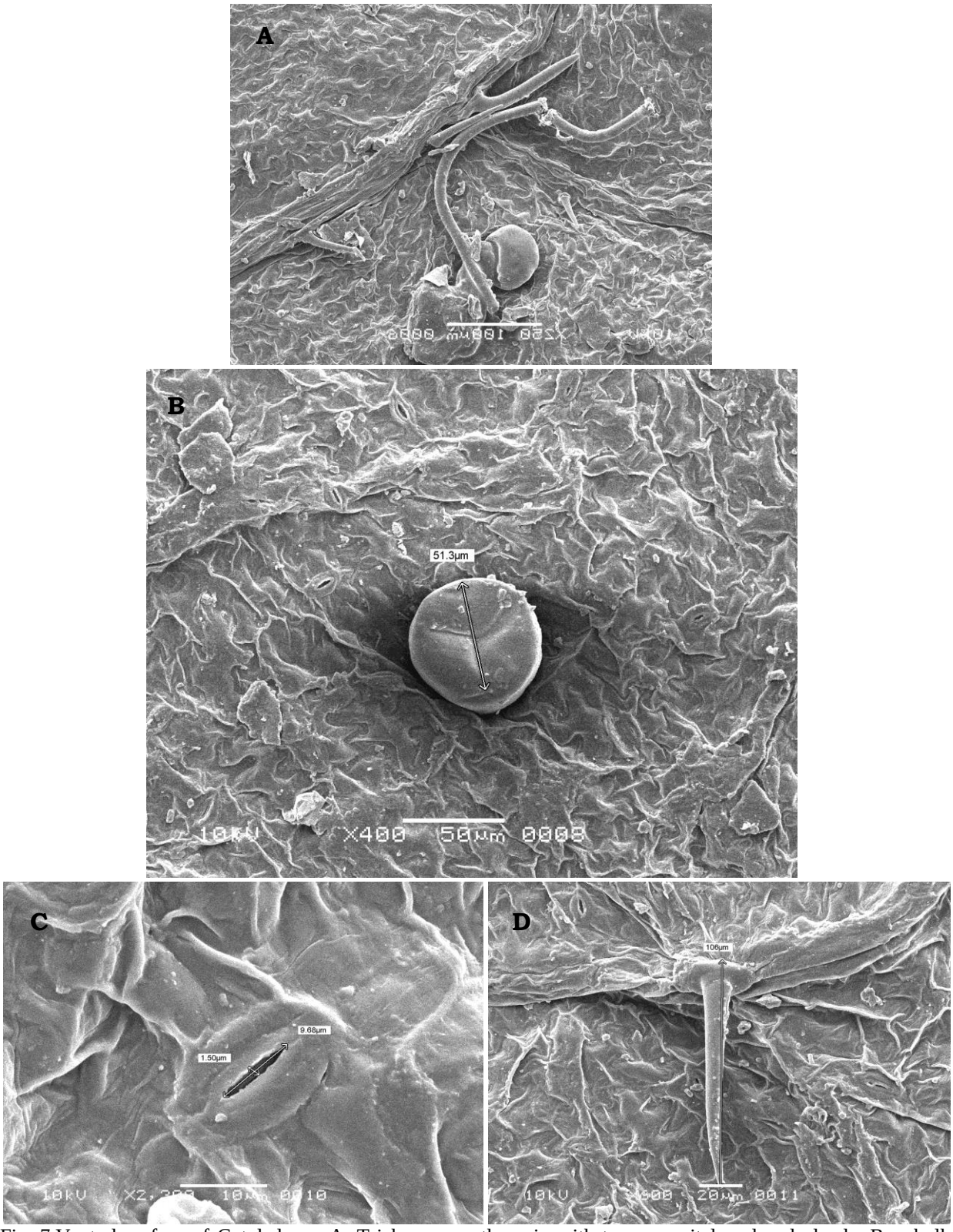


Fig. 7.Ventral surface of Cotyledon – A, Trichomes on the vein with two oppositely- placed glands; B, a bulky somewhat spherical but almost flat-topped gland in the depression, when seen from the top (the top tier is composed of three cells); C, a stoma (9.68 x 1.50µm pore size) and D, a trichome (106 µm in length).

Fig. 8. Dorsal leaf surface-Bulbous-based trichomes with sharply-pointed and elongated conical apical cells.

Hypocotyl

Long, cylindrical, pubescent.

Epicotyl

Epicotylar part of seedling is pubescent and parts of seedling (stem, petiole, leaves, calyces and pods bear trichome - glandular (resin glands) and eglandular both (Fig. 5A and B). The sinuous epidermal cells around the base cell of trichomes are arranged radially (Fig. 3D).

Cotyledon

Cotyledons are two, petiolate, wide elliptic or oblong-orbicular in shape, apex round with apex angle obtuse. The cotyledonary base is embayed in sinus with cotyledon base extension of c 0.25 cm. Five-nerved at base (Fig. 2B). Nerves are lighter in colour. Cotyledons are more or less equal in size (257 and 267 mm^2, respectively) and they stay with seedling for quit sometime. The seedling we studied had still borne cotyledons (and three leaves only) when entered the reproductive phase under drought conditions and produced two pods (Fig. 1). It indicated to the ruderal nature of *R. minima*. Curtailment of shoot under stress and diversion of resources into flowers, short life cycle, occurrence in disturbed areas and early production of flowers suggest to the ruderal nature of a species (Grime, 1977, 1979).

Cotyledons of *R. minima* are amphitrichomic and amphistomatic. Conical trichomes of dorsal surface are bulbous at the base and pointed at apex. On dorsal surface the glands are present but somewhat infrequently (Fig. 6A). Stomata generally of paracytic type (Fig. 6B). Glands on ventral surface are stalked, situated in depression and provided with a bulky head measuring 51.3 µm (Fig. 7B). Stomata on ventral surface are also paracytic and are as large as on dorsal surface (Fig. 7C).Trichomes on ventral surface are relatively longer (Fig. 7D).

Leaves

Leaves alternate, trifoliolate, herbaceous; margins entire, upper surface dull, lower surface pale green. The leaflets are rhomboid, ovate or sub-orbicular. Leaves are amphitrichomic (Fig. 8 and 9). Trichomes are bulbous-based and apically straight-sharply-pointed, curved or round. They and much larger on the veins and margins of the leaf (Fig. 8-11). There are golden to orange-red glands (secretory trichomes) on the underside of leaf (Fig. 5Aii and 5Aiii, 10, 11, 12). In lateral leaflets apex is acute (apex angle 70-84^o); the base of leaflets obtuse (base angle 91-100^o). In terminal leaflets the apex angle was mostly broad acute (82-88^o) but in one terminal leaflet apex angle was larger than right angle (110^o). The base angle of terminal leaflets was always obtuse (98–108^o). Petiolules pubescent; stipules caduceus, densely pubescent; Stipels present. The lateral leaflets asymmetrical, elliptical-ovate, and significantly smaller than the terminal leaflet. In the seedling studied, the lateral leaflets varied in size from 79 mm^2 to 417 mm^2 whereas terminal leaflets varied from 555 to 802 mm^2 (Table 1).

Primary and secondary venation prominent. The venation in leaflets of *R. minima* appeared to be camptodromous. There were three veins arising from the base of lamina in lateral as well as terminal leaflet (Fig. 3A, B and C). The secondary adjacent veins from these veins were arching distally towards margin where they anstomose with each other. According to Weyland (1968), the venation pattern in tribe Phaseoleae is known to vary only slightly and the prominent venation is camptodromous. In *Rhynchosia pringlej* each lateral vein is prominent, arching distally towards the margin, where it joins with the distal adjacent lateral vein. Branching loops of these lateral veins form a series of diminishing loops. Some 26 out of 53 species of Papilionoideae (Leguminosae) had brachidodromous veins on the same lamina as well (Weyland, 1968).

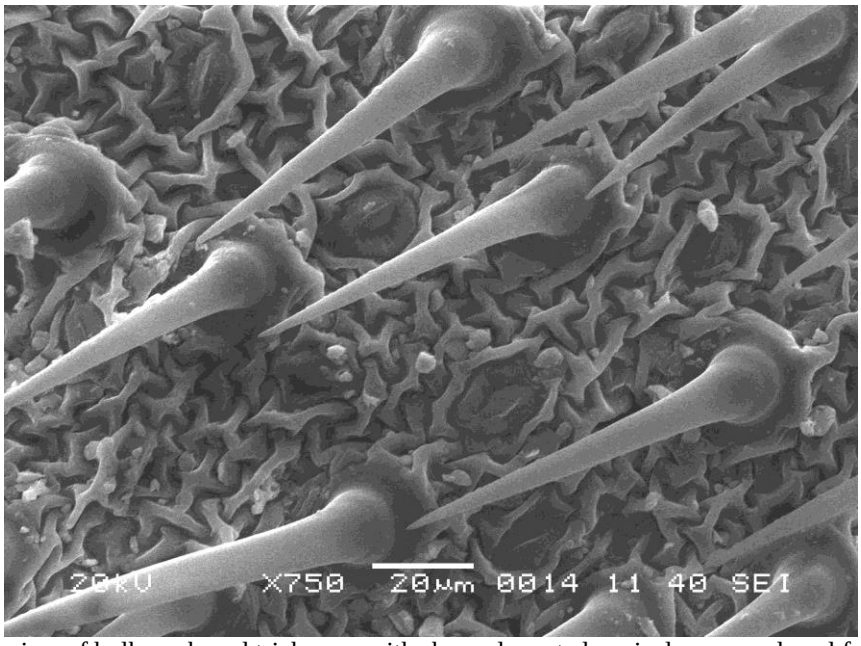


Fig. 9. Close up view of bulbous-based trichomes with sharp elongated conical apex on dorsal foliar surface and some scattered stomata.

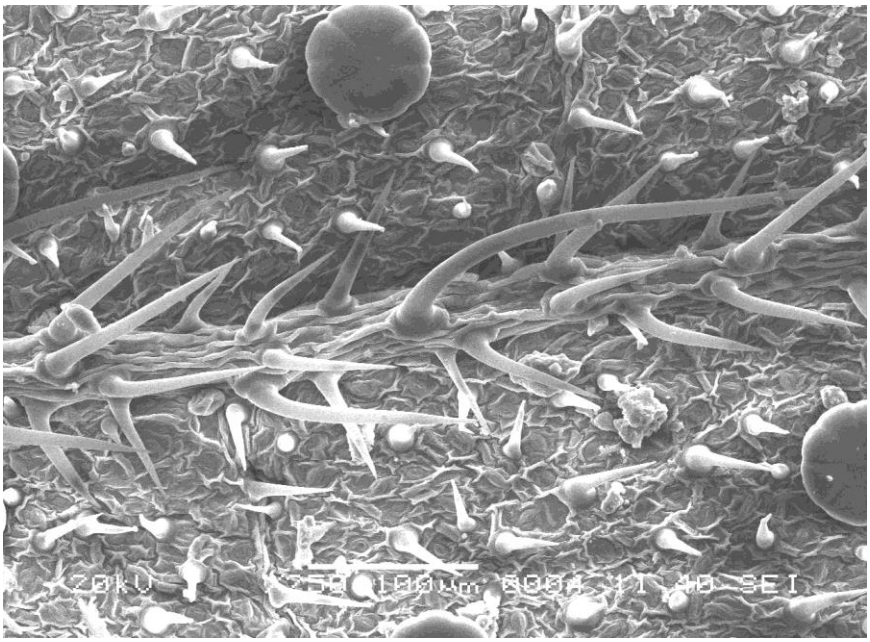


Fig. 10. Ventral surface of leaf -Straight and curved non-glandular trichomes on the veins are much larger in size than those on the laminar islands. Two glandular trichomes (glands) are also visible.

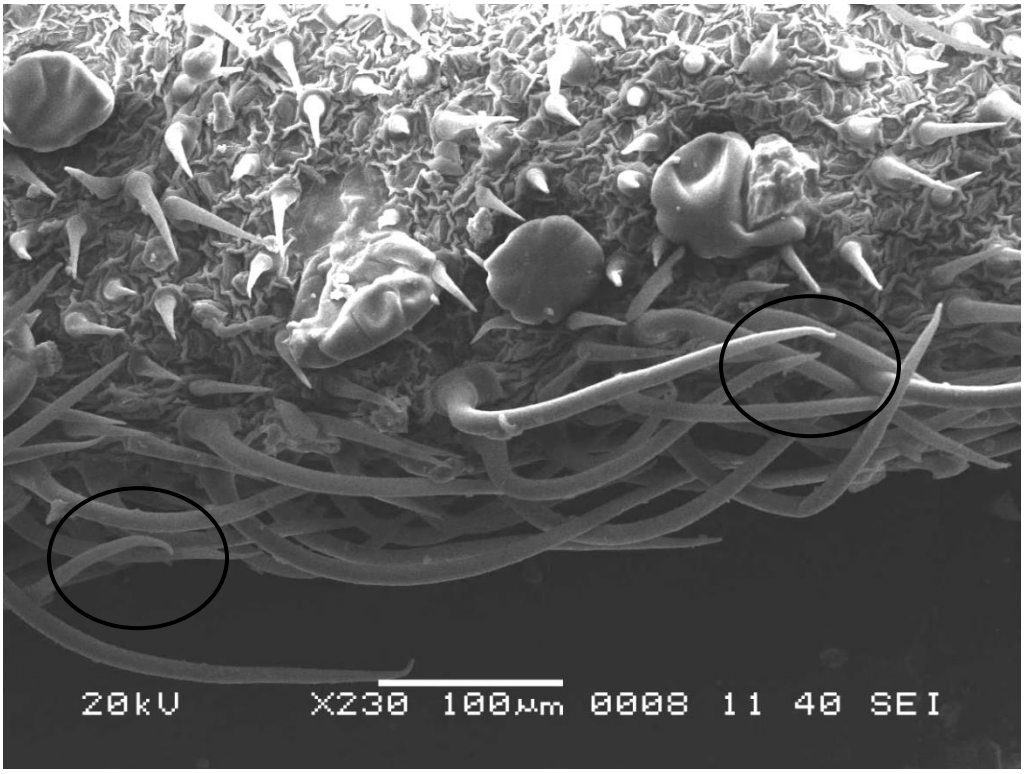


Fig. 11. Ventral surface of leaf. Long curved non-glandular trichomes arising from margins of the leaf (appressed with the margin) with some glands scattered near margin (ventral surface). Some trichomes are with curved apices (as shown in circles).

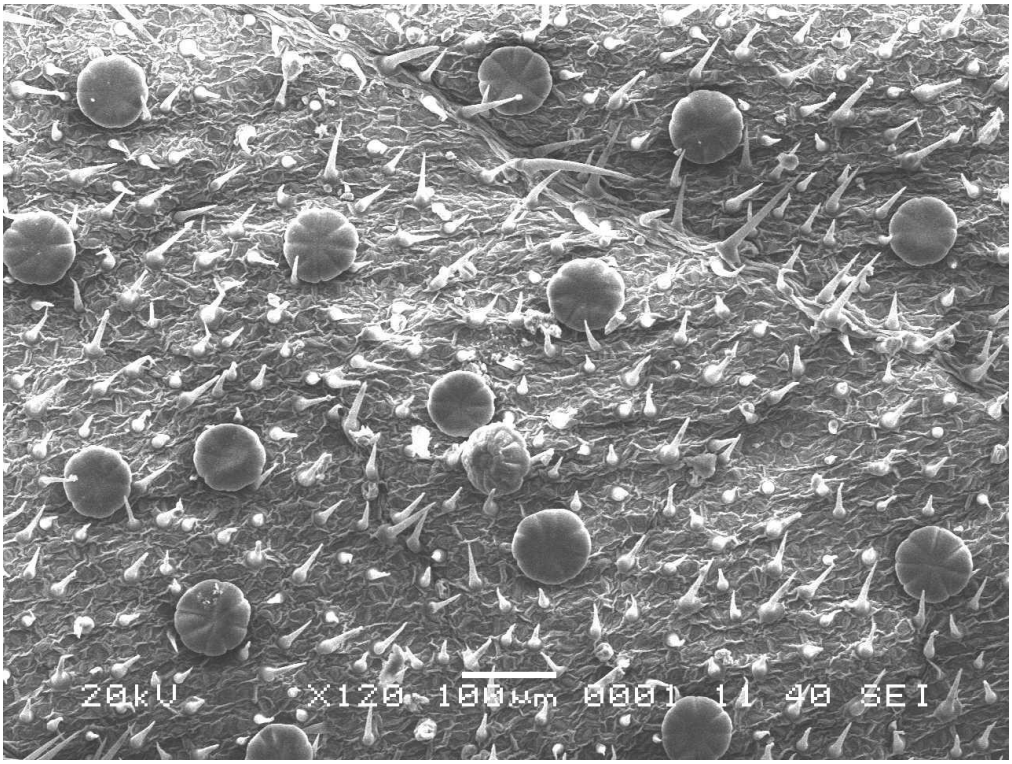


Fig. 12. Ventral surface of leaf – General view showing glands scattered amongst the sharply pointed bulbous based trichomes.

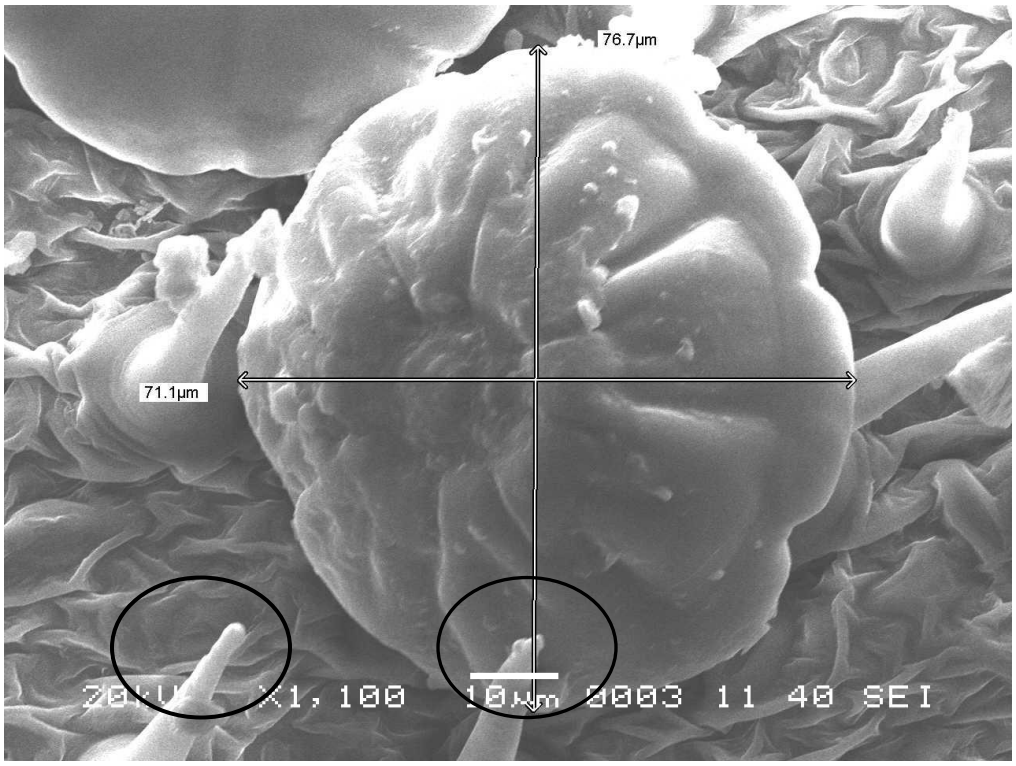


Fig.13. Ventral surface of leaf - Close up view of gland on the lamina amongst the trichomes. The gland is provided with 8 cells arranged around a central cell. The gland admeasured 71.1 to 76.7 µm in diameter. The circles indicate bulbous-based trichomes with elongated but rounded or beaked apical cell (possibly glandular).

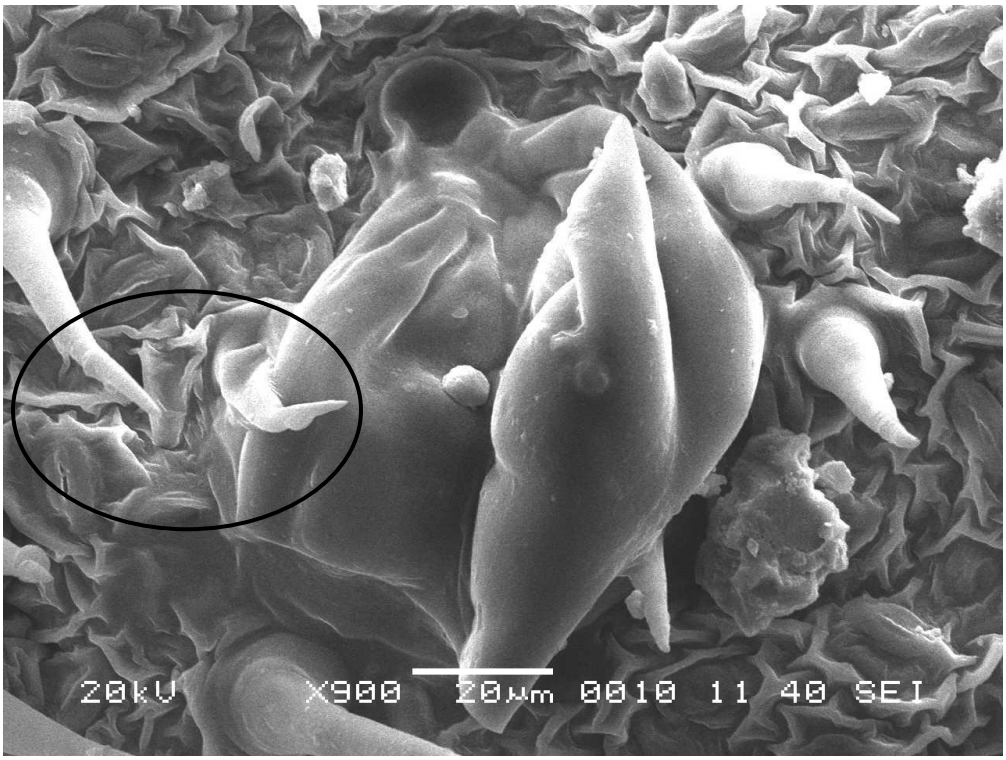


Fig. 14. A dried collapsing gland showing basal attachment – c. 15 um in diameter. Trichomes with elongated apical cell, is possibly glandular (in circle).

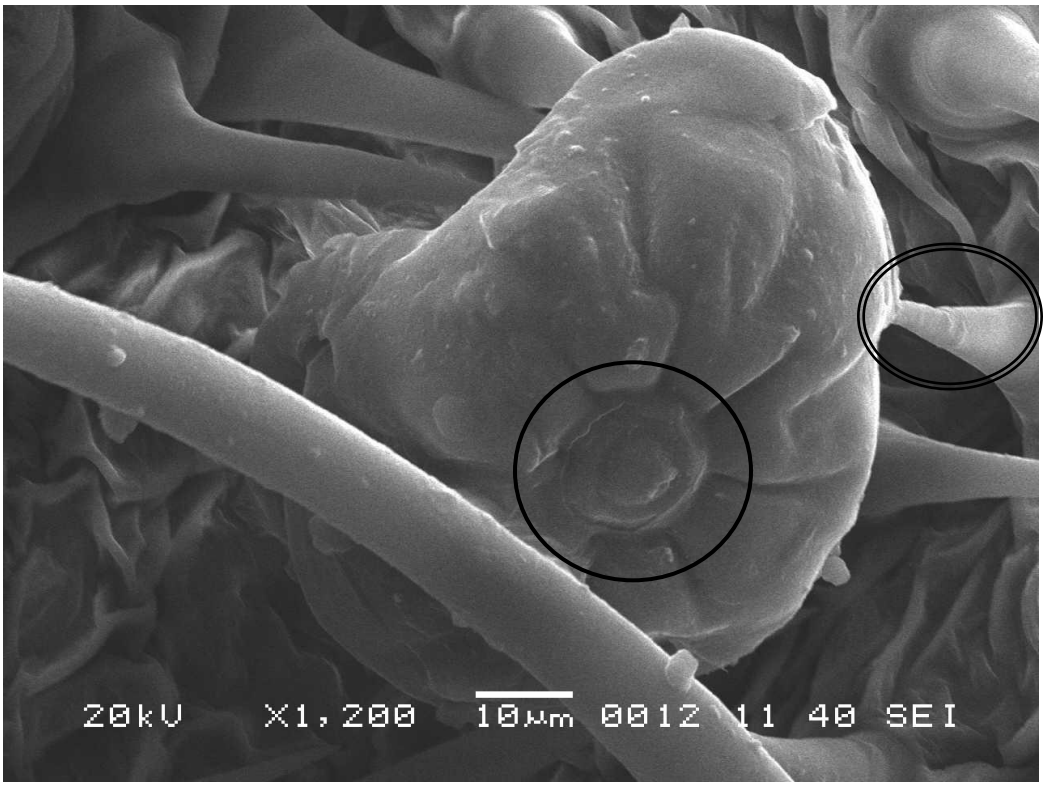


Fig. 15. SEMs showing an umbrella-like gland (with 8 peripheral cells surrounding a central circular cell (shown in single-lined circle). It is presumably distal end of the stalk. A bulbous-based trichome with elongated terminal cells (presumably glandular) is shown in a double-lined circle).

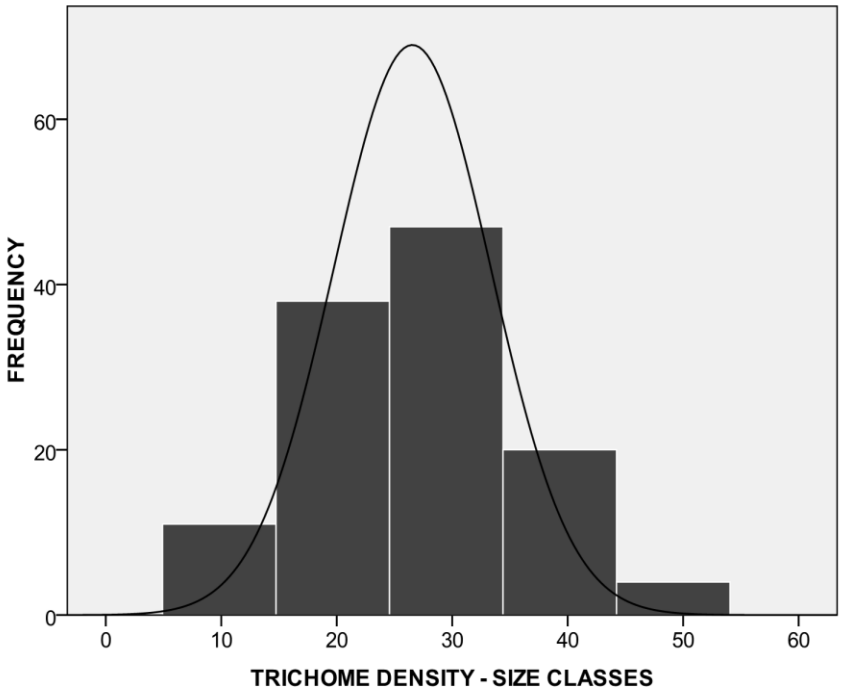


Fig. 16. Trichome density per mm^2 on ventral surface of lamina. Distribution is asymmetrical (platykurtic).

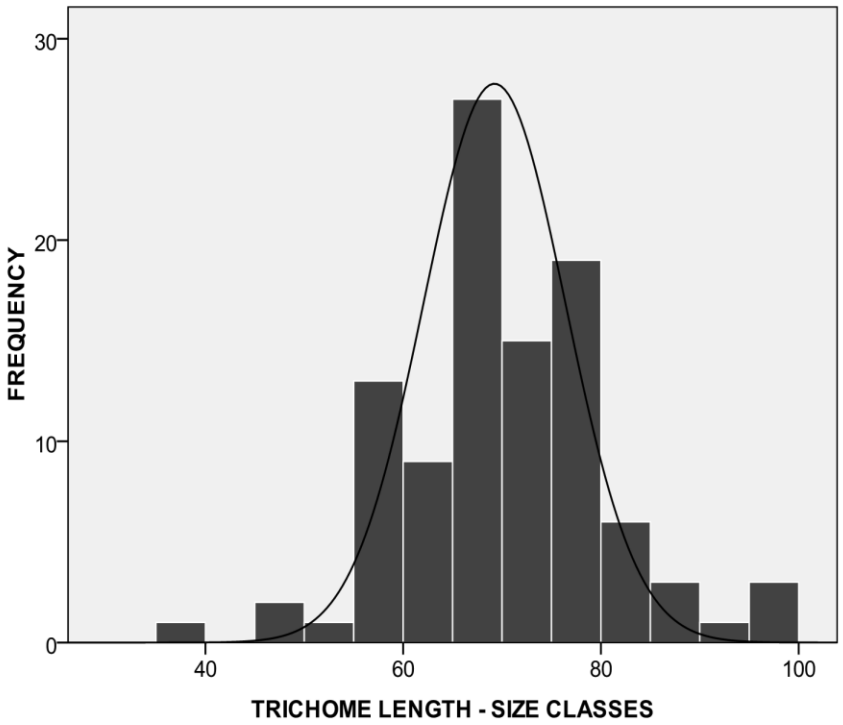


Fig. 17. Length of Trichomes (µm) distributed on ventral surface of lamina (exclusive those present on veins or margins.). Normal distribution.

Trichomes

Trichomes are present throughout the plant body of *R. minima* – on stem, cotyledons, leaves, petioles, calyces and fruits. Cotyledons and leaves both were amphitrichomic. The leaves of tropical species appear to be generally amphitrichomic. Khan *et al*. (2013) had studied 51 tropical species (no legume included) and found 38 spp. to be amphitrichomic and 13 hypotrichomic. There were both types of trichomes – glandular and eglandular. Vergas *et al*. (2015) studied apical leaflets of seven species of genus *Rhynchosia* for trichome type in herborized materials. They also described two types of trichomes in *Rhynchosia* – Glandular or secretory and Eglandular. Besides eglandular trichomes, Vergas *et al.* (2015) have described four types of secretory trichomes from the two surfaces of leaflets of *R. minima* and referred to them as novelties in the secretory structures of the genus *Rhynchosia*.

I. Spherical capitate trichomes with circular apical cells with stalk (unicellular)
II. Ellipsoid capitate trichome (large terminal secretory cells and unicellular stalk.
III. Bulky capitate trichome (large terminal secretary cells arranged in epidermal depressions.
IV. Bulbous-based trichomes with cells apically elongated and terminal slightly rounded basal cells large.

In our studies of *R. minima* seedling, we could find following types of trichomes.

Type 1. Orange-brown spherical capitate glandular trichome with umbrella like head (top tier three celled) attached to the cotyledonary epidermis in a depression Cotyledons (Fig. 6 and 7).

Type 2. Orange-brown bulky, capitate glandular trichome with umbrella like head composed with eight peripheral cells around the central cell, attaching to the surface through a unicellular stalk (ventral surface of leaflet Fig. 12, 13, and 14). They were also present on young stem, petiole, calyces and developing fruits (Fig. 4, 5 and 6).

Type 3. Long trichome with bulbous base and apical cells elongated and terminal cell rounded (glandular - seen on leaflet, Fig. 13).

Type 4. Long trichomes with bulbous base and apical cell elongated and sharply pointed (Eglandular seen on leaflet, Fig. 9, 10, 11). Presumably protective in function.

Type 5. Short trichomes with bulbous base and straight sharply pointed apex. Eglandular, leaflet, Fig. 10). Presumably protective in function.

Density of bulbous-based protective eglandular trichomes as determined on ventral side of a large leaflet (of 802 mm^2 in area) averaged to be 26.87 ± 0.861 trichomes per mm^2 (N = 120) varying 39.09% and distributed non-normally (KS-z = 2.204, p < 0.0001, with little platykurtosis (Fig. 16). In 70.8 % of the observations, the trichome density varied between 11 and 30 trichomes per mm^2.

Frequently trichomes were attenuate and uniseriate. In 70% of the observations, length of the bulbous eglandular trichomes ranged between 61 and 70 µm. The base of trichome was around 20 µm in diameter. Trichome were found to be longer on the veins and much longer on the margins of the leaflets (Fig. 8, 9 10 and 11). Trichomes length in laminar islands averaged to 69.72 ± 0.105 um (N = 100) varying by 15.08% (39.4-98.4 um). It distributed normally (KS-z 0.906, P < 0.385) (Fig. 17).

Special Glandular Trichomes or Nectaries or Resin glands

Rhynchosia minima is a nectariferous plant. The brown orange or reddish glandular trichomes are present all over the plant surface but more copiously on the underside of leaf (Fig. 4, 5, 6 and 7) and the enlarged view of them is given in in Fig.13, 14 and 15). The glandular spherical capitate and stalked trichomes of *R. minima* are also described as gland (Ali, 1977), nectary (Bhattacharya and Maheshwari, 1970) or "Resin glands" (http:///www.tropicalgrasslands.asn.au/legume...). These nectaries (Type 1 and 2 trichomes as given above) appear as minute dots on the ventral surface of the leaflets. They are discoidal in shape and stalked. They may be seen on cotyledon, stem, petiole, calyx and developing fruits also (Fig. 4 and 5).The diameter of the foliar glands in head region averaged to 80.85 ± 1.55 µm varying 12.1%. Some 67.5% of the glands ranged in head size from 71to 90 µm. None of the gland observed was lesser than 60µm in head size (Fig. 18). Bhattacharya and Maheshwari (1970) have reported that these glands are not associated with vein endings of the leaflets and are covered with cuticular layer. In the early hours of the morning secretion occurs from them in the form of glistening drops. We observed these glands in *R. minima* to be very closely arranged in young leaves (Fig. 5Aii), as also observed by Bhattacharya and Maheshwari (1970). The nectaries later get dispersed with some distances among them. In our studies, the density of these glands on young leaf of c 1cm^2 was observed to be 914.9 glands per cm^2. In 62% of the observations, gland density ranged from 700 to 1000 glands per cm^2 and this parameter distributed normally (KS-z: 1.047, p < 0.229, NS). On larger leaf of 6 cm^2 in size, the glands were quite lesser in number (132 glands per cm^2) (Fig. 20 A and B). In 61% of the observations the density on larger leaf ranged between 151 and 200 glands per cm^2. The distribution of gland density was, therefore, symmetrical in young leaf and asymmetrical in larger leaf. Such a pattern of distribution may be due to foliar expansion and removal of glands with maturity of leaf owing to random events. Single-celled stalk of the glands (Fig. 14, 15) should make them quite vulnerable to detach from the foliar surface. However, the secretion of these glands coupled with the sharply- pointed non-glandular trichomes should provide considerable protection to younger leaves from insects.

The glands sometimes impart a reddish shade to the surface of cotyledons. Both surfaces of cotyledons were observed to have such bulky glands in depressions (Fig. 6 and 7). Such glands on the ventral surface of cotyledon appeared to have bulky spherical head of diameter c 51 µm and composed of three cells in apical tier when seen from the top. It greatly resembles the three-tiered gland (3 cells in each tier) described by Bhattacharya and Maheshwari (1970, Fig. 2 (Y, page 14) and reproduced here as Fig. 19 (A) from the foliar surface. They have not investigated the cotyledons.

The foliar glands were larger (80.85 ± 1.55 µm) than cotyledonary glands (c 51.3 µm). Foliar glands are bulky capitate and their head region is composed of eight peripheral cells surrounding a central circular cell (possibly stalk cell or the distal end of the stalk) (Fig. 13-15). The development and structure of foliar nectaries in *R. minima* and some other genera of Fam. Papilionaceae are described by Bhattacharya and Maheshwari (1970). The full-grown nectary in *R. minima* was also reported by them to show eight cells in its apical region in Transverse section (Fig. 2 (Za, page 14 of Bhattacharya and Maheshwari, 1970; reproduced here as Fig. 19(B). The absence of the central cell in the mid of the apical head of the nectary in their studies may presumably be attributed the lower order of magnification employed in their studies (X 768) as compared to that employed in the present studies by SEM (X 1100/1200). Bhattacharya and Maheshwari reported while senescing the upper tier cells lose cytoplasm and cell walls are disorganized (Fig. 19 C and D). The structure of nectaries of *R. minima* is reported to be somewhat similar to *Vicia faba*. They are non-vascularized and do not contain crystals. Extra-floral nectaries unlike Mimosaceae and Caesalpiniaceae are considered to be uncommon in Papilionaceae. They occur in tribes Vicieae, Phasoleae, Galegeae, Trifolieae, Hedysaraceae, Delbergieae, Genisteae and Loeae. But they are absent in the tribes Podalyrieae, Sophoreae and Swartzieae. Such nectaries are taxonomically very important in genus *Rhynchosia*, which has been divided into three groups by Bhattacharya and Maheshwari (1970). Nectaries are absent in *R. filipes* Benth. and *R. pseudo-cajan* Cambers. Nectaries are present on the lower surface only in *R. acutissima* Twaites, *R. capitate* DC., *R.*

cyanosperma Benth., *R. densiflora* DC. *R. falconeri* Baker, *R. minima* DC, *R. nummularia* DC, *R. rufescens* DC. *R. suaveolens* DC. and *R. viscosa*. Nectaries are present on both surfaces of leaf e.g., *R. arvensis* Bath. Ex Baker. Large elongated glands are also known to occur on Cercideae leaf surfaces. *Bauhinia* (55 taxa), *Cercis* (1sp.), *Phanera* (1 sp.), *Piliostigma* (2 spp.), *Schnella* (19 spp.) and *Tylosema* (1 sp.) were studied by Duarte-Almeida *et al.* (2015). Forty two *Bauhinia* taxa had glands on abaxial surface. From humid forests *Bauhinia* spp. had no gland surface. Density of glands was higher on species from "Cerrado" (a savanna ecosystem) and "Caatinga" (a semi-arid ecosystem from Northwest Brazil.

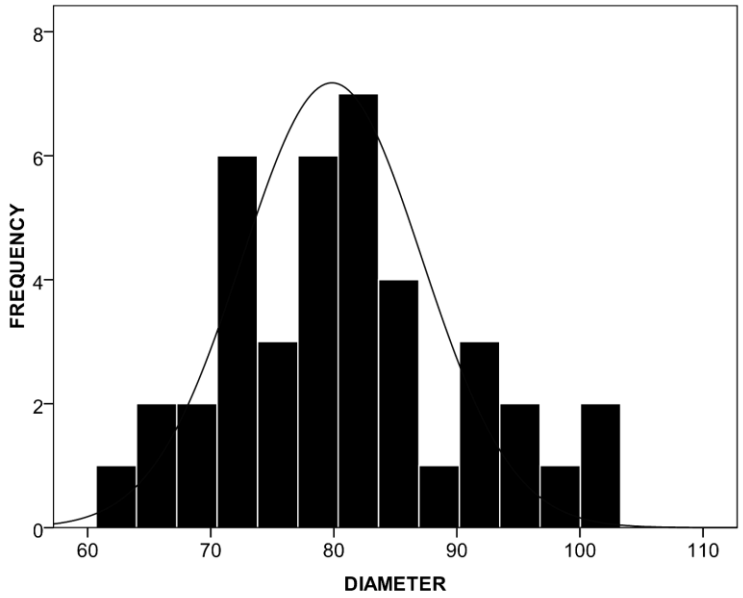


Fig. 18. Diameter (µm) of glands in head region on the ventral surface of leaf.

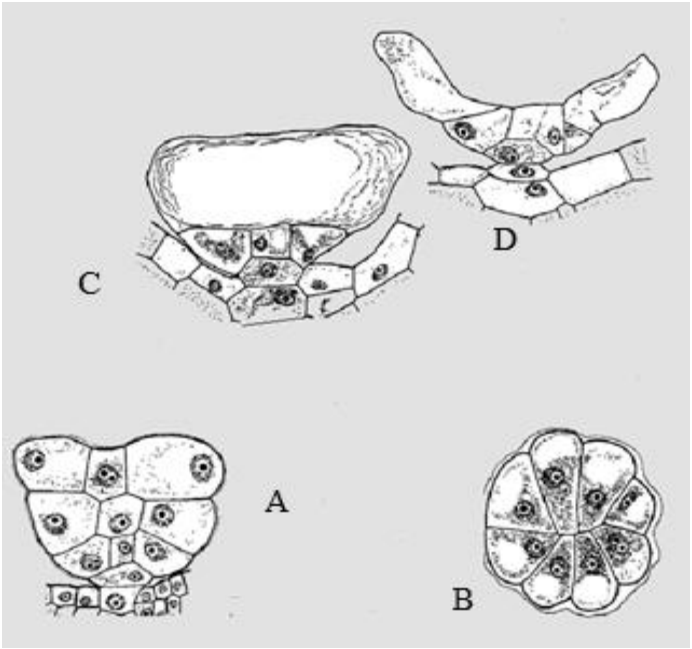


Fig. 19. Morphological structure of foliar nectary of *R. minima* as given by Bhattacharya and Maheshwari (1970) at magnification: X 768. Mature nectary showing three tiers of three cells each subtended by a single cell (A). A transverse section of nectary at this stage shows 8 cells (B). C, Mature nectary showing remnants of cytoplasm in the upper tier. The cell walls have become disorganized. D, Enlarged view of a nectary.

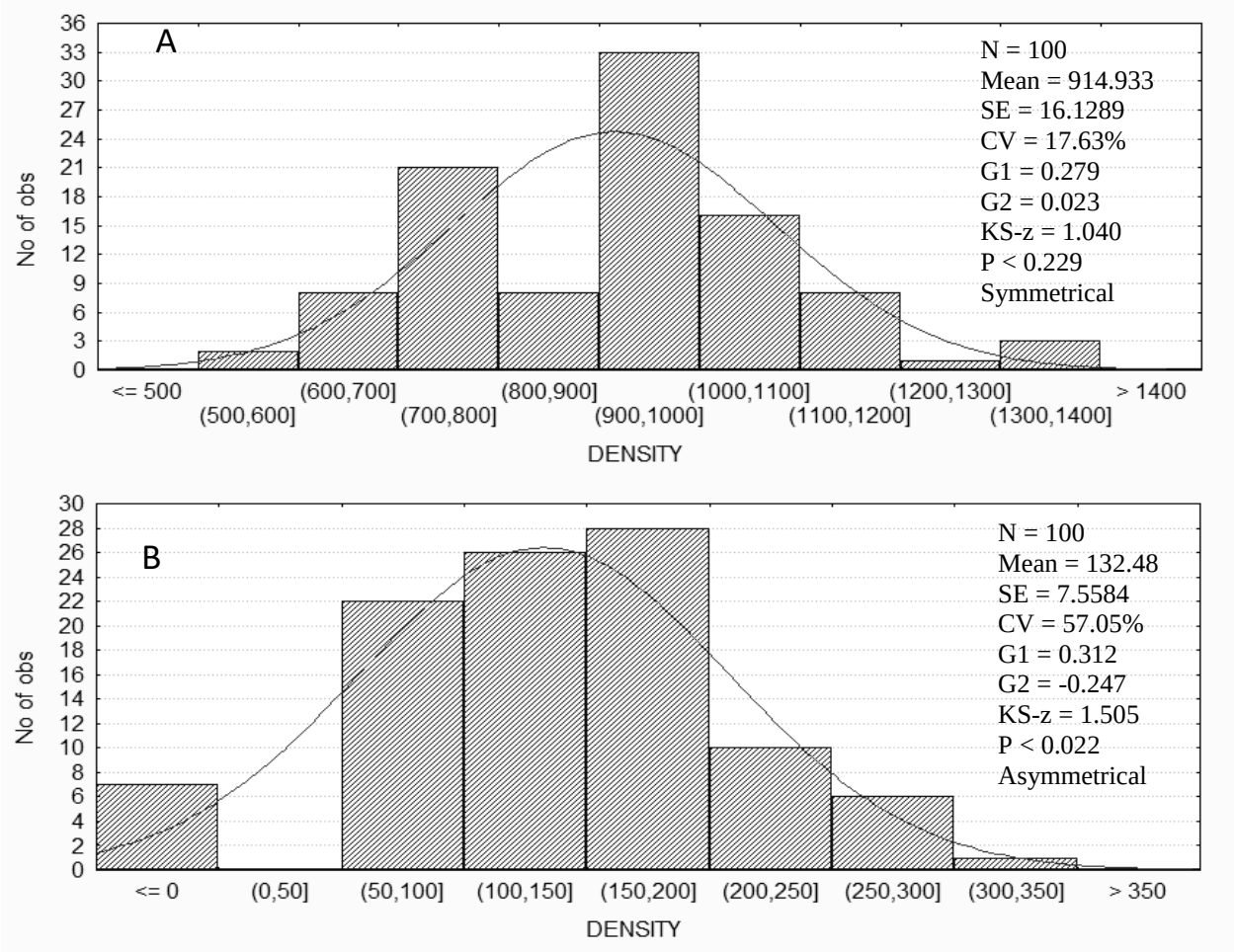


Fig. 20. Frequency distribution of density of glands per cm^2 on the ventral surface of young (A, around 1 cm^2 in size) and mature (B, around 6 cm^2 in size) leaf of *R. minima.* G1 = skewness and G2 = kurtosis. The errors of skewness and kurtosis, Sg1 and Sg2, in each case were 0.241 and 0.478, respectively, for N = 100.

Stomata

In present studies, *R. minima* showed a variety of stomatal types. Paracytic stomata were found on adaxial and abaxial surfaces of cotyledons (Fig. 6 and 7). There were, however, paracytic, anisocytic, staurocytic and anomocytic types of arrangements of subsidiaries in foliar stomata (Fig. 21-23). Parveen *et al.* (2007) reported only anomocytic stomata in *R. minima*. Metcalfe and Chalk (1979) have reported paracytic, anomocytic and parallelocytic but not anisocytic stomata from Papilionaceae. According to Tripathi and Mondal (2012) three stomatal types of Leguminales are paracytic, anisocytic and anomocytic – found in various combinations in Caesalpiniaceae, Mimosaceae and Fabaceae. The basic type of stoma in *R. minima* in our studies, however, appeared to be paracytic one which is considered to give rise to other stomatal types. Stace (1966) opined that many of the genera may have basically paracytic subsidiary cells but that extra walls may develop and thus giving the appearance of an anomocytic state. According to Stace (1966) the occurrence of anomocytic, anisocytic and paracytic stomata on one leaf of *Anopyxis* (Boodle and Fritsch in Metcalfe and Chalk, 1950) is probably explicable in this way. The studies into the seedling structure of *Cassia fistula* (D. Khan, unpublished) are supportive to the phenomenon of transformation of a paracytic stoma into anisocytic one by formation of a cell wall in a subsidiary cell. Paracytic stomata are, however, the most frequent in several Papilionaceous plants (Metcalfe and Chalk, 1950, 1979; Bokhari and Dasti, 199; Freire *et al.*, 2005; Almeida, 2010, 2011; Tripathi and Mondal, 2012; Khan *et al.*, 2014, 2015a and b).

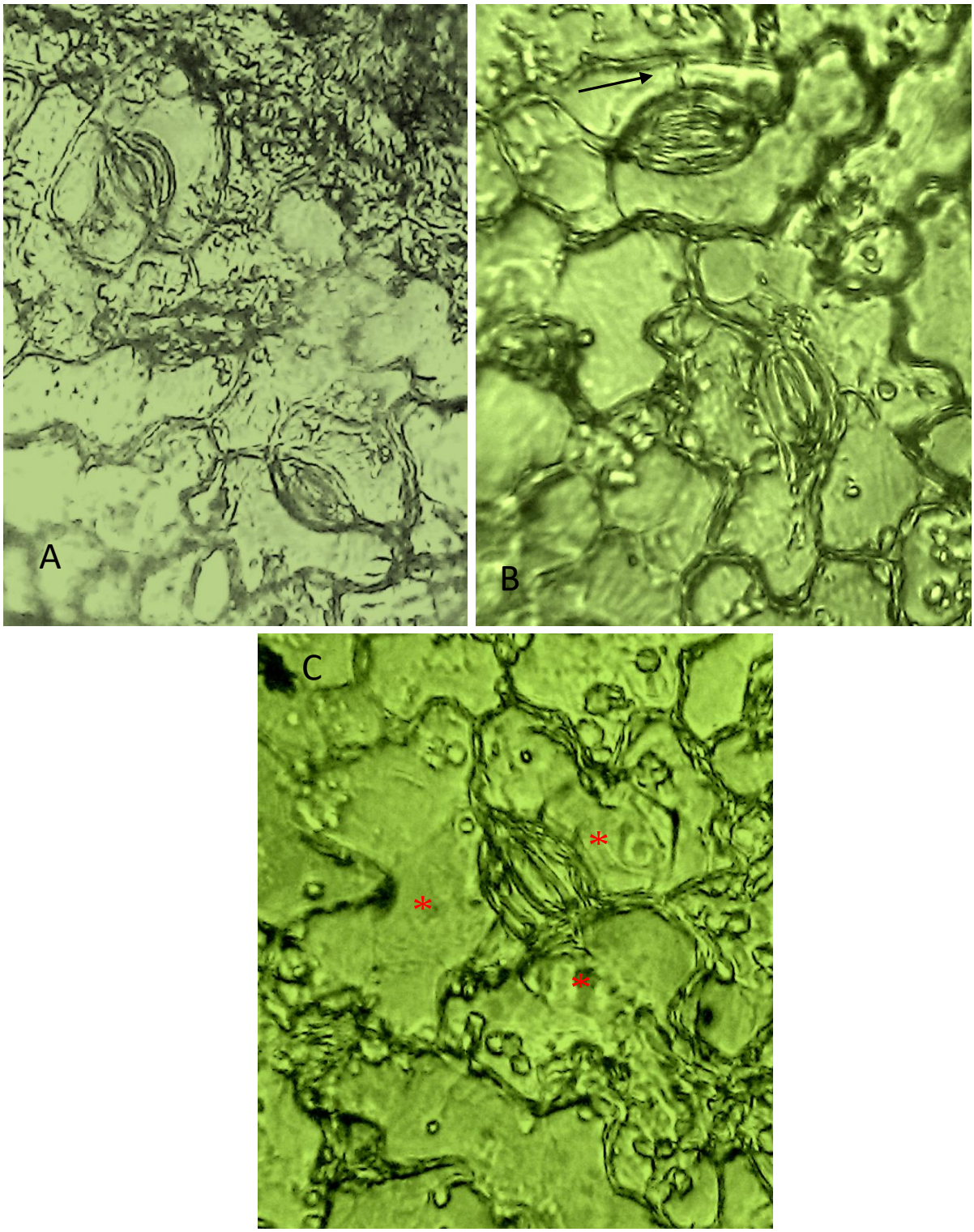


Fig. 21. Stomatal types of *R. minima*. A, Two paracytic stomata are clearly visible. B, a paracytic stoma (bottom) and the other paracytic stomata turning into an anisocytic stoma due to a wall development in the upper subsidiary cell (shown by an arrow). C, An anisocytic stoma. Subsidiaries and ground epidermal cells are sinuous in their anticlinal contours.

There were also some abnormal stomata in *R. minima*. A half stoma associated contiguously with a paracytic stoma (Fig. 24). The stomatal abnormalities may be the result of environmental perturbations as suggested by several workers (Car and car, 1990; Gan et al., 2010). It has been suggested by Croxidale (2000) that structure,

development and patterning of stomata on the leaf surface is the function of complex processes. This should be viewed from evolutionary, physiological, ecological and organ point of view. Local flora need to be investigated from such point of view.

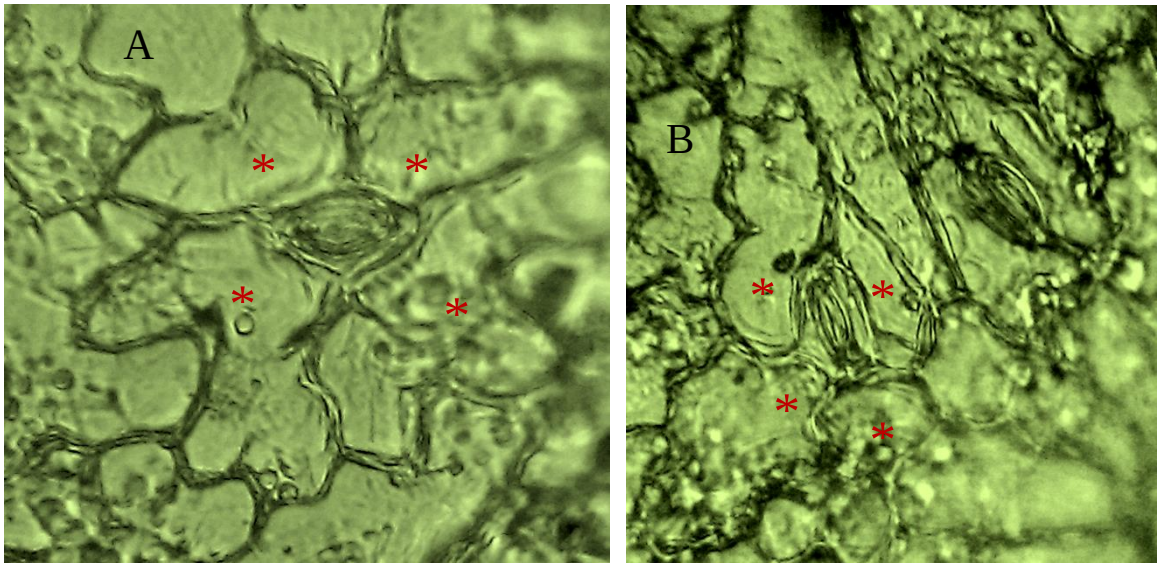


Fig. 22. Rare staurocytic type of arrangement of subsidiaries in *R. minima*.

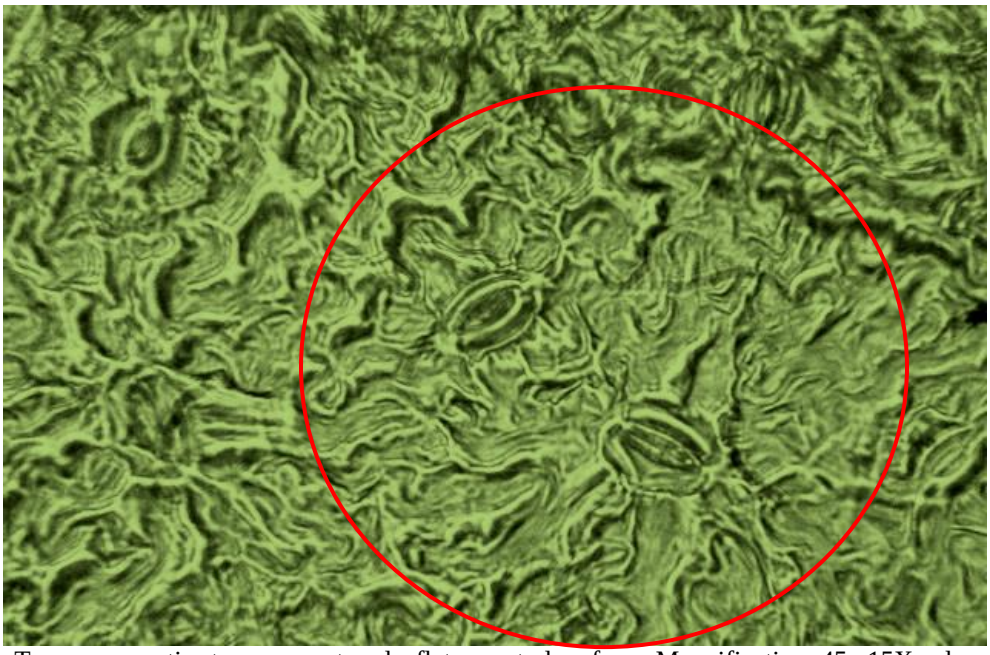


Fig. 23. Two anomocytic stoma on mature leaflet – ventral surface. Magnification: 45 x15Xand zoom 2X.

R. minima leaflets are amphistomatic with much larger number of stomata on ventral surface (283.3 ± 4.390 stomata per mm^2, N = 100, CV = 38.6%) as compared to the dorsal surface (48.06 ± 1.853 stomata per mm^2, N = 100, CV = 38.6%). On dorsal surface the stomatal density was distributed asymmetrically (positively skewed and leptokurtic, KS-z = 1.667, $p < 0.008$). Around 76% of observations on stomatal density fell in the size class of 21-60 stomata per mm^2. On ventral surface, in 74% of the observations the stomatal density fell between 250 and 300 stomata per mm^2 (Fig. 25).

The stomatal size on abaxial surface of *R. minima* leaf, based on observations of 60 stomata, averaged to 18.53 ± 0.36 x 13.17 ± 0.30 µm. In more than 72% of the observations, stomatal length (pore + guard cells) fell between

15.1 and 20 µm. The predominating class of stomatal width (pore + guard cells) was 10.1 – 16 µm. Stomata on adaxial surface were comparable in size (19.76 ± 0.22 x 11.19 ± 0.18 µm, N = 60) to that on the abaxial surface.

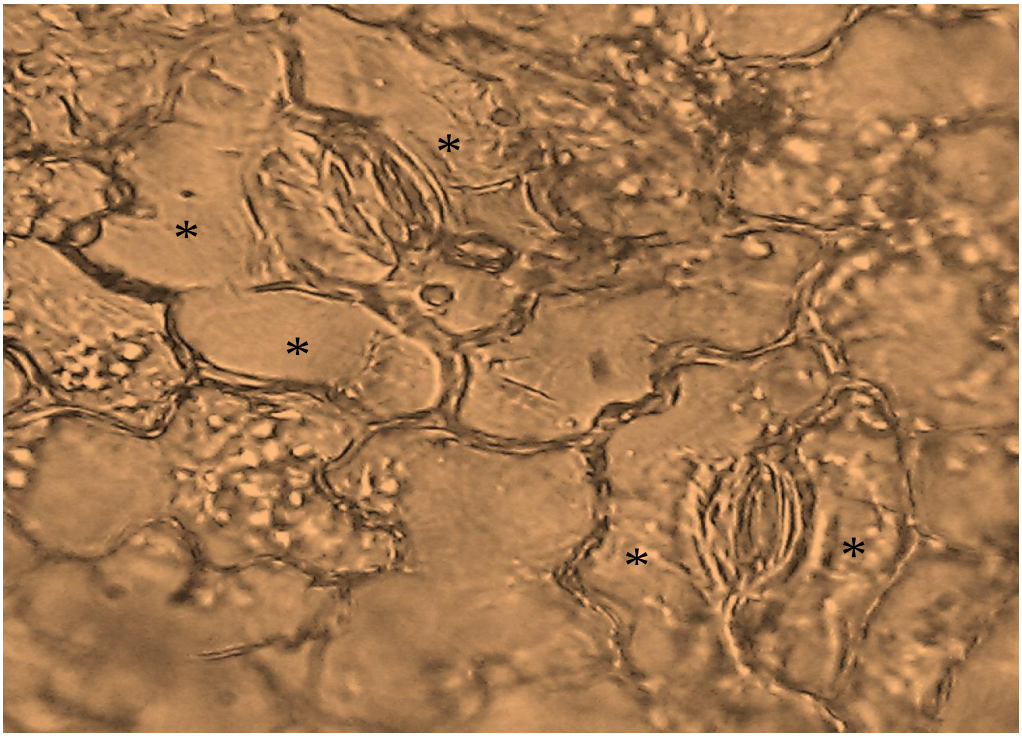


Fig. 24. Besides a normal paracytic stoma in right lower corner, an abnormal stoma may be seen in the upper left corner. It is surrounded with three subsidiaries and provided with a half stoma (single guard cell) besides an adjacent normal stomatal pore.

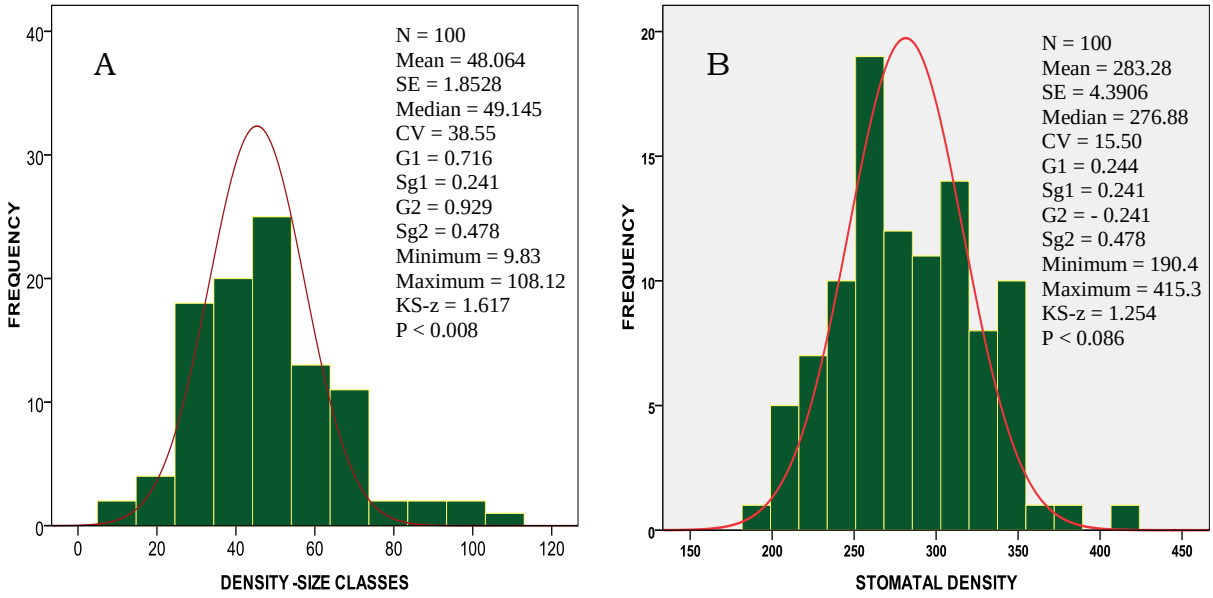
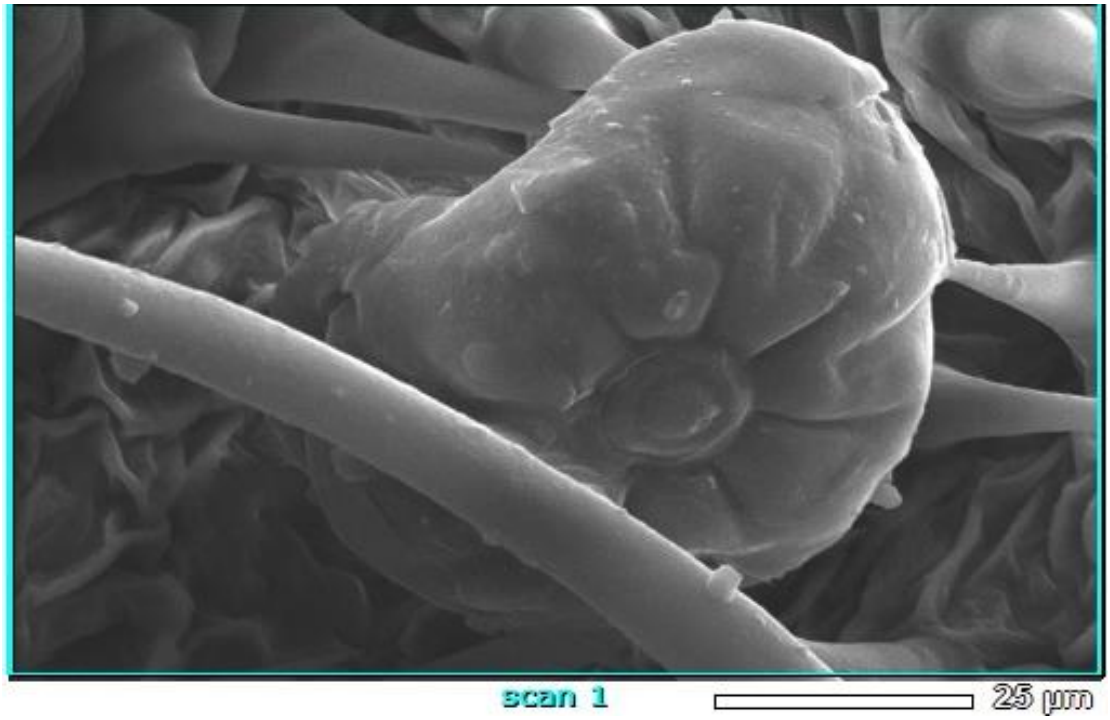
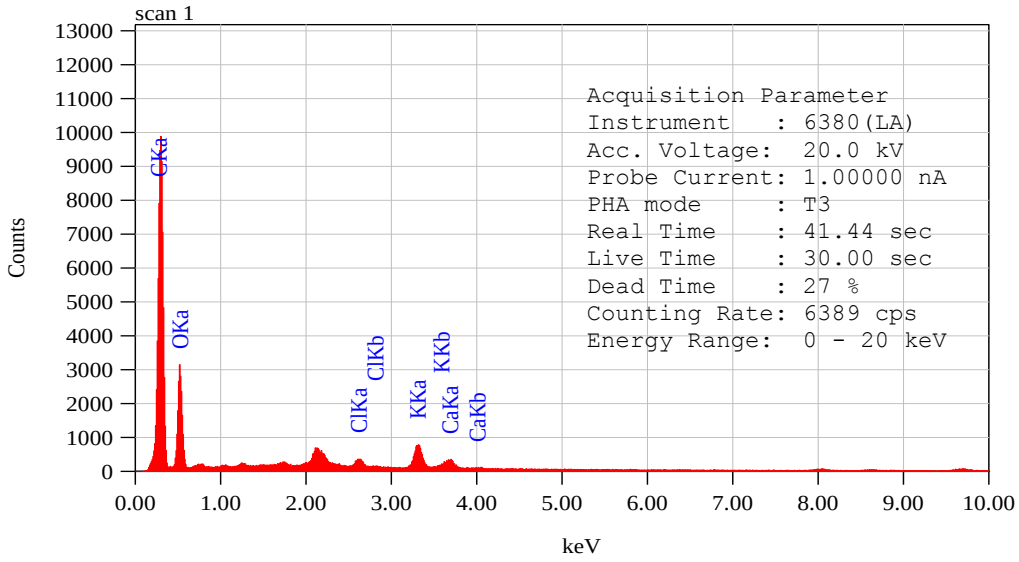


Fig. 25. Frequency distribution of stomatal density per mm^2 on dorsal and ventral surface of mature leaf of *R. minima*.





ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.6366

Element	(keV)	mass%	Error%	At%	Compound	mass%	Cation	K
C K	0.277	53.42	0.26	61.60				56.3506
O K	0.525	42.78	1.39	37.04				35.7435
Cl K	2.621	0.64	0.37	0.25				1.3525
K K	3.312	2.29	0.52	0.81				4.7238
Ca K	3.690	0.87	0.61	0.30				1.8296
Total		100.00		100.00				

JED-2300 AnalysisStation JEOL

Fig. 26. Elemental analysis of gland and the adjoining area of R. *minima* leaf.

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. The result of scan for the foliar region containing an extra-floral nectary is presented in Fig.26. In scan, the dominating element was carbon (53.42 ± 0.26%), followed by oxygen (42.78 ± 1.39%), Calcium (0.87 ± 0.34%), Potassium (2.29 ± 0.52%), Magnesium (2.27 ± 0.29%) and Chlorine (0.64 ± 0.37 %).

Barnes (1996) has presented data on nitrogen and macro- and micronutrients from the harvested herbage of *R. minima* in Nyankpala trials (Ghana) and analyzed according to AOAC (1970) methods and expressed as mg/kg biomass dry weight. The content of Nitrogen was 1.81mg/kg, that of Ca (7171 mg /kg), P (899 mg/kg), Na (103.96 mg/kg), K (3198 mg/ kg), Mg (326 mg/kg), Mn (395 mg/kg), Zn (39 mg/kg), Cu (7 mg/kg), and Fe (760 mg/kg). R. minima appeared to be potassiophillic as K was much higher in concentration in its forage than that of Na. Barnes (1996) reported much higher concentration of K in *Vigna unguiculata* also.

It may be mentioned that although EDS do not detect single-shelled element (hydrogen) but detects Oxygen and Carbon which are not usually estimated in agronomic studies. Larger proportion of foliar carbon and oxygen in EDS determination may be due to higher carbohydrates concentration. The aqueous extract of *R. minima* is reported to contain alkaloids, flavonoids, tannins and terpenoids and steroids and glycosides in ether and chloroform extracts (Mali and Mahele, 2008).

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