Chapter 4

RESULTS

4.1.1 Taxonomy and distribution:

Kurz (1873) validated the name *Brucea mollis* provided by Wallich in 1847 based on a material collected from Sylhet (now Bangladesh). While describing the species Kurz (1873) referred Wallich Catalog without mentioning the number. There are two sets of herbarium sheets bearing the numbers 8483A and 8483B housed in Kew Herbarium and another one sheet bearing the numbers 8483A and 8483B in British Museum of Natural History (BM). Out of the specimens housed at Kew, two sheets (K00112562, K000651340) has only one specimen and the rest have both 8483A and 8483B specimens together on each (K000651339 & K000651338, K001125625 & K001125626, BM000788472 & BM000946697). All these specimens are from male plants only. Later on Bennett (1875) cited the Catalogue number of Wallich (Wall.Cat.8483) and provided a brief description of the leaflet, inflorescence and the fruit. Referring Griffith he also mentioned the distribution of the species as subtropical eastern Himalaya, Sikkim and Bhutan. Kurz (1873) described the inflorescence as racemose but Bennett (1875) appended the description of the inflorescence and fruit by defining the inflorescence as axillary panicle and the fruit by adding size, colour and texture. Subsequently, Kurz (1877) again dealt with the species with fragmentary description of the leaf, leaflet, flower and fruit. Gamble (1881) classified the members Simarubeae into two Tribes *viz*., Simarubeaeand Picramnieae where he included *B. mollis* under the tribe Simarubeae referring Bennette (1875) and Kurz (1877).

In 1883, *B. mollis* was given a new name *B. luzoniensis* by a France geographer Paul Vidal de la Blache without any description (Vidal, 1883). Merrill (1906) while referring *B. luzoniensis* mentioned the species as endemic to Philippine but without any description. As such, *B. luzoniensis* remain as *nom. nud.*

In 1906, Merrill described *B. mollis* under the name *B. membranacea* as a new species under the genus based on a specimen of male plant only from Luzon in Philippines collected by R. Meyer in 1905 (US01108292 & US00101771). However, he wrongly cited the authority of the genus as J. S. Muell. His description about the plant was imperfect pertaining to arrangement leaves, features of leaflet margin, length of inflorescence, flower colour and features of staminate and pistilate flowers. Merrill (1906) considered that *B. membranacea* differs from *B. luzoniensis* in having broader petals, very short stamen and smaller leaves and leaflets. However, he considered that both the species are similar in having short inflorescence. In 1908, Merrill and Rolfe merged *B. luzoniensis* with *B. mollis* as conspecific and corrected the authority of the genus as J. S. Mill. Lecomte (1911) wrongly cited the Wall Catalogue number as 8433 while describing *B. mollis*. He also described a new variety *viz. B. mollis* var. *tonkinensis* H. Lec. based on specimens of male plants (P01817289 & P01817290) collected from Vietnam which differs from *B. mollis* with much developed cymes (Lecomte, 1911). However, F. Gagnepain raised the status of *B. mollis* var. *tonkinensis* H. Lec. to a species (*cf.* Ho, 2000). In 1915, Merrill again established a new species *B. macrobotrys* on the basis of differences in size of leaflets, inflorescence and fruits even though he admitted that this new species is allied to *B. mollis* and *B. luzoniensis*. Further, he was also sceptical about the status of his newly established species. In 1917, Merrill described another new species *B. stenophylla* on the basis of number, size and arrangement of

leaflets, features of leaflet margin and size of inflorescence based on specimens (26396, Oteyza and Garcia, 11.11.1916) collected from Luzon, Benguet and Baguio of Philippine by Oteyza and Garcia. However, in an endnote Merrill mentioned the close relatedness of *B. stenophylla* with *B. mollis, B. luzoniensis* and *B. membranacea* (Merril, 1923). Merril (1923) referred *B. macrobotrys* and *B. stenophylla* as separate species from *B. mollis* without any descriptions but included *B. luzoniensis* and *B. membranacea* as synonyms of *B. mollis*. Li (1943) described a new species *B. acuminata* on the basis of a material collected by S.P. Ko from Kwangsi, Ching His district of China being different in size and shape of leaflets and length of inflorescence from *B. mollis* and *B. mollis* var. *tonkinensis.*

The first approach for a comprehensive description of the species *B. mollis* was made by Nootebom (1962) with specific key for the species under the genus. He described the taxonomy, habit, distribution and ecology of the plant along with a note where he reduced *B. macrobotrys* and *B. stenophylla* to synonyms of *B. mollis*. Interestingly, he mentioned that flowers and inflorescence of *B. mollis* and *B. javanica* are similar and are white, creamy or red in colour. He provided illustrated distribution of *B. mollis* as East Himalayas, Burma, Siam (Thailand), Laos, Cambodia, Hainan to Malaysia and throughout the Philippines. But unfortunately Nootebom (1962) also described the species based only the male plants.

Brandis (1906) briefly described *B. mollis* on the basis of the material collected by him from Thoungyeen valley in 1862. He noted the distribution as Sikkim, Khasi hills, Manipur and Burma (Myanmar).

Hua and Thomas (2008) for the first time described the species on the basis of both male and female plants and referred an illustration of the plant made by Yu

Hanping (1997).They also reduced *B. acuminate* and *B. mollis* var. *tonkinensis* to synonyms of *B. mollis.*

Brucea mollis Wall. [Cat. (1848) 8483] *ex* Kurz, J. As. Soc. Beng. 42(2):64, 1873; Benn. Fl. Br. Ind. 1: 521, 1875; Kurz, Forest Fl. British Bur. 1:201-202, 1877; Gamble, Indian Timbers, 63, 1881; Clarke, J. Linn Soc. 25:11, 1890; Pottinger and Prain, Rec. Bot. Surv. India, 1(11):234, 1898; Merr. *et* Rolfe, Philip. J. Sc. 3. Bot. 104, 1908; Lecomte H., Notulae Systematicae:104,1909; Lecomte, Fl. Gén. I-C.1: 698, 1911; Rodger A., List Trees, Shrub, and Principal Climbers, etc.Recorded from Burma, 2:28, 1921; Merr.En. Philip. 2: 347, 1923; Craib, Fl. Siam.En.1: 241, 1926; Merr.*et* Chun, Sunyatsenia, 5: 89, 1940; Santapau and Abdulali, J. Bombay Nat. His. Soc. 58(1):164, 1961; Noot. *et* Leyden, Fl. Mal. Ser. I. 6 (2): 193,1962; Gupta *etal*., Rev. Ind. Med. Pl. 4 : 431, 2004; Ho, Illus. Fl. of Vietnam, 2: 383, 2000; Brandis, Indian Trees:127, 1906; Hua *et* Thomas, Fl. China. 11: 103, 2008. *B. luzoniensis* Vidal Sinops.Atlas, 19 t. 26, f. B, 1883; Merr. Publ. Gov. Philip. No35: 26, 1906; Merr. Philip. J. Sc. 1 Suppl. 70, 1906. *B. membranacea* Merr. Philip. J. Sc. 1 Suppl. 70, 1906. *B. macrobotrys* Merr. Philip. J. Sc. 10 (Bot.): 19, 1915; Merr. En. Philip. 2: 347, 1923. *B. stenophylla* Merr. Philip. J. Sc. 12 (Bot.): 274, 1917. *B. acuminata* Li, J. Arn.Arb. 24: 445, 1943, *ex descr.*

Vern. Name: Quinine, Bapkehu (Karbi); Makamara, Suga (Philippines); Bauru or Baru (Savara); Kosangi chettu (Telegu).

Shrub or small tree, perennial, polycarpic, usually 0.5-3 m tall. Stem slender, radius 2-4 cm, pale brown, densely white lenticellate, glabrous, straight, stout, nodes 5-20 cm apart, internodes towards the base longer than that towards the apex, each node bears single leaf, leaf scars of fallen leaves prominent (Pl.1 A-D). Branches terete, dark green or pale brown, densely white lenticellate, glabrous.Branchlets

green or yellow, puberulent. Root much branched, cylindrical and thick, prominent secondary growth present. Leaves deciduous, arranged in 1/3 phyllotaxy, alternate, imparipinnate, 30 – 40 cm long, 20 – 25 cm broad; rachis light green, densely pubescent, orientation apical and exmedial, margin convex, shape symmetrical, form narrow obovate, apex acute, obtuse or rounded, basal acute or obtuse; venation craspedodromous; petiolules densely puberulent, about 4-6 mm long, inflated, petiolar attachment marginal. Leaflets 7, 9 or 11 or 13, 3 – 16 cm long, 2.5 – 7 cm wide and 100 – 150 µm thick, membranous, opposite; yellow tomentose when young, puberulent or glabrescent, dark green or yellowish green on the adaxial surface and light green on the abaxial surface, shining when mature; orientation apical, exmedial, margin convex, shape lanceolate-obovate or narrowly obovate, narrow elliptical, narrow or wide oblong or occasionally round or bilobed; apex acuminate or irregularly round or wide obtuse, base either asymmetrical acute or obtuse; margin entire or slightly undulating; venation pinnate, camptodromous, weak brochidodromous or rarely actinodromous, lateral veins 5-12 pairs, abaxially conspicuously prominent; petiolules normal puberulent, 4-6 mm long, attachment marginal. Several types of trichomes present. Plants dioecious, flower unisexual, minute. Male inflorescence axillary raceme, 15-163 cm long, densely puberulent, remification acropetal up to 3^0-4^0, inflorescence bears a small brown stipule at the base. Male flowers born on secondary much developed cyme, perfect, 2-3 mm in diam, tetramerous, dichlamydeous, heterochlamydeous, biseriate, aposepalous, apopetalous, polyandrous, actinomorphic; sepals green, pubescent, triangular, petals greenish white, spoon shaped, pubescent, longer than stamens, stamens 4-6. Disc partly flattish and partly globose, four lobed. All the floral parts are free. Anther exserted, basifixed, latrorse. Filament 400-450 µm long. Anther lobe 400-450 µm

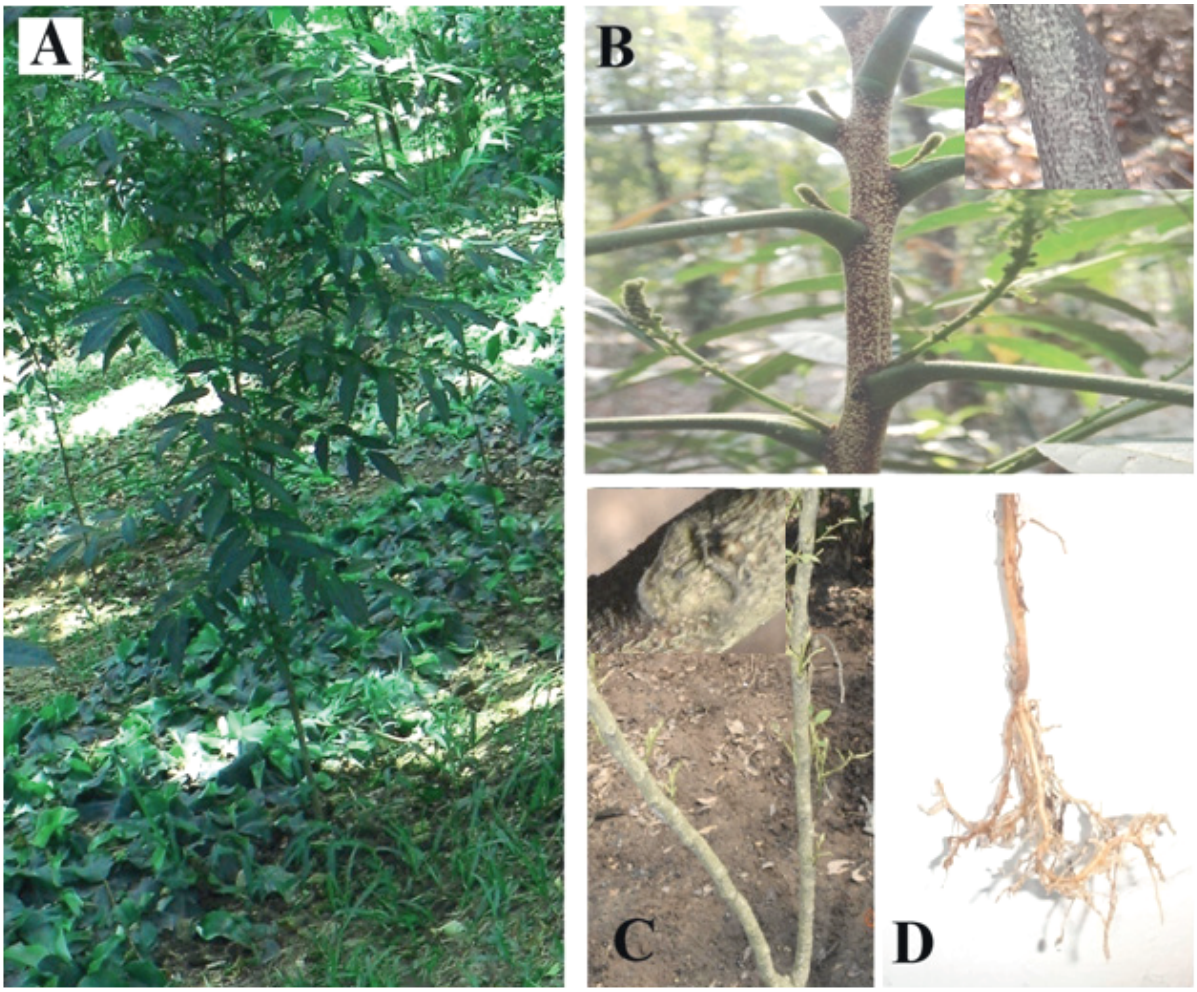


PLATE 1: Habit and vegetative parts of *B. mollis*. A. Habit, B. White spots over the stem, C. Stem exhibiting leaf scars, D. Root.

long and 180-190 µm in diam. Pollens tricolpate, surface raticulate, size 31.8×29.2 µm. Female axillary racemes 16-40 cm long, puberulent, ramification is not frequent like male, observed up to 2^0. Female flowers either solitary or born on reduced secondary cyme, inconspicuous, 2-3 mm long with 3-4 mm pedicel, no odour or scent, perfect, tetramerous, actinomorphic, dichlamydeous, heterochlamydeous, biseriate, aposepalous, apopetalous, tepals 8 arranged in two whorls, densely puberulent. Pistil apocarpus, carpels 4, green, pubescent, ovary unilocular, hypogynous, style short curved, extended at the right angle to the ovary, stigma fleshy, dries up immediately after pollination. All the floral whorls are free. Disk green, flattish, shallow. Druparium monocarp, ovoid, 7-12 mm long and 6-8 mm broad or 2 fruits develop from single flower. Pedicel 3-4 mm. Mature fruit globose, slightly tapering at both the ends, green while young and bright red, orange when fully ripe. Exocarp becomes scarcely reticulated and red brown when dry. Apomixis rarely observed in male flowers.

Habitat: The species is growing on shady hill slopes inside forests, thickets or along road sides.

Note: *Brucea mollis* was established by Kurz on the basis of a material collected by Wallich in 1847 from Sylhet (now Bangladesh). There are two sets of herbarium sheets collected by Wallich bearing the number 8483A and 8483B housed in Kew Herbarium and another one sheet bearing the number 8483A and 8483B in British Museum of Natural History (BM). Out of the specimens housed at Kew, only two sheets (K001125627 & K000651340) has only one specimen and the rest have both 8483A and 8483B specimens together on each (K000651339 & K000651338, K001125625 & K001125626, BM000788472 & BM000946697). The set housed in British Museum of Natural History (BM) the specimen bearing the number

BM000788472 is marked as Isotype by H. P. Nooteboom while dealing with Flora Malesiana in 1961. But Kurz did not designate any material as Holotype. Therefore, the selection of Isotype by H. P. Nooteboom is erroneous. Further, all the description of the species provided so far *viz.*Vidal (1883) and Merrill (1906, 1908, 1915 and 1917) were based on male plants only.It is only in 2008 Li provided the description of the female flowers. But till now none of the workers described that the species is a dioecious one. Under such circumstances Lectotypification and at the same time epitypification become essential for proper representation of the species. After thorough study of all the original materials (K001125627, K000651339 & K000651338, K001125625 & K001125626, K000651340, BM000788472 & BM000946697) it was found that the material deposited at Kew (K001125627) with Wallich collection 8483B agreed with the description in the protologue and hence it is selected as Lectotype.

As the species is a dioceous one and all the description (including the protologue) so far provided were based on male plants only and the lectotype selected by the present investigator is also a specimen from a male plant only an epitype is also designated here.

Lectotype: (designated here): Sylhet, 1847, *E. J. C. Wallich* 8483 B (K001125627) (Pl.2A).

Epitype: Karbi Anglong, 05.04.2016, *D. Kakati* 015 (GUBH 18417) (Pl.2B).

Specimens examined:

Department of Botany, Gauhati University, Gopinath Bordoloi Nagar, Jalukbari, Kamrup, Assam, India 04.09.2014 *D. Kakati* 005 (GUBH); Recreation Park, Diphu, Karbi Anglong District, Assam, India 06.11.2014, *D. Kakati* 007 (GUBH); Manja to Diphu, Karbi Anglong District, Assam, India 06.11.2014, *D.*

PLATE 2: A. Lectotype (8483 B) and B. Epitype (015)

Kakati 008 (GUBH); Diphu, Karbi Anglong District, Assam, India 17.12.2014, *D. Kakati* 011 (GUBH); Diphu, Karbi Anglong District, Assam, India02.02.2015, *D. Kakati* 016 (GUBH); Near Lahorijan Reserve Forest, Diphu, Karbi Anglong District, Assam, India 24.04.2015, *D. Kakati* 021 (GUBH); Near Lahorijan Reserve Forest, Diphu, Karbi Anglong District, Assam, India 24.04.2015, *D. Kakati* 032 (GUBH);NEDFi R & D Centre, Khetri, Nagaon, Assam, India 26.06.2015, *D. Kakati* 045 (GUBH);Diphu, Karbi Anglong District, Assam 05.04.2016, *D. Kakati* 052 (GUBH); Diphu, Karbi Anglong District, Assam 05.04.2016, *D. Kakati* 061 (GUBH); Samar, Philippine April 1914, *M. Ramos* 00044336 (HUH);Samar, Philippine April 1914, *M. Ramos* 00101771 (USNH);Lamao River, Mt. Mariveles, Province of Bhutan, Luzon March 1905, *R. Meyer* 01108292 (USNH);Lamao River, Mt. Mariveles, Province of Bhutan, Luzon March 1905, *R. Meyer* 00101770 (USNH);Sikkim. [Lama] hiles, Alt. 609.60121932., s.n.,*J. D. Hooker*(K);Philippines April 1914, *M. Ramos* 1626 (BM); Samar, Philippines 01.04.1914, *M. Ramos* BS1626 (L); Samar island, Philippines April 1914, *M. Ramos* 1626 (GH); India s.n.,*N. Wallich* 8483B (BM); H. Bot. Calcuttae s.n.,*s. coll* 8483 (K); Thailand 16.03.1905, *C. C. Hosseus* 432 (M); Vietnam 23.05.1884, *H. F. Bon* 2556 (P); Cayayan Valley, forested slopes, Philippines April 1914, *M. Ramos* 1626 (MO); Sylhet s.n., *F. De Silva* 8483 (K); Siam, Thailand 19.11.1922, *Garrett, Henry Burton Guest* TCD0013426 (TCD); Tonkin méridional, Kiên Khê, in collib. Dông Hâm, Vietnam 23.05.1884, *H. F. Bon* P01817290 (P); India s.n., *N. Wallich* 8483a (BM); Siam, Chiengmai, Thailand 02.04.1911, *Kerr, G. Arthur Francis* 1750 (TCD); Sylhet s.n., *F. De Silva* 8483 (K).

World distribution: Martaban and upper Tenasserim of Burma (Myanmar); Samar, Cauayan valley (Philippines); Syllhet (Bangladesh); subtropical eastern Himalaya,

Sikkim (India); Bhutan; Cambodia; China; Laos; Malaysia; Myanmar; Myanmar; Nepal; Thailand; Vietnam (Pullaiah, 2006) (Fig. 4.1.1).

Distribution in India: Arunachal Pradesh, Assam (Karbi-Anglong), Darjeeling, West Manipur, Meghalaya, Nagaland, Sikkim (Fig. 4.1.2)

Ditribution in Karbi Anglong: Recreation park, Diphu; on the way from Manja to Lahorijan forest (Fig. 4.1.2).

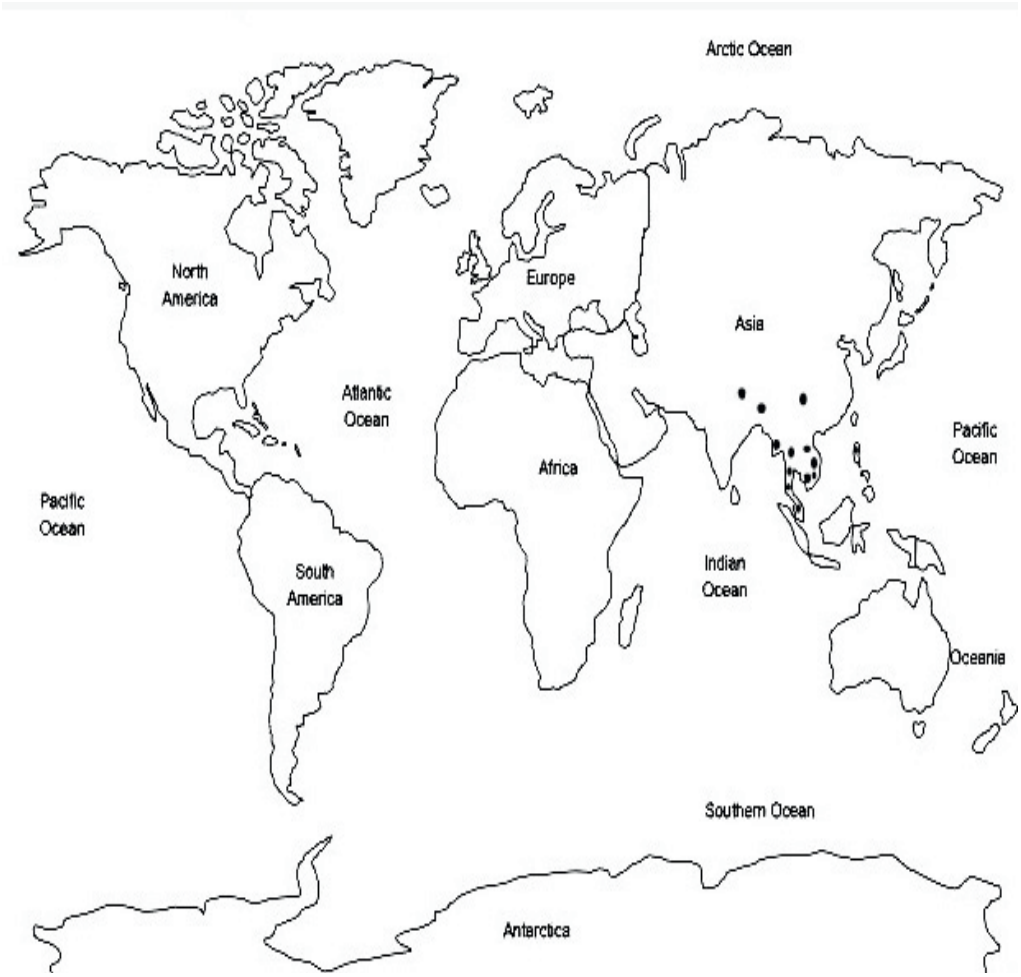


Fig 4.1.1 World distribution of *B. mollis* (http://atlantislsc.com/map-world-black-and-white/map-world-black-and-white-25-unique-world-map-printable-ideas-on-pinterest/)

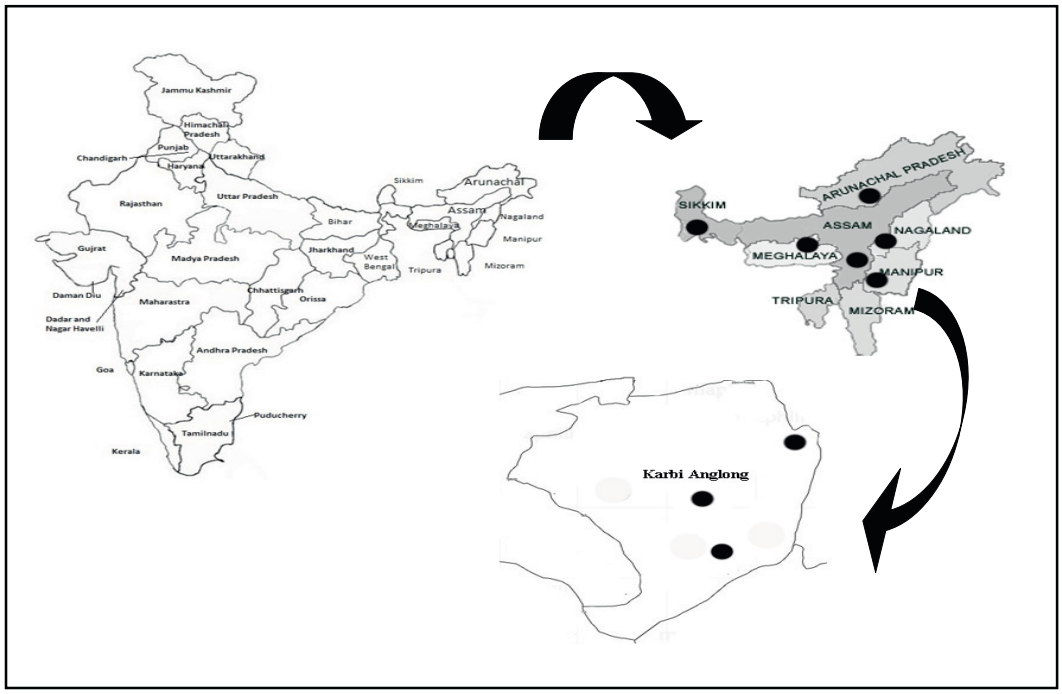


Fig 4.1.2: Distribution of *B. mollis* in India (https://www.google.co.in/imgres)

4.1.2 Macro and micro morphology of vegetative parts:

4.1.2.1 Macro Morphology:

4.1.2.1 .1 Root:

Root of *B. mollis* is composed of tap root system. Secondary roots are much branched, cylindrical and thick, off white showing prominent secondary growth. Root hairs zone is 25 – 30 mm above the root tip (Pl.1A).

4.1.2.1. 2 Stem:

Stem of *B. mollis* is slender with 2 to 4 cm radius, pale brownish or dark green having white spots spread over the stem, lenticellate, glabrous, straight and stout (Pl.1B,1C). Nodes at 5 to 20 cm, internodes towards the base longer than that towards the apex, each node bears single leaf. Leaf scars of fallen leaves are prominent. Branches terete, dark green or pale brownish, lenticellate and glabrous, tender branches pubescent with short pale hairs.Lateral shoots develop from nodes.

4.1.2.1.3 Leaf:

Leaves pinnately compound, alternate, arranged in 1/3 phyllotaxy, imparipinnate, 30-50 cm long, 20 – 25 cm broad; rachis light green, pubescent;

petiolules inflated, puberulent, about 6 mm long (Pl.3A-F). Margin of the leaf convex, shape symmetrical, form narrow obovate (l/w ± SD = 1.82 cm ± 0.17 cm). Apical angle acute, obtuse or rounded. Basal angle acute or obtuse. Various types of simple glandular and non glandular trichomes and glands present over the petiole. Petiolar attachment marginal.

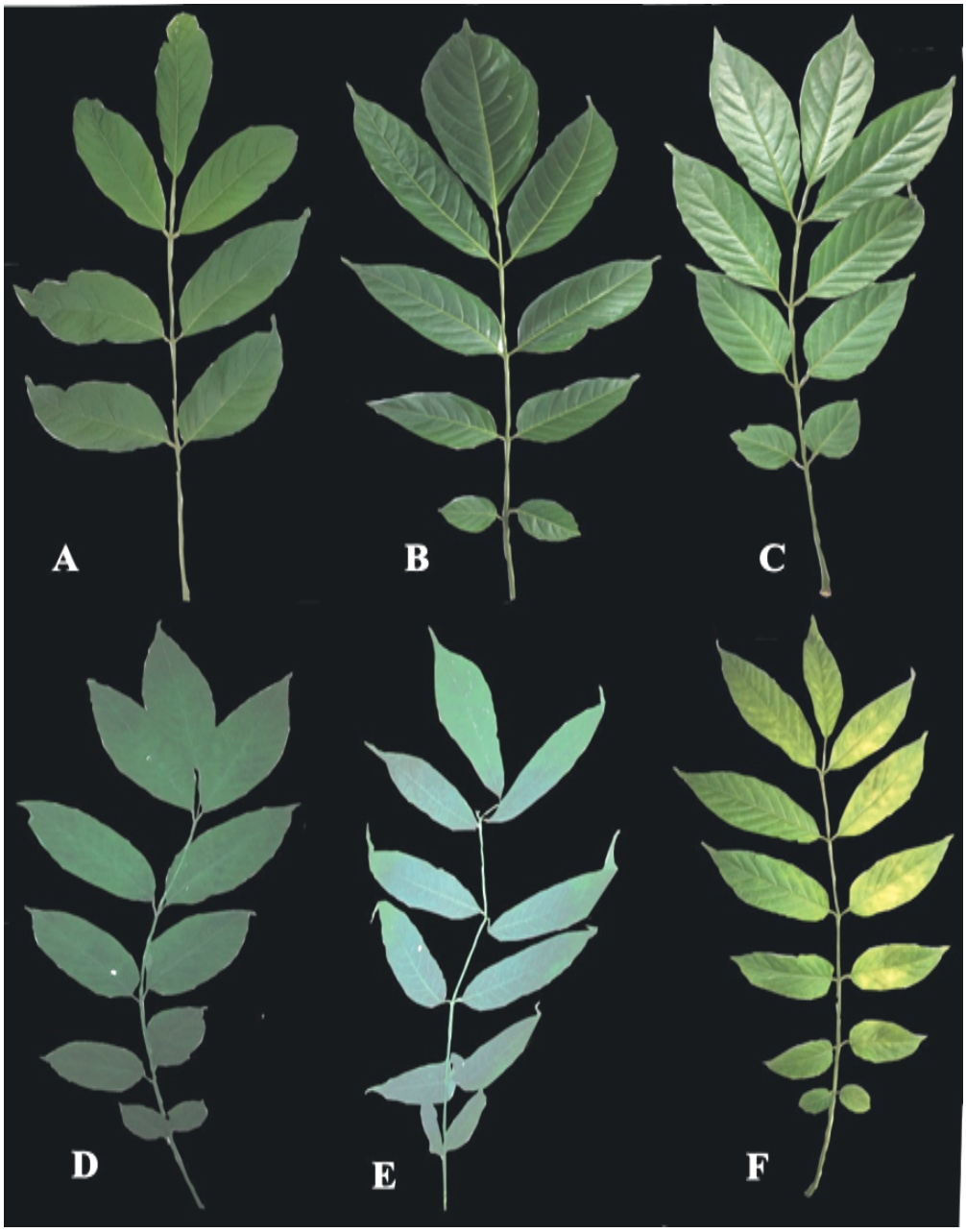
4.1.2.1.4 Leaflet:

There is no homogeneity in size and shape of leaflets. It exhibits a series of transitional forms in shape and size of leaflets which ranges from round, elliptical to ovoid. Altogether six variations in the shape of leaflets have been observed. Leaflet number varies from 7, 9, 11 to13, membranous, opposite, size and shape variable. Petiolules normal, puberulent, about 6 mm long (Pl.3A-F). Leaflets dark green or often yellowish green on the adaxial surface and light green on the abaxial surface, shining when mature. Orientation apical, exmedial, margin convex, entire or undulating. Both halves of lamina on either sides of the midrib are symmetrical, except at base which is occasionally asymmetrical. Shape varies from lanceolate-obovate or narrowly obovate, narrow elliptical, narrow or wide oblong or occasionally round or bilobed. Sizes of leaflets in a leaf are either equal or reduced proportionately towards the base and equal or proportionately reduced or often larger towards the apex. The lower two leaflets of leaves are usually smaller and rounded or similar to the other. Depending on laminar size blade of leaflets falls into either microphyll or nanophyll class (Table-1). Apex and base of the leaflets also shows great variations in their shape. Apex acuminate or irregularly round or bilobed and wide obtuse. Base is either asymmetrical acute or obtuse. Petiolular attachment marginal.There is no homogeneity in size and shape of leaflets and exhibits a number of transitional forms (Pl.3G-N).

Table: 1 Variations of size and shape of leaflets of *B. mollis.*

Leaf	Length($l=l_m+l_a+l_b$)cm	Width (w) cm	l:w	Shape		Laminar size(l*w*2/3) cm^2	Blade class	Apex angle	Apex shape	Base angle	Base shape
				Clas s	Sub class						
G	14+0+0=14	3.8	3.68	Obovate	Lanceolate	35.11	Microphyll	Acute	Acuminate	Asymmetrical acute	Narrow convex
H	13+0+0=13	3	4.33	Elliptical	Narrow elliptical	25.74	Microphyll	Acute	Acuminate	Asymmetrical acute	Cuneate
I	12.5+0+0=12.5	3.5	3.57	Oblong	Narrow oblong	29.45	Microphyll	Acute	Acuminate	obtuse	Wide convex
J	8.5+0+0=8.5	3.5	2.42	Obovate	Narrow obovate	19.6	Nanophyll	Acute	Acuminate	obtuse	Nearly truncate
K	6.8+0+0=6.8	3.2	2.13	Obovate	Narrow obovate	14.3	Nanophyll	Acute	Acuminate	obtuse	Nearly truncate
L	4.2+0+0=4.2	3.2	1.31	Special	Round	8.8	Nanophyll	Wide obtuse	Rounded	obtuse	Rounded
M	1.5+1+0=2.5	3	1.16	Special	Bilobed	4.95	Nanophyll	Wide obtuse	Emarinate	obtuse	Rounded
N	3+0+0=3	1.8	1.66	Oblong	Wide oblong	3.54	Nanophyll	Obtuse	Convex	obtuse	Wide convex

(lm= Midvein length, la=Apical extension length, lb= Basal extension length)



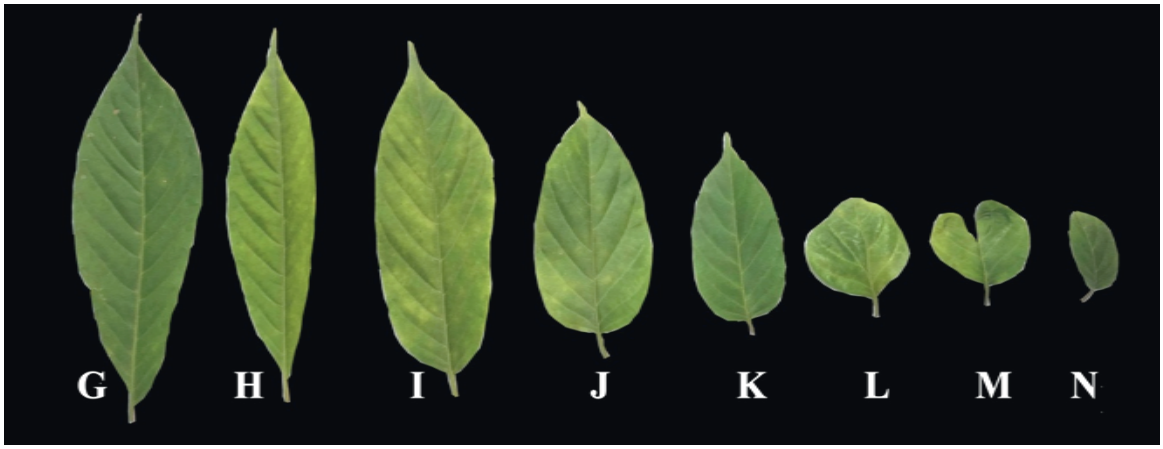


PLATE 3: A-F. Range of variation of leaves. G-N. Range of variation in size and shape of leaflets

4.1.2.1.5 Raised Marginal Glandular Nodule:

Preliminary examination shows that the raised marginal glandular nodules (RMGN) of fairly uniform size and shape are present uniseriately on the abaxial side along the margin of the lamina at the ends of major and minor veins (Pl.13A). These can be observed with naked eyes as slightly raised tips (Pl.13B-C).

4.1.2.2 Micro Morphology:

4.1.2.2.1 Leaf venation:

Venation pattern of the leaf is craspedodromous (Pl.4A). Lateral veins of leaflets vary from 5 to 12 on either sides of the midrib, opposite at the base and alternate towards apex. Venation pinnate, type Camptodromous, sub type weak brochidodromous (Pl.3G) or rarely actinodromous (Pl.3L), position of the first point of primary vein radiation suprabasal, vein development perfect, reticulate (Pl.3L) or vein development imperfect, reticulate (Pl.3M).

Primary vein (1°) or midrib straight, unbranched, rarely branched (F), considered weak having size= 0.023 cm ± 0.0051cm. Secondary vein (2°) or first order lateral (FOL) forms nearly uniform, abruptly curved, angle of divergence wide acute (65-80°), loop forming branches enclosed by 3° and 4° arches (Pl.4D). Marginal veins form loops (Pl.4C). Higher order venation observed up to fourth order externals or 5^0 ramifications. Intersecondary veins (I) or extralaterals prominent, strong, short and simple, originating from the medial 1° vein interspersed among the secondary veins in interlateral segment (Pl.4A).

Tertiary venation (3°) or first order externals prominent. Angles of tertiary origin on the exmedial side of the secondary veins when compared with that on the admedial side of the secondary veins two combinations observed (Table 2). 3° veins alternate or opposite, usually joining each other with an offset, weakly percurrent and forming sinuous or lattice (Pl.4E). 3° vein angle to 1° oblique and decreasing exmedially.

Table 2: Analysis of tertiary vein origin:

	Angle of 3° origin on the exmedial side of 2°'s		
Angle of 3° origin on the admedial side of the 2°'s		Acute	Right
	Obtuse	AO	RO

Distinct higher order venations up to quaternary (4°) or third order externals and quinternaries (5°) or fourth order externals observed. 4° and 5° veins proportionally reduced in width and course orthogonal (Pl.4B). 4° veins get reticulated regularly and polygonaly which anastomose with other veins to form similar polygons. 5° veins are dichotomizing. Marginal ultimate venation looped (Pl.4C).

Loops lateral, external and internal basiscopic and have different bases and arches comprises of various combinations (Pl.4D). Lateral and external loops lack distortion and elongated widthwise *i.e.* perpendicular to the midrib. Internal basiscopic loops may be elongated both in width and length *i.e.* parallel to the midrib or randomly within lateral loop (Table 3).

Table 3: Vein architecture of leaflet of *B. mollis*

1° vein catagory	2° vein catagory	2° vein spacing	2° vein angle	Inter 2° veins	3° vein course	3° vein angle to 1°	3° vein angle variability	4° vein catagory	5° vein catagory	Areolation
Pinnate	Weak brochiodomous	Irregular increasing or decreasing 2wards base	Wide acute	Strong, short and simple	Weakly percurrent and sinuous	Oblique	Decreasing exmedially	Orthogonal	Orthogonal	Moderately developed

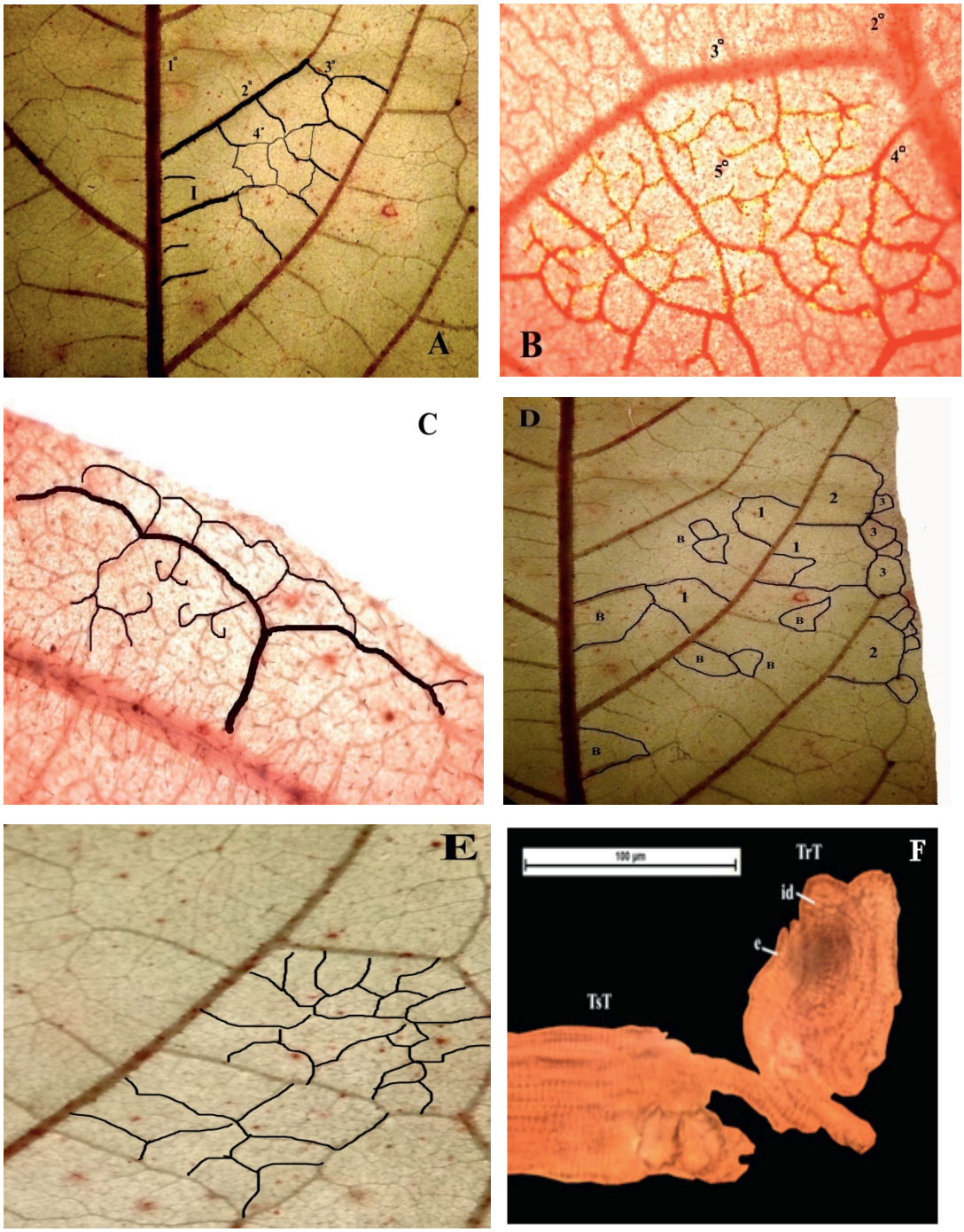


PLATE 4: Venation pattern: A. 1°, 2°, 3° and 4° vein architecture with prominent intersecondaries (I); B. Areole with ultimate dichotomizing vein terminals; C. Looped marginal venation; D-E Higher order venation architecture, D. Loops, E. Lattice; F. Accessory elements in leaf, Transfusion tracheids (TsT), terminal tracheary (TrT), idioblast (id), independent trachied ending (e).

Vein endings:

Number of vein endings in each areole numerous and ultimate veins dichotomously branched and curved which are either symmetrical or asymmetrical.Veinlets commonly uniseriate, short, thin, branched twice or thrice (Pl.4B).

Leaf rank:

As per the description of LAWG the leaflet rank of *B. mollis* falls in the rank 3r (Table 4).

Table 4: Attributes for determining leaflet rank of *B. mollis*

Elements		Attributes (result)
1° Course		Regular
2° vein	Course	Regular
	Angle of origin	Regular
	Spacing	Regular
Intercostal area		Shape similar
3° veins	Course	Regular
	Resolution from 2°	Good
	Resolution from 4°	Good
Areolation	Shape	Almost regular
	Size	Almost regular
	Orientation	Imperfect
Vein order with excurrent branching		5°
Blade petiole separation		Good

Terminal Tracheids (TrT):

Terminal trachieds ends independently (Pl.4F) or form terminal tracheary idioblasts (Pl. 4F). Tracheary idioblasts uniseriate, isodiametric or elongated and

occur as bundle of juxtaposed dilated tracheids lie at right angles to a bundle of elongated uniseriate tracheids at vein endings.

Transfusion tracheids (TsT):

Transfusion tracheids long or short with spiral or reticulate thickening which occur along the borders of veins and vein endings (Pl.4F).

4.1.2.2.2 Stomata:

Lamina is hypostomatic with randomly oriented paracytic stomata (Pl.5A-F). Subsidiary cells distinct, chlorophyllous, may or may not be of equal size. Guard cells equal in size, elliptical, pore elongated. Stomatal frequency (SF) is 16.9% and stomatal index (SI) is 13.1. Length of the stomatal pore is 11.4 ± 1.1 µm, size of subsidiary cells 20.6 ± 0.8 µm x 5.66 ± 0.5 µm, size of the guard cells 14.46 ± 1 µm x 19.2 ± 0.5 µm.

4.1.2.2.3 Trichome in leaf:

Distribution, occurrence and micro morphological features of trichomes occurring in different parts of the leaf of *B. mollis* have been studied (Table 5). The result of LM and SEM analysis exhibit the presence of twenty different types of trichomes in the *B. mollis.* Itis postulated that the presence of trichomes is positively correlated with the accumulation of secondary metabolites in different plant parts (Rusydi *et al.,* 2013).

Leaf observed under LM exhibits only capitate and simple long unicellular trichomes (Pl. 6 A, B). Frequency of trichome is more in abaxial than adaxial surface. Both long and short non glandular unicellular or multicellular trichomes are more in midribs and veins than on both surfaces of lamina (Pl.6E,F).

Abaxial surface have numerous simple short unicellular trichomes (Pl.7C,D). On the lower epidermis non glandular trichomes are simple short or long unicellular

and simple short or long multicellular with pointed tips (Pl.8A-H). The former is composed of a large basal cell and an elongated cell. Later is composed of five to ten cells. Bright orange, red, yellow or black cellular depositions are observed in both types of hairs under LM. The ornamentation of surface of the trichomes under SEM appears to be echinate or adorned with micropapillae (Pl. 9I). Fusiform and peltate glands are observed in lower epidermis under LM (Pl.9A-B). Under SEM capitate, stipitate, peltate, fusiform, patelliform and cupular glandular trichomes are observed in lower epidermis (Pl.9C-L). Capitate trichome may be sessile or stalked. Sessile capitate trichomes have large clavate head without any basal cell or stalk (Pl.9D). Stalked capitate trichome (Pl.9C) has one basal cell, one or two celled stalk and a large wrinkled unicellular head. Stipitate trichomes may or may not have a swollen basal cell (Pl.9E-F). Peltate trichomes may be calcified, wrinkled sunken, wrinkled sub sessile (Pl.9G-I). The peltate trichomes consist of one or two basal epidermal cells with a very short stalk and a large spherical head. Peltate trichomes are copious on the abaxial surface than capitate trichomes. A fusiform glandular trichome is composed of one basal cell, one thin stalk cell and two layers of head cells. Cupular trichome is cup shaped. Patelliform are flat and copular forms are cup shaped trichomes which secretes nectaries. One unidentified type of peltate trichome is also observed under SEM (Pl.9L).

Under LM mature peltate trichome of abaxial surface consist of a four celled secretory head assembly enclosed in a smooth cuticle (Pl.9B). Head cells are based on a central stalk. The elevated cuticle of the head possibly comprised of accumulated secretory material. It caused the cuticle to swell up and give a globular appearance.

Adaxial surface have simple non glandular multicellular long and a few short trichomes enclosed in thick cuticle (Pl.10A-D). Frequency of trichome is less than the abaxial surface. It is composed of large basal cell and three to five long cells. There are bright orange, red, yellow or black cellular depositions. Under LM mature peltate trichomes and epidermal pores are observed (Pl.11A-C). Under SEM the surface ornamentation of the trichomes are smooth, rough or echinate (Pl.11D-E). Sometimes covers with fungal hyphae. Under SEM various types of glandular trichomes are observed which are capitate, stipitate, peltate, digitiform, pateliform and cupular types in adaxial surface (Pl.12A-I). Capitate trichomes may have smooth folded head or calcified large head with one cell stalk and a basal cell. Stipitate trichomes have three to four stalk cells and a large folded head. Peltate trichomes are round with smooth or rough heads with micropapillae. Digitiform glandular trichomes have a large basal cell, three to four round stalk cells and an apical pointed secretory cell. Their number is less than other glandular types of trichomes. Patelliform and cupular trichomes are also observed under SEM.

Under LM head of peltate trichomes appears to be assembly of two, three, four, five or six secretory cells enclosed in a smooth cuticle (Pl. 12 A). Red orange and black depositions are prominent in the peltate trichomes.

Rachis of the leaf has simple non glandular long or short multicellular trichomes enclosed in thick cuticle. They may be three to sixteen cells long. Bright coloured cellular depositions are observed under LM (Pl. 12 J-K).

4.1.2.2.4 Trichome in Petiole:

In petiole both non glandular and glandular trichomes are present. There are two types of simple non-glandular trichomes *viz*., short and long simple unicellular and simple multicellular. The former is composed of one large basal cell and one

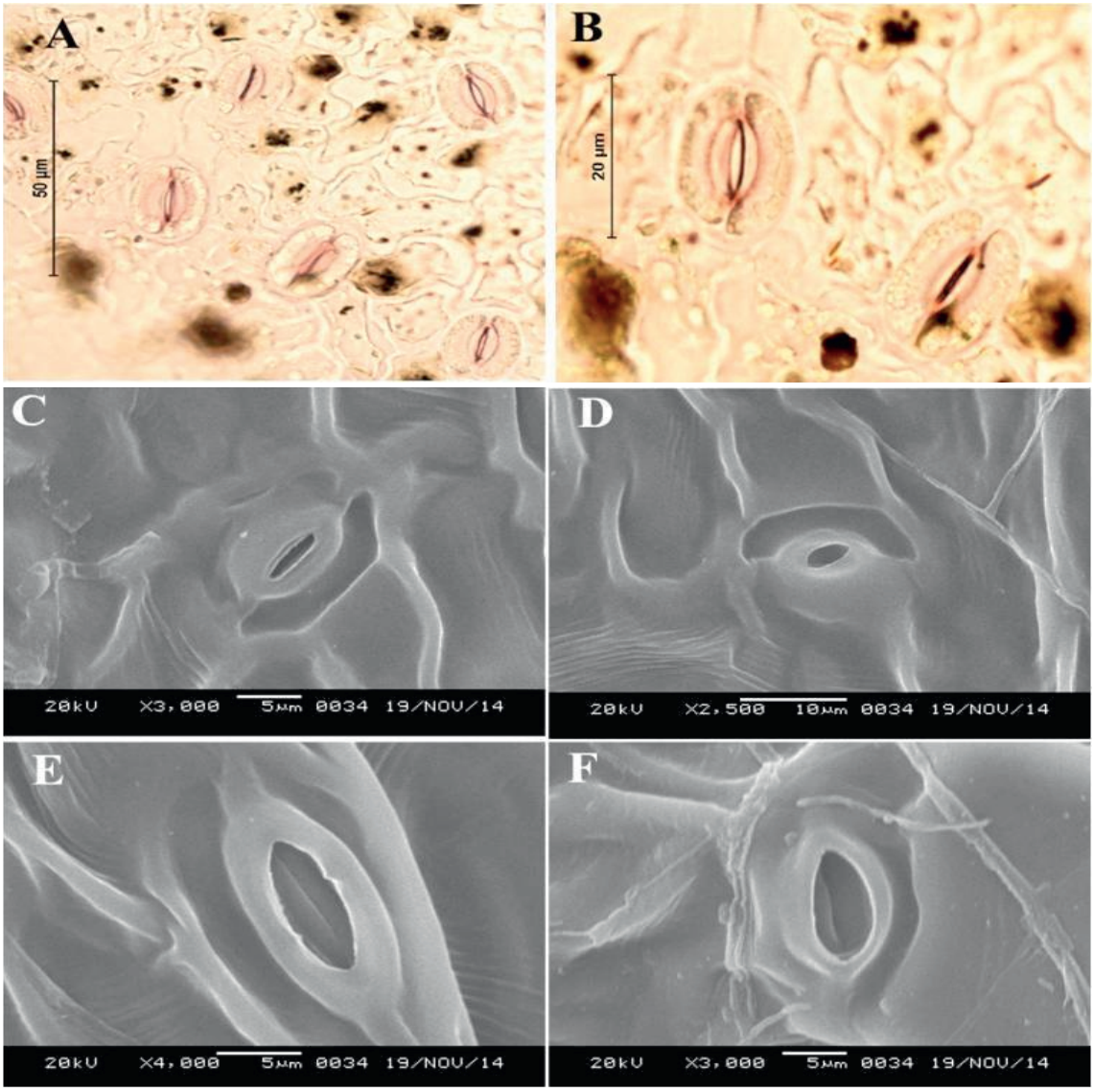


PLATE 5: Paracytic stomata of leaf under LM (A-B) and SEM (C-F)

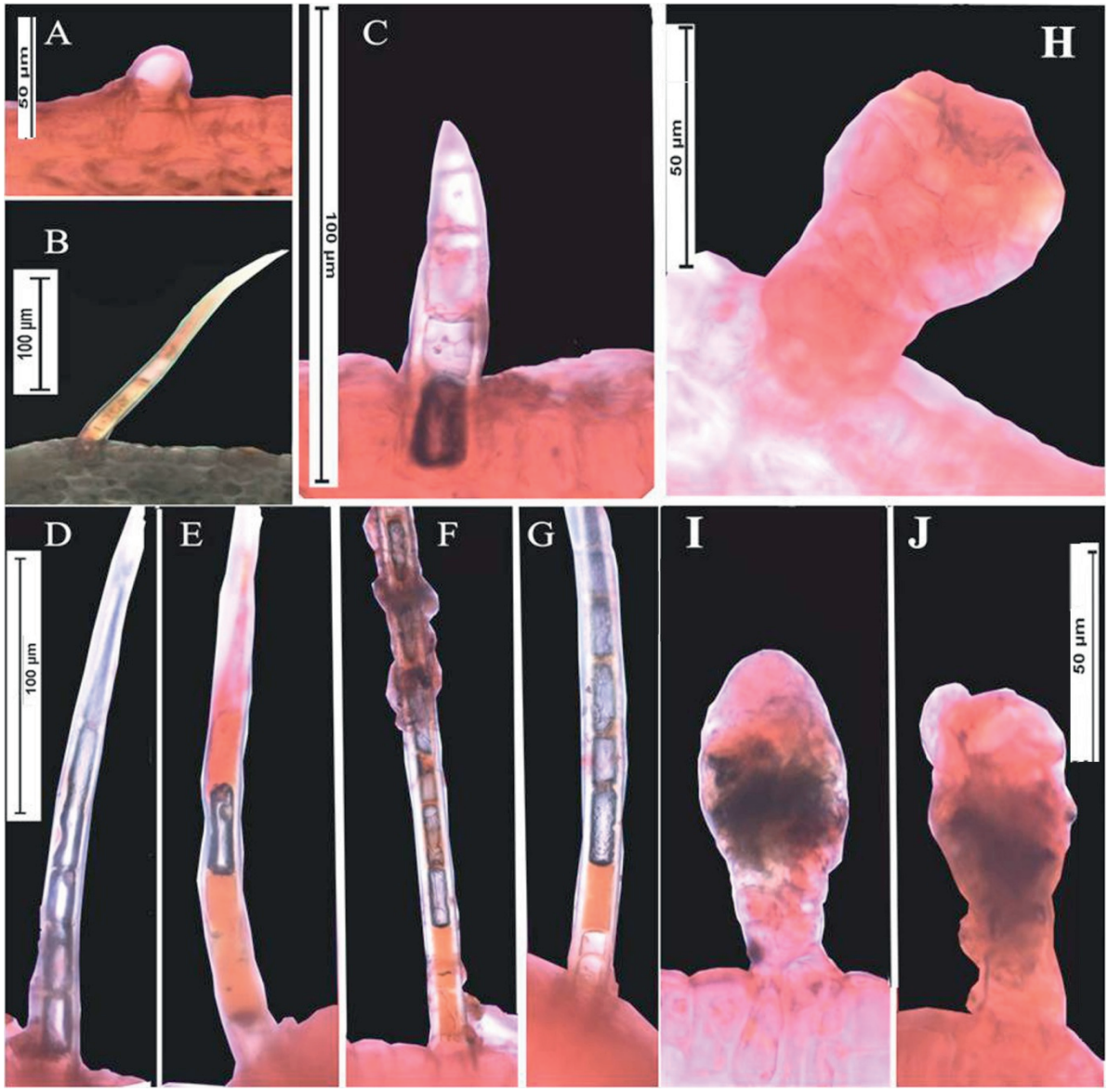


PLATE 6: Micromorphology of trichomes present in the petiole under LM. A. Short unicellular trichome, B. Long unicellular trichome, C.Short multicellular trichome, D-G. Long multicllular trichome with bright red, orange, yellow and black secondary metabollite depositions, H. Capitate trichome, I-J. Stipitate trichome.

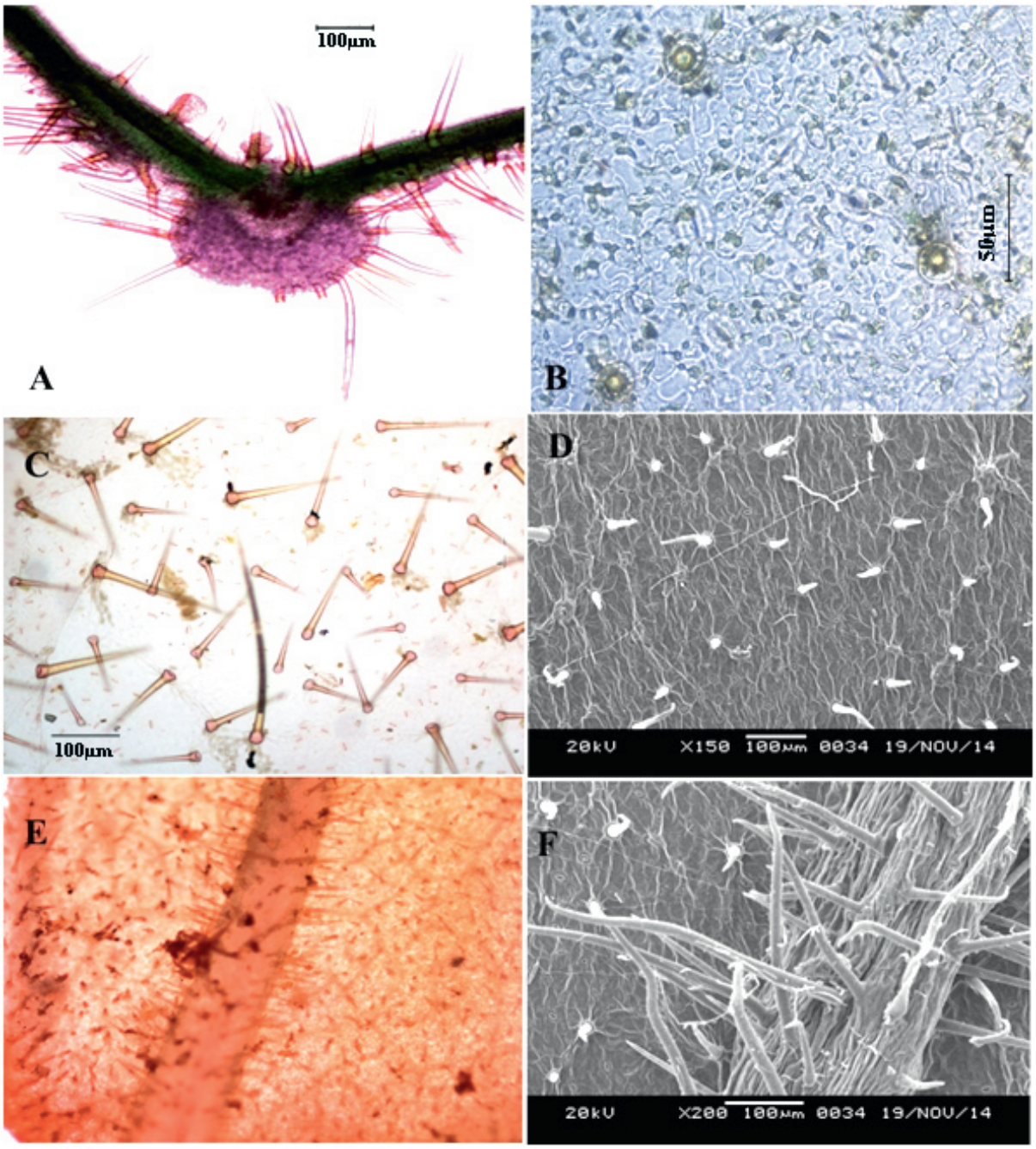


PLATE 7: Distribution of trichomes in abaxial surface of the leaf. A. TS. of young leaf different types of trichomes, B. Mature peltate trichomes, C-D. Non glandular trichomes under LM (10x) and under SEM, E-F. Non glandular trichome in the veins under LM (4.5x) and SEM.

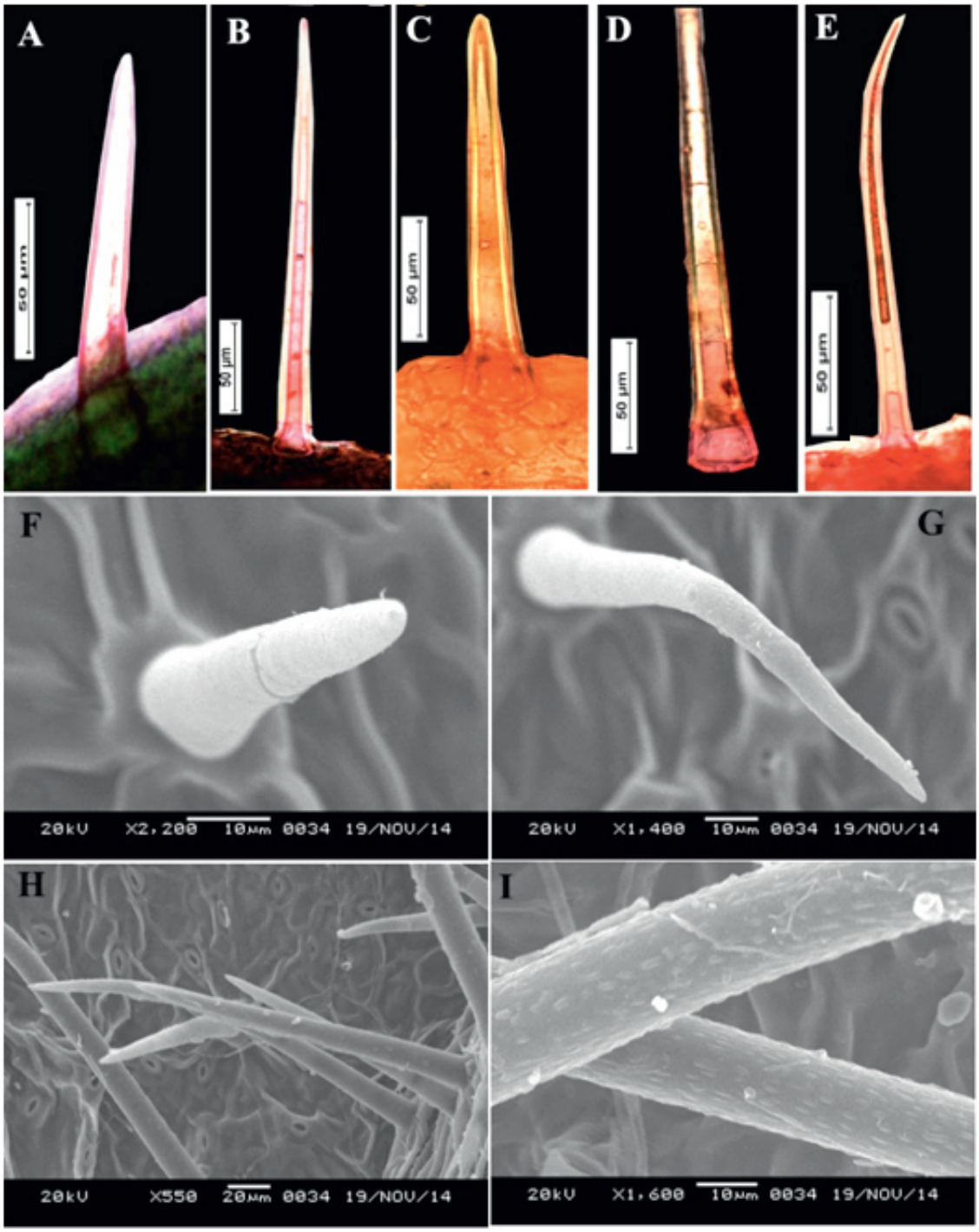


PLATE 8: Simple non glandular trichomes present in LE of leaf under LM (A-E) and SEM (F-I). A. Simple short unicellular non glandular trichome, B. Simple long unicellular non glandular trichome, C. Simple short multicellular non glandular trichome, D-E. Simple long multicellular non glandular trichome, F. Simple short smooth walled non glandular trichome, G-H. Simple long smooth walled non glandular trichome, I. Simple non glandular trichome ornamented with micropapillae.

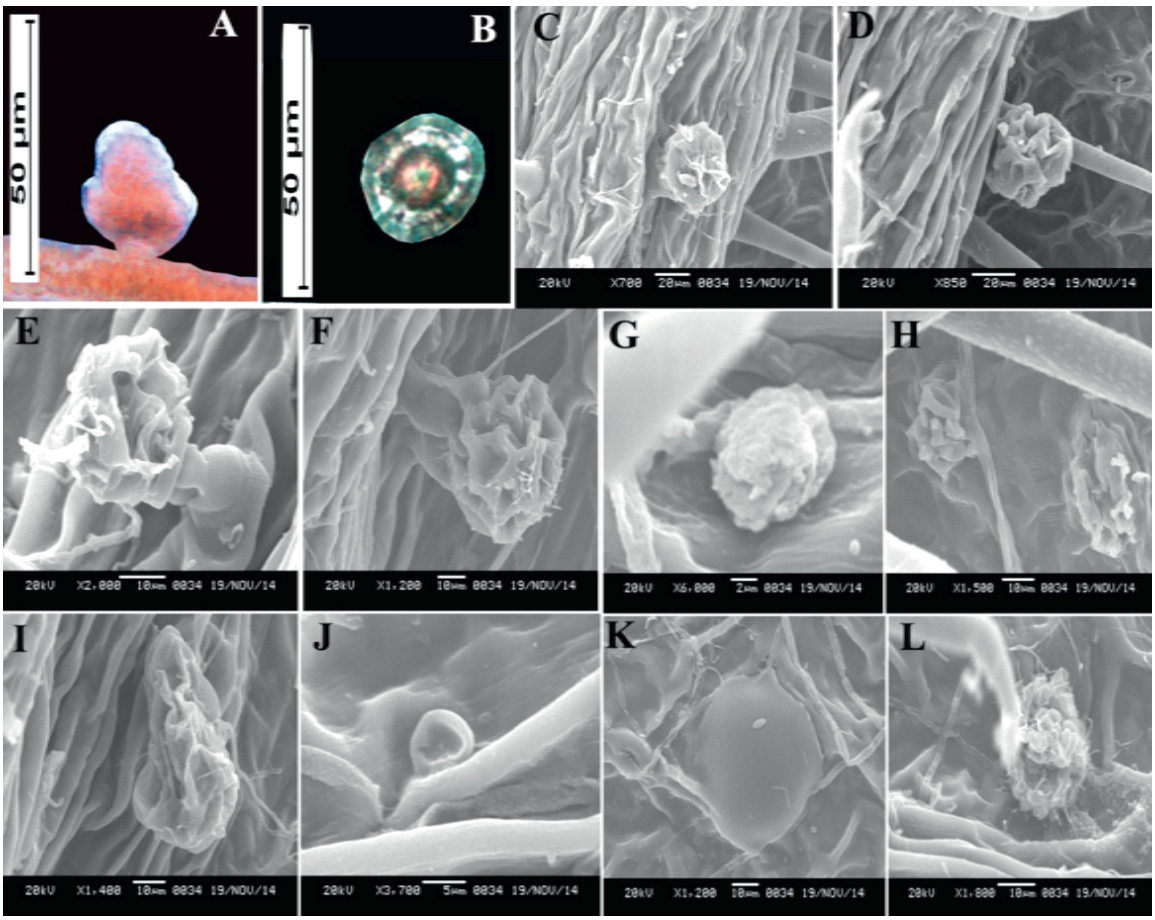


PLATE 9: Simple glandular trichomes on abaxial surface of leaf under LM (A-B) and SEM (C-M). A. Fusiform trichome, B. Mature peltate trichome, C. Stalked capitate trichome, D. Sessile capitate trichome, E. Stipitate trichome with swollen base, F. Stipitate trichome without swollen base, G. Calcified peltate trichome, H. Wrinkled sunken peltate trichome, I. Wrinkled sub sessile peltate trichome, J. Cupular trichome, K. Patelliform trichome, L. Unknown peltate trichome.

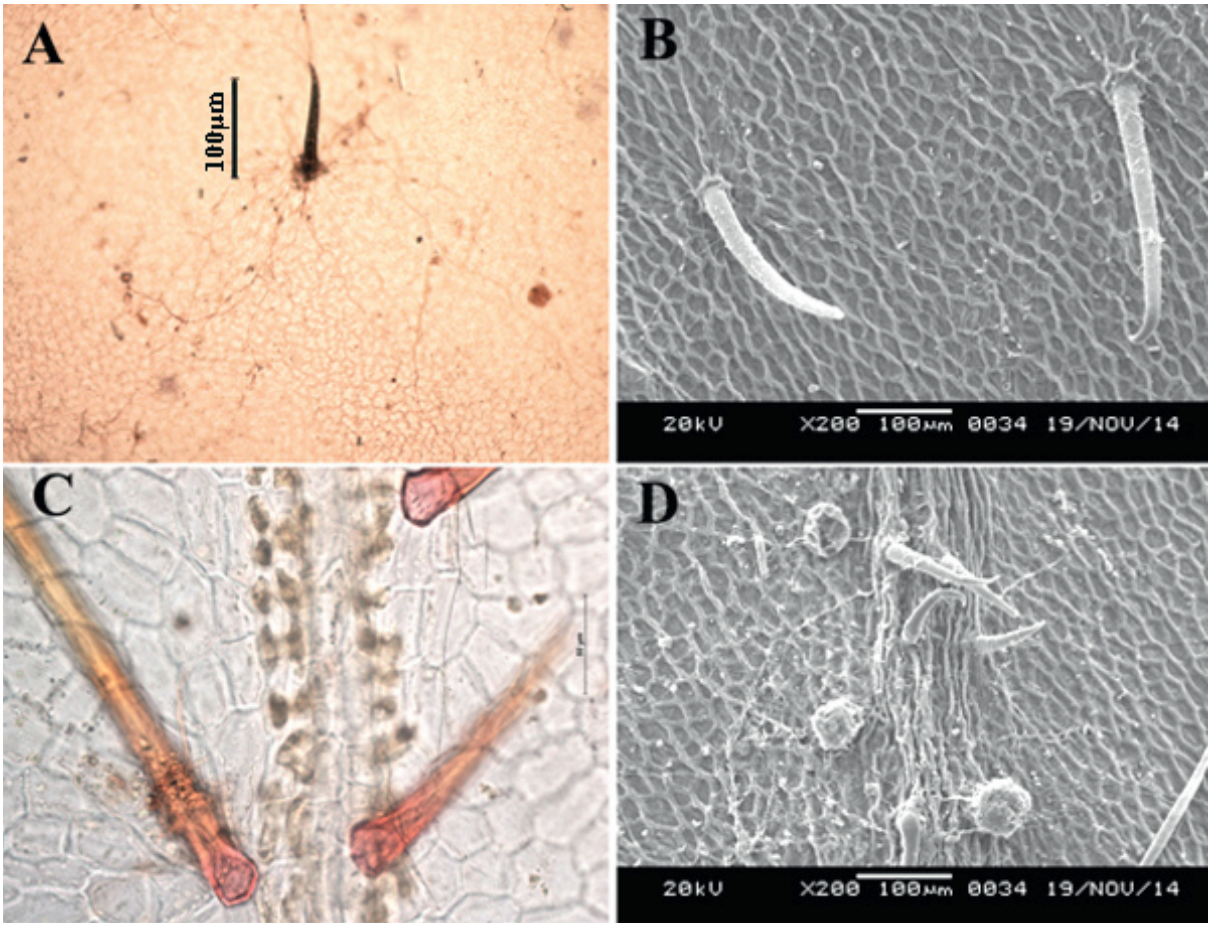


PLATE 10: Distribution of trichomes on Adaxial surface of leaf and mid rib under LM (A,C) and SEM (B,D). A-B. Lamina, C-D. Midrib.

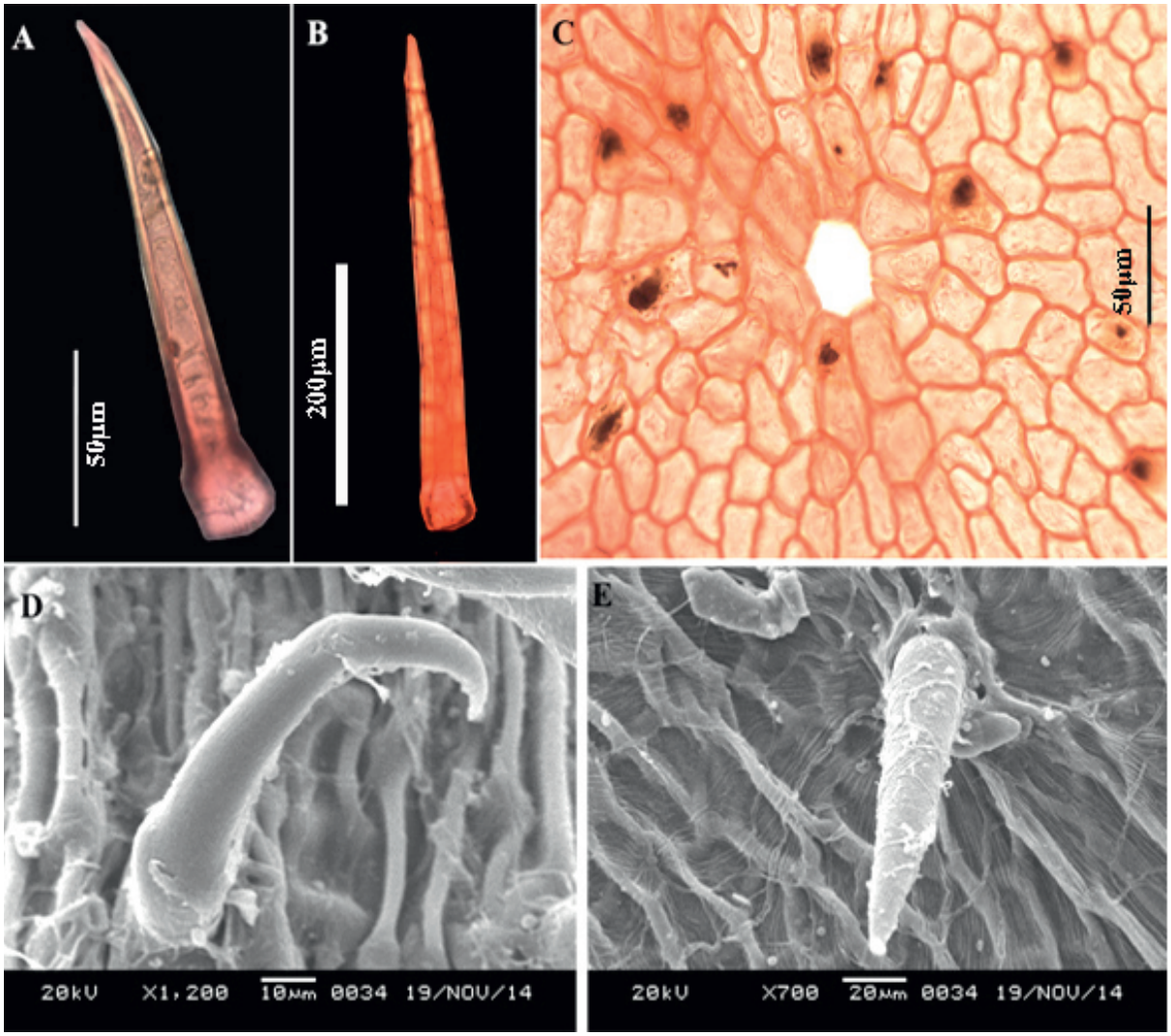
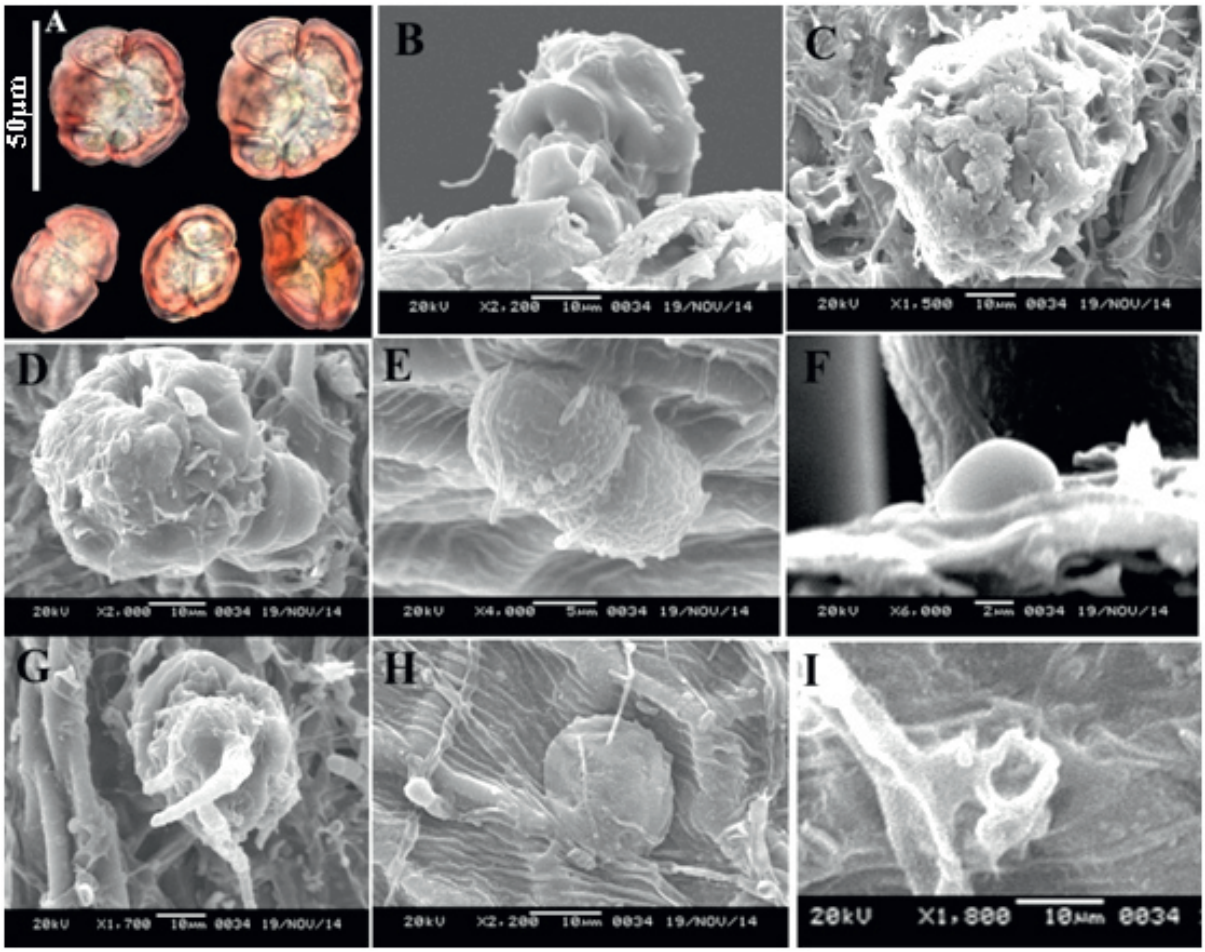


PLATE 11: Simple multicellular trichome and epidermal pore on adaxial surface under LM (A-C) and SEM (D-E). A. Simple non glandular multicellular short trichome, B. Simple non glandular multicellular long trichome, C. Epidermal pore, D. Simple non glandular multicellular trichome with smooth wall, E. Simple non glandular multicellular trichome with rough wall.



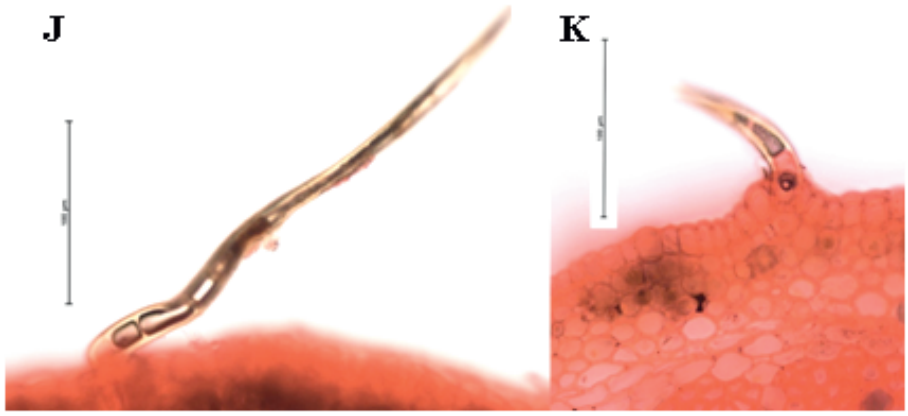


PLATE 12: Glandular trichomes on adaxial surface and rachis under LM (A) and SEM (B-I). A. Six, five, two, three and four peltate trichomes. B. Stalked, smooth headed capitate trichome, C. Stalked, calcified headed capitate trichome, D. Stipitate trichome, E. Peltate trichome with micropapillate head. F. Peltate trichome with smooth head, G. Digitiform trichome, H. Patelliform trichome. I. Cupular trichome, J. Long simple multicellular non glandular trichome of mid rib, K. Long simple multicellular non glandular trichome of mid rib.

short or elongated cell (Pl.5A-G), whereas the later consists of three to 12 cells. Both types of trichomes have thick cuticle and bright red, black or orange coloured cellular deposits. Tips of short non glandular trichomes are ovate. But that of long uni- or multi-cellular trichomes have pointed tips.

Glandular trichomes in petiole are either capitate or stipitate (Pl.6H-J). The stalk of capitate trichomes is one or two celled. While secretory head is formed by 9-16 cells. Heads are not covered by any cuticle. Black cellular deposits have been observed in the heads. Stipitate trichomes are similar to capitate ones but they have long stalks composed of six to eight cells which elongate during the development of the trichome. Head of stipitate trichomes are composed of six to ten cells and are covered with a thin cuticle. Heavy deposition of black cellular exudates is prominent in stipitate trichomes as compared to the capitate ones. Sometimes both glandular and non glandular trichomes also present in clusters. Frequency of non glandular trichome is more than the glandular ones in petiole.

Table 5: Distribution of types of trichome in different parts of *B. mollis*

Type	UE	LE	Petiole	Rachis
Non glandular				
Short unicellular	-	+	+	-
Long Unicellular	-	+	+	-
Short multicellular	+	+	+	+
Long multicellular	+	+	+	+
Glandular				
Capitate sessile glandular trichome	-	+	-	-
Capitate stalked glandular trichome with	+	+	+	-

smooth/folded/wrinkle head				
Capitate stalked glandular trichome with calcified head	+	-	-	-
Stipitate glandular trichome with swollen basal cell	-	+	-	-
Stipitate glandular trichome without swollen basal cell	+	+	+	-
Peltate trichome with calcified head	-	+	-	-
Peltate trichome with smooth head	+	-	-	-
Peltate trichome with micropapillate head	+	-	-	-
Wrinkled sunken peltate trichome	-	+	-	-
Wrinkled sub sessile peltate trichome	-	+	-	-
Fusiform glandular trichome	-	+	-	-
Patelliform glandular trichome	+	+	-	-
Cupular glandular trichome	+	+	-	-
Digitiform glandular trichome	+	-	-	-
Unknown peltate type I	-	+	-	-

LE-lower epidermis, UE-upper epidermis

4.1.2.2.5 Raised marginal glandular nodule:

Raised Marginal glandular nodulesare submerged in leaf tissue. They are neither completely internal nor enclosed completely by the leaf epidermis, rather the dome shaped head bulges out from leaf tissue (Pl.13B).

Under LM dome of the RMGN can be observed distinctly as black core of secretory cells covered with a crown of non glandular multicellular trichomes (Pl. 13 B- D). The dome consists of aggregated secretory cells which got separated from the

adaxial epidermis by a layer of flattened palisade parenchyma cells forming the cup (Pl. 14C). Therefore, the structure can also be considered as submerged nodule instead of gland. The diameter of the cup is 188.6 μ ± 4.3μ. The height of the dome is 266.4μ ± 11.3μ. Leaf clearings and vertical sections of a leaf exhibit vascularization of the nodule.

The gland does not have any opening to the exterior, although a fissure is seen at the neck of the groove (Pl.14B). Lumps of bluish black depositions with few translucent spaces within the nodule can be distinctly seen under low resolution (Pl.15A). When observed under high resolution bluish black lumps are found to be made up of aggregation and accumulation of small granules while the translucent areas are nothing but the spaces consisting of the tiny granules themselves surrounding the polygonal pulpy secretory cells. At the base of the nodule there is large number of bluish black druses (Pl.15B-C). The laminar tissue contains large clumps of dark black secretions surrounding the black nodule (Pl.15 D-E). They are constituted with the similar bluish black granules that are found within the nodule.

Study of the RMGN under SEM confirmed that there is no porous opening present in the nodule to the exterior (Pl.16A-F).The elevated top of the dome shaped nodule is smooth (Pl.16D) and at the same time the elevated part of the nodule is not continuous with the adaxial laminar surface. At the junction of the two there is a gap which is filled by folding fissures having openings to the exterior (Pl.16E).

Development of the RMGN begins as a spherical dark spot at vein end probably formed by secretory cells (Pl.17A). It gradually started expanding, changes its colour to translucent and forming a schizogenous sac gap (Pl.17 B). There is no evidence of cell lysis or enlargement and for which the development can be considered as schizogenous. Parenchymatous lining present below the nodule

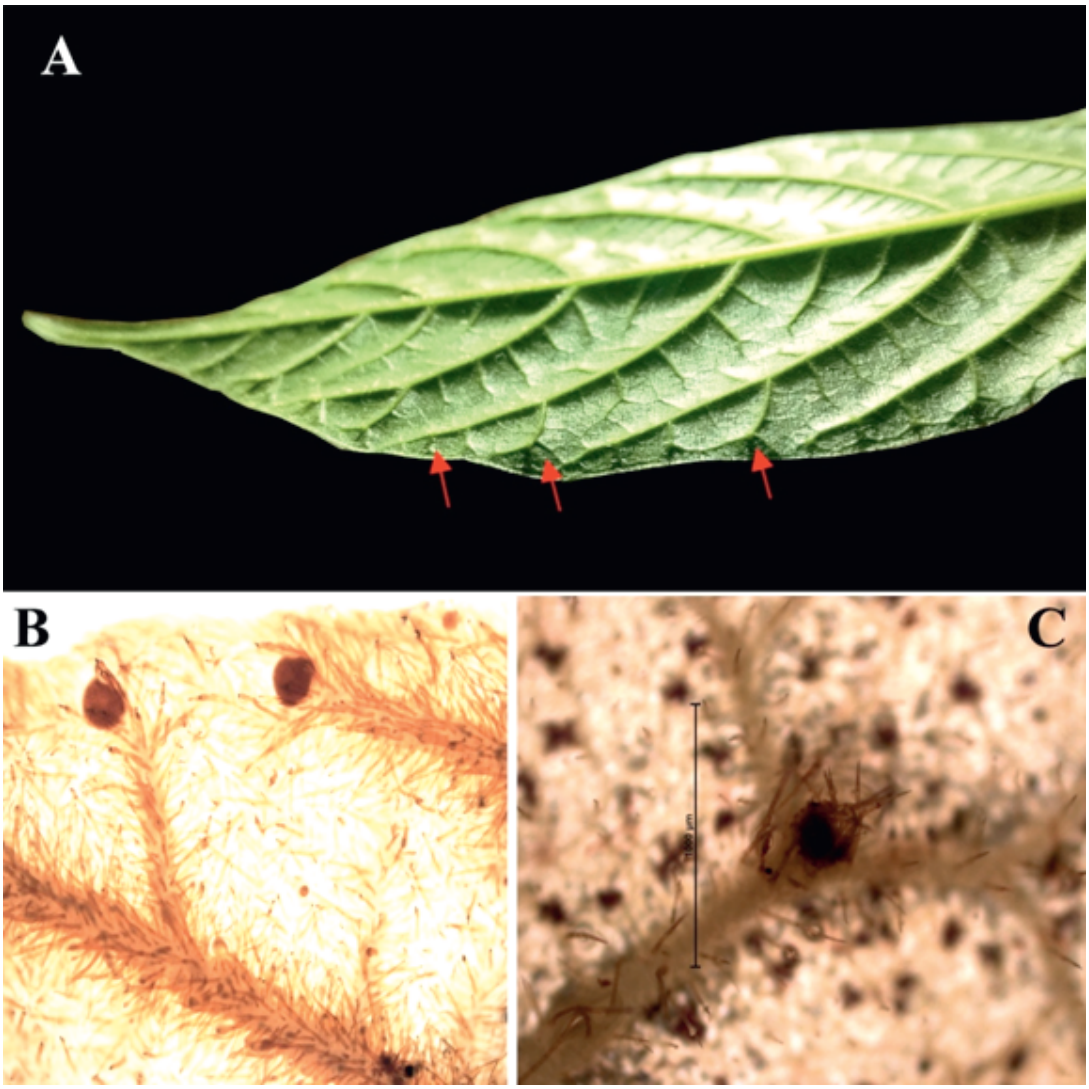


PLATE 13: Raised marginal glandular nodule. A- RMGN at the ends of major veins, B. RMGN with under LM (10x), C. RMGN crowned with trichomes under LM (10x).

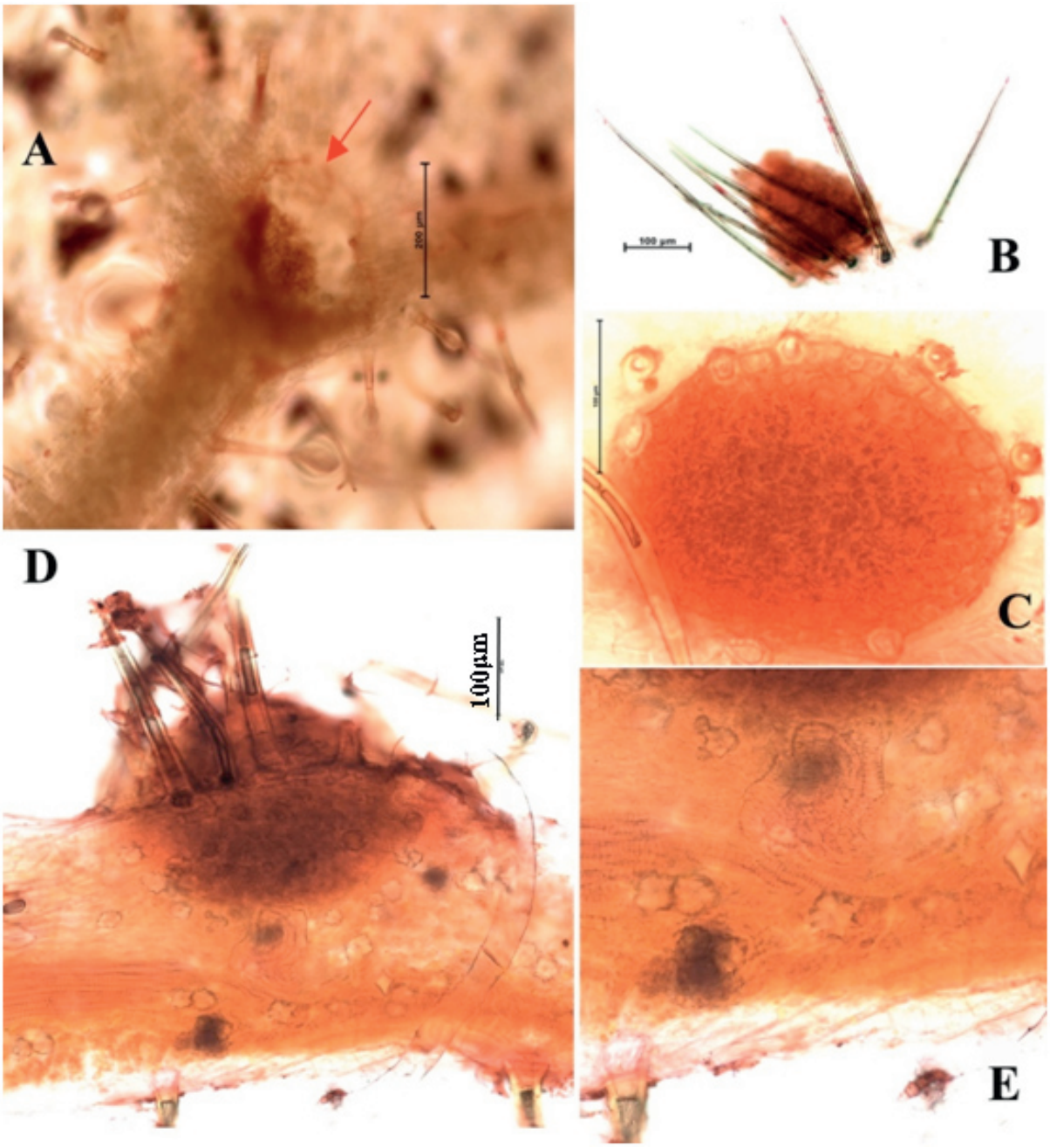


PLATE 14: RMGN under LM. A. Groove of the nodule after removal of the secretory tissue and trichomes, 10x. B. Dome shaped secretory tissue with trichomes, 4x, C. Groove with organized parenchymatous layer after leaf clearing, 10x, D. VS of the gland, 40x, E. Vein ends at the base of the nodule, 40x.

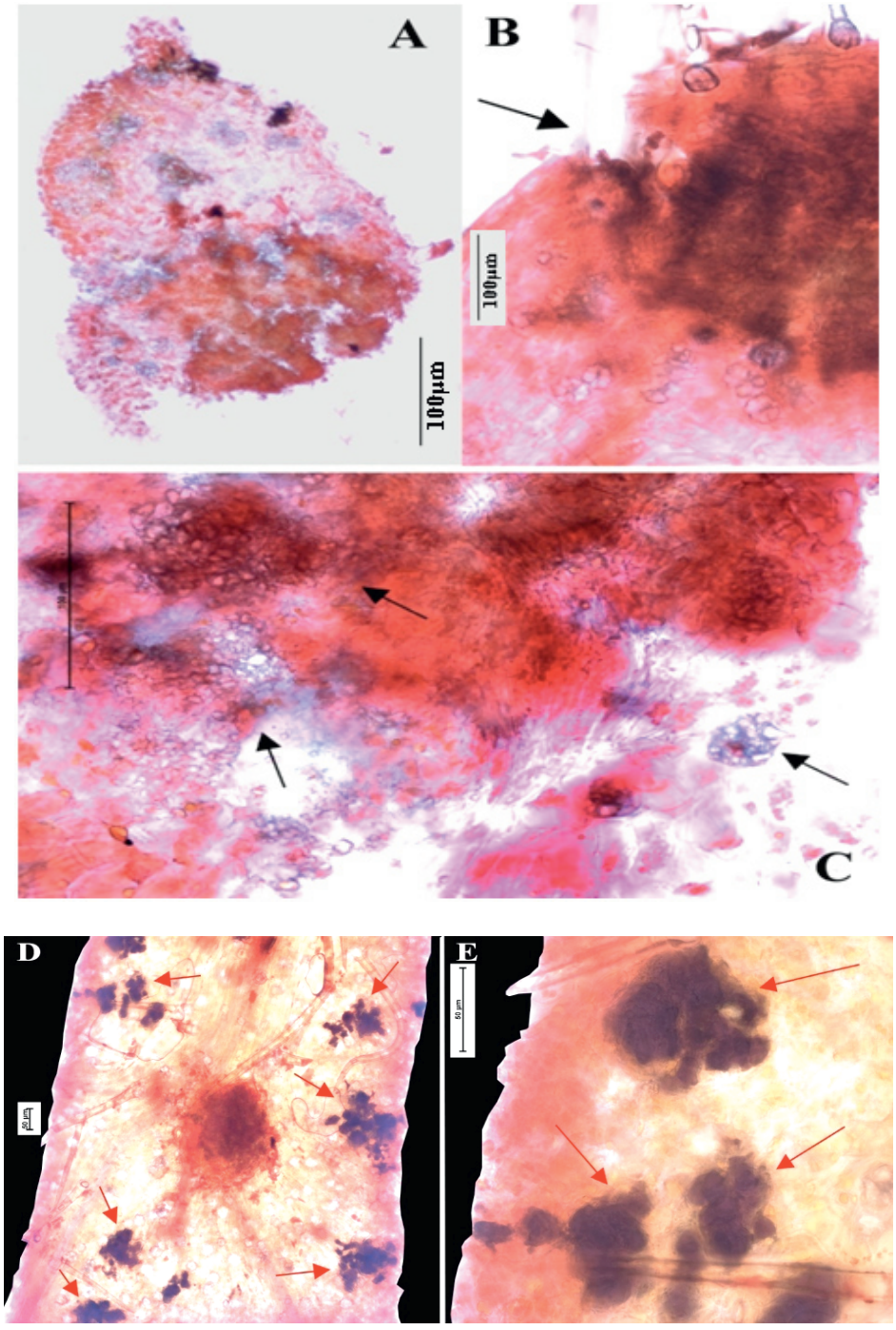


PLATE 15: Secretory tissue in RMGN. A. Secretory tissue mass, 10x, B. Fissure at the neck of the groove, 40x, C. Bluish black granules, blurry mucilage tissue and bright orange secretion, 40x; D-E. Black secretory mass in the leaf tissue under LM, 10x, 40x.

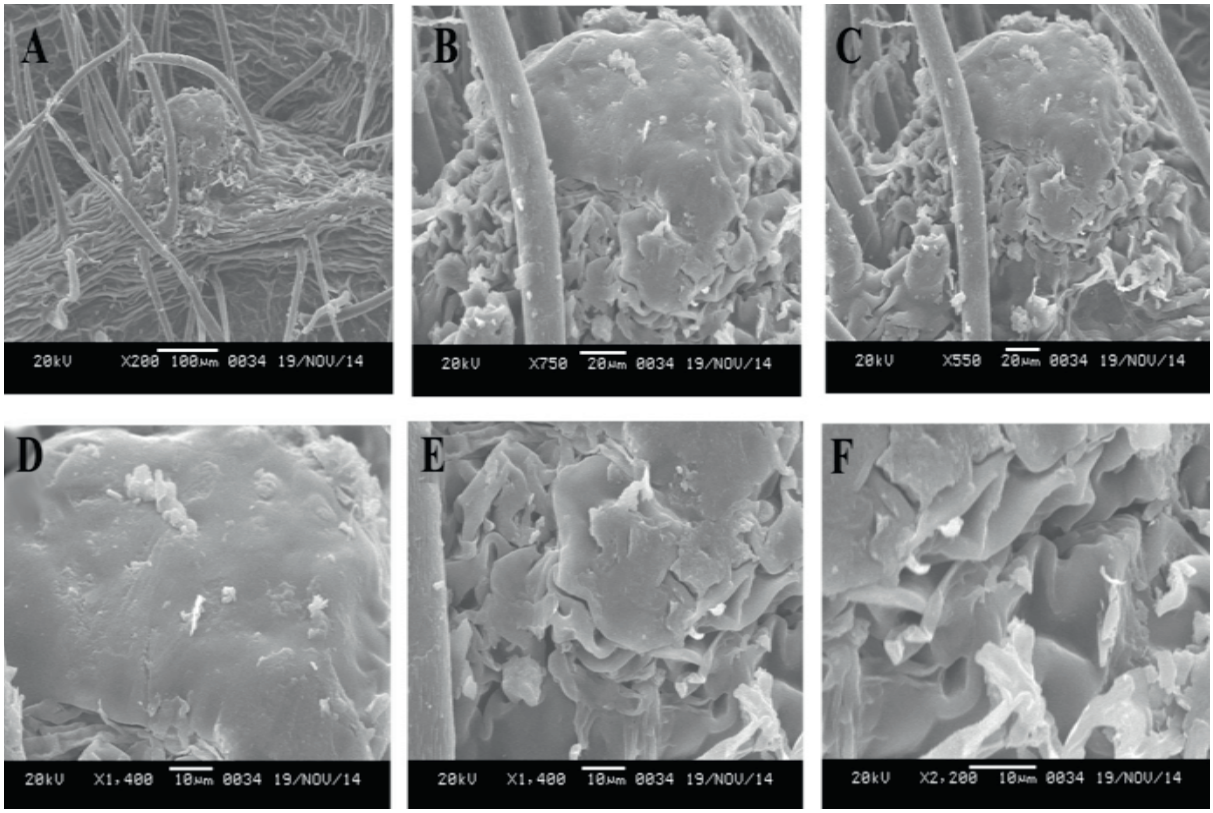


PLATE 16: RMGN under SEM. A. RMGN at the junction of secondary vein, B-D. Top view of the nodule exhibiting smooth surface, E-F. Folding fissures at the junction of the elevated part of the nodule and the base.

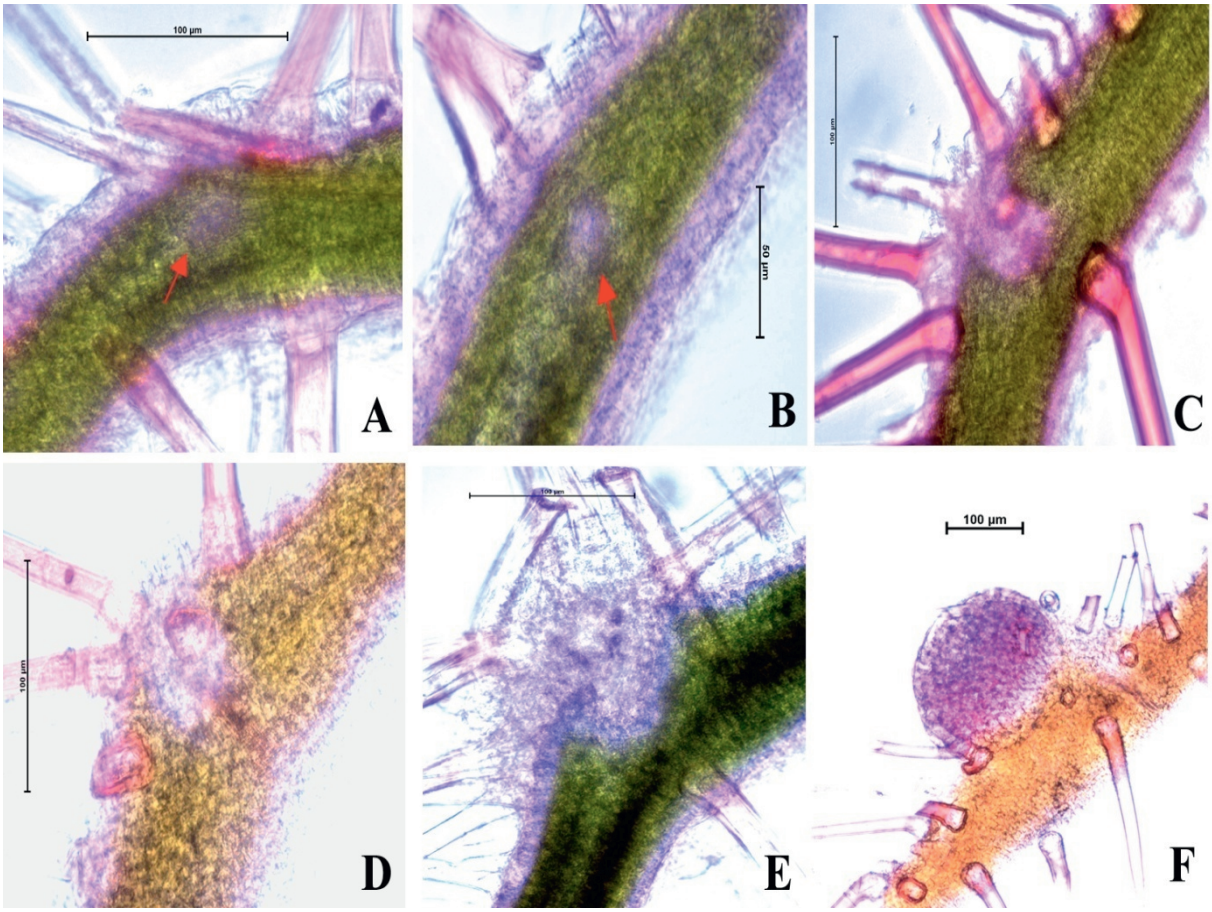


PLATE 17: Stages of development of RMGN. A. Initiation spot, B. Translucent schizogenous sac gap, C-E. expansion of the nodule towards adaxial epidermis, F. Mature RMGN

separated RMGN from the leaf mesophyll. Formation and aggregation of translucent secretory cells inside the schizogenous sac continues resulting into the expansion of the nodule towards adaxial epidermis (Pl.17C-E). Finally, the nodule emerges out from the leaf tissue (Pl.17F). Traces of vascular tissue can be seen at the base of the nodule.

4.1.3 Floral morphology and anatomy:

Plants are dioecious, flower unisexual, minute, born in long raceme, emerged from the axiles of leaf. Morphology and anatomy of the different floral parts are studied (Table 6).

4.1.3.1 Male flower:

Male inflorescence cymose, 15-163 cm long, densely covered with hairs. The average number of male flowers per inflorescence is 80.32 ± 18.4 with a minimum 29 and maximum 127 (Pl.18A-B). Floral parts contain different glands which may produce nectar like substance which often attract black ants as visitors. Inflorescence bears a small brown stipule at the base.

Ramification in the inflorescence is acropetal up to 3° and 4° resulting into primary, secondary and tertiary branching. Primary and secondary ramification occur acropetaly but the tertiary or terminal ramification is basepetal bearing two small flowers in lateral branches (Pl.19 A-B).

4.1.3.1.1Macro morphology:

A mature male flower is inconspicuous, perfect, 2-3 mm in diameter, with no odour or scent, tetramerous, dichlamydeous, heterochlamydeous, biseriate, aposepalous, apopetalous, polyandrous, actinomorphic (Pl.19A). Sepals green, pubescent, triangular, petals greenish white, spoon shaped, pubescent, longer than

stamens, stamens 4 to 6. Disk partly flattish and partly globose. All the floral parts are free.

Sepals are 0.5 mm long and 0.3 mm in breadth, more or less triangular shaped, posses several glandular hairs (Pl.19D). Petals are 0.8 to 1.2 mm long and 0.3 mm in breadth, elliptical in shape. Disk green, globose, four lobed formed by the fusion of aborted ovaries and the tissue of the floral disc. There is cliff at the center which makes the four lobes distinct. Stamens are inserted between lobes of disk.

Androecium consists of 4, 5 or 6 stamens (Pl.18C-E). Anther exserted, basifixed, latrorse (Pl. 20A-B). Filament is 400-450 µm long. Anther lobes are 400-450 µm long and 180-190 µm in diameter. Epidermis surrounds the sporogenous tissue mass which produces numerous pollen grains. Connective is thick enough and has a diameter of 60-75 µm. Each anther lobe produces about 120-160x4=520 no. of pollens per anther lobe. Pollens tricolpate, surface raticulate, size 31.8 x 29.2 µ (Pl.20C-D).

4.1.3.1.2 Micro morphology:

Petals and sepals are covered by several glandular hairs.Petals characteristically posses one or two large stipitate trichome about 50 µm high at its base. Multicellular glandular hairs are of two types- short (50-65 µm) and long (100-200 µm) (Pl.19D,E).

Sepals exhibit uni and multicellular non glandular trichomes and multicellular capitate trichomes, stomata paracytic. Small stomatal pores are about 8.5-10 µm long. Guard cells chlorophyllus (Pl. 21 E-I). 300-450 µm long glandular hairs are scattered throughout the surface of the sepal (Pl.21A-D).

PLATE 18: A-B. Inflorescence C-E. Male flower with four, five and six stamens.

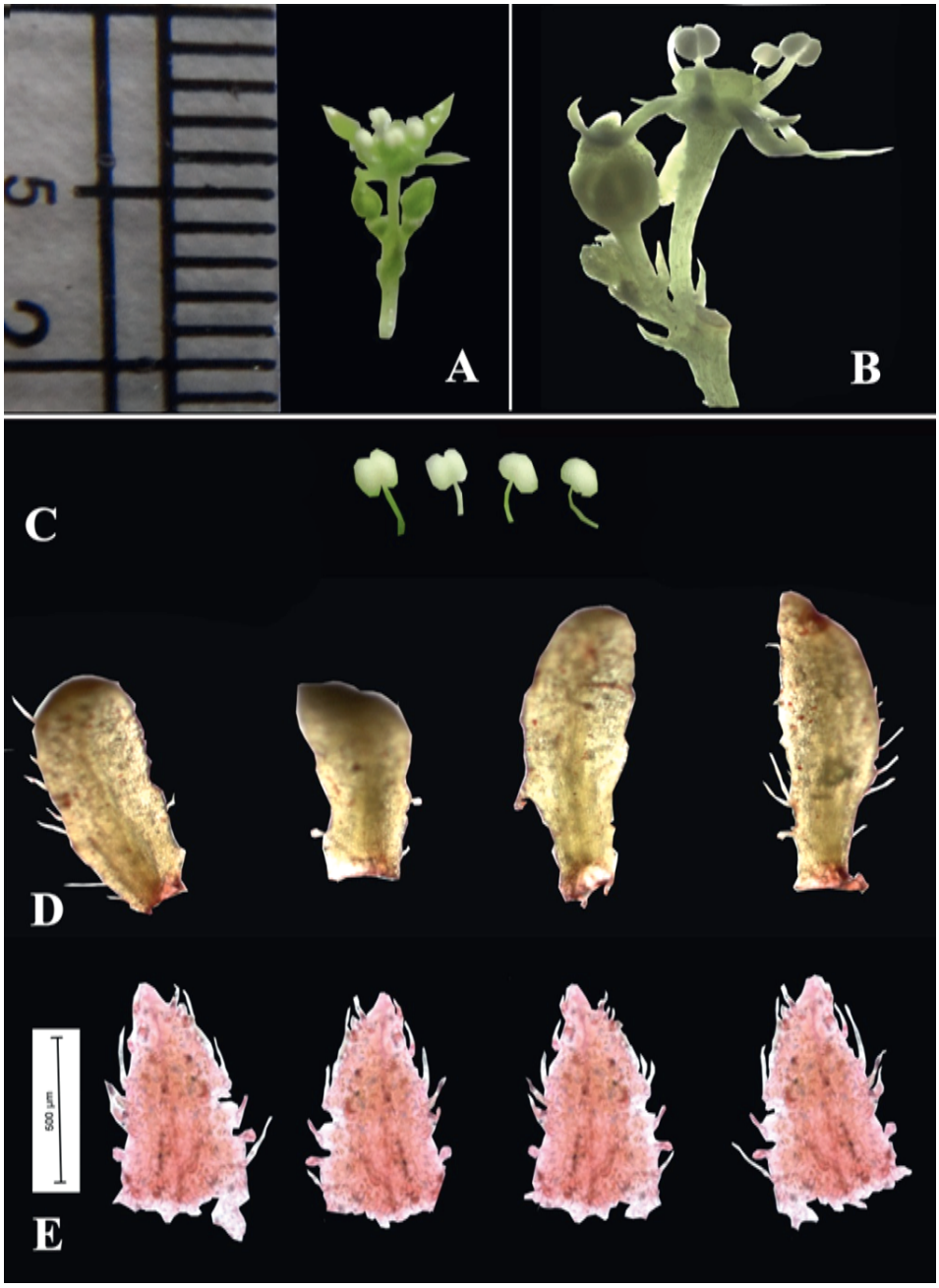


PLATE 19: A - B. Male flower C. Anther, D. Sepal, E. Petal.

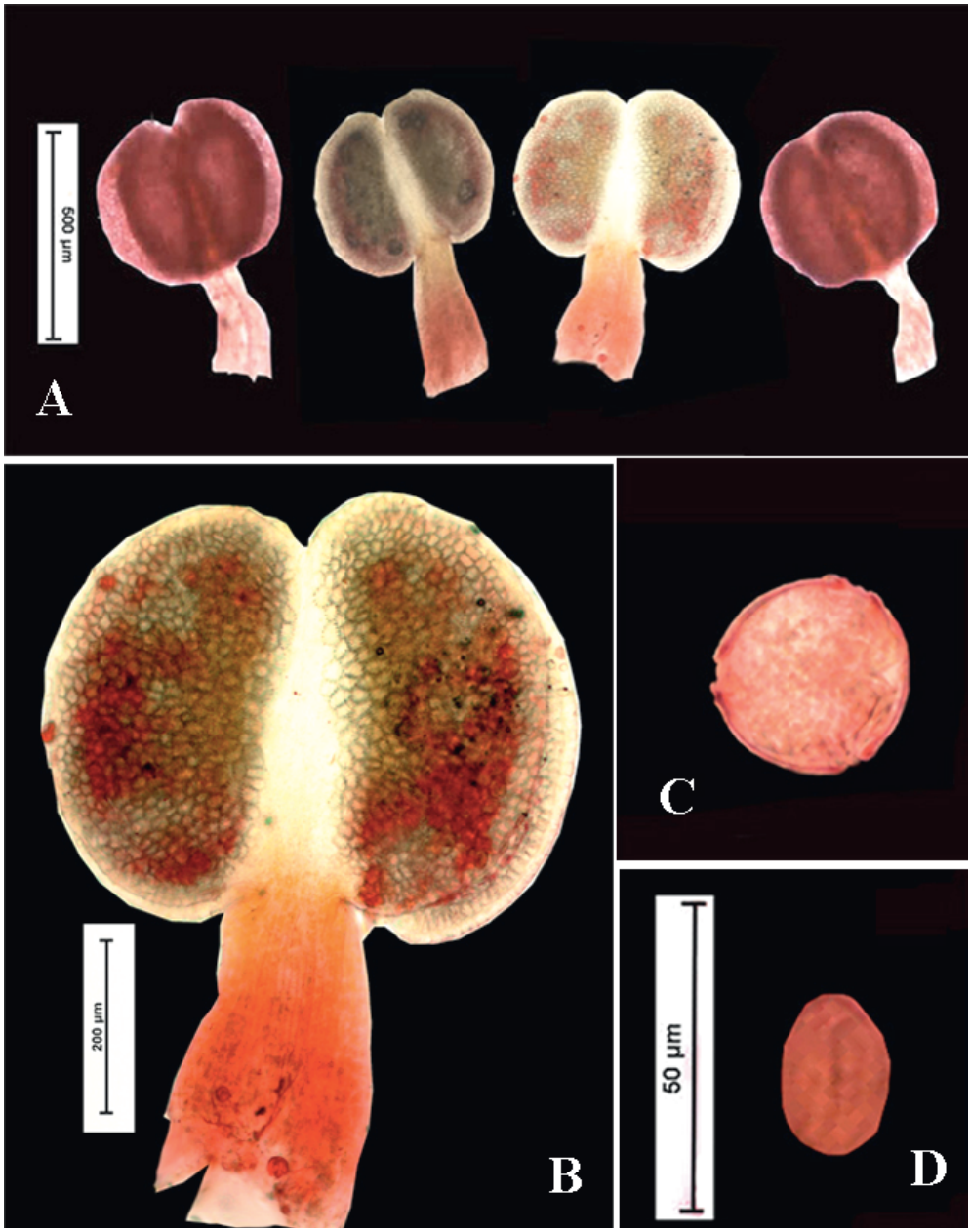


PLATE 20: A- B. Individual stamen, 100x; C-D. Pollen, 400x.

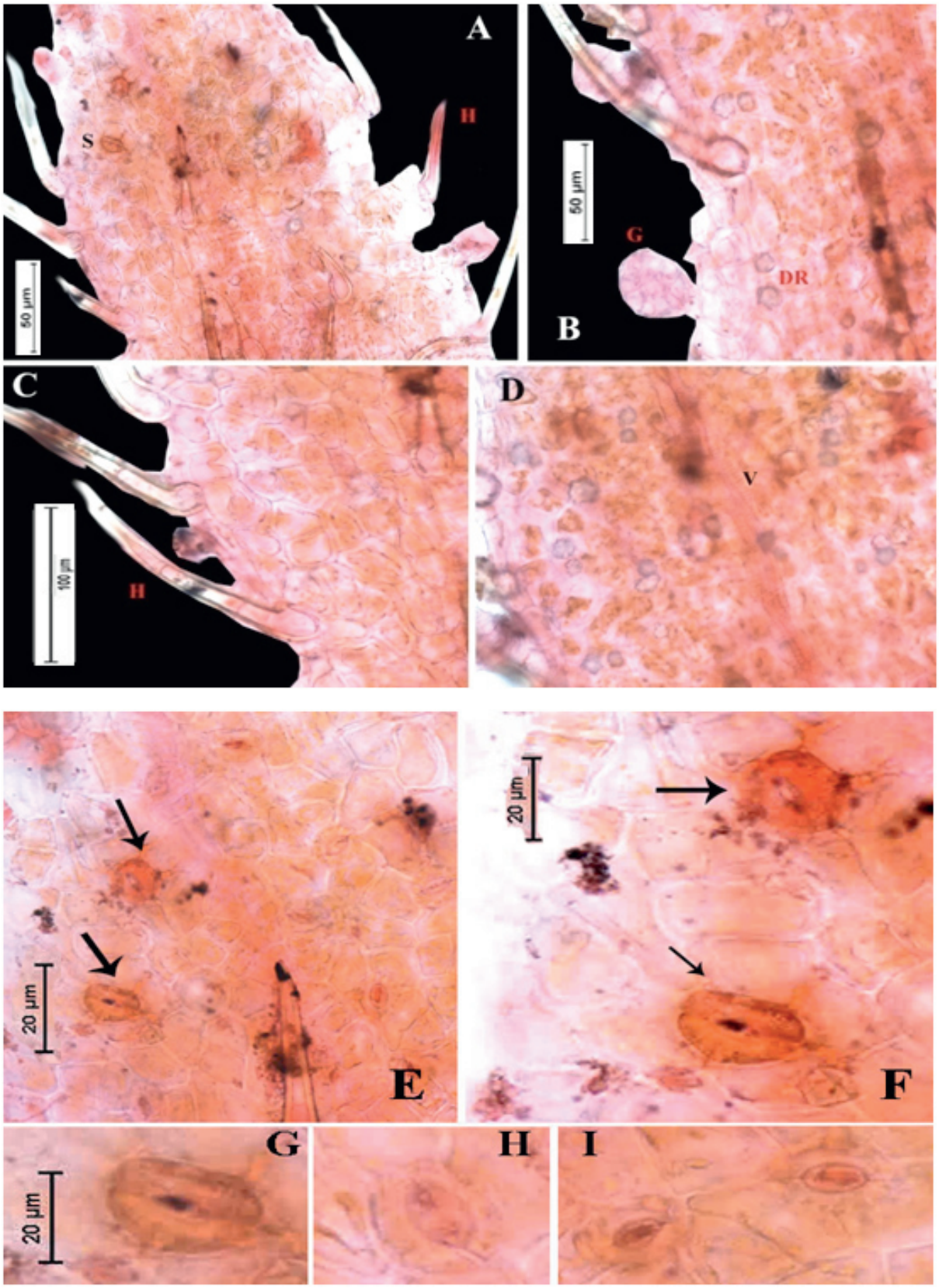


PLATE 21: A-I. Micromorphology and anatomy of sepal, Epidermal Hair (H), Glands (G), Scalariformed vessels (V) and stomata (arrowheads), 400x

Petals exhibit uni and multicellular non glandular trichomes and capitate and stipitate glandular trichomes (Pl.22 C-D), functional paracytic stomata present with well developed guard cells (Pl.22E-H).

Multicellular non glandular trichomes and capitate trichomes are present throughout the epidermis of consolidated intrastaminal floral disc (Pl.24A).

4.1.3.1.3 Anatomy of male flower:

Sepal:

Cells quadrangular or polygonal, vascular traces prominent (Pl.22A-D). Epidermis parenchymatous, uniseriate. Intercellular space absent.Cell wall thick. Cells polygonal. Single trace of scalariform xylem present through the centre. Supporting tissue absent. Secretory cavities are also present. Druses of secondary metabolites are scattered throughout the sepal.

Petal:

Cells are more or less quadrangular, triangular and sometimes polygonal vascular traces prominent. Epidermis is parenchymatous, uniseriate (Pl.22A). Intercellular space absent. Cell wall thin and delicate. Single trace of scalariform xylem present through the centre (Pl.22B). Supporting tissue absent. Secretory cells present. Druses of secondary metabolites are scattered throughout the petal.

Stamen:

Anther is tetrasporangiate, but fused to result in two large locules when get matured (Pl. 23A). Epidermis uniseriate, made up of small rectangular, flattened cells with straight walls (Pl23.C-D). Cuticle smooth and thin. Endothecium thick, uniseriate and cells radially elongated. Characteristics depositions of fibrous bands of lignocellulosic secondary thickenings arising from the inner tangential walls are present (Pl.23D). Epidermis and endothecium constitutively about 40 µm thick.

Middle layers and tapetum were crushed and flattened. Stomata absent. There were no secretory cavities in the anther. Mature pollens loosely arranged within anther sac. Connective and short filament is composed of elongated parenchyma cells (Pl.23B).

Consolidated intrastaminal floral disc in male flower:

L.S. of the floral disc exhibit a globose structure (Pl.24C). Epidermis uniseriate, parenchymatous, pubescent (Pl.24B). Inner mass is composed of unspecialized, round, binucleate parenchymatous cells (Pl. 23G). Functional paracytic stomata present (Pl. 24F). At the centre of the structure there is a cone shaped cliff containing blackish alkaloid deposits (Pl. 25A). At the centre of each lobe there is a distinct locule containing blackish intercellular depositions (Pl.24D-E). Druses of alkaloids are present. Vascularization reduced. Ends of scalariformed trachieds are prominent (Pl.25D-E). Successive T. S. of the disc at regular interval reveals unbranched traces of vascular cylinder radiates at the base of the consolidated disc (Pl.25F-I). Remnants of traces ended blindly in the cortex of the receptacle below the cliff.

4.1.3.2 Female flower:

Female inflorescence is cymose, 16-40 cm long,densely covered with hairs, average number of flowers per inflorescence is 35.2 ± 11.7 with a minimum 8 and maximum 55 (Pl.26 A-B). Ramification in inflorescence is not frequent like male, sometimes observed up to 2°. Flower born acropetaly on the rachis. Secondary and tertiary lateral flowers are born basepetaly, but mostly do not persist till maturity.

4.1.3.2.1 Macro morphology:

A mature female flower is inconspicuous with no odour or scent, perfect, tetramerous, dichlamydeous, heterochlamydeous, biseriate, aposepalous,

apopetalous, composed of eight inconspicuous tepals arranged in two whorls, flower actinomorphic, hypogynous (Pl.26C). A mature flower is 2 to 3mm long with 3 to 4mm pedicel. Pistil apocarpus, consist of 4 carpels, ovary unilocular, hypogynous, style short curved, extended at the right angle to the ovary, stigma fleshy (Pl.26D-E). Stigma dries up immediately after pollination. All the floral whorls are free. Outer tepals are green, triangular, 900-1500µm in length and 600-700µm in breadth (Pl.26F-G).

Inner tepals are also green, triangular, 1000-2000µm long and 600-750µm broad. Disk green, flattish, shallow. Carpals are arranged over the disk.Tepals absent in mature flower.

Gynoecium consists of four green, hairy short carpels. Each carpel is 1-3 mm long, consists of a large ovary, short curvy style and a stigmatic crest extended along the style (Pl.27A). Pollen is received on stigmatic crest, consisting of papillose surface. Elongated stigma is the adaptation for wind pollination in advanced forms. Each ovary is unilocular. Stigmatic crest dries up immediately after pollination.

4.1.3.2.2 Micro morphology:

Tepals are densely covered with hairs (Pl.26H-M). Uni and multi cellular non glandular trichomes present are 150-500µm long. Capitate trichomes are 50–55µm long and 30-35µm in wide. Filiform trichomes are about 200µm long. Peltate glandular trichomes also present. They are concentrated towards the mid vein of tepal. Different shaped, stalked or sessile papillae are also present (20µm-60µm).

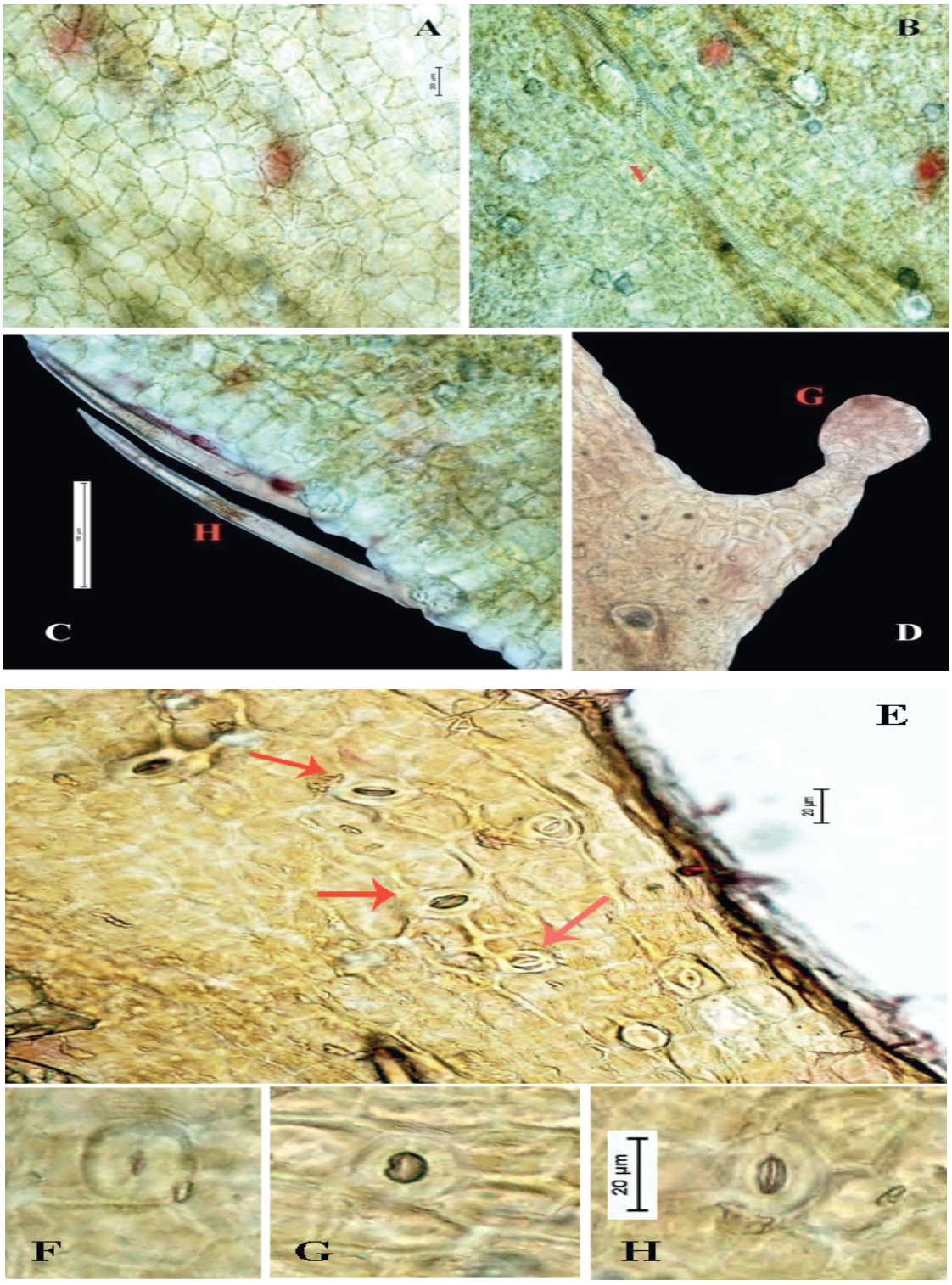


PLATE 22: Micromorphology and anatomy of petal. A. Epidermal cells, 400x; B. Vascular traces (V), 400x; C. Hairs (H), 400x; D. Gland (G), 400x; E-H. Stomata (arrowheads), 400x.

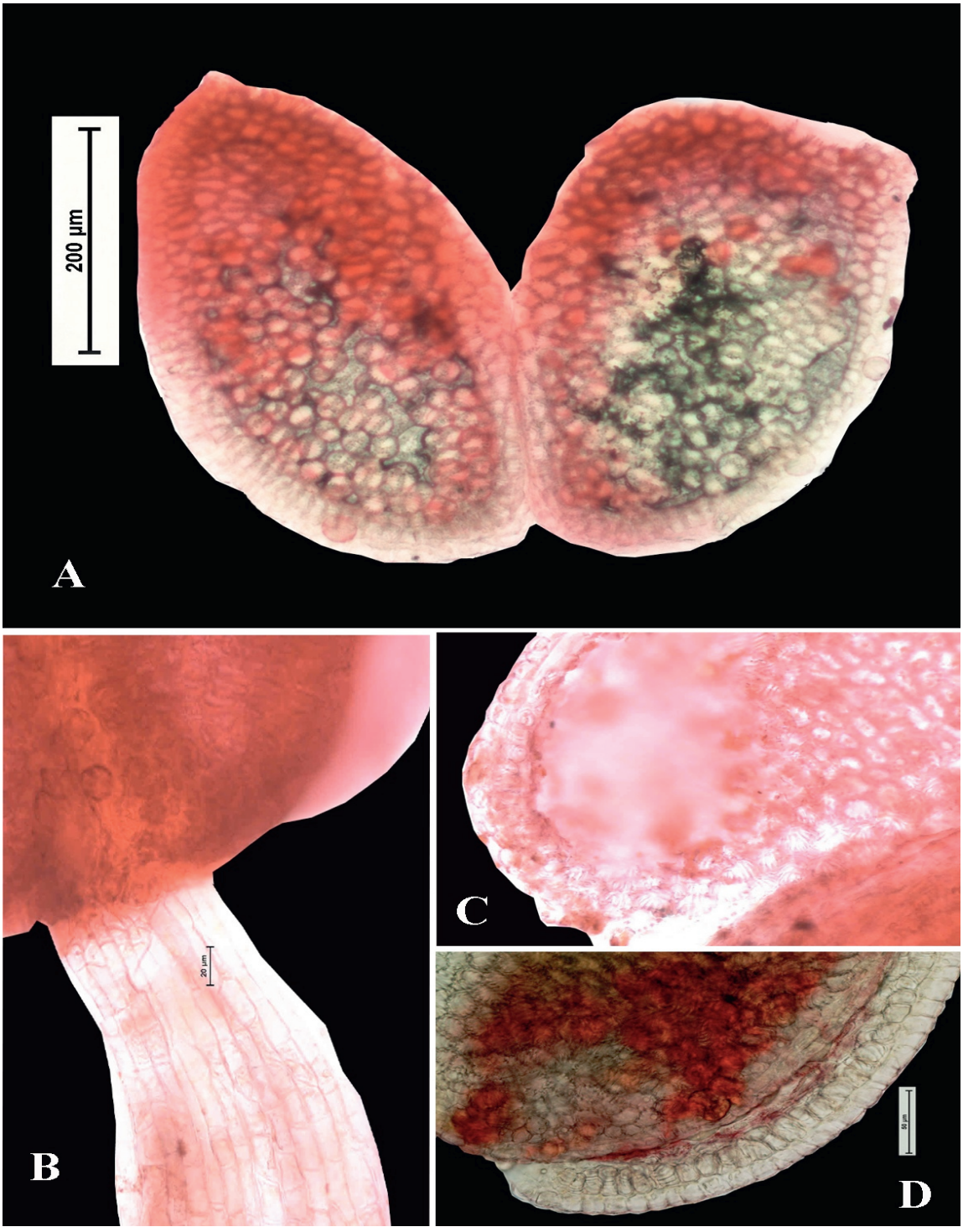


PLATE 23: Stamen.A.TS of anther lobe, 100x; B. LS of filament, 100x; C-D. Sporogenous tissue (LM), 400x.

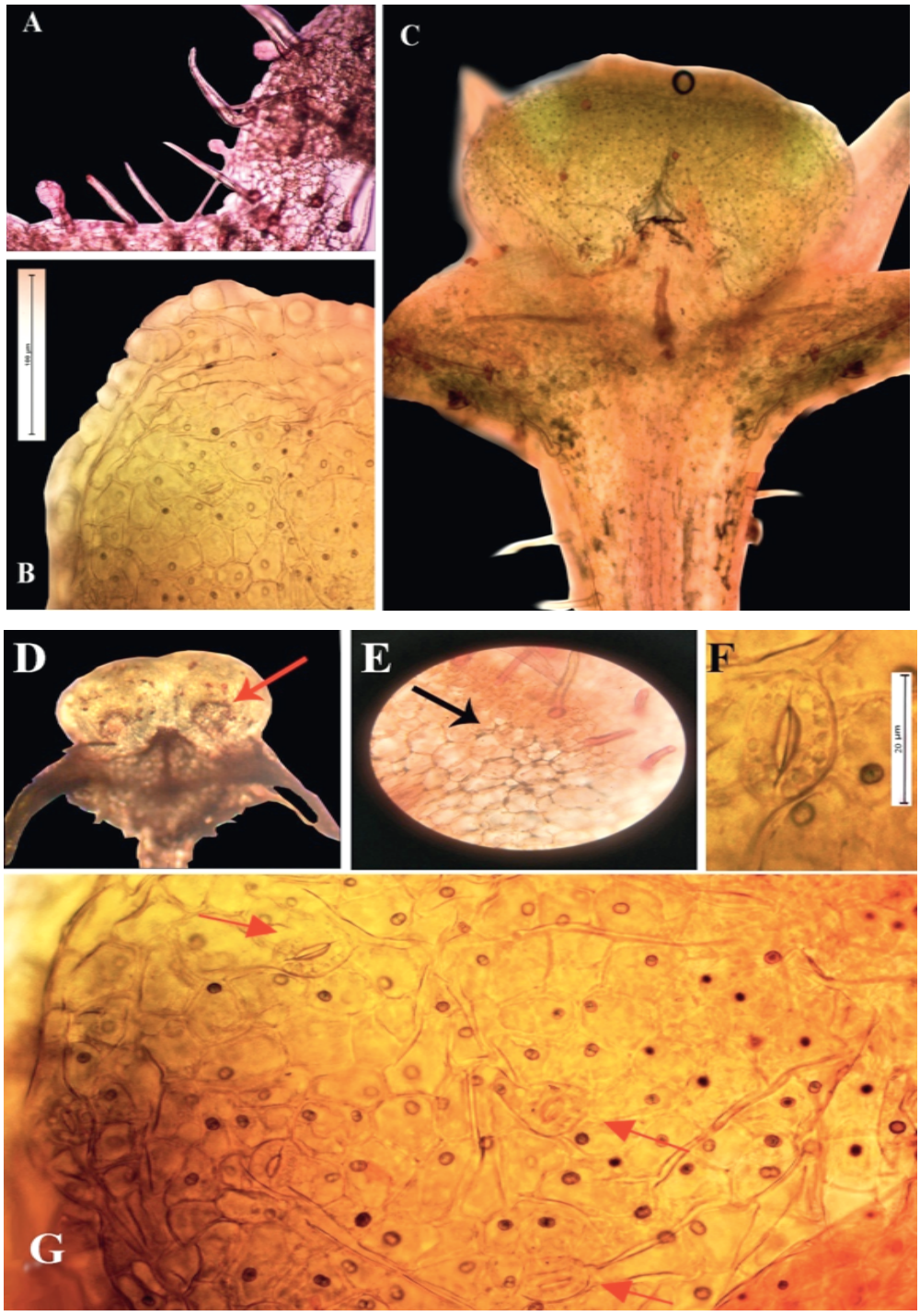


PLATE 24: Male floral disc. A. Glandular and non glandular epidermal hairs, 100x; B. L.S. of uniseriate epidermis, 400x; C. L.S. of floral disc, 400x; D-E. L.S. inner locule containing intercellular deposits (arrowheads), 400x; F. Stomata present in the epidermis of floral disc, 400x; G. T.S. Binuclelate cells (arrowheads), 400x.

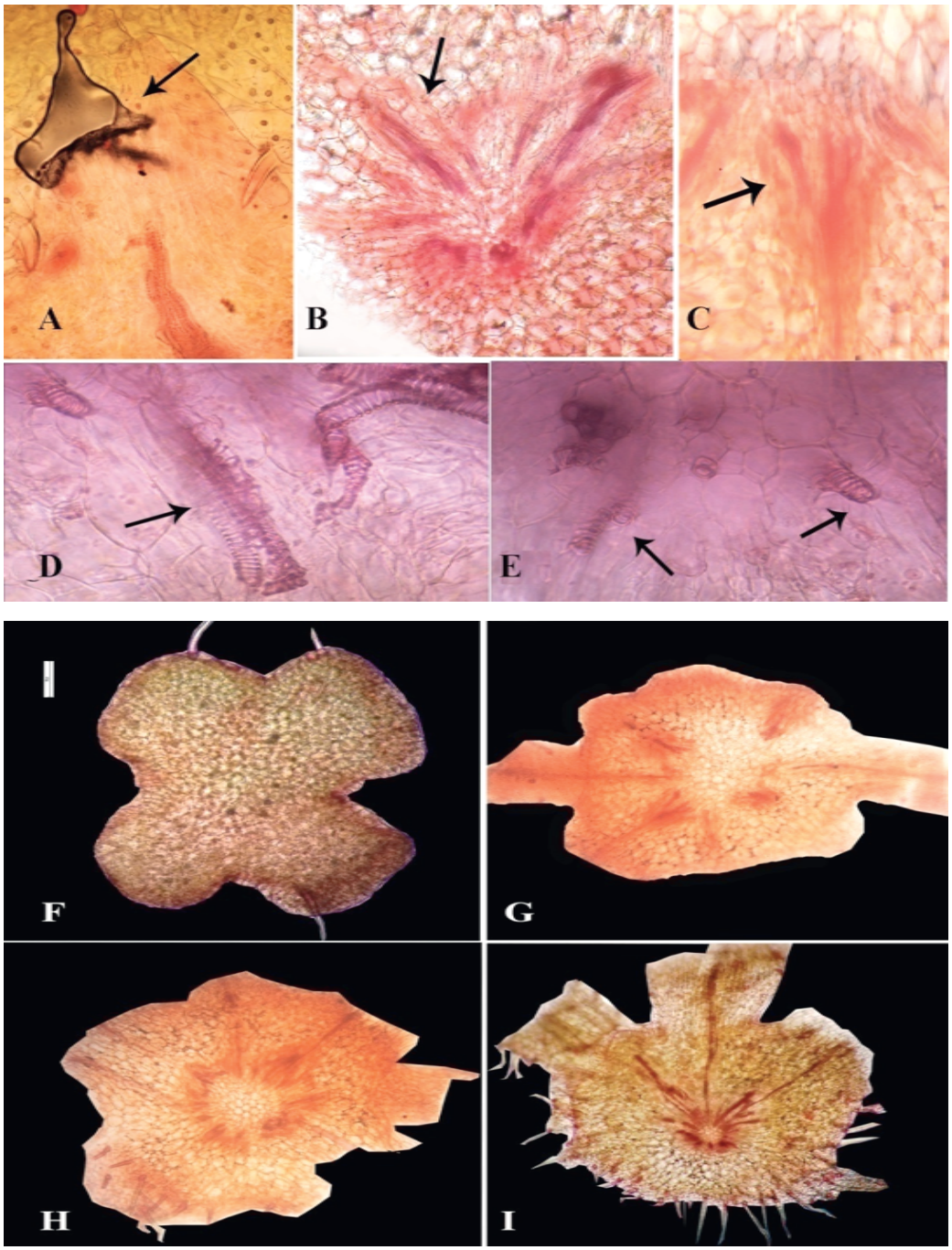


PLATE 25: Anatomical male floral disc. A. The cone shaped cliff at the centre of the floral disc, 400x; B-C. Vascular elements, and their branching present, 400x; D-E. Scalariformed trachieds, 400x; F-I. Successive TS of the disc from top to bottom at regular interval exhibiting branching of vascular traces, 400x.

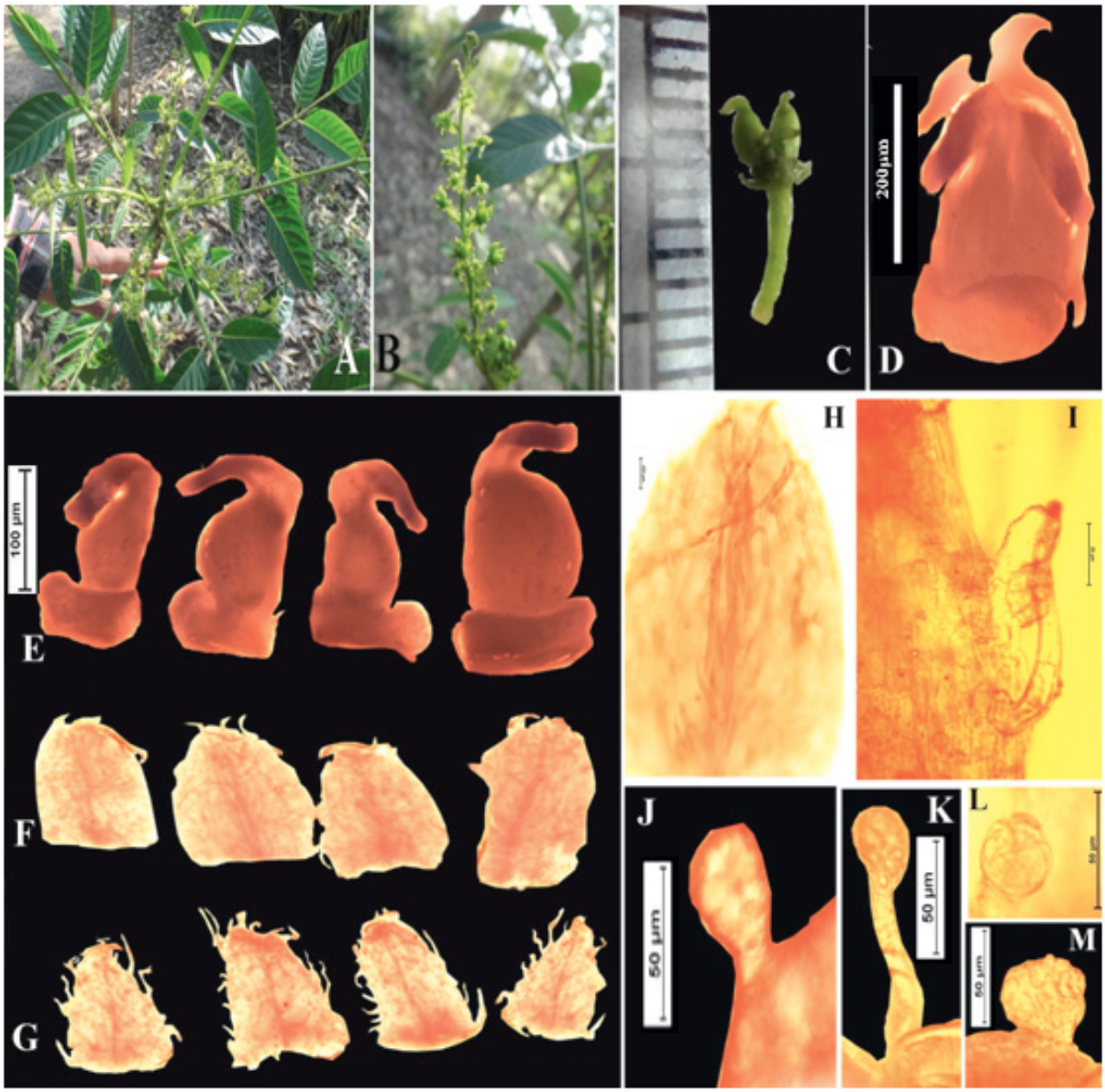


PLATE 26: Female inflorescence and flower, A-B. Inflorecence, C. Complete flower, D-E. Carpels, F-G. Tepals, H-M. different types of glandular and non glandular hairs present in tepals.

4.1.3.2.3 Anatomy of female flower:

Tepal:

Tepals inconspicuous, ephemeral. Epidermis uniseriate composed of unspecialized tabloid, spherical or elongated parenchyma. Stomata absent. Vascular tissue totally diminished (Pl.26H).

Table 6: Quantitative features of flower of *B. mollis*

Floral part	Male		Female	
	Range	Average ± SD	Range	Average ± SD
Pedicel length	2100 µm -2000 µm	2040 µm ± 54.7	4000µm-4500µm	4506µm± 8.9
Sepal length	852 µm - 775 µm	820 µm ± 48.1	1400µm-1550 µm	1480µm ± 57
Sepal breadth	310 µm- 405 µm	389.4µm ± 9.44 µm	700 µm -770 µm	700µm± 44.7
Petal length	1470 µm- 1520µm	1494 µm ± 20.7	1350µm-1550 µm	1491µm±78.6
Petal breadth	320 µm- 330 µm	331 µm ± 11.4	1100µm-1210 µm	1158.33 µm ± 45.2
Stamen				
Filament length	370 µm- 390 µm	376 µm ± 8.9		
Anther length	500 µm - 520 µm	508 µm ± 8.3		
Anther breadth	310 µm- 320 µm	314 µm ± 5.4		
Pollen length	24 µm - 25 µm	24.4 µm ± 0.54		
Pollen diameter	30 µm -32 µm	30.6 µm ± 0.89		
Carpel				
Ovary length			850µm-1550µm	1290µm±277.03
Ovary breadth			750µm- 850 µm	796µm ± 36.4
Style length			950µm-1010µm	988µm ± 23.8
Stigma length			190µm-210 µm	198µm ± 8.3

Gynoecium:

Gynoecium consists of four carpels. Ovary is unilocular, superior, uniovular (Pl.27A). The ovary wall is composed of 2 to 3 layers unspecialized irregular shaped parenchymatous cells. Cuticle is smooth, thin. Epidermis parenchymatous, covered with unicellular non glandular trichomes. Ovule is solitary, orthotrpous, bitagmic, placentation marginal. Ovule also crassinucelate having massive cell layers between nucellar epidermis and megaspore. Both the integuments have distinct cucticles (Pl.27B). Nucellus massive. Style is solid, massive and long. Vascular traces reduced with very less branching extended only to the base of the style up to micropyle. But do not enter into the style. Nectaries occur as dots in the apex of the ovule *i.e.* at the base of the style (Pl.27C). Secretory cavities occur in the parenchymatic ovarian mesophyll.

4.1.3.3 Fruit:

Morphology:

The fruit of *B. mollis* born in dangling axillary bunches. The fruits produced in groups of 1- 6 on each bunch (Pl.28A-D). The fleshy drupe fruits were ovoid in shape, monocarp *i.e.* druparium. 7-12 mm long 6-8 mm broad. Mostly 1 or 2 fruits develop from a single flower. Pedicel 3-4 mm. Mature fruit is globose, slightly tapering at both the ends, green when young and became orange, red to blackish-red when ripe. It consisted of a thin shining epicarp when young, fleshy mesocarp, hard and stony endorcarp. The seed consisted of two large expanded cotyledons.Exocarp becomes scarcely reticulated and red brown and dry when mature. Fruits of the same bunch get ripe simultaneously. Thus, the lack of variation in the time of fruit maturity within the bunch serves to maximize the competition for substrate for successful seed germination at the forest floor.

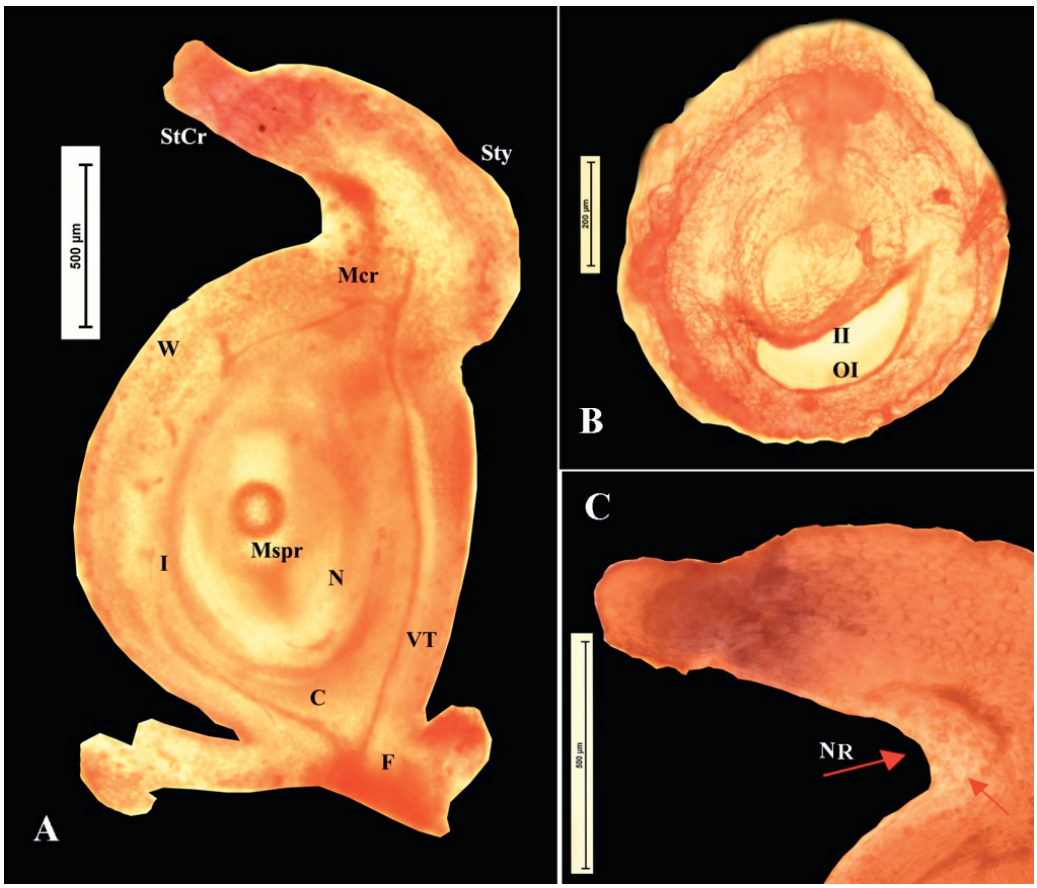
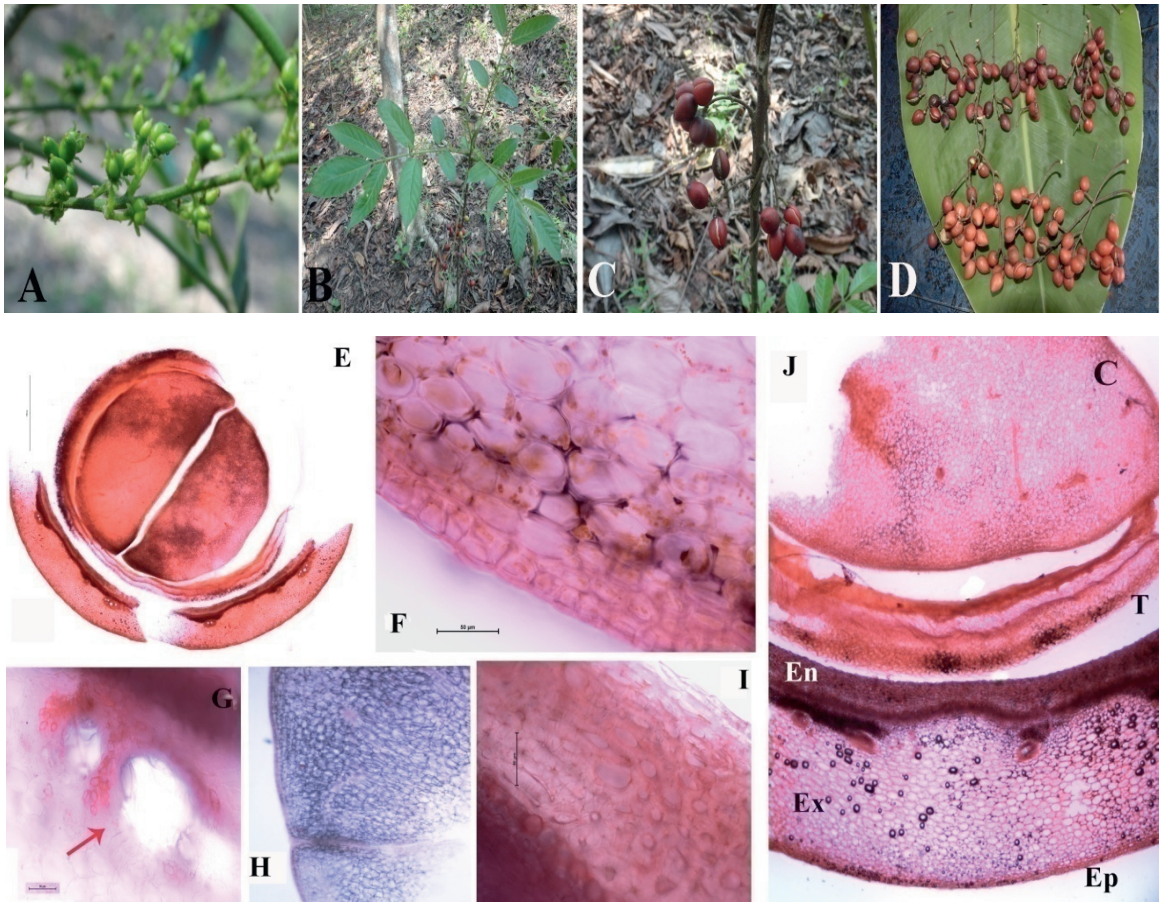


PLATE 27: Anatomical of carpel. A. LS of carpel, 100x, B. TS of Carpel, 100x, C. Stigmatic crest, 400x.Stigmatic crest (StrCr), style (Sty), Micropyle (Mcr), ovary wall (W), Nucellus (N), Megasprocyte (Mspr), Chalaza (C), Funiculus (F), Vascular trace (VT), Integument (I), Inner integument (II), Outerintegument (OI), Nectary (NR).



PLACE 28: Morphological and anatomical of fruit. A. Immature fruit B-D. Ripe fruit. E- J. TS. of fruit. F. Uniseriate epidermis and intercellular space filled with secondary metabolites, 400x; G. Large secretory canal surrounded by vascular trace in pericarp, 400x; H. Cells of cotyledons heavily deposited with secondary metabolites, 400x; I. Dry and raticulate endocarp, 400x; J. TS. of fruit, 400x. C-Cotyledon, T- Testa, En-endocarp, Ex-Exocarp, Ep- Epidermis

Anatomy:

T.S. of fruit is circular in outline (Pl.28E,J). Epidermis composed of uniseriate polygonal parenchymatous cells with thin cuticle (Pl.28F), followed by fleshy succulent pericarp enclosing the hard stone layer. Cotyledons are unequal. Exocarp many layered, fleshy, cells are round, polygonal, thin walled parenchyma. Endocarp dry fleshy crushed reticulate, undulating outside and entire inside (Pl. 28 I). There are 8 sets of secretory canals present outside the undulating endocarp, arranged alternately in one and two pairs (Pl.28G). Each canal is surrounded by vascular traces. Testa thin, dry, membranaceous, many layered, fibrous. Cotyledons planoconvex, heavily deposited with black intercellular deposits (Pl.28H). Endosperm absent.

4.1.3.4 Anatomy of rachis:

T.S. of rachis circular in outline (Pl.29A). Vascular bundles arrange in a ring enclosing the large parenchymatous pith (Pl.29E). Multicellular non glandular trichomes present on epidermis (Pl.29B-C). Epidermis is uniseriate, composed of barrel shaped parenchyma cells followed by chlorenchymatous hypodermis (Pl.29E). Next to it lays collenchymatous cortex. Cambium 9-10 celled thick, cuts off patches of phloem outside and ring of xylem inside (Pl.29E).Xylem consists of trachieds and large vessels. Secretory canals present (Pl.29D,F). Druses of alkaloids and oil drops present in the pith (Pl.29G).

4.2 Floral biology:

4.2.1 Phenology of flowering:

The male and the female plants in *B. mollis* exhibit two distinct trends in their phenology. In male plants flowering initiated in the first week of February and reaches its blooming peak in the first week of March, which lasted till the last week

of April (Fig. 4.2.1, Fig. 4.2.3). Although from first week of May flowering in male plants started to decline but it continues till December. The male plants remain in vegetative stage or without flowering only in the month of January and thus the male plants remain in flowering stage for about eleven months.

On the other hand the flowering of female plants of *B. mollis* confined only for a period of 15-25 days. In female plants flowering initiated from the first week of March and the flowers get matured by the second week. Flowering reaches its maximum by the third and fourth weeks of March and then started a declining trend till the first week of April when fruit setting started (Fig. 4.2.2, Fig. 4.2.3).The fruits require a long period for maturity in the month of February next year*i.e.* a period of about eleven months.So in female plants blooming peak is only two to three weeks. Thus the flowering period of female plants lasted for about three weeks with blooming peak of only two to three weeks.

During blooming peak the male plants have maximum of 85-95 flowers per inflorescence and female plants have maximum of 35 to 45 flowers per inflorescence.

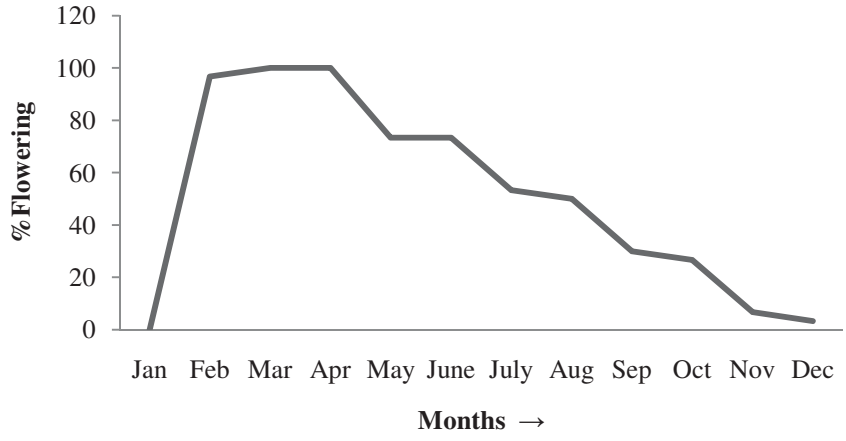


Fig. 4.2.1: Blooming peak in male plants

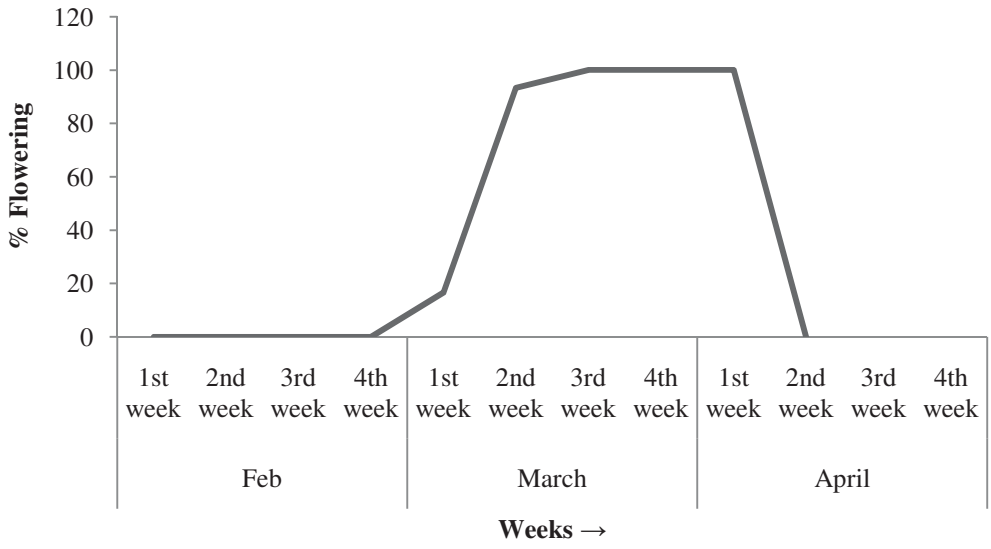


Fig. 4.2.2: Blooming peak in female flower

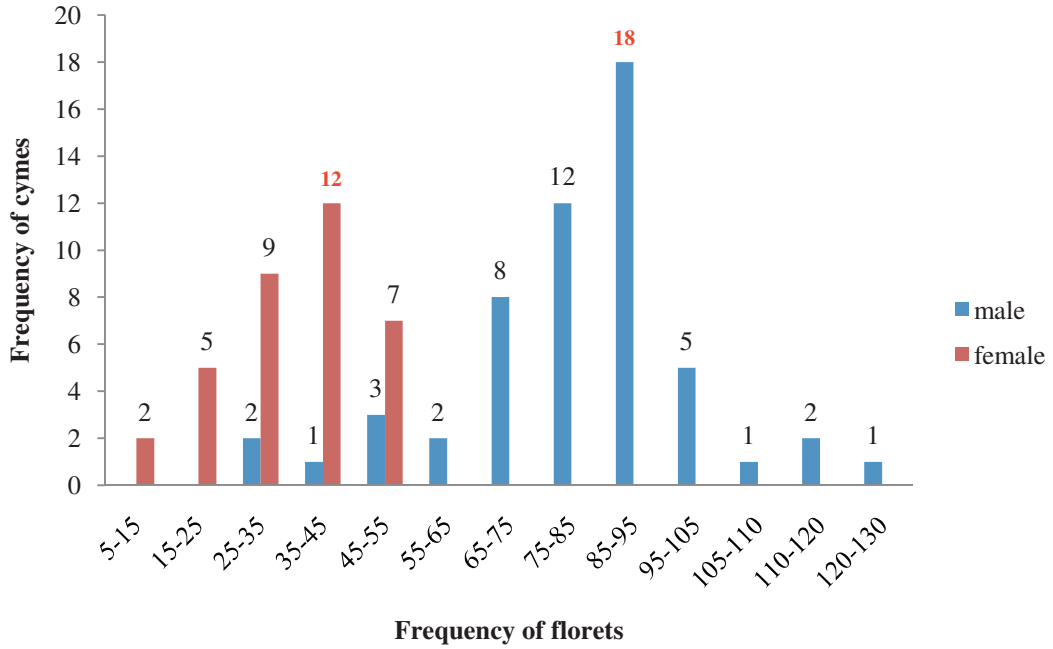


Fig. 4.2.3: Frequency of male and female flowers per cyme during Blooming peak

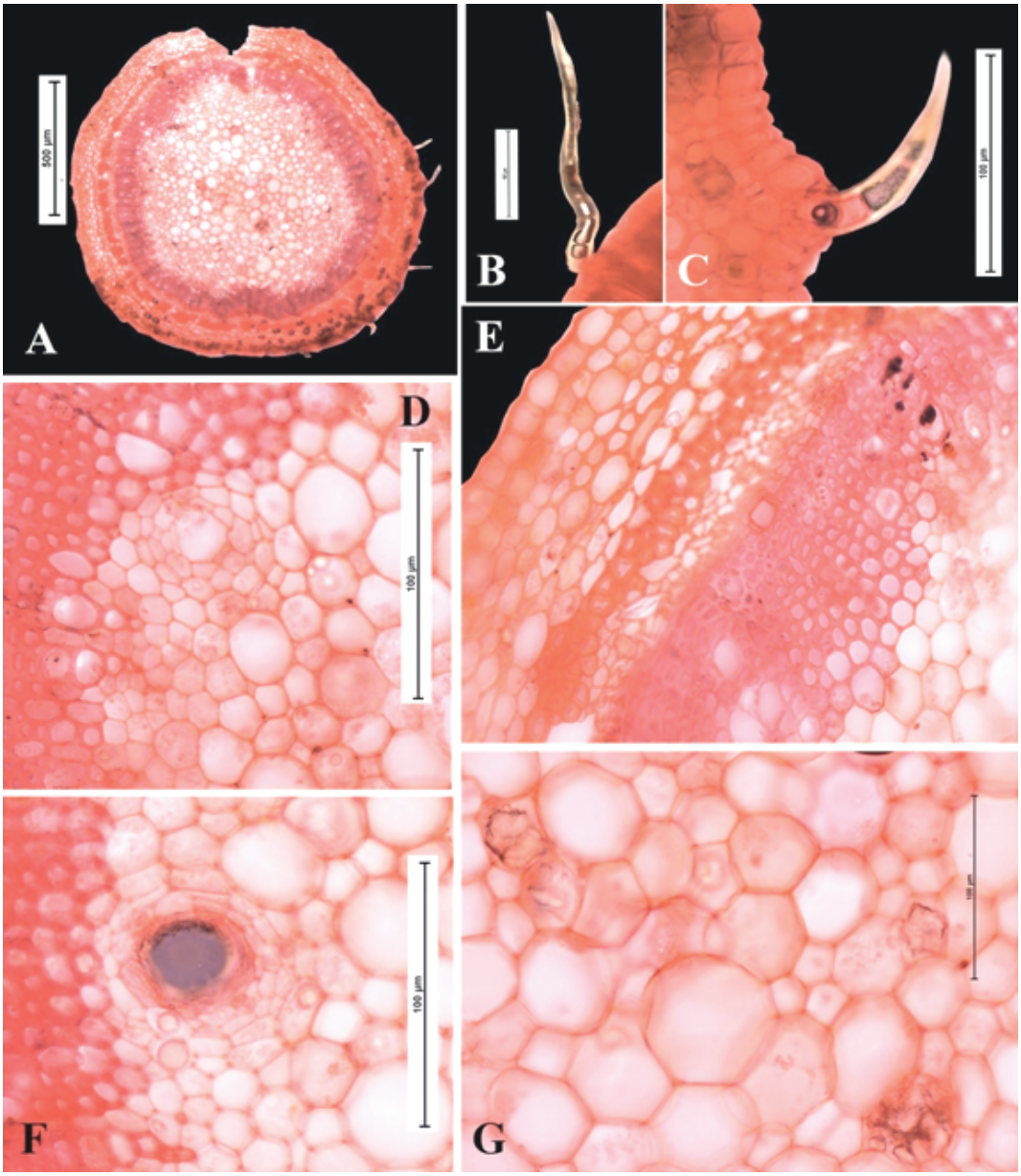


PLATE 29: Anatomy of rachis. B.A-G. TS. of Rachis. A. TS. of rachis, 40x; B-C. Glandular hair, 400x; D. Secretory canal in the periphery of pith, 400x; E. Vascular cylinder of rachis, 400x; F. Secretory canal filled with black secretion, 400x; Druses of calcium oxalate and oil drops in pith, 400x.

4.2.2 Anthesis:

Flowering period:

Opening of flowers in the cyme occur acropetaly. In male the two basal flowers opens basepetaly after the anthesis of the terminal flower. Anthesis is both diurenal and nocturnal (Fig. 5.2.4). Anthesis starts in the early morning from 4:30 AM and lasts till 9 AM. Then it stopped and again occurs in the afternoon from 4 PM till 6:30 PM for a short period. The life span of a single flower is 10- 15 days, after that it dries up. Anthesis of a single floret is long, lasted for 1-1.5 days in male and 2-5 days in female.

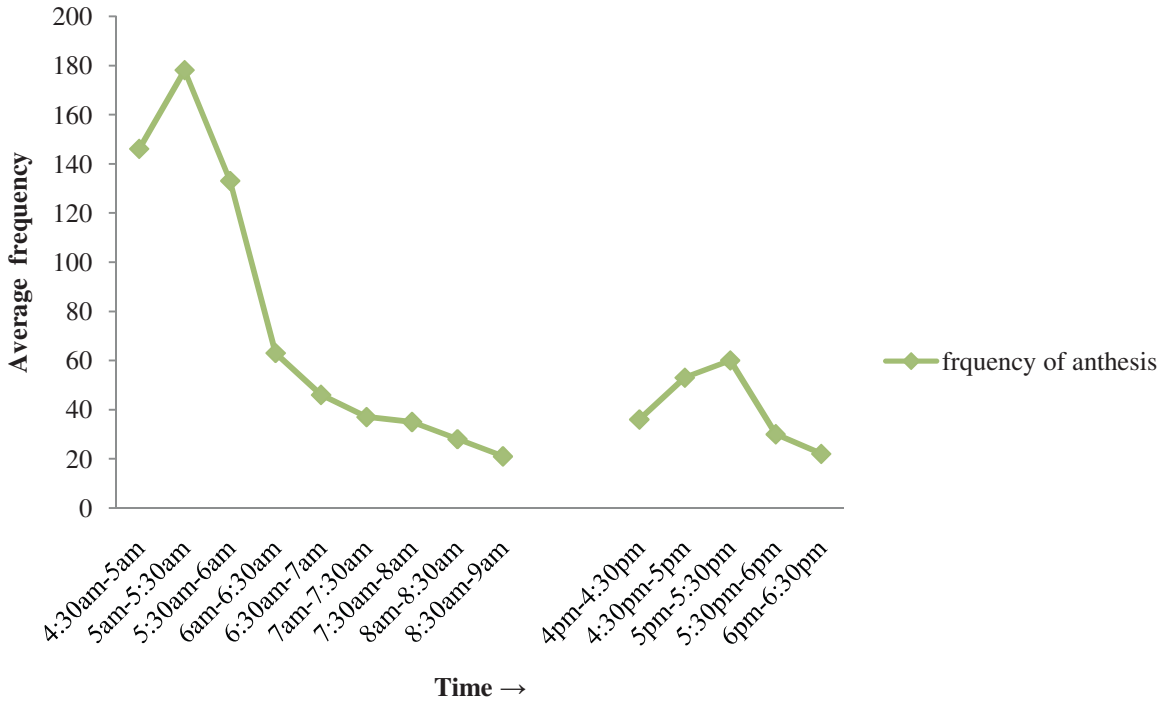


Fig. 4.2.4: Pattern and average frequency of anthesis

4.2.3 Flowering dynamics of reproductive whorls:

4.2.3.1 Stages of stamen development*:*

Flowering dynamic of stamen development can be divided into nine broad stages (Table 7, Pl. 30).

Table 7: Developmental stages of anther in *B.mollis*

Stage	Developmental stage	Developmental activity
1	Anther initiation	Primordial initiated
2	Start of anther differentiation	Sepals start opening and primordia develops four furrows to form four heads.
3	Development of anther lobe	Each head developed in to four separate lobes, floral disc primordia initiated.
4	Initiation of filament	Initiation of filament begins, growth of floral disc continues and vessel ends become prominent.
5	Initiation of connective formation	Filament growth continues and anther lobes are more prominent with development of the connective.
6	Tissue differentiation begins within anther	Tissue inside anther lobes differentiated into outer layer and inner tissue mass,
7	Formation of sporogenous tissue begins	Sporogenous tissue differentiated within anther lobe
8	Pollen formation begins	Pedicel elongation continues, connective becomes fleshy and prominent, anther lobes well developed, sporogenous tissue differentiated into pollens, filaments continues elongation and floral disc becomes distinct.
9	Maturation of anther	Anthers fully matured with prominent lobes, pollens, connectives and filament.

Development and differentiation of anther in *B. mollis* can be considered to complete in nine stages (Table 7; Fig. Pl.30). Floral organogenesis begins with the

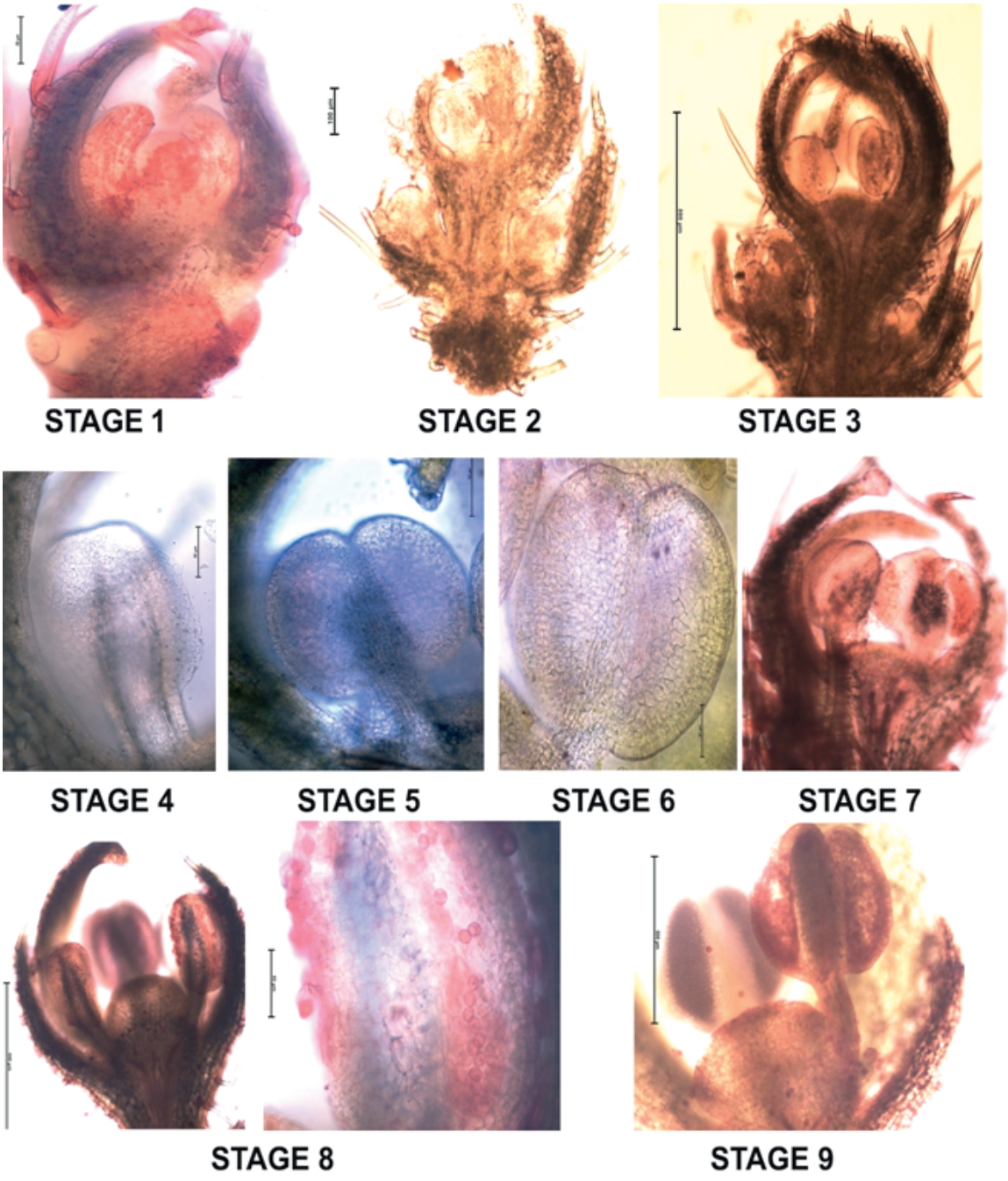


PLATE 30: Stages of development of anther

initiation of four primordia of sepals followed by four primordia of petals. The broad, thick, dome shaped apex of the male flowers remain enclosed within the calyx and corolla. Development of stamen starts with the differentiation of anther primordia (Pl.30 Stage 1). As the anther primordia continue its development, the floral disc also differentiated to form four lobes (Pl.30 Stage 2-3). In subsequent development the entire apical meristem is involved in formation of the floral disc. This is followed by the differentiation and development of the filament and connective (Pl. 30 Stage 4-5). Subsequently, the tissue differentiation begins within the anther lobe resulting into the formation of prominent outer and inner walls enclosing the sporogenous tissue (Pl.30 Stage 6-7). Sporogenous tissue later differentiated to form pollens (Pl.30 Stage 8). Maturation of stamen still continues until the pollens get mature (Pl.30 Stage 9).

4.2.3.2 Stages of development of carpel*:*

Table 8: Developmental stages of carpel in *B.mollis*

Stage	Developmental stage	Developmental activity
1	Carpel initiation	Primordia emerge and remain attached laterally, enclosed in sepals.
2	Style initiation	Style formation initiate by formation of separate bubble in between the four carpel primordia.
3	Differentiation of ovary	Sepals start opening, separation bubble starts widening. Ovary walls start differentiation inside the primordia.
4	Stigmatic crest formation	Carpels get separated, style bend at an angle of 60° to the ovary, style become fleshy and turgid, stigmatic crest starts developing.
5	Ovule formation	Style further bend to form 90° angle on the ovary, become more turgid and stout, ovule develops with prominent integuments and nucellus tissue, calyx and corolla fully open.
6	Changes after pollination	After pollination, stigmatic crest dries up, style become flaccid, ovaries enlarged.

Flowering dynamic of carpel can be divided into five distinct stages (Pl.31). Carpel initiation begins with emergence of four primordia at the apical meristem of the female floral bud (Pl. 31 Stage 1). They remain enclosed in sepal and petal. This is followed by the initiation of erect style from the apex of the primordia (Pl. 31 Stage 2). At this stage a separation bubble forms in between the four adjacent carpel primordia. Sepals start opening. Ovary walls start differentiation at the distal end of the carpel primordia (Pl. 31 Stage 3). The separation bubble enlarges resulting in to the separation of the carpels. Subsequently, the carpels get completely separated (Pl.31 Stage 4), style elongates and bend at 60° to the ovary, becomes fleshy and turgid. Stigmatic crest starts developing in this stage. Calyx and corolla opens completely exposing the carpels with developed ovules (Pl. 31 Stage 5). Style further bends to form a 90° angle on the ovary. Ovary differentiated in to integuments and nucellus tissue.

The changes that take place after pollination is considered as a different stage characterized by the dried up stigmatic crest, flaccid style and enlarged ovary (Pl.31 Stage 6).

4.2.4 Pollination:

Pollination syndrome of *Brucea mollis* is found to be ambophily *i.e.* pollination by both wind and insect. Wind is the primary means for pollination in the plant. Morphological traits indicating anemophily includes inconspicuous petal in male flower and absent of showy petal in female flower, exposed anthers and stigma, and elongated exposed hanging cymes.

But the plant is very often visited by ants, and they are also found to play a crucial role in pollination. Various traits favorable for insect pollination are also present in *B. mollis*, such as inflorescences with many closely ranked and short

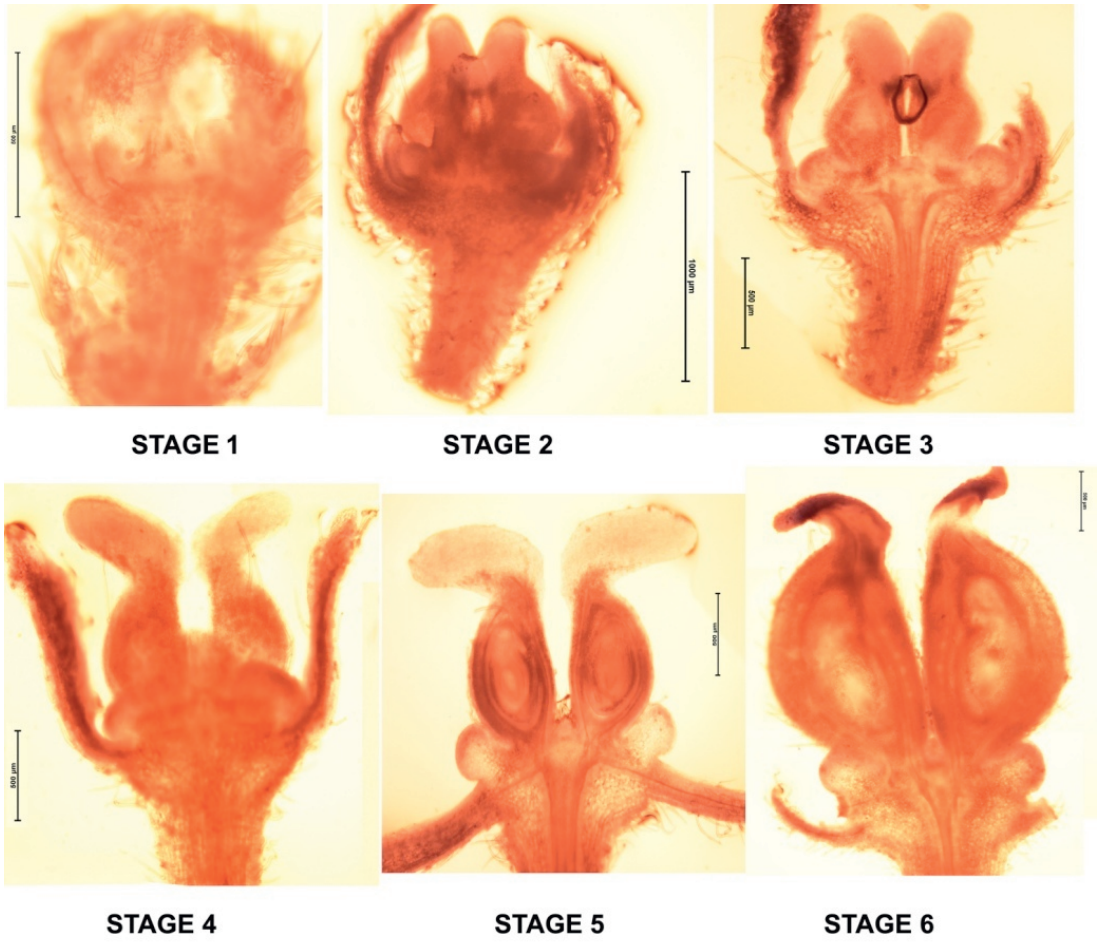


PLATE 31: Stages of development of carpel

pedicel flowers, long anthesis of a single flower and concentrated blooming peak in a single month, petals having several glands and sticky pollens. Therefore, the pollination in *B. mollis* is transitional between anemophily and entomophily.

4.2.5 Fertilization:

Fertilization in *B. mollis* is porogamic *i.e.* fertilized by micropyle end (Pl.32A). Micropylar end get closed just after fertilization (Pl.32D). Fleshy stigmatic crest also get dried (Pl.32B-C). After fertilization pericarp starts developing and ovary metamorphosed into fruit. Fertilized ovary is heavily deposited with black cellular deposits.

4.2.6 Apomixis:

In *B. mollis* apomixis is also observed. Parthenocarpic fruit was recorded in male plants during the month of March and April (Pl.32E-F). These fruits are smaller than normal. They last only for 10-15 days and then got dried and fell off. Parthenocarpic fruits are developed from the sterile ovarian tissue of the floral disc of male flowers. Stem sucker formation is also not uncommon in *B. mollis* (Pl.32G).

4.3 Anatomy of vegetative parts:

4.3.1 Anatomy of Root:

T. S. of root is circular in outline (Pl.33A). Secondary growth resulted in the formation of bark. Periderm is 10-20 layer, phellem layer is dark coloured, ruptured, lenticels prominent (Pl.33C). Cortex composed of several layers of parenenchyma, traversed by loose xylem fibers, heavily deposited with dark brown or black cellular deposition. Druses and microsphenoidal calcium oxalate crystals with granules of other alkaloids are distinct within the cells (Pl.33B, D). Cortex is followed by 2-3 layer cambium which cutting off xylem rays inside forming the vascular cylinder (Pl.33D). Pith is obliterated with vascular tissue. Cambium composed of 5-6 layers

producing scattered phloem outside and large vascular cylinder inside. Vascular cylinder is traversed by medullary rays. Trachieds are oval or rectangular and vessels are circular in T.S. Medullary rays are heavily deposited with microsphenoidal granules.

4.3.2 Anatomy of Stem:

T.S. of stem is circular (Pl.34A). Multicellular simple trichomes present on the epidermis which are 100-200 µm long and contain bright orange deposition. Epidermis gets ruptured due to bark formation (Pl.34B). Periderm uneven in thickness, few to many layered, cells square or rectangular, occasionally get detached, heavily deposited with bright coloured alkaloids. Lenticels are frequent (Pl.34D). Cortex is of uniform thickness, comprises of several outer layers of oval chlorenchyma cells, followed by layers of round to oval collenchymatous hypodermis. Endodermis and pericycle are not distinguishable. Cambium composed of 8-10 layered rectangular cells producing patches of phloem outside and a ring of xylem inside and these are traversed by elongated radiating medullary rays. Vascular bundles are conjoint collateral open. Trachieds are mostly hexagonal or pentagonal in TS. Microsphenoidal calcium oxalate crystals are heavily deposited throughout the trachied. Vessel cells appear circular in cross section with a range of varying diameter (Pl.34E). Secretory canals are present towards the centre of the vascular cylinder (Pl.34C). Pith is large and comprises of large polygonal or circular parenchyma cells with dark brown deposition of alkaloids. Druses of calcium oxalate and oil drops are present in the parenchymatous cells of pith (Pl.34F).

4.3.3 Anatomy of Leaf:

V.S. of lamina exhibits uniseriate upper and lower epidermis with barrel shaped cells (Pl.35A,B). Adaxial epidermis covered with a thin layer of cuticle and

the cells are irregular polygonal, pentagonal or hexagonal. The adaxial epidermis devoid of stomata but with multicellular trichomes, the frequently of which is less than abaxial surface. Abaxial epidermal cells are irregular in shaped with radiating margin. Both stomata and multicellular glandular trichomes present and the frequency of trichomeis more than the adaxial surface. In between the upper and lower epidermises mesophyll cells are arranged in rows which are differentiated into 1-2 layered palisade parenchyma just below the adaxial epidermis followed by 4-5 layered spongy parenchyma with intercellular spaces. Chloroplast present in all mesophyll cells but concentration is ore in palisade cells. Collateral vascular bundles are of different size. Xylem ensheathed with parenchyma cells dispersed throughout the mesophyll (Pl.35C). Alkaloid deposition in the mesophyll tissue appears in black, bright yellow or pale yellow. Microsphenoidal crystals are embedded in palisade and spongy tissues (Pl.35B).

Anatomy of leaf under SEM:

Micromorphological study of the V.S. of leaf by SEM in 1400X magnification exhibits long, stacked palisade and loose spongy parenchyma cells with a layers of adaxial epidermis (Pl.35D, E). Upper epidermal cells appear polygonal, midrib exhibit nodulated longitudinal folds.

Midrib:

T.S. of midrib is convex and contour on both the surfaces, covered with simple and glandular trichomes (Pl.36A). Epidermis is uniseriate with round or cuboidal parenchyma cells followed by 3-4 layers of small, oval or round collenchyma cells. Ground tissue is composed of round, oval and pentagonal parenchyma cells. Two lateral patches of 2-3 layers chlorenchymatous mesophyll

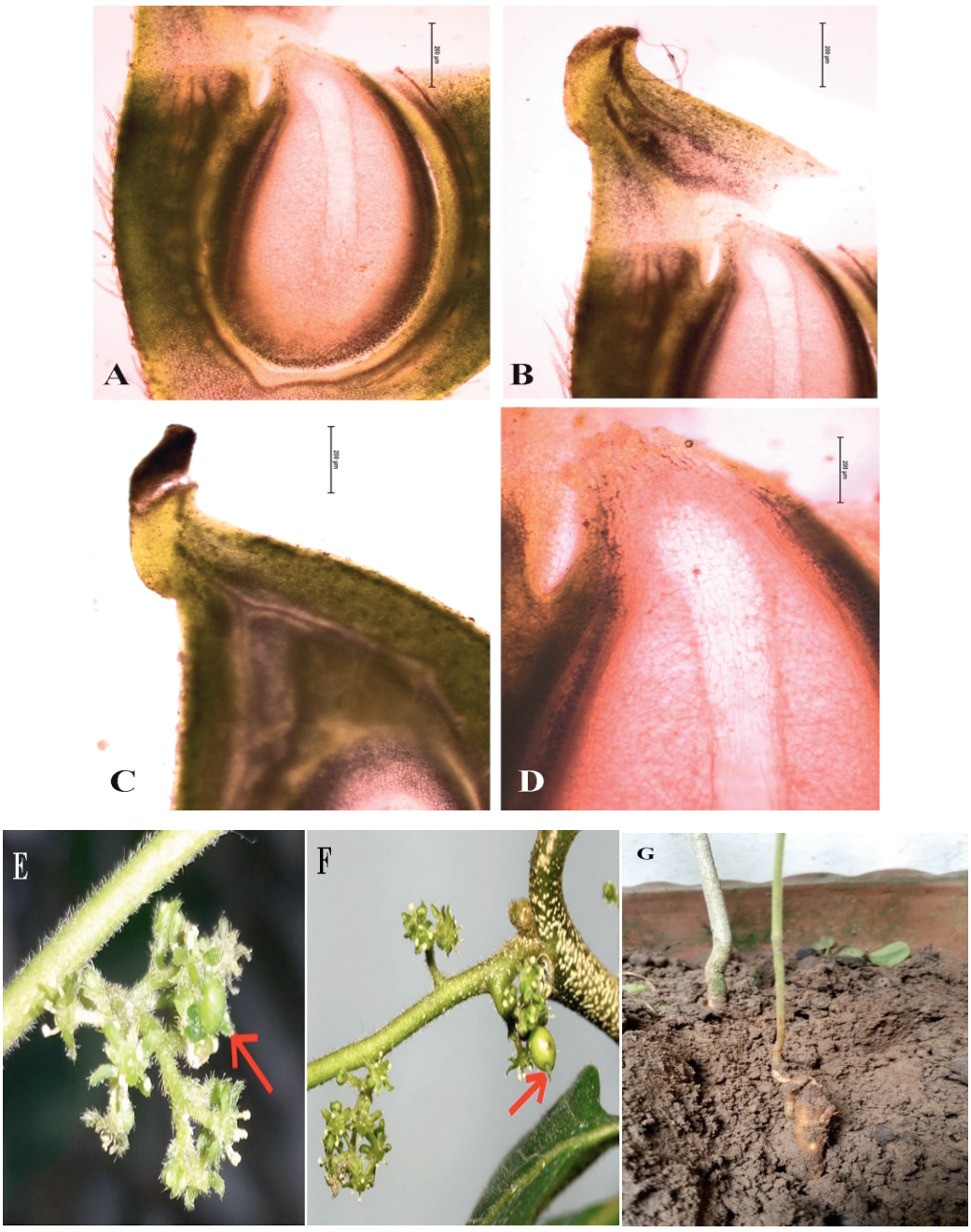


PLATE 32: Fertilization and apomixis. A. Porogamic fertilization, 400x; B. Fleshy stigmatic crest before fertilization, 400x; C. Dried stigmatic crest after fertilization, 400x; D. closed micropyle after fertilization, 400x; E-F. Parthenocarpic fruit developed in male plant; G. Sucker.

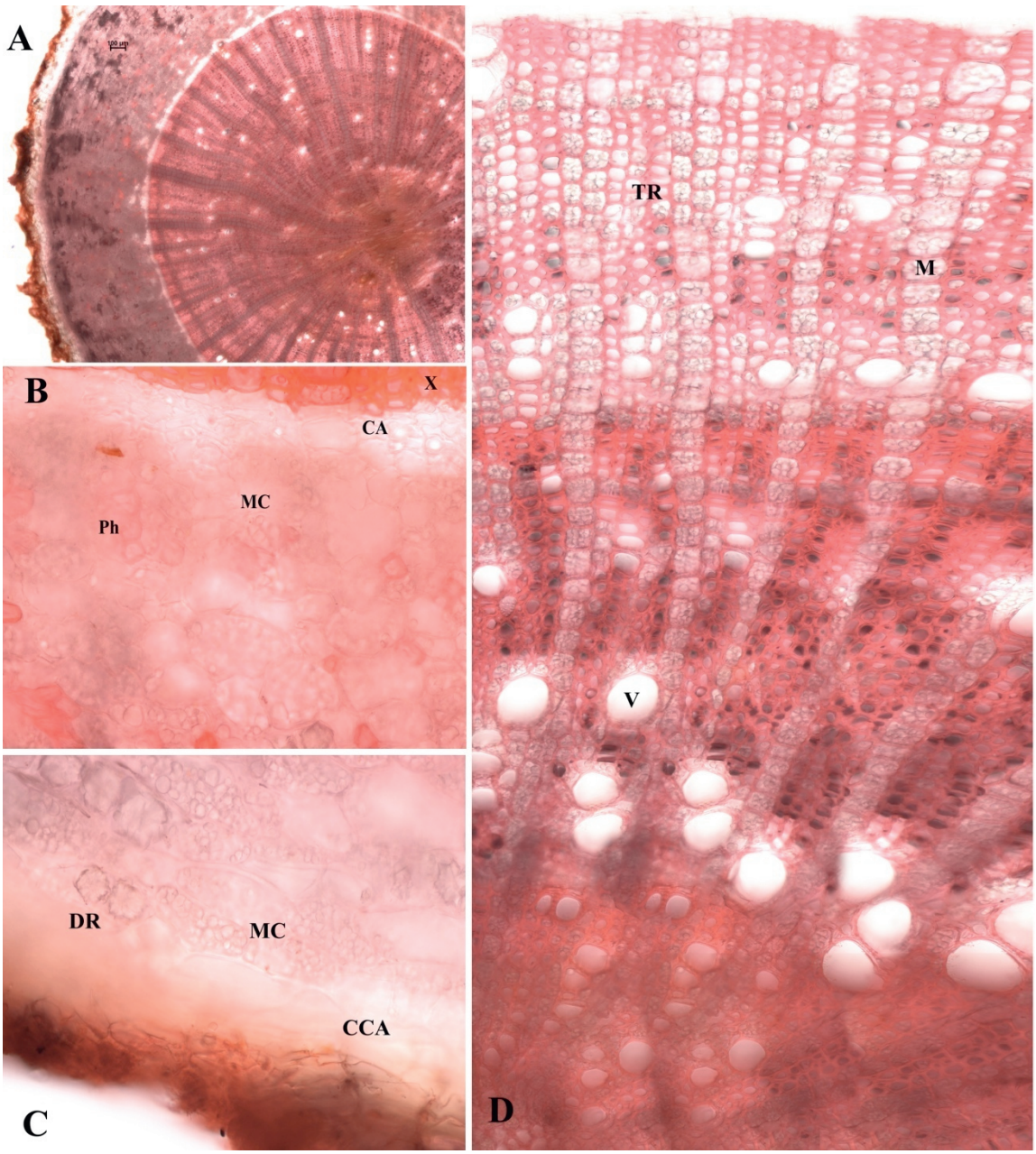


PLATE 33: Anatomy of root. A. T.S of root, 10x; B. Vascular cambium, Microsphenoidal crystal of alkaloids and scattered phloem in cortex, 40x layer near bark, 40x; C. Cork cambium, Calcium oxalate druses and Microsphenoidal crystal, 40x; D. Vascular cylinder, 40x. Cambium (CA), Cork Cambium (CCA), Vessels (V) and Trachieds (TR), Calcium oxalate druse (DR) and Microsphenoidal crystal (MC), Xylem (X), Medullary ray.

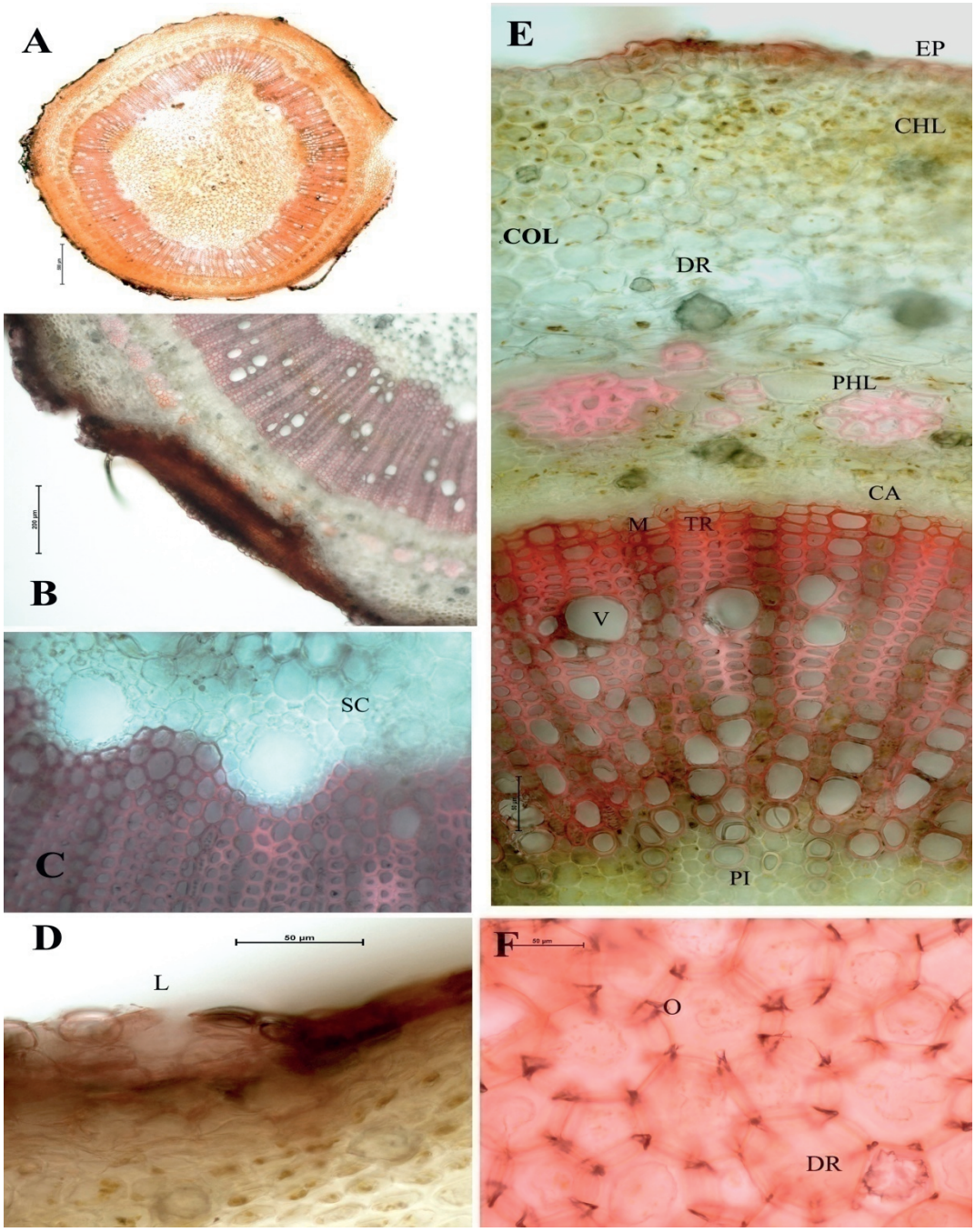


PLATE 34: Anatomy of stem. A. T.S of stem, 40x; B. Bark with depositionof secondary metabolites, 100x; C. Secretory canal at the edge of vascular cylinder, 400x; D. Lenticel in bark, 400x; E. T.S of stem, 400x; F. Pith with intercellular deposits, oil drops and druses of calcium oxalate, 400x. Oil drops (O) and Calcium oxalate Druse (DR), Lenticel (L), Secretory canal (SC), Epidermis (EP), Chlorenchyma (CHL), Collenchyma (COL), Phloem (PHL), Cambium (CA), Trachied (TR), Vessel (V), Pith (PI), Medullary ray (M).

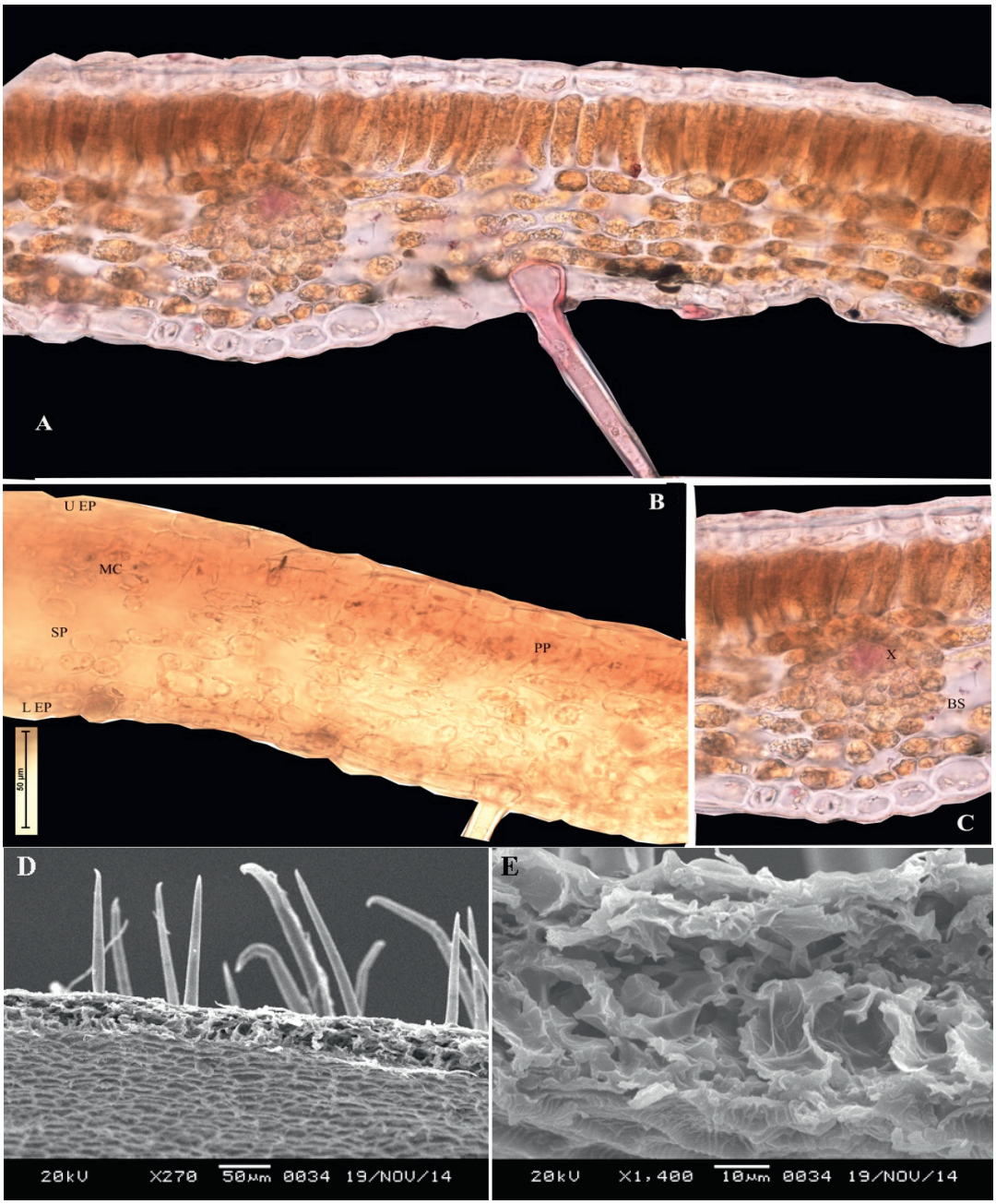


PLATE 35: Anatomy of leaf under LM and SEM. A. V.S. of leaf, 400x; B. V. S. of Lamina, 400x; C. Vascular Bundle, 400x; D. V.S. of leaf under SEM; E. Mesophyll cells under SEM. Upper epidermis (U EP), Lower epidermis (L EP), Palisade parenchyma (PP), Spongy parenchyma (SP), Microsphenoidal crystal (MC) of calcium oxalate, xylem (X), bundle sheath (BS).

are present on the adaxial side. Vascular bundles arranged as a large arch on the abaxial side with patches of phloem outside and rows of xylem towards the centre (Pl.36B-C). Xylem is endarch. Two small complementary patches of xylem are present on the adaxial side. Druses and microsphenoidal crystals of calcium oxalate are present in the parenchyma cells near the vascular bundle. Granules of yellow and orange chemicals are alsopresent throughout the parenchymatous tissue. Large secretory canal of about 5.5 µm diameter present at the centre of the mid rib (Pl. 36D).

Petiole:

T.S. of petiole is more or less circular in outline, convex and contour on both the surfaces, covered with simple, short multicellular, glandular trichomes with heavy deposition of black substances probably alkaloid(Pl.37A-G). Cells of epidermis are uniseriate and comprised of barrel shaped parenchyma lying under a thin layer of cuticle. It is followed by layers of small, oval or round collenchymatous hypodermis. Ground tissue composed of round, oval or pentagonal parenchyma cells withdruses and microsphenoidal crystals of calcium oxalate and bright orange granules of secondary metabolites. Two lateral patches of chlorenchyma present on the adaxial side that diminishes towards abaxial surface which is an important anatomical character of the species.In the centre vascular bundles are arranged in a ring comprising of 55-65 xylem rays. Layer of phloem is present following the ground tissue. Metaxylem in the centre is surrounded by protoxylem on both the sides. Parenchymatous pith contains a large secretory canal of about 50 µm diameter surrounded by concentric rings of parenchyma cells (Pl.37G).

Raised marginal glandular nodules:

Hand sections and clearing of leaves exhibit that the structure is submerged in leaf tissues. They are neither completely sunken nor enclosed completely by the leaf epidermis, rather the dome shaped head bulges out from leaf tissue (Pl.38A).

The underlying cup of uniseriate flattened parenchymatous cells hold the dome shaped loose secretory tissue mass (Pl.38A-B). The dome consists of aggregated secretory cells which got separated from the adaxial epidermis by a layer of flattened palisade parenchyma cells forming the cup. Major and minor vein endings having distinct spiral thickening are present just below the structure, few forming idioblasts also (Pl.38B).

Cells within the nodule are of two types- polygonal pulpy secretory cells and elongated cells (Pl.38C). Blurry mucilage tissue and few granules of bright orange secretions are also present in between the secretory cells of the RMGN. Large number of bluish black microsphenoidal crystals, druses and lumps are scattered throughout the tissue of the nodule.

4. 4 Seed germination and viability:

The seeds of *B. mollis* were subjected to different germination tests in order to determine the rate of germination and viability and the effect of different conditions and effectors (Pl.39A-E). The percentage germination *i.e.* the viability and the days to germinate varies with various conditions.

4.4.1 Effect of methods and season on germination:

Mature ripe and unripe seeds with fruit wall intact have not germinated. Mature ripe seeds without fruit wall germinated faster than the mature unripe seeds in soil. Both mature ripe and unripe seeds started germination within 10-15 days. The mature ripe seeds exhibit maximum 85.6±0.57% germination while only 41±1%

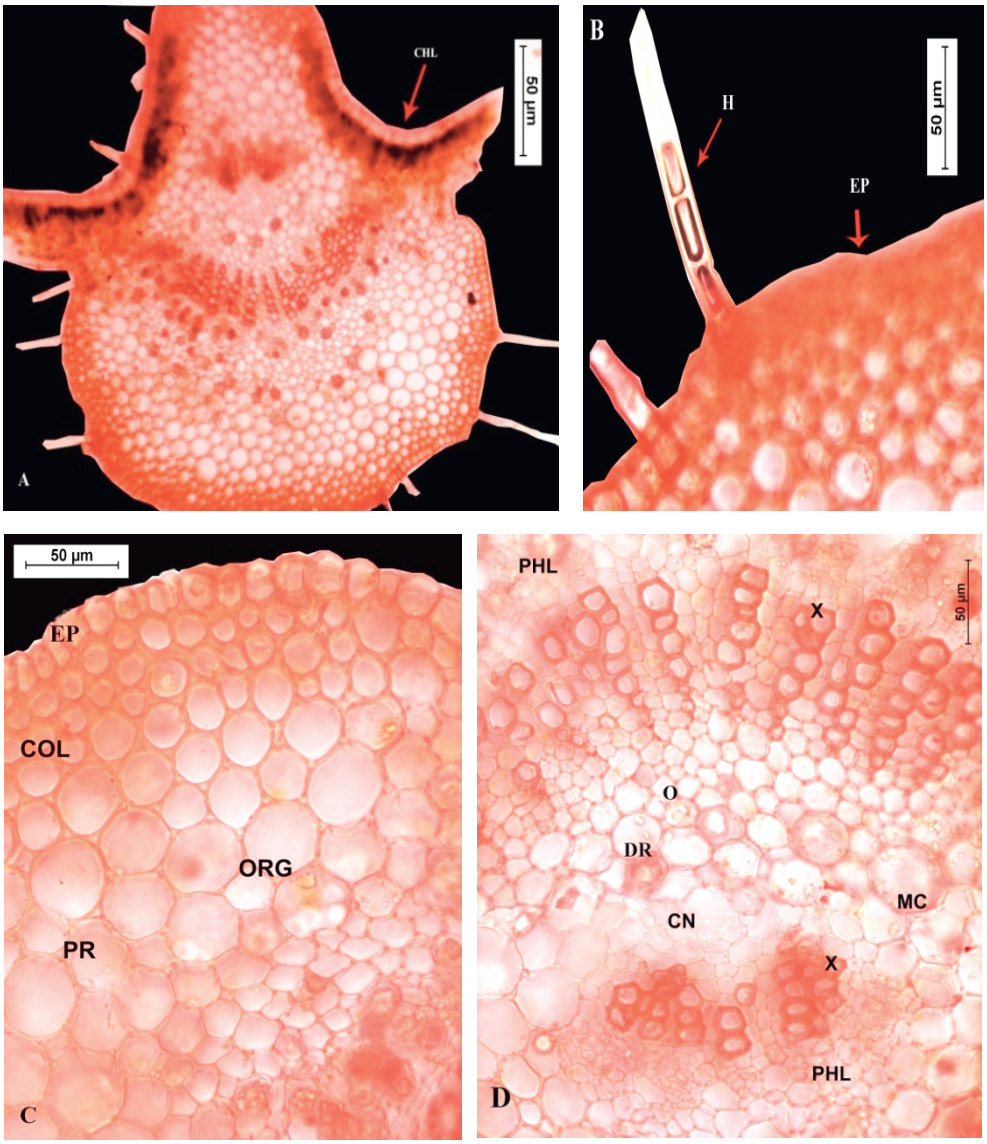


PLATE 36: Anatomy of midrib. A. T.S of midrib, 100x; B. Epidermal hair, 400x; C-D. T.S of midrib with cortex and vascular bundle, 400x. Epidermal hair (H), Chlorenchyma (CHL), Epidermis (EP), Collenchyma (COL), Parenchyma (PR), Phloem (PHL), Xylem (X), Central canal (CN), Volatile oil drop (O), Druse (DR) and Microsphenoidal crystal (MC) of Calcium oxalate, Orange colored granules of secondary metabolites (ORG).

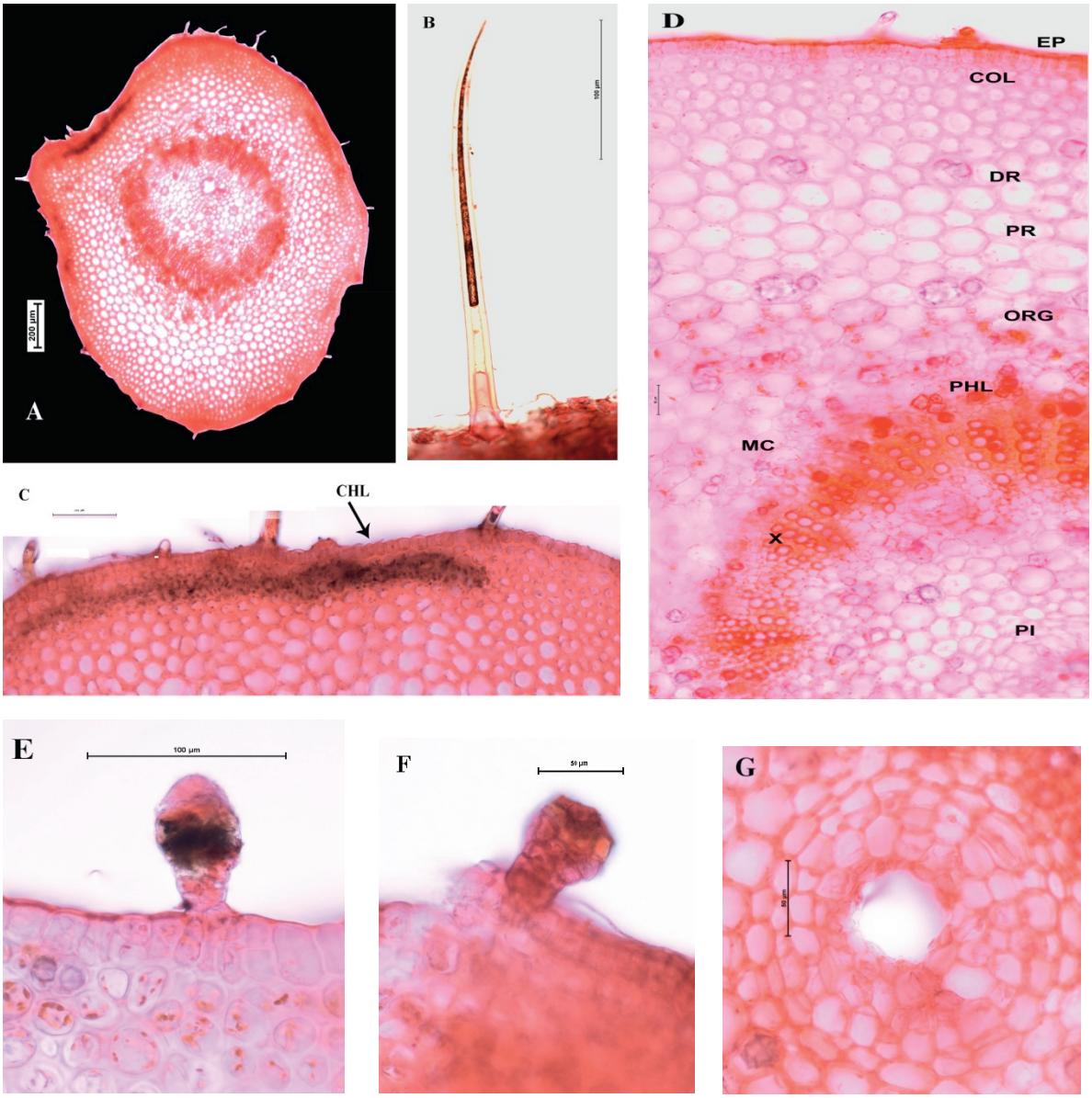


PLATE 37: Anatomy of petiole. A. T.s of petiole, 100x; B. Multicellular long hair with deposition of alkaloid, 400x; C. 400x; D. T.S of petiole, 400x; E-F. Glandular capitate trichome, 400x; G. Secretory canal surrounded by concentric rings of parenchymatous cells, 400x. Chlorenchyma (CHL), Calcium oxalate druse (DR), Microsphenoidal crystal (MC) of calcium oxalate, Epidermis (EP), Collenchyma (COL), Parenchyma (PR), Phloem (PHL), Xylem (X), Pith (PI), Orange colored granules (ORG).

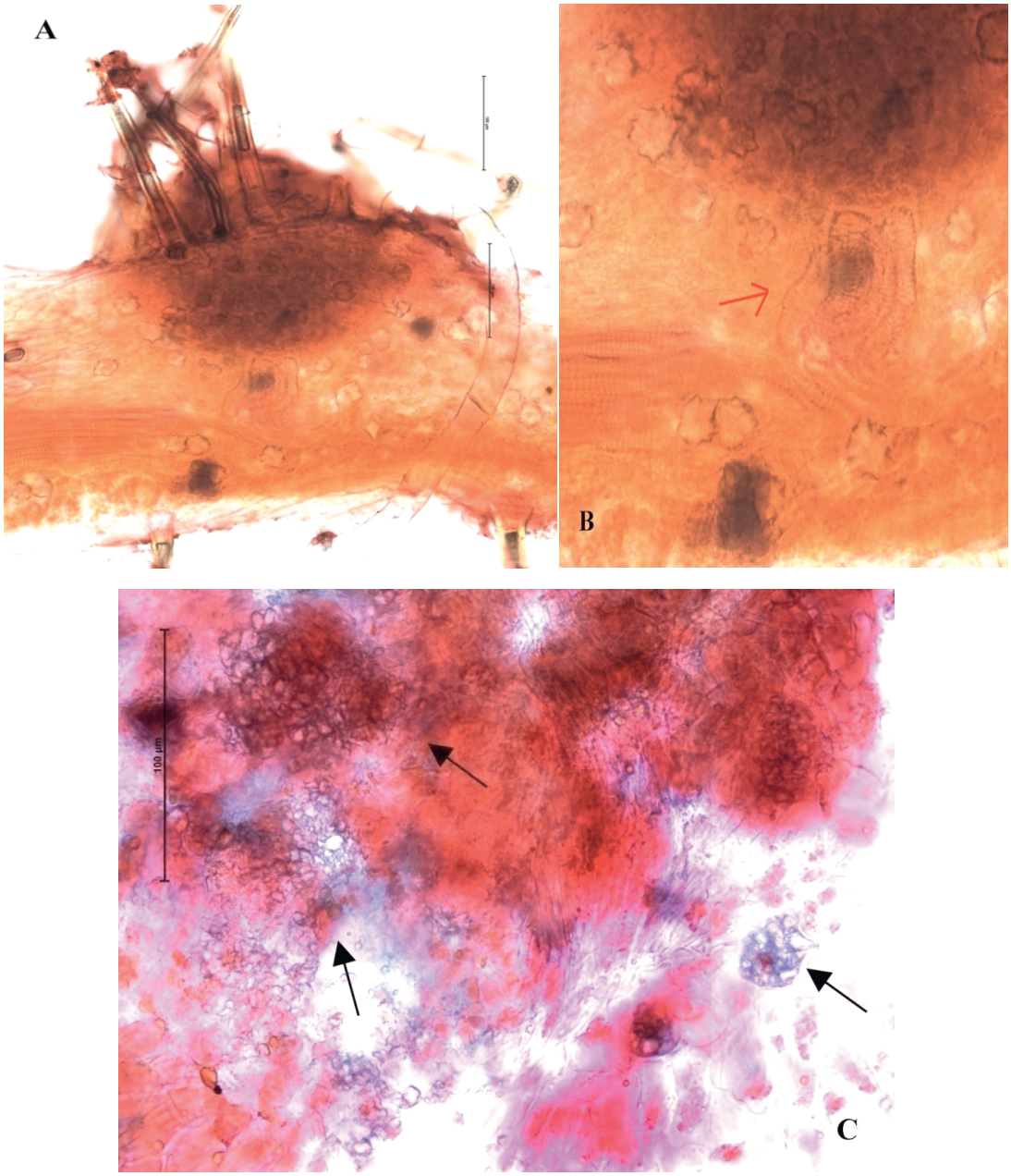


PLATE 38: Anatomy of Raised marginal glandular nodule leaf. A. V. S. of the tip of a secondary vein with large marginal gland and a crown of trichomes around it, 400; B. Vein terminal at the base of the nodule, 400x; C. T.S. of RMGN exhibiting different cell types, 400x.

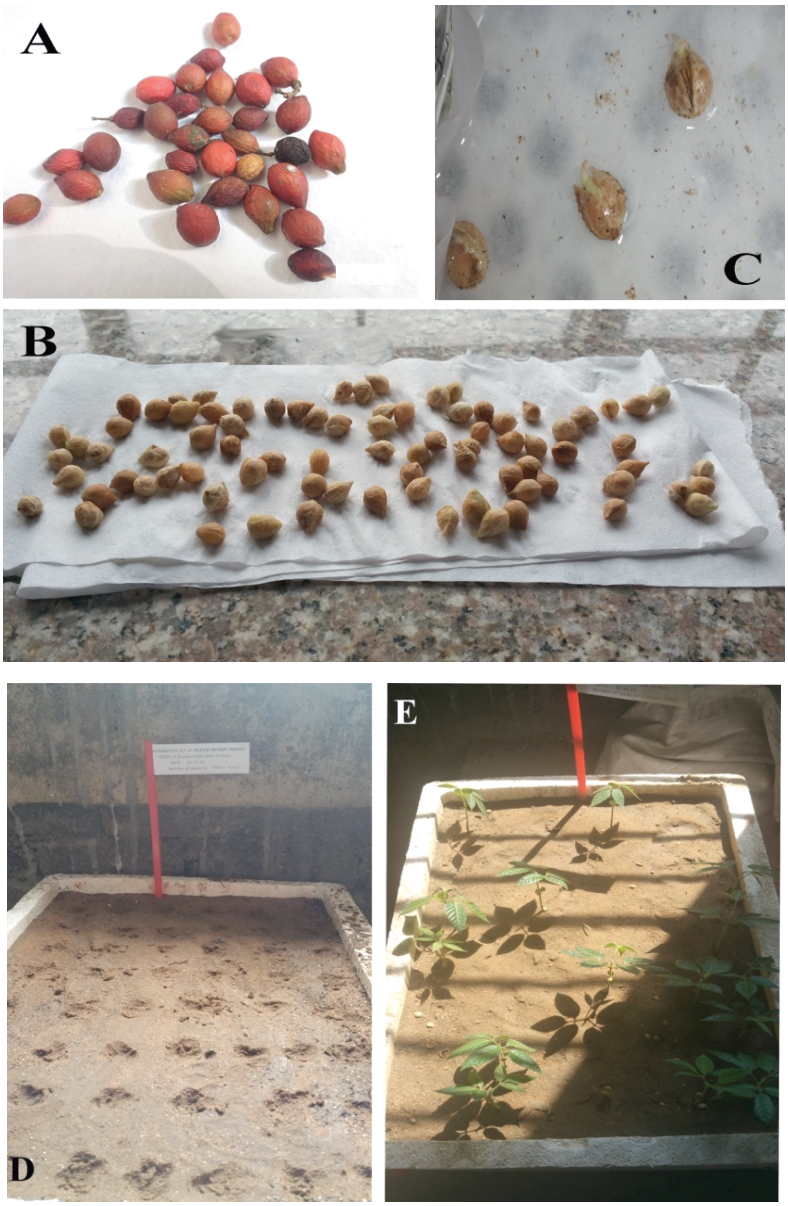


PLATE 39: Seed germination. A. Fruit with intact wall, B. Germinating seeds, C. Seeds after removing fruit, D. Seeds in seed bed, E. Seedling.

of the mature unripe seeds got germinated. Seeds in summer show better germination percentage than that in Winter (Fig.4.4.1). Though mature ripe seeds exhibits better germination rate (Fig.4.4.2) mature unripe seed germinate faster exhibiting higher CVG (Table: 9).

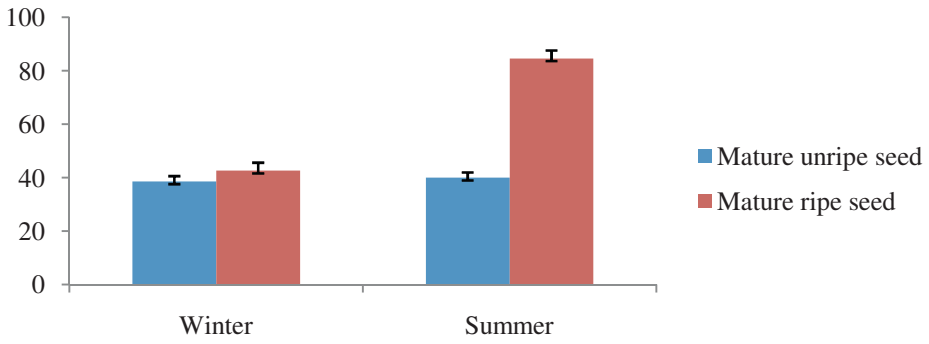


Fig. 4.4.1: Germination percentage of mature ripe seed and mature unripe seed in Winter and Summer ($p < 0.05$)

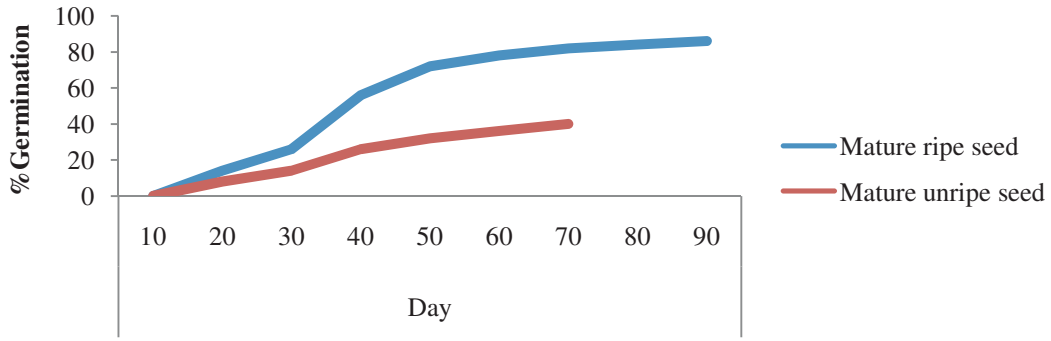


Fig. 4.4.2: Germination of mature ripe and mature unripe seeds

Table 9: CVG of mature ripe and mature unripe seeds

	Winter	Summer
Mature ripe seed	0.015	0.0234
Mature unripe seed	0.016	0.02580

Seeds sown in soil and sand mixture in field germinated better and faster than that germinated in Petri plates inside laboratory conditions (Fig.4.4.3, Fig.4.4.4, Table 10).

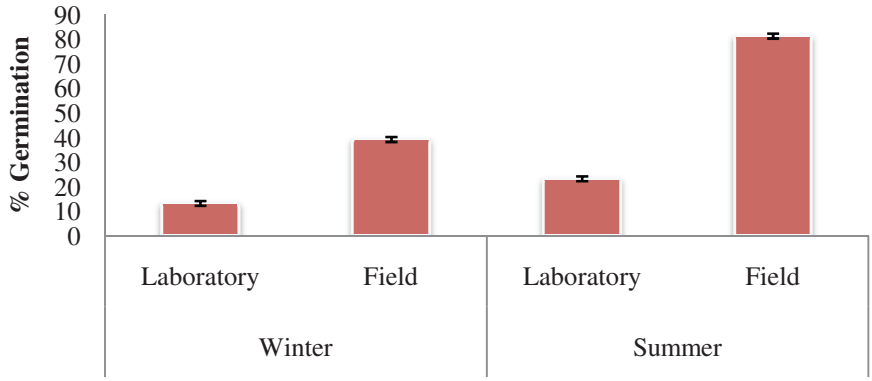


Fig. 4.4.3: Germination in Laboratory and soil ($p < 0.05$)

Table 10: CVG in Laboratory and soil

		CVG
Winter	Laboratory	0.025
	Field	0.016
Summer	Laboratory	0.066
	Field	0.022

4.4.2 Influence of fruit wall on germination:

Seeds without fruit wall in 1:1 soil and sand mixture start germinating from 10-15 days in summer and 28-31 days in winter. They continued to germinate until 90 days after sowing. Seeds with fruit wall intact did not germinate (Table:11).

Table 11: Effect of fruit wall in seed germination

	Duration of germination (Days)					
	Petri plate		Soil:Sand (1:1)		GA treatment 300 ppm)	
	December	May	December	May	December	May
Seeds with fruit wall intact	No germination	No germination	No germination	No germination	No germination	No germination
Seeds where fruit wall removed (days after sowing)	35-36	12-13	78-80	59-80	17-22	40-43

4.4.3 Influence of Gibberellic acid in germination:

Seeds of *B. mollis* treated with three different concentrations of GA *viz.* 100 ppm, 200 ppm and 300 ppm exhibits no significant difference in germination percentages (Fig.4.4.6, Table 13). The germination percentage is similar to that of the seeds grown in the field.

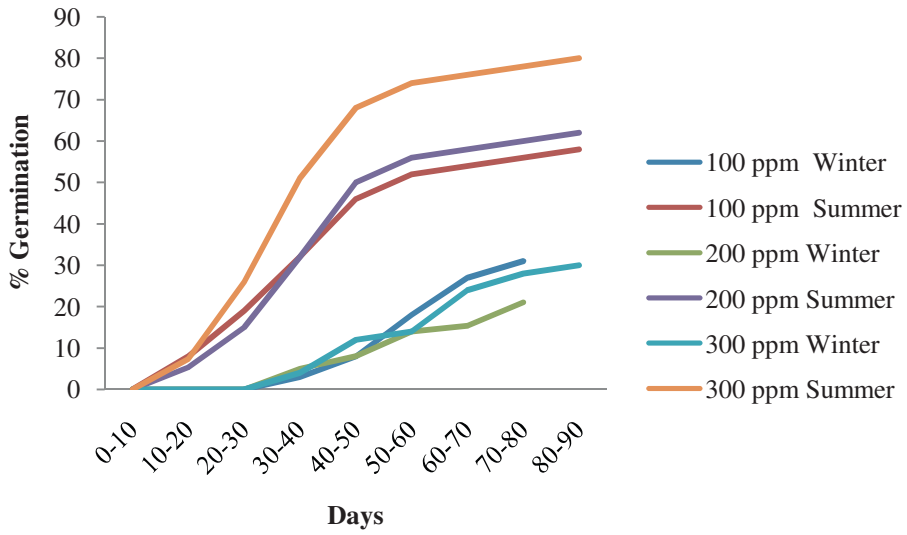


Fig. 4.4.4: Effect of different concentrations of GA *i.e.* 100 ppm, 200 ppm and 300 ppm on germination

Table 12: CVG of seeds treated with different concentrations of GA

100 ppm		200 ppm		300 ppm	
Winter	Summer	Winter	Summer	Winter	Summer
0.017	0.025	0.013	0.026	0.017	0.026

4.4.4 Seed Viability:

Seed viability test of *B. mollis* using 2, 3, 5-triphenyltetrazolium chloride (TTC) exhibits 76.6 ± 15.2 % viability which coincide with the germination experiments carried out.

4.5 Traditional uses:

The plant is popularly known as ‘quinine’ or ‘Bapkehu’ among the ethnic communities of Karbi Anglong. All the respondents who knew the plant has inherited the knowledge from the elders of their family. The plant is not cultivated by anyone. They used to collect it from wild whenever required. The plant is reported to be scarce even in the areas of Karbi Anglong where it occurs.

Ethnic people uses fresh fruit or root decoction of *Brucea mollis* when they suffer from fever of any kind including malaria. They use to take one or two fresh fruit as per the severity of infliction. To prepare the root decoction 4-5 cm of root is taken, bark is removed carefully, wash it, crushed and then boiled in a glass of water for half an hour. Sometimes instead of decoction infusion is used. One or two tablespoonful of the decanted liquid is taken twice or thrice daily depending on the severity of the fever. Overdose of root decoction is reported to cause temporary paralysis. However, such adverse effect is usually treated with fruit juice of *Garcinia pedunculata* and *Averrhoa carambola.*

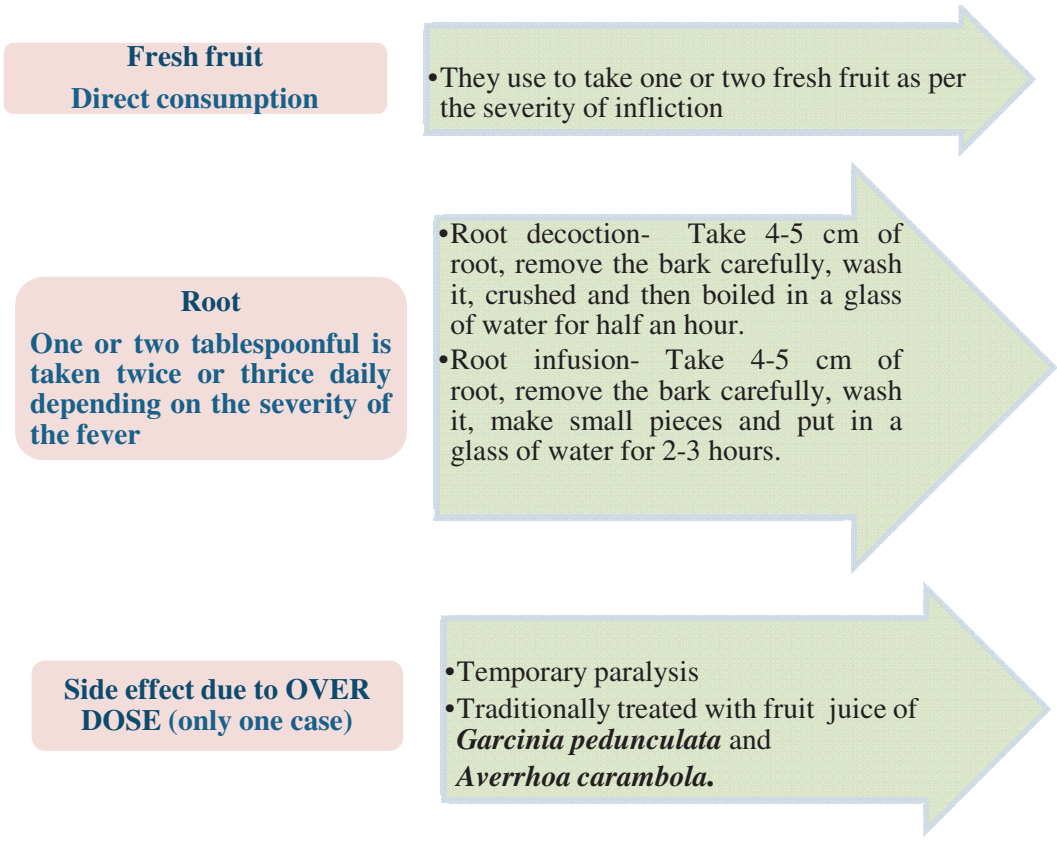


Fig. 4.5: Traditional method of using *B. mollis* and possible side effect