RESEARCH ARTICLE

Studies on micromorphology of leaves in some members of genus *piper* linn.

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ABSTRACT

Stomata, the vital gate between plant and atmosphere may play a central role in plant vegetation responses to environmental conditions, which have been and are being investigated from molecular and whole plant perspectives, as well as at ecosystem and global levels. Stomatal diversity in foliar epidermis has great value in plant systematic studies. The present work deals with the anatomical investigations with respect to stomatal complex in genus *Piper* belonging to family Piperaceae where an attempt has been made to recognize the taxonomic value of genus *Piper.* Stomatal complex of eight species of genus *Piper* has been studied for the present work. The species are as follows: *P. chaba, P. pedicellosum, P. sylvestre, P. talboti, P. boehmeriaefolium, P. lonchites, P. miniatum, P. galeatum*. Three types of stomata were observed: paratetracytic, staurocytic and anisocytic. All the species show the presence of stomata on the lower epidermis only. *P. galeatum* was noticed with combination of two types of stomata on its epidermis. Rest all the species were found to show single type of stomata. The observations suggest the predominance of paratetracytic stomata followed by and staurocytic and anisocytic type. Detailed study of foliar trichomes in the above mentioned species showed abundance in glandular trichomes. Under glandular trichomes, types of unmodified epidermal cells and modified epidermal cells were found to be persistent. Interestingly, eglandular trichomes of type trichome apex obtuse were also noticed in two species. *P. boehmeriaefolium* was been observed with both glandular as well as eglandular type of trichomes.

Keywords: *Piper*, Stomatal complex, Paratetracytic, trichomes, taxonomic value, Intrageneric classification.

INTRODUCTION

A stoma is a microscopic pore on the surface of land plants. It is surrounded by a pair of specialized epidermal cells called guard cells, which act as a turgor-driven valve that open and close the pores in

response to given environmental conditions. Transpiration via stomata supplies water and minerals to the entire plant system. When a plant encounters adverse environmental conditions, such as drought, a plant hormone called abscisic acid triggers stomata to shut tightly in order to prevent plants from dehydration and wilting. Many workers such as Edeoga (1991), Edeoga and Osawe (1996), Mbagwu and Edeoga (2006), Nwachukwu and Mbagwu (2006) stressed that epidermal and cuticular traits of plants epidermal cells, type and arrangement of stomata, size and shape of trichomes and number of vascular bundles could serve as vital tools in solving taxonomic problems in Angiosperms. Stomatogenesis has long been studied by morphologists, physiologists and taxonomist .The morphology and ontogenies of taxa are important in intrageneric systematics. Diversity in stomata types, even on the same surface of an organ, indicates the weakness in using stomata as a taxonomic character (Pant and Kidwai, 1964). Inspite of diversity, the most frequent stomata type can be used as a taxonomic character. Apart from physiognomic characters, anatomical properties of plant parts are sources for taxonomic inferences in different groups of flowering plants (Edeoga et al., 2007; Guimeraes et al., 2007; Kaplan et al., 2007; Keshavarzi and Zare, 2006). Despite the immense economic importance of the legumes and the physiological importance of the stomatal apertures, reports on the frequency and the structure of the stomata are lacking or incomplete for many species.

Piperaceae are grouped in about 8-12 genera and about 1200- 5000 species; are pantropical in distribution; and confined to the tropical and subtropical portions of the world. It is commonly known s pepper family. these are mostly herbs and shrubs but there are also small trees and woody climbers. The genus *Piper,*Linn., the largest in the fmily Piperaceae, occurs throughout the tropical and subtropical regions of the hemispheres. As conventionally construed, *Piper* is a large pantropical genus. More than 3000 species have been recorded (Rahiman and Nair, 1983), but because of the large number of species, wide distribution, very small, achlamydous and closely aggregated flowers, many unisexual species, and lack of critical phyletic study (Hooker, 1886; Lawrence, 1951), an acceptable species concept could not be established till date. Pipers are generally perennial shrubs bearing adventitious or sometimes epiphytic roots. Leaves are alternate, petiolated and with deciduous stipules. Leaf blade is simple, ovate, lanceolate or elliptic. Leaves are pinnately costateor multicostate. Inflorescence is spike or catkin; oppositifolius. Flowers are usually dioecious; unisexual; sessile; with the peltate or copular bracts. Stamens are 1-4 with 2-celled anthers. Ovary is superior; 1-celled, free with solitary ovule. Fruit is sessile, oblong or globose, pulpy, reen, red, yellow, drupe or berry. Seeds are with thin testa. Pipers are of huge economic and medicinal significance. Boiled stem and leaves of Piper are used as medicine while roots are used to cure stomachache, common cold, etc. the aromatic leaf is a masticatory used with lime, catechu, arecanut and other species, and sometimes with tobacco act as a stimulant, intoxicant, carminative, astringent, aphrodisiac and antiseptic. Leaf juice has fungicidal and nematodicidal properties due to the presence of essential oil. Seeds powdered and given with honey are used in cough, cold and asthma.

There is meager anatomical work carried out in genus *Piper* of piperaceae. Family piperaceae has undergone considerable changes in the circumscription of various taxanomic ranks. Howard (1973) has considered the family as the most difficult family. In spite of all the efforts carried out by the taxonomists, many species could not be placed comfortably in the classification on the basis of available morphological data. A number of species are very intimate with the leaf morphological characters. Hence, in the present study, attempt has been made to recognize the taxonomic value of the stomatal complex and trichomes. Variations in stomata and foliar trichomes of eight species of genus *Piper* has been studied for the present

work. The species are as follows: *P. chaba, P. pedicellosum, P. sylvestre, P. talboti, P. boehmeriaefolium, P. lonchites, P. miniatum, P. galeatum*.

MATERIAL AND METHODS:

Plant material, fresh and preserved, was collected from various states such as Maharashtra, Tamil Nadu, Kerala, Assam, Meghalaya, and West Bengal. Majority of plants were however obtained from Meghalaya. Most of the species are procured through B.S.I., Eastern circle, Shilong and few from C.N.H. and Indian Botanical Garden, Howrah. The identifications were checked with reference to various floras and standard Indian Herbaria. The detailed study of the various species of *Piper* was carried out in the laboratory using both dissecting and compound binocular microscope.

I. To study the stomata, various methods were tried for the mounting of lamina showing the surface layers in surface views.

1) Simple scraping technique employed by Jordell Laboratories in U.K. as described by Metcalfe (1963) was tried.
2) Some pieces of lamina were boiled in 5- 15% concentrated HNO_3 till the leaf showed blister formation on its surface. The lamina was then thoroughly washed and the blisters with epidermis was peeled off.
3) The boiling of the lamina was also tried in 5% NAOH/ KOH and the remaining procedure followed was the same as above.
4) 5% $CuSO_4$ solution was taken, in which some pieces of lamina were boiled for 10-15 minutes. While boiling, two to three ml of concentrated HNO_3 was added. Blisters were formed on the leaf surface. As in the above procedure, the lamina pieces were washed and the blistered epidermal surface was peeled off.

   The peeled epidermis from the above methods was then stained with Safranine or Haematosylin solution and mounted in 80%-85% glycerine.

II. To study the foliar trichomes in several lamina, cleared with sodium hypochlorite solution were observed directly under the compound microscope.

The camera lucida sketches of the stomata and trichomes at x 450 magnification were drawn by using Erma Camera Lucida. Finally, the text figures of the stomata were prepared.

RESULT AND DISCUSSION:

The different types of stomata have been reported on the same surface of an organ in diverse angiospermic families (Tognini,1897; Loftfield, 1921; Sen, 1958; Pant and Kidwai, 1964; Paliwal, 1965;Pant and Mehra, 1965; Inamdar 1969; Inamdar and Patel, 1971; and Bahadur et.al. 1971). Stomata are always restricted to the lower epidermis in *Piper* (Metcalf and Chalk, 1950). Interestingly, a few stomata are also noted on upper epidermis of *Piper leptosachya*, chapm. (Yuncker and Gray, 1934). Most of the stomata are surrounded by a rosette of many epidermal cells, while some are cruciferous in nature (Metcalf and Chalk, 1950). However, stephanocytic type of stomatal apparatus have been found to be characteristic for chloranthaceae, Saururaceae and piperaceae (Baranov, 1987). The epidermal characters of plants in systematic studies in distinguishing certain groups of plants have been used (Stace,1965; Ramayya and Rajgopal, 1968, 1971). The mature stomata of 8 species of *Piper* have been studied. The results obtained indicates the presence of paratetracytic, staurocytic and anisocytic type of stomata. All the eight species showed the stomata at their lower epidermis only. Only one species amongst all was noticed with the combination of two types of stomata.

The types of stomata observed in the studied species of Piper are as follows:

Paratetracytic type: The guard cells elongated and kidney shaped. The epidermal cells are pentagonal, hexagonal and polygonal in shape.

Staurocytic type: The guard cells are kidney shaped in appearance. The epidermal cells are almost in shape, with a rounded cell wall pattern.

Anisocytic type: The guard cells are slightly elongated and kidney shaped in appearance. The epidermal cells are pentagonal and hexagonal in shape.

Paratetracytic and Staurocytic type: The guard cells are large and kidney shaped in appearance. The epidermal cells are larger in size as compared to the lower epidermis. They are pentagonal and also isodiametric in shape having a rounded cell wall pattern.

KEY BASED ON STOMATAL COMPLEX:

Type 1: Paratetracytic type:

P. chaba - Lower epidermis
P. pedicellosum - Lower epidermis
P. sylvestre - Lower epidermis
P. talboti - Lower epidermis

Type 2: Anisocytic type: :

P. boehmeriaefolium - Lower epidermis
P. lonchites - Lower epidermis

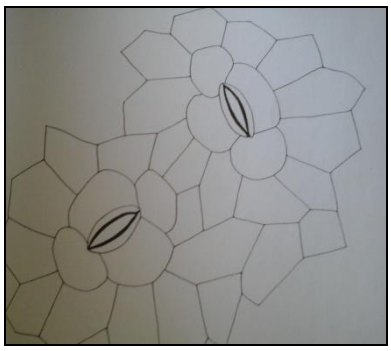
Type 3: staurocytic type:

P. miniatum - Lower epidermis

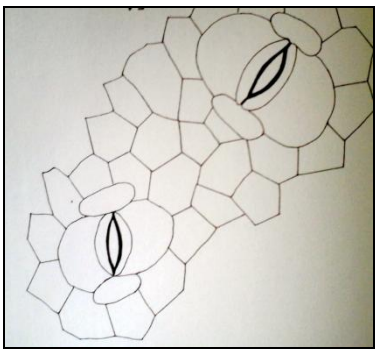
Type 4: paratetracytic and Staurocytic :

P. galeatum - Lower epidermis

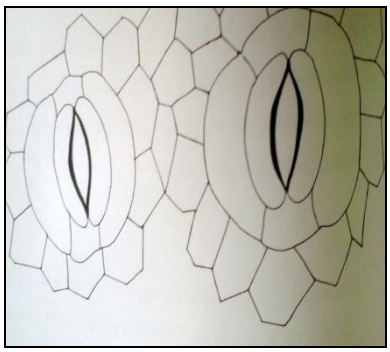
Type 1: Paratetracytic type:



P. chaba

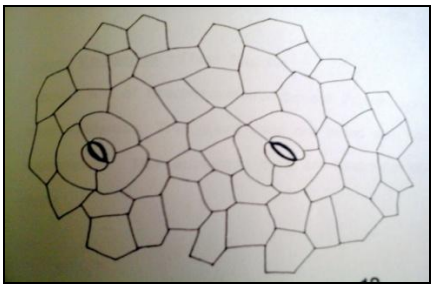


P. pedicellosum

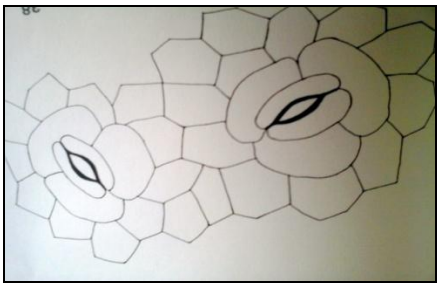


P. sylvestre & P. talboti

Type 2: Anisocytic type:

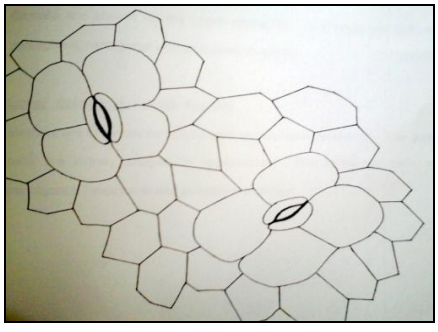


P. boehmeriaefolium



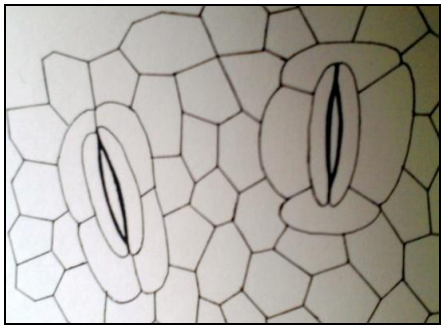
P. lonchites

Type 3: staurocytic type:



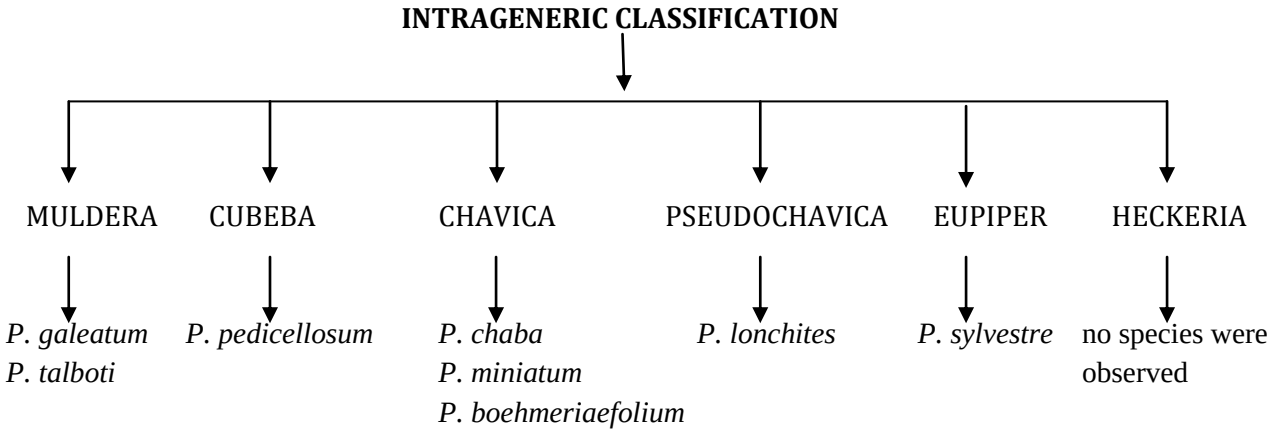
P. miniatum

Type 4: paratetracytic and Staurocytic :



P. galeatum

Intrageneric classification of genus *Piper* given by hooker:



According to intrageneric classification of *Piper* Section 1, Muldera consists of *P. galeatum* and *P. talboti* of which P. galeatum shows paratetracytic and staurocytic types of stomata while in *P. talboti* paratetracytic ype of stomata are observed. Section 2, Cubeba consists of *P. pedicellosum* shows paratetracytic type of stomata. Section 3 Chavica shows maximum species namely *P. chaba, P. miniatum, P. boehmeriaefolium*. *P. chaba* shows paratetracytic type of stomata and *P. miniatum* shows staurocytic type of stomata. In *P. boehmeriae folium* anisocytic type of stomata was observed. *P. lonchoites* categorized under section 4 Pseudochavica shows anisocytic type of stomata. *P. sylvestre* categorized under section 5 Eupiper shows paratetracytic type of stomata. No species were categorized under section 6 Heckeria.

The structure and development of trichomes and glands have been studied in many angiosperm families (DeBary, 1884; Solereder, 1928; Netolitzky, 1932; Cowan, 1950; Farooq, 1963; Pant and Banerji, 1965; Inamdar, 1967, 1968; Inamdar and Patel, 1973; Roe, 1971; Ramayya, 1972; Singh, Jain and Sharma, 1974; Lowell and Lucansky, 1986). Metcalfe and Chalk (1950) have reported 14 eglandular and 5 glandular type of trichomes in Polemoniales. They have also observed glistening pearl glands in Piper sporadically, which are usually filled with protein and oil. Inamdar and Patel (1973) have recorded the structure, ontogeny and classification of trichomes in 40 genera and 112 species of the Polemoniales, and have found eglandular, glandular and eglandular cum glandular types of trichomes. In the present research work, trichomes of all eight Piper species were examined and it has been observed that piper show the presence of either eglandular trichomes or glandular or both. Interestingly, two species of *Piper* were found with both the types of trichomes.

The types of trichomes observed in the studied species of Piper are as follows:

A. EGLANDULAR TRICHOMES:

a) Trichome Apex Obtuse:

i. Trichomes are eglandular, simple and of unicellular, conical type, with an obtuse apex and a rounded base. The outer walls are concave and sinuous in nature. (eg)

ii. Trichomes are long and eglandular, simple and of unicellular type, with almost parallel outer walls showing a slightly obtuse apex and a rounded base. (eg)

B. GLANDULAR TRICHOMES:

a) Unmodified Epidermal Cells:

i. Trichomes are glandular , multicellular and of peltate type. The trichome basal cell is with unmodified epidermal cells and a thickened, polygonal trichome base.

b) Modified Epidermal Cells:

i. Trichomes are glandular , multicellular and of peltate type. The trichome basal cell is with modified radially thickened epidermal cells and a thickened, polygonal trichome base.

KEY BASED ON TRICHOME COMPLEX :

A. EGLANDULAR TRICHOMES:

a) Trichome Apex Obtuse:

- *P. boehmeriaefolium*
- *P. miniatum*

B. GLANDULAR TRICHOMES:

a) Unmodified Epidermal Cells:

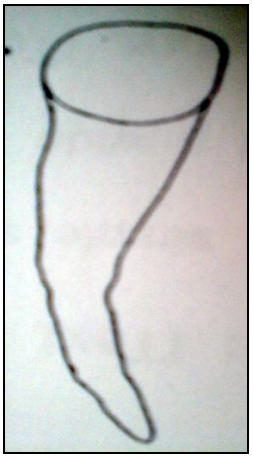
- *P. boehmeriaefolium*
- *P. miniatum*
- *P. chaba*
- *P. galeatum*
- *P. lonchites*
- *P. pedicellosum*
- *P. sylvestre*

b) Modified Epidermal Cells:

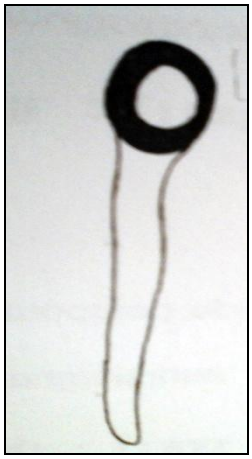
- *P. talboti*

A. EGLANDULAR TRICHOMES:

a. Trichome Apex Obtuse:



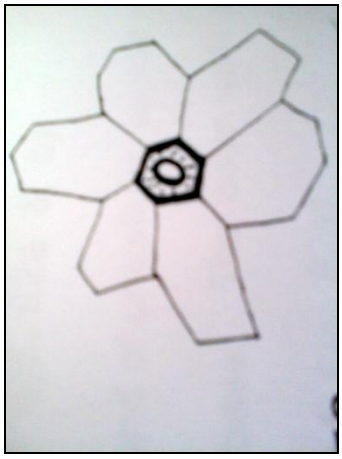
P. boehmeriaefolium



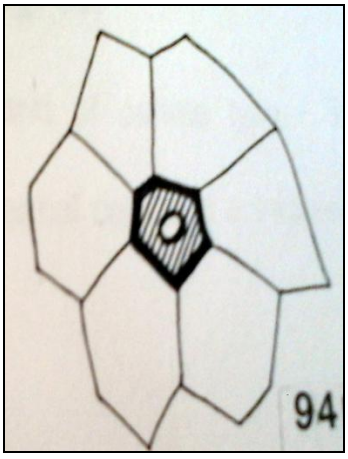
P. miniatum

B. GLANDULAR TRICHOMES:

a.Unmodified Epidermal Cells:



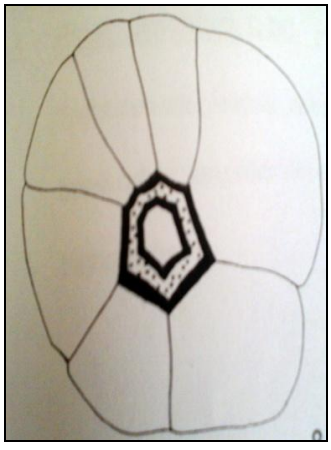
P. boehmeriaefolium



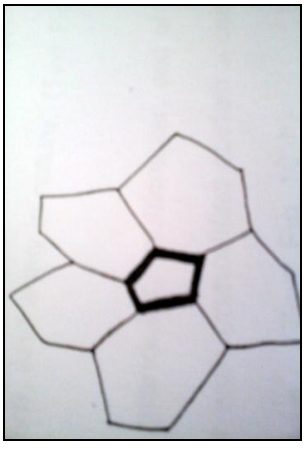
P. miniatum

A. GLANDULAR TRICHOMES:

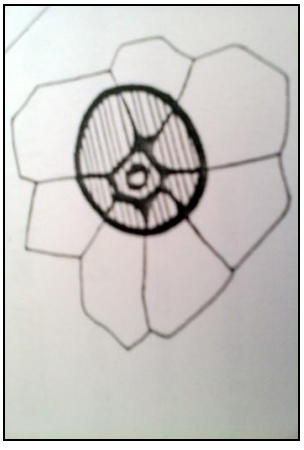
a. Unmodified Epidermal Cells:



P. chaba



P. pedicellosum

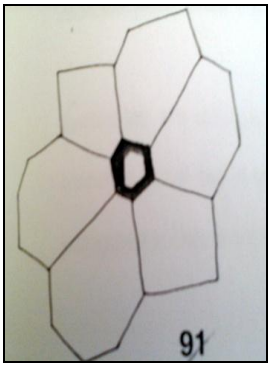


P. sylvestre

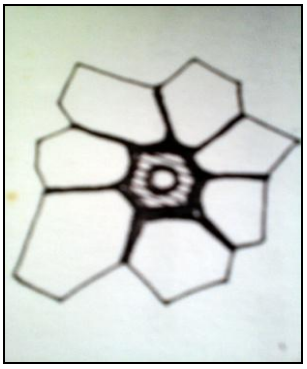
P. galeatum

A. GLANDULAR TRICHOMES:

b. Unmodified Epidermal Cells



P. lonchites



b)Modified epidermal cell: *P. talboti*

CONCLUSION

The present work deals with the laminar anatomical investigations on genus *Piper* of family Piperaceae. 12 species of Piper have been studied in the present work. The family Piperaceae has undergone considerable changes in the circumscription of various taxonomic ranks. From taxonomic point of view, Howard (1973) has considered Piperaceae as the most difficult family. The vegetative morphological characters diagnosed family piperaceae and genus *Piper* to a certain extent, but a large number of species are very intimate by their leaf morphological characters which posed difficulty in determining the group. However, the intrageneric classification of *Piper* (Hooker, 1883) was mainly based on reproductive morphological characters. Hence, an attempt has been made to recognize the taxonomic value of stomatal complex. The leaf anatomical studies is applicable to pharmacognosical importance as well. In the present work, seven species were found to show single type of stomata, however, *P. galeatum* was noticed with combination of two types of stomata. From the intrageneric classification, it has been found that the artificial key to the stomatal complex suggests the predominance of Paratetracytic type of stomata (5 species) followed by staurocytic and anisocytic type of stomata (2 species each). The Paratetracytic types of stomata are also characteristically observed in Section III – Chavica, which covers the majority of the studied species. Detailed study of foliar trichomes have shown abundance of glandular trichomes (unmodified epidermal cells type) in most of the species studied. Hence, the study of the micromorphology of leaves piperaceae members has been concluded to be quite significant taxonomically.

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