



Leaf epidermal anatomy of the genus *Silene* (Caryophyllaceae) from Pakistan

Sumaira SAHREEN^{*1}, Mir Ajab KHAN², Muhammad Rashid KHAN³, Rahmat Ali KHAN³

¹ Botanical Sciences Division, Pakistan Museum of Natural History, Islamabad, Pakistan

² Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan

³ Department of Biochemistry, Quaid-i-Azam University, Islamabad, Pakistan

Abstract

Anatomical studies of 16 species of the genus *Silene* have been carried out from Pakistan by light microscopy. There is considerable variation in leaf epidermal anatomy in the species of *Silene*. In anatomical studies type of stomata, shape and size of epidermal cells, trichomes and crystals are important to identify the taxa at specific level. In almost all the species abaxial surface was different from the adaxial surfaces. Diacytic type of stomata is the diagnostic character of family Caryophyllaceae. In *Silene* basic type of stomata is diacytic but some other stomatal types are also present such as anisocytic and anomocytic. *S.arenosa* can be easily distinguished by cristarque type of crystal. Epidermal cells of *S. moorcroftiana* are larger in size i.e.125 µm in length while in *S. indica* length of epidermal cell is 30µm. In *S. villosa* 4 - 5 - celled glandular hairs are abundantly present that is its species- specific character. The study suggests that the genus *Silene* has both primitive as well as advanced characters. A dichotomous key is constructed for the species identification using the characters that have been studied.

Keywords: *Silene*, Caryophyllaceae, Stomata, Epidermis, Trichomes

1. Introduction

Silene is one of the largest genera of flowering plants in the world consisting of about 700 species (Greuter, 1995; Jurgens et al., 2002; Jurgens, 2004). In Pakistan the genus is represented by 28 species (Ghazanfar and Nasir, 1986). Arora and Panday (1996) and Bakshi (1984) reported the medicinal properties of genus *Silene*.

Anatomical features are of particular value to identify small scraps of plant material (Stace, 1980). Anatomical or endomorphic characters are not appreciably influenced by environmental changes and are basically uniform from one group to another (Bokhari, 1987). Different workers (Ahmad and Safa, 1995; Ahmad, 1997; Aranbari and Colares, 1993; Ataslar, 2004) have utilized epidermal and stomatal characteristics in the systematic studies of some families with some success. Rashid et al. (1987) have worked out the epidermal anatomy of some members of Family Convolvulaceae and Solanaceae. Similarly, Das (2002) worked on the ontogeny of stomata of some Indian mangroves. Gilani et al. (2002) investigated the leaf epidermal anatomy of selected *Digitaria* species. Metcalfe and Chalk (1950) studied the anatomy of caryophyllaceae and reported that generally the stomata are diacytic i.e. caryophyllaceous type but exceptions where stomata are anisocytic. Uniseriate glandular hairs were reported in *Silene*. Calcium oxalate crystals are found commonly in *Diathus* and *Silene*.

Davis (1967) investigated the anatomy of Caryophyllaceae and reported that the glandular and eglandular hairs are of diagnostic value in Caryophyllaceae. The previous anatomy studied on Caryophyllaceae had been done by Metcalfe and Chalk (1983). Akhter and Syed (2006) studied epidermal structures as taxonomic features in some members of Acanthaceae. Jafari et al. (2008) have utilized epidermal characters in the systematic studies of *Silene* species in Iran. Yildiz and Minareci (2008) reported glandular hairs and stomata on both surfaces of leaves of *Silene urvillei*.

* Corresponding author / Haberleşmeden sorumlu yazar: sumairasahreen@gmail.com

Although anatomical evidence has played an important role in discerning natural groups and understanding phylogenetic relationships, however there is a great need for knowledge of anatomical investigation in many plant families including Caryophyllaceae especially from Pakistan. The present study was undertaken to investigate the epidermal structure and to find out whether they can be used for taxonomic identification of some species in genus *Silene*. In this paper leaf epidermal anatomy of the genus *Silene* from Pakistan has been presented for the first time.

2. Material and methods

The anatomical investigations are based on the herbarium specimen obtained from Quaid-i-Azam University, Islamabad (ISL). For epidermal studies Shultze's method of maceration with improved technique was followed (Subrahmanyam, 1996). In a test tube, leaves were boiled with 4 ml of concentrated nitric acid to which 2g of potassium chloride and 1ml of distilled water was added as the epidermis was separated in the form of a thin pellicle, the content of the test tube were emptied in water. Epidermal strips were washed with water and placed on glass slide then poured one to two drops of lactic acid and covered with cover slip. The prepared slides were studied under the light microscope. Permanent slides for anatomical reference collection have been deposited in the Plant Taxonomy Lab, Department of Plant Sciences, Quaid-i-Azam University, Islamabad. Terminology used is after Easu (1965). The following species were studied:

<i>S. apetala</i>	<i>S. arenosa</i>	<i>S. brahuica</i>	<i>S. citrina</i>
<i>S. conoidea</i>	<i>S. falconariana</i>	<i>S. indica</i>	<i>S. kunawerensis.</i>
<i>S. longisepala</i>	<i>S. moorcroftiana</i>	<i>S. ovalifolia</i>	<i>S. pseudo-verticellata</i>
<i>S. tenuis</i>	<i>S. villosa</i>	<i>S. viscosa</i>	<i>S. vulgaris</i>

3. Results

Table 1 summarizes the features of leaf epidermal cells from the taxa examined. Light micrographs of *Silene* species are presented in Figs.1-17.

Size and Shape of Epidermal cells: The shape of epidermal cells is irregular, rectangular and polygonal with smooth, slightly wavy, wavy and highly undulating walls.

10 species viz. *S. apetala*, *S. citrina*, *S. conoidea*, *S. falconariana*, *S. indica*, *S. kunawerensis*, *S. moorcroftiana*, *S. pseudoverticillata* and *S. tenuis* and *S. viscosa* have irregular shape of cells. Similarly, 4 species viz. *S. arenosa*, *S. ovalifolia*, *S. villosa* and *S. vulgaris* have polygonal cells. Epidermal cells of *S. brahuica* and *S. longisepala* are rectangular.

The size of epidermal cells of the species of *Silene* ranges from 30×18µm to 125×65µm. There is great variation in the size of the epidermal cells. Area of epidermal cell appear to be the largest (125×65µm) in *S. moorcroftiana*, and the smallest (30×18µm) in *S. indica*.

Stomata: Stomata are of different types. Eight species viz. *S. arenosa*, *S. brahuica*, *S. conoidea*, *S. kunawerensis*, *S. longisepala*, *S. tenuis*, *S. viscosa* and *S. vulgaris* have diacytic type of stomata while rest of the species have variety of stomata i.e. diacytic, anomocytic and anisocytic. *S. arenosa*, *S. viscosa* and *S. vulgaris* have less number of stomata on adaxial surface as compare to abaxial surface.

Trichomes: Trichomes are absent in *S. apetala* and *S. vulgaris*. Trichomes range from glandular to non- glandular and unicellular to multicellular hairs. *S. longisepala* and *S. moorcroftiana* have same 2-3 celled glandular hairs. Similarly, *S. arenosa*, *S. falconariana*, *S. kunawerensis*, *S. ovalifolia* and *S. viscosa* have non glandular hairs.

Crystals: *S. arenosa*, *S. conoidea*, *S. moorcroftiana*, *S. tenuis*, *S. viscosa* and *S. vulgaris* have different type of crystals on epidermal surface. *S. arenosa* have cristarque cell.

Based on the present observation and variation shown in epidermal structures following key is proposed for identification of investigated species.

Key to *Silene* species

- 1 + Cristarque cell is present..... 1. *S. arenosa*
- Cristarque cell is absent..... 2
- 2 + Trichomes absent, numerous stomata on both surfaces..... 2. *S. apetala*
- Trichomes present, less stomata on adaxial surface..... 3
- 3 + 3-4 celled nonglandular hairs on both surfaces..... 3. *S. viscosa*
- 5-6 celled glandular hairs on abaxial surface..... 4
- 4 + Abaxial and adaxial surfaces have 5-6 celled and 1-3 celled glandular hairs respectively 4. *S. brahuica*
- Nonglandular hairs are present..... 5
- 5 + Highly undulating walls of epidermal cells with blunt ended trichomes..... 5. *S. kunawerensis*
- Smooth walls of epidermal cells with non-blunt ended trichomes..... 6
- 6 + Non-glandular trichomes abundant on abaxial and less on adaxial surface 6. *S. vulgaris*
- Non-glandular trichomes abundant on both surfaces..... 7
- 7 + 5-6 celled nonglandular hairs with polygonal epidermal cells shape 7. *S. ovalifolia*
- 2-3 celled glandular hairs with rectangular epidermal cells shape 8
- 8 + 2-3 celled glandular hairs with smooth walled epidermal cells 8. *S. longisepala*
- 2-3 celled glandular hairs with wavy walled epidermal cells 9
- 9 + Trichomes abundantly present on both surfaces..... 9. *S. citrina*
- Trichomes occasionally present on both surfaces..... 10
- 10 + 1-celled trichome with highly undulating epidermal cell wall..... 10. *S. tenuis*
- 4-5 celled trichome with smooth epidermal cell wall..... 11
- 11 + 4-5 celled glandular hairs..... 11. *S. villosa*
- Trichomes absent..... 12
- 12 + Epidermal wall of abaxial surface wavy..... 12. *S. vulgaris*
- Epidermal wall of abaxial surface smooth to slightly wavy..... 13
- 13 + Diacytic type of stomata with glandular and non-glandular hairs..... 13. *S. conoidea*
- Diacytic, anomocytic and anisocytic stomata with glandular hairs..... 14
- 14 + Rudimentary trichome present..... 14. *S. pseudovercillata*
- Rudimentary trichome absent 15
- 15 + Highly undulating wall of epidermal cells on adaxial surface..... 15. *S. indica*
- Wavy wall of epidermal cells on adaxial surface..... 16. *S. moorcroftiana*

4. Discussion

The present study was restricted mainly to stomatal complex, trichomes and its types, epidermal cell shape and cystoliths i.e. crystals. The results indicate certain facts of taxonomic and phylogenetic importance. The presence of trichomes in different species of genus *Silene* is obvious. Different types of trichomes are found in these species ranging from unicellular, peltate non-glandular to multicellular shaggy and glandular long multiseriate hairs. The leaf epidermal anatomy of different species of Caryophyllaceae has proved to be of much importance in the identification at species level. Davis and Heywood (1963) emphasized the use of anatomical characters, as these are reliable and fairly constant within a taxon. In some species there is difference in the adaxial and abaxial surface. This character has been observed in a few species.

In *Silene* basic type of stomata is diacytic but some other stomatal types are also present such as anisocytic and anomocytic. The present study matches with the result of Grewal (2000) who reported diacytic type of stomata in Caryophyllaceae. Warming (1920) reported that caryophyllaceous type of stomata is found in certain species of Caryophyllaceae with elongated epidermal cells. Stomata sometimes tend to be of the Cruciferous type in these species. In Caryophyllaceae genera can be segregated on the basis of smooth and highly undulating wall of epidermal cells. Jafari et al. (2008) reported crenate subsidiary cells in *S. conoidea* and also entire walls in some species of *Silene*. The present studies are also in agreement with their findings as in *S. conoidea* smooth to slightly undulating wall is found on both surfaces. In some species of *Silene* there are smooth walls like *S. arenosa*, *S. apetala*, *S. brahuica*, *S. longisepala*, *S. ovalifolia* and *S. villosa* on both abaxial and adaxial surfaces. Abaxial and adaxial surfaces of *S. tenuis* and *S. kunawerensis* have highly undulating walls. In *S. indica* and *S. citrina* abaxial surfaces have highly undulating wall with irregular shape of cells while adaxial surfaces have smooth wall with angular cells. In *S. falconariana* abaxial and adaxial both surfaces have smooth walls with polygonal shape of cells and numerous stomata. While *S. viscosa* have wavy walls on abaxial surface and smooth wall on adaxial surface and less stomata.

Table 1: Leaf Epidermal Anatomical Features of *Silene* species

Taxon	Type of stomata	Stomata on abaxial	Stomata on adaxial	Trichome on abaxial	Trichome on adaxial	Shape of crystal	Wall of epidermal cell on abaxial	Wall of epidermal cell on adaxial	Shape of epidermal cell
<i>Silene apetala</i>	Diacytic , Anisocytic, anomocytic	Numerous	Numerous	absent	Absent	Absent	Smooth	Smooth	Irregular, rectangular
<i>Silene arenosa</i>	Diacytic	Numerous	Less	Short non-glandular blunt end	Short non-glandular blunt end	Cristarque cell	Smooth	Smooth	Polygonal
<i>Silene brahuica</i>	Diacytic	Numerous	Numerous	5-6 celled, glandular	1-3 celled glandular	Absent	Smooth	Smooth	rectangular
<i>Silene citrine</i>	Diacytic , anisocytic	Numerous	Numerous	5- 6 celled abundant, non-glandular +	1 celled abundant, glandular non	Absent	Smooth	Highly sinuous	Irregular ,polygonal
<i>Silene conoidea</i>	Diacytic	Numerous	Numerous	3 -4 celled abundant, non-glandular +	3 -4 celled less, non glandular +	Present	Smooth to slightly wavy	Smooth to slightly wavy	Irregular
<i>Silene falconariana</i>	Diacytic , Anisocytic, anomocytic	Numerous	Numerous	abundant, non-glandular	less, non glandular	Absent	Smooth to slightly wavy	Smooth	Irregular
<i>Silene indica</i>	Diacytic , Anisocytic ,anomocytic	Numerous	Numerous	1-celled, occasionally present	1-celled occasionally present	Absent	Smooth	Highly undulating wall	Irregular, angular
<i>Silene kunawerensis</i>	Diacytic	Numerous	Numerous	3 -4 celled non glandular , apex blunt	3 -4 celled non glandular , apex blunt	Absent	Highly undulating wall	Highly undulating wall	Irregular
<i>Silene longisepala</i>	Diacytic	Numerous	Numerous	2- 3 celled, glandular	2- 3 celled, glandular	Absent	Smooth	Smooth	Rectangular angular
<i>Silene moorcroftiana</i>	Diacytic , anisocytic	Numerous	Numerous	2- 3 celled, glandular	2- 3 celled, glandular	Present	± wavy wall	Wavy wall	Irregular angular
<i>Silene ovalifolia</i>	Diacytic , Anisocytic ,anomocytic	Numerous	Numerous	5- 6 celled abundant, non-glandular	5- 6 celled abundant, non -glandular	Absent	± smooth	± smooth	polygonal
<i>Silene pseudoverticillata</i>	Diacytic , Anisocytic, anomocytic	Numerous	Numerous	Rudimentary hairs	Rudimentary hairs	Absent	± wavy wall	Wavy wall	Irregular angular
<i>Silene tenuis</i>	Diacytic	Numerous	Numerous	1-celled occasionally present	1-celled occasionally present	Present	Highly undulating wall	Highly undulating wall	Irregular
<i>Silene villosa</i>	Diacytic , Anisocytic ,anomocytic	Numerous	Numerous	4- 5 celled glandular	4- 5 celled glandular	Absent	Smooth	Smooth	polygonal
<i>Silene viscosa</i>	Diacytic	Numerous	Less	3 -4 celled abundant, non-glandular	3 -4 celled less non-glandular	Present	Smooth	wavy wall	Irregular
<i>Silene vulgaris</i>	Diacytic	Numerous	Less	Absent	Absent	Present	± wavy wall	Smooth	polygonal

±= more or less



Fig 1: Abaxial surface of *Silene apetala* showing stomata and epidermal cells

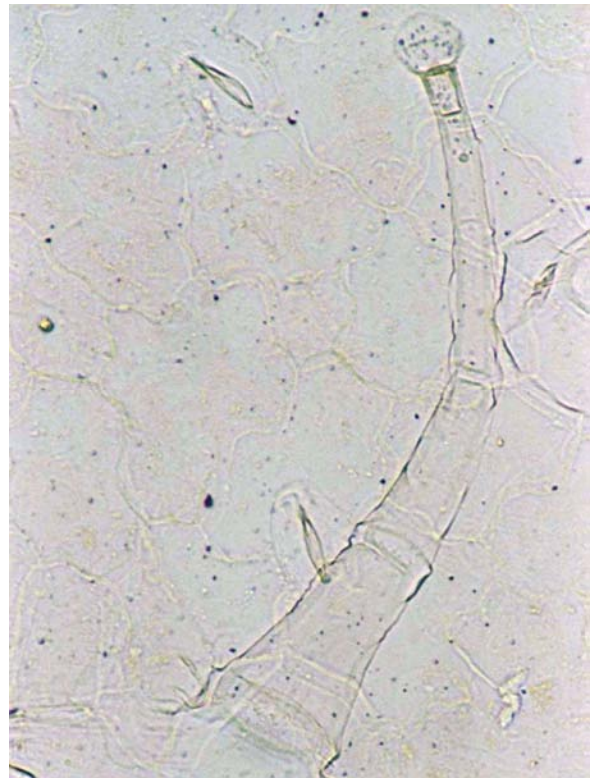


Fig 3: Abaxial surface of: *Silene brahuica* showing 5-6 celled glandular hair

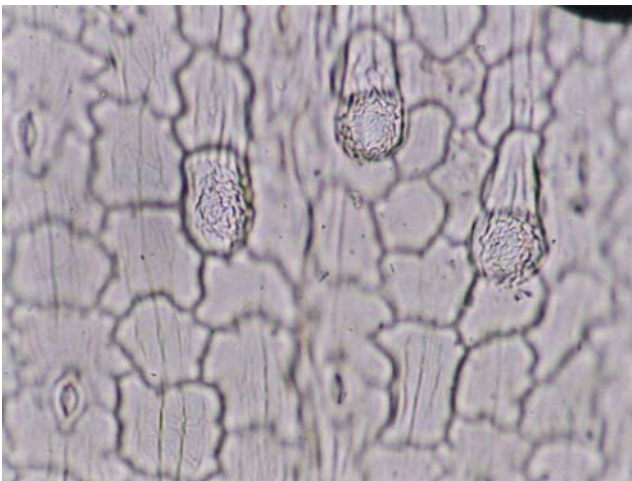


Fig 2: Adaxial surface of *Silene arenosa* showing cristate cells, stomata and epidermal cells

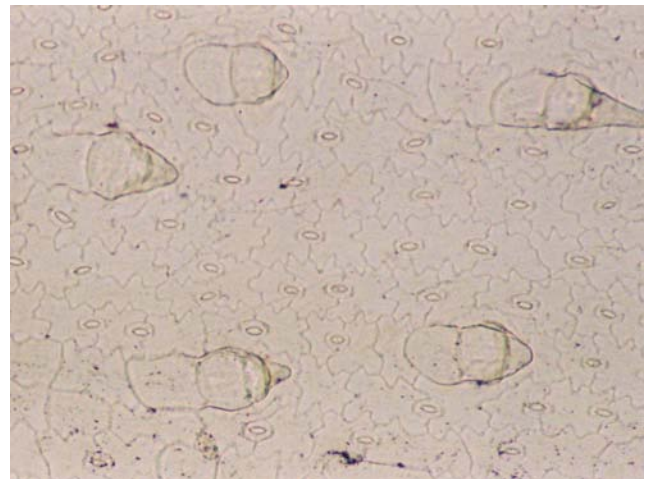


Fig 4: Adaxial surface of *Silene citrina* showing non glandular trichome, stomata and epidermal cells

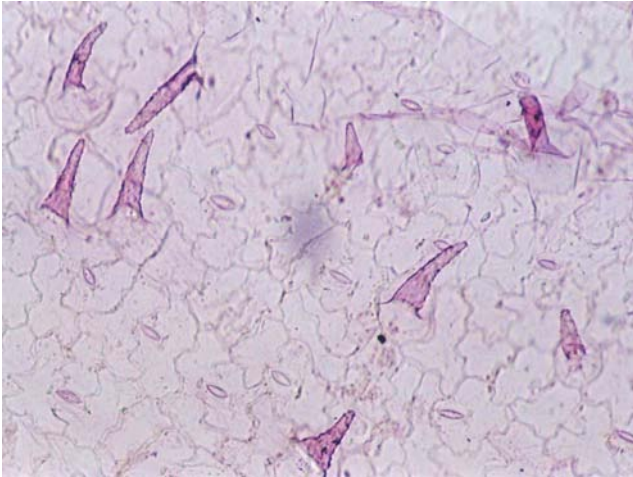


Fig 5: *Silene conoidea* showing trichomes, stomata and epidermal cells

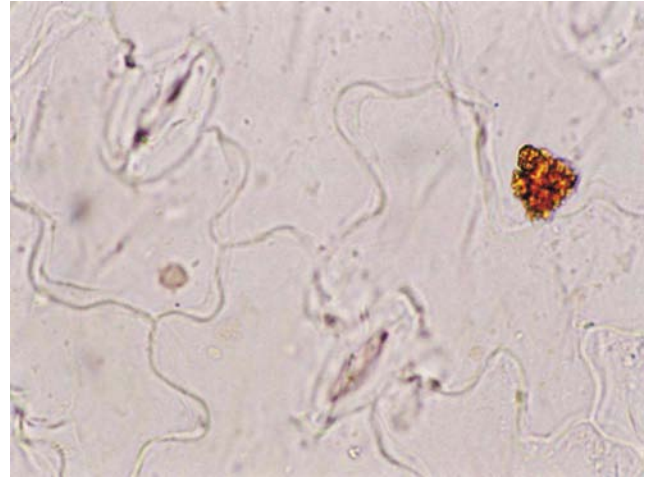


Fig. 7: Adaxial surface of *Silene indica* showing stomata and highly undulating wall



Fig 6: Abaxial surface of *Silene falconariana* showing trichomes, stomata and epidermal cells



Fig. 8: *Silene kunawerensis* showing 3-4 celled non glandular hairs, stomata and epidermal cells

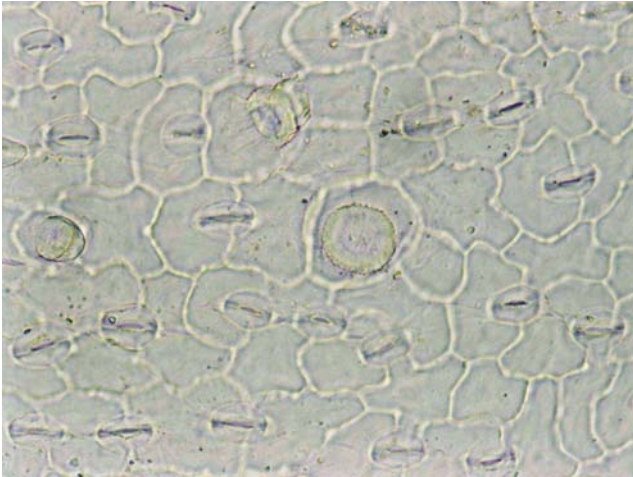


Fig. 9: *Silene longisepala* showing stomata and epidermal cells

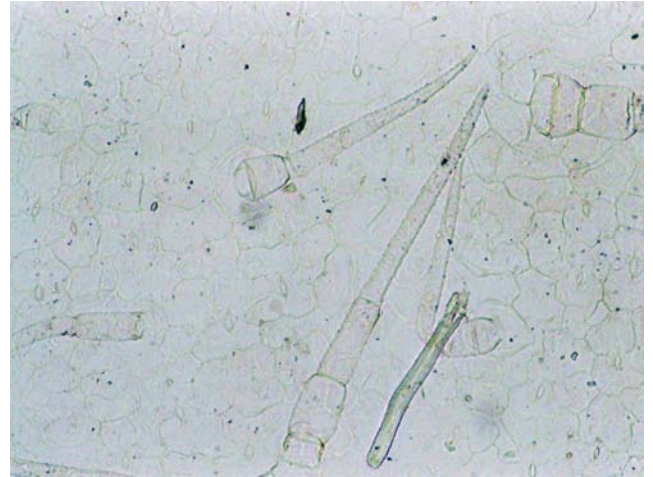


Fig. 11: *Silene ovalifolia* showing 5-6 celled non glandular hairs

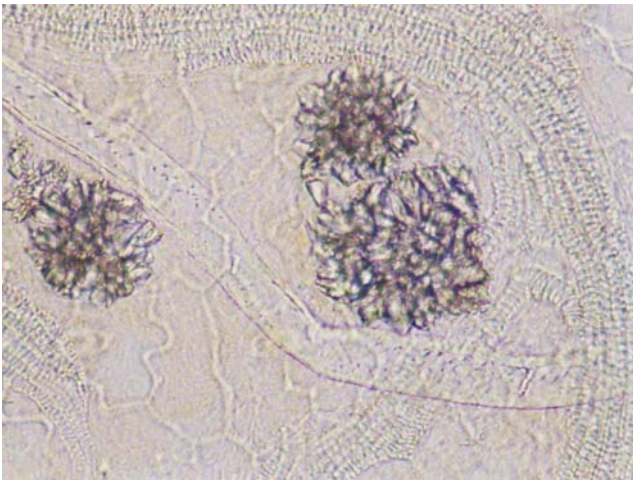


Fig. 10: Abaxial surface of *Silene moorcroftiana* showing crystals and epidermal cells

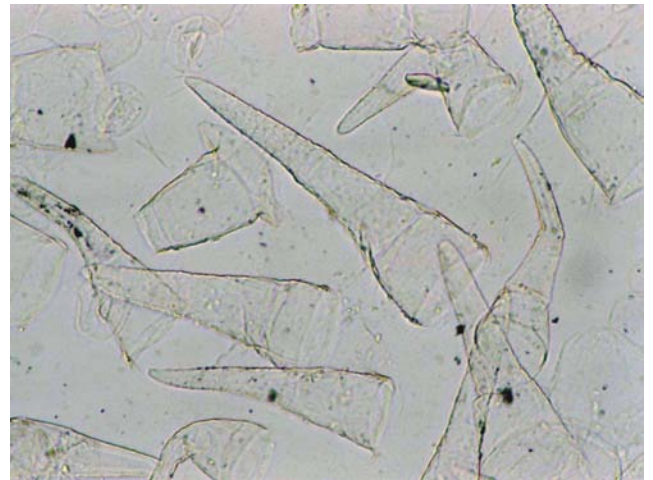


Fig. 12: *Silene pseudoverticillata* showing trichomes



Fig. 13: *Silene tenuis* showing stomata and epidermal cells



Fig. 15: *Silene viscosa* showing non glandular hairs



Fig. 14: *Silene villosa* showing 4-5 celled glandular hairs , stomata and epidermal cells

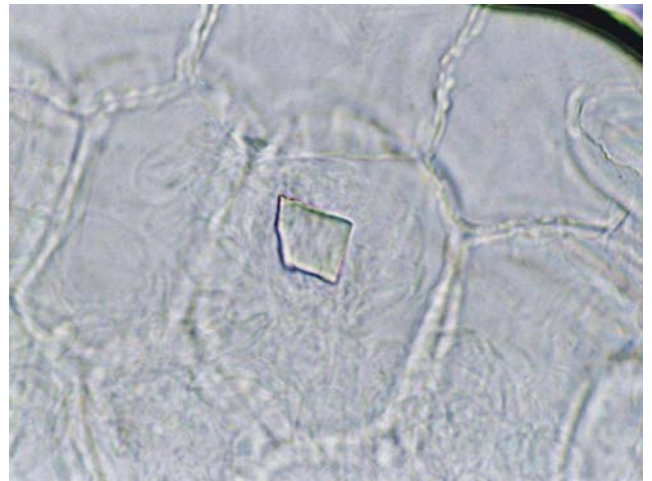


Fig. 16: *Silene viscosa* showing crystal

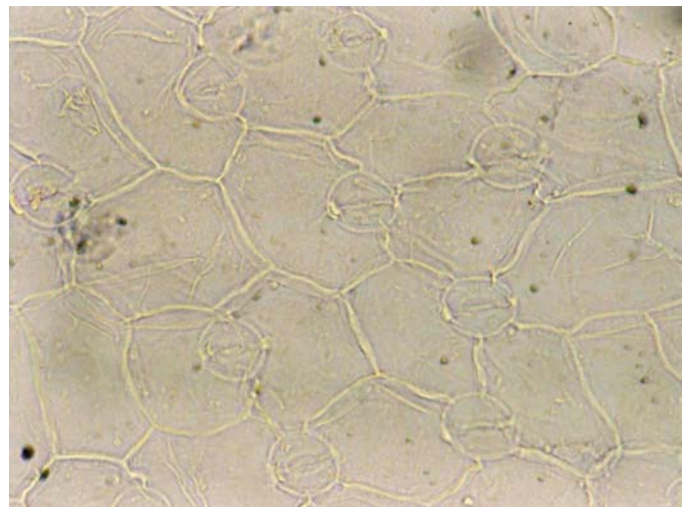


Fig. 17: *Silene vulgaris* showing stomata and epidermal cells

Stomatal variation in some cases is helpful at higher level of taxonomic hierarchy (Stace, 1965). *S. vulgaris* and *S.arenosa* have less number of stomata on adaxial surface as compare to abaxial.

Epidermal cells of *S. tenuis* are larger in size i.e.132 µm in length while in *S. vulgaris* length of epidermal cell is 30µm.This shows considerable variation between the two species.

Stomatal features are taxonomically important for identification and delimitation of various taxa at different taxonomic levels. 31 distinct patterns are found only in Pteriophytes (Dilcher, 1974). Diacytic type on both surfaces of stomata on both surfaces are found in *S. arenosa*, *S. brahuica*, *S. longisepala*, *S. conoidea*, *S. vulgaris* and *S. viscosa*. Rest of the studied species have diacytic type of stomata along with anisocytic and anomocytic.

Trichome morphology and distribution can yield important clues regarding specific, generic, tribal and subfamilial relationship. In a number of instances, hairs are species- specific. The use of pubescence characters to support generic relationships or differences established on the basis of other characters have been established in a number of diverse families, including Asteraceae, Icacinaceae, Goodeniaceae, Ericaceae and Graminae (Dickison, 2000). In *S. indica*, *S. tenuis*, *S. apetala* and *S. vulgaris* trichomes are occasionally found. In *S. villosa* 4 – 5 - celled glandular hairs are abundantly present. In *S. conoidea* 3 – 4 - celled non-glandular hairs are abundant on abaxial while less on adaxial surface. In *S. brahuica* 5 – 6 - celled non-glandular hairs are found on abaxial while 1 – 3 - celled trichomes are present on adaxial surface. In *S. citrina* 5 – 6 - celled glandular and non-glandular hairs are found commonly while on adaxial surface 1-celled hairs are common.

Metcalf and Chalk (1950) reported that uniseriate hairs with a glandular cell at apex recorded in *Silene* and hair structure believed to be of specific diagnostic value in different species of Caryophyllaceae. In *S. ovalifolia* 5 – 6 celled hook-like hairs with acute tip are abundantly present. In *S. longisepala* and *S. moorcroftiana* 2 – 3 - celled glandular hairs are found on both surfaces. These results are in conformity with that of Yildiz & Minareci (2008). In *S. arenosa* short hairs with mucronate or blunt ends are found. In *S. pseudoverticellata* rudimentary trichomes are present.

Stace (1980) found that trichome anatomy is of immense importance in the family Combretaceae and it proved helpful in classification at all levels from the family down to the separation of species and even varieties.

Crystalline substances of varied form and chemical composition are found in the cells, cell walls and intercellular spaces of plant tissues. An unusual crystal cell of special taxonomic significance is the cristarque cell. Cristarque cell are highly diagnostic because they occur only in few families (Dickison, 2000). Metcalfe and Chalk (1950) reported that calcium oxalates are commonly present in the form of large conspicuous cluster crystals in many genera and species including *Silene*. The abundance of the crystals some times varies within single species in specimens from different localities.

In *S. arenosa* cristarque cells are present on adaxial surface that is the species-specific character. Different types of crystals are found in *S. vulgaris*, *S. conoidea*, *S. tenuis*, *S. viscosa* and *S. moorcroftiana*. These anatomical features observed on the leaves are consistent with those of Metcalfe and Chalk (1950).

From this preliminary study of genus *Silene* it appears that it is an advanced genus having mostly anomocytic, anisocytic and diacytic stomata, and unicellular to shaggy multiseriate trichomes. The present results are in agreement with Takhtajan (1980), who reported that stomata with two subsidiary cells are more primitive features and those without subsidiary cells are advanced features.

It is concluded that anatomical epidermal features can help in identification and classification of taxa up to the species level in the genus *Silene*.

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