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MICROMORPHOLOGICAL INVESTIGATION OF FOLIAR ANATOMY OF *FAGOPYRUM* MILL., AND *RUMEX* L. OF POLYGONACEAE

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Abstract

Leaf epidermal studies have been carried out on eleven species belonging to *Fagopyrum* Mill., and *Rumex* L. of the family Polygonaceae. Comprehensive micromorphological studies of *Fagopyrum* Mill., and *Rumex* L. species have been made for the first time. The use of light microscopy has made possible in depth to study leaf surface features such as shape of epidermal cells, stomatal pattern, their distribution on adaxial and abaxial leaf surface and trichome types. Epidermal cell shapes are variable but generally polygonal. Five different stomatal patterns are reported for Polygonaceae. Variation among glandular and non glandular trichomes is also noted. Crystalliferous cells are recorded for the first time in *Rumex nepalensis* Spreng. This anatomical study has taxonomic importance, on the basis of which identification keys are prepared

Introduction

Polygonaceae is a cosmopolitan family containing approximately 1,200 species in 48 genera (Freeman & Reveal, 2005). They are group of morphologically different herbs, shrubs, small trees or climbers characterized by simple leaves with ochrear stipules, unilocular ovary and endospermic seeds (Hutchinson & DaLziel, 1954; Brummitt, 1992). Regardless of the dissimilarities, Polygonaceae is considered as monophyletic assemblage of plants by several authors (Chase *et al*., 1993; Lledo *et al*., 1998; Lamb-Frye & Kron, 2003). Members of the family are disseminated worldwide, from the tropics to the arctic, but most of the species are concentrated in the northern temperate region (Heywood, 1978). In Pakistan family is represented by 19 genera and 103 species (Qaiser, 2001).

The genus *Fagopyrum* Mill., consists of 16 species, a few of which have been newly discovered (Ohnishi, 1998) and only 4 species are found in Pakistan (Qaiser, 2001). The genus is distinguished by its petaloid non accrescent tepals, stamens eight in number, filaments flattened, three short and curved styles and trigonous, beaked and angular fruit (Decraene & Akeroyd, 1988; Decraene *et al*., 2000).

Rumex L. is a genus of more than 200 species, distributed in temperate regions particularly in the northern areas of both parts of the world represented in Pakistan by 15 species and two hybrids (Rechinger, 2001). It is characterized by the presence racemose or paniculate inflorescence, 6 tepals in two whorls, inner whorl enlarged in fruiting stage (valve) and midvein form tubercles (Li *et al.*, 2003).

In this study 4 species of *Fagopyrum* Mill., and 7 species of *Rumex* L. were included for foliar epidermal investigation. This is the first report on the investigation of the foliar anatomy of these two genera and these studies have adequately revealed the reliability of foliar epidermal characters. Inamdar (1971) worked on epidermal structure and stomatal development of *R. hastatus* D.Don, *R. dentatus* L. and *F. debotrys* (D. Don.). Hara & Hong (2001) investigated tepal surface micromorphological characters in the genus *Fagopyrum* Mill. Ayodele and Olwokudejo (2006) reported leaf anatomy of two West African species of *Rumex* L. (*R. abyssinicus* Jacq. and *R. bequaertii* De Willd).

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The main objectives of this survey, is to add more information about leaf anatomy so as to distinguish taxa of the two genera on the basis of both qualitative and quantitative characters of epidermal cells, stomata, glandular and non glandular trichomes. In addition, to determine the differences in epidermal cells shape, stomata and trichomes types at upper and lower epidermis and examine which type is most common in the species of *Fagopyrum* Mill., and *Rumex* L.

Materials and Methods

Dried leaves of representative specimens from Quaid-i-Azam University herbarium, Islamabad, Pakistan, of the two genera of Polygonaceae listed in Table 1 were used for anatomical studies. Dried leaves were placed in boiling water for few minutes to soften until they became unfolded and ready for epidermal scrapping. Leaf samples were prepared according to modified method of Cotton (1974), who followed Clark's (1960) technique. The leaves were placed in a tube filled with 88% Lactic acid kept hot in boiling water bath (Model, Memmert-91126-FRG, Germany) for about 30 to 40 minutes. Lactic acid softens the leaf due to which it was possible to scrap the leaf surface with sharp scalpel. Slides of both abaxial and adaxial surfaces of leaf were prepared and mounted in clean 88% Lactic acid. Both qualitative and quantitative micromorphological foliar characteristics were observed using LM. Microhistological photographs of both surfaces were taken by Nikon (FX-35) Camera equipped light microscope.

Results and discussion

This study includes shape of leaf epidermal cells, stomatal types and trichomes (glandular and non glandular) on adaxial and abaxial surfaces both quantitatively and qualitatively, although qualitative characters play key role in the identification of species as compared to quantitative characters. Epidermal characters have potential taxonomic significance and are helpful as an additional taxonomic character (Stace, 1965; Baronova 1992).

The characteristics of leaf epidermis of *Fagopyrum* Mill. and *Rumex* L. species of the Polygonaceae under LM observations are listed in Tables 2 and 3.

Fagopyrum Mill.

Foliar anatomical investigations of *Fagopyrum* Mill. species under observation are made for the first time. Among the taxa of *Fagopyrum* Mill., the shape of epidermal cells vary from smooth and thick walled pentagonal, hexagonal, heptagonal, polygonal cells to irregular cells with undulating walls. Epidermal cells n *F. gilesii* are irregularly shaped on adaxial surface while smooth walled polygonal cells on abaxial surface (Table 2). Rest of species are with tetrahedral, pentagonal, hexagonal epidermal cells with smooth and thick walls on adaxial surface and irregular cells with undulate, sinuate walls on abaxial surface showing mesomorphic nature of the species (Stace, 1965). Quantitative data indicate smallest cell size in *F. tataricum* adaxial surface (25-47.5×12.5-20 µm) and largest in *F. esculentum* adaxial surface (50-90×25-75 µm).

The size and shape of stomata are taxonomically important characters (Tahir & Rajput, 2009). Leaves in the genus are amphistomatic, common type of stomata are paracytic. Staurocytic pattern is seen only in *F. tataricum* along with anomocytic type (Figs. 1 & 2). Inamdar (1971) reported anomocytic, anisocytic and paracytic stomata in *F. debotrys*. Quantitatively, stomatal length on adaxial surface of *F. gilesii*, *F. debotrys*, *F. esculentum* and abaxial surface of *F. tataricum* is more or less same.

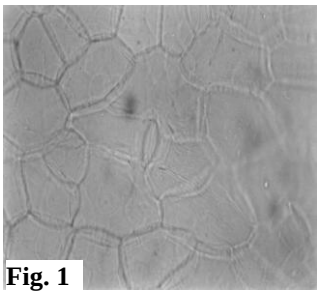


Fig. 1

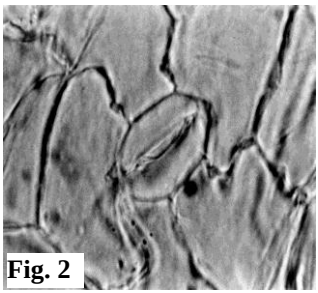


Fig. 2

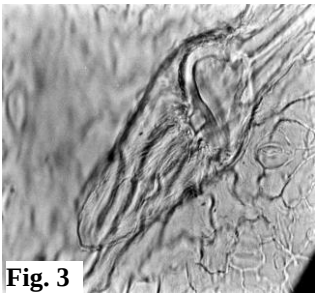
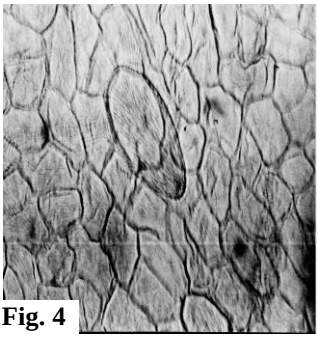


Fig. 3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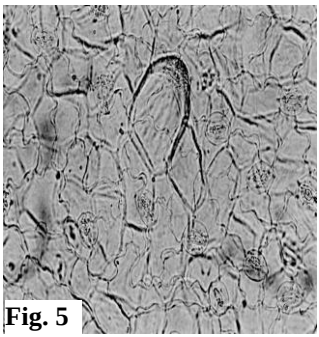


Fig. 5

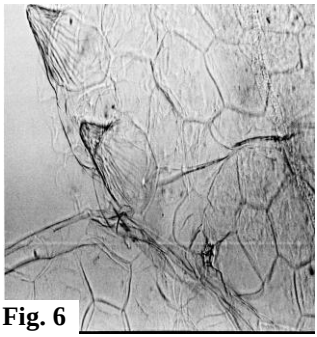


Fig. 6

Fig. 1. Anomocytic stomata in *F. tataricum* (400X).
Fig. 2. Staurocytic stomata in *F. tataricum* (1000X).
Fig. 3. Papillae with basal partition in *F. debotrys* (1000X).
Fig. 4. Baloon shaped papillae in *F. esculentum* (400X).
Fig. 5. Papillae without partition in *F. tataricum* (400X).
Fig. 6. Papillae and 2-celled glandular trichome in *F. debotrys* (200X).

Epidermal glands are of significant importance in relative investigations in angiosperms, which originate as of epidermal cells (Werker, 2000). Metcalfe and Chalk (1950) provided little information about trichomes in Polygonaceae. Eglandular trichomes in the form of papillae are present in all taxa of *Fagopyrum* Mill. except *F. gilesii* and are useful for the species delimitation. These are of three different types:

1. Angular papillae with central or basal partition in *F. debotrys* (Figs. 3 and 6).
2. Baloon shaped papillae in *F.esculentum* (Fig. 4).
3. Papillae without partition but with striations in *F.tataricum* (Fig. 5).

Inamdar (1971) recorded unicellular and bicellular peg like trichomes in *F. debotrys*. Hong (2001) during his investigation on tepal surface micromorphology reported papillate tepal epidermises and suggested their role in entomophilly. Presence of papillae is considered to be an ancient trait (Glover and Martin, 2002). The length of blunt ended papillae without partition but with striations varies from 130-160 µm in *F. tataricum* abaxial surface while width of papillae in all species is almost same (Table 2). Therefore all four species of *Fagopyrum* Mill. can be distinguished from each other on the basis of presence or absence of papillae and type of papillae. Among the glandular trichomes, 2-celled thick walled peltate trichomes are observed in all species of *Fagopyrum* Mill. with variation in the distribution pattern on both surfaces. In *F. tataricum*, size of trichome is more or less same on both leaf surfaces and varies from 12.5-20×10-25 µm. Largest trichomes are noted in *F. esculentum* abaxial surface i-e, 45-62.5×37.5-62.5 µm (Table 2).

Key to *Fagopyrum* Mill. Species

1a: Epidermal cells on adaxial surface irregular, papillae absent ……. 1. *F. gilesii*
1b: Epidermal cells on adaxial surface not irregular, papillae present …….. 2
2a: Stomata anomocytic and striated angular papillae with basal or central partition …….. 2. *F. debotrys*
2b: Stomata anomocytic, staurocytic or paracytic, papillae balloon shaped or blunt ended without partition …… 3
3a: Stomata paracytic, shape of papillae is balloon like ..…… 3. *F. esculentum*
3b: Stomata anomocytic and staurocytic, papillae blunt ended without partition but with striations ……. 4. *F. tataricum*

***Rumex* L.**

The present investigation represents first detailed qualitative and quantitative study of leaf epidermis in *Rumex* L. species. Joshi (1935) worked on the anatomy of *Rumex* L. with respect to the morphology of the internal bundles and the origin of the internal phloem. Inamdar (1971) worked on epidermal structure and stomatal development of *R. hastatus* and *R. dentatus*. Ayodele and Olwokudejo (2006) studied epidermal anatomy of *R. abyssinicus* and *R. bequaertii* in West Africa.

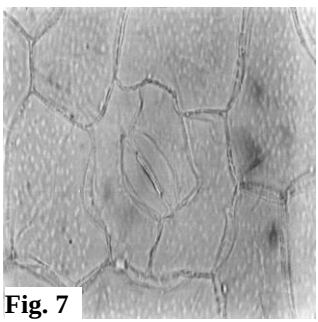
In comparison to the majority of the species of *Fagopyrum* Mill. where irregular epidermal cells with undulating walls are restricted to abaxial surface, in *R. acetosa, R. chalepensis* (Fig. 8), *R. dentatus* and *R.nepalensis* adaxial cells are irregular in shape with undulating walls similar to *F. gilesii*. In *R. vesicarius* and *R. hastatus* cells are polygonal on both while pentagonal and hexagonal shape cells are seen in *R. patientia* leaf surfaces (Table 3). Thick and pitted walls which are characteristic features of the genus *Polygonum* L. is noted only in *R. nepalensis*. Size of epidermal cells varies from 35-70×25-40 µm on adaxial surface of *R. patientia* which appears to be minimum while maximum in *R. vesicarius* abaxial surface i-e., 50-125×30-100 µm (Table 3).

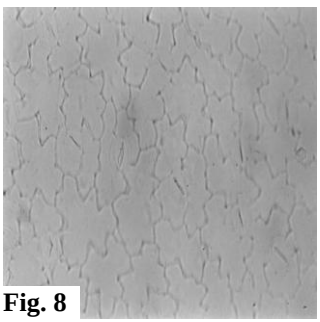
Stomata are distributed on both leaf surfaces and pericytic is the most common type of the stomata followed by anisocytic and staurocytic type (Fig. 7 and 9). More than one type of stomata are also present on the same surface of single species i-e., pericytic and anisocytic stomata are seen in *R. acetosa*, *R. dentatus* and *R. patientia*. The presence of different stomatal types on the same surface of single species is an important taxonomic character (Ayodele and Olwokudejo (2006). Staurocytic stomata are noted in *R. patientia* which can serve as its distinguishing character (Fig. 9). Inamdar (1971) reported paracytic stomata in *R. dentatus* and *R. hastatus*. Ayodele and Olwokudejo (2006) noted anomocytic and diacytic stomata in *R. bequaertii*. Recently Ahmad *et al.,* (2009) reported amphianisocytic stomatal pattern in *R. vesicarius* which does not correspond to the present findings of pericytic stomata for the same species. Size of stomata on both epidermises of *R. hastatus* is nearly same (17.5-33×10-15 µm) while stomatal length on adaxial surfaces of *R. dentatus* and *R. nepalensis* is of same range (35-40 µm).

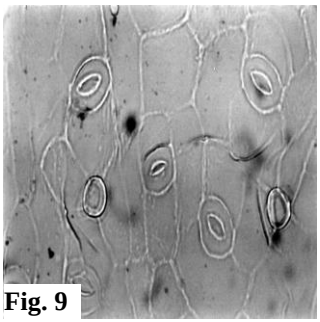
Glandular trichomes could be considered facultative salt glands and they may be part of apparatus of dispersion of extreme radiation (Tattini and Gucci, 1999). Non glandular trichomes are totally absent in the taxa of *Rumex* L. while glandular trichomes are peltate and 1-4 celled centrally (Fig 9, 10 and 11). *R. hastatus* has 2-4 celled peltate trichomes on both surfaces, *R. nepalensis* possesses 1, 2 and 4-celled trichome only on abaxial surface and *R. vesicarius* has 2 and 4-celled trichome on abaxial surface while adaxial surface possesses only 2-celled trichome. Quantitative data shows that the length of 4-celled trichomes is somewhat greater on the abaxial surface of *R. vesicarius* and *R. nepalensis*

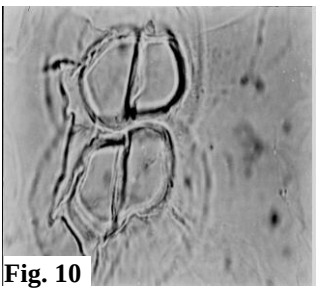
(25-40 µm) than that of *R. hastatus* abaxial surface. 2-celled peltate trichome on adaxial surface of *R. chalepensis* is 10-20×5-15 µm which is recorded to be smallest in size (Table 3). 3-celled peltate trichomes of *R. hastatus* were of same size on both leaf surfaces (Table 3).

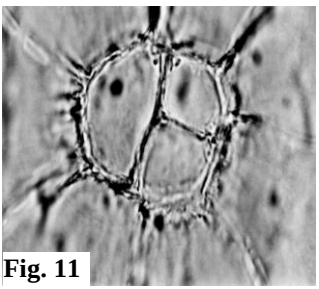
One of the important characters noted in *R. nepalensis* is the presence of crystalliferous cells in epidermis, not seen in any other genus of Polygonaceae and other *Rumex* L. species under observation (Fig. 12 and 13). So it can be distinguished from rest of *Rumex* L. species. Crystalliferous cells in epidermal cells were observed for some species of Polygonaceae by Solereder (1908), Inamdar (1971) and Lersten and Curtis (1992). Stern and Carlsward (2006) in their anatomical and systematic study of *Oncidiinae* (Orchidaceae) also observed crystalliferous, circular cells throughout the mesophyll.

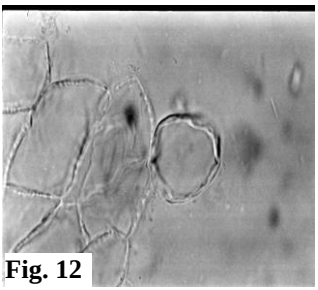












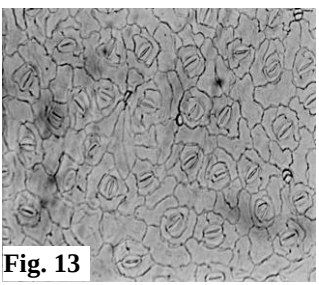


Fig. 7. Anisocytic stomata in *R. acetosa* (1000X).
Fig. 8. Irregular cells with undulating walls in *R. chalepensis* (400X).
Fig. 9. Staurocytic stomata and 1-celled peltate trichome in *R. patientia* (400X).
Fig. 10. 2-celled peltate trichomes lying side by side in *R. hastatus* (1000X).
Fig. 11. 3-celled peltate trichomes in *R. hastatus* (1000X).
Fig. 12. An enlarged crystalliferous cell in *R. nepalensis* (1000X).
Fig. 13. Low focus crystallifeous cells in *R. nepalensis* (200X).

Key to *Rumex* L. species

1a: Epidermal cells on adaxial surface pentagonal and hexagonal, stomata staurocytic type …….. 1. *R. patientia*
1b: Epidermal cells on adaxial surface irregular or pentagonal, hexagonal or polygonal, stomata not staurocytic type ……..……. 2
2a: Epidermal cells pentagonal, hexagonal and polygonal on abaxial surface, large sized epidermal cells on adaxial surface 97×60 µm …..…….. 2. *R. dentatus*
2b: Abaxial epidermal cells polygonal, average size of adaxial cells smaller than that of 97×60 µm ……..…… 3
3a: Peltate glands absent on adaxial surface, crystalliferous cells present ……..... …….. 3. *R. nepalensis*
3b: Peltate glands present on adaxial surface, crystalliferous cells absent …… 4
4a: Stomata anisocytic and pericytic, length of epidermal cells on both surfaces same …… 4. *R. acetosa*
4b: Stomata only of pericytic type, epidermal cells not of same length on both leaf surfaces ……..……. 5
5a: Epidermal cells on adaxial surface irregular in outline, peltate trichomes only 2-celled ……..…… 5. *R. chalepensis*
5b Epidermal cells on adaxial surface polygonal, peltate trichomes 2-4 celled ……..……. 6
6a: 3-celled peltate trichomes present, same width of stomata on adaxial and abaxial surface (10-15 µm) ……...… 6. *R. hastatus*
6b: 3-celled peltate trichomes absent, different width of stomata on both surfaces …… 7. *R. vesicarius*

Conclusion

The present paper presents number of vital micromorphological features and most of these characters are extremely stable in two genera. Most of the anatomical characters are not decisive features at specific level. Nevethless, there are some differences that are of worth in recognition and delimitation of the taxa.

The study of epidermal surfaces both qualitatively and quantitatively reveals some new types of stomata described in present work which are not described previously, such as staurocytic type and pericytic type.

Recent work shows that these genera cannot be distinguished on the basis of trichome type, although to some extent e.g., presence of papillae in *Fagopyrum* Mill. species which serve as their distinguishing character by their presence or absence and type of papillae.

During the present survey crystalliferous cells are recorded for the first time in *R.nepalensis* and characterize the species, not already described for *Rumex* L. species. Overall epidermal characters are useful in identification of different taxa in Polygonaceae.

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