Full Length Article



Systematic Value of Foliar Anatomical Features in *Polygonum* species, Family Polygonaceae from Pakistan

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ABSTRACT

A study of foliar epidermis in thirteen species of *Polygonum* was performed. The study revealed some distinctive anatomical features such as epidermal cells outline, single or mixed type of stomata on the single leaf surface and presence or absence of trichomes adaxially and abaxially. The shape of epidermal cells was variable; stomata were constant in most of the species and one-three celled glandular peltate trichomes were examined in few species, while non-glandular trichomes were totally absent. Although the analyzed epidermal characters have systematic value, they are to likely be stable at specific level.

Key Words: Polygonaceae; Trichomes; Anaisocytic; Staurocytic; Peltate

INTRODUCTION

Polygonaceae is a large dicot family of 48 genera and 1200 species (Freeman & Reveal, 2005; Sanchez & Kron, 2008). In Pakistan it is represented by 19 genera and 103 species (Qaiser, 2001).

Polygonum L. comprises of about 60 species, distributed nearly worldwide and in Pakistan represented by 20 species (Qaiser, 2001). The genus is characterized by prostrate habit, sometimes subprostrate or erect, alternate leaves, presence of ochreae on nodes, axillary flowers, tepals with only one main vein, stamen in two whorls, outer smaller in size than inner, filaments swollen at the base and absence of nectaries (Ronse Decraene & Akeroyd, 1988).

The systematic potential of foliar anatomical features is well recognized in botanical literature (Stace, 1965). For instance, epidermal characters of different genera of Polygonaceae have been studied by Metcalfe and Chalk (1950), Inamdar (1971), Kapoor *et al.* (1971), Mitchell (1971), Lersten and Curtis (1992), Ayodele and Olowokudejo (2006) and Yasmin *et al.* (2009) and found that epidermal characters are increasingly important source of taxonomic value. The intention here is to present a more precise characterization of the foliar epidermal anatomy with light microscopy and to identify the taxonomic potential of the characters in order to identify different species of the genus.

MATERIALS AND METHODS

Dried leaves (4-5) of representative specimens from

Quaid-i-Azam University Herbarium, Islamabad, Pakistan of Polygonum L. of the family Polygonaceae given in Table I, were used for anatomical studies. Dried leaves were placed in boiling water for few minutes so that they became soft and unfolded and then 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 min. 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 surface 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.

Cluster analysis. A dendrogram was constructed by unweighted paired group method with EUCLIDEAN option, using MVSP software version 3.13 (Kovach, 2007).

RESULTS AND DISCUSSION

In the present study foliar anatomy of 13 species of the genus *Polygonum* L. was analyzed by its qualitative and quantitative characters. In the taxa of *Polygonum* L. the epidermal cells varied from tetrahedral, pentagonal, hexagonal and heptagonal, polygonal benzene ring like cells with thick pitted walls to irregular in shape with undulating walls (Fig. 1 A, E-G). In *P. cognatum*, epidermal cells on adaxial surface were polygonal, while irregularly shaped

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B. P. cognatum. Two-celled peltate trichomes (1000X)

Fig. 1. Light micrographs (LM) of foliar anatomy of selected species of the genus Polygonum L.

A. *P. cognatum.* A Polygonal, thick and pitted walled cells with anisocytic stomata (400X)



D. *P. arenastrum.* **D.** Epidermal cells with anisocytic stomata (200X).



E. *P. sarobiense.* Irregularly shaped epidermal cells (200X)

C. P. cognatum. Three-celled peltate gland with radiating walls (1000X)



F. *P. plebijum*. Irregular pitted epidermal cells with pericytic stomata (400X)



G. *P.polycnemoides.* Benzene ring shaped epidermal cells with anisocytic stomata (400X)





H. *P. polycnemoides*. Three-celled peltate glands (400X)





I. *P. patulum.* Epidermal cells shape and anisocytic stomata (200X)



Species	Locality	District	Collector name	Acc. No
P.aviculare L.	Kawas	Quetta	Manzoor and Maqsood	69130
	Near Babusar top	Hazara	M.N. Choudhari et al.	122645
P. paronychioides C.A. May. ex Hohen	Chanbhri	Muzaffarabad	Shahzad et al.	940070
	Batakundi	Hazara	Mir Ajab and M. Afzal	74462
P. afghanicum Meisn	Landi Kotal	Khyber agency	M. Tanveer and Dilawar	106327
P. olivascens Rech. f. & Schiman-Czeika	Kolpur	Quetta	Manzoor and Maqsood	54286
P. rottboellioides Jaub. & Spach	4 miles on the way to Naltar	Northern areas	M.N. CHoudhari et al.	117938
P. molliaforme Boiss	Gitti Das	Hazara	Iqbal Dar <i>et al</i> .	09230
P. patulum M. Bieb.	Nagar	Gilgit	Mir Ajab and M. Afzal	58927
	Mistar	Chitral	Wali-Ur-Rehman and Subhan	116202
P. polycnemoides Jaub. & Spach	Dudnial (stony soil)	Muzaffarabad	Shahzad and Ayaz	69157
	Uacub	Chitral	Wali-Ur-Rehman	116176
P. cognatum Meisn	Babusar top	Hazara	Iqbal Dar <i>et al</i> .	09245
P. sarobiense Rech. f.	Meiragean	Chitral	Wali-Ur-Rehman	116235
P. plebijum R. Br.	Dhulli	Poonch	Bashir Ahmad and Javed	109471
* •	Kot Jamal	Mirpur	Shahzad and Arif	49423
P. effusum Meisn.	Bajur	Dir	Ghulam Farooq	26448
	Nawal Nadi	Poonch	M.N. Choudhari et al.	26507
P. arenastrum Boreau	Nall	Khuzdar	Muqarrab Shah and Dilawar	108251

Table I. List of species investigated for anatomical studies with location, district Collector name and accession numbers

cells on its abaxial surface. Irregular cells with pitted, thick and undulating walls on both leaf epidermises were observed in P. sarobiense, P. plebijum, P. effusum, P. olivascens and P. paronychioides. Avodele and Olowokudejo (2006) reported striated polygonal cells in P. plebijum, 50-100 µm wide on adaxial surface and 55-100 µm on abaxial surface. P. rottboellioides and P. molliaforme were characterized by tetrahedral, pentagonal and hexagonal epidermal cells. At specific level epidermal cell size is helpful to some extent for the distinction of species. Maximum cell size was recorded on the abaxial surface of P. polycnemoides (100-130×35-55 µm) and P. effusum adaxial (90-120×30-100 µm) and abaxial surface (100-120×25-125 µm), while minimum in P. paronychioides adaxial (30-55×25-35 µm), abaxial surface (30-65×15-30 µm) and P. rottboellioides adaxial surface (25-60×20-30 µm). In most of the species of Polygonum L. length of epidermal cells was more or less double the width of cells (Fig. 3, Table II).

Leaves in Polygonum L. were amphistomatic with different frequency of stomata distribution on both leaf surfaces and also more than one type of stomata were noted on the single leaf surface. Stomatal pattern is quite variable in the family Polygonaceae (Inamdar, 1971; Mitchell, 1971; Haraldson, 1978) and could not be used to delimit taxa (Lersten & Curtis, 1992). During the present study four stomatal types were recorded in genus (Anisocytic, paracytic, pericytic & staurocytic). Anisocytic was the most prevalent type of stomata in the genus (Fig. 1A, D, G, I). P. rottboellioides is the only species with staurocytic type of stomata along with common anisocytic type. Pericytic stomata were recorded in P. plebijum along with anisocytic type (Fig. 1F). Kapoor et al. (1971) noted anomocytic, anisocytic and paracytic type of stomata in P. plebijum, while Ayodele and Olowokudejo (2006) reported only large anisocytic stomata. Recently, Ahmad et al. (2009) suggested diacytic and amphianisocytic stomata for the same species. Anisocytic and paracytic stomatal pattern was observed in *P. arenastrum* and *P. molliaeforme*. Average stomata size varied between $15\times6 \ \mu\text{m}$ (*P. aviculare* adaxial surface) to $32\times21 \ \mu\text{m}$ (*P. polycnemoides* adaxial surface). In *P. patulum* size of stomata was of same range on both surfaces $(15-25\times10-15 \ \mu\text{m})$.

Schotsman (1950) proposed that epidermal glands of Polygonum L. are reliable characters for the identification of species. They originate from epidermal cells and are of significant importance in the study of angiosperms (Werker, 2000). Different taxa can be identified on the basis of trichomes (Munsif et al., 2007). In the genus Polygonum L. non-glandular trichomes were totally absent, while centrally one to three-celled peltate glands with 5-6 subsidiary cells were present in few species (Fig. 1B-C, H). In P. rottboellioides, P. paronychioides, P. arenastrum, P. afghanicum, P. patulum, P. aviculare, P. molliaeforme, P. effusum and P. olivascens no trichomes were seen. Lersten and Curtis (1992) reported the presence of glandular trichomes in P. paronychioides. P. cognatum can be identified due to the presence of one to three-celled peltate glands. In P. polycnemoides centrally one and three-celled peltate trichomes were in close proximity to stomata (Lersten & Curtis, 1992; reported the absence of glands). In P. plebijum two-celled peltate trichomes were present only on abaxial surface. Avodele and Olowokudejo (2006) did not see any type of peltate trichomes on both epidermises of West African P. plebijum. Trichome size considerably showed variation in different taxa of Polygonum L. However, the size of three-celled trichomes in *P. cognatum* adaxial surface (37.5×23 µm) and in P. polycnemoides adaxial surface (34×38 µm) was recorded to be the maximum. Size of one-celled trichomes in *P. sarobiense* was same on both surfaces (Table II).

In order to show the relationship among different

Table II. Summary of qualitative and quantitative epidermal characteristics of genus *Polygonum* L. (All measurements are in μ m)

Foliar characters	Р.	Р.	Р.	Р.	P.rottboelli	Р.	Р.	Р.	Р.	Р.	Р.	Р.	Р.
	cognatum	arenastrum	sarobiense	plebijum	oides	aviculare	molliaeforme	paronychioides	polycnemoides	patulum	effusum	afghanicum	olivascens
Ordinary epidermal cells	Polygonal cells with	Hexagonal, heptagonal	Irregular shape cells	Irregular in shape	Tetrahedral,	Pentagonal	Tetrahedral,	Irregular cells with undulating	Hexagonal cells making	Hexagonal, benzene ring	Irregular in shape with	Large tubular	Irregular in shape with
(Adaxial/	pitted and	smooth walled.	with thick.	slightly	hexagonal.	and	hexagonal in	and pitted thick	benzene ring	like cells with	nitted	in shape with	undulating
Abaxial)	thick walls/	slightly irregular	pitted and	pitted and	pitted	polygonal/	out line,	walls/Irregular	like structure /	straight thick	walls/Irrgul	thick pitted	walls/
	Irregular	and not much	undulating	undulating	walls/	Pentagonal	pitted walls	shape cells with	Benzene ring	and slightly	ar cells	walls /	Irregular
	shaped cells	elongated/Hexag	walls/	walls /	Tetrahedral,	, hexagonal	with slight	pitted	like hexagonal	pitted walls/		Irregular cells	shape cells
	with pitted	onal, heptagonal	Irregularly	Irregular in chopo	pentagonal,	and	undulation/	undulating	cells	Hexagonal colle with		with	
	walls		snapeu cens	in snape	cells with	porygonai	pentagonal.	walls		slightly pitted		and pitted	
					pitted walls		hexagonal			walls		walls	
Stomata	Anisocytic /	Anisocytic,	Anisocytic/	Anisocytic	Anisocytic,	Paracytic/	Anisocytic,	Anisocytic/	Anisocytic/	Anisocytic/	Anisocytic/	Anisocytic/	Anisocytic
(Adaxial/	Anisocytic,	paracytic/Anisoc	Anisocytic	, pericytic/	staurocytic/	Paracytic	paracytic /	Anisocytic	Anisocytic	Anisocytic	Anisocytic	Anisocytic	/Anisocyti
Abaxial)	frequently	ytic and		Anisocytic	Anisocytic,		Anisocytic,						с
	distributed	stomata are		, pericytic	staurocytic		paracytic						
		equally											
		distributed on											
		both surfaces											
Non-glandular	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/
(Adaxial/	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Abaxial)													
Glandular	One to three-	Absent/	One-celled	Absent/	Absent/	Absent /	Absent/	Absent/	One and three-	Absent/	Absent/	Absent/	Absent/
trichomes	celled peltate	Absent	peltate /	Two-	Absent	Absent	Absent	Absent	celled peltate	Absent	Absent	Absent	Absent
(Adaxiai/ Abavial)	striations/One		one-celled	neltate					giands / same				
1100.100)	-celled peltate		pende	gland					surface				
Length of	52±1.5	80±7.07	55±11.6	69±8.42	39±5.78	50±6.51	42±5.38	37±5.04	97±4.35	52±7.17	102±5.00	80±10.20	72±5.14
ordinary	(40-75)/	(60-100)/	(40-100)/	(50-90)/	(25-60)/	(35-70)/	(25-55)/	(30-55)/	(85-110)/	(35-70)/	(90-120)/	(60-100)/	(60-90)/
epidermal cells	52±1.8	73±13.5	54±1.87	91±9.79	45±5.70	52±7.51	49±6.20	42±6.44	112±5.8	56±8.06	106±4.00	65±12.41	68±13.0
in µm (Adaxiai /Abayial)	(35-75)	(33-95)	(50-60)	(70-125)	(33-63)	(35-80)	(33-03)	(30-65)	(100-150)	(40-80)	(100-120)	(35-95)	(45-100)
Width of	35±3.53	31±2.44	33±3.39	36±5.07	25±2.23	32±3.74	25±2.2	30±1.58	44±1.87	27±1.22	72±12.41	30±1.58	42±3.3
ordinary	(25-45)/	(25-35)/	(25-45)/	(25-45)/	(20-30)/	(25-45)/	(20-30)/	(25-35)/	(40-50)/	(25-30)/	(30-100)/	(25-35)/	(30-50)/
epidermal cells	26±1.0	34±4.22	32±3.39	36±6.20	25±2.23	27.5±1.2	30±1.5	21±2.50	44±3.31	31±4.0	70±23.3	32.5±4.78	34±4.8
in µm Adaxiai/ Abayial	(25-30)	(25-45)	(25-40)	(20-55)	(25-55)	(25-30)	(25-35)	(15-30)	(33-33)	(20-40)	(25-125)	(25-45)	(25-50)
Length of	30±1.5	13±2.2	24±4.84	18±1.22	17±1.22	15±2.2	17±0.5	31.5±3.3	32±3.74	17±2.69	25.5±0.3	20±2.88	28±0.55
stomata in µm	(25-35)/	(10-15)/	(15-40)/	(15-20)/	(15-20)/	(10-20)/	(15-20)/	(25-42.5)/	(20-40)/	(15-25)/	(25-26.5)/	(15-25)/25	(25-30)/
Adaxial/	25±2.53	25	23±4.4	20±2.23	21±2.10	27±1.2	20±2.2	16 ±0.5	22±1.22	17±2.69	20±1.22		20±2.23
Abaxiai	(15-35)	14:100	(10-35)	(15-25)	(15-25)	(25-30)	(15-25)	(15-20)	(20-25)	(15-25)	(15-25)	12 - 1 - 22	(15-25)
stomata in um	(10-20)/	(10-15)/	(10-20)/	(10-15)/	(10-15)/	(5-10)/	(10-15)/	21±2.91 (15-30)/	21±2.44 (15-25)/	(10-15)/	(10-15)/	(10-15)/	(15-20)/
Adaxial/	22±1.22	18±1.22	20±1.58	12±3.0	11±1.00	14±1.00	12±1.22	12±1.22	12±1.22	12±1.22	12±1.22	17.5±1.35	13±1.22
Abaxial	(20-25)	(15-20)	(15-25)	(5-20)	(10-15)	(10-15)	(10-15)	(10-15)	(10-15)	(10-15)	(10-15)	(15-20)	(10-15)
Length of non	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/
giandular trichomes in um	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Adaxial /													
Abaxial													
Width of non	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/	Absent/
giandular trichomes in um	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Adaxial /													
Abaxial				_									
Length of	One-celled	Absent/	One celled-	Two-	Absent/	Absent/	Absent/	Absent/	One-celled	Absent/	Absent/	Absent/	Absent/
trichomes in um	25)/22.5+0.9	Absent	(15-20)/	Absent/	Ausem	Absent	Absent	Absent	(10-15)	Absent	AUSCIII	Absent	AUSCIII
Adaxial /	(20-25)		18±1.2	12±1.2					Three-celled				
Abaxial	Two-celled		(15-20)	(10-15)					34±3.67 (25-				
	24±3.64 (20-								45)/21±2.50				
	Three-celled								(13-30)				
	37.5±1.2 (35-												
	40)/Absent		_	_					_				
Width of	One celled	Absent/	One celled	Two-	Absent/	Absent/	Absent/	Absent/	One-celled	Absent/	Absent/	Absent/	Absent/
giandular trichomes in um	7.3±1./ (3- 15)/ 9.5+1 7	AUSCIII	v15±1.22 (10-15)/	Absent/	Ausent	Ausent	AUSCHI	AUSent	(20-25)/	AUSCHI	Ausent	Absent	Ausent
Adaxial /	(5-15)		13±1.22	10±1.11					14±1.87				
Abaxial	Two-celled		(10-15)	(7.5-12.5)					(10-20)				
	21±0.5 (20-								Three-celled				
	Three-celled								(30-45)/				
	23±1.2 (20-								22±2.5				
	25)/Absent								(15-30)				

*Mean values followed by min-max in parentheses. $\pm =$ Standard error

species of *Polygonum* L., cluster analysis was performed. A dendrogram was made on the basis of length / width of epidermal cells, presence or absence of stomata, non-glandular and glandular trichomes on adaxial and abaxial surface. *P. aviculare* and *P. cognatum* were found to be

closely related to each other. *P. molliaeforme*, *P. rottboellioides* pair and *P. patulum*, *P. sarobiense* pair were equally similar. This analysis proved that foliar anatomical investigations are taxonomically significant in understanding the relationships between the species (Fig. 2).

Fig. 2. Cluster analysis of different species of *Polygonum* L. based on foliar anatomical characters





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Adaxial epidermal celk length Abaxial epidermal celk length Adaxial epidermal celk width Abaxial epidermal celk width



Key to the species of *Polygonum*

1a. Epidermal cells polygonal on adaxial surface, one to three-celled peltate trichomes present on adaxial leaf surface......1. *P. cognatum*

2a. Epidermal cells hexagonal and heptagonal in outline, same range of stomata length and width on adaxial surface i-e $10-15 \times 10-15 \,\mu\text{m}$2. *P. arenastrum*

4a.	Two-celled	peltate	trichome	s present	only on
abaxial	surface,	stomata		anisocytic	and
pericytic.				4. P. J	olebijum
4b.	Two-celled	peltate ti	richomes of	on both su	rfaces or
1					~

6b. Epidermal cells tetrahedral, pentagonal, hexagonal, polygonal or irregular on both leaf surfaces, width of stomata on adaxial surface more than $10 \,\mu m$7

7a. Epidermall cells tetrahedral, pentagonal and hexagonal on both leaf surfaces, anisocytic and paracytic stomata on the same leaf surface......7. *P. molliaeforme*

9a. One and three-celled peltate trichomes present, epidermal cells length on abaxial surface varies from 100-130 μm......9. *P. polycnemoides*.
9b. Peltat trichome absent and length of epidermal

10a. Epidermal cells hexagonal in outline; equal sized stomata on both leaf surfaces......10. *P. patulum*.

10b. Epidermal cells irregular in outline; size of stomata different on both leaf surfaces......11

11a. Both leaf surfaces with nearly equal sized epidermal cells, same range of stomata width on both surfaces i-e, 10-15 µm.....11. *P. effusum*

CONCLUSION

Based on present study, it would appear that although there is considerable variation in the qualitative foliar anatomical characters at specific level, but insufficient grounds for using these to recognize different taxa. Nevertheless, it is clear from cluster analysis that the combination of qualitative and quantitative anatomical features is potentially significant to identify species.

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