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Anatomical variations in stomatal attributes of selected species of family Asteraceae

Muhammad Asif Tahir[1], **Rizwan Sarwar**[2], **Sajid Safeer**[2]*, **Imran Hamza**[1], and **Muhammad Faraz Khan**[2]

[1] University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan.
[2] PMAS Arid Agriculture University, Rawalpindi, Pakistan.
*Author for correspondence: Sajidsafeersardar@gmail.com.

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In order to document stomatal characters which are significant in taxonomy, a detailed microscopic study on leaf epidermis of the selected taxa from Asteraceae was carried out. The plant species were *Sonchus oleraceus*, *Bidens bipinnata*, *Centaurea iberica*, *Conyza bonariensis*, *Helianthus annuus*, *Lectuca serriola*, *Parthenium hysterophorus*, *Tagetes erecta*, *Cosmos sulphureus*, *Launaea procumbens*, *Zinnia elegan*, *Galinsoga parviflora* and *Conyza canadensis*. All the species were amphistomatic and four types of stomata i.e., tetracytic, anomocytic, anisocytic and tricytic were recognized. Tetracytic stomatal type was dominant followed by anomocytic. Stomatal density was high on abaxial epidermis than adaxial epidermis except in *Conyza bonariensis* and *Conyza canadensis*. Highest stomatal density was in *Tagetes erecta* while lowest in *Conyza canadensis*. In lower epidermis stomatal index was higher in *Sonchus oleraceus* followed by *Bidens bipinnata* and *Tagetes erecta* while in upper epidermis highest index was shown by *Cosmos sulphureus*. Stomatal aperture and guard cell size and density were also significant features in these species.

Highlighted Conclusion
The study indicates the significance of micromorphological characters in identification of plant species.

Asteraceae is the largest family of vascular plants includes 13 tribes (Adams 1963), 1,100 genera and 25,000 species (Barroso 1986). In Pakistan it is represented by 142 genera and 620 species (Nasir et al 1972). The family is worldwide in distribution, easily recognizable and highly advanced. The species of Asteraceae are woody herbs or shrubs and some are trees and climbers (Olorode 1984). The leaves may be simple, pinnately lobed, opposite or alternate. The anatomical diversity is commonly observed in the members of Asteraceae. The anatomical characters are stomatal distribution on both the surfaces of leaves, the position of guard cells (Solereder 1908).

Stomata are epidermal pores, each surrounded by a pair of guard cells. The exchange of gases such as carbon dioxide and oxygen between plant and environment is the main function of epidermal pores (Perveen et al. 2007). On the basis of presence of stomata, the leaves may be amphistomatic, epistomatic or hypostomatic. Amphistomatic leaves were found in the plants of family Polygonaceae with hexacytic and paracytic stomata in the upper epidermis while tetracytic, anisocytic and hexacytic stomata in the lower epidermis (Hameed et al. 2008). The leaves of amphistomatous plants may have equal frequency of the stomata on both the leaf surfaces or it may be higher in abaxial surface (Ticha 1982). The evidences from fossil angiosperms and many other seed plants suggest that, anomocytic stomata is an ancestral type from which other stomatal types have been evolved. Actinocytic stomata firstly evolved from anomocytic type (Carpenter 2005). The leaves present on top of plants have higher stomatal density on both upper and lower epidermis. There are no significant differences between length and width ratio of stomatal aperture of adaxial and abaxial stomata (Furukawa 1992). Seven types of stomata in dicots have been recognized in which amphianisocytic type was frequently observed (Ahmed et al. 2009). Amphianisocytic type of stomata was reported in family Asteraceae and it was concluded that stomatal variety is helpful character in taxonomic studies of plants (Ahmed et al. 2009). During the explanation of stomatal types in some dicots of Karachi, six stomatal types i.e. anomocytic, diacytic, paracytic, anisocytic, parallelocytic and

cyclocytichave been found by Perveen et al. (2007). In Euphorbiaceae, the leaves are of hypostomatic to amphistomatic type. The stomatal characters such as type, occurrence, frequency, size and index were calculated. It was concluded that the predominant type of stomata was anomocytic followed by the paracytic, hexacytic and anisocytic complex types (Thakur and Patil 2011).

The density of ordinary epidermal cells and stomata were lower in the epidermis of a shade leaf than a sun leaf having similar insertion point (Poole et al. 1996). The stomatal density was found to be variable and positively correlated with length of guard cells and stomatal pores (Brewer and Nunez 2007). The stomata and epidermal cells may be on the same level in epidermis or stomata may be raised or sunken (Essau 2006). There are many variations in size, shape and arrangement of leaves on the stem. These variations are a source of interest for a taxonomist. Dicotyledonous leaves with broad lamina have large number of stomata on adaxial surface. Many of the monocotyledonous leaves with nearly vertical position may have similar distribution of stomata on both surfaces. Submerged leaves are devoid of stomata while floating leaves have stomata on upper epidermis only (Eames and Macdaniels 2000). The anatomical attributes that can be observed in the members of family Asteraceae are stomatal types, trichomes, papillae, hydathodes and vascular bundles (Metcalfe and Chalk 1979). In angiosperms, leaf is anatomically diagnostic organ and it has a variety of anatomical characters that can be employed for classification of plants (Stace 1984).

Distribution of stomata depends on the environmental conditions of plant habitat. Stomata are not present on the leaves of aquatic submerged plants. These are present only on upper surface of floating leaves while on both surfaces in aerial leaves. Stomata remain closed during water deficient condition and open under favorable condition of photosynthesis (Zeiger et al. 1987).

MATERIAL AND METHODS

Collection of study materials. Selected plant species of family Asteraceae were collected from District Muzaffarabad Azad Jammu and Kashmir. Thirteen species viz. *Sonchus oleraceus* L., *Bidens bipinnata* L., *Centauria iberica* Spreng., *Conyza bonariensis* (L.) Cronq., *Helianthus annuus* L., *Lectuca serriola* L., *Parthenium hysterophorus* L., *Tagetes erecta* L., *Cosmos sulphureus* Cav., *Launaea procumbens* Roxb., *Zinnia elegans* Jacq., *Galinsoga parviflora* Cav. and *Conyza canadensis* (L.) Cronq. These plants were identified with the help of Flora of Pakistan (Ali and Qaiser 2007).

Isolation of epidermis. Fresh leaves were immersed in water to prevent dehydration. The abaxial and adaxial epidermis of each specimen was separated with the help of razor, needles and forceps (Hameed et al. 2008). In some species a film of transparent nail polish was directly applied to the leaf surfaces. After drying, the impression provided excellent detail of epidermis (Perveen et al. 2007).

Staining, mounting and microscopic examination. A small portion of epidermis was stained in one percent aqueous solution of safranine for about 4-5 minutes (Abdulrahaman and Oladele 2005). The stained material was kept in a series of alcoholic solution of different concentrations and mounted in Canada balsam for microscopic examination (Hameed et al. 2008). Observations were carried out at x400 using a Swift Microscope (M7000D, Japan) and photographs were taken at the same power using an IRMECO GmbH Microscope (Model IM-900, Germany) fitted with camera.

Determination of stomatal complex types. Stomatal complex types were examined on abaxial and adaxial epidermis of selected species. Terminology used in respect of stomatal types followed Prabhakar (2004).

Determination of stomatal density and index (%). Stomatal density was determined as the number of stomata per square millimeter and stomatal index was determined as; number of stomata per square millimeter divided by number of ordinary epidermal cells per square millimeter plus number of stomata per square millimeter multiplied by 100 (Saadu et al. 2009).

Size of stomatal pore and guard cells (µm). Average length and width of stomatal pore and guard cells on abaxial and adaxial epidermis was measured using eyepiece micrometer (Khan et al. 2011).

Statistical analysis. Five replicates were used for each parameter and the quantitative results were subjected to MS Excel program. Mean and standard deviation were calculated.

RESULTS

Distributed in four tribes and twelve genera, thirteen selected species of Asteraceae were studied for microscopic details of stomatal attributes in leaf epidermis.

Stomatal complex types. The selected species were amphistomatic with same or different stomatal types on abaxial and adaxial epidermis. Four types of stomatal complexes namely tetracytic, anomocytic, anisocytyic and tricytic were observed. The tetracyticstomatal type was dominant followed by anomocytic, anisocytic and tricytic, respectively. More than one stomatal type was investigated in selected species except *Helianthus annuus* with anomocytic stomatal type (Table 1).

Table 1. Stomatal types, density and index in selected Asteraceae species.

Plant species	Stomatal type		Stomatal density Mean±SD		Stomatal index(%) Mean±SD	
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Sonchus oleraceus	Tetracytic, Tricytic	Anomocytic, Tetracytic	332.06±8.92	81.18±12.75	36.52±0.94	17.12±1.48
Bidens bipinnata	Tetracytic	Anisocytic	403.53±6.70	103.53±13.55	31.79±1.79	17.99±2.53
Centauria iberica	Anisocytic	Anisocytic, Tetracytic	191.77±8.92	132.94±12.20	19.51±0.94	9.92±0.84
Conyza bonariensis	Anomocytic	Tetracytic	161.18±8.92	187.06±9.67	17.44±1.12	16.20±0.98
Helianthus annuus	Anomocytic	Anomocytic	208.23±8.92	178.82±14.17	22.38±1.07	19.39±1.49
Lectuca serriola	Tetracytic	Tetracytic, Tricytic	265.88±12.75	158.84±16.13	18.83±0.61	20.97±2.09
Parthenium hysterophorus	Anomocytic	Tetracytic	361.18±13.54	217.65±17.15	23.84±1.05	14.93±1.00
Tagetes erecta	Anisocytic Tetracytic, Anomocytic	Anisocytic, Tetracytic	414.11±15.34	328.23±21.77	26.11±0.69	20.69±1.34
Cosmos sulphureus	Anisocytic	Anisocytic	283.53±14.04	161.18±12.20	25.34±6.77	24.18±1.15
Launaea procumbens	Anomocytic, Tetracytic	Tetracytic	160.00±10.52	129.41±18.60	22.12±1.35	16.06±2.05
Zinnia elegans	Tetracytic	Tetracytic, Anisocytic	183.53±7.67	136.47±17.84	13.31±0.52	10.30±1.26
Galinsoga parviflora	Anomocytic, Tetracytic	Tetracytic	171.76±10.52	60.00±7.67	23.33±1.26	9.72±1.29
Conyza Canadensis	Anomocytic, Anisocytic	Anisocytic, Tricytic	152.94±11.76	178.82±10.69	15.28±1.22	18.68±1.26

Stomatal density. Stomatal density was higher on abaxial epidermis than adaxial epidermis in most species. The highest density of stomata on abaxial surface was recorded in *Tagetes erecta* (414.11) followed by *Bidens bipinnata* (403.53) and *Parthenium hysterophorus* (361.18) respectively. The highest stomatal density on adaxial surface was observed in *Tagetes erecta* (328.23) followed by *Parthenium hysterophorus* (217.65) and *Conyza bonariensis* (187.06) respectively. The lowest stomatal density was observed in *Conyza canadensis* (152.94) on abaxial epidermis while in *Galinsoga parviflora* (60) on adaxial epidermis. Two species, *Conyza bonariensis* and *C. canadensis* have shown higher stomatal density on adaxial than abaxial epidermis (Table 1). It was interesting because stomatal density of terrestrial plants is generally higher on abaxial epidermis.

Stomatal index (%). Stomatal index was higher in *Sonchus oleraceus* (36.52%) followed by *Bidens bipinnata* (31.79%) and *Tagetes erecta* (26.11%) in lower epidermis while in upper epidermis highest index was shown by *Cosmos sulphureus* (24.18%) *Lectuca serriola* (20.97%) and *Tagetes erecta* (20.69%). Two species, *Lectuca serriola* and *Conyza Canadensis* showed high stomatal index on upper epidermis than on lower epidermis (Table 1).

Stomatal pore size (µm). The largest pore was measured in *Helianthus annuus* (21.12µm) followed by *Cosmos sulphureus* (18.08µm) and *Launaea procumbens* (16.92µm) on abaxial epidermis while the pore length was smaller on abaxial epidermis of *Sonchus oleraceus* (10.37µm). Wider stomatal pores were observed in abaxial epidermis of *Centauria iberica* (4.20µm), *Helianthus annuus* (3.99µm) and *Sonchus oleraceus* (3.08µm) while in the adaxial epidermis of *Zinnia elegans* (4.47µm), *Sonchus oleraceus* (3.99µm) and *Bidens bipinnata* (3.99µm), respectively (Table 2).

Table 2. Size of stomatal aperture and guard cells in selected Asteraceae species.

Plant species	Stomatal aperture size (µm) Mean±SD				Guard cell size (µm) Mean±SD			
	Abaxial		Adaxial		Abaxial		Adaxial	
	Length	Width	Length	Width	Length	Width	Length	Width
Sonchus oleraceus	10.37±1.11	3.08±0.85	16.91±2.41	3.99±1.09	20.38±2.66	5.75±3.99	25.16±2.92	7.08±5.85
Bidens bipinnata	10.74±1.84	2.66±1.34	14.90±1.73	3.99±1.09	19.15±4.06	4.73±2.13	24.21±3.03	6.65±3.99
Centauria iberica	13.35±1.05	4.20±1.13	11.97±2.33	2.98±1.08	20.48±1.52	5.85±3.99	18.35±2.65	5.58±4.52
Conyza bonariensis	14.21±3.14	2.87±1.21	13.09±0.74	2.44±1.08	21.60±2.21	5.91±3.99	18.67±2.14	5.27±2.93
Helianthus annuus	21.12±2.00	3.99±1.16	19.47±2.14	2.82±0.64	27.93±3.30	5.59±3.46	22.72±3.39	5.43±4.79
Lectuca serriola	12.40±2.70	2.02±0.85	18.04±4.89	2.98±0.83	36.55±1.64	6.60±5.59	24.74±5.06	5.06±3.19
Parthenium hysterophorus	15.05±1.63	2.50±0.30	13.51±2.17	2.81±0.40	22.13±3.10	5.64±5.05	19.03±3.70	5.11±3.99
Tagetes erecta	15.54±3.83	2.07±0.95	13.83±1.20	2.87±0.35	21.28±2.05	4.73±3.72	17.50±2.50	3.83±2.66
Cosmos sulphureus	18.08±2.08	2.50±0.44	18.30±4.03	2.76±1.11	28.20±2.42	5.59±3.46	24.53±3.05	5.11±3.72
Launaea procumbens	16.92±3.89	2.71±0.52	18.25±3.02	3.51±1.14	24.89±3.64	6.22±5.32	25.27±3.36	4.63±3.99
Zinnia elegans	15.21±2.71	3.03±1.52	12.82±1.31	4.47±1.10	20.64±3.58	5.32±4.79	20.43±2.79	4.68±3.99
Galinsoga parviflora	11.33±1.48	2.23±0.66	12.50±1.67	1.54±0.69	17.45±1.10	3.97±3.19	18.62±1.55	3.08±2.39
Conyza canadensis	13.35±1.58	2.02±0.85	17.56±3.27	3.46±0.60	21.81±3.59	5.27±3.99	21.76±2.04	4.26±3.46

Guard cell size (µm). Largest guard cell was found on abaxial epidermis of *Lectuca serriola* (36.55µm) followed by *Cosmos sulphureus* (28.20µm) and *Helianthus annuus* (27.93µm) while on the adaxial epidermis, *Launaea procumbens* (25.27µm) showed the largest guard cell followed by *Sonchus oleraceus* (25.16µm) and *Lectuca serriola* (24.74µm). The length of guard cells in *Conyza canadensis* was almost similar on both the leaf surfaces. The width of guard cells was recorded to be highest in *Lectuca serriola* (6.60), *Launaea procumbens* (6.22) and *Conyza bonariensis* (5.91) on abaxial epidermis whereas *Sonchus oleraceus* (7.08) *Bidens bipinnata* (6.65) and *Centauria iberica* (5.58) have highest width on abaxial epidermis in selected species (Table 2).

DISCUSSION

The stomatal anatomy of leaf epidermis has been studied in detail to measure the degree of variations in selected species of Asteraceae. These species were amphistomatic with tetracytic, anomocytic, anisocytic and tricytic stomatal complex types. Adedeji and Jewoola (2008) reported anomocytic and anisocytyic stomatal types in Asteraceae. All the selected species showed more than one type of stomata except *Helianthus annuus*, coinciding with earlier investigation on genus *Ficus* (Kong 2001). Stomatal aperture length varied from 10.37µm to 21.12µm and 11.97µm to 19.47µm on abaxial and adaxial epidermis, respectively. The width of aperture varied from 2.02µm to 4.20µm and 1.54µm to 4.47µm on abaxial and adaxial epidermis, respectively coinciding with the findings in Polygonaceae as stated by Hameed et al. (2008). There is some correlation between stomatal length on abaxial and adaxial epidermis showing anatomical relationship between the leaf surfaces. The maximum length of guard cell occurred in *Lectuca serriola* and *Cosmos sulphureus* in lower while *Launaea procumbens* and *Sonchus oleraceus* in upper epidermis, respectively .These observations resemble to the findings of Akcin and Binzet (2010) that anatomical features varied in upper and lower epidermis in Boraginaceae. The higher stomatal index occurred in *Sonchus oleraceus* in lower epidermis while in the upper epidermis of *Cosmos sulphureus*. Stomatal index ranges from 9.72% to 36.52% in selected species. It is investigated that stomatal index was higher on lower epidermis of some species while on upper epidermis of other species as reported in Onasma species of family Boraginaceae (Akcin and Binzet 2010).

Variations were observed on abaxial and adaxial epidermis of the same species while most of the stomatal features were stable in most of the species. The stomatal complex types, density, index, size of pore and guard cell have shown significant differences. These variations are helpful to differentiate these species. It is concluded that leaf stomatal attributes may be significant taxonomic tools in the identification of these species.

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