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COMPARATIVE FOLIAR MICROMORPHOLOGICAL STUDIES OF SOME SPECIES OF ASTERACEAE FROM ALPINE ZONE OF DEOSAI PLATEAU, WESTERN HIMALAYAS

A. Bano, M. Ahmad, M. Zafar, S. Sultana and M. A. Khan

Department of Plant Sciences, Quaid-i-Azam University Islamabad Pakistan
Corresponding Author e-mail: abidabano86@gmail.com

ABSTRACT

This study concerns the evaluation of foliar epidermal anatomical characteristics of twelve species of Asteraceae by light microscopy (LM). The plant materials were collected from various localities of Deosai plateau. Three stomata types including anisocytic, actinocytic and anomocytic have been found in the family. Stomata type is Anisocytic in *Artemisia persica*, Actinocytic in *Circium falconerii* and *Erigeron multiradiatus* while anomocytic in rest of the nine species. The stomata type seems to be constant character at genus level. Leaf epidermal features like shape of epidermal cells and trichomes are found useful taxonomic tools. The epidermal cells were irregular or polygonal with straight or undulate walls. Two species of *Saussurea* viz., *Saussurea nepalensis* and *S. obvallata* are distinguishable on the basis of shape of epidermal cells. The pattern of walls is similar on both abaxial and adaxial sides in all species except in *Senecio chrysanthemoides* where it is weakly undulate on abaxial side and heavily undulate on adaxial side. The diversity in the foliar trichomes is of taxonomic importance for discrimination of taxa at specific level. *Artemisia persica* was unique in being the only species with stellate trichome. *Aster himalaicus* can be delimited from other species by the possession of J-shaped trichomes while long multicellular trichomes were found in *Conyza japonica* and *Senecio chrysanthmoides*. The results of foliar micromorphological features were comparatively described and quantitative parameters were also presented which proved to be helpful for differentiation of selected species.

Key words: Foliar epidermal anatomy, Asteraceae, Deosai plateau.

INTRODUCTION

The Deosai Plateau is located at 30°00 N, 75°30 E in the north of the main Himalayan range in northern Pakistan, and is 4115 m above mean sea level (Fig. 1). It is one of the highest plateaus in the world, and is well above the tree line. The plateau covers an area of almost 5000 km^2. It is surrounded by the Himalayas and lies close to the magnificent Karakorum mountain range which includes K2, the second highest peak in the world at 8611 m. Approximately 340 plant species belonging to 142 genera and 36 families have been recorded from Deosai Plateau. This high biodiversity is due to the topography, its location at the junction of four major mountain ranges and local adaptation of the indigenous plant/animal species (Woods *et al*., 1997). Sultana *et al*. (2007) researched the altitudinal distribution of grasses, sedges and rushes of the Deosai Plateau. These authors found that majority of species are present between 3500 m to 4500 m altitude due to the availability of moisture and a favorable climate. Bano *et al*. (2014) provided information of 50 medicinal plant species of Deosai Plateau used to treat different ailments. Bano *et al*. (2012) carried out the pollen morphology of some endemic species of *Pedicularis* from Deosai plateau. Ahmad *et al*. (2013) carried out the pollen morphology of ten species of Asteraceae from Deosai plateau and deduced that the selected species are well established in the area. A total of 114 plant species belonging to 28 families were found around Sheosar Lake (Shaheen and Qureshi, 2011).

The Family Asteraceae comprises 1530 genera and 2300 species distributed within 3 subfamilies and 17 tribes (Ghafoor, 2002). It is a cosmopolitan family, which in terms of size is the largest in Pakistan, comprising 650 species in 15 tribes including some in cultivation (Ghafoor, 2002).

The taxonomic position of a number of families are made on the basis of leaf epidermis and is one of the most important taxonomic characters from the biosystematic point of view (Stace, 1984; Jones, 1986). Davis and Heywood (1963) emphasized the use of anatomical characters as these are fairly constant and reliable within a taxon. The epidermis have a number of important diagnostic character that provide valuable clues for identification like size, shape and orientation of stomata, guard cells and subsidiary cells, distinctive or specialized form of trichomes, structural peculiarities of epidermal cell walls (Dickison, 2000). Metcalfe and Chalk (1950) gave a sparse description of the general anatomy of the family Asteraceae. Inceer and Ozean (2011) considered leaf anatomy as an additional taxonomic tool for 18 taxa of Asteraceea in Turkey. Adedeji and Jewoolao (2008) concluded that leaf epidermal characters are taxonomically important in twelve species of Asteraceae. Makbul *et al*. (2008) suggested that anatomical features are more important

than palynological ones in explaining variation among the examined taxa.

Very limited data exists on the leaf epidermal anatomical studies of family Asteraceae from Pakistan. Qureshi *et al.* (2002) carried out taxonomic studies of six species of genus *Sonchus* (Asteraceae) from Pakistan. Ahmad (2005) conducted morphological and anatomical studies of 23 species of genus *Saussurea* (Asteraceae) from Pakistan. Hayat *et al*. (2009) found that foliar trichomes of genus *Artemisia* are good taxonomic markers. Hayat *et al*. (2010) studied stomatal variation in 24 taxa of genus *Artemisia* which can be utilized to solve the taxonomic issues within the genus. There are no reports on leaf anatomical studies of Asteraceae from the alpine zone of Deosai plateau and its adjacent areas. The present study is aimed at identifying and describing the foliar anatomical diversity of indigenous species of Asteraceae by light microscopy (LM). The systematic potential of leaf anatomical features of some plant groups has been reported but there is a lack of attention to work solely on this feature and elaborate its significance. The pin point objectives of the current study were to (1) observe quantitative and qualitative features of leaf epidermal cells (2) compare the variation of different stomatal types and trichomes in the selected species (3) elaborate the taxonomic value of foliar epidermal anatomical characters based on trichome morphology, stomata and epidermal cells diversity.

MATERIALS AND METHODS

The plants were collected from various localities of Deosai plateau during summer 2012 and 2013. They were identified through available literature (Nasir and Ali, 1970-2002) and also comparing with preserved specimens in the herbarium of Quaid-i-Azam University, Islamabad (ISL).

Dried leaves were placed in boiling water for few minutes to soften them until they became unfolded and ready for epidermal scraping. Dried leaves were used for anatomical studies following the procedure of Cotton (1974) and Clark (1960). The dried leaves were placed in a test tube, filled with 88% lactic acid and kept hot in a boiling water bath for about 50 - 60 minutes. Lactic acid softens the tissue of leaf due to which peeling off is made possible. To prepare the abaxial surface, the leaf were placed keeping its adaxial surface upward and then it were flooded with 88% cold lactic acid. The adaxial epidermis were cut across the leaf using a sharp scalpel blade and scraped away together with the mesophyll cells until only the abaxial epidermis of the leaf remained on the slide. The epidermis was placed, outside uppermost and mounted in clean 88% lactic acid. A similar procedure was followed to prepare the adaxial epidermis. The parameters studied were shape and margin of epidermal cells, number of epidermal cells on abaxial and adaxial sides, length and width of leaf epidermis and trichomes, the presence and absence of stomata on each epidermis, type and size of stomata. Microphotographs were taken by using CCD digital camera (DK 5000) fitted on Leica light microscope (DM 1000). Stomatal index was calculated using the formula of Salisbury (1972). SI= S/(S+E) ×100, where S denotes the number of stomata per unit area and E the number of epidermal cells of the same area.

RESULTS AND DISCUSSION

The micromorphological features of foliar epidermis of selected species were analyzed by its qualitative and quantitative characters (Table 1 and 2).

The shape of epidermal cells is polygonal or irregular with straight or undulate walls. The species of the same genus are distinguishable on the basis of shape of epidermal cells. Two species viz., *Saussurea nepalensis* and *S. obvallata* have very different epidermal cells between the abaxial and adaxial sides. In *Saussurea nepalensis* the shape of cells are polygonal and isodiametric on abaxial epidermis (Fig. 2q) and penta, hexa or polygonal on adaxial side (Fig. 2r). In *S. obvallata* the shape of cells are irregular on abaxial side (Fig. 2s) and polygonal on adaxial side (Fig. 2t). Similarly the shape of epidermal cells is polygonal on abaxial side (Fig. 2i) and penta or hexagonal on adaxial side of *Cremanthodium ellisii* (Fig. 2j). In *Cremanthodium reniforme*, the shape of epidermal cell is irregular on abaxial side (Fig. 2k) and polygonal on adaxial side (Fig. 2l). So the shape of epidermal cells can be used to distinguish species of the same genus. The pattern of walls is similar on both abaxial and adaxial sides in all species except in *Senecio chrysanthemoides* where it is weakly undulate on abaxial side (Fig. 2u) and heavily undulate on adaxial side (Fig. 2v). The size of epidermal cells is highest in *Cremanthodium ewersii*and smallest in *Artemisia persica* on both adaxial and abaxial surfaces.

The majority of the taxa are amphistomatic except *Senecio chrysanthmoides* and two species of *Cremanthodium* where they are present on abaxial surface only. Most of the studied taxa have anomocytic type of stomata. However anisocytic stomata are observed in *Artemisia persica* on abaxial surface (Fig. 2a) and actinocytic on both surfaces of *Circium falconerii* (Fig. 2e, f) and *Erigeron multiradiatus* (Fig. 2m, n). The type of stomata is same on both abaxial and adaxial surfaces when present. All the species have less number of stomata on adaxial surface as compare to abaxial surface. Stomatal size although quantitative is also important. Stomatal size is consistently higher on the abaxial surface than on the adaxial surface in all the species studied. The stomatal size is highest in *Saussurea nepalensis* and lowest in *Jurinea dolomiaea* on both

abaxial and adaxial sides. The stomatal index ranges between 11.7 – 19.5 on abaxial side and 5.5- 13.6 on adaxial side. On the adaxial surface, it is highest in *Aster himalaicus* (13.6) and lowest in *Erigeron multiradiatus* (5.5) while on the abaxial surface it is highest in *Circium falconerii* (19.5) and lowest in *Conyza japonica* (10.4).

Trichomes are mostly present on adaxial surface in majority of the taxa except in *Aster himalaicus* and *Conyza japonica* where it is present on both surfaces. In *Aster himalaicus*, JShaped non glandular trichomes are observed on adaxial side (Fig. 2d) while on abaxial side trichomes are unicellular with long thin and pointed apical cells (Fig. 2c). Non glandular trichomes are present in *Conyza japonica* on adaxial side (Fig. 2h) which is bicellular to multicellular with long thin and pointed apical cells infrequently short and pointed too. Sessile glandular trichomes are present on abaxial side of *Conyza japonica* (Fig. 2g). In *Senecio chrysanthmoides* 3-4 celled non glandular trichomes are present on the adaxial surface (Fig. 2v). In *Artemisia persica* trichomes are glandular with unicellular head and multicellular trichomes on adaxial side (Fig. 2b). In *Erigeron multiradiatus* non glandular trichomes are present on adaxial surface which are bicellular to multicellular (Fig 2n). In *Jurinea dolomiaea*, trichomes are unicellular with long thin and pointed apical cell on adaxial side (Fig2p).

The anatomical features of some indigenous Asteraceous species are presented here for the first time from alpine zone of Deosai plateau. The present results are in agreement with data previously reported by Metcalfe and Chalk (1979) for the family Asteraceae. However, the size of some anatomical characters differs. Ahmad *et al*. (2010) noted valuable intergeneric and interspecific variations in the pattern of epidermal cells that can be used to as an important taxonomic tool to identify many species. The shape of epidermal cells is polygonal or irregular with straight or undulate walls in the selected taxa of Asteraceae. Measurement of leaf epidermal cell length and width are found useful aids in distinguishing varieties with similar flowering date in perennial ryegrass (Wilkins and Sabanci, 1990). The size of epidermal cells shows considerable variation between the two species of *Saussurea*. The epidermal cells of *Saussurea obvallata* are larger in size i.e. 72.5µm in length while in *Saussurea nepalensis* length of epidermal cells is 40µm.

The structural feature of individual stoma may be important, but equally valuable is their pattern of distribution while presence or absence of stomata on the upper leaf surface is often a good diagnostic character (Sonibare*et al*. 2005). The results of a wide range of stomatal size in the species studied agree with the observations of Wilkinson (1971) in his study of some families. Inceer and Ozean, 2011 reported anomocytic stomata in 18 taxa of Asteraceae. The present results are in agreement with these previous reports as majority of the taxa have anomocytic stomata. However, anisocytic stomata are observed in *Artemisia persica* on abaxial surface. Hayat *et al*. (2010) also reported anisocytic stomata type in *Artemisia*. The shape of the pair of guard cells as seen in surface view, i.e., stomatal shape is of taxonomic value as reported by Adedeji (2004). Stomatal shape of the species studied varied between circular and elliptic. According to Olatunji (1983), stomatal index can be helpful for species delimitation. The stomatal index ranges between 11.7 – 19.5 on abaxial side and 5.5- 13.6 on adaxial side in the present study.

At the specific level**,** studies of trichomeshave been found useful by many workers (Adedeji, 2004; Adedeji*et al*., 2007). According to Metcalfe and Chalk (1979), the species, genera or even whole families can frequently delimit by the presence of particular type of trichome. Both glandular and non glandular trichomes are present in the studied taxa. The majority of the taxa including *Aster himalaicus, Conyza japonica, Erigeron multiradiatus, Jurinea dolomiaea* and *Senecio chrysanthemoides* have non glandular trichomes present on adaxial sides. The glandular trichomes are present in *Artemisia persica* on adaxial side and *Conyza japonica* on abaxial side. Hayat *et al*. (2009) reported 16 types of glandular and non glandular foliar trichomes in *Artemisia* and found that foliar trichomes of genus *Artemisia* are good taxonomic features and can be used for the discrimination of different taxa within the genus.

The present study revealed that foliar anatomical characters including size and shape of epidermal cells and trichomes are potentially significant to delimit the taxa at specific level. The two species of *Saussurea* and *Cremanthodium* can be easily differentiated on the basis of shape of epidermal cells. The size of epidermal cells shows considerable variation between the two species of *Saussurea.* The diversity in the foliar trichomes also serves as a valuable taxonomic tool at specific level. However, the type of stomata seems to be constant at genus level. The diversity of leaf epidermal features was based on the variation of stomata types, morphology of epidermal cells and trichome. Although each feature on its own has systematic value, the combination of some of these characteristics proved to be helpful for identification of species. However, there is a need of detail study of these micromorphological features using scanning electron microscopy (SEM) to resolve taxonomic conflicts as these characters proved to be very useful from identification and classification point of view.

Table 1. Qualitative characters of foliar epidermal cells of twelve Asteraceous species

	Abaxial epidermis			Adaxial epidermis			
Name of species	**Shape of cells**	**Pattern of walls**	**Stomata type**	**Shape of cells**	**Pattern of walls**	**Stomata type**	**Trichomes**
Artemisia persica Boiss.	Polygonal	Straight	Anisocytic	Polygonal	Straight	Anomocytic	Stellate glandular
*Aster himalaicus*C.B. Clarke	Irregular	Heavily undulate	Anomocytic	Irregular	Heavily undulate	Anomocytic	2-3 celled non glandular
Circium falconerii (Hook. f.) Petr.	Polygonal	Straight	Actinocytic	Polygonal	Straight	Absent	Absent
Conyza japonica (Thunb.) Less.	Polygonal	Straight	Anomocytic	Polygonal	Weakly undulate	Anomocytic	3-4 celled non glandular
Cremanthodium ellisii (J. D. Hooker) Kitamura	Polygonal	Straight	Anomocytic	Penta& hexagonal	Straight	Absent	Absent
Cremanthodium reniforme (Candolle) Bentham	Irregular	Weakly undulate	Anomocytic	Polygonal	Weakly undulate	Absent	Absent
Erigeron multiradiatus (Lindl. Ex DC.) Benth.	Irregular	Straight	Actinocytic	Irregular	Straight	Actinocytic	3 celled non glandular
*Jurineadolomiaea*Boiss.	Polygonal	Straight	Anomocytic	Polygonal	Straight	Anomocytic	1 celled non glandular
Saussurea nepalensis Spreng.	Polygonal & isodiametric	Straight	Anomocytic	Penta, hexa& polygonal	Straight	Anomocytic	Absent
Saussurea obvallata(Candolle) Schultz	Irregular	Heavily undulate	Anomocytic	Polygonal	Weakly undulate	Absent	Absent
Senecio chrysanthemoides DC. Prodr.	Polygonal	Weakly undulate	Anomocytic	Polygonal	Heavily undulate	Anomocytic	2-3 celled non glandular
Taraxacum tibatinum Hand. Mazz.	Irregular	Heavily undulate	Anomocytic	Irregular	Heavily undulate	Anomocytic	Absent

Table 2. Quantitative characters of foliar epidermal cells of twelve Asteraceous species

	Abaxial epidermis			Adaxial epidermis			
Name of species	**Size of cells (µm) L×W**	**Size of stomata (µm) L×W**	**Stomatal index (%)**	**Size of cells (µm) L×W**	**Size of stomata(µm) L×W**	**Stomatal index (%)**	**Trichomes (µm)**
Artemisia persica	25(22.5-27) ×20(16.5-23)	22(19.5-23) ×7(5.5-8)	14.7	23 (19.5-25) × 19 (16.5-21)	21(19.5-23) ×6.5 (4.5-7)	8.2	162.5(145-170) × 10 (7.5-12)
Aster himalaicus	65(57.5-69) × 22.5(17.5-25)	32.5(30.5-34) × 17.5 (15.5-19)	12.6	61(57.5-64)× 19.5 (16-21.5)	29.5(26-32.5)×16 (13-17.5)	13.6	250(215-275) ×15 (11.5-17)
Circium falconerii	39(32.5-43) ×34(30-36.5)	22.5(19.5-24) × 17.5 (15.5-19)	19.5	36(34.5-37) × 29.5 (26.5-32)	19.5(17-21.5)×15 (12.5-16)	7.4	Absent
Conyza japonica	42(37.5-45) ×39(33.5-43)	27.5(24.5-29) × 15 (13-16)	10.4	39(36.5-42) ×37 (35-39.5)	26 (24.5-27) × 13 (11.5-15)	9.4	112.5(91-121.5) ×15 (11.5-17)
Cremanthodium ellisii	75(69.5-78) × 37.5 (33.5-39)	35(32.5-36) × 27.5 (25.5-28.5)	13.8	69 (66.5-72)× 33.5 (30.5-35)	Absent	_	Absent
Cremanthodium raniforme	65(61-66.5) ×29(23.5-32)	34(29.5-36) ×26.5 (23.5-29)	18.2	61.5(57-64)× 26 (23.5-29)	Absent	_	Absent
Erigeron multiradiatus	39 (37.5-42) ×31(29-34.5)	35(33.5-37) × 21 (19.5-23)	16.5	36(35.5-39)× 27 (26.5-30)	31.5(29-34.5) × 18 (16.5-21)	5.5	80 (60-99.5) × 15 (11.5-17)
Jurinea dolomiaea	38(32.5-43) ×33(30-36.5)	21.5(19.5-24) × 16.5 (15.5-19)	18.5	35(33-36.5) × 29 (26.5-32)	18.5(16.5-21)×14 (12.5-16)	6.4	110 (91-119.5) × 14 (11.5-17)
Saussurea nepalensis	40(37.5-42) ×32.5(29-34.5)	36(33.5-37) × 22.5 (19.5-23.5)	17.6	37(35.5-39)× 28.5 (26.5-30)	32.5(29-34.5) × 19 (16.5-21)	6.4	Absent
Saussurea obvallata	72.5(67.5-74) ×24 (21.5-27)	27.5(24.5-29) × 32.5 (29.5-34)	15.3	67.5(63.5-72) ×21 (19.5-23)	25 (23-27.5)× 29.5 (27.5-31)	12.5	Absent
Senecio chrysanthemoides	67.5(63-69.5) ×50(47-53.5)	27.5(25.5-29) ×25 (23.5-26.5)	11.7	64 (59.5-67) × 47.5 (44.5-49)	Absent	_	150(130-170) ×14 (9.5-16)
Taraxacum tibatinum	52.5(48-54.5) ×22.5(18.5-24.5)	27.5(25-29.5) ×17.5 (15.5-19)	16.9	49(46.5-51)× 18.5 (15.5-21)	23(21-25.5)×15.5 (13.5-17)	6.5	Absent

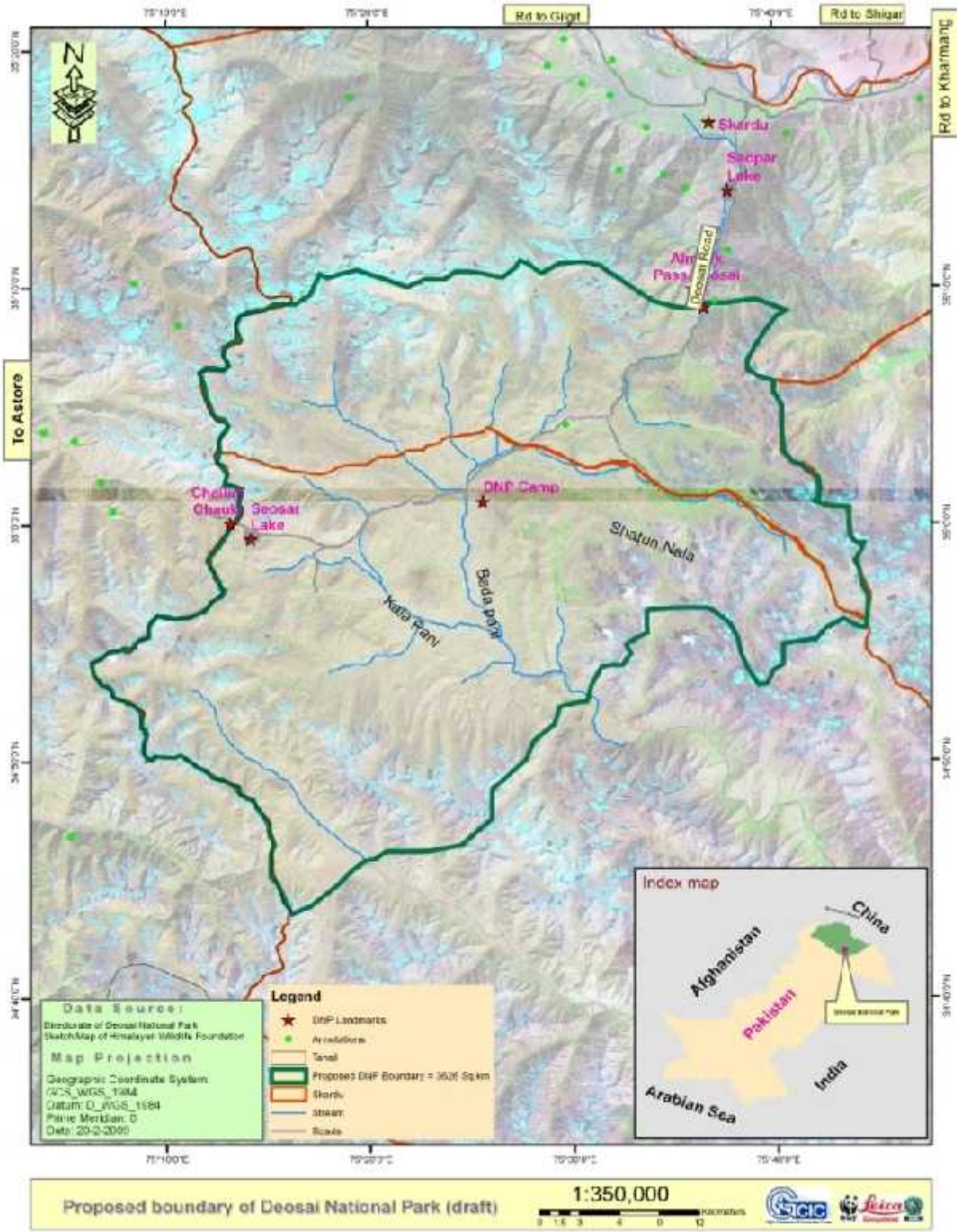

Fig. 1. Map of Deosai Plateau (Source: World Wildlife Fund)

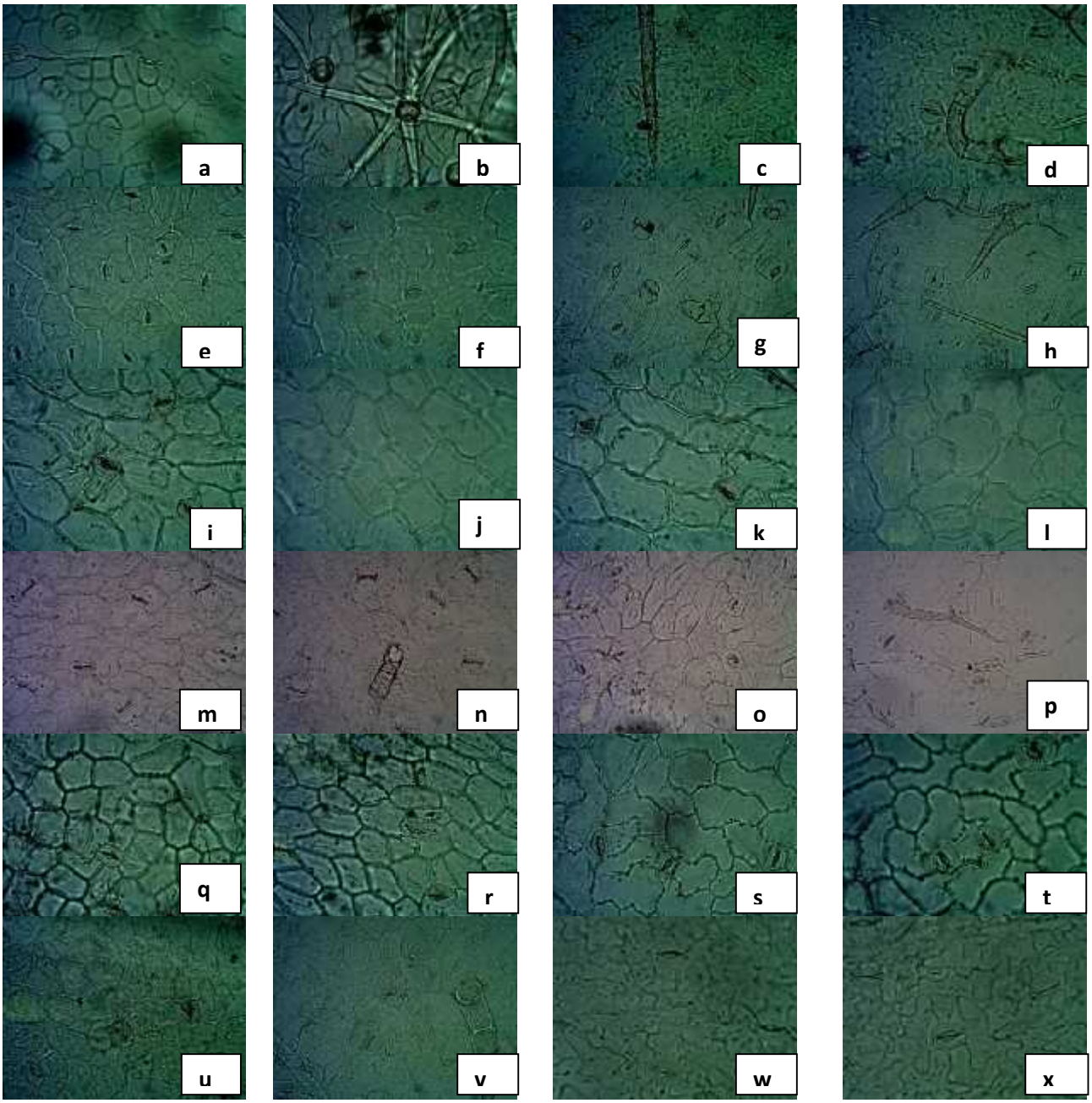

Fig. 2. Light micrographs (LM) of shape of cells, pattern of walls, stomata and trichomes (20x) (a)Abaxial surface of *Artemisia persica* (b)Adaxial surface of *A. persica*(c)Abaxial surface of *Aster himalaicus* (d)Adaxial surfaceof *A. himalaicus*(e)Abaxial surface of *Circiumfalconeri*(f)Adaxial surface of *C. falconeri*(g)Abaxial surface of *Conyza japonica* (h)Adaxial surface of *C. japonica* (i)Abaxial surface of *Cremanthodiumellisii* (j)Adaxial surface of *C. ellisii*(k)Abaxial surface of *C. raniforme* (l)Adaxial surface of *C. raniforme* (m)Abaxial surface of *Erigeron multiradiatus* (n)Adaxial surface of *E. multiradiatus* (o)Abaxial surface of *Jurineadolomiaea* (p)Adaxial surface of *J. dolomiaea* (q) Abaxial surface of*Saussureanepalensis* (r) Adaxial surface of *S. nepalensis*(s)Abaxial surface of *S. obvallata* (t)Adaxial surface of *S. obvallata*(u)Abaxial surface of *Seneciochrysanthemoides* (v)Adaxial surface of *S. chrysanthemoides*(w)Abaxial surface of *Taraxacumtibatinum*(x)Adaxial surface of *T. tibatinum*

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