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LEAF ANATOMICAL ADAPTATIONS OF SOME HALOPHYTIC AND XEROPHYTIC SEDGES OF THE PUNJAB

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Abstract

Some members of family Cyperaceae inhabit a variety of habitat types in the Punjab region including polluted and brackish wetlands, desert and semi-desert habitats, river and canal banks, wastelands, agricultural fields, etc. Five sedge species (*Bulboschoenus affinis, Cyperus alternifolia*, *C. conglumeratus*, *Fimbristylis dichotoma*, and *Schoenoplectus litoralis*), representatives of distinct habitat types, were studied to investigate the leaf anatomical modifications to withstand environmental stresses. *Cyperus alternifolia* is the cultivated species of this family, whereas, *Cyperus conglumeratus* a xeropbhytic species of Cholistan desert. The later was found to have adapted some specific anatomical modifications like extensive sclerification, stomata located only on abaxial leaf surface, and high proportion of parenchyma. *Fimbristylis dichotoma* recorded from the saline waterlogged areas showed high proportion of aerenchyma in leaves. *Bulboschoenus affinis* and *Schoenoplectus litoralis* recorded from the brackish Kalar Kahar Lake, showed highly developed bulliform cells and extensive aerenchyma development. Generally, the anatomical features were the representatives of specific habitat types and degree of stress tolerance in these species.

Introduction

Family Cyperaceae contains about 70 genera and 4000 species, distributed throughout the world. It is represented by 22 genera and 179 species in Pakistan, of which many of them are weedy species (Kukkonen, 2001). Sedges are found growing in a variety of habitats like polluted soils, hyper-saline waters, dryland salinities; however, many are associated with wetlands, or with poor soils (Khan & Qaiser, 2006).

Generally, in the members of family Cyperaceae, epidermal cells of adaxial surface are much larger than those of abaxial surface (Sharma & Mehra, 1972). Bulliform cells in the midrib region are very well developed in most taxa (Sonnenberg & Botha, 1992). Vascular bundles are collateral and bundle sheath is double-layered, outer layer parenchymatous and inner highly sclerified and fibrous (Zhang *et al*., 1998). Silica bodies (phytoliths) have also been reported in the epidermis of many species of Cyperaceae (Starr & Ford, 2001), whereas stomata are paracytic with two subsidiary cells lying parallel to stomatal pore (Bagniewska-Zadworna & Zenkteler, 2006).

In family Cyperaceae, both C_3 and C_4 photosynthetic forms are common (Sonnenberg & Botha, 1992). Two different types of photosynthetic cells have been described in C_4 sedges, the primary carbon assimilation cells (PCA), which are the positional equivalent of mesophyll cells, and the photosynthetic carbon reduction cells (PCR), the positional equivalent to bundle sheath cells (Soros & Dengler, 2001). On the basis of anatomical features, C_4 sedges have been further divided into 4 subtypes, which are based on the presence or absence of parenchymatous bundle sheath layer and the position of PCR tissue in leaves (Bruhl & Perry, 1995).

Anatomical features of different plant organs are considered an adaptive response to habitat ecology of a certain species (Grigore & Toma, 2007). Therefore, it was hypothesized that native species of family Cyperaceae must have developed specific leaf anatomical features, which have enabled them to survive under harsh climatic conditions like salinity and drought. The present investigation was focused on detailed study of leaf tissue architecture in these species from ecologically very diverse habitats.

Material and Methods

Five species of family Cyperaceae [*Bolboschoenus affinis* (Roth) Drobov, *Cyperus alternifolius* L., *Cyperus conglumeratus* Rottb., *Fimbristylis dichotoma* (L.)Vahl, *Schoenoplectus litoralis* (Schrad.) Palla] were collected from diverse habitats from the Punjab province. *Bulbochoenis affinis* and *S. litoralis* were collected from the hypersaline Kallar Kahar Lake, whereas *F. dichotoma* from a salt-affected habitat near Sahianwala, Faisalabad (Table 1). *Cyperus conglumeratus* was collected from a sand dune of the Cholistan desert. *Cyperus alternifolius,* was collected from the Botanic Garden, University of Agriculture, Faisalabad.

For anatomical studies, a 2 cm piece of leaf and bract from the base was taken and preserved in FAA (formalin acetic alcohol) solution for 48 h. The fixative contained v/v formalin 5%, acetic acid 10%, ethyl alcohol 50% and distilled water 35%. Plant material was later transferred to acetic alcohol solution (v/v acetic acid 25% and ethyl alcohol 75%) for long-term preservation. Free-hand sectioning technique was used to prepare double-stained (safranin and fast green) permanent slides. Camera photographs were taken by Carl-Ziess camera-equipped compound microscope. Anatomical characteristics relating to leaf dermal, parenchymatous, mechanical, and vascular tissues were recorded using ocular micrometer, which was calibrated with a stage micro-meter. The data were subjected to analysis of variance for comparison of means (Steel *et al*., 1997).

Table 1. Details of some species of family Cyperaceae collected from the Punjab

Species	Collection details	Habitat ecology
Bolboschoenus affinis (Roth) Drobov (Alkali bulrush)	Kalar Kahar Lake, Chalwal 32, 46, 30.50N, 72, 42, 01.08E Alt: 643m	An aquatic halophyte (Lillebo *et al*., 2003; Khan & Qaiser, 2006) It is adapted to oligotrophic waters and an important food for waterfowl and other wildlife (Dykyjová, 1986)
Cyperus alternifolius L. (Umbrella sedge)	Old Bot. Garden, Univ. Agri., Fasailabad 31, 25, 43.66N, 73, 04, 16.50E Alt: 187m	Native to the swamps of Madagascar, but cultivated in botanical gardens throughout the world (Ball *et al*., 2002)
Cyperus conglumeratus Rottb. (Monjhan)	Kot Murid, Rahim Yar Khan 28, 22, 03.02N, 70, 45, 52,54E Alt: 93m	A xerophytic plant (Abulfatih, 2003), distributed in coastal and desert habitats in Pakistan (Kukkonen, 2001)
Fimbristylis dichotoma (L.) Vahl (Forked fimbry)	Sahianwala, Faisalabad 31, 35, 51.41N, 73, 14, 32.74E Alt: 189m	A halophytic species (Nemoto & Panchaban, 1991), distributed in grass lands along river, and as a weed in all tropical and subtropical countries (Siwakoti & Tiwari, 2007)
Schoenoplectus litoralis (Schrad.) Palla (Coast club-rush)	Kalar Kahar Lake, Chakwal 32, 46, 29.89N, 72, 42, 51.26E Alt: 643m	An aquatic halophyte (Khan & Qaiser, 2006)

Results

Leaf anatomy: Leaf shape was conical in three species, *Bulboschoenus affinis, Fimbristylis dichotoma* and *Schoenoplectus litoralis* (Table 2 and Fig. 1). These three species also showed highly developed bulliform cells on adaxial surface of leaf midrib, and prominent chlorenchyma along with patches of sclerified hypodermis on abaxial leaf surface of midrib. In addition, these species had two distinct layers of bundle sheath around vascular bundle, the outer with large parenchymatous cells and inner sclerified and fibrous. Large aerenchyma was recorded in the lamina of these species between two successive vascular bundles. Moreover, the size of epidermal cells was different on both adaxial and abaxial surfaces, and those on abaxial surface were much larger than the abaxial cells. The size of metaxylem vessels was also greatly increased in these species.

Table 2. Anatomical characteristics of some species of family Cyperaceae from the Punjab

Species	*Bolboschoenus affinis*	*Cyperus alternifolius*	*Cyperus conglumeratus*	*Fimbristylis dichotoma*	*Schoenoplectus litoralis*
Leaf anatomical parameters					
Midrib thickness (µm)	7146.82c	3484.68a	5261.32b	8289.98e	7697.00cd
Lamina thickness (µm)	5228.52c	2556.50a	3518.34b	5775.64de	5653.32d
Adaxial epidermis thickness (µm)	326.78de	253.14b	320.81d	161.89a	260.92bc
Abaxial epidermis thickness (µm)	81.70a	284.38c	270.64b	323.77d	286.97c
Bulliform thickness (µm)	898.65d	390.66b	248.61a	485.66c	956.71e
Sclerenchymatous thickness (µm)	408.48c	162.00a	285.69b	971.32e	695.79d
Vascular bundle area (μm^2)	0.42c	0.11a	0.29b	0.57d	0.94e
Metaxylem area (μm^2)	104628.72e	21613.25a	77356.51b	83664.96c	99236.19d
Phloem area (μm^2)	124413.69e	55082.81a	72816.12b	93298.47d	83916.80c
Adaxial stomatal density	21.05b	31.14d	35.62e	29.88c	19.41a
Adaxial stomatal area (μm^2)	59702.98e	36629.81c	44317.32d	34465.99b	32541.71a
Abaxial stomatal density	44.11c	0.00a	0.00a	17.32b	44.90c
Abaxial stomatal area (μm^2)	39664.23d	0.00a	0.00a	33071.24b	38831.23c
Bract anatomical parameters					
Midrib thickness (µm)	3435.43b	2618.98a	4357.81d	4133.16c	4266.96cd
Lamina thickness (µm)	1554.12a	2027.60c	3625. 45e	1701.89b	2612.42d
Adaxial epidermis thickness (µm)	81.80a	337.93e	183.52d	143.13b	161.24c
Abaxial epidermis thickness (µm)	93.29a	109.45b	168.73e	142.08d	117.08c
Bulliform thickness (µm)	399.75b	1098.28e	173.53a	486.25c	696.65d
Sclerenchymatous thickness (µm)	363.59d	168.97a	257.82b	324.17c	435.40e
Vascular bundle area (μm^2)	1.07a	1.14b	2.19e	1.25c	1.57d
Metaxylem area (μm^2)	33908.51c	48749.22d	33491.62c	26763.78b	22332.49a
Phloem area (μm^2)	43908.51c	66665.28d	44537.84c	27939.63a	39578.08b
Adaxial stomatal density	26.06c	64.19e	30.72d	24.00b	13.87a
Adaxial stomatal area (μm^2)	32931.38c	32499.48c	47136.84d	27416.24a	28997.48b
Abaxial stomatal density	48.12d	00.00a	00.00a	15.72b	23.87c
Abaxial stomatal area (μm^2)	38664.23d	00.00a	00.00a	34261.82b	36997.48c

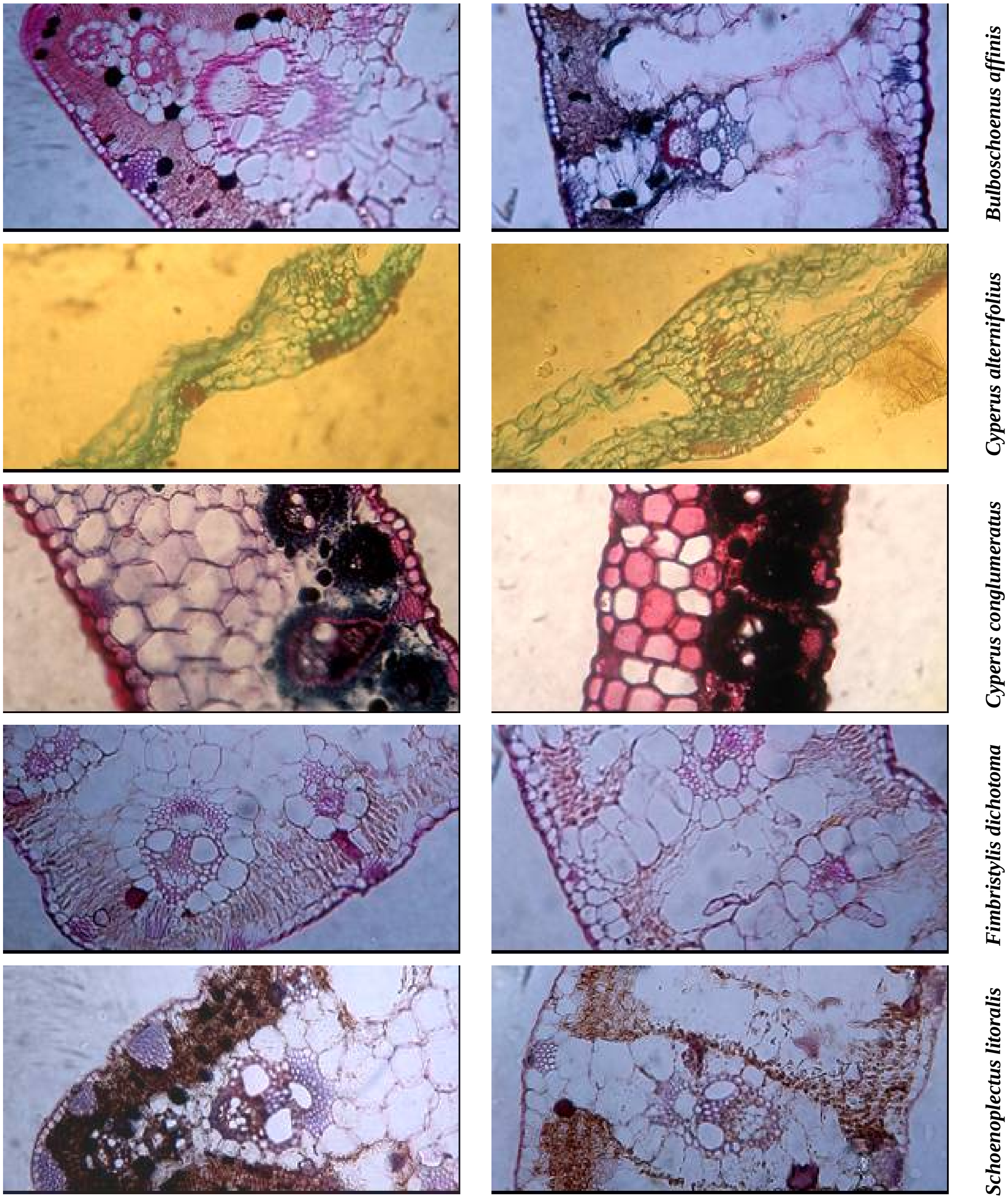

Fig. 1. TS of leaf of some species of family Cyperaceae from the Punjab.

In contrast, *Cyperus conglumeratus* from the desert habitat showed no prominent bulliform cells on the adaxial surface and aerenchyma was totally absent from the leaves. The ratio of cortical parenchyma was relatively higher than those recorded in other species. Sclerification of the vascular tissues was the maximum in this species. *Cyperus alternifolius*, the cultivated ornamental, had no prominent midrib, but high proportion of aerenchyma in the laminar region. Additionally, small patches of hypodermal sclerenchyma were recorded on adaxial leaf surface.

Stomata were absent from the abaxial surface of the leaf in two species, *C. conglumeratus* and *C. alternifolius.* However, the maximum density was recorded on the adaxial surface in *B. affinis,* which also had the maximum stomatal area. *Schoenoplectus litoralis* also had high stomatal density, but stomatal area was relatively lower as compared to that recorded in *B. affinis.* On abaxial surface, the maximum stomatal density was recorded in *C. conglumeratus* and stomatal area in *B. affinis.*

Bract anatomy: Conical-shaped midrib was recorded in *B. affinis, F. dichotoma* and *S. litoralis,* whereas, the midrib in *C. alternifolius* was round in cross-section (Table 2 and Fig. 2). The adaxial epidermal cells were considerably larger than those at the abaxial surface, only in one species, *C. alternifolius.* Well-developed bulliform cells were observed in all species except in *C. conglumeratus.* Aerenchyma was recorded only in two species, *C. alternifolius* and *F. dichotoma*. A double-layered bundle sheath was noticed in the bract of all species, however, in *C. alternifolius* the inner layer was highly sclerified. Moreover, an intensive sclerification was recorded in this species at both sides of vascular bundles. Intensive sclerification was recorded in the vascular region of *C. conglumeratus.*

Stomatal area and density in bracts presented almost a similar pattern as were recorded in the case of leaf (Fig. 3). Stamata were recorded only at abaxial surface in *C. alternifolius* and *C. conglumeratus.* Stamatal density was the maximum in *B. affinis* at the adaxial surface and *C. alternifolius* at the abaxial surface, whereas, stomatal area was the maximum in *B. affinis* at the abaxial surface and in *C. alternifolius* at the abaxial surface.

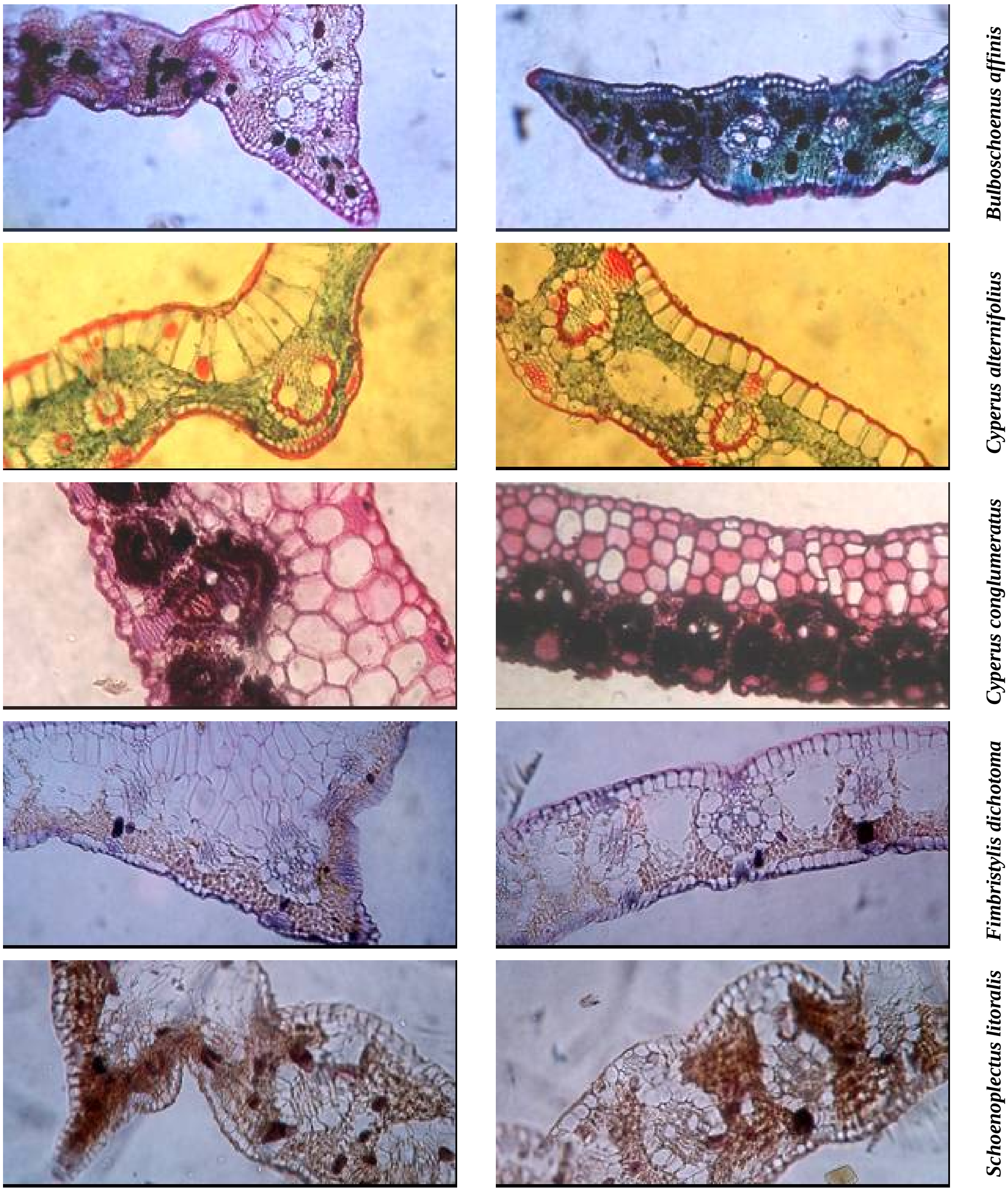

Fig. 2. TS of bract of some species of family Cyperaceae from the Punjab.

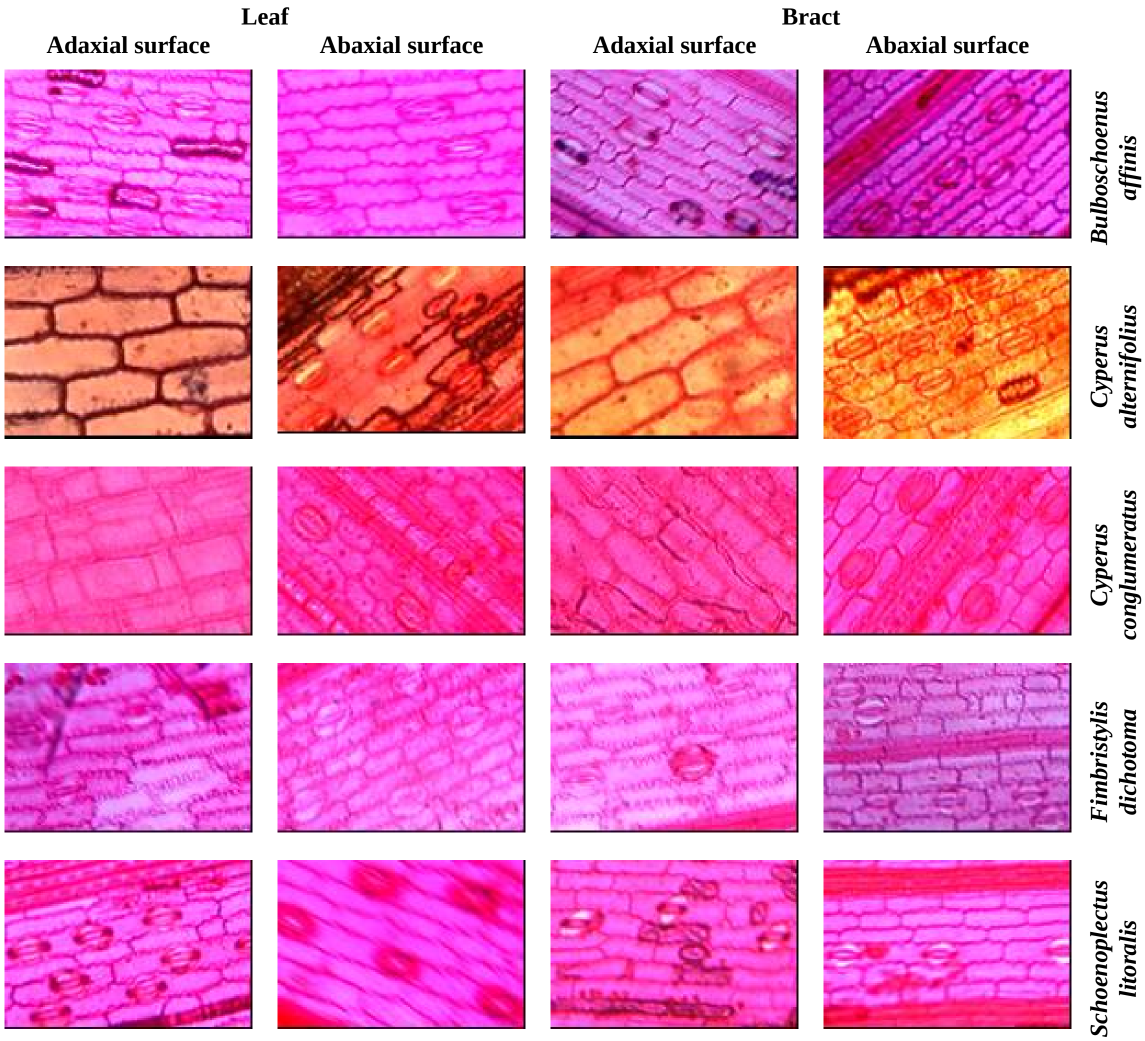

Fig. 3. Leaf and bract surface studies of some species of family Cyperaceae from the Punjab

Discussion

Bulboschoenus affinis and *Schoenoplectus litoralis* aquatic halophytes were collected from hypersaline waters of Kalar Kahar Lake. *Cyperus alternifolia,* recorded previously from aquatic habitats of Madagascar (Lillebo *et al*., 2003) was the only cultivated species found in the Punjab region. *Cyperus conglumeratus,* a xerophytic species was among very few species of Cyperaceae inhabiting the desert habitat (Abulfatih, 2003). *Fimbristylis dichotoma* was also a halophytic species and had been reported to be widely distributed in tropical and subtropical countries (Siwakoti & Tiwari, 2007).

The species from salt affected aquatic habitats (*B. affinis* and *S. litoralis*) showed almost similar structural modifications in their leaves and bracts. However, thicknesses of leaf and bract were relatively greater in *B. affinis*. Moreover, metaxylem and phloem areas were the maximum in this species, whereas sclerenchyma thickness, bulliform area and vascular bundle area were the maximum in *S. litoralis*. Moreover, stomatal density and its area, particularly on adaxial leaf surfaces were the maximum in *B. affinis*. *Schoenoplectus litoralis* also showed large stomata with high density on the adaxial leaf surface. The frequency of aerenchyma in leaves of this species was also quite high. Anatomical modifications in two species, *B. affinis* and *S. litoralis* are very specific, which might have enabled them to inhabit highly saline and waterlogged conditions. Aerenchyma and high density and size of stomatal complex can improve gas exchange more effectively, especially under saline and anaerobic conditions of the soil (Muhlenbock *et al*., 2007; Naz *et al*., 2010). High proportion of parenchymatous bundle sheath around the vascular tissues and bulliform cells may increase water storing capacity, as well as the space for dumping off toxic ions like Na^+ and Cl^- (Nishioka *et al*., 1996; Hameed *et al*., 2009).

Fimbristylis dichotoma, a widely distributed halophytic species, showed similar types of anatomical modifications, as were recorded in other aquatic halophytes. This species shares waterlogged saline habitats as well as dryland salinity. Smaller stomata in this species can control turgor pressure and are the important modifications for tolerating physiological drought conditions (Kulkarni *et al*., 2008). Moreover, thick lamina and midrib with high proportion of aerenchyma may improve the succulence by enhancing water storing ability, but also the translocation of oxygen and salts via aerenchyma (Colmer *et al*., 2009). This again plays a critical role in tolerating a variety of environmental stresses like drought and salinity stresses (Hameed *et al*., 2009).

A xerophytic species, *C. conglumeratus,* showed very specific modifications, which might have enabled it to tolerate extremes of aridity as well as high salinities to some extent. Absence of stomata from the adaxial surface, extensive sclerifications around the vascular tissues and high proportion of stomata in both bract and leaf were the modifications towards water conservation via prevention of water loss through plant surface and storing additional water (Hameed *et al*., 2010). These modifications are vital for the survival of species under extreme aridity as well as in saline coastal areas.

The introduced cultivated species (*C. alternifolius*) showed modifications for only aquatic habitat, and not for saline and anaerobic conditions. The presence of aerenchyma and greatly enlarged epidermal cells can improve gaseous exchange and water storage to some extent (Muhlenbock *et al*., 2007).

Overall, most of the anatomical modifications like presence of aerenchyma, enlarged parenchyma (e.g. bundle sheath and bulliform cells), extensive sclerification, and size and orientation of stomata in leaf are the anatomical markers for stress tolerance. Therefore, it was concluded that leaf anatomical features can be affectively used in evaluating degree of stress tolerance in different species from diverse ecological habitats.

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