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Leaf anatomy of two genera of tribe Eragrostideae (Poaceae) from Mandal forest of Kedarnath wildlife sanctuary, Uttarakhand, India

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Abstract
The Poaceae is a grass family importantly covering all the cereals. The grasses are so vital in the economy of nations and particularly in an agricultural country like ours, where the provision of food and fodder is a constant problem for the populace and it is surprising that more time and energy have not been devoted to the study of the Poaceae. This tribe is characterized by unspecialized spikelets usually with several florets, 3-veined lemmas, and a rather cartilaginous texture, and also by a ciliate ligule, although there are exceptions to all these characters. Leaf anatomical investigations of two Generas belonging to tribe Eragrostideae (Poaceae) were carried out from. Maximum length of long cells was observed in genus *Eragrostis.* Microhairs with hemispherical distal cell, saddle shaped silica bodies and bulliform cells deeply penetrating the mesophyll are the diagnostic characters, which justify both the species in the same tribe.

Keywords: spikelets, ligule, erogrostideae, bulliform, mesophylls etc

1. Introduction
Poaceae is one of the largest families among the angiosperms, and is represented in every phytogeographic region in the world. It comprises about 10,000 species and 651 genera. It is divided into six sub families [9]. The family encompasses tremendous ecological, morphological, physiological, and generic diversity and is divided into 651 [9] to 765 genera [18]. In the classification of grasses proposed by Grass Phylogeny Working Group (GPWG) the genera have been put into forty two tribes and twelve subfamilies.
In Hooker's Flora British India, (1883) [3] the monocotyledons, so, far as it relates to the present Indian political boundary, comparised 424 genera, 4081 species, 14 sub-species and 378 varieties. After that [17] mentioned 246 genera and 2564 species, as well as, new records of plants from the present Indian political boundary. [1] developed a system with the incorporation of chromosome morphology and anatomy of leaf epidermis in his classification. [23, 28], and [6] have made useful contributions to this system.
The leaf epidermal anatomy provides extensive taxonomic data related to grasses. Epidermal traits i.e. epidermal cells, stomata and hairs have proved to be an important tool in delimitation of taxa in many plant families [25, 19, 2, 29]. It is confirmed that leaf anatomical features can help to elucidate taxonomic relationships at different levels [20, 10, 8] and these leaf anatomical characters are of great value in grass systematic and characterization of broad groups within the grasses, particularly subfamilies and tribes.
Occurrence of sclerenchyma and bundle sheath (Kranz Sheath), the width of sclerenchyma, the indumentum of leaves and length and frequency of epidermal basis are important features that can identify relationship among the genera of Poaceae [11, 16, 33]. Characters such as the thickness of the leaf, the number and arrangement of vascular bundles might be systematically useful, and characters such as the distribution of prickles may be relatively stable or environmentally variable [14]. The position of vascular bundles in the blades appears to be a useful diagnostic character above the generic level [13].
Grasses in tribe Eragrostideae look morphologically similar and there is confusion in the identification, differentiation and delimitation of species, genera and the tribe based on morphological description. Anatomical studies could be an important tool to resolve the taxonomic problems within the tribe, so the purpose of the present study is to identify and explore the foliar anatomical diversity in the tribe Eragrostideae that may prove helpful in the identification and differentiation at specific and generic level.

2. Material and Methods
The present study was under taken to assess a anatomical study of two grasses of tribe Eragrostideae. Plant material was collected from Mandal Forest division of Kedarnath Wildlife Sanctuary during field visits. The grass specimens were identified with the help of recent and relevant literature [3, 5, 21, 15, 26]. The herbarium specimens were matched with authentic specimens lodged at Herbarium, Forest Research Institute, Dehradun (D.D.).

2.1 Preparation of Permanent Slides
2.1.1 Fixation
Leaf samples were collected from the Kedarnath wildlife sanctuary at the time of specimen collection. Fixation was the first step for kranz anatomy in which tissue was preserved for anatomical study. The leaf samples were preserved in FAA (formalin acetic acid), made of formaldehyde, alcohol and acetic acid [4] and marked them same number as herbarium specimen.

Composition of FAA

95% ethyl alcohol	=	30.0 ml
Formalin	=	10.0 ml
Glacial acetic acid	=	2.0 ml
Distilled water	=	60.0 ml

2.1.2 Dehydration & Clearing

The collected samples were carried to Plant Physiology laboratory in glass sample bottles. To prepare the leaf samples for paraffin embedding, the leaf samples were washed with distilled water and then dehydrated using serial concentrations of ethyl alcohol 50%, 70%, 90%, 95% and absolute alcohol respectively. All water content was removed with this dehydration process, which would be immiscible with paraffin wax. The samples were kept for one day in each concentration. After removal of water a clearing agent xylene was used because xylene is miscible with both 100% alcohol and paraffin, and makes a bridge between alcohol and paraffin. For clearing, the samples were transferred into pure xylene for overnight.

2.1.3 Paraffin Embedding

Wax embedding was carried out in an oven adjusted at 60^0C where the leaf samples were transferred every 40 minutes from a mixture of wax and xylene, into pure wax and finally into another container or pure paraffin wax. The melted wax, containing the plant segments were poured into a handmade mold, cooled in water and trimmed.

2.1.4 Sectioning

The segments were sectioned transversally using a rotatory microtome (LEICA RM 2145), adjusted at 8-10 microns (µm). Using a brush, the ribons of sections were collected on glass slides, which had been wetted with egg albumin to keep the sections attached to the slides. The slides were left overnight on a hot plate to give maximum expansions of the tissues.

2.1.5 Dewaxing & Staining

Dewaxing was done by immersing the slides with their sections in pure xylene for one minute. To remove the xylene the sections were then dehydrated by transferring them into series of ethyl alcohol concentrations 100%, 95%, 90%, 70% and 50% respectively and stained by flooding them with safranin stain dissolved in 50% ethyl alcohol after staining washed them with distilled water. They were then dehydrated back into 50%, 70%, 90%, 95% and 100% ethyl alcohol respectively and stained with fast green stain dissolved in absolute ethyl alcohol, washed in clove oil, covered with a drop of Canada balsam, and covered with a cover slip. The prepared slides were left for drying in an oven adjusted at 60° C for at least three days.

Fixation, dehydration, clearing, block preparation of leaf samples in paraffin wax and staining was done in Plant Physiology laboratory of Botany Division while microtomy of samples was completed in Forest Pathology Division of Forest Research Institute, Dehradun.

2.1.6 Microscopial Examination

The prepared permanent slides were examined using (Leica Dialux 22 EB) light microscope. The eye piece lens was (x10) whereas the objective lenses were (x4, x10 and x25). The prepared slides were photographed using (Leitz Dialux 20) light microscope fitted with Wild (PMPS II) camera, using Kodak coloured films 36 *ExP*. 24 x 36mm ISO 100/210.

3. Results & Discussion

Table 1: Eragrostideae

1	**Spikelets in open, contracted or spikelike panicles**	**Eragrostis**
+	Spikelets sessile or very shortly pedicelled, loosely to densely Imbricate in digitate or racemosely arranged spikes or spikelike racemes, very rarely in solitary spikes	(2)
2	Spikes persistent	Eleusine
+	Spikes deciduous at maturity	(3)

1. Eleusine indica (L.) Gaertner, Fruct. Sem. 1: 8. 1789; Hook. f. in Fl. Brit. India 7: 293. 1896; Bor, GBCIP 493. 1960; Babu, Herb. Fl. D.Dun 609. 1977; Naithani, F. Chamoli 2: 741. 1985; Gaur, Fl. Garhwal 663. 1999. *Cynosurus indicus* L., Sp. Pl. 72. 1753.

Vernacular Name: Jharnpriya-Kodu, Hindi- Mandla. English-Crab Grass.

Fl. & Fr.: July to November.

Description

Annuals; culms erect or ascending, 30-60cm high, branched, glabrous, leafy; nodes glabrous. Leaves linear, 15-30x0.3cm, scabrid above; sheath flattened, ciliate on the margins; ligule short, truncate membrane, scarious or ciliate. Spikes subdigitate, rarely digitate, compressed, slender, narrow, 4-5, 7-10x0.5cm; spikelets 3-5 flowered, pale green, compressed, glabrous or hairy, 5mm long, 2 seriate.

Lower glume lanceolate-subulate, 3.5mm long, 1 nerved, with winged keel; upper glume 4mm long, many keeled. Lowest lemma ovate-subacute, 4mm long, 3 nerved; palea oblong, 2.5mm long, 2 keeled. Grains oblong-globose, rugose, brown, about 1mm across. Collected from Kanchula Kharak area (2650m).

Leaf Anatomy

Mesophyll cells with radiate chlorenchyma; traversed by column of colourless cells. Leaf blade adaxially flat; with more constant in size. Midrib conspicuous; with one bundle only. Bulliforms present in discrete, regular adaxial groups; associate with colourless cells to form deeply penetrating fans. PCR sheath outline uneven. PCR sheath extensions absent. PCR cells without suberised lamella. The concentric arrangement of mesophyll and bundle sheath indicates that *Eleusine indica* have anatomic feature of C_4 plants (Fig. 1 a, b & c).

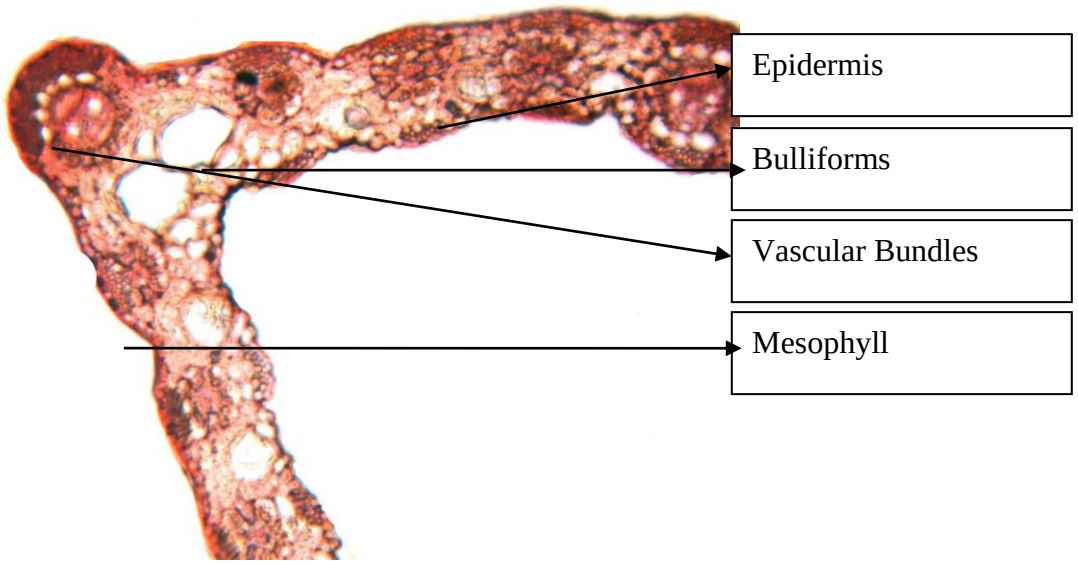
2. Eragrostis nigra Nees ex Steudel, Syn. Pl. Glum. 1: 267.

1854; Hook. f. in Fl. Brit. India 7: 324. 1896; Bor, GBCIP 511. 1960; Naithani, Fl. Chamoli 2: 743. 1985; Gaur, Fl. Garhwal 664. 1999.
Fl. & Fr.: June to September.

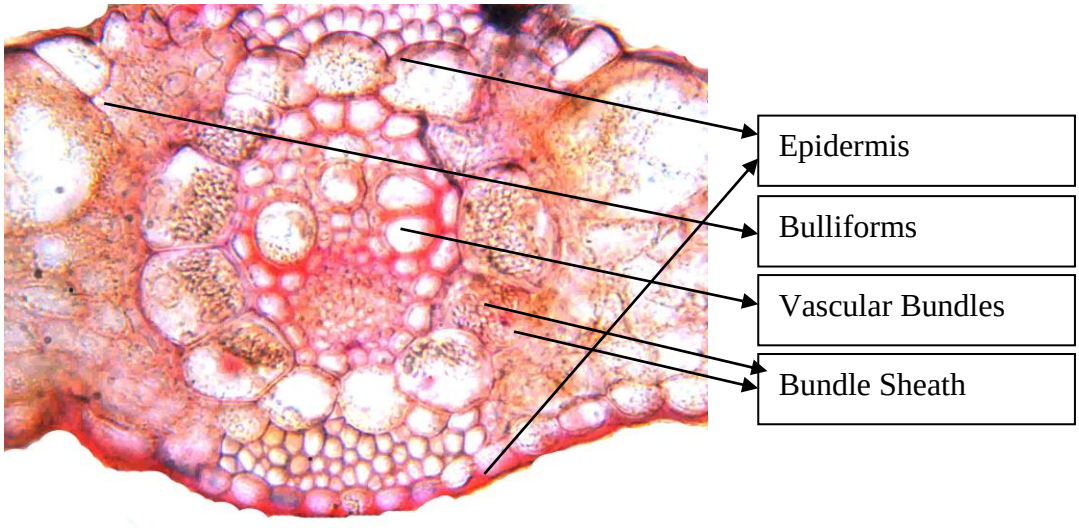
Description
Perennials; culms slender, erect, 15-90cm high, stout and leafy at base, glabrous; node glabrous. Leaves flat, linear, 10-20x0.2-0.5cm, scabrid-hairy; sheath glabrous, striate, mouth bearded; ligule short, ciliate rim.
Panicles effuse, spreading, 15-30cm long; branches many in whorls or fascicles; spikelets 3-9 flowered, scattered, slate-gray to black, 4.5x2mm. glumes compressed, scarious, glabrous, acute, 1 nerved; lower glume 1.5mm long; upper glume 2mm long. Lemmas ovate-concave, acute, 2mm long, 3 nerved; palea obtuse, denticulate, 1.8mm long. Grains minute, oblong. Collected from Deoria Taal and Tungnath area (up to 3450m).

Leaf Anatomy
Mesophyll with radiate chlorenchyma. Leaf blade with distinct, prominent adaxial ribs to adaxially flat. Midrib not readily distinguishable; with one bundle only. Bulliforms present in discrete, regular adaxial groups; associated with colourless cells to form deeply penetrating fans. All the vascular bundles accompanied by sclerenchyma. PCR sheath outlines uneven. PCR sheath extensions present. The concentric arrangement of mesophyll and bundle sheath indicates that *Eragrostis nigra* have anatomic feature of C_4 plants (Fig. 2 a, b & c).

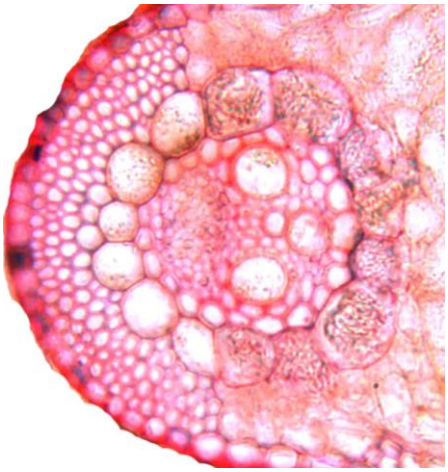
4. Figures



(a)

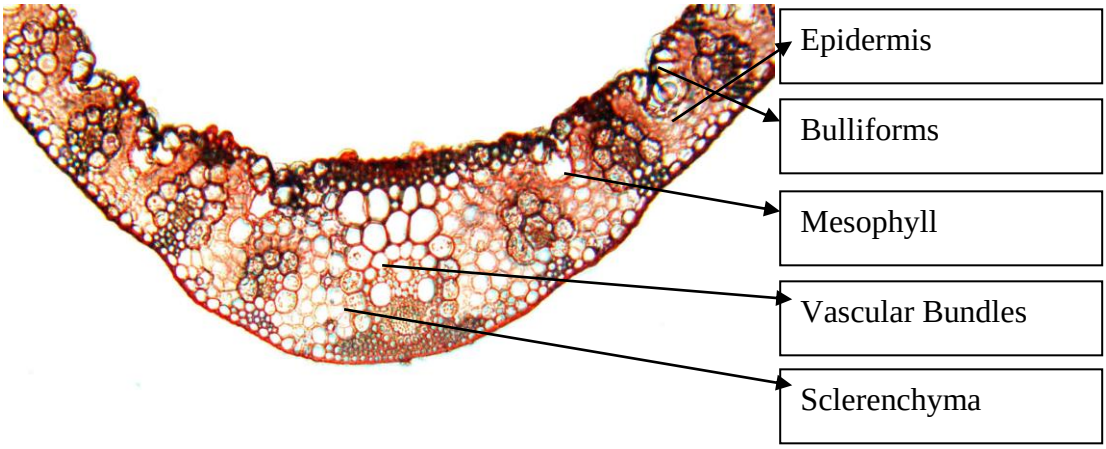


(b)

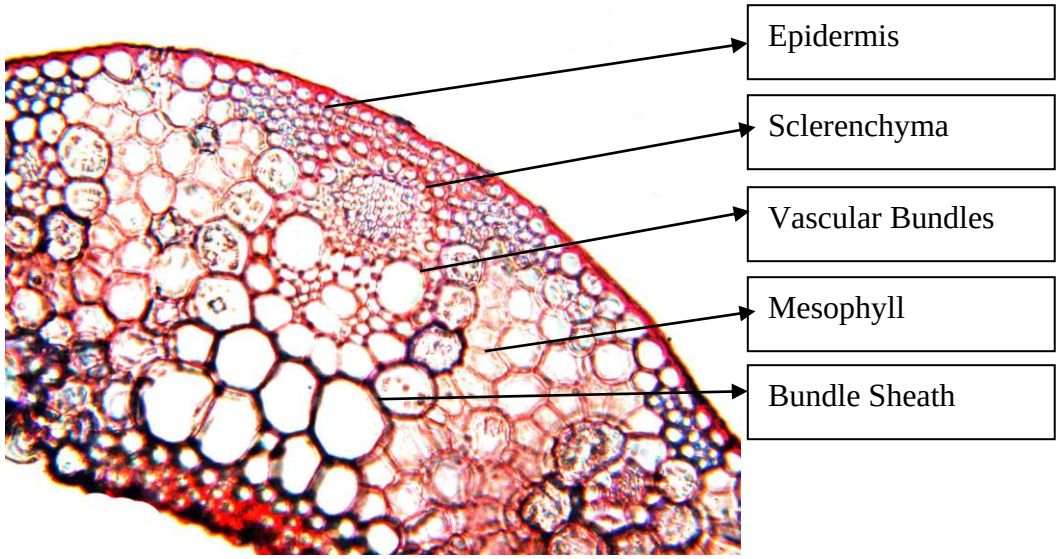


(c) Midrib with single vascular bundle

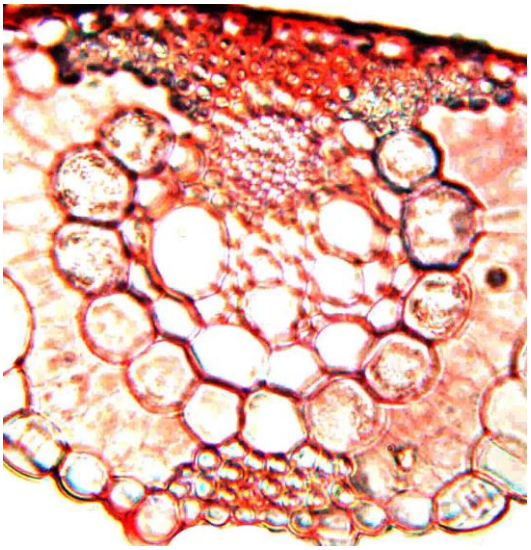
Fig 1: a, b & c Leaf Anatomy of *Eleusine indica*



(a)



(b)



(c) Single vascular bundle with

Fig 2: a, b & c Leaf Anatomy of *Eragrostis nigra*

5. Conclusion

Garhwal Himalaya has been a centre of floristic as well as ecological studies from past hundred of years. The first authentic attempt to collect the plants from Garhwal Himalaya was made by an English officer Thomas Hardwicke in 1796. He collected large number of plants from Alaknanda Valley [7]. Before the later part of 19th century taxonomists were confined to the use of the features of reproductive organs, as floral characters were considered to provide the most valuable characters to taxonomic affinities [22], but the taxonomists have posed many problems by using the traditional methods based on grass morphology. Of all the non reproductive organs, leaf is the most widely used part in plant taxonomy [27], and leaf epidermis is the second most important character parallel with cytology for solving taxonomic problems.

Transverse sections of grass leaves are also helpful in the identification and taxonomic delimitation of grasses. [12] first studied the transverse sections of grass lamina, and used the character of the position of bulliform cells in relation to the vascular bundle for identification purposes.

Occurrence of sclerenchyma and bundle sheath (Kranz Sheath), the width of sclerenchyma, the indumentums of leaves and length and frequency of epidermal basis are features of prime importance that can identify relationship among the genera of Poaceae [11, 16, 14] pointed out that characters such as the thickness of the leaf, the number and arrangement of vascular bundles might be systematically useful, and characters such as the distribution of prickles may be relatively stable or environmentally variable. [13] also observed that the position of vascular bundles in the blades appeared to be a useful diagnostic character above the generic level.

Both genera from the tribe Eragrostideae i.e., *Eleusine* and *Eragrostis*, look morphologically similar but anatomical studies are helpful in their differentiation and identification, when correlated with their morphological characters. Inter-costal long cells in all the species of different genera in the tribe are with thin sinuous or moderately thick sinuous walls. Long cells in genus *Eragrostis* have maximum length ranging from 90-165µm. [20] also observed that long cells are obscured by papillae in this species. Microhairs of grasses are characteristic bicellular trichomes, commonly found on the leaves but also occurring elsewhere in the plant [24, 30]. They are lacking in the subfamily Pooideae but almost universally present in other subfamilies and their presence or absence is widely used as a taxonomic character [31, 32]. The genus *Eragrostis* is recognized by bicellular microhairs with hemispherical distal cell.

According to [14] adaxial parenchyma in keel region is rarely found in Chloridoideae, and never been found in Pooideae. In most species keel is round and conspicuous but *Eleusine indica* differs by having V.shaped keel. Chlorenchyma cells are radially arranged around the vascular bundles and bulliform cells are in fan shaped or irregular groups deeply penetrating into the mesophyll.

The present study showed that different species exhibit variations in different anatomical characters which are valuable in their identification and differentiation, while there are some characters which are similar in all species of the tribe, e.g., saddle shaped silica bodies, microhairs with hemispherical distal cell and bulliform cells deeply penetrating the mesophyll are the characteristic of this tribe, which justify to place all these species in the same tribe.

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