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TAXONOMIC AND ECOLOGICAL IMPLICATIONS OF CULM HISTOLOGY IN FAMILY POACEAE

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Abstract

Culm histology is significant diagnostic feature for the distinguishing and delimitation of taxa at species level. This research is conducted on culm anatomy of twenty-six grasses from arid to semiarid regions of Baluchistan (Saharo-Sindian ecoregion). Sections were prepared using shandon microtome. The outcomes revealed significant diagnostic qualitative and quantitative anatomical characteristics including number and arrangement of major and peripheral vascular bundles, central and marginal cavities, culm shape and outline, shapes and number of different types of cells, sclerification in vascular bundles. Culm shapes were terete, elliptical, quadrangular, and semiterete. In all studied taxa collateral closed vascular bundles were examined. Highest number of collective vascular bundles (55) and largest culm diameter was observed in *C. divisus* 2982.2µm length and 2500.6µm width. The smallest culm diameter was observed in *A. cyanantha* 401.2 µm. Vascular bundles were in the rows of 1-4. Taxonomic key based on qualitative anatomical features significantly discriminate the taxa down to species level. Quantitative variables are statistically analysed, collected them into a matrix and analysed their ability to delimit the studied taxa. PCA is strongly linked for epidermis, vascular bundles, sclerenchyma length and width. Loading and correlation plots determined highest correlation in culm diameter, chlorenchyma, parenchyma, sclerenchyma, vascular bundles and epidermis. Xylem exhibited minimum while phloem was negatively correlated. UPGMA dendrogram and PCA revealed the highest association among species under the same genera *Aristida, Cenchrus, Dactyloctenium,* except species of genus *Eremopyrum*, that showed wide variations on ordination. Highly significant difference among the means in ANOVA provided distinct quantitative features that were important for the discrimination among the studied taxa when combined with qualitative characters.

Key words: Anatomy, Vascularization, Grasses, Baluchistan.

Introduction

Grasses belong to the economically important family Poaceae (Makesh *et al.,* 2022). They are soil stabilizers as they play key role in recycling of eroded soil. Physical and chemical properties of the soil define the grass types in specific floristic regions (Ahmad *et al.,* 2009). Twenty-three percent (23%) of the earth vegetation cover is occupied by grasses (Singh & Parabia, 2003). Worldwide, 10000 species and 620 genera of Poaceae have been reported including 492 species and 158 genera gathered in 26 tribes from Pakistan (Cope, 1982). It is a homogenous group of monocots and easily recognizable family in the field (Anjum & Muhammad, 2012). Poaceae ranks first in abundance among the families. According to the classification of Barnhart (1895), in Pakistan it is represented by five subfamilies comprising of annual or perennial herbs, rarely shrubs and trees (Raees *et al.,* 2017).

To archaebotanists, grasses are of significance for the examination of taxonomic history as they provide various resources for humans, including main food crops. They can survive varied climatic conditions including species surviving in saline, arid, and other harsh environmental conditions due to their greater adaptability (Nazish & Althobaiti, 2022). It includes medicinally important plants e.g. *Cyandon dactylon* (anabolic, antiseptic), *S. italica* (treat chicken pox) (Shamah *et al.,* 2019). Plant histology was essential in classifying two major groups (vascular and non-vascular plants) (Mousa *et al.,* 2021). Petiole anatomical features were successfully manipulated in separation of borassoid palms possessed undivided phloem in petiole from coryphoid palms which have two separate phloem strands in vascular bundles. The presence or absence of vessels, the development of their end walls, and distribution in different parts of plant were found to be significant parameters in phylogeny and taxonomy (Erwin & Stockey, 1991).

Previously, the classification of Poaceae members into tribes was completely relied on the morphology of inflorescence, now, the systematics distribution is heavily based on cryptic characteristics. Characters based on the anatomy of plants are the most important among these cryptic features (Tropicos | Name - !!Poaceae Barnhart). Variations in stem microhairs and nodal vascular plexus assisted in the delimiting Poaceae species into major groups. Macro prickles and the shapes and diameters of vascular bundles, and epidermis were found significant in distinguishing the studied taxa in Poaceae (Al-Khafaji & Al-Bermani, 2014).

Anatomy of different organs of grasses have been studied. Comparative leaf anatomical features of grasses that have significant taxonomic value (Ellis, 1979) were studied to indicate adaptations in different ecological regions (Rafique *et al.,* 2021). Leaf blade morpho-

anatomical studies of bamboos and anatomical adaptations in stem, root and leaf to survive in ecological constraints were investigated (Rafique *et al.,* 2021; Leandro *et al.,* 2020; Mousa *et al.,* 2021). Qualitative and quantitative detailed taxonomic study of stem of Poaceae species helped in characterization of the taxa (Al-Khafaji & Al-Bermani, 2014). Stem of Poaceous taxa of range lands was comparatively analysed based on sclerenchyma and vascular bundles (Makesh *et al.,* 2022). Anatomical evolution in leaf and stem with respect to arid zone was analysed by Shamah *et al.,* (2019).

The effects of climatic variations and weather conditions on stem anatomical features during summer and monsoon were studied by Makesh *et al.,* (2022). The findings were found positive in the delimitation of studied Poaceae taxa. Although the plastic responses, the number of vascular bundles, cortical layers and xylem vessel length were helpful in separating the species. Variations existed in anatomy of grasses in various climatic conditions, these changes helped in characterization taxa up to generic level in grasses (Makesh *et al.,* 2022).

Baluchistan has a land area of 347193 km^2 making it the largest province of Pakistan. It comprises 43% land area of the country. Minimum and maximum temperature range from -10 to 50^oC (Saleem, 1990). Climate of Baluchistan is arid to semiarid, with annual mean 250mm precipitation (Saleem, 1990). 94% of the area of Baluchistan consists of range lands, more than half of the range land area is covered by grasses *Cymbopogon* and *Chrysopogon*, grazed by variety of herbivores. Rain fall of western depression is prevalent in Baluchistan. Climate of Baluchistan is favourable for rearing livestock and fruit production. Availability of water have direct impact on the production of wild flora. Due to its arid climate and high exposure, Baluchistan province is among the most vulnerable to droughts (Jamro *et al.,* 2020). Culm is the stem in the grasses supporting the inflorescence. In this research anatomical studies of grasses are documented first time from Baluchistan. The research will examine the microanatomical variations and similarities that will assist in the delimitation of studied taxa. The study focuses to mark the anatomical features of the grasses in the arid zone Baluchistan province, Pakistan.

Material and Methods

Sampling: Collection of grass species was carried during March 2022 to May 2022, from southern regions of Baluchistan including districts Mastung, Kalat, Kharan, Lasbella, Khuzdar, Hingol national park (Makran) (Fig. 1). Specimens were gathered, preserved, and fumigated. Identification of plants was done by one of us (MA), (Quaid-i-Azam university, herbarium of Pakistan) and Flora of Pakistan (www. efloras.org/). International Plant Name Index (IPNI) and the Plant List (TPL) are utilized for plant names confirmation. Specimens were submitted to herbarium of Pakistan (ISL) QAU with assigned voucher numbers (Table 1).

Table 1. The investigated taxa of Poaceae from Baluchistan province of Saharo-Sindian sub-region.

S.No.	Name of species	Accession number	Locality	Habitat	Elevation (m)	Collector name
1.	*Aristida adscensionis* L.	133255 (ISL) | QAU	Makran	Muddy soil	1633	Bibi Sadia, Amjad Khan
2.	*Aristida cyanantha* Steud.	133256 (ISL) | QAU	Mastung	Silty soil	1440	Bibi Sadia, Wajia
3.	*Aristida funiculate* Trin. & Rupr.	133257 (ISL) | QAU	Yateabad	Gravely soil	1040	Bibi Sadia
4.	*Arundo donax* L.	133258 (ISL) | QAU	Sanjavi	Muddy soil	1100	Bibi Sadia
5.	*Boissiera squarrosa* (Sol.) Nevski	133259 (ISL) | QAU	Kalat	Sandy soil	1800	Bibi Sadia
6.	*Cenchrus setigerus* Vahl	133260 (ISL) | QAU	Hingol, Bela	Lower hills and plains	65	Bibi Sadia, Salman
7.	*Chloris barbata* Sw.	133261 (ISL) | QAU	Othal, Bela	Muddy soil	850	Bibi Sadia, Salman
8.	*Chrysopogon aucheri* (Boiss.) Stapf	133262 (ISL) | QAU	Hingol, Kalat, Makran	Rocky slopes	1800	Bibi Sadia, Amjad Khan
9.	*Cymbopogon martini* (Roxb.) W. Watson.	133263 (ISL) | QAU	Khuzdar, Hingol	Muddy soil	853	Bibi Sadia
10.	*Dactyloctenium aristatum* Link, Hort.	133264 (ISL) | QAU	Bela	Sandy soil	850	Bibi Sadia, Wajia
11.	*Dactyloctenium scindicum* Boiss.	133265 (ISL) | QAU	Bela Othal	Gravely soil	60	Bibi Sadia
12.	*Diplachne fusca* (L.) P. Beauv.	133266 (ISL) | QAU	Hubchowki	Muddy soil	20	Bibi Sadia
13.	*Enneapogon persicus* Boiss.	133267 (ISL) | QAU	Mastung, Hingol	Sandy soil	1650	Bibi Sadia, Salman
14.	*Eragrostis curvula* (Schrad.) Nees	133268 (ISL) | QAU	Kalat, Mastung	Silty soil	1700	Bibi Sadia
15.	*Eremopyrum bonaepartis* (Spreng.) Nevski	133269 (ISL) | QAU	Kalat	Muddy soil	1800	Bibi Sadia, Salman
16.	*Eremopyrum distans* (K. Koch) Nevski	133270 (ISL) | QAU	Mastung, Sibi	Sandy soil	2500	Bibi Sadia, Wajia
17.	*Hordeum marinum* subsp. *gussoneanum* (Parl.) Thell.	133271 (ISL) | QAU	Mastung	Gravely soil	1500	Bibi Sadia, Wajia
18.	*Imperata cylindrica* (L.) Raeusch	133272 (ISL) | QAU	Makran	Semi-sandy soil	2000	Bibi Sadia
19.	*Leptothrium senegalense* (Kunth) Clayton	133273 (ISL) | QAU	Kund maleer	Sandy soil	960	Bibi Sadia, Wajia
20.	*Panicum antidotale* Retz.	133274 (ISL) | QAU	Bela	Sandy soil	63	Bibi Sadia
21.	*Cenchrus divisus* (Gmel.) Henr.	133275 (ISL) | QAU	Makran, Panjgur	Sandy rocky soil	960	Bibi Sadia
22.	*Cenchrus flaccidus* Griseb.	133276 (ISL) | QAU	Kalat, Kharan	Muddy soil	1700-700	Bibi Sadia
23.	*Phalaris minor* Retz.	133277 (ISL) | QAU	Hingol	Gravely soil	1750	Bibi Sadia
24.	*Piptatherum baluchistanicum* Freitag	133278 (ISL) | QAU	Kalat, Hingol	Sandy soil	2500	Bibi Sadia, Salman
25.	*Schismus arabicus* Nees	133279 (ISL) | QAU	Makran, Kalat	Mountains	2124	Bibi Sadia
26.	*Tetrapogon villosus* Desf.	133280 (ISL) | QAU	Makran, Hingol	Mountain slopes	1751	Bibi Sadia, Wajia

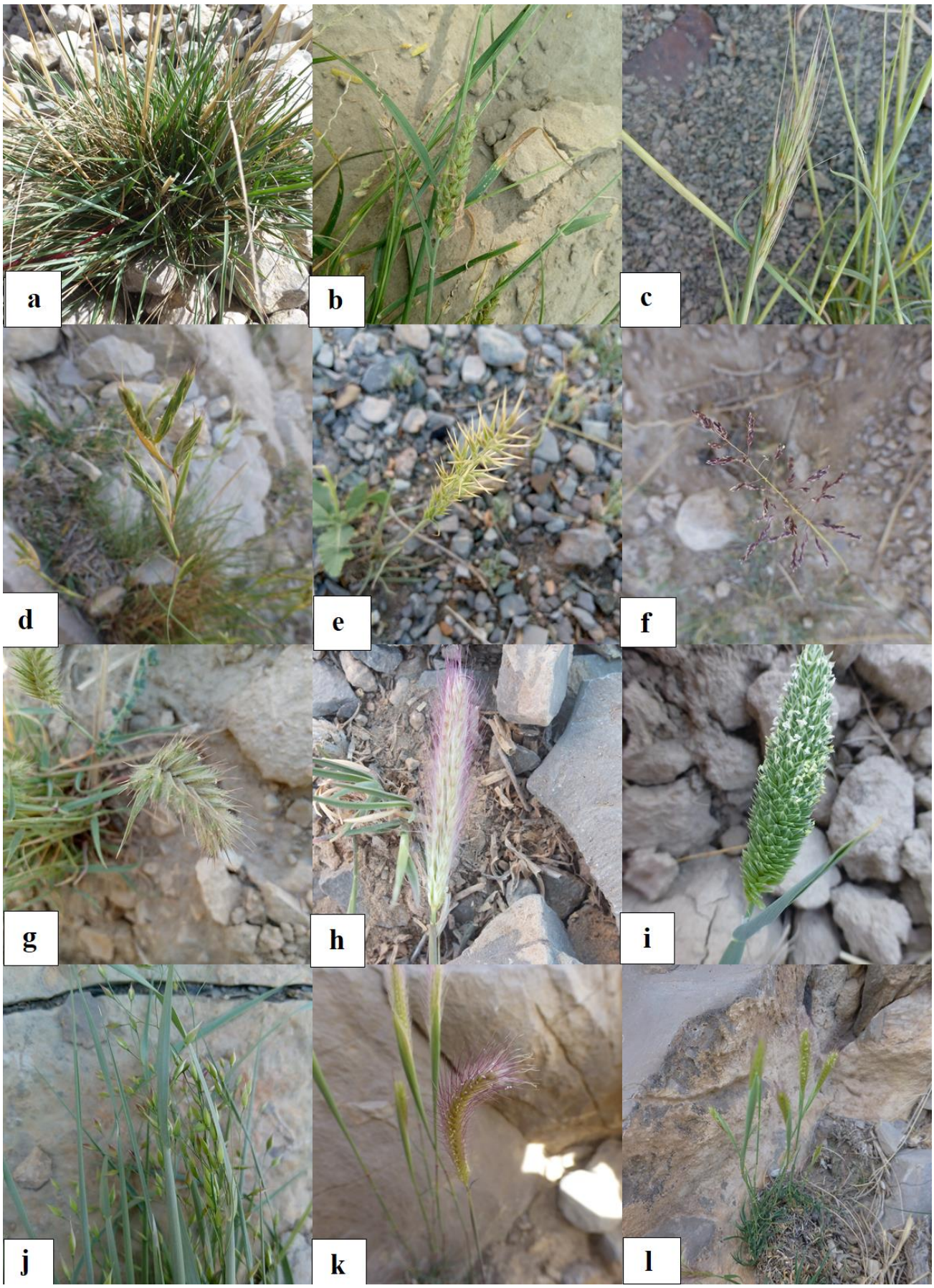

Fig. 1. Field photographs of (**a**) *Arundo donax* (**b**) *Cenchrus setigerus* (**c**) *Chrysopogon aucheri* (**d**) *Cymbopogon martini* (**e**) *Enneapogon persicus* (**f**) *Eragrostis curvula* (**g**) *Eremopyrum distans* (**h**) *Hordeum marinum* (**i**) *Phalaris minor* (**j**) *Piptatherum baluchistanicum* (k) (l) *Tetrapogon villosus*.

Anatomical preparation technique: For the anatomical studies methodology of Akhtar *et al.,* (2022) is used. Sections of the culm are taken from the region that supports the inflorescence.

Section cutting: Culm samples are treated with 10% saline formal solution twice (four hours) for fixation. Methanol in different concentrations 70%, 80%, 90%, 100% and again 100% (each for one hour) was used for dehydration. Xylol was then applied (two changes for one hour) for dealcoholisation followed by impregnation using wax at 58-62ºC (twice for one hour). Culm samples were then embedded via section cutting at 3–5-micron thickness with the use of microtomy. The melting of prepared slides is finally carried out at 62ºC.

Staining: Deparaffinization was carried out for 5 minutes with twice via xylol. Then methanol in different concentrations (100 %, 90% and 70%, one minute each) was applied for rehydration. Washed the slides for one minute with tap water. Then for five minutes the haematoxylin basic stain was applied and washed with tap water for one minute. The slides were then dipped in acid alcohol (1%) and washed for one minute using tap water. Further 1% eosin is applied to the slide for 30-60 seconds, and then washed for one minute with tap water. Methanol in different dilutions (70%, 80%, 90%, & 100% twice) was used for final dehydration for 30 seconds each. The slides were then cleared with xylol with two times for one minute each. Slides were mounted using dibutylphthalate polystyrene xylene (DPX) and then labelled. Prepared slides were observed under Nikon and Meiji (Japan) light microscope. Micrographs were taken by using LEICA-DM-1000 light microscope with fitted camera of Meiji infinity DK-5000. The standard Kellogg, (2015) terminology of Poaceae anatomy was followed. For the cavities the description of Yang *et al.,* (2011) was used.

Statistical analysis: The mean calculated values for each studied character were drawn from 10 observed readings. Statistical analysis was carried out via PAST (4.03) (Hammer *et al.,* 2001). Principal component analysis (PCA) and Unweighted Pair Group Method (UPGMA) dendrogram was constructed based on culm to examine relationship among the studied taxa. Analysis of variance among means was analyzed by multiple samples ANOVA at $p<0.01$ level of significance.

Taxonomic key: The observed anatomical qualitative features of culm were then used to construct identification key for the separation of taxa.

Table 2. Qualitative characteristics based on culm anatomical features of selected Poaceous taxa.

S.No.	Plant name	*Epidermal layers*	*Number of Major VB*	*Number of peripheral VB*	*Number of chlorenchyma layer*	*Number of sclernchyma layer*	*Bundle sheath made up of*		*Ground parenchyma*	*Central Cavity*	*Cortical /Marginal cavities*	*Sclerenchymatou Hypodermal strands*
							MS	PS				
1.	*Aristida adscensionis* L.	1	8	11	2	3	+	+	++	-	-	+
2.	*Aristida cyanantha* Steud.	1	16	22	7	7	+	+	+++	-	+	+
3.	*Aristida funiculate* Trin. & Rupr.	2	19	23	4	5	+	+	+	+	+	+
4.	*Arundo donax* L.	1	12	21	6	5	+	+	++	-	-	+
5.	*Boissiera squarrosa* (Sol.) Nevski	2	10	17	5	4	+	+	+	+	+	+
6.	*Cenchrus setigerus* Vahl	1	19	9	4	2	+	+	++	-	+	+
7.	*Chloris barbata* Sw.	2	14	14	4	3	+	-	+	+	-	+
8.	*Chrysopogon aucheri* (Boiss.) Stapf	1	8	13	3	3	+	+	++	-	-	+
9.	*Cymbopogon martini* (Roxb.) W.Watson.	1	9	6	4	4	+	+	+	+	+	+
10.	*Dactyloctenium aristatum* Link, Hort.	1	20	10	9	2	+	-	++	-	-	+
11.	*Dactyloctenium scindicum* Boiss.	1	15	12	4	3	+	-	+	+	-	+
12.	*Diplachne fusca* (L.) P. Beauv.	1	15	22	9	1	+	+	+	+	+	+
13.	*Enneapogon persicus* Boiss.	1	12	7	4	4	+	+	+	+	-	+
14.	*Eragrostis curvula* (Schrad.) Nees	2	8	12	3	10	+	-	+++	-	-	+
15.	*Eremopyrum bonaepartis* (Spreng.) Nevski	1	5	22	2	7	+	-	++	-	+	+
16.	*Eremopyrum distans* (K. Koch) Nevski	1	13	15	4	8	+	+	++	-	-	+
17.	*Hordeum marinum* subsp. *gussoneanum* (Parl.) Thell.	1	11	12	4	8	+	+	++	-	-	+
18.	*Imperata cylindrica* (L.) Raeusch	2	12	9	4	2	+	+	++	-	+	+
19.	*Leptothrium senegalense* (Kunth) Clayton	1	10	10	4	4	+	+	+++	-	-	+
20.	*Panicum antidotale* Retz.	1	12	10	5	4	+	+	++	-	+	+
21.	*Cenchrus divisus* (Gmel.) Henr.	1	42	13	8	5	+	-	++	-	-	+
22.	*Cenchrus flaccidus* Griseb.	1	13	21	5	5	+	+	++	-	-	+
23.	*Phalaris minor* Retz.	1	13	14	4	3	+	+	++	-	+	+
24.	*Piptatherum baluchistanicum* Freitag	1	14	14	7	6	+	+	+	+	-	+
25.	*Schismus arabicus* Nees	1	12		5	5	+	-	+	+	-	+
26.	*Tetrapogon villosus* Desf.	1	10	9	4	5	+	-	++	-	-	+

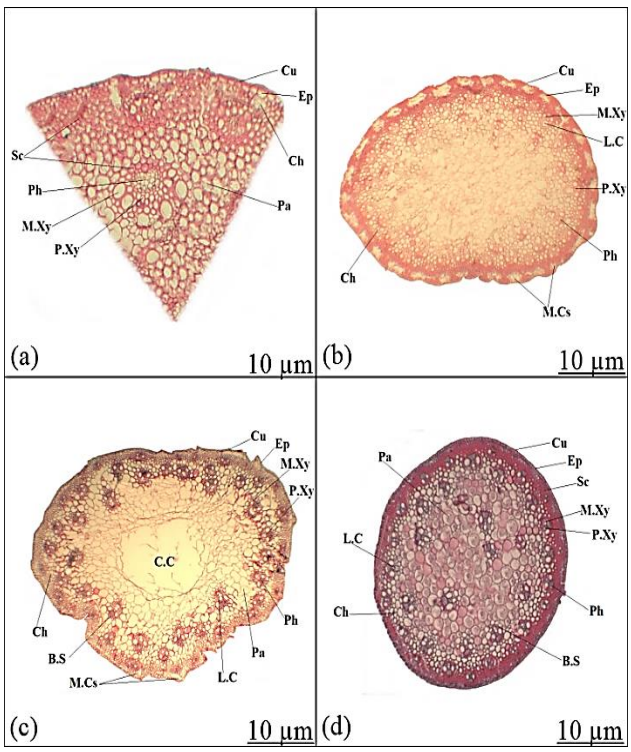

Fig. 2. Culm anatomy of (a) *Aristida adscensionis* (b) *Aristida cyanantha* (c) *Aristida funiculata* (d) *Arundo donax* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: Bundle sheath, L.C: Lysigenous cavity).

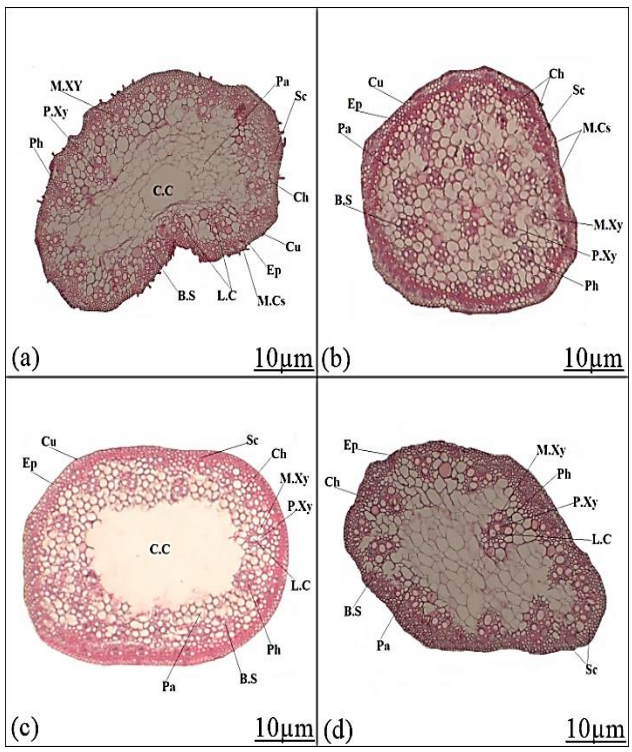

Fig. 3. Culm anatomy of (a) *Boissiera squarrosa* (b) *Cenchrus setigerus* (c) *Chloris barbata* (d) *Chrysopogon aucheri* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: Bundle sheath, L.C: Lysigenous cavity).

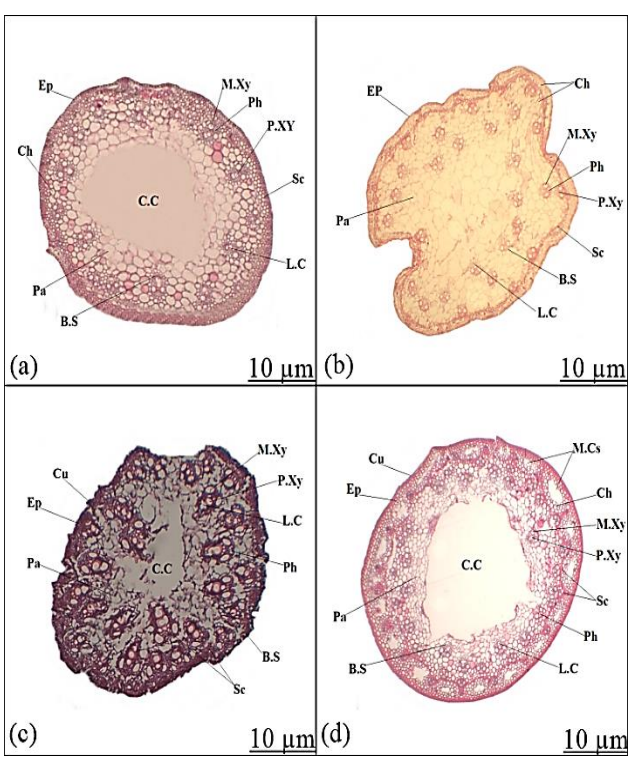

Fig. 4. Culm anatomy of (a) *Cymbopogon martini* (b) *Dactyloctenium aristatum* (c) *Dactyloctenium scindicum* (d) *Diplachne fusca* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: Bundle sheath, L.C: Lysigenous cavity).

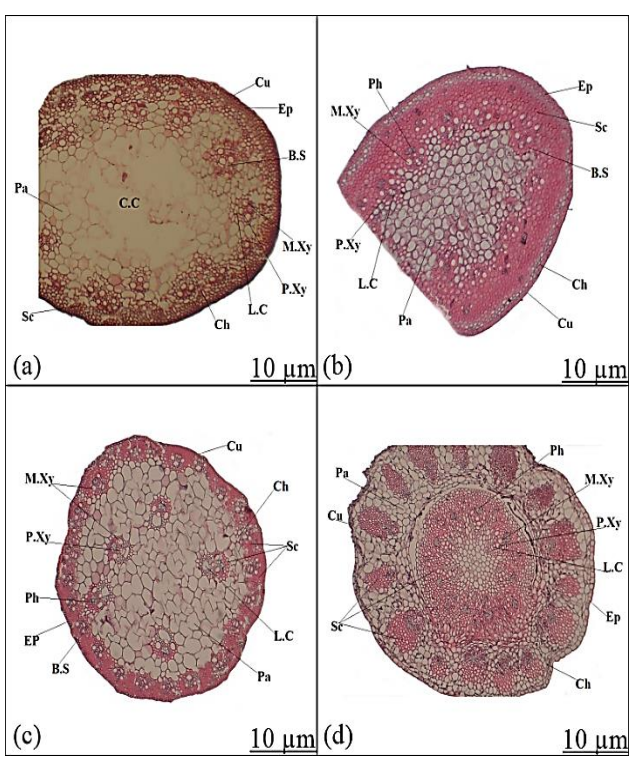

Fig. 5. Culm anatomy of (a) *Enneapogon persicus* (b) *Eragrostis curvula* (c) *Eremopyrum bonaepartis* (d) *Eremopyrum distans* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: Bundle sheath, L.C: Lysigenous cavity).

Table 3. Qualitative characteristics based on culm anatomical features of selected Poaceous taxa.

S.No.	Species name	Culm shape	Epidermis shape	Sclerenchymatous hypodermis shape	Parenchyma shape	Chlorenchyma shape	Vb Arrangments	Cuticle structure	Xylem shape	Phloem shape
1.	*Aristida adscensionis* L.	Terete	Square to Rectangular	Hexagonal	Hexagonal	Annular	Two Rows	Smooth	Oval	Angular
2.	*Aristida cyanantha* Steud.	Elliptical	Square to Rectangular	Angular	Angular to Isodiametric	Isodiametric	Three rows	Smooth	Round	Angular
3.	*Aristida funiculate* Trin. & Rupr.	Semiterete	Angular	Hexagonal	Irregular	Angular	Three rows	Smooth	Round to Oval(M) Isodiametric(P)	Tetra to hexagonal
4.	*Arundo donax* L.	Terete	Angular	Tetra to Hexagonal	Round	Angular	Scattered	Smooth	Oval to Round (M) Isodiametric(P)	Angular
5.	*Boissiera squarrosa* (Sol.) Nevski	Elliptical	Rectangular to Angular	Tetra to Hexagonal	Angular to Isodiametric	Lamellar to Annular	Two rows	Undulated	Round to Oval	Angular
6.	*Cenchrus setigerus* Vahl	Terete	Angular	Angular	Isodiametric	Angular	Scattered	Smooth	Round (M) Isodiametric(P)	Angular
7.	*Chloris barbata* Sw.	Quadrangular	Rectangular	Angular	Isodiametric	Lamellar	Two rows	Smooth	Round	Angular
8.	*Chrysopogon aucheri* (Boiss.) Stapf	Elliptical	Square	Tetra to Hexagonal	Hexagonal	Lamellar	Scattered	Smooth	Round (M) Oval (P)	Angular
9.	*Cymbopogon martini* (Roxb.) W. Watson.	Terete	Rectangular to Angular	Hexagonal	Round to Isodiametric	Angular	Single rows	Smooth	Round	Rectangular to Hexagonal
10.	*Dactyloctenium aristatum* Link, Hort.	Quadrangular	Rectangular	Tetra to Hexagonal	Irregular	Angular	Scattered	Smooth	Round to Oval	Angular
11.	*Dactyloctenium scindicum* Boiss.	Semiterete	Angular	Angular	Irregular	Angular	Two rows	Undulated	Oval (M) Round (P)	Rectangular to Hexagonal
12.	*Diplachne fusca* (L.) P. Beauv.	Terete	Angular	Triangular to Hexagonal	Angular	Angular	Four rows	Smooth	Round	Tetra to hexagonal
13.	*Enneapogon persicus* Boiss.	Elliptical	Round	Triangular to Hexagonal	Hexagonal	Angular	Two rows	Smooth	Round	Rectangular
14.	*Eragrostis curvula* (Schrad.) Nees	Elliptical	Rectangular	Hexagonal	Round to Isodiametric	Angular	Two rows	Smooth	Round	Hexagonal
15.	*Eremopyrum bonaepartis* (Spreng.) Nevski	Terete	Round to Angular	Hexagonal	Tetra to Isodiametric	Angular	Scattered	Smooth	Round to Oval (M) Round to Isodiametric (P)	Tetra to Hexagonal
16.	*Eremopyrum distans* (K. Koch) Nevski	Terete	Rectangular	Tetra to Hexagonal	Irregular	Isodiametric	Three rows	Smooth	Round	Tetra to Hexagonal
17.	*Hordeum marinum* subsp. *gussoneanum* (Parl.) Thell.	Terete	Square to Rectangular	Angular	Irregular	Lamellar	Two rows	Smooth	Round	Tetragonal
18.	*Imperata cylindrica* (L.) Raeusch	Elliptical	Angular	Angular	Irregular	Angular	Scattered	Undulated	Oval (M) Isodiametric (P)	Tetra to Hexagonal
19.	*Leptothrium senegalense* (Kunth) Clayton	Elliptical	Rectangular to Angular	Hexagonal	Irregular	Lamellar	Two rows	Smooth	Round	Tetragonal
20.	*Panicum antidotale* Retz.	Terete	Round	Hexagonal	Isodiametric	Lacunar	Scattered	Undulated	Round & Oval	Tetragonal
21.	*Cenchrus divisus* (Gmel.) Henr.	Terete	Square	Angular	Round to Angular	Hexagonal	Scattered	Smooth	Round	Tetragonal
22.	*Cenchrus flaccidus* Griseb.	Terete	Round	Angular	Round to Oval	Isodiametric	Scattered	Smooth	Round (M) Oval (P)	Triangular
23.	*Phalaris minor* Retz.	Quadrangular	Square	Tetra to Hexagonal	Irregular	Angular	Three rows	Smooth	Round	Tetra to Hexagonal
24.	*Piptatherum baluchistanicum* Freitag	Elliptical	Square	Hexagonal	Isodiametric	Angular	Three rows	Smooth	Round (M) Isodiametric (P)	Tetra to Hexagonal
25.	*Schismus arabicus* Nees	Elliptical	Rectangular	Angular	Irregular	Lamellar	Single row	Smooth	Round & Oval	Tetragonal
26.	*Tetrapogon villosus* Desf.	Terete	Round	Hexagonal to Isodiametric	Isodiametric	Annular	Two rows	Smooth	Round (M) Isodiametric (P)	Angular

Table 4. Quantitative data of histological properties of studied Poaceous species.

S. No.	Plant species	L. W	Epidermal cell (µm)	Chlorenchyma (µm)	Parenchyma (µm)	Sclerenchyma (µm)	Vascular bundle (µm)	Xylem (µm)	Phloem (µm)	Culm (µm)
			Min-Max = Mean ± SE							
1.	*Aristida adscensionis* L.	L	12-17=14.8 ± 0.82689177	11.75-13=12.35 ± 0.231840462	13.75-17.25=16.25 ± 0.637377439	7.75-9.75=8.75 ± 0.395284708	75.25-87=83.1 ± 2.074548144	31.5-37.25=35.5 ± 1.030776406	30.25-35.5=33.55 ± 0.916515139	975-1001=985.6 ± 4.445222154
		W	10.25-13.5=12.35 ± 0.584166072	9.5-12=10.6 ± 0.491172068	17.25-19.25=18.15 ± 0.340954542	7.75-9=8.45 ± 0.215058132	47-50.25=48.4 ± 0.635413251	34-35.5=34.7 ± 0.242383993	32.75-35.75=34.35 ± 0.6254998	938-988=970.6 ± 10.52425769
2.	*Aristida cyanantha* Steud	L	8.75-9.75=9.25 ± 0.176776695	12.75-14.75=13.95 ± 0.348209707	15.5-17.75=16.45 = 0.398434436	5.25-7.75=6.6 ± 0.471699057	50.25-55.5=52.15 ± 0.982980163	38.5-40.5=39.5 ± 0.395284708	37.25-40.25=38.45 ± 0.496235831	255-302=280.2 ± 9.366963222
		W	5.25-7.75=6.4 ± 0.465026881	11.75-12.75=12.2 ± 0.16583124	13-14.75=13.55 ± 0.310241841	6.5-7.25=6.85 ± 0.15	25.25-32=29.25 ± 1.535008143	31.25-33.5=32.2 ± 0.413823634	29.75-33.5=32 ± 0.702673466	393-408=401.2 ± 2.853068524
3.	*Aristida funiculate* Trin. & Rupr.	L	13.25-14.5=13.95 ± 0.215058132	15.75-17=16.25 ± 0.237170825	24.75-27.75=26.2 ± 0.489897949	7-9=8.25 ± 0.353553391	51.25-77=63.6 ± 4.248234928	26.75-32=29.55 ± 0.856592085	30.25-33=32.2 ± 0.502493781	907-1003=972.8 ± 18.81063529
		W	10.75-12.25=11.6 ± 0.26925824	15.75-17=16.25 ± 0.237170825	21.75-23.25=22.45 ± 0.289395923	7-9=8.15= 0.331662479	61-63.25=62.4 ± 0.407737661	32.25-34.25=33.3 ± 0.357071421	28.25-32.25=30.15 ± 0.846315544	796-823=808.8 ± 5.228766585
4.	*Arundo donax* L.	L	15.75-19.5=17.7 ± 0.629483916	21.75-24.5=23.3 ± 0.588430115	27.25-31.25=29.35 ± 0.645174395	13.5-15.75=14.95 ± 0.398434436	105.25-122=111.4 ± 2.991237202	23.5-24.75=2 ± 0.262202212	23.25-25.25=24.05 ± 0.365718471	1902-1988=1962.6 ± 15.64161117
		W	14.5-17.25=15.55 ± 0.48347699	17.75-19.25=18.45 ± 0.289395923	23-24.5=23.6 ± 0.291547595	12-13.75=12.75 ± 0.306186218	62.25-69.5=66.25 ± 1.425219281	20.25-22=21.05 ± 0.289395923	30.25-32.75=31.35 ± 0.465026881	962-988=976.2 ± 4.38634244
5.	*Boissiera squarrosa* (Sol.) Nevski	L	12.25-13.75=13 ± 0.25	12.75-15.75=14.3 ± 0.604152299	19.75-22=21.05 ± 0.443001129	5.75-7.75=6.85 ± 0.340954542	97.75-110.75=104.85 ± 2.080564827	19.75-23=21.85 ± 0.573367247	24.25-26.5=25.3 ± 0.413823634	1008-1158=1109.4 ± 26.88977501
		W	10.25-12.25=11.25 ± 0.395284708	10.25-12.25=11.55 ± 0.348209707	32.75-35.75=34.55 ± 0.598957428	4.5-5.75=5.15 ± 0.231840462	63.75-72.75=68.3 ± 1.560048076	19.25-21.75=20 ± 0.447213595	21-22=21.5 ± 0.176776695	887-931=906.2 ± 7.206941099
6.	*Cenchrus setigerus* Vahl	L	18-19.5=18.8 ± 0.289395923	16.25-18.75=17.15 ± 0.437321392	36-39.75=38.25 ± 0.617454452	15.25-19.75=16.45 ± 0.834415963	90.25-97.75=93.85 ± 1.391042774	37-38.75=38 ± 0.370809924	31.75-38.75=34.9 ± 1.25399362	1505-1607=1562.2 ± 22.79122638
		W	14.5-17.25=15.55 ± 0.48347699	12.25-13.25=12.8 ± 0.16583124	25.5-38.75=29.95 ± 2.276785014	11-13.25=12.1 ± 0.451386752	54.75-63.75=59.2 ± 1.560048076	31.25-36.5=33.65 ± 0.944060379	27-33=28.95 ± 1.044030651	966-988=978.2 ± 4.079215611
7.	*Chloris barbata* Sw.	L	8.75-11=9.95 ± 0.413823634	12.75-17.25=15.35 ± 1.014273139	25.75-29.75=26.95 ± 0.73058196	7-9.5=8.3 ± 0.508674749	83.25-99.75=92.55 ± 2.934067143	33-37.25=34.6 ± 0.792937576	36.75-38.5=37.65 ± 0.340954542	1308-1388=1346.4 ± 13.05603309
		W	7.25-9.5=8.15 ± 0.375832409	11.5-12.75=12.05 ± 0.215058132	19.75-22=21.05 ± 0.398434436	5.25-7.75=6.55 ± 0.496235831	54.5-64=58.25 ± 1.57718103	30.75-33.25=32.2 ± 0.421307489	28.75-32=30.4 ± 0.545435606	788-821=801.8 ± 5.8
8.	*Chrysopogon aucheri* (Boiss.) Stapf	L	19-22=20.55 ± 0.577711	16.75-19.75=17.75 ± 0.541987085	30.5-38.75=33.65 ± 1.509552914	17.75-18.75=18.25 ± 0.176776695	100.25-113=107.4 ± 2.188892414	38.25-39.75=38.95 ± 0.289395923	41.25-42.25=41.75 ± 0.176776695	805-815=808.2 ± 1.854723699
		W	16.7-19=17.55 ± 0.398434436	16-17.25=16.65 ± 0.231840462	19-24.5=21.75 ± 0.974679434	15.25-16.25=15.75 ± 0.176776695	75.75-85.5=80.35 ± 2.00093728	23.25-24.25=23.75 ± 0.176776695	33-34.75=33.95 ± 0.348209707	695-709=703.2 ± 2.973213749
9.	*Cymbopogon martini* (Roxb.) W.Watson.	L	11.5-13.75=12.45 ± 0.382426464	17.25-19.5=18.15 ± 0.375832409	32.25-37=34.5 ± 0.832916562	12.75-14.75=13.9 ± 0.331662479	99.5-108=102.8 ± 1.709166464	30.75-35.5=33.7 ± 0.9367497	32.25-35.75=34.4 ± 0.696419414	1469-1505=1482.6 ± 6.392182726
		W	13.75-15.25=14.45 ± 0.266926956	14.25-15.5=14.85 ± 0.231840462	21.75-25.5=23.3 ± 0.73058196	9.75-12=11.15 ± 0.407737661	66.75-72.75=69.5 ± 1.204159458	29.75-31.5=30.6 ± 0.291547595	32.75-35.75=34.4 ± 0.556776436	921-988=952.4 ± 12.51239386
10.	*Dactyloctenium aristatum* Link, Hort.	L	14.5-16.25=15.45 ± 0.348209707	12.75-15.5=13.85 ± 0.465026881	15.75-19.25=17.65 ± 0.594768863	12.25-14=13.15 ± 0.322102468	90.5-101.25=96.85 ± 1.991544627	43.5-47=45.15 ± 0.687386354	38.25-41.75=39.25 ± 0.637377439	1276-1308=1293.8 ± 5.877074102
		W	12.75-15.75=13.9 ± 0.509901951	13.75-16=15.15 ± 0.407737661	13.25-15.5=14.45 ± 0.456891672	15.25-16.25=15.75 ± 0.176776695	68.25-75.75=72.8 ± 1.374772708	38.5-40.75=39.7 ± 0.390512484	35.25-39.25=37.45 ± 0.751664819	751-788=766 ± 6.473020933
11.	*Dactyloctenium scindicum* Boiss.	L	10.25-13=11.75 ± 0.575543222	13.5-18=15.05 ± 0.80389676	15.25-18.25=16.5 ± 0.670820393	12-13.75=13 ± 0.306186218	80.25-88.75=84.4 ± 1.372953022	23-28.5=26.25 ± 1.051784198	30.5-33.75=32.25 ± 0.575543222	1794-1824=1808 ± 5.394441584
		W	11-13.75=12.6 ± 0.471699057	12-13.25=12.65 ± 0.231840462	14.5-15.5=15.1 ± 0.20310096	8.75-9.75=9.25 ± 0.176776695	101-112.75=106.8 ± 1.893079502	30.25-33.25=32.1 ± 0.509901951	25.25-31.75=29 ± 1.112429773	882-922=904.4 ± 6.682813779
12.	*Diplachne fusca* (L.) P. Beauv.	L	19.75-23=21.25 ± 0.675462804	16.5-20.5=18.85 ± 0.823862853	25.75-27=26.5 ± 0.223606798	13.75-17.75=15.85 ± 0.823862853	70.25-75.75=73.5 ± 1.06653645	19-22.25=20.4 ± 0.709753478	26-34.75=30.8 ± 1.664707181	2001-2101=2043.6 ± 23.06859337
		W	17-19.25=18.15 ± 0.451386752	15.75-18.25=17.25 ± 0.480884602	21.75-23.25=22.55 ± 0.289395923	12-13.25=12.65 ± 0.231840462	49.25-50.5=49.85 ± 0.231840462	27.25-31=29.55 ± 0.677310859	38.25-41.75=39.75 ± 0.77055175	1478-1503=1493.8 ± 4.726520919
13.	*Enneapogon persicus* Boiss.	L	7-9.75=8.25 ± 0.575543222	14.5-15.75=15.15 ± 0.231840462	15.25-17=16.25 ± 0.32596012	12-13=12.55 ± 0.183711731	75.75-87.75=82.9 ± 2.180309611	39.5-47=43 ± 1.459880132	25.75-32.25=30.35 ± 1.20052072	583-618=601.4 ± 5.626721959
		W	6.75-8=7.35 ± 0.231840462	11.75-13.25=12.4 ± 0.26925824	18.75-20.5=19.45 ± 0.310241841	10.25-12.75=11.4 ± 0.430116263	47-58.25=50.3 ± 2.059126028	2.5-39.75=31.15 ± 7.174869337	26-31=29.55 ± 0.913099118	288-305=296.6 ± 3.140063694

Table 4. (Cont'd.).

S. No.	Plant species	L. W	Epidermal cell (µm)	Chlorenchyma (µm)	Parenchyma (µm)	Sclerenchyma (µm)	Vascular bundle (µm)	Xylem (µm)	Phloem (µm)	Culm (µm)
14.	*Eragrostis curvula* (Schrad.) Nees	L	5.25-8.75=6.25 ± 0.637377439	13.25-16.75=14.85 ± 0.654790043	22.25-25.25=24.1 ± 0.497493719	12.75-13.5=13.2 ± 0.145773797	99.75-112=104.15 ± 2.22289226	34-39.5=37.2 ± 1.010569147	33.25-38.5=36.3 ± 1.073545528	1352-1369=1360.8 ± 3.168595904
		W	5.25-9=7.15 ± 0.709753478	7.75-10.75=9.25 ± 0.518411034	18.25-21.75=20.1 ± 0.567890835	9.25-12.25=10.2 ± 0.538516481	43.5-47.25=45.2 ± 0.80389676	39.75-42.25=41.15 ± 0.509901951	32-34.5=33.75 ± 0.447213595	756-801=788 ± 8.300602388
15.	*Eremopyrum bonaepartis* (Spreng.) Nevski	L	8.5-12=10.3 ± 0.588430115	15.25-22=17.15 ± 1.261447581	11.75-13.75=12.55 ± 0.365718471	7.75-9.75=8.65 ± 0.407737661	100.25-115.75=109.1 ± 2.744995446	18.5-20.75=19.65 ± 0.451386752	19.5-20.75=20.15 ± 0.231840462	784-884=828.6 ± 22.5756506
		W	8-9.75=9.05 ± 0.310241841	12.25-14.75=13.35 ± 0.430116263	10.25-12.25=11.2 ± 0.339116499	5.25-7.25=6.45 ± 0.365718471	44-50.5=47.15 ± 1.27377392	17.75-19.75=18.65 ± 0.340954542	17-19.75=18.45 ± 0.566789202	521-578=550.2 ± 10.70233619
16.	*Eremopyrum distans* (K. Koch) Nevski	L	19.75-22=20.85 ± 0.375832409	15.75-19.25=17.9 ± 0.640312424	19.75-22.25=21.45 ± 0.443001129	13.25-15.5=14.35 ± 0.437321392	88.75-97.75=91.45 ± 1.672199151	22-34.25=29.05 ± 2.280898946	23-29=25.3 ± 1.044030651	1439-1488=1465.4 ± 9.43716059
		W	14.25-17=15.5 ± 0.474341649	12.75-15.5=14.35 ± 0.527967802	17-19.25=17.9 ± 0.4	11.75-13.75=12.5 ± 0.353553391	63.5-68.5=65.35 ± 0.896521054	37-41=38.9 ± 0.678232998	22.25-24.5=23.55 ± 0.40620192	1008-1124=1093 ± 21.65871649
17.	*Hordeum marinum* subsp. *gussoneanum* (Parl.) Thell.	L	18.25-20.5=19.15 ± 0.422788363	16.5-18=17.2 ± 0.289395923	18.25-20.25=18.9 ± 0.384057287	11-12=11.55 ± 0.16583124	82-88.75=84.4 ± 1.21346611	26-39.5=34.1 ± 2.767670501	26.5-32=28.5 ± 0.990580638	1488-1554=1509.4 ± 11.48303096
		W	15.75-17.75=16.85 ± 0.340954542	12.75-15.5=14 ± 0.575543222	15.75-20.25=17.75 ± 0.724568837	10.25-12.75=11.3 ± 0.502493781	65.75-72=67.95 ± 1.099431671	31-34.25=32.45 ± 0.532681894	2.5-34.25=26.85 ± 6.100512274	593-661=611.8 ± 12.43945336
18.	*Imperata cylindrica* (L.) Raeusch	L	19.25-22.25=20.7 ± 0.604152299	13.5-15.25=14.45 ± 0.289395923	15.25-16.25=15.9 ± 0.187082869	11.25-13.5=12.65 ± 0.407737661	76.25-82.75=79.35 ± 1.20052072	24.25-32=26.4 ± 1.428723206	26.5-31.75=29.05 ± 1.044030651	1105-1205=1148.4 ± 16.95169608
		W	16.5-19.75=18.5 ± 0.60724789	14-16=14.9 ± 0.407737661	12.75-14.75=13.8 ± 0.339116499	10.25-11.25 ± 10.75±0.176776695	67-71.25=68.8 ± 0.838152731	28.5-38.25=33.95 ± 1.730967937	29.5-33.75=31.75 ± 0.720243015	673-703=691.8 ± 5.462600113
19.	*Leptothrium senegalense* (Kunth) Clayton	L	14.5-16.25=15.45 ± 0.348209707	13.75-15.75=15 ± 0.353553391	15.75-19.25=17.65 ± 0.594768863	12.25-14.25=13.4 ± 0.384057287	91.25-101.25=96.7 ± 1.984943324	33-38=35.1 ± 0.816241386	34.75-35.75=35.35 ± 0.16955825	1276-1308=1293.8 ± 5.877074102
		W	12.75-15.75=13.9 ± 0.509901951	10.25-12.25=11.25 ± 0.379143772	13.25-15.5=14.45 ± 0.456891672	10.25-11.75=11.15 ± 0.257390754	68.25-75.75=72.8 ± 1.374772708	33.5-35.5=34.55 ± 0.398434436	30.25-35.25=33.1 ± 0.9	751-788=766 ± 6.473020933
20.	*Panicum antidotale* Retz.	L	12-13.5=12.7 ± 0.266926956	13-15.25=13.95 ± 0.443001129	17.75-19.5=18.4 ± 0.302076149	10.25-11.5=10.9 ± 0.231840462	150.25-159=153.75 ± 1.594913791	29.5-34=31.8 ± 0.933407735	36.75-39.75=38 ± 0.512347538	1305-1329=1316 ± 4.847679857
		W	10.25-12.25=11.35 ± 0.375832409	12.75-13.75=13.35 ± 0.16955825	12.75-15.25=13.8 ± 0.502493781	7.75-9.75=8.65 ± 0.407737661	94.75-110.25=101.65 ± 2.537961781	34.25-39=36.65 ± 0.970180396	24-27.25=24.8 ± 0.619475585	531-564=550 ± 5.991660872
21.	*Cenchrus divisus* Gmel.) Henr.	L	9-10.5=9.8 ± 0.266926956	18-22=20.05 ± 0.784219357	31.75-34.75=33.45 ± 0.649037749	11.75-14.75=12.7 ± 0.538516481	91.5-100.25=95.95 ± 1.579952531	46-49.75=47.5 ± 0.711512474	32.75-36.75=34.75 ± 0.745821695	2899-3008=2982.2 ± 20.86959511
		W	7-9.5=8.1 ± 0.491172068	14-17.25=16.15 ± 0.594768863	22-24.5=23.3 ± 0.470372193	9-10.75=9.85 ± 0.302076149	69-73=71.15 ± 0.853668554	36-39.25=37.55 ± 0.677310859	24.75-28.5=26.9 ± 0.687386354	2491-2508=2500.6 ± 2.942787794
22.	*Cenchrus flaccidus* Griseb.	L	16-18.25=17.1 ± 0.430116263	16.75-18.5=17.5 ± 0.32596012	18.25-20.25=19.25 ± 0.32596012	10.75-12.75=11.45 ± 0.348209707	72-75.75=73.95 ± 0.739087275	26.25-38.75=31.4 ± 2.111871208	29.5-36.75=33.6 ± 1.343037602	966-1001=982.4 ± 7.131619732
		W	14.75-16.5=15.7 ± 0.320156212	19.75-21=20.45 ± 0.215058132	15.75-17=16.45 ± 0.215058132	8-10.25=9.2 ± 0.413823634	54.75-63.75=58.25 ± 1.693738469	38.75-44.25=41.25 ± 0.977880361	31-37=34.05 ± 1.162970335	793-808=798.6 ± 2.731300057
23.	*Phalaris minor* Retz.	L	8-10.5=9.35 ± 0.515994186	14.25-17.75=15.7 ± 0.743303437	22-24.5=23.35 ± 0.515994186	10.25-11.25=10.75 ± 0.176776695	207-230.75=223.6 ± 4.375071428	33.75-36.5=35.15 ± 0.451386752	22.75-24.75=23.65 ± 0.375832409	1908-2008=1972.4 ± 19.12223836
		W	7.75-9.75=8.5 ± 0.353553391	9.25-12=10.55 ± 0.496235831	15.75-19.75=18.2 ± 0.743303437	8-10.25=9.35 ± 0.375832409	126-135.75=129.85 ± 1.789902232	21.25-24.5=22.85 ± 0.645174395	25.5-28.75=27.05 ± 0.604152299	504-533=514.8 ± 5.462600113
24.	*Piptatherum baluchistanicum* Freitag	L	10.5-12=11.3 ± 0.289395923	12.75-15.5=14.05 ± 0.555652769	24-25.75=24.75 ± 0.32596012	12.75-15.25=14.1 ± 0.444409721	100.25-113=107.8 ± 2.314897406	34.25-39.5=35.9 ± 0.950657667	33.25-37.75=35.7 ± 0.860232527	901-988=940 ± 15.85875153
		W	9-10.25=9.55 ± 0.215058132	8.75-10.75=9.6 ± 0.331662479	18-20.5=19.1 ± 0.527967802	11.25-12.25=11.75 ± 0.176776695	73.75-85.25=79.6 ± 2.141261311	39.25-42.25=40.2 ± 0.538516481	31.75-34.25=32.85 ± 0.527967802	777-801=792.2 ± 4.397726685
25.	*Schismus arabicus* Nees	L	7-9.5=8 ± 0.454147553	12.75-14.75=13.55 ± 0.348209707	15.25-16.5=15.95 ± 0.215058132	10.25-12.75=11.5 ± 0.425734659	96.25-100.75=98.7 ± 0.923309266	33.75-37.25=35.85 ± 0.718505393	43.75-47=45.1 ± 0.615426681	577-614=600.2 ± 6.319810124
		W	5.75-7.25=6.3 ± 0.254950976	11.25-12.25=11.75 ± 0.176776695	12-14.75=13 ± 0.487339717	8.5-10.25=9.45 ± 0.289395923	49.5-57=53.4 ± 1.266392514	39.25-40.75=40.1 ± 0.26925824	38.75-40.75=39.7 ± 0.357071421	244-304=287.2 ± 11.0516967
26.	*Tetrapogon villosus* Desf.	L	4.25-5.5=4.85 ± 0.231840462	17.25-19.5=18.3 ± 0.390512484	30.25-34.75=32.5 ± 0.911729126	15.25-16.25=15.75 ± 0.176776695	63.75-75.25=68.9 ± 1.896707674	33.25-37.25=35.15 ± 0.796868873	36.75-38.25=37.4 ± 0.26925824	1189-1203=1197.8 ± 2.477902339
		W	5.25-6=5.55 ± 0.145773797	14.5-15.75=15.15 ± 0.231840462	28.25-30.5=29.65 ± 0.392109679	12.75-13.75=13.25 ± 0.176776695	87-91.5=88.8 ± 0.834415963	30.25-37.25=32.9 ± 1.218605761	30.25-34.5=32.95 ± 0.755810823	466-501=484.6 ± 6.021627687

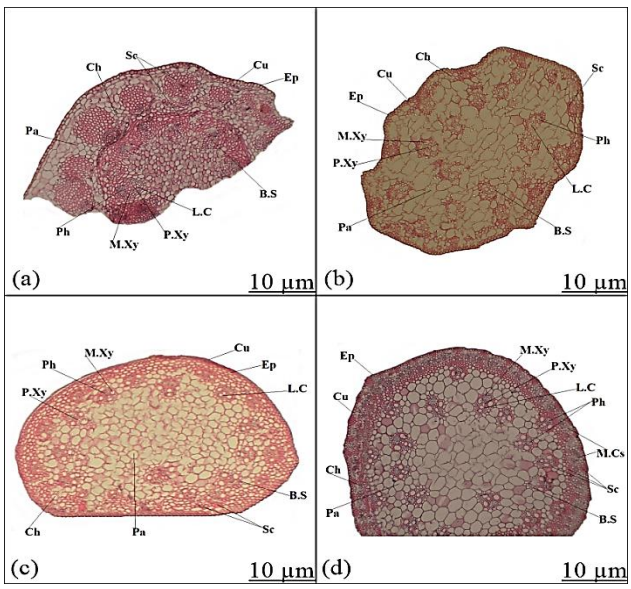

Fig. 6. Culm anatomy of (a) *Hordeum marinum* (b) *Imperata cylindrica* (c) *Leptothrium senegalense* (d) *Panicum antidotale* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: Bundle sheath, L.C: Lysigenous cavity).

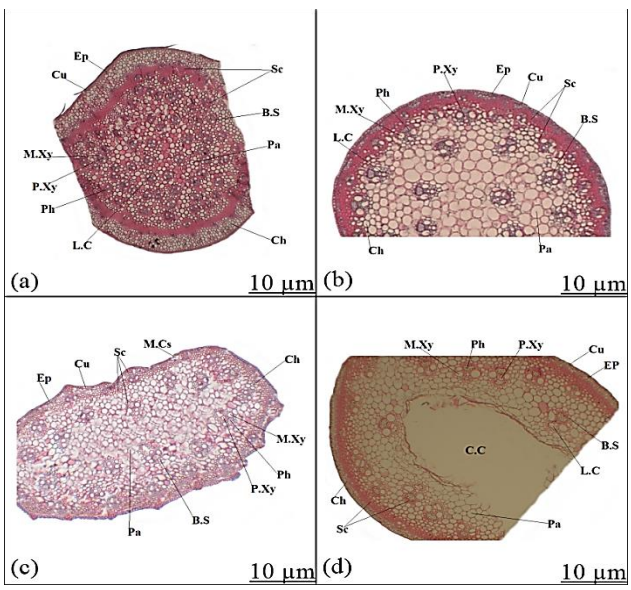

Fig. 7. Culm anatomy of (a) *Cenchrus divisus* (b) *Cenchrus flaccidus* (c) *Phalaris minor* (d) *Piptatherum baluchistanicum* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: Bundle sheath, L.C: Lysigenous cavity).

Results

The details of anatomical examinations of culm of studied species are given below.

Cuticle and epidermis: Thick cuticle was present in *A. adscensionis, C. setigerus, D. scindicum, D. fusca, L. senegalense, C. divisus, P. baluchistanicum* and *T. villosus* (Figs. 2-8). Epidermal cells are compactly arranged in single row in 21 species (Table 2). In *A. funiculata, B. squarrosa, C. barbata, E. curvula* and *I. cylindrica* epidermis was two layered. The shape of epidermal cells is square, rectangular, angular and round (Table 3). Maximum size of epidermis was *D. fusca* 21.25 µm length and 18.15 µm width. Smallest epidermal cell was noted in *T.* villosus 4.85 µm length and 5.55 µm width (Table 4).

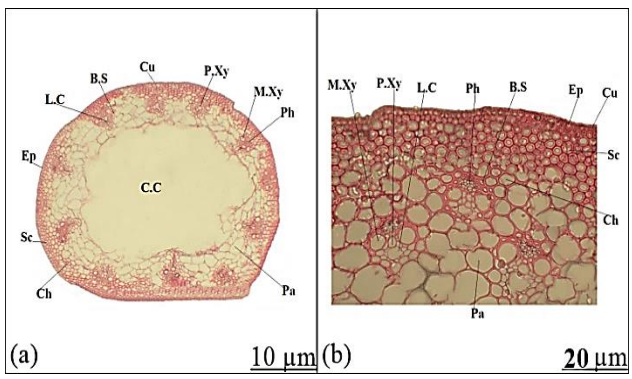

Fig. 8. Culm anatomy of (a) *Schismus arabicus* (b) *Tetrapogon villosus* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: Bundle sheath, L.C: Lysigenous cavity).

Hypodermis and sclerenchyma: Hypodermis was sclerenchymatous in studied taxa with maximum number of layers 10 in *E. curvula* and minimum of one layer in *D. fusca* (Figs. 4, 5). Sclerenchyma cells shapes were from tri, tetra to hexagonal and isodiametric. In *E. distans* and *H. marinum* sclerenchyma strands were found with each vascular bundle. Maximum size of sclerenchyma cell was observed in *C. aucheri* 18.25 µm length and 15.75 µm width. Sclerenchyma cell was smallest in *A. cyanantha* with 6.6 µm length and *B. squarrosa* 5.15 µm width (Table 4).

Parenchyma and chlorenchyma: Ground parenchyma was dense/ compact in *A. cyanantha, E. curvula* and *L. senegalense*. Round, angular, isodiameteric, hexagonal and irregular shapes of parenchyma are observed. Parenchyma cells were smallest in *E. bonaepartis* with length 12.55 µm and width 11.2 µm. *L. senegalense* had maximum cells length 38.25 µm and *B. squarrosa* 34.55 µm width. Chlorenchyma were lamellar, annular, isodiameteric, lacunar, angular and hexagonal (Table 3). *C. divisus* was noted with maximum chlorenchyma cell length of 20.05 µm length and width 20.45 µm in *C. flaccidus*. Smallest chlorenchyma cell was observed in *A. adscensionis* 12.35 µm (length) and *E. curvula* 9.25 µm (width) (Table 4).

Vascularization and cavities: Vascular bundles were collateral closed. Protoxylem lacuna (lysigenous cavity) arose at the inner side of the protoxylem. Bundle sheath surrounded the vascular bundles. It was sclerenchymatous with parenchyma layer. Vascular bundles were arranged in single to four circles or in some plants somewhat scattered arrangement. The vascularization was categorized as major and peripheral vascular bundles. Maximum number of major VB was 42 in *C. divisus* whereas minimum number was 5 in *E. bonaepartis*. *A. funiculate* had maximum number of peripheral vascular bundles 23, whereas *Cymbopogon martini* had minimum number 6. Among the studied taxa 9

species had hollow culm (central cavity) in cross section, and ten species had marginal / cortical cavities (Table 2).

Statistical analysis: Statistical examination of anatomical parameters disclosed that component 1 and 2 accounted for 89.746% and 10.023% sum of squares variance in PCA analysis. Species of similar genera were clustered, closed with exception of *Eremopyrum*. Members of *Aristida* were in close cluster with each other. PCA positively linked Ep.L, Ep.W, Ch.L, Ch.W, Pa.L, Pa.W, Vb.L, Vb.W, Cu.L, Cu.W in cluster while Xy.L, Xy.W and Ph.L, Ph.W are negatively corelated. Outcomes were in the agreement of Mohtashamian *et al.*, (2022), they determined that family Sapindaceae anatomical features (parenchyma length/width, collenchyma length/width, VB) were significant for discrimination and analysing variations. UPGMA dendrogram resulted in two clades, one major cluster with 24 and second with two taxa. Akhtar *et al.*, (2022), observed that diverse taxa expressed more correlation with each other. *C. setigerus* and *C. flaccidus* were marked with differences in quantitative anatomical features compared to *C. divisus*. *Aristida* species were gathered in the same cluster above the two clades. *Eremopyrum* species were in the different clusters (Figs. 11, 12).

Discussion

Culm anatomy is documented in Poaceous taxa for the first time from Southern Baluchistan. The microanatomical structures are significant for differentiating the studied grasses (Apóstolo *et al.,* 2022). Hassan *et al.,* (2022) examined 18 anatomical attributes in the delimitation of seven *Aegilops* species. Anatomy of stem and internodes impart a judgemental significance on the classification of *Aegilpos* taxa. Shape of the culm in the current studied grasses is terete in 12 species, elliptical in 9, quadrangular in 3, and semiterete in 2 species (Kellogg, 2015). Al-Khafaji & Al-Bermani, (2014) documented terete and crescent shapes of the stem in grasses. Culm diameter, vascular bundles shape, diameter, number, distribution and thickness of sclerenchyma are taxonomic tools successfully implicated in discrimination of species of the same genus in the examined Poaceae taxa (Al-Khafaji & Al-Bermani, 2014). The comparative examination revealed that the grasses of the arid regions adapted terete shape culm.

Similarly, in this study the studied taxa of genus *Aristida, Cenchrus, Dactyloctenium* and *Eremopyrum* were observed with difference in culm shapes, shapes of parenchyma, sclerenchyma, chlorenchyma, number of major and peripheral vascular bundles, number of chlorenchyma layers, central and marginal cavities. These variations were specific to each taxon, thus highly significant in the identification and delimitation of studied taxa. The distinct qualitative and quantitative features for all the studied taxa are given in the Tables 2-4 for their separation.

In the current study majority of the grasses possessed smooth cuticle in contrast to undulated one (Wooller, 2002). Thickness and structure of the cuticle were the additional parameters in delimitation of taxa. Thick cuticle was present in *A. adscensionis, C. setigerus, D. scindicum, D. fusca, L. senegalense, C. divisus, P. baluchistanicum* and *T. villous.* Epidermal or endodermal cells in compact arrangement beneath the cuticle is present in Poaceous taxa in single or double layers in square, rectangular, angular and round shapes. Previously thicker epidermis in *Eleusine indica, Paspalidium flavidum* and *Setaria pumila* was reported as strengthening character in their identification (Rafique *et al.,* 2021). In poaceous species photosynthetic tissue chlorenchyma was prominent. Layers of chlorenchyma cells and their diameter are found significant in specification of taxa. Shamah *et al.,* (2019) reported spherical to oval epidermal cells with thick cuticle in stem and a continuous cylinder formed in ground tissues consisting of chlorenchyma strands followed by sclerenchyma surrounding vascular bundles. Sclerenchyma layer in the culms of most of the Poaceae is bounded to epidermis either continuous or discontinuous pattern (Al-Khafaji & Al-Bermani, 2014). Maximum thickness in terms of number of layers of sclerenchyma was observed in *E. curvula.* Whereas *E. bonaepartis, A. donax, C. divisus, C. flaccidus* were also observed with thick sclernchyma. In the studied species sclerenchymatous hypodermis was present. The examined plants showed variations in sclerenchyma strands and number of layers. Sclerenchyma is marked in cortex region, below the epidermis, with vascular bundles. *E. distans* and *H. marinum* are marked with dense sclerenchyma strands in vascular bundles. This trait may be resulted due to less water availability in the arid and semiarid regions. Anatomical traits employed as tools for taxonomy in Bambusa species as strong relation was recorded for microstructures and delimitation of taxa. Dense sclerification in culm specifically in vascular region and inner side of epidermis are distinctive anatomical characters in Bamboos (Apóstolo *et al.,* 2022).

Vascular bundles in the studied grasses are scattered in rings, each surrounded by bundle sheath. Proto and meta xylem (present in Y shape) are distinguished. Protoxylem lacuna or lysigenous cavity is prominent. Vascular bundles are categorized in major and peripheral. Major vascular bundles are larger in size and present towards centre whereas peripheral are arranged at margins and comparatively smaller in size. The number of vascular bundles, their size and arrangement are diagnostic characters for the distinction of examined grass species (Yang *et al.,* 2014). Bundle sheath surrounding the vascular bundles are generally made up of sclerenchyma, called as mestome sheath, it is accompanied by parenchymatous sheath. Shamah *et al.,* (2019) studied that each vascular bundle surrounded by single sclerenchyma bundle sheath, scattered in ground tissue. Larger vascular bundles in *E. tenella* and *D. bipinnata* are significant for their distinction. The key adaptability is phenotypic plasticity which is of taxonomic, environmental, and ecological significance of that trait (Wells *et al.,* 2000). Air spaces are referred as aerenchyma (highly organised with distinct patterns of development) or cavities (large irregular, mostly lysigenous). In grasses these cavities are present in cortex or pith. The studied Poaceous taxa are marked and distinguished for the presence of distinct type of air spaces. Central cavities are characterized in 9 while marginal / peripheral cavities in 10 grasses. *Schismus arabicus* possessed largest central cavity, followed by *Piptatherum baluchistanicum. Aristida cyanantha* and *Diplachne fusca* are marked with highest number of

marginal cavities. Grasses develop central cavities at internodes, may remain solid or combination of solid and hollow (McKim, 2019).

PCA score plots determine clusters of the variables categories and helps to visualize trends in data set in a novel system of axes (Kim *et al.,* 2015). Correlation loading plots for determination of correlation among the mean values, are necessary and strengthen principal component analysis. Uga *et al.,* (2009) studied relationship among the root anatomical traits via PCA plots. These plots contributed to the identification of studied trees. Loading plots determine the positive and negative correlation while correlation of loading plots help to interpret correlation between PCs and variables via presenting significant levels and visualization of explained variance (Kim *et al.,* 2015). Highest correlation is found in culm diameter, chlorenchyma, parenchyma, sclerenchyma, vascular bundles and epidermis respectively. Phloem is negatively correlated with other anatomical traits; little correlation is exhibited with xylem. Xylem is the least positively correlated parameter (Figs. 9-10). Variance among the means is determined via multiple samples ANOVA. Analysis of variance (Multiple sample ANOVA) determine the variations among the data set of more than two independent variables. In this study the p value is less than 0.05 alpha level, while the obtained value is greater than this value. Hence it is concluded that there exists highly statistically significant difference among the means of studied characteristics, this can be used as systematic approach for the delimitation of taxa.

Culm anatomical features have significant implications in modern taxonomy and ecology. Siqueiros-Delgado, (2007) demonstrated the application of culm anatomy in *Bouteloua* and relatives (Gramineae) and stated that there were very few valuable characters. Characterization of the culm anatomy of *Guadua angustifolia* showed its significance in taxonomy of Poaceae (Londoño *et al.,* 2002). Quantitative characterization and morpho-anatomical studies were successfully employed in correct characterization of each studied Bamboos species (Apóstolo *et al.,* 2022). Depending on the cross-sectional diameter and type (solid or hollow) of the stem, the quantitative and qualitative traits have substantially helped to isolate the species. The sclerenchyma tissue thickness varied among studied species. Similar differences existed between the parenchyma tissues in terms of thickness, tissue type and structure, ranging from regular annular to semi-annular or annular tissue types and between tiny, medium, and large clusters of tissue. The number of vascular bundles, their diameters, and the diameter of a single vessel differed significantly between species (Mousa *et al.,* 2021). A taxonomic key based on anatomical characteristics has been effectively used to identify species (Mabel *et al.,* 2013). By examining anatomical features that aid in enabling the classification of complex species, taxonomic keys analyse the differences between species. In this study the anatomical features of the culm are successfully employed in the separation of studied grasses (Table 5).

Positive correlation is determined at generic level in *Aristida, Dactyloctenium, Cenchrus* based on quantitative features such as diameters of epidermis, chlorenchyma, parenchyma, xylem phloem, vascular bundles, culm. While *Eremopyrum* species expressed variations and were divergent in cluster and principal component analysis. Instead, features such as marginal and central cavities, vascular bundles number and arrangement, shapes and number of layers of epidermis, chlorenchyma, sclerenchyma are varied both at generic and species level. These will help in the identification of grass species in the absence of morphological and floral evidence. Previously morpho-anatomical features were successfully employed in identification, differentiation and characterization of Bambusa species utilizing both qualitative (cells shapes, types, surface) and quantitative (cells layers, diameter) features (Apóstolo *et al.,* 2022). The studies have shown that culm anatomy characters can be used to distinguish between different subfamilies, tribes, and genera within Poaceae. For instance, the arrangement of vascular bundles is a diagnostic feature that can be used to distinguish between the subfamilies Panicoideae and Chloridoideae (Banan *et al.,* 2019). Similarly, the presence or absence of bulliform cells in the epidermis and the arrangement of vascular bundles can be used to distinguish between different genera within the subfamily Pooideae (Kumar & Nautiyal, 2017). In conclusion, this study documents the significant anatomical features of studied grasses. The findings can be effectively applied to taxonomical investigations in the taxa placement, evolutionary studies, and the ecological impacts on the anatomical features. Anatomical characteristics that are distinctive and species explicit can assist to carry out species level discrimination.

Ecological implications of culm anatomy: Environmental gradients along with functional traits shape the distribution of species and differences in their occurrence. In arid areas with water scarcity, grass cuticle is one of the significant features that help in survival. The thicker yet differential permeable cuticle in stem help grasses growth in various conditions as it prevent evaporation of water and act as barrier to pathogens (Shamah *et al.,* 2019). It is regarded as paleocological trait for the study of grasses in lake sediments. Cuticle analysis from fossil record is alternative to reconstruct the grass flora previously dominated in the area (Wooller, 2002). Thick epidermis indicates the adaptability of grasses in saline and drought conditions by playing significant role in conservation of water (De Micco & Aronne, 2012). Similarly compact arrangement of cells in epidermis and its thickness is a mark for limited water supply and it as an adaptive feature. Drought and salinity design thicker epidermis in grasses. Thicker epidermis in *Eleusine indica, Paspalidium flavidum* and *Setaria pumila* is reported as an adaptation to salinity and drought stress (Rafique *et al.,* 2021).

Shamah *et al.,* (2019) characterized drought tolerance adaptations in anatomy, helping the survival in arid and semiarid regions. Increased and developed sclerenchyma in grasses help in mechanical support and tolerate stress conditions (Rafique *et al.,* 2021). Increased sclerification is

significant feature in stress tolerant species. It provides strength, resist water loss, and protect plant tissues (Rafique *et al.,* 2021). Structural modifications in microanatomical features are used to assess the tolerance to environmental stresses. In dry environments plants with these adaptations can be used to pasture area and increase the vegetation cover (Apóstolo *et al.,* 2022). These variations help to evaluate the grasses and their adaptability (Rafique *et al.,* 2021). Salinity resulted in increased number of vascular bundles, sclerification, decreased metaxylem, phloem, sheath in *Leptochloa fusca* (Ola *et al.,* 2012). Cavities in the roots of *Paspalum distichum* is an advantage in flooded conditions (Yang *et al.,* 2011). Air spaces help the plants to survive in stressed conditions. Pith cavities and small marginal / cortical cavities studied in 4 grasses regarded as adaptive feature to tolerate flood conditions and these types of species may help to restore the degraded ecosystems (Yang *et al.,* 2011).

Table 5. Dichotomous key based on culm anatomical characters of Poaceous taxa.

Link character	Leads	Characters	Taxa/ Go to link character
1	+	Culm quadrangular	2
	-	Culm non-quadrangular	4
2	+	Epidermis cell square	*Phalaris minor*
	-	Epidermis cell rectangular	3
3	+	Angular sclerenchyma cell in hypodermis	*Chloris barbata*
	-	Tetra to hexagonal sclerenchyma cell in hypodermis	*Dactyloctenium aristatum*
4	+	Culm semiterete	5
	-	Culm non-semiterete	6
5	+	Three rows of vascular bundles	*Aristida funiculata*
	-	Two rows of vascular bundles	*Dactyloctenium scindicum*
6	+	Culm elliptical	7
	-	Culm terete	14
7	+	Vb rows	8
	-	Vb scattered	13
8	+	Vb single row	*Schismus arabicus*
	-	Vb two or three rows	9
9	+	Vb two rows	10
	-	Vb three rows	*Piptatherum baluchistanicum*
10	+	Epidermis cell round	*Enneapogon persicus*
	-	Epidermis cell not round	11
11	+	Cuticle undulated	*Boissiera squarrosa*
	-	Cuticle smooth	12
12	+	Angular chlorenchyma cell	*Eragrostis curvula*
	-	Lamellar chlorenchyma cell	*Leptothrium senegalense*
13	+	Hexagonal parenchyma cell	*Chrysopogon aucheri*
	-	Irregular parenchyma cell	*Imperata cylindrica*
14	+	Phloem angular	15
	-	Phloem not angular	17
15	+	Vb two rows	*Tetrapogon villosus*
	-	Vb scattered	16
16	+	Parenchyma cell round	*Arundo donax*
	-	Parenchyma cell isodiametric	*Cenchrus setigerus*
17	+	Phloem cell triangular	*Cenchrus flaccidus*
	-	Phloem cell not triangular	18
18	+	Chlorenchyma cell lamellar	*Hordeum marinum* subsp. *gussoneanum*
	-	Chlorenchyma cell non lamellar	19
19	+	Vb rows	20
	-	Vb scattered	22
20	+	Vb single row	*Cymbopogon martini*
	-	Vb more than one row	21
21	+	Vb three rows	*Eremopyrum distans*
	-	Vb four rows	*Diplachne fusca*
22	+	Parenchyma cell square	*Cenchrus divisus*
	-	Parenchyma cell not square	23
23	+	Cuticle smooth	*Eremopyrum bonaepartis*
	-	Cuticle undulated	*Panicum antidotale*

ANOVA results.

Test for equal means					
	Sum of sqrs.	**df**	**Mean square**	**F**	**p value**
Between groups:	5.17154E07	15	3.44769E06	111.5	1.756E-132
Within groups:	1.23678E07	400	30919.5	($p<0.01$)	
Total:	6.40832E07	415		1E-05	

Note: The Table 1 and 4 may be kept as supplementary data

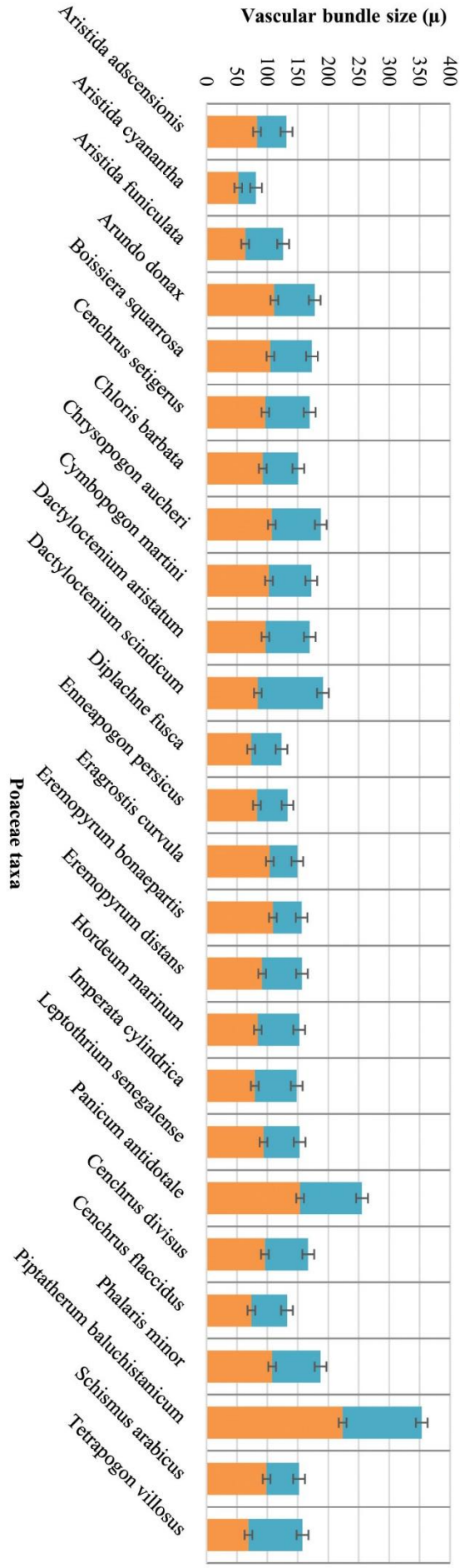

Fig. 9. Variations in mean values of vascular bundles length and width.

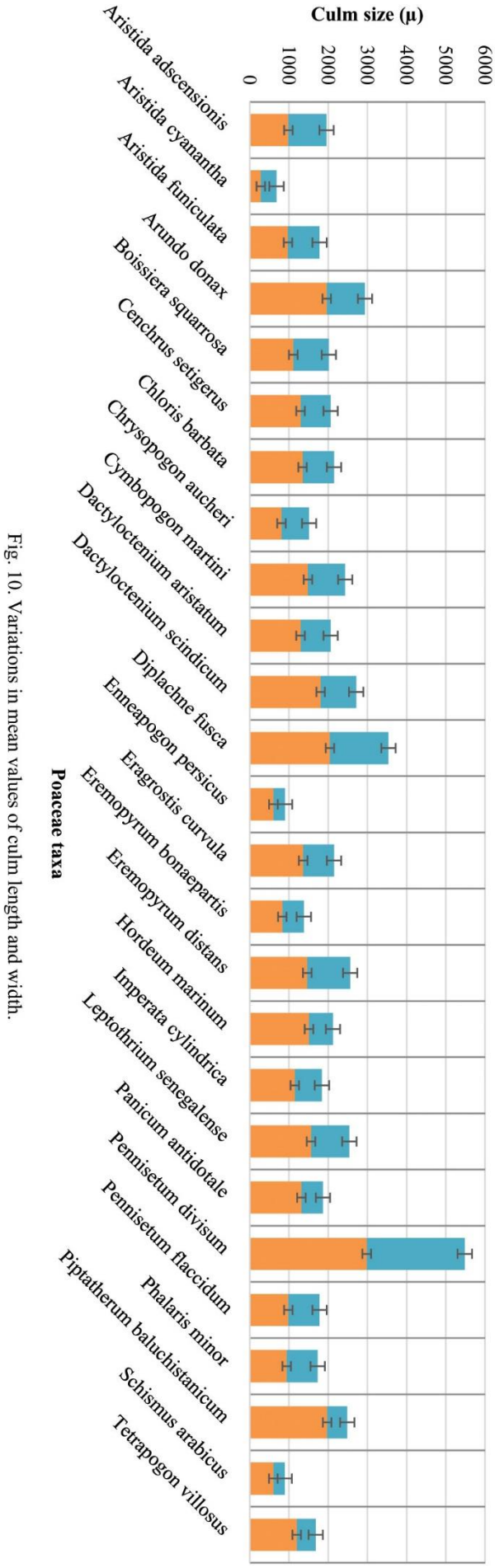

Fig. 10. Variations in mean values of culm length and width.

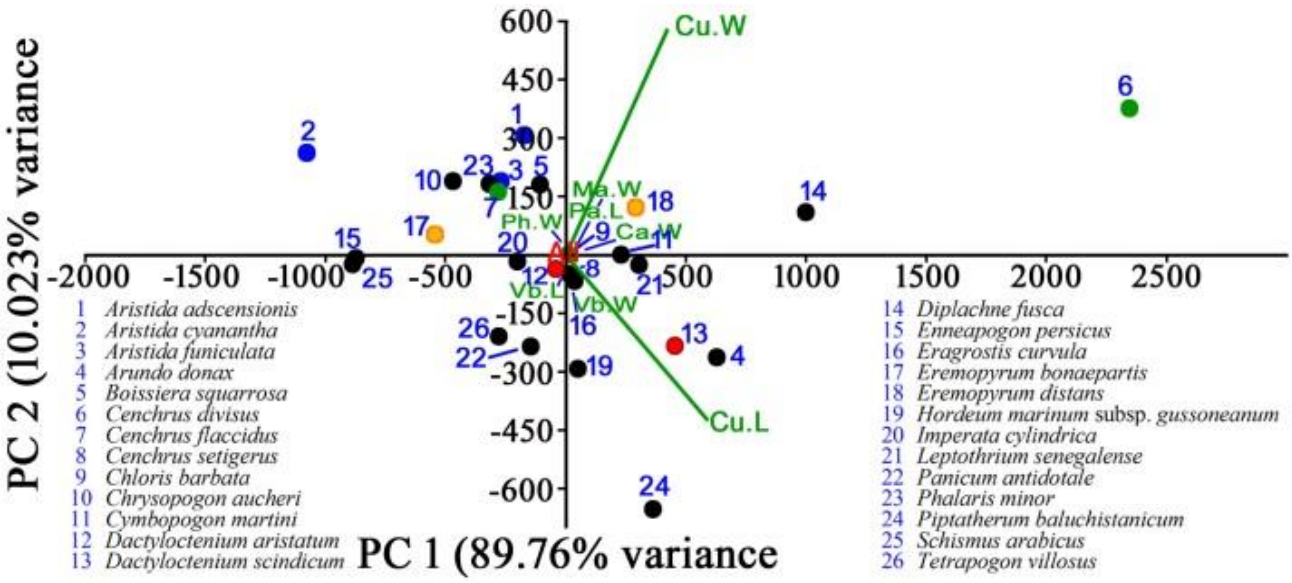

Fig. 11. Utility of culm features in discriminating among species of grasses by PCA (length and width of epidermis, parenchyma, chlorenchyma, xylem, phloem, vascular bundles, culm, sclerenchyma).

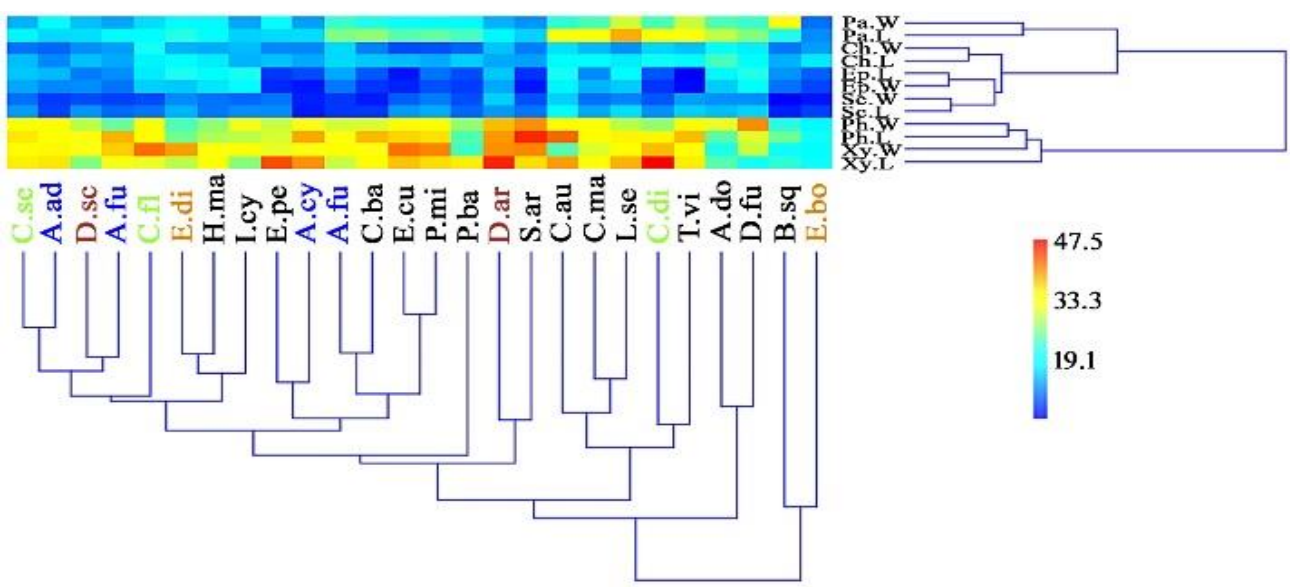

Fig. 12. Dendrogram showing the similarity index of Poaceous taxa based on quantitative parameters of culm.

Conclusion

Culm anatomy is a significant character as it delimits the Poaceae taxa via providing a set of morphological characters that are largely independent of the external morphology. Features such as number, arrangement, and size of vascular bundles, presence or absence of sclerenchyma cells in the cortex, degree of sclerification, arrangement of epidermal cells can be used to distinguish between different grasses and provide valuable insights into their evolutionary relationships and ecological adaptations. The dense sclerenchyma is one of the adaptive features among some of the studied species of this arid region. Whereas quantitative features of culm anatomy may be useful for the identification of different species, particularly when combined with qualitative features. However, it is important to note that these features can vary depending on environmental factors, so it is important to consider multiple characteristics when identifying grasses.

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