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 km² making it the largest province of Pakistan. It comprises 43% land area of the country. Minimum and maximum temperature range from -10 to 50°C (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 58-62°C (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 62°C.

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	l on cul	m anato	omical f	eatures	of sele	ected I	Poaceo	us taxa	ì.		
S.No.	Plant name	Epidermal layers	Number of Major VB	Number of peripheral VB	Number of chlorenchyma layer	Number of sclernchyma layer		Bundle sneath made up of	Ground parenchyma	Central Cavity	Cortical /Marginal cavities	Sclerenchymatou Hypodermal strands
		pide	qun_l	lumb	/ump	/nmb	MS	PS	irour	entr	ortic	clere
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		2	5	5	+	-	+	+	-	+
26.	Tetrapogon villosus Desf.	1	10	9	4	5	+	-	++	-	-	+

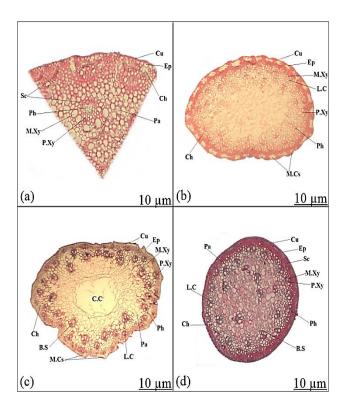


Fig. 2. Culm anatomy of (a) *Aristida adscensionis* (b) *Aristida cyanantha* (c) *Aristida funiculata* (d) *Arundo donax* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: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

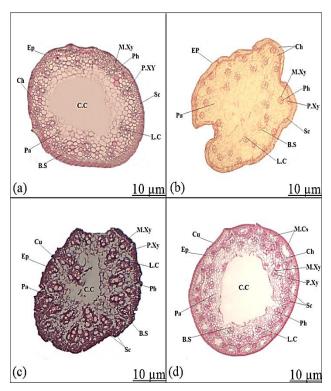


Fig. 4. Culm anatomy of (a) Cymbopogon martini (b) Dactyloctenium aristatum (c) Dactyloctenium scindicum (d) Diplachne fusca (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities,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

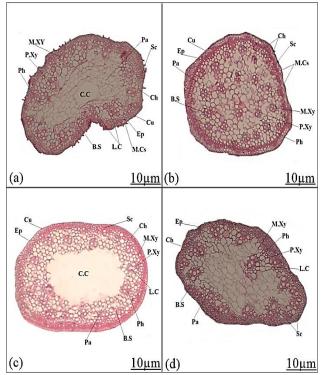


Fig. 3. Culm anatomy of (a) *Boissiera squarrosa* (b) *Cenchrus setigerus* (c) *Chloris barbata* (d) *Chrysopogon aucheri* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: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

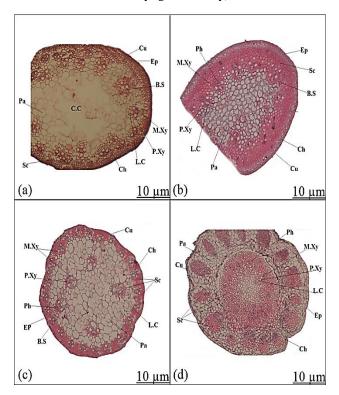


Fig. 5. Culm anatomy of (a) Enneapogon persicus (b) Eragrostis curvula (c) Eremopyrum bonaepartis (d) Eremopyrum distans (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: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

S.No.	S.No. Species name	Culm shape	Epidermis shape	Sclerenchymatous hypodermis shape	Parenchyma shape	Chlorenchyma shape	Vb Arrangments	Cuticle structure	Xylem shape	Phloem shape
-:	Aristida adscensionis L.	Terete	Square to Rectangular	Hexagonal	Hexagonal	Annular	Two Rows	Smooth	Oval	Angular
5.	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	Tetra to Hexagonal	Angular to Isodiametric	Lamellar to Annular	Two rows	Undulated	Round to Oval	Angular
9 7	Cenchrus setigerus Vahl	Terete	Angular Rectanonlar	Angular	Isodiametric Isodiametric	Angular I amellar	Scattered	Smooth	Round (M) Isodiametric(P)	Angular
. %	Chrysopogon aucheri (Boiss.) Stanf	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
Ξ	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
4.	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
,		E	-	Hexagonal to			3			

				Table 4. Quantitative	Table 4. Quantitative data of histological properties of studied Poaceous species.	roperties of studied P	oaceous species.			
S. No.	. Plant species	L. W	Epidermal cell (µm)	Chlorenchyma (um)	Parenchyma (um)	Scierenchyma (um)	Vascular bundle (µm)	Xylem (um)	Phloem (um)	Culm (um)
			,		,	Max =	Mean ± SE	,	,	,
		Γ	12-17=14.8 ± 0.82689177	$11.75 - 13 = 12.35 \pm 0.231840462$	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
-i	Aristida adscensionis L.	A	10.25-13.5=12.35	9.5-12=10.6 ±	17.25-19.25=18.15 ±	7.75-9=8.45 ±	47-50.25=48.4 ±	34-35.5=34.7 ±	32.75-35.75=34.35	938-988=970.6 ±
		_	± 0.384100072 8.75-9.75=9.25 ±	12.75-14.75=13.95 ±	15.5 - 17.75 = 16.45 =	$5.25-7.75=6.6 \pm$	50.25-55.5=52.15 ±	38.5-40.5=39.5 ±	37.25-40.25=38.45	10.32423769 255-302=280.2 ±
2.	Aristida cvanantha Steud	נ	0.176776695	0.348209707	0.398434436	0.471699057	0.982980163	0.395284708	± 0.496235831	9.366963222
		W	$5.25 - 7.75 = 6.4 \pm 0.465026881$	$11.75 - 12.75 = 12.2 \pm 0.16583124$	$13-14.75=13.55 \pm 0.310241841$	6.5 -7.25= 6.85 ± 0.15	$25.25 - 32 = 29.25 \pm 1.535008143$	$31.25-33.5=32.2 \pm 0.413823634$	$29.75 - 33.5 = 32 \pm 0.702673466$	$393-408=401.2 \pm 2.853068524$
	Anistida funimiato	J	13.25-14.5=13.95	$15.75 - 17 = 16.25 \pm 0.237170825$	24.75-27.75=26.2 ±	7-9=8.25 ±	$51.25-77=63.6 \pm 4.248234928$	26.75-32=29.55 ±	$30.25 - 33 = 32.2 \pm 0.2003781$	$907 - 1003 = 972.8 \pm 18.81063529$
ÿ.	Trin. & Rupr.	×	10.75-12.25=11.6	$15.75 - 17 = 16.25 \pm 0.237170825$	21.75-23.25=22.45 ± 0.280305023	7-9=8.15=	$61-63.25=62.4 \pm 0.407737661$	32.25-34.25=33.3	28.25-32.25=30.15 + 0.846315544	796-823=808.8 ± 5.228766585
		Γ	15.75-19.5=17.7 ±	$21.75-24.5=23.3 \pm 0.588430115$	27.25-31.25=29.35 ± 0.645174305	13.5-15.75=14.95 ± 0.308434436	105.25-122=111.4 ±	23.5-24.75=2 ±	23.25-25.25=24.05	1902-1988=1962.6 + 15 64161117
4.	Arundo donax L.	8	14.5-17.25=15.55 + 0.48347699	17.75-19.25=18.45 ± 0.289395923	$23-24.5=23.6 \pm 0.291547595$	12-13.75=12.75 ± 0.306186218	62.25-69.5=66.25 ± 1 42519281	20.25-22=21.05 ± 0.289395923	30.25-32.75=31.35	962-988=976.2 ± 4 38634244
	Roiceiora contanoca	Γ	12.25-13.75=13 ±	12.75-15.75=14.3 ± 0.604152299	19.75-22=21.05 ±	5.75-7.75=6.85 ± 0.340954542	97.75-110.75=104.85	19.75-23=21.85 ± 0.573367247	24.25-26.5=25.3 ±	1008-1158=1109.4
5.	(Sol.) Nevski	A	10.25-12.25=11.25	10.25-12.25=11.55 ±	32.75-35.75=34.55 ±	4.5-5.75=5.15 ±	63.75-72.75=68.3 ±	19.25-21.75=20 ±	21-22=21.5 ±	887-931=906.2 ±
			$\pm 0.395284/08$ $18-195=188$	0.348209/0/	36-39 75=38 25 ±	0.231840462	1.560048076	$0.44/213595$ 37-38 75=38 \pm	31 75-38 75=34 9	7.206941099
9	Conchrus sotioorus Vahl	L	0.289395923	0.437321392	0.617454452	0.834415963	1.391042774	0.370809924	± 1.25399362	± 22.79122638
5	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	×	14.5 - 17.25 = 15.55 ± 0.48347699	$12.25 - 13.25 = 12.8 \pm 0.16583124$	25.5-38.75=29.95 ± 2.276785014	$11-13.25=12.1 \pm 0.451386752$	$54.75-63.75=59.2 \pm 1.560048076$	31.25-36.5=33.65 ± 0.944060379	$27-33=28.95 \pm 1.044030651$	$966-988=978.2 \pm 4.079215611$
		Γ	8.75-11=9.95 ±	12.75-17.25=15.35 ±	25.75-29.75=26.95 ±	7-9.5=8.3 ±	83.25-99.75=92.55 ±	33-37.25=34.6 ±	36.75-38.5=37.65	1308-1388=1346.4
7.	Chloris barbata Sw.	A	7.25-9.5=8.15 ±	$11.5 - 12.75 = 12.05 \pm 0.000$	19.75-22=21.05 ±	5.25-7.75=6.55 ±	54.5-64=58.25 ±	30.75-33.25=32.2	$28.75-32=30.4 \pm 0.00000000000000000000000000000000$	788-821=801.8 ±
		٠	0.5/5852409 $19-22=20.55 \pm$	0.215038132 $16.75-19.75=17.75 \pm$	$30.5-38.75=33.65 \pm$	0.496253831 17.75-18.75=18.25 ±	1.5 / 18103 $100.25 - 113 = 107.4 \pm$	$\pm 0.42130/489$ 38.25-39.75=38.95	41.25-42.25=41.75	805-815=808.2 ±
×	Chrysopogon aucheri	_	0.577711		1.509552914	0.176776695		± 0.289395923	± 0.176776695	1.854723699
5	(Boiss.) Stapf	×	$16.7 - 19 = 17.55 \pm 0.398434436$	$16-17.25=16.65 \pm 0.231840462$	$19-24.5=21.75 \pm 0.974679434$	$15.25 - 16.25 = 15.75 \pm 0.176776695$	$75.75-85.5=80.35 \pm 2.00093728$	23.25-24.25=23.75 ± 0.176776695	$33-34.75=33.95 \pm 0.348209707$	$695-709=703.2 \pm 2.973213749$
c	Cymbopogon martini	Τ	11.5-13.75=12.45 ± 0.382426464	$17.25 - 19.5 = 18.15 \pm 0.375832409$	$32.25-37=34.5 \pm 0.832916562$	12.75-14.75=13.9 ± 0.331662479	$99.5 - 108 = 102.8 \pm 1.709166464$	30.75-35.5=33.7 ± 0.9367497	32.25-35.75=34.4 ± 0.696419414	1469-1505=1482.6 ± 6.392182726
γ.	(Roxb.) W.Watson.	M	13.75-15.25=14.45 ± 0.266926956	$14.25 - 15.5 = 14.85 \pm 0.231840462$	$21.75-25.5=23.3 \pm 0.73058196$	$9.75 - 12 = 11.15 \pm 0.407737661$	$66.75 - 72.75 = 69.5 \pm 1.204159458$	29.75-31.5=30.6 ± 0.291547595	32.75-35.75=34.4 ± 0.556776436	921-988=952.4 ± 12.51239386
	Dactyloctenium aristatum	T	14.5 - 16.25 = 15.45 ± 0.348209707	$12.75 - 15.5 = 13.85 \pm 0.465026881$	$15.75 - 19.25 = 17.65 \pm 0.594768863$	$12.25 \text{-} 14 \text{=} 13.15 \pm 0.322102468$	90.5-101.25=96.85 ± 1 991544627	$43.5-47=45.15 \pm 0.687386354$	38.25-41.75=39.25 ± 0.637377439	1276 - 1308 = 1293.8 ± 5.877074102
10.	Link, Hort.	M	12.75 - 15.75 = 13.9 ± 0.509901951	$13.75 - 16 = 15.15 \pm 0.407737661$	$13.25 - 15.5 = 14.45 \pm 0.456891672$	$15.25 - 16.25 = 15.75 \pm 0.176776695$	$68.25 - 75.75 = 72.8 \pm 1.374772708$	38.5-40.75=39.7 ± 0.390512484	35.25-39.25=37.45 ± 0.751664819	$751-788=766 \pm 6.473020933$
	Dactyloctonium soindicum	Γ	10.25-13=11.75 ± 0.575543222	13.5-18=15.05 ±	15.25-18.25=16.5 ± 0.670820393	12-13.75=13 ± 0.306186218	80.25-88.75=84.4 ±	$23-28.5=26.25 \pm 1.051784198$	30.5-33.75=32.25	1794-1824=1808 ± 5 394441584
ij		×	11-13.75=12.6 ±	12-13.25=12.65 ±	14.5-15.5=15.1 ±	8.75-9.75=9.25 ±	$101 - 112.75 = 106.8 \pm 1000.000$	30.25-33.25=32.1	25.25-31.75=29 ±	882-922=904.4 ±
		Ľ	$0.4/109903/$ $19.75-23=21.25 \pm$	0.231840462 $16.5-20.5=18.85 \pm$	$25.75-27=26.5 \pm$	13.75-17.75=15.85 ±	70.25-75.75=73.5 ±	\pm 0.309901931	$26-34.75=30.8 \pm$	0.082813779 $2001-2101=2043.6$
12.	Diplachne fusca (L.) P. Beauv.		0.675462804 $17-1925=1815+$	0.823862853	0.223606798	0.823862853 $12-13.25=12.65+$	1.06653645	0.709753478	38 25-41 75=39 75	± 23.06859337
		>	0.451386752	0.480884602	0.289395923	0.231840462	0.231840462	0.677310859	± 0.77055175	± 4.726520919
12	Paramone mountain Doice	L	$7 - 9.75 = 8.25 \pm 0.575543222$	$14.5 - 15.75 = 15.15 \pm 0.231840462$	$15.25 - 17 = 16.25 \pm 0.32596012$	$12-13=12.55 \pm 0.183711731$	75.75-87.75=82.9 \pm 2.180309611	$39.5-47=43 \pm 1.459880132$	25.75-32.25=30.35 ± 1.20052072	$583-618=601.4 \pm 5.626721959$
.5.	Enneapogon persicus Boiss.	W	$6.75-8=7.35 \pm 0.231840462$	$11.75 - 13.25 = 12.4 \pm 0.26925824$	$18.75 - 20.5 = 19.45 \pm 0.310241841$	$10.25 - 12.75 = 11.4 \pm 0.430116263$	$47-58.25=50.3 \pm 2.059126028$	2.5-39.75=31.15 ± 7.174869337	$26-31=29.55 \pm 0.913099118$	288-305=296.6 ± 3 140063694
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					Table 4. (Cont'd.)	nt'd.).				
S. No.	S. No. Plant species	L. W	Epidermal cell (μm)	Chlorenchyma (μm)	Parenchyma (µm)	Sclerenchyma (μm)	Vascular bundle (μm)	Xylem (µm)	Phloem (µm)	Culm (µm)
	Fraorostis curaula	ı	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 ±	34-39.5=37.2 ± 1 010569147	33.25-38.5=36.3 ± 1 073545528	1352 - 1369 = 1360.8 ± 3.168595904
14.	(Schrad.) Nees	W	$5.25-9=7.15 \pm 0.709753478$	7.75-10.75=9.25 \pm 0.518411034	$18.25 - 21.75 = 20.1 \pm 0.567890835$	$9.25 - 12.25 = 10.2 \pm 0.538516481$	$43.5-47.25=45.2 \pm 0.80389676$	39.75-42.25=41.15 ± 0.509901951	$32-34.5=33.75 \pm 0.447213595$	$756-801=788 \pm 8300602388$
	Eremonvrum honaepartis	Γ	$8.5 - 12 = 10.3 \pm 0.588430115$	$15.25-22=17.15 \pm 1.261447581$	11.75-13.75=12.55 ± 0.365718471	$7.75-9.75=8.65 \pm 0.407737661$	100.25 - 115.75 = 109.1 ± 2.744995446	18.5 - 20.75 = 19.65 ± 0.451386752	19.5-20.75=20.15 ± 0.231840462	$784-884=828.6 \pm 22.5756506$
15.	(Spreng.) Nevski	×	$8-9.75=9.05 \pm 0.310241841$	$12.25 - 14.75 = 13.35 \pm 0.430116263$	$10.25 - 12.25 = 11.2 \pm 0.339116499$	$5.25-7.25=6.45 \pm 0.365718471$	$44-50.5=47.15 \pm 1.27377392$	17.75-19.75=18.65 ± 0.340954542	$17-19.75=18.45 \pm 0.566789202$	$521-578=550.2 \pm 10.70233619$
Š	Eremonvrum distans	Γ	$19.75-22=20.85 \pm 0.375832409$	$15.75 - 19.25 = 17.9 \pm 0.640312424$	$19.75 - 22.25 = 21.45 \pm 0.443001129$	$13.25 - 15.5 = 14.35 \pm 0.437321392$	88.75-97.75=91.45 ± 1.672199151	22-34.25=29.05 ± 2.280898946	$23-29=25.3 \pm 1.044030651$	1439 - 1488 = 1465.4 ± 9.43716059
16.	(K. Koch) Nevski	A	$14.25 - 17 = 15.5 \pm 0.474341649$	$12.75 - 15.5 = 14.35 \pm 0.527967802$	$17-19.25=17.9\pm0.4$	11.75-13.75=12.5 ± 0.35353391	$63.5 - 68.5 = 65.35 \pm 0.896521054$	$37-41=38.9 \pm 0.678232998$	22.25-24.5=23.55 ± 0.40620192	$1008 - 1124 = 1093 \pm 21.65871649$
-	Hordeum marinum subsp.	Γ	18.25-20.5=19.15 ± 0.422788363	$16.5 - 18 = 17.2 \pm 0.289395923$	$18.2520.25\text{=}18.9 \pm \\ 0.384057287$	$11-12=11.55 \pm 0.16583124$	$82-88.75=84.4 \pm 1.21346611$	26-39.5=34.1 ± 2.767670501	$26.5 - 32 = 28.5 \pm 0.990580638$	$1488 - 1554 = 1509.4$ ± 11.48303096
17.	gussoneanum (Parl.) Thell.	×	15.75-17.75=16.85 ± 0.340954542	$12.75-15.5=14 \pm 0.575543222$	15.75-20.25=17.75 ± 0.724568837	$10.25 - 12.75 = 11.3 \pm 0.502493781$	$65.75 - 72 = 67.95 \pm 1.099431671$	31-34.25=32.45 ± 0.532681894	2.5-34.25=26.85 ± 6.100512274	593-661=611.8 ± 12.43945336
0	Imperata cylindrica (L.)	Γ	19.25-22.25=20.7 ± 0.604152299	13.5-15.25=14.45 \pm 0.289395923	$15.25 - 16.25 = 15.9 \pm 0.187082869$	11.25-13.5=12.65 \pm 0.407737661	$76.25-82.75=79.35 \pm 1.20052072$	$24.25-32=26.4 \pm 1.428723206$	26.5-31.75=29.05 ± 1.044030651	$1105-1205=1148.4$ ± 16.95169608
	Raeusch	W	$16.5 - 19.75 = 18.5 \pm 0.60724789$	$14-16=14.9 \pm 0.407737661$	$12.75 - 14.75 = 13.8 \pm 0.339116499$	10.25-11.25 ± 10.75±0.176776695	$67\text{-}71.25\text{=}68.8 \pm 0.838152731$	28.5-38.25=33.95 ± 1.730967937	29.5-33.75=31.75 ± 0.720243015	$673-703=691.8 \pm 5.462600113$
-	Leptothrium senegalense	Γ	14.5 - 16.25 = 15.45 ± 0.348209707	$13.75 - 15.75 = 15 \pm 0.353553391$	$15.75 - 19.25 = 17.65 \pm 0.594768863$	$12.25 - 14.25 = 13.4 \pm 0.384057287$	91.25-101.25=96.7 ± 1.984943324	$33-38=35.1 \pm 0.816241386$	34.75-35.75=35.35 ± 0.16955825	1276-1308=1293.8 ± 5.877074102
	(Kunth) Clayton	M	12.75-15.75=13.9 ± 0.509901951	10.25-12.25=11.25 ± 0.379143772	$13.25 15.5 \text{=-} 14.45 \pm 0.456891672$	$10.25 - 11.75 = 11.15 \pm 0.257390754$	68.25-75.75=72.8 ± 1.374772708	33.5-35.5=34.55 ± 0.398434436	30.25 - 35.25 = 33.1 ± 0.9	$751-788=766 \pm 6.473020933$
ć	D	Γ	$12-13.5=12.7 \pm 0.266926956$	$13-15.25=13.95 \pm 0.443001129$	$17.75-19.5=18.4 \pm 0.302076149$	$10.25 - 11.5 = 10.9 \pm 0.231840462$	150.25-159=153.75 ± 1.594913791	$29.5-34=31.8 \pm 0.933407735$	$36.75-39.75=38 \pm 0.512347538$	1305-1329=1316 \pm 4.847679857
70.	Fanicum antidotale Ketz.	M	10.25 - 12.25 = 11.35 ± 0.375832409	12.75-13.75=13.35 ± 0.16955825	$12.75 - 15.25 = 13.8 \pm 0.502493781$	7.75-9.75=8.65 \pm 0.407737661	94.75-110.25=101.65 ± 2.537961781	34.25-39=36.65 ± 0.970180396	$24-27.25=24.8 \pm 0.619475585$	$531-564=550 \pm 5.991660872$
7	Cenchrus divisus	Γ	$9 - 10.5 = 9.8 \pm 0.266926956$	$18-22=20.05 \pm 0.784219357$	$31.75-34.75=33.45 \pm 0.649037749$	11.75-14.75=12.7 ± 0.538516481	91.5-100.25=95.95 ± 1.579952531	$46-49.75=47.5 \pm 0.711512474$	32.75-36.75=34.75 ± 0.745821695	2899-3008=2982.2 ± 20.86959511
.1.7	Gmel.) Henr.	×	$7-9.5=8.1 \pm 0.491172068$	$14\text{-}17.25\text{=}16.15 \pm 0.594768863$	$22-24.5=23.3\pm0.470372193$	$9 10.75 \text{=-} 9.85 \pm 0.302076149}$	$69-73=71.15 \pm 0.853668554$	36-39.25=37.55 ± 0.677310859	24.75-28.5=26.9 ± 0.687386354	2491-2508=2500.6 ± 2.942787794
ξ	Joseph Daniel	Γ	$16-18.25=17.1 \pm 0.430116263$	$16.75 - 18.5 = 17.5 \pm 0.32596012$	$18.25-20.25=19.25 \pm 0.32596012$	$10.75 - 12.75 = 11.45 \pm 0.348209707$	72-75.75=73.95 ± 0.739087275	26.25-38.75=31.4 ± 2.111871208	29.5-36.75=33.6 ± 1.343037602	$966-1001=982.4 \pm 7.131619732$
77	Centra s jucciaus Ouseo.	×	$14.75 - 16.5 = 15.7 \pm 0.320156212$	$19.75 - 21 = 20.45 \pm 0.215058132$	$15.75 17 16.45 \pm 0.215058132$	$8-10.25=9.2 \pm 0.413823634$	54.75-63.75=58.25 ± 1.693738469	38.75-44.25=41.25 ± 0.977880361	$31-37=34.05 \pm 1.162970335$	793-808=798.6 \pm 2.731300057
6		Γ	$8-10.5=9.35 \pm 0.515994186$	14.25-17.75=15.7 ± 0.743303437	$22-24.5=23.35 \pm 0.515994186$	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
73.	rhdiaris minor Ketz.	W	$7.75-9.75=8.5 \pm 0.353553391$	$9.25 - 12 = 10.55 \pm 0.496235831$	15.75-19.75=18.2 ± 0.743303437	$8-10.25=9.35 \pm 0.375832409$	126-135.75=129.85 ± 1.789902232	21.25-24.5=22.85 ± 0.645174395	25.5-28.75=27.05 ± 0.604152299	$504-533=514.8 \pm 5.462600113$
5	Piptatherum baluchistanicum	Γ	$10.5\text{-}12\text{=}11.3 \pm 0.289395923$	12.75-15.5=14.05 ± 0.555652769	24-25.75=24.75 ± 0.32596012	12.75-15.25=14.1 ± 0.444409721	$100.25 - 113 = 107.8 \pm 2.314897406$	34.25-39.5=35.9 ± 0.950657667	33.25-37.75=35.7 ± 0.860232527	$901-988=940 \pm 15.85875153$
,	Freitag	8	$9 10.25 \text{=-} 9.55 \pm 0.215058132$	$8.75 - 10.75 = 9.6 \pm 0.331662479$	$18-20.5=19.1 \pm 0.527967802$	11.25-12.25=11.75 \pm 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 \pm 4.397726685
30	Colisional and biographics	Γ	$7 - 9.5 = 8 \pm 0.454147553$	12.75-14.75=13.55 ± 0.348209707	$15.25 16.5 \text{=-} 15.95 \pm \\ 0.215058132$	$10.25-12.75=11.5 \pm 0.425734659$	$96.25 - 100.75 = 98.7 \pm 0.923309266$	33.75-37.25=35.85 ± 0.718505393	$43.75-47=45.1 \pm 0.615426681$	$577-614=600.2 \pm 6.319810124$
	Schismus arabicas inces	M	$5.75-7.25=6.3 \pm 0.254950976$	11.25-12.25=11.75 ± 0.176776695	$12-14.75=13 \pm 0.487339717$	$8.5 10.25 = 9.45 \pm 0.289395923$	$49.5-57=53.4 \pm 1.266392514$	39.25-40.75=40.1 ± 0.26925824	$38.75 - 40.75 = 39.7 \pm 0.357071421$	$244-304=287.2 \pm 11.0516967$
ć	3 - Q - 11:	Γ	$4.25-5.5=4.85\pm0.231840462$	$17.25 - 19.5 = 18.3 \pm 0.390512484$	$30.25 - 34.75 = 32.5 \pm 0.911729126$	$15.25 - 16.25 = 15.75 \pm 0.176776695$	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
70.	rerapogon vinosus Dest.	×	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	$87-91.5=88.8 \pm 0.834415963$	30.25-37.25=32.9 ± 1.218605761	30.25-34.5=32.95 ± 0.755810823	$466-501=484.6 \pm 6.021627687$

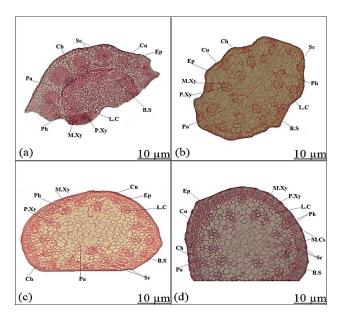


Fig. 6. Culm anatomy of (a) *Hordeum marinum* (b) *Imperata cylindrica* (c) *Leptothrium senegalense* (d) *Panicum antidotale* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities,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

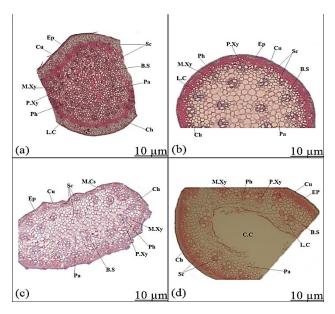


Fig. 7. Culm anatomy of (a) *Cenchrus divisus* (b) *Cenchrus flaccidus* (c) *Phalaris minor* (d) *Piptatherum baluchistanicum* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, 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 μ m length and 18.15 μ m width. Smallest epidermal cell was noted in T. villosus 4.85 μ m length and 5.55 μ m width (Table 4).

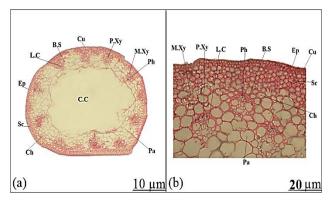


Fig. 8. Culm anatomy of (a) *Schismus arabicus* (b) *Tetrapogon villosus* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: Bundle sheath, L.C: Lysigenous cavity).

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 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 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 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 crosssectional 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 semiannular 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 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, Dactvloctenium, 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 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.

	Table 5.	Dichotomous key based on culm anatomical char	
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

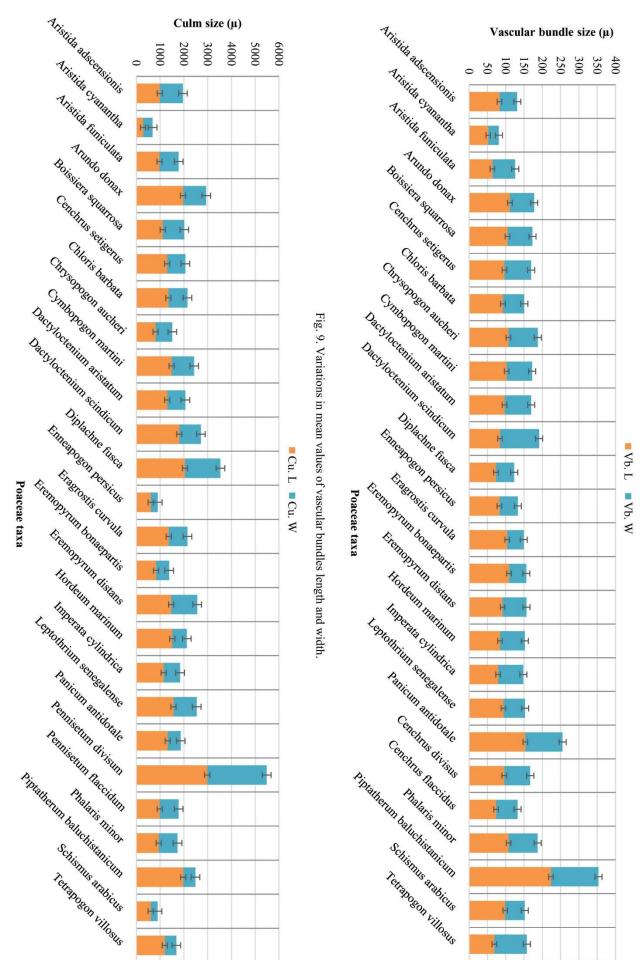


Fig. 10. Variations in mean values of culm length and width

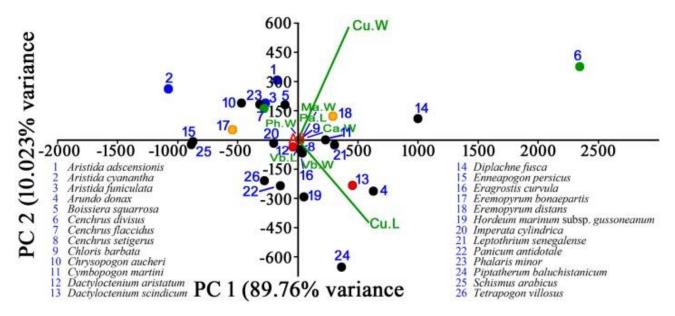


Fig. 11. Utility of culm features in discriminating among species of grasses by PCA (length and width of epidermis, parenchyma, chlorenchyma, xylem, phloem, vascular bundles, culm, sclerenchyma).

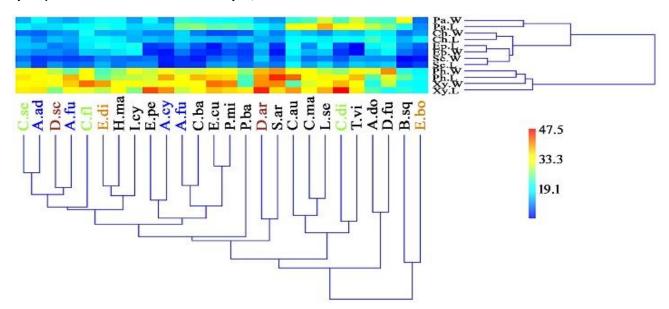


Fig. 12. Dendrogram showing the similarity index of Poaceous taxa based on quantitative parameters of culm.

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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