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Micromorphological variations and taxonomic implications of caryopses of some grasses from Pakistan

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Summary: In this study, 13 taxa of Poaceae were studied regarding morphology of caryopses. Micro- and macromorphological characters were observed in detail by light microscope (LM) and scanning electron microscope (SEM). Size of caryopses in selected taxa ranged between 0.5–9.2 mm length and 0.7–4 mm width. Different colors of caryopses (whitish brown, yellow, green and brown) with linear oblate, obovate, round, shallowly obtriangular and elliptic shapes were recorded. Depressed and grooved hila were observed. Dorsiventral and lateral types of caryopsis compression were found. Significant patterns were noted on the surface, such as rugose, scabrate, reticulate, striate, scaberulous and papillate. Bulges, silica cells, prickles, spines, bicellular micro hairs and granules were analyzed as epicuticular projections. Periclinal and anticlinal wall patterns, texture and thickness were studied. The present study focuses on caryopsis morphology of Poaceae and their taxonomical importance in the identification of thirteen species.

Keywords: caryopsis characters, microscopy, morphology, Poaceae, taxonomy

Grasses are distributed more commonly than any other taxa of flowering plants. They have a great adaptability, which enables them to grow under different conditions. They occur frequently in the semi-arid prairies of the American continent, steppes of Asia and the savannas of Africa. Poaceae are economically very important, because of providing cereal species as well as forage plants for animals (Gautam et al. 2018). Taxa of Poaceae constitute a natural homogenous group of plants. Undoubtedly, it is one of the most fascinating families of all flowering plants and it is highly recognized by humans as well as animals due to its great diversity and adaptability. The value and culture of cereals have been successfully appreciated since the prehistoric age. The members of cereals are highly adaptable and therefore they are able to survive in a variety of habitats and different climatic conditions. Scientists made great efforts to distinguish taxa properly and ultimately to classify about 10 000 species of the grass family. Many systematic attempts have been done to classify these species based on morphological characteristics, such as the spikelet (Ahmad et al. 2010).

Cereals are one of the biggest and significant classes of flowering plants comprising about 700 genera and around 1100 species (Usma et al. 2019). Regarding the number of genera, they take the third position after Orchidaceae and Compositae, and the fifth position after Leguminosae, Compositae, Rubiaceae and Orchidaceae in the number of species (Peeters et al. 2004). According to Chen & Peterson (2006), Poaceae constitute around 11 000 species and 700 genera, 60 tribes and six subfamilies that are widespread. In Pakistan, there are 158 genera and about 492 species, 26 tribes and 5 subfamilies (Cope 1982).

Spikelet is the most prominent character of grasses, which is not present in other families except Cyperaceae. The spikelet consists of two distichous and alternate bracts (glumes or empty bracts)

at the base attached by a floret or florets. The florets are arranged in alternating or dichotomous manner on the rhachilla (tough or joint axis). Palea and lemma (a pair of flowering bracts) are attached to each floret. It consists of the perianth that contains 2–3 small scales (lodicules), a single or two alternate whorls of stamens and a central ovary with a pair of rarely solitary or lateral styles that usually have plumose stigmata (Khine 2016). Grasses are used for various products like sugar, grain, paper, spices, perfume and other things of daily needs. The majority of the world's population is dependent on their staple food on grains of cultivated grasses comprising wheat, rice, sorghum, corn, etc. Grasses supply directly or indirectly the larger part of man's nutritional needs as cereals or as fodder for animals. Grasses are playing a vital role in the conservation of soil erosion. The economic importance of grasses can be understood from the fact that man has been growing cereals for food since ancient times (Singh 2008). Grasses are ethnobotanically very important as they are the main source of fodder and forage for cattle (Samreen et al. 2015).

Caryopsis, an indehiscent single-seeded fruit, comprises a fused pericarp and gives rise to a monocarpellary uniovulate ovary. The grass pericarp is very thin consisting of only a few cell layers: a thick-walled outer epidermis, a few parenchymatous cell layers with vascular bundles and an inconspicuous inner epidermis (Rudall et al. 2005). The micro-structural pattern examination of the seed coat through SEM and stereoscope is a trustworthy approach for analyzing phylogenetic relationships and solving taxonomic issues (Koul et al. 2000). Characteristics of caryopses, not regarding the embryo, have been investigated by many scientists, e.g. in tribe Chloridoideae, caryopses of 45 genera were studied using micro-morphological features (Liu et al. 2005).

Scanning electron microscopy is of great taxonomic significance for identification of micromorphological characters of plants (Esfandani-Bozchaloyi & Zaman 2018). In Pakistan, there is no comprehensive report regarding caryopsis morphology of grasses available. The current study is the first one. The objective of this study is to investigate and evaluate various micro- and macromorphological characters of caryopses of the selected species of Poaceae by using both LM and SEM techniques.

Materials and methods

Collection and identification. Thirteen Poaceae specimens having dried as well as mature caryopses were gathered from various regions in Pakistan (Table 1). Flora of Pakistan was used for species identification, while the taxonomic names were confirmed with the plant list (www.theplantlist.org). Herbarium samples along with their voucher numbers are deposited in the Herbarium of Pakistan [ISL], Quaid I Azam University (Islamabad) (Table 1). Structural measurements and observations were examined taking into consideration shape, color, size, ornamentation, dimension, stylopodium, epicuticular projections, etc. along with general appearance of caryopses.

Scanning Electron Microscopy (SEM). SEM observations followed the protocol of Butt et al. (2018) with few modifications. A sticky tape was used to mount the caryopses directly on the stub. The samples were analyzed by SEM (Model JEOL JSM5910) after being sputter-coated with gold-palladium in the Central Resources Library (CRL), Department of Physics, University of Peshawar, Pakistan. Polaroid P/N 665 film was used to photograph the examined samples. Surface characteristics were explained according to Barthlott et al. (1998). Snow's (1998) terminologies were used to describe these shapes. Caryopsis shape is of little or no taxonomic value.

Table 1. List of the studied taxa.

No.	Taxon	Collection site	Voucher No.	Collectors
1	*Cenchrus echinatus* L.	Chakwal	AU-67	Anwer, Nomana
2	*Dactyloctenium aegyptium* (L.) Willd.	Islamabad	AU-33	Anwer
3	*Dactyloctenium scindicum* Boiss.	Salt range area	AU-55	Anwer
4	*Dichanthium annulatum* (Forssk.) Stapf.	Islamabad	AU 57	Anwer
5	*Desmostachya bipinnata* (L.) Stapf.	Chakwal	AU-149	Anwer, Nomana
6	*Eleusine indica* (L.) Gaertn.	Rawalpindi	AU-61	Anwer
7	*Enneapogon persicus* Boiss.	Chakwal	AU-29	Anwer, Nomana
8	*Oryza sativa* L.	Jhelum	AU-45	Anwer, Kanwal
9	*Poa annua* L.	Chakwal	AU-125	Anwer, Nomana
10	*Poa angustifolia* L.	Islamabad	AU-91	Anwer
11	*Saccharum spontaneum* L.	Chakwal	AU-137	Anwer
12	*Sorghum halepense* (L.) Pers.	Chakwal	AU-93	Anwer
13	*Triticum aestivum* L.	Chakwal	AU-84	Anwer

Results

Variations in caryopsis size. Caryopsis size in selected specimens of Poaceae is varying. Minimum caryopsis length was observed for *Poa annua* (0.5 mm), while *Enneapogon persicus* has a maximum length of 9.2 mm. Caryopses of *Triticum aestivum* have a maximum width of 4 mm, while the minimum value was recorded for *Poa annua* and *Eleusine indica* (0.7 mm). The length and width of the remaining species fall in the intermediate category. In terms of the number of caryopses per 1 gram, *Poa annua* contains the greatest number (968), while *Triticum aestivum* has the smallest number (73) (Table 2). The monographs of the studied species are given in Figs 1, 2 and 3.

Variations in qualitative features of caryopses. Colour of the caryopses vary from species to species (Table 2). Caryopses of studied grasses have five different shapes. *Poa annua*, *Dactyloctenium aegyptium* and *D. scindicum* have rounded caryopses, while *Desmostachya bipinnata* has ovate caryopses. Oblong caryopses were found in *Sorghum halepense*, *Saccharum spontaneum*, *Eleusine indica* and *Dicanthium annulatum*. The most variable shape was observed for *Oryza sativa*, which is linear. The rest of the species has obovate caryopses.

Depressed and grooved hilum types were recorded in selected Poaceae specimens. *Dactyloctenium aegyptium*, *D. scindicum*, *Dichanthium annulatum*, *Enneapogon persicus* and *Triticum aestivum* have a grooved hilum, while *Desmostachya bipinnata*, *Poa annua*, *Saccharum spontaneum* and *Sorghum halepense* have depressed hila. In other species, a hilum was not visible (Table 2). Compression types in the studied taxa were either lateral or dorso-ventral. The caryopses of *Enneapogon persicus*, *Oryza sativa*, *Poa angustifolia*, *Sorghum halepense* and *Saccharum spontaneum* were dorsiventrally compressed. In the remaining species, caryopses were laterally compressed (Table 2).

Caryopses features observed through scanning electron microscopy. Nine different types of surface features were observed. *Cenchrus echinatus* has a striate surface, *Oryza sativa* a papillate ornamentation. Rugose surface was observed in *Sorghum halepense* and *Poa angustifolia*, while *Saccharum spontaneum* has a reticulate-rugose surface. *Dactyloctenium aegyptium* and *D. scindicum*

Table 2. Qualitative and quantitative features of caryopses studied by using LM. NV= not visible, L/W= length to width ratio.

No.	Taxon	Colour	Shape	Hilum	Compression	Length | Width in mm		L/W	No. of caryopses / g
1	*Cenchrus echinatus* L.	green	obovate	NV	lateral	4	1	4	433
2	*Dactyloctenium aegyptium* (L.) Willd.	light brown	rounded	grooved	lateral	1	1	1	793
3	*Dactyloctenium scindicum* Boiss.	light brown	rounded	grooved	lateral	0.8	0.8	1	811
4	*Dichanthium annulatum* (Forssk.)Stapf.	purplish	oblong	grooved	lateral	4	1	4	555
5	*Desmostachya bipinnata* (L.) Stapf.	yellow	ovate	depressed	lateral	1.2	1	1.2	655
6	*Eleusine indica* (L.) Gaertn.	yellowish green	oblong	NV	lateral	2.1	0.7	3	892
7	*Enneapogon persicus* Boiss.	yellow	widely obovate	grooved	dorso-ventral	9.2	2	4.6	289
8	*Oryza sativa* L.	white	linear	NV	dorso-ventral	6.5	1.5	4.3	119
9	*Poa annua* L.	brown	rounded	depressed	dorso-ventral	0.5	0.7	3	962
10	*Poa angustifolia* L.	green	obovate	NV	lateral	2	0.8	2.5	968
11	*Saccharum spontaneum* L.	reddish brown	widely oblong	depressed	dorso-ventral	1.1	0.7	1.57	753
12	*Sorghum halepense* (L.) Pers.	yellowish green	oblong	depressed	dorso-ventral	4.5	2	2.25	393
13	*Triticum aestivum* L.	brown	widely obovate	grooved	lateral	7	4	1.75	73

have a foveolate surface. *Enneapogon persicus* has rugulate surface ornamentation of caryopsis. *Desmostachya bipinnata* has a papillate-striate surface, while *Eleusine indica* has papillate rugose surface. The remaining species have a reticulate surface of caryopses (Table 3).

Bulges were the most common projection type observed in *Poa annua*, *Desmostachya bipinnata* and *Cenchrus echinatus.* Hairs were also present in *Cenchrus echinatus* as epicuticular projections. Silica bodies were only present in *Eleusine indica*. The rest of the species were devoid of epicuticular projections (Table 3). No significant differences were recorded in cell arrangement and cell outline. The cell outline in the analyzed taxa was in-line or random. The examined panicoids have irregular and regular arrangement of cells (Table 3).

Variations in cell wall pattern. Periclinal and anticlinal walls were examined in terms of cell pattern surface, thickness and level. The anticlinal wall was thin in *Poa angustifolia* and *Desmostachya bipinnata*, while in other species anticlinal wall was thick. Three types of anticlinal-wall patterns were noted: entire, wavy and inconspicious. *Cenchrus echinatus*, *Poa annua*, *Saccharum spontaneum* and *Triticum aestivum* have entire anticlinal walls, while *Oryza sativa* and *Desmostachya bipinnata* have inconspicious anticlinal walls. The rest of the species have wavy anticlinal walls (Table 3). The periclinal wall's surface is coarse, medium, fine or glabrous. *Desmostachya bipinnata* has

Table 3. Micromorphological features of caryopses using scanning electron microscopy. A.W.T. = anticlinal wall thickness, A.W.P. = anticlinal wall protuberance, P.W.S. = periclinal wall surface, P.W.L. = periclinal wall level

No.	Taxon	Surface pattern	Stylopodium	Epicuticular projection	Cell outline	Cell arrangement	A.W.T.	A.W.P.	P.W.S.	P.W.L.
1	*Cenchrus echinatus* L.	striate	style base with tuft of hairs	bulges and hairs	random	irregular	thick	entire depressed	coarse	elevated
2	*Dactyloctenium aegyptium* (L.) Willd.	foveolate	style base persistent	absent	in rows	regular	thick	slightly wavy	glabrous	depressed
3	*Dactyloctenium scindicum* Boiss.	foveolate	style base persistent	absent	in rows	irregular	thick	slightly wavy	glabrous	depressed
4	*Dichanthium annulatum* (Forssk.) Stapf.	reticulate	persistent with tuft of hairs	absent	in rows	regular	thick	wavy	glabrous	depressed
5	*Desmostachya bipinnata* (L.) Stapf.	papillate-striate	persistent	bulges	random	irregular	thin	inconspicious	medium	flat
6	*Eleusine indica* (L.) Gaertn.	papillate-rugose	persistent	silica bodies	random	regular	thick	wavy	glabrous	depressed
7	*Enneapogon persicus* Boiss.	regulate	persistent	absent	random	irregular	thick	wavy	glabrous	depressed
8	*Oryza sativa* L.	papillate	style base persistent	absent	random	irregular	thick	inconspicious	glabrous	depressed
9	*Poa annua* L.	reticulate	persistent	absent	in rows	regular	thick	entire	glabrous	flat
10	*Poa angustifolia* L.	rugose	persistent	bulges	in rows	irregular	slightly thin	wavy	glabrous	depressed
11	*Saccharum spontaneum* L.	reticulate-rugose	style base persistent	absent	random	irregular	thick	entire	glabrous	flat
12	*Sorghum halepense* (L.) Pers.	rugose	persistent	absent	in rows	irregular	thick	wavy	glabrous	elevated
13	*Triticum aestivum* L.	reticulate	persistent with tuft of hairs	absent	in rows	regular	thick	entire elevated	fine	depressed pinched

a medium texture of the periclinal wall and *Cenchrus echinatus* has a coarse texture. *Triticum aestivum* has a fine patterned periclinal wall. Glabrous periclinal walls were present in the rest of the species. We found three types of periclinal walls: depressed, flat and elevated. *Cenchrus echinatus* and *Sorghum halepense* have elevated periclinal wall, whereas *Saccharum spontaneum*, *Poa annua* and *Desmostachya bipinnata* have flat periclinal walls (Table 3).

Discussion

An important tool in analyzing different ranks of Poaceae is caryopsis morphology, which shows significant characters of species delimitation in grasses (Dhara et al. 2013). Caryopses of cereals are complex structured tissues comprising one embryo enclosed in a single seed and layers of integuments which cover the embryo and an endosperm rich in carbohydrates. Morphological

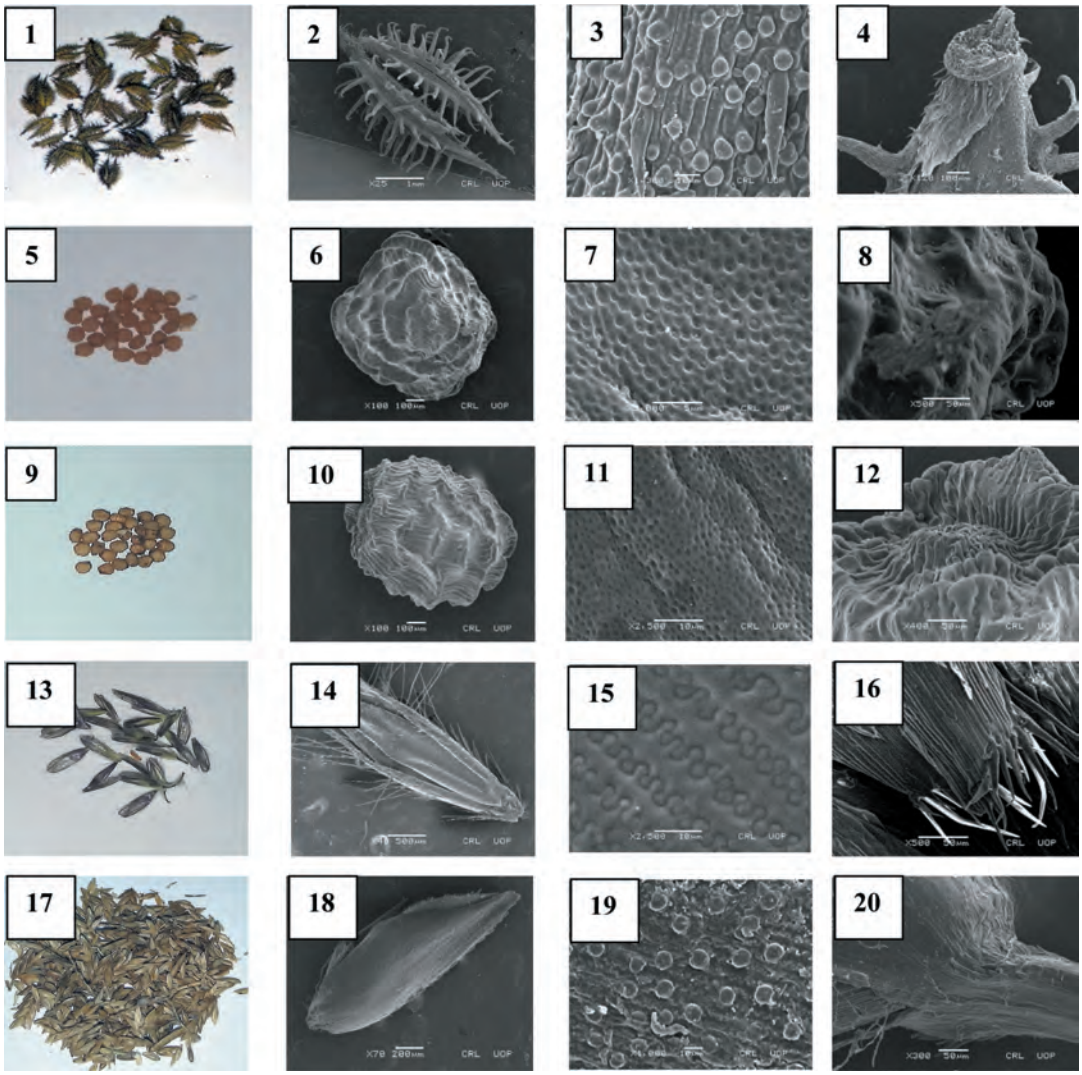

Figure 1. *Cenchrus echinatus.* (1) caryopses, (2) general view, (3) surface view, (4) stylopodium. *Dactylolactenium aegyptium.* (5) caryopses, (6) general view, (7) surface view, (8) stylopodium. *Dactylolactenium scindicum.* (9) caryopses, (10) general view, (11) surface view, (12) stylopodium. *Dicanthium annulatum.* (13) caryopses, (14) general view, (15) surface view, (16) stylopodium. *Desmostachya bipinnata.* (17) caryopses, (18) general view, (19) surface view, (20) stylopodium.

characters are crucial to further dissect the caryopses on molecular and genetic levels, as they are characteristic of Poaceae and serve as the main source of carbohydrates in human nutrition (Drea et al. 2005).

Caryopsis size serves as a significant taxonomic tool to examine different taxa of Poaceae (Liu et al. 2005). Lengths of caryopsis and lemma are linked together. The majority of the floret area in most of the members of grasses is covered by mature caryopses. This character is significant regarding the final use of cereals. Caryopsis size and endosperm proportion have a great influence on the germination and growth of the seedling. The lesser caryopsis size and endosperm proportion are, the higher will be the chances of germination and seedling emergence (Huang et al. 2004). In the present study, *Dactyloctenium scindicum*, *D. aegyptium*, *D. bipinnata*, *Saccharum spontaneum*

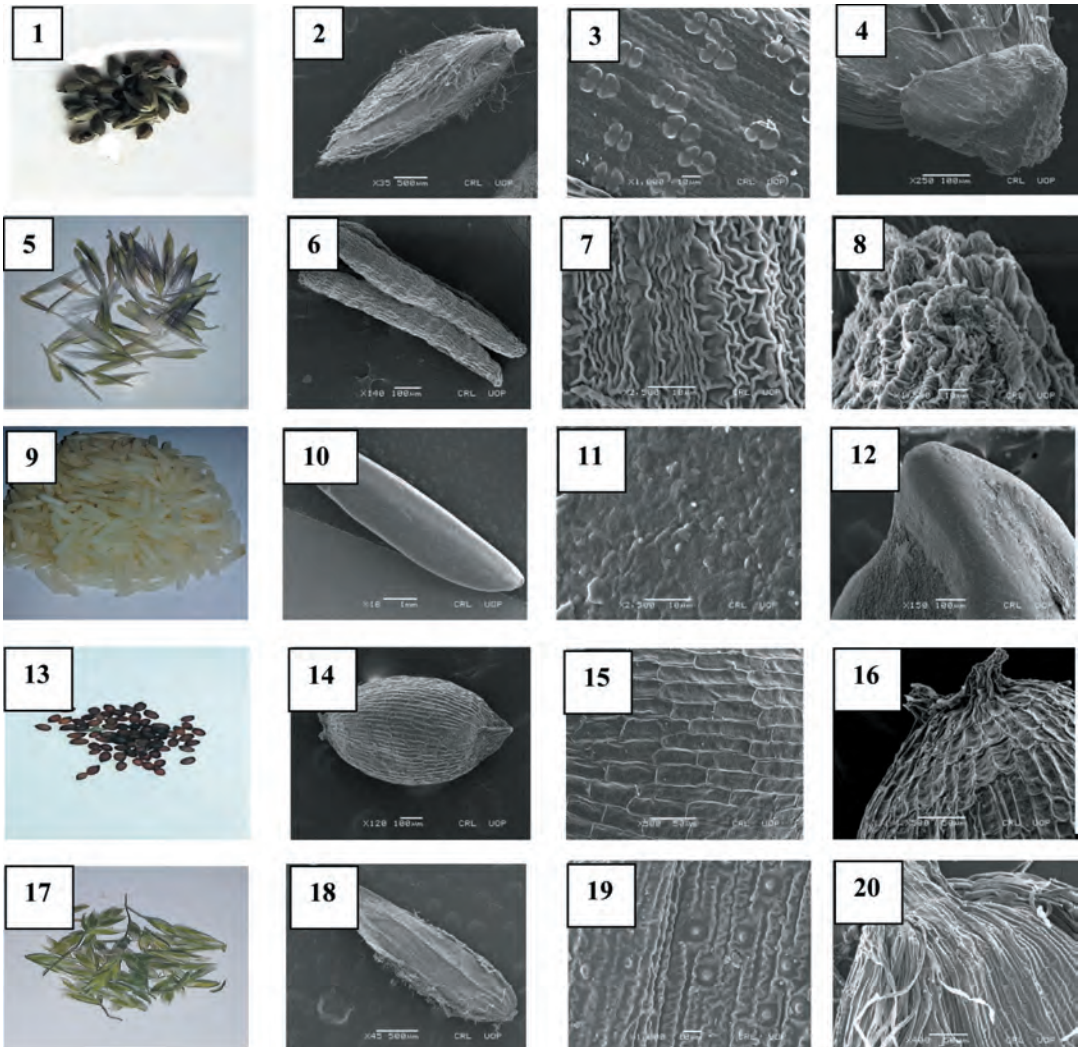

Figure 2. *Eleusine indica.* (1) caryopses, (2) general view, (3) surface view, (4) stylopodium. *Enneopogan persicus.* (5) caryopses, (6) general view, (7) surface view, (8) stylopodium. *Oryza sativa.* (9) caryopses, (10) general view, (11) surface view, (12) stylopodium. *Poa annua.* (13) caryopses, (14) general view, (15) surface view, (16) stylopodium. *Poa angustifolia.* (17) caryopses, (18) general view, (19) surface view, (20) stylopodium.

and *Triticum aestivum* have a smaller length to width ratio: 1, 1, 1.2, 1.57 and 1.75 respectively. Among the studied taxa, these plants may have a higher germination frequency than the others.

Some of the vital taxonomic characters in various taxa of Poaceae are compression-type, hilum-position, colour and shape of caryopses (Vivek et al. 2015). Caryopsis color is associated with the anthocyanin composition and contents in the cereals (Zykin et al. 2018). The various colours in the studied taxa indicate the presence of anthocyanin and different pigments, which may provide clues to the researchers to further evaluate these grasses phytochemically. Caryopsis traits including size, shape, colour, yield and germination are important features for designing cultivation experiments. In a few reports, the colour difference among grass caryopses is not a significant taxonomic character, as this feature is constant in all subfamilies of grasses. Four types of stylopodium patterns were observed: style base persistent with a tuft of hair (*Dichanthium*

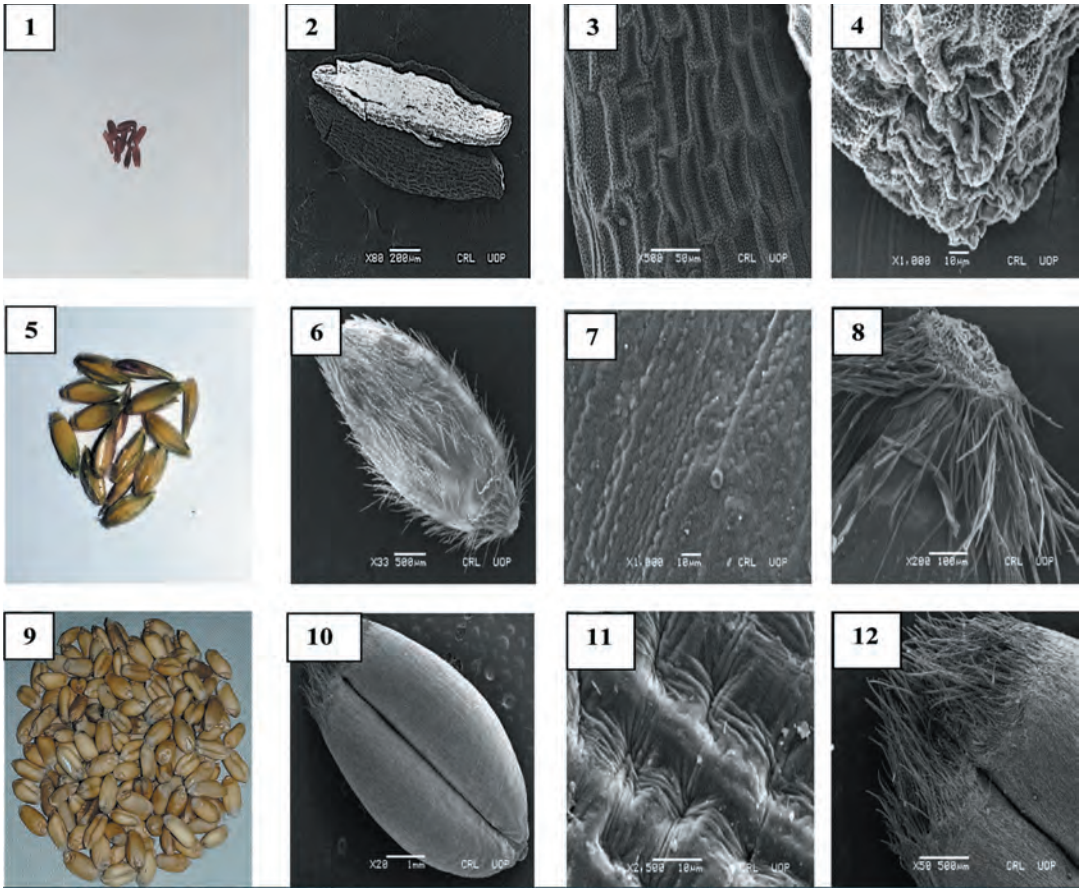

Figure 3. *Saccharum spontaneum.* (1) caryopses, (2) general view, (3) surface view, (4) stylopodium. *Sorghum halepense.* (5) caryopses, (6) general view, (7) surface view, (8) stylopodium. *Triticum aestivum.* (9) caryopses, (10) general view, (11) surface view, (12) stylopodium.

annulatum and *Cenchrus echinatus*), style base persistent (*Saccharum spontaneum*, *Oryza sativa*, *Dactyloctenium aegyptium* and *Dactyloctenium scindicum*), style persistent (*Sorghum halepense*, *Poa angustifolia*, *Poa annua*, *Enneapogon persicus*, *Eleusine indica* and *Desmostachya bipinnata*) and style persistent with hair tuft (*Triticum aestivum*).

One of the differentiating factors in Poaceae is the appearance and position of the hilum as it can be seen in the caryopsis outline. Caryopsis size and anatomy are the new potentials and valuable characters (Costea & Tardif 2002). Caryopsis compression could be used in combination with different features to show differences in various grass taxa (Barker 1994). Surface ornamentation patterns and other micromorphological features may provide additional taxonomic information (Pinar et al. 2007). Well defined epicuticular projections having a specific shape are regarded as a vital taxonomic principle (Barthlott et al. 1998). But in the present study, no differences between the analyzed taxa could be seen in epicuticular projections. One of the most remarkable characteristics of caryopses is the stylopodium. Distinctive characters at the species level are differences within the periclinal wall and anticlinal wall pattern (Luqman et al. 2019). The present study showed the detailed morphological features of the caryopses of some grasses from Pakistan by means of scanning electron microscopyl, which might be valuable in the correct identification of these grasses to provide useful information for cultivation experiments.

Conclusion

The current study demonstrates the use of SEM as an important tool for the investigation of caryopsis morphology of Poaceae, because detailed epicuticular projections and surface sculpture patterns are revealed. Vital distinctive features for morphological characterization of Poaceae caryopses are presented which may be used for identification. The use of advanced microscopic techniques is suggested to further analyze the internal characters of caryopses as they are helpful in establishing a phylogenetic relationship among the studied taxa.

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