International Journal of Theoretical and Applied Research (IJTAR)
ISSN: 2812-5878

Homepage: https://ijtar.journals.ekb.eg

Original article

Pollen Grains and Caryopsis Features of Selected Poaceae species and Their Taxonomic Significance

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ARTICLE INFO

Received 20/05/2023
Revised 09/11/2023
Accepted 11/11/2023

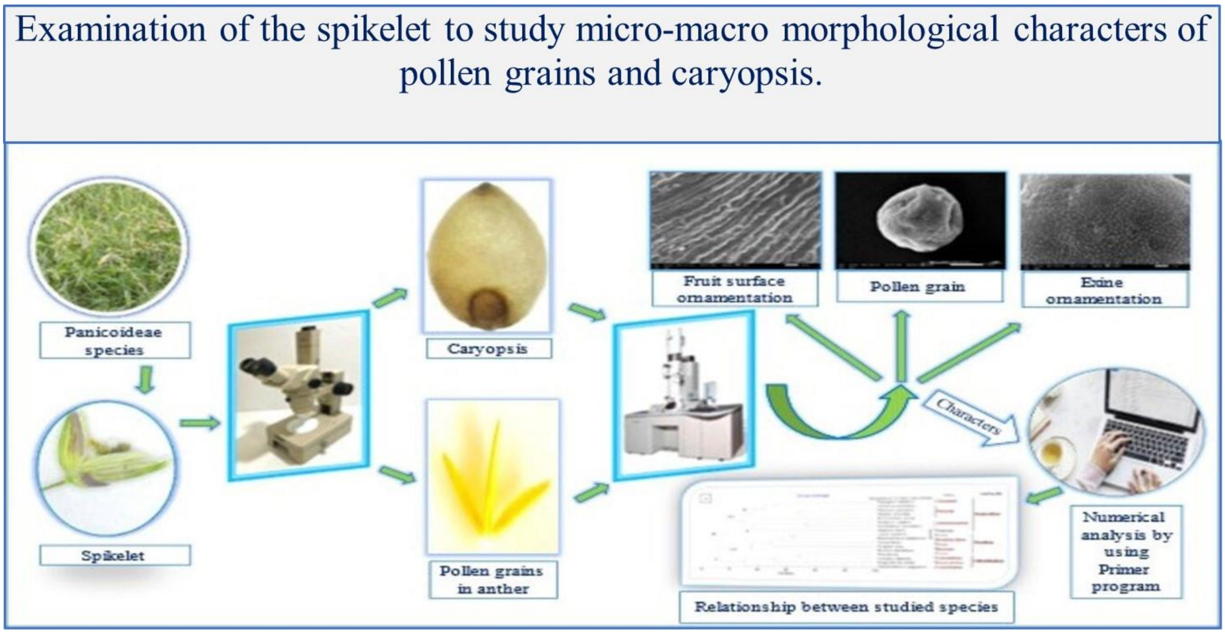
Keywords

Pollen grains
Caryopsis
Numerical analysis and
Poaceae

ABSTRACT

Pollen grains and caryopsis morphology of seventeen species belongs to seventeen genera, eight tribes and three subfamilies of Poaceae were studied by using light and scanning electron microscopes. Morphological characters of pollen grains and caryopsis coat are very important traits which can be used at taxonomic level. Pollen grains are mostly oblate-spheroidal, spheroidal and prolate-spheroidal while recorded six shapes elliptic, oblong, ovate, broad ovate, obovate and rounded for caryopsis. On the basis of exine ornamentations five distinct pollen types are recognized (verrucate-granulate, areolate-granulate, micro gemmate, micro gemmate-granulate and granulate) and nine different patterns for caryopsis surface coat (reticulate, rugose, scalariform, striate, reticulate - foveate, reticulate-rugose, reticulate-striate, ruminate and striate -favulariate). The size, shape, exine thickening, exine ornamentation, operculum and annulus diameter of pollen grains are very important characteristics for delimiting the species. Palynology do not support the tribal and generic classification, whereas the morphology of caryopsis is crucial for identifying the taxa in the family Poaceae.

Graphical abstract



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DOI: 10.21608/IJTAR.2023.212100.1059

Special issue "selected papers from the 2nd International Conference on Basic and Applied Science (2nd IACBAS-2023)"

1. Introduction

The Poaceae family, commonly known as grasses, is one of the largest families of flowering plants and the second most diverse family among Monocotyledons. It comprises approximately 700 genera and about 10,000 species [1,2,3]. Recently, it comprised about 11,000–12,000 species divided among about 750–770 genera [4,5]. In Egypt, Poaceae is represented by 284 species (including 44 cultivated species) in 110 genera and 19 tribes [6]. Also, it is represented by 284 native and naturalized species belonging to 103 genera, 22 tribes and 7 subfamilies [7]. According to the plant list (2022) the family is composed of about 759 genera and11554 species. They are ubiquitous and play a significant role in the Earth's ecosystems. The family members, which may be found on every continent are the most global of all higher plants. They can be found anywhere, from mountain summits to seashores, including the polar regions and the equator. Also, in freshwater and saltwater marshes, streams, ponds, rain forests, parched slopes, deserts, and tundra, they can be found [8]. They also are dominant, covering an estimated 40% of the Earth's land surface [9] and represent all the three ecological types as mesophytes, hydrophytes and xerophytes.

Poaceae plays a significant role in our daily life and economy of Egypt for more than 12,000 years [10]. Their members are providing us with our staple cereals as *Eragrostis*, *Hordeum, Oryza*, *Sorghum*, *Triticum,* and *Zea*. Also providing us with sugar crops as *Saccharum*, reeds as *Arundo* and *Phragmites*, building and nicety materials as *Bambusa* [1,11], *Arundo, Phragmitis,* and *Themeda* produce large amount of cellulose, which yield excellent material for paper pulp. Also, fibrous remains of sugarcane are used in the manufacture of paper, cardboard and biofuels. Certain grasses are used by people for curing various ailments such as oil yielding-species belonging to *Cymbopogon* and *Cynodon dactylon* are utilized for the treatment of dysentery, fever, anemia and bowl complaints. *Hordeum, Oryza, lolium, Triticum, Avena* and *Zea* are used in production of alcohol, starch, and vinegar in different parts of world. Recently, they become important sources of raw material for the biomass and bioenergy industry such as Arundo and Saccharum [12,13].

The morphological structure of pollen grains and caryopsis have been used as taxonomic and evolutionary markers in plants. Studies on the pollen grains and caryopsis of some species of Poaceae using light and scanning electron microscopes have provided valuable insights into the morphology and structure of these plants that are differentiated from others.

pollen morphology is one of the significant tools in solving taxonomic problems of the family, generic or specific level and has become part of the multidisciplinary and collective approach in plant systematic and evolution. Similar to floral morphology, palynology of the family is also remarkably uniform. However, palynology does not correspond with tribal and generic classification, it is significantly helpful at the specific level. Pollen morphology of the family Poaceae were examined by using different apparatus as LM, SEM and TEM by many scientists as [14-22].

Fruit is very important for species identification based on diagnostic features. The type of fruit in poaceae is caryopsis. The caryopsis morphology is long used traditionally as one of the very instructive taxonomic characters to determine the- identity e.g., [23-27] of many genera, species and infra-specific categories in the family Poaceae. Non-embryo characters of the caryopses had been ignored researchers, yet they can offer imperative information on phylogenetic relationships [24]. Non-embryo characters of the caryopses had been investigated by certain authors e.g., [28-30]. Also, caryopsis morphology was examined by many authors e.g., [31-37]. For the Egyptian taxa of Poaceae there are only few studies. Most studies have focused mainly on a systematic revision [38-40]. The scan of pollen grains and caryopsis for Poaceae species growing in Egypt is not yet correctly projected, accordingly, the aim of present study is to evaluates the significance taxonomic importance of pollen grains and caryopsis morphological characteristics in classification and identification level.

2. Materials and Methods

1-Plant material: Seventeen species of Poaceae belonging to 8 tribes according to [7] were collected fresh from different localities in Egypt as shown in (Table 1).The materials studied were identified according to plant key of [6,7,41]. Reference herbarium specimens of studied species were prepared and kept in the herbarium of Botany and Microbiology Department, Faculty of Science, Al-Azhar University (Girls).

2-Sample preparation: details for pollen grains and caryopsis were examined using light microscope (LM). For pollen grains, rapid method preparation was used by using 5% NAOH and safranin solution then pollen grains were mounted in glycerin jelly and observed under light microscope (E40, 0.65) using the 15x eye lenses and photographed [42]. Five measurements per specimen were taken for polar axis (PA), equatorial diameter (ED), PA/ED ratios, pore and annulus diameters, exine thickness were calculated. For fruit morphology, 3-5 were examined under binocular stereo microscopy (Olympus-SZ40-PT) coupled with digital camera. The size, shape, color, ventral face, compression, hilum, embryo and stylopodium of caryopsis morphology were recorded. The details of pollen grains and caryopsis morphology were investigated in electron scanning microscope (SEM) with the use of a JEOL microscope (JSM IT200) with a voltage of 20 KV. The anther suspended in a drop of water to assist release of pollen grains which were transferred to a metallic stub using double sided cello tape, coated with gold, then examined under SEM and photographed. Also, two mature caryopses of each species were cleaned and mounted directly on a metallic stub using double sided cello tape, coated with gold, then examined under SEM and photographed. The terminology of [43] was followed for pollen grains characters and the terminology of [24,26,44] was assumed for caryopsis coat characters.

3-Data analysis: The characters of the pollen grains and fruit for the studied species were exposed to the numerical analysis using the PRIMER ver.6 program.

4- Data collection: the data about pollen grains and caryopsis were collected from taxonomic books, theses and publication researches that is related to this study.

Table 1. List of studied species with their description and geographical distribution.

Subfamilies (Ibrahim et al. 2016)	Tribe	Species name	Description	Collecting region	Date
Chloridoideae	Cynodonteae	*Cynodon dactylon* (L.) Pers.	perennial	Nasr City-Cairo	1-2021
	Cynodonteae	*Dactyloctenium aegyptium* (L.) Willd.	annual	Nasr City-Cairo	8-2020
	Eragrostideae	*Eragrostis tenuifolia* (A. Rich.) Hochst ex Steud.	perennial	Nasr City-Cairo	1-2021
Panicoideae	Andropogoneae	*Dichanthium annulatum* (Forssk.) Stapf	perennial	Nasr City and El-Zohriya garden -Cairo	11-2020 3-2021
	Andropogoneae	*Sorghum virgatum* (Hack.) Stapf	annual	Nahia- El Giza	3-2021
	Paniceae	*Digitaria ciliaris* (Retz.) Koeler	annual	Nasr City-Cairo	9-2021
	Paniceae	*Echinochloa colona* (L.) Link	annual	El Monofia	5-2021
	Paniceae	*Cenchrus echinatus* L.	annual	Nasr City-Cairo	7-2020
	Paniceae	*Setaria verticillata* (L.) P. Beauv.	annual	El Monofia	4-2021
	Paniceae	*Panicum coloratum* L.	perennial	Nasr City-Cairo	9-2020
	Paspaleae	*Paspalum dilatatum* Poir.	perennial	Orman garden-El Giza and Nasr City-Cairo	3-2021
Pooideae	Brachypodieae	*Brachypodium distachyon* (L.) P. Beauv.	annual	Marsa Matrouh	2-2021
	Bromeae	*Bromus catharticus* Vahl	Short-lived perennial	Nasr City-Cairo and Belbes-El Sharkia	4-2021
	Poeae	*Avena fatua* L.	annual	Marsa Matrouh, Belbes-El Sharkia	2-2021 4-2021
	Poeae	*Lolium perenne* L.	perennial	Matrouh -Alexandria road and Nasr City-Cairo	2-2021 4-2021
	Poeae	*Phalaris minor* Retz.	annual	Matrouh -Alexandria road and Nahia-El Giza	2-2021 3-2021
	Poeae	*Poa annua* L.	annual	El Monofia	4-2021

3. Results

The pollen grains and caryopsis morphological characters for seventeen species of Poaceae are summarized in **Tables (2-4) and Figures (1-4).**

Pollen grains features:

The pollen grains shape recorded three types; prolate spheroidal, spheroidal and oblate-spheroidal. The main type is prolate spheroidal recorded in eleven studied species [*Eragrostis tenuifolia, Dichanthium annulatum*, *Sorghum virgatum, Digitaria ciliaris, Echinochloa colona, Panicum coloratum, Paspalum dilatatum, Bromus catharticus, Avena fatua, Lolium perenne* and *Phalaris minor*], spheroidal in [*Cynodon dactylon, Cenchrus echinatus, Setaria verticillata, Brachypodium distachyon* and *Poa annua*] and oblate-spheroidal only in [*Dactyloctenium aegyptium*].

The apertures number is monoaperture except in [*Eragrostis tenuifolia, Echinochloa colona, Cenchrus echinatus, Panicum coloratum, Paspalum dilatatum* and *Phalaris minor*] are diapertures. The annulus is distinct in most studied species but reduced in [*Cynodon dactylon, Dactyloctenium aegyptium, Digitaria ciliaris, Panicum coloratum, Brachypodium distachyon* and *Bromus catharticus*].

The operculum is sunken in [*Cynodon dactylon, Dactyloctenium aegyptium, Eragrostis tenuifolia, Dichanthium annulatum, Sorghum virgatum, Digitaria ciliaris, Lolium perenne* and *Poa annua*] and at a level in all the reminder. The pore diameter is narrow in [*Cynodon dactylon, Dactyloctenium aegyptium, Dichanthium annulatum, Setaria verticillata, Panicum coloratum, Paspalum dilatatum, Lolium perenne* and *Phalaris minor*] and wide in the reminder. The annulus diameter is wide in most studied species but narrow in [*Cynodon dactylon, Dactyloctenium aegyptium, Eragrostis tenuifolia, Digitaria ciliaris, Paspalum dilatatum, Lolium perenne* and *Poa annua*].

Table 2. Macro and micro morphological features of pollen grains for studied species.

Character / Species	Pollen shape:1=oblate-spheroidal (88-99), 2=spheroidal (100), 3=prolate spheroidal (101-114)	Apertures number:1= monoaperture, 2= diapertures	Annulus:1=distinct, 2=reduced	Operculum:1=at a level, 2=sunken	Polar diameter (µm)		Equatorial diameter (µm)		PA/ED × 100
					Average	Stander	Average	Stander	
Cynodon dactylon	2	1	2	2	29.348	±1.366	29.285	±0.868	100.21
Dactyloctenium aegyptium	1	1	2	2	32.520	±2.449	32.795	±2.239	99.159
Eragrostis tenuifolia	3	2	1	2	29.599	±1.366	28.952	±1.814	102.234
Dichanthium annulatum	3	1	1	2	31.773	±3.647	30.327	±2.964	104.76
Sorghum virgatum	3	1	1	2	38.923	±2.243	34.384	±1.462	113.19
Digitaria ciliaris	3	1	2	2	41.317	±4.12	41.125	±4.22	100.46
Echinochloa colona	3	2	1	1	29.895	±0.751	29.577	±0.797	101.07
Cenchrus echinatus	2	2	1	1	47.991	±6.259	48.124	±6.345	99.722
Setaria verticillata	2	1	1	1	36.686	±2.9	36.840	±3.078	99.582
Panicum coloratum	3	2	2	1	41.763	±2.198	39.828	±1.687	104.85
Paspalum dilatatum	3	2	1	1	39.245	±2.04	38.44	±3.122	102.09
Brachypodium distachyon	2	1	2	1	38.085	±2.005	38.285	±2.099	99.476
Bromus catharticus	3	1	2	1	43.006	±3.806	42.444	±3.403	101.32
Avena fatua	3	1	1	1	44.513	±3.245	40.257	±4.26	110.57
Lolium perenne	3	1	1	2	41.709	±3.979	38.711	±1.348	107.74
Phalaris minor	3	2	1	1	41.944	±1.164	39.529	±1.69	106.10
Poa annua	2	1	1	2	26.687	±1.323	26.66	±1.558	100.10

Table 2 cont.: Macro and micro morphological features of pollen grains for studied species.

Character / Species	Pore diameter (µm)			Annulus diameter (µm)			Exine thickness (µm)			Pollen Surface: 1=irregular in-folding, 2=regular in-folding, 3= non-infolded, 4=sunken	Surface sculpturing (ornamentation): 1=verrucate-granulate, 2=areolate-granulate, 3=micro gemmate, 4=micro gemmate-granulate, 5=granulate
	Pore: 1-narrow (1.5-2.5 µm), 2- wide (2.6-4µm).	Average	Stander	Annulus: 1- narrow (5-7 µm), 2- wide (7.1-10 µm).	Average	Stander	Exine thickness: 1- Thin (0.5-1 µm), 2- thick (1.1-2 µm)	Average	Stander		
Cynodon dactylon	1	1.7	±0.235	1	5	±0.208	1	0.98	±0.227	3	2
Dactyloctenium aegyptium	1	2.4	±0.26	1	6.1	±0.728	1	0.76	±0.227	2	1
Eragrostis tenuifolia	2	2.6	±0.2	1	6	±0.385	1	0.80	±0.302	1	5
Dichanthium annulatum	1	2.1	±0.228	2	7.3	±0.346	2	1.44	±0.251	2	1
Sorghum virgatum	2	2.9	±0.355	2	8.2	±0.443	2	1.34	±0.244	2	1
Digitaria ciliaris	2	2.7	±0.156	1	6.8	±0.195	2	1.41	±0.229	3	1
Echinochloa colona	2	2.8	±0.16	2	7.6	±0.424	1	0.94	±0.327	1	2
Cenchrus echinatus	2	3.6	±0.321	2	9.3	±0.405	2	1.59	±0.174	2	1
Setaria verticillata	1	1.8	±0.13	2	8.0	±0.445	2	1.03	±0.17^	1	1
Panicum coloratum	1	2.3	±0.107	2	8.1	±0.907	2	1.53	±0.403	1	1
Paspalum dilatatum	1	2	±0.36	1	5.9	±0.883	1	0.94	±0.089	1	1
Brachypodium distachyon	2	3.2	±0.674	2	8.0	±0.802	2	1.19	±0.351	2	4
Bromus catharticus	2	2.6	±0.088	2	7.2	±0.368	2	1.12	±0.345	1	3
Avena fatua	2	3.9	±0.116	2	10.3	±0.617	1	0.98	±0.302	4	3
Lolium perenne	1	2	±0.348	1	6.5	±0.352	2	1.59	±0.386	3	4
Phalaris minor	1	2.4	±0.298	2	7.45	±0.249	2	1.20	±0.413	3	2
Poa annua	2	2.6	±0.033	1	6.67	±0.247	1	0.67	±0.199	2	2

The exine is thick in most studied species but thin in [*Cynodon dactylon, Dactyloctenium aegyptium, Eragrostis tenuifolia, Echinochloa colona, Paspalum dilatatum*, *Lolium perenne* and *Poa annua*]. The surface of pollen grains is in-folding in most studied species except in [*Cynodon dactylon*, *Digitaria ciliaris*, *Lolium perenne* and *Phalaris minor*] are non infolded and sunken only in [*Avena fatua*].

The sculpturing ornamentation of pollen grains recorded by SEM for the studied species shows five different types:

1- Verrucate-granulate in *Dactyloctenium aegyptium, Dichanthium annulatum*, *Sorghum virgatum, Digitaria ciliaris, Cenchrus echinatus, Setaria verticillata, Panicum coloratum* and *Paspalum dilatatum*.

2- Areolate-granulate recorded in *Cynodon dactylon*, *Echinochloa colona*, *Phalaris minor* and *Poa annua*.
3- Micro-gemmate noted in *Bromus catharticus* and *Avena fatua*.
4- Micro-gemmate-granulate in *Brachypodium distachyon* and *Lolium perenne*.
5- granulate in *Eragrostis tenuifolia*.

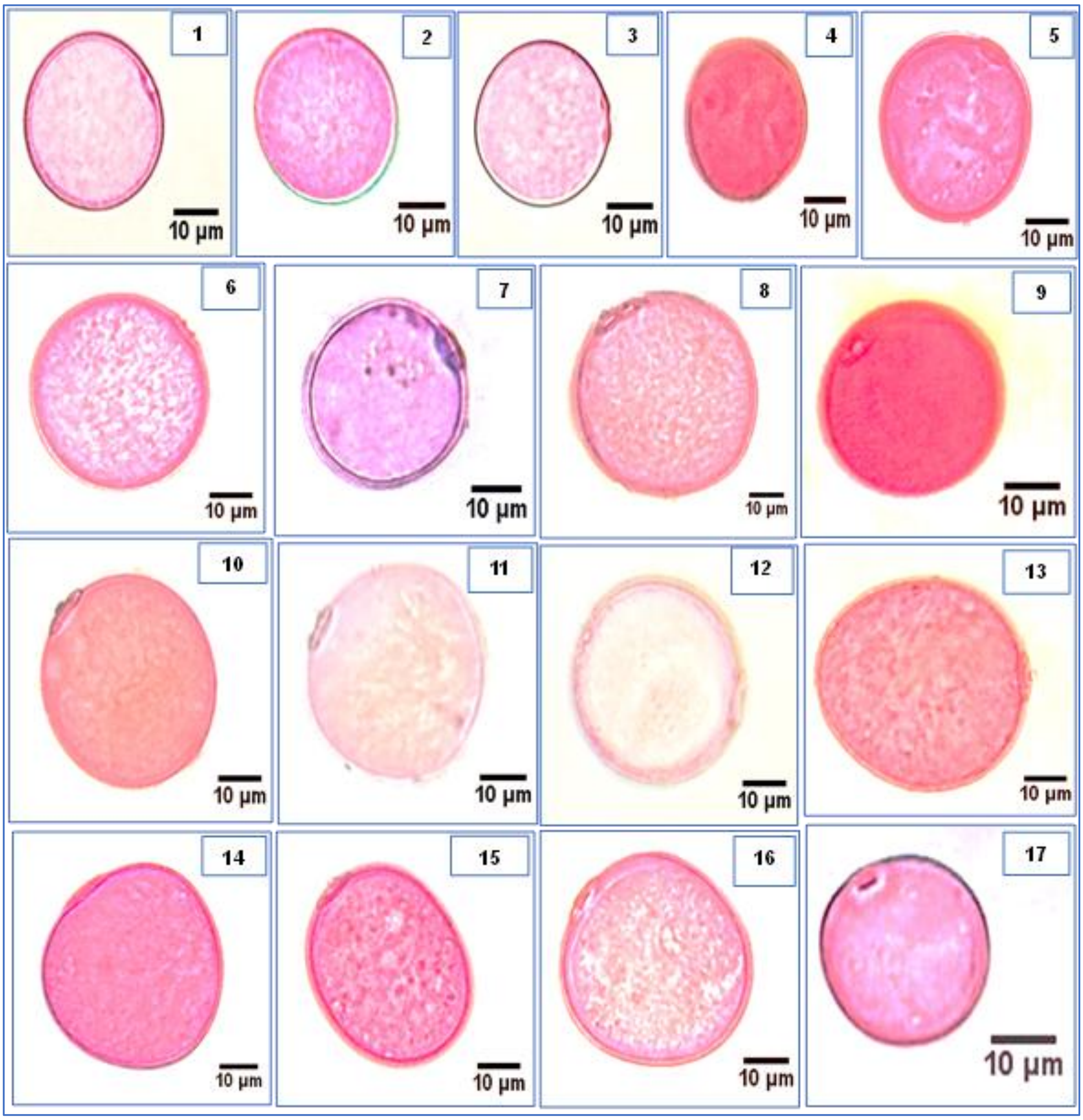

Figure (1): Pollen grains morphology of studied species of Poaceae as revered by light microscope. 1. *Cynodon dactylon*, 2. *Dactyloctenium aegyptium*, 3. *Eragrostis tenuifolia*, 4. *Dichanthium annulatum*, 5. *Sorghum virgatum*, 6. *Digitaria ciliaris*, 7. *Echinochloa colona*, 8. *Cenchrus echinatus*, 9. *Setaria verticillata*, 10. *Panicum coloratum*, 11. *Paspalum dilatatum*, 12. *Brachypodium distachyon*, 13. *Bromus catharticus*, 14. *Avena fatua*, 15. *Lolium perenne*, 16. *Phalaris minor* and 17. *Poa annua*.

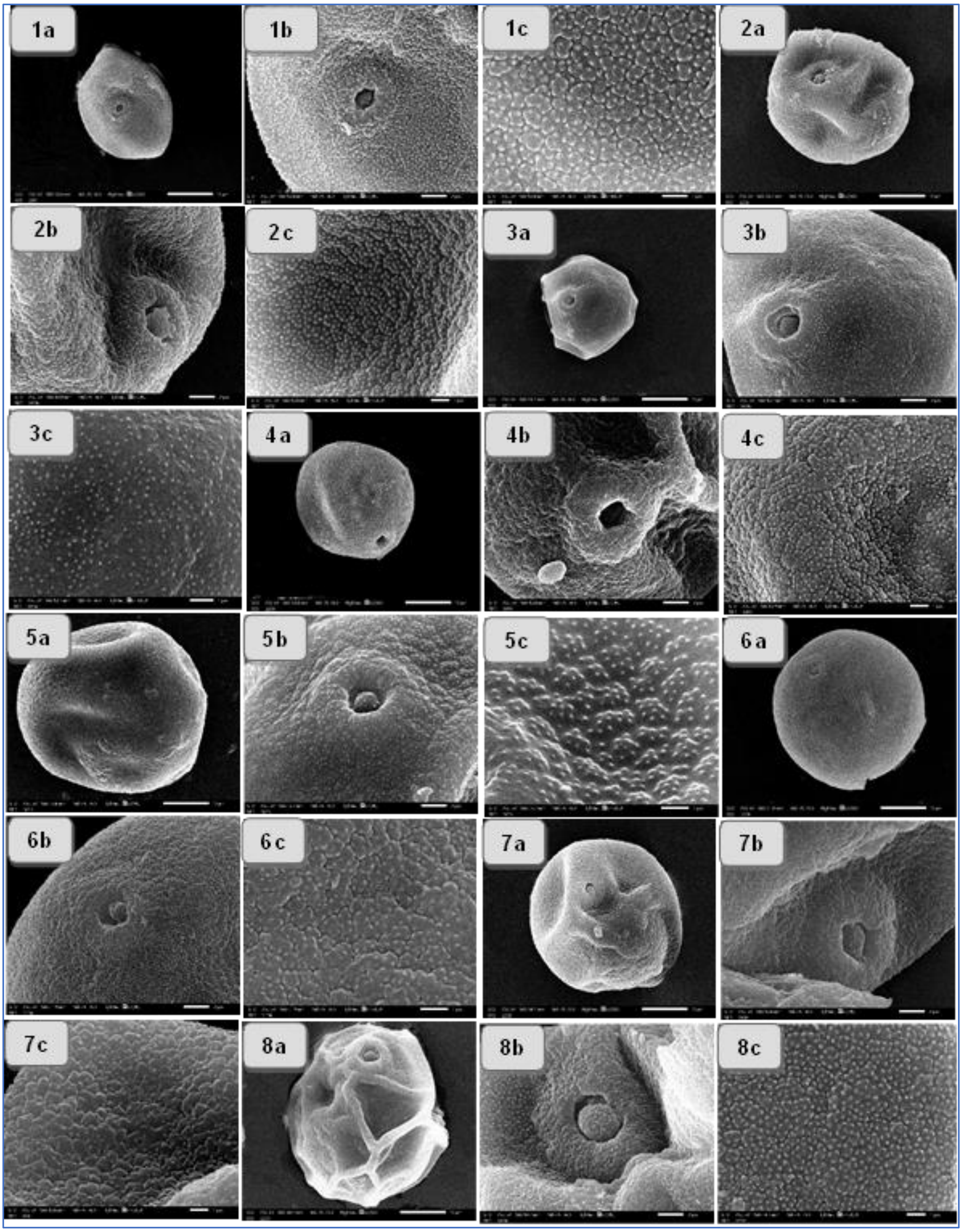

Figure (2): Pollen grains morphology of studied species of Poaceae as revered by SEM.1. *Cynodon dactylon*, 2. *Dactyloctenium aegyptium*, 3. *Eragrostis tenuifolia*, 4. *Dichanthium annulatum*, 5. *Sorghum virgatum*, 6. *Digitaria ciliaris*, 7. *Echinochloa colona*, 8. *Cenchrus echinatus*.

a-Pollen shape b- Pore shape c- Exine ornamentation

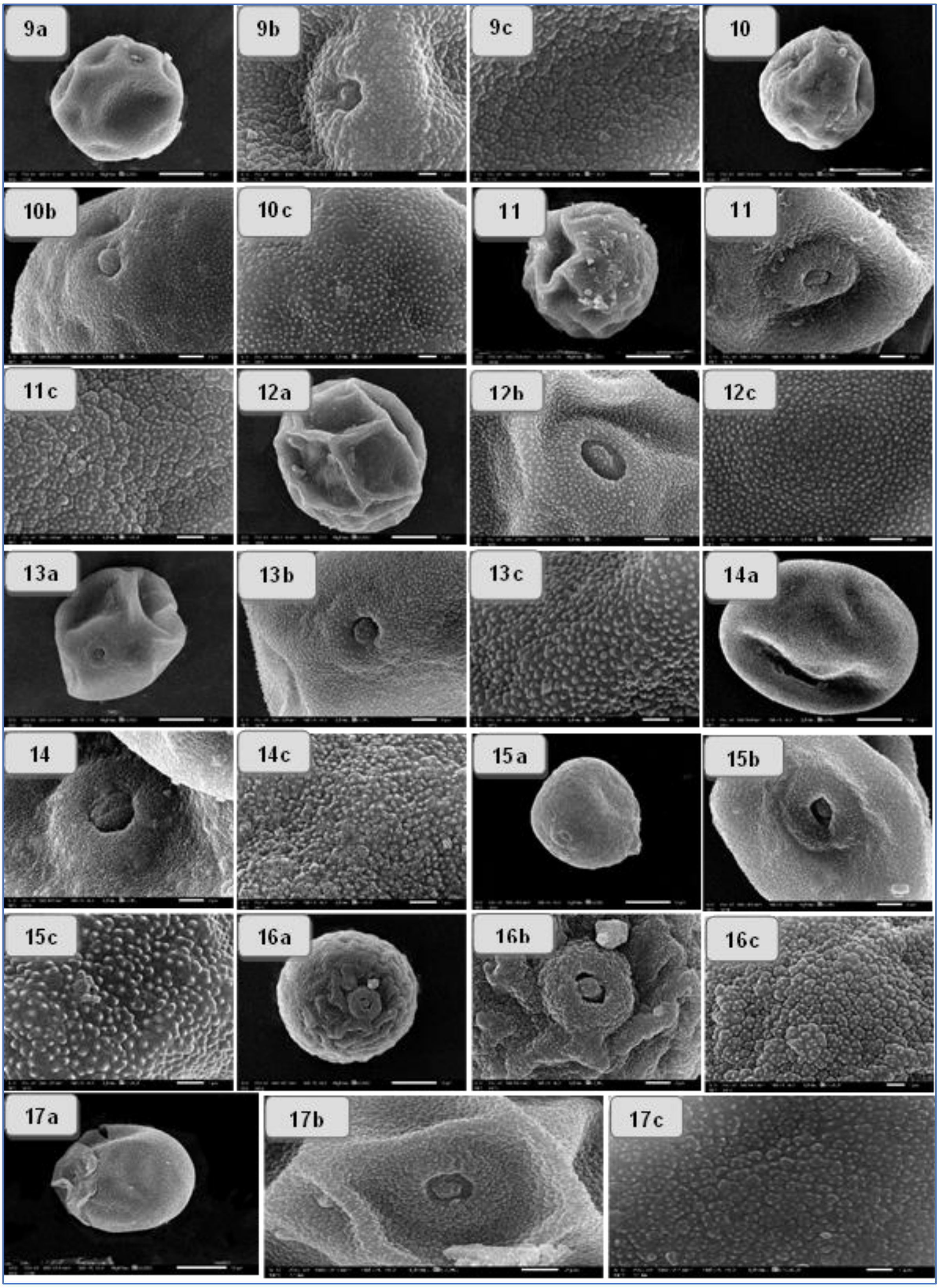

Figure (2) Cont.: Pollen grains morphology of studied species of Poaceae as revered by SEM. 9. *Setaria verticillata*, 10. *Panicum coloratum*, 11. *Paspalum dilatatum*, 12. *Brachypodium distachyon*, 13. *Bromus catharticus*, 14. *Avena fatua*, 15. *Lolium perenne*, 16. *Phalaris minor* and 17. *Poa annua*.

a-Pollen shape b- Pore shape c- Exine ornamentation

Caryopsis features:

The shape of caryopsis recorded six different types ranged between elliptic in [*Cynodon dactylon, Dichanthium annulatum, Sorghum virgatum, Digitaria ciliaris* and *Poa annua*], ovate in [*Echinochloa colona, Setaria verticillata, Panicum coloratum* and *Phalaris minor*], oblong in [*Eragrostis tenuifolia, Brachypodium distachyon, Bromus catharticus, Avena fatua* and *Lolium perenne*], obovate in [*Cenchrus echinatus*], broad ovate in [*Paspalum dilatatum*] and rounded only in [*Dactyloctenium aegyptium*]. The caryopsis texture is glabrous in most studied species while hairy in [*Avena fatua*] and crinkled in [*Dactyloctenium aegyptium*]. The caryopsis color is brown or light brown in most studied species except in [*Digitaria ciliaris, Echinochloa colona, Cenchrus echinatus, Setaria verticillata* and *Panicum coloratum*] are creamy.

The caryopsis size ranged from very small, small or large; very small (1-1.5 mm.) in [*Dactyloctenium aegyptium, Eragrostis tenuifolia, Echinochloa colona, Setaria verticillata* and *Panicum coloratum*], small (1.6-3mm.) in [*Cynodon dactylon, Dichanthium annulatum, Digitaria ciliaris, Cenchrus echinatus, Paspalum dilatatum, Phalaris minor* and *Poa annua*] and large (3.1- 5 mm.) in [*Sorghum virgatum, Brachypodium distachyon, Bromus catharticus, Avena fatua* and *Lolium perenne*].

The ventral face shows three different types; concave in [*Dactyloctenium aegyptium, Digitaria ciliaris, Echinochloa colona, Brachypodium distachyon, Bromus catharticus, Avena fatua, Lolium perenne, Phalaris minor* and *Poa annua*], convex in [*Cynodon dactylon, Eragrostis tenuifolia, Dichanthium annulatum, Sorghum virgatum* and *Cenchrus echinatus*] and flat in [*Setaria verticillata, Panicum coloratum* and *Paspalum dilatatum*].

On the other hand, the hilum recorded three shapes; oval as in [*Dichanthium annulatum, Sorghum virgatum, Digitaria ciliaris, Echinochloa colona, Cenchrus echinatus, Setaria verticillata, Panicum coloratum* and *Paspalum dilatatum*], linear in [*Brachypodium distachyon, Bromus catharticus, Avena fatua, Lolium perenne* and *Phalaris minor*] and punctiform in [*Cynodon dactylon, Dactyloctenium aegyptium, Eragrostis tenuifolia* and *Poa annua*]. Position of hilum is terminal in most studied species but central in [*Dactyloctenium aegyptium, Brachypodium distachyon, Bromus catharticus, Avena fatua, Lolium perenne* and *Phalaris minor*]. The hilum level is slightly depressed in [*Dichanthium annulatum, Sorghum virgatum, Digitaria ciliaris, Echinochloa colona, Cenchrus echinatus, Avena fatua, Lolium perenne* and *Phalaris minor*], raised in [*Cynodon dactylon, Dactyloctenium aegyptium, Eragrostis tenuifolia* and *Poa annua*], flat in [*Setaria verticillata, Panicum coloratum* and *Paspalum dilatatum*] and grooved in [*Brachypodium distachyon* and *Bromus catharticus*].

The embryo is large ($\geq {}^{1}/_{2}$ caryopsis length) in [*Cynodon dactylon, Dactyloctenium aegyptium, Eragrostis tenuifolia, Dichanthium annulatum, Sorghum virgatum, Digitaria ciliaris, Echinochloa colona, Cenchrus echinatus, Setaria verticillata* and *Panicum coloratum*] and small ($< {}^{1}/_{2}$ caryopsis length) in [*Paspalum dilatatum, Brachypodium distachyon, Bromus catharticus, Avena fatua, Lolium perenne, Phalaris minor* and *Poa annua*]. The stylopodium is absent in [*Dactyloctenium aegyptium, Digitaria ciliaris, Echinochloa colona, Setaria verticillata, Panicum coloratum, Brachypodium distachyon, Bromus catharticus, Avena fatua* and *Lolium perenne*] and present in [*Cynodon dactylon, Eragrostis tenuifolia, Dichanthium annulatum, Sorghum virgatum, Cenchrus echinatus, Paspalum dilatatum, Phalaris minor* and *Poa annua*].

According to the caryopsis coat patterns seen through SEM, fruit of the studied species showed nine different types; reticulate as in [*Dactyloctenium aegyptium, Digitaria ciliaris, Cenchrus echinatus* and *Poa annua*], rugose in [*Cynodon dactylon, Setaria verticillata* and *Paspalum dilatatum*], scalariform in [*Eragrostis tenuifolia, Panicum coloratum* and *Phalaris minor*], striate in [*Brachypodium distachyon* and *Lolium perenne*], reticulate-foveate in [*Bromus catharticus*], reticulate-rugose in [*Sorghum virgatum*], reticulate-striate in [*Dichanthium annulatum*], ruminate in [*Echinochloa colona*] and striate-favulariate in [*Avena fatua*].

The studied species demonstrated heterogeneous characteristics of outer epidermal cell shape as shown in Table 4; elongated in [*Cynodon dactylon, Eragrostis tenuifolia, Panicum coloratum, Brachypodium distachyon, Avena fatua, Lolium perenne* and *Phalaris minor*], irregular elongated in [*Dichanthium annulatum, Sorghum virgatum, Echinochloa colona, Cenchrus echinatus, Setaria verticillata* and *Paspalum dilatatum*], polygonal in [*Dactyloctenium aegyptium* and *Poa annua*], irregular polygonal in [*Digitaria ciliaris*] and isodiametric in [*Bromus catharticus*].

The species showed difference in the anticlinal wall shape as revealed; it is straight-undulate as in [*Dactyloctenium aegyptium, Eragrostis tenuifolia, Sorghum virgatum, Digitaria ciliaris, Cenchrus echinatus, Paspalum dilatatum* and *Poa annua*], straight in [*Cynodon dactylon, Brachypodium distachyon, Avena fatua, Lolium perenne* and *Phalaris minor*], straight-sinuous in [*Panicum coloratum*], undulate in [*Echinochloa colona* and *Setaria verticillata*], circular in [*Bromus catharticus*] and sinuous in [*Dichanthium annulatum*].

Anticlinal wall level is concave as in [*Bromus catharticus*], flat-raised in [*Lolium perenne*] and raised in the remainders. Anticlinal wall texture is warty in [*Sorghum virgatum* and *Bromus catharticus*] and smooth in the rests. The thickness of anticlinal wall is thick in [*Dactyloctenium aegyptium, Eragrostis tenuifolia, Dichanthium annulatum, Sorghum virgatum, Digitaria ciliaris, Cenchrus echinatus, Panicum coloratum, Paspalum dilatatum, Bromus catharticus, Avena fatua* and *Poa annua*], thin in [*Echinochloa colona, Setaria verticillata, Brachypodium distachyon, Lolium perenne* and *Phalaris minor*] and very thick only in [*Cynodon dactylon*].

The periclinal wall level is concave as in [*Cynodon dactylon, Dactyloctenium aegyptium, Eragrostis* *tenuifolia*, *Dichanthium annulatum*, *Sorghum virgatum*, *Digitaria ciliaris*, *Panicum coloratum*, *Brachypodium distachyon*, *Avena fatua* and *Poa annua*], flat-concave in [*Cenchrus echinatus*, *Setaria verticillata*, *Paspalum dilatatum* and *Lolium perenne*], flat in [*Echinochloa colona* and *Phalaris minor*], convex only in [*Bromus catharticus*]. Periclinal wall texture is smooth in [*Cynodon dactylon, Dactyloctenium aegyptium, Dichanthium annulatum*, *Digitaria ciliaris*, *Echinochloa colona*, *Cenchrus echinatus*, *Setaria verticillata*, *Panicum coloratum*, *Brachypodium distachyon*, *Avena fatua*, *Lolium perenne*, *Phalaris minor* and *Poa annua*], rough in [*Sorghum virgatum, Paspalum dilatatum* and *Bromus catharticus*] and smooth-striate only in [*Eragrostis tenuifolia*].

Table 3. Macro-morphological characters of caryopsis for studied species.

Character / Species	Shape: 1=elliptic, 2= oblong, 3=ovate 4=obovate, 5= broad ovate, 6= round	Texture: 1=glabrous, 2=hairy, 3=wrinkled	Color: 1=brown, 2=light brown, 3=creamy	Caryopsis size: 1- very small (1-1.5 mm.), 2- small (1.6-3mm.), 3- large (3.1- 5 mm.).	Length Mm		Wide Mm		L / W ratio
					Average	Stander	Average	Stander	
Cynodon dactylon	1	1	1	2	1.78	±0.116	0.68	±0.04	2.62
Dactyloctenium aegyptium	6	3	1	1	0.78	±0.132	0.78	±0.172	1
Eragrostis tenuifolia	2	1	1	1	1.12	±0.097	0.48	±0.074	2.33
Dichanthium annulatum	1	1	2	2	1.78	±0.292	0.68	±0.116	2.62
Sorghum virgatum	1	1	1	3	3.7	±0.451	1.46	±0.08	2.53
Digitaria ciliaris	1	1	3	2	1.9	±0.063	0.76	±0.135	2.5
Echinochloa colona	3	1	3	1	1.52	±0.146	0.8	±0.167	1.9
Cenchrus echinatus	4	1	3	2	2.46	±0.101	1.42	±0.074	1.73
Setaria verticillata	3	1	3	1	1.12	±0.074	0.66	±0.12	1.70
Panicum coloratum	3	1	3	1	1.5	±0.063	0.88	±0.074	1.70
Paspalum dilatatum	5	1	1	2	1.7	±0.089	1.44	±0.048	1.18
Brachypodium distachyon	2	1	1	3	6.16	±0.185	1.16	±0.162	5.31
Bromus catharticus	2	1	1	3	5.92	±0.466	1.5	±0.15	3.95
Avena fatua	2	2	2	3	6.76	±1.22	1.28	±0.633	5.28
Lolium perenne	2	1	2	3	4.26	±0.048	1.26	±0.048	3.38
Phalaris minor	3	1	1	2	2.72	±0.146	1.18	±0.16	2.31
Poa annua	1	1	1	2	1.56	±0.048	0.44	±0.048	3.55

Table (3) cont.: Macro-morphological characters of caryopses for studied species.

Character / Species	Ventral face:1=concave, 2=convex, 3=flat	Compression		Hilum			Embryo size: 1=large (≥ 1/2 caryopsis length), 2=small (<1/2 caryopsis length)	Stylopodium: 1=absent, 2=present
		extent: 1=lateral, 2=ventral, 3=not compressed, 4=dorsal	depth: 1=slight, 2=none, 3=strong	shape: 1=oval, 2=linear, 3=punctiform	position: 1=terminal, 2=central	level: 1=slightly depressed, 2=raised, 3=flat, 4=grooved		
Cynodon dactylon	2	1	1	3	1	2	1	2
Dactyloctenium aegyptium	1	1	1	3	2	2	1	1
Eragrostis tenuifolia	2	1	1	3	1	2	1	2
Dichanthium annulatum	2	4	1	1	1	1	1	2
Sorghum virgatum	2	3	2	1	1	1	1	2
Digitaria ciliaris	1	2	1	1	1	1	1	1
Echinochloa colona	1	2	1	1	1	1	1	1
Cenchrus echinatus	2	4	1	1	1	1	1	2
Setaria verticillata	3	2	1	1	1	3	1	٢
Panicum coloratum	3	3	2	1	1	3	1	1
Paspalum dilatatum	3	2	1	1	1	3	2	2
Brachypodium distachyon	1	3	2	2	2	4	2	1
Bromus catharticus	1	1	3	2	2	4	2	1
Avena fatua	1	3	2	2	2	1	2	1
Lolium perenne	1	2	1	2	2	1	2	1
Phalaris minor	1	1	3	2	2	1	2	2
Poa annua	1	1	1	3	1	2	2	2

Numerical analysis:

The numerical analysis was performed by using PRIMER (software, version 6) statistical program to the obtained data of pollen grains and caryopsis morphological features of 34 character (14 character for pollen grains and 20 characters for caryopsis) with 97character states (table 2-4) to establish the relationships between the studied species.

The dendrogram obtained from pollen grains characters only **(Fig. 5a)** showed that; The studied species of *Eragrostis tenuifolia* separated from the reminder species in cluster (**I**) and the cluster (**II**) divided into two groups each group classified into two subgroups. Group one (**G1**) included three species; *Dactyloctenium aegyptium*, *Cynodon dactylon* and *Poa annua* in subgroup **(A)** and four species; *Lolium perenne*, *Digitaria ciliaris*, *Dichanthium annulatum*, and *Sorghum virgatum* in subgroup **(B).** Group two (**G2**) included six species in subgroup (**A**), *Echinochloa colona*, *Paspalum dilatatum*, *Phalaris minor*, *Panicum coloratum*, *Cenchrus echinatus* and *Setaria verticillata* while the subgroup (**B**) contains three species, *Avena fatua*, *Brachypodium distachyon* and *Bromus catharticus*.

The results obtained from fruit (caryopsis) characters only **(Fig. 5b)** also divided the studied species into two main clusters. Cluster **(I)** included four species; *Dactyloctenium aegyptium*, *Eragrostis tenuifolia*, *Cynodon dactylon* and *Poa annua*. The second cluster **(II)** contain the reminder studied species and divided into two groups; group **(G1)** contain five species; *Bromus catharticus*, *Phalaris minor*, *Avena fatua*, *Brachypodium distachyon* and *Lolium perenne* while the group **(G2)** contain the rest eight studied species and divided into two subgroups. The subgroup

(**A**) contains two species; *Digitaria ciliaris* and *Sorghum virgatum* while the subgroup (**B**) contains the reminder species.

The dendrogram **(Fig.5c)** produced from the combined data of pollen grains and caryopsis showed that; species were classified into two major cluster; Cluster **(I)** included four species; *Dactyloctenium aegyptium*, *Eragrostis tenuifolia*, *Cynodon dactylon* and *Poa annua*. The second cluster **(II)** contain the reminder studied species which divided into two groups; group **(1)** contain five species; *Bromus catharticus*, *Phalaris minor*, *Avena fatua*, *Brachypodium distachyon* and *Lolium perenne* while the group **(2)** contain the rest eight studied species and divided into two subgroups. The subgroup (**A**) contains three species; *Digitaria ciliaris*, *Dichanthium annulatum*, and *Sorghum virgatum* while the subgroup (**B**) contains the reminders.

Table 4. Micro-morphological characters of caryopses for studied species.

Character / Species	Fruit Coat pattern sculpturing: 1=reticulate,2=rugose, 3=scalariform, 4=striate, 5=reticulate - foveate, 6=reticulate-rugose, 7=reticulate-striate, 8=ruminate, 9=striate -favulariate	Outer epidermal cell shape: 1=elongated, 2=irregular elongated, 3=polygonal, 4=irregular polygonal, 5=isodiametric	Anticlinal wall: Shape: 1=straight-undulate, 2=straight, 3=undulate, 4=circular, 5= sinuous, 6= straight-sinuous	Anticlinal wall: Level: 1=raised, 2=concave, 3=flat-raised	Anticlinal wall: Texture: 1=smooth, 2=warty	Anticlinal wall: Thickness: 1=thick, 2=thin 3=very thick	Periclinal wall: level:1=concave, 2=flat-concave, 3=flat, 4=convex	Periclinal wall: Texture: 1=smooth, 2=rough, 3=smooth-striate
Cynodon dactylon	2	1	2	1	1	3	1	1
Dactyloctenium aegyptium	1	3	1	1	1	1	1	1
Eragrostis tenuifolia	3	1	1	1	1	1	1	3
Dichanthium annulatum	7	2	5	1	1	1	1	1
Sorghum virgatum	6	2	1	1	2	1	1	2
Digitaria ciliaris	1	4	1	1	1	1	1	1
Echinochloa colona	8	2	3	1	1	2	3	1
Cenchrus echinatus	1	2	1	1	1	1	2	1
Setaria verticillata	2	2	3	1	1	2	2	1
Panicum coloratum	3	1	6	1	1	1	1	1
Paspalum dilatatum	2	2	1	1	1	1	2	2
Brachypodium distachyon	4	1	2	1	1	2	1	1
Bromus catharticus	5	5	4	2	2	1	4	2
Avena fatua	9	1	2	1	1	1	1	1
Lolium perenne	4	1	2	3	١	2	2	1
Phalaris minor	3	1	2	1	١	2	3	1
Poa annua	1	3	1	1	1	1	1	1

Figure (3): Caryopsis morphology of studied species of Poaceae as revered by light microscope. 1. *Cynodon dactylon*, 2. *Dactyloctenium aegyptium*, 3. *Eragrostis tenuifolia*, 4. *Dichanthium annulatum*, 5. *Sorghum virgatum*, 6. *Digitaria ciliaris*, 7. *Echinochloa colona*, 8. *Cenchrus echinatus*, 9. *Setaria verticillata*, 10. *Panicum coloratum*, 11. *Paspalum dilatatum*, 12. *Brachypodium distachyon*, 13. *Bromus catharticus*, 14. *Avena fatua*, 15. *Lolium perenne*, 16. *Phalaris minor* and 17. *Poa annua*.

A-Ventral view b- Dorsal view

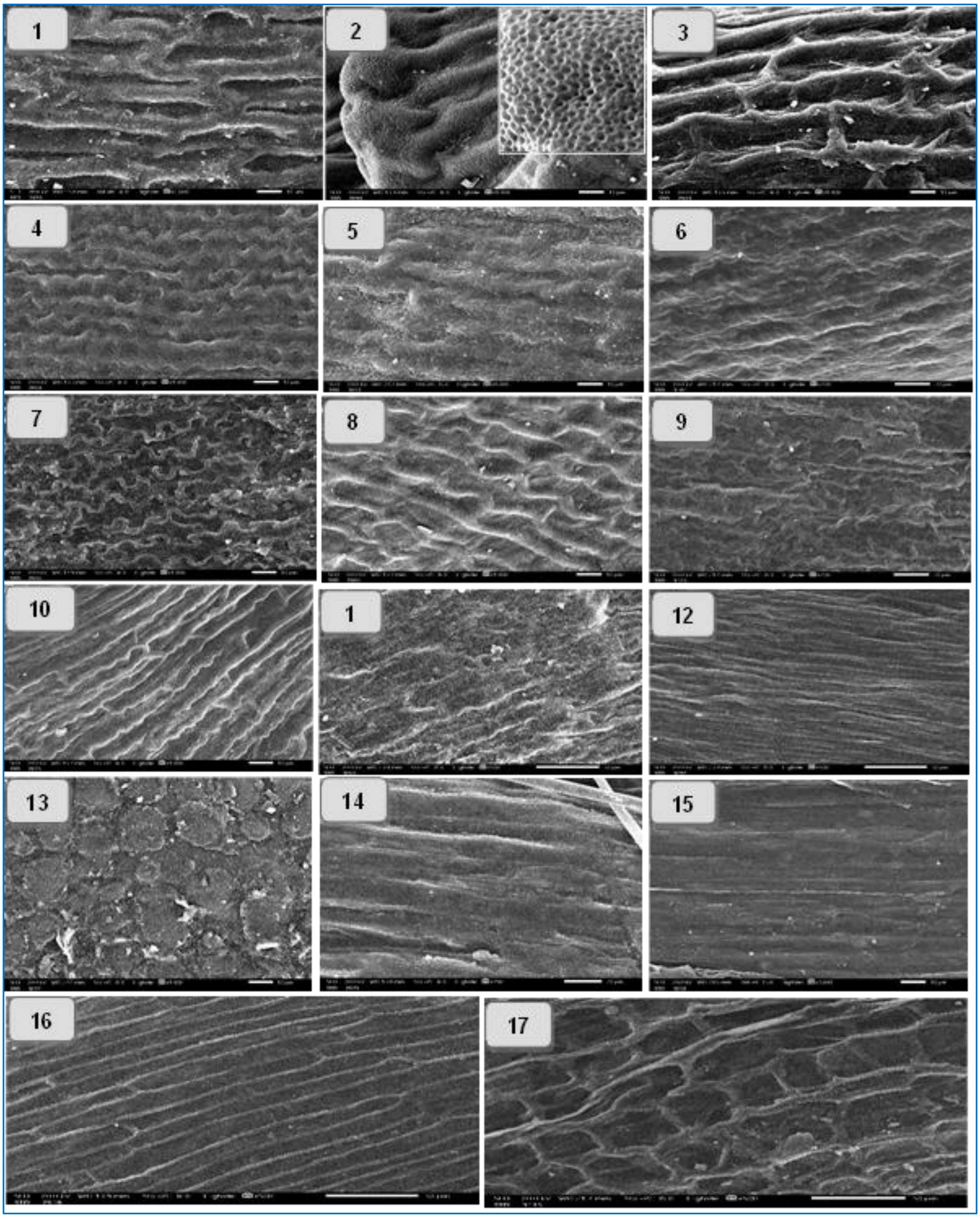

Figure (4): Caryopsis morphology of studied species of Poaceae as revered by SEM. 1. *Cynodon dactylon*, 2. *Dactyloctenium aegyptium*, 3. *Eragrostis tenuifolia*, 4. *Dichanthium annulatum*, 5. *Sorghum virgatum*, 6. *Digitaria ciliaris*, 7. *Echinochloa colona*, 8. *Cenchrus echinatus*, 9. *Setaria verticillata*, 10. *Panicum coloratum*, 11. *Paspalum dilatatum*, 12. *Brachypodium distachyon*, 13. *Bromus catharticus*, 14. *Avena fatua*, 15. *Llium perenne*, 16. *Phalaris minor* and 17. *Poa annua*.

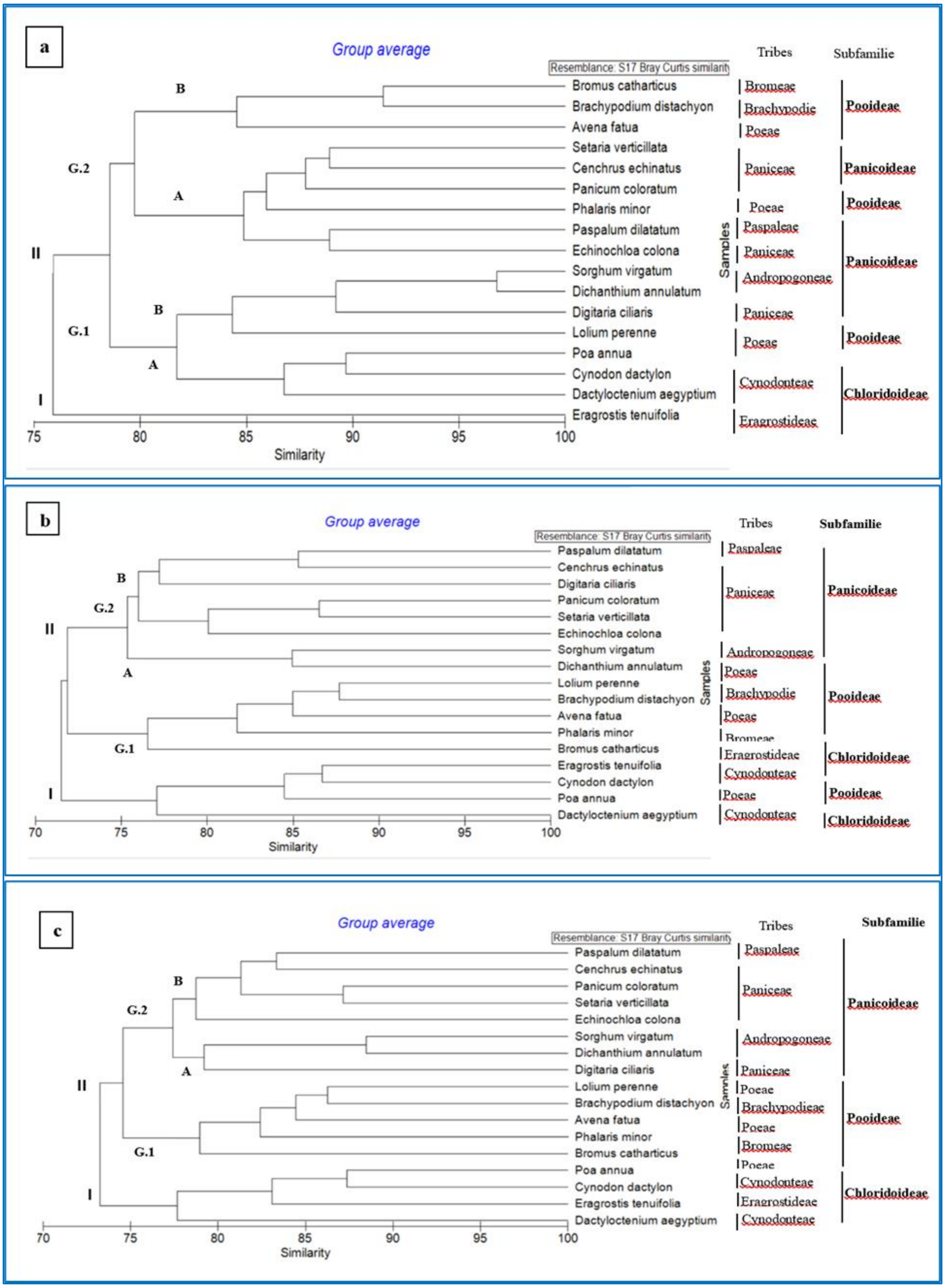

Figure (5) : Dendrograms showing the interrelationships between studied species of Poaceae based on pollen grains and caryopsis characters by using PRIMER Program. (a) Pollen grains characters, (b) Caryopsis characters, (c) Combined pollen grains and caryopsis characters

4. Discussion

Palynology is one of the major corrections used by the modern taxonomist for the identification and differentiation of closely related species. Pollen morphology acts as an additional and significant tool for taxonomic description and implication.

Poaceae is a stenopalynous family as reported in other palynological studies [45-49]. The pollen grains are generally apolar, monoporate–diporate rarely triporate. Surface ornamentation is generally areolate, areolate cumscabrate, or simply scabrate, [50,51]. According to [52], Poaceae pollen were evolved from monosulcate-monoporate to operculate. [14] reported non-annulated pollen in the genus *Pariana*. [53] reported monoporate, heteropolar, prolate-spheroidal and operculate-annulate pollen, with mixed scabrate type exine and insular type in the genus *Eremopyrum*. The pollen grains of studied species have oblate-spheroidal, spheroidal and prolate-spheroidal shape, prolate-spheroidal is the dominant shape for the studied species while four species appeared oblate-spheroidal and three appeared shape spheroidal, this is in agreement with [20]. The aperture for pollen grains of the studied species are monoporate or diporate with distinct or reduced annulus, which agree with [19,22]. Exine ornamentation is an important feature from an evolutionary and phylogenetic point of view [54]. The study of the exine surface of Poaceae pollen grains under SEM shows variations among the studied species where it is ranged from verrucate-granulate, areolate-granulate, micro gemmate, micro gemmate-granulate to granulate. This is different from those results obtained by [19] who in his study reported that exine of Poaceae pollen grains usually areolate-scabrate, rarely areolate cum scabrate. The morphological characters of caryopsis offer new awareness about some significant features that helps in identifying diverse sections of family Poaceae [32,55]. The different shapes of caryopsis play an important role for differentiating between the different species of Poaceae [32]. Some of the vital taxonomic characters in various taxa of Poaceae are compression-type, hilum position, color and shape of caryopsis [56]. In the present study the caryopsis recorded different shapes ranged from elliptic, oblong, ovate, broad ovate, obovate and rounded which help to distinguish between the studied species, this is in agreement with [36,38]. The color for studied species showed small difference ranging from brown, light brown, to creamy and this partially agree with [35]. Hilum shape ranged from oval, linear to punctiform while position showed terminal or central. A majority of species showed large embryo size and the reminder small. This is different from those results obtained by [33] who in their study reported that hilum shape is V shaped while position is basal and embryo is large. The ventral face of caryopsis recorded three types; convex, concave and flat while compression is ranged from lateral, ventral, not compressed, to dorsal. The stylopodium is absent in some species and present in the reminder. This is in agreement with [26]. The caryopsis texture usually glabrous, except *Avena fatua* which is hairy and *Dactyloctenium aegyptium* wrinkled. Sculpturing of seed plays important role in determining different species [57]. The caryopsis coat pattern sculpturing showed variation ranged from reticulate, rugose, scalariform, striate, reticulate-foveate, reticulate-rugose reticulate-striate, ruminate to striate - favulariate and this is partially agreeing with [38]. Distinct characters at the species level are variations within the anticlinal wall and periclinal wall pattern [58]. The grass family has been divided in several subfamilies ranging from two according to [59] to 13 according to [60]. Recently The Grass Phylogeny Working Group [61,62] recognized 12 subfamilies with different tribes. In the present study the studied species represented into three subfamilies and eight tribes [7]. The result obtained from the numerical analysis by using pollen grains characters show that the morphology of pollen grains as, size, shape, exine thickening, exine ornamentation, operculum and annulus diameter are very important characters for delimiting the species but not support the subfamilies and tribal classification while the morphology of caryopsis plays an important role in identification of species in family Poaceae and can be used for subfamilies and tribal classification level.

5. Conclusion

The study focuses on the examination of pollen grains and caryopsis (grain) features in several species belonging to the Poaceae family and discusses their taxonomic relevance. Morphological characters of pollen grains and caryopsis coat are very important at taxonomic level. The shape, size, exine ornamentation, operculum and annulus diameter for pollen grains are very important for species identification. Also, shape, color and sculpture of caryopsis coat play an important role in identification and classification of Poaceae species.

Suggest possible future research directions:

we will expand the study to include DNA analysis of species

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