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Research Article

Pollen Micromorphology among Amaranthaceous Species from Desert Rangeland: Exine Stratification and their Taxonomic Significance

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The aim of the study was to visualize the micromorphology of Amaranthaceous pollen using scanning electron microscopy collected from the Thal Desert. Field collection was conducted from July to September 2021. A total of 14 taxa of the family Amaranthaceae were collected which belong to nine genera. *Achyranthes aspera*, *Aerva javanica*, *Aerva lanata*, *Amaranthus graecizans*, *Amaranthus retroflexus*, *Amaranthus viridis*, *Bassia indica*, *Chenopodium album*, *Chenopodium ficifolium*, *Chenopodium murale*, *Digera muricata*, *Haloxylon stocksii*, *Salsola tragus*, and *Suaeda fruticosa* were studied in terms of pollen morphotypes. Pollen were acetolyzed and observed under optical and scanning microscopy. Qualitative and quantitative characters were measured to analyze the pollen to uncover its taxonomic significance. Qualitative characters observed were the shape of pollen in polar and equatorial views; the most frequent shape observed was spheroidal in the polar view, whereas in the equatorial view, prolate spheroidal was the dominant shape. Exine ornamentation is the key characteristic of pollen which is very helpful, and eight different types of ornamentations were observed in collected taxa: smooth sparsely granulate, scabrate-spinulose, microspinulose perforate, microechinate scabrate to metareticulate, granulate, nanospinulate, granulate-spinulose perforate, granulate-perforate echinate, and microechinate perforate. Periporate-type aperture was observed among all taxa. Mesoporia, ektexinous bodies, and tectum features also show variations among Amaranthaceous grains. In quantitative character, *A. retroflexus* recorded highest in polar view 26.3 μm and the lowest was of *C. album* 12.2 μm. Highest P/E index ratio was recorded in *S. fruticosa* (1.12) whereas the lowest for *D. muricta* (0.94). Exine thickness was highest in *S. tragus* 2.15 μm and lowest in *A. graecizans* 0.78 μm. The maximum number of pores was recorded as 32-36 in *D. muricata*. Artificial taxonomic keys were constructed based on findings that reinforce the importance of the micromorphological ultrastructural diversity of pollen among Amaranthaceous taxa. It was concluded that the descriptions of pollen morphotypes presented greatly contribute to our understanding of desert species identification.

1. Introduction

The study of systematic traits representing dicot species has proven to be quite useful in taxonomy at various hierarchical levels [1]. Therefore, our research builds on past studies of the Thal Desert [2–4]; however, the majority of the above-reported studies from Thal dryland present only a checklist of plant species along with ethnomedicinal documentation without any focus on micromorphological traits of dicot angiosperms. With respect to the spectrum of systematic traits, exploration of desert dicot angiosperms has been ignored for many reasons. Various characters have been used in delimiting the Amaranthaceous taxa, but not much attention has been drawn to evaluating pollen ultrastructure using scanning microscopy. It is obvious that precise and detailed analysis of various features of pollen stratification will support accurate identification. However, the palynomorph micromorphology using SEM in the Amaranthaceae has not been investigated, which may have taxonomic implications.

For a long time, the Amaranthaceae and Chenopodiaceae families were recognized to be closely related. Previous morphological and phylogenetic research indicated that Amaranthaceae and Chenopodiaceae were thought to represent one evolutionary lineage [5]. Group et al. [6] combined both families and placed them in the Caryophyllales order. Amaranthaceae has 180 genera and 2500 species that are found all over the world. The majority of them are perennial and annual herbs. From the tropics to the subtropics, Amaranthaceous species can be found in a wide range of ecological habitats [7–9]. This family is extremely important ecologically and commercially, especially in harsh habitats such as deserts, semideserts, and salt marshes. The species is usually the dominant plant group and provides valuable fodder for grazing cattle [10]. The seeds, roots, stems, and fruits of Amrанthaceous plants were used by herbalists in the Thal Desert for medicinal purposes [11].

Pollen morphological similarities are evident at both generic and specific levels. Commonly, the pollen grains of Amaranthaceae are periporate, the pollen grain shape differs from spheroidal to subspheroidal, and the exine is either perforate or imperforate and has a stimulating more or less similar sculpture, structures, and aperture with a persistent or a deciduous operculum [12]. In the dimensions of the grains, clear differences have been exhibited at the generic level by the biometric analysis, and no severe deviation has been observed between the studied species of Amaranthaceae and this may validate the work of [13], who specified that pollen grains both of the same and closely related species tend to be alike if they face constant environmental factors, and the degree to which they are alike is a degree of their close relationship. Nowicke and Skvarla [14] also apprehended the same estimation. For the family Amaranthaceae, the study of pollen morphology in the determination of generic affinity has proved very valuable, and the extensive classification of the family was undoubtedly highlighted by the general pollen survey [7]. Current studies have considered that pollen of Chenopodiaceae is mostly not distinguishable from species to species like from *Chenopodium album* to *Amaranthus graecizans*. From the studies on the pollen grains of Chenopodiaceae, it is well known that the grains are in general circular and polyporate.

Palynomorphological examination offers a way to play a role in the systematic classification and identification of plant species at the microscopy level. Amaranthaceous taxa were identified by comparing the microscopic characteristics of various specimens in order to detect differences among them [15]. Advanced microscopy techniques like scanning electron microscopy (SEM) are applied to analyze the morphological traits of plants and their organs. SEM can be used to differentiate between micromorphological traits of closely related taxa. Scanning electron microscopy of pollen is also used to create new terminology for describing pollen ornamentation developing a numerical approach to pollen sculpturing and even computer analysis of the exine [16]. Amaranthaceae exhibits the highest pollen morphological diversity in the whole order Caryophyllales [17]. Pollen studies were carried out at different taxonomic ranks (from family to species), and several characters (both quantitative and qualitative) were considered. Qualitative characters include pollen and shape type, exine sculpturing, pore membrane sculpturing, pore borders, convexness of mesopori, and shape of spines. Quantitative characters include pollen diameter, pore number, diameter and density, exine thickness, interporal distance, punctate/spinule ratio, spinule height and density, and punctae density. These quantitative features show a broad range of overlap, and as a consequence, they appear to be not good to distinguish taxonomic groups [18]. Pollen with a microspinose, punctate tectum is the rule, and rarely, the punctate are annulated. The variation in size, shape, and number of ektexinous bodies covering the pores provide most of the morphological variation observed in the pores of Amaranthaceae pollen [19]. Previous studies [20, 21] have confirmed that in *Amaranthus* taxa, flowers lack nectar glands and pollen grains are small (diameter 18 to 28 μm), usually with 30 to 45 pores uniformly distributed on their surface. Pollen grains of dioecious species have a larger number of apertures on the visible surface. However, three important parameters can be pointed out by Angelini et al. [22]; the first two are biometrical parameters (interporal distance and exine thickness) and the third one is a morphological microechini occurance on pollen surface.

In order to better understand the taxonomic relevance of the family Amaranthaceae, we have conducted a thorough pollen morphometric study using scanning bioimaging technique to examine pollen samples. The palynomorph identification provides accurate taxonomic characterization for better understanding the Amaranthaceous systematics. Currently, this study aims at reporting the pollen ultrastructural variations using scanning electron microscopy among dominant Amaranthaceous taxa from Thal Desert rangeland. We also depict the pollen spectrum in desert vegetation to uncover the taxonomic significance of Amaranthaceous species.

2. Materials and Methods

2.1. Plant Sampling. Field visits to Thal Desert areas were conducted during the flowering season March and August in 2021 to collect Amaranthaceous taxa (Figure 1). During fieldwork, field data such as date of collection, plant habit, and voucher number were noted (Table 1). Amaranthaceous species, along with fresh pollen samples, were collected, pressed, dried, poisoned, mounted, and submitted to Pakistan's Herbarium (ISL). The International Plant Names Index (https://www.ipni.org/) was used to validate the names of Amaranthaceous taxa. Specimens were identified using Flora of Pakistan and verified by comparing them to already deposited herbarium specimens.

2.2. Slide Preparations via Acetolysis Process. The acetolysis process introduced by [23] was followed by a few modifications. Anthers from dried specimens were transferred to microscopic slides with forceps. Because some species of flowers are so small that an anther cannot be separated as a whole, the entire flower was photographed on a slide. One to two drops of acetic acid were poured into the slide, and then, plant material was cursed by using a glass rod for about 1 minute. The debris was removed from the slide by using a needle. A drop of glycerin jelly was poured on the slide and then covered with a coverslip. Permanent nail varnish was used to make the slide permanent. A total of 4–5 slides were prepared for each specimen. A well-prepared slide was used for calculations of morphopalynological features under the microscope Nikon (Japan eyepiece WF40X-18MM), and reading was taken on 40x magnification. For each qualitative trait, 15 readings were measured to calculate the mean value. The following measures were recorded for each species: polar axis diameter, equatorial diameter, pore size, interporal distance, pore density, and exine thickness.

2.3. Scanning Microscopic Imaging Analysis. For the three-dimensional (3D) ultrastructure of pollen grains and stratification of exine wall, scanning electron microscopy, an important tool in the field of palynology, was employed. Pollen stubs of the dicot species were prepared for SEM analysis using Majeed et al.'s [24] protocol. EM was used to examine the external ornamentation in depth. The pollen was first acetolyzed and then placed in 90% ethanol for SEM. They were then positioned on gold-palladium-coated iron stubs. Photographs of pollen were taken through SEM Model JEOL JSM5910. Pollen characters were described using the terminologies of [25, 26].

2.4. Statistical Analysis. Data was analyzed statistically to find out the mean, maximum, minimum, and standard error using SPSS 16.00 statistical software. For statistical analysis of mean (minimum-maximum) ± SE, almost 15 to 20 readings were taken for each parameter [27].

2.4.1. P/E Index. P/E ratio is determined based on equatorial diameter of same pollen as given by Butt et al. [28].

$$\text{P/E ratio} = \frac{P}{E} \times 100, \tag{1}$$

where P is the polar diameter and E is the equatorial diameter.

2.4.2. Pollen Fertility and Sterility (%). Using the following formula, the fertility and sterility percentage were calculated [29].

$$\text{Fertility} = \frac{F}{F} + S \times 100. \tag{2}$$

F represents the number of fertile, whereas S is the number of sterile pollen on ocular.

$$\text{Sterility} = \frac{S}{S} + F \times 100. \tag{3}$$

S is the number of sterile pollen, and F represens the number of fertile pollen on ocular.

2.4.3. Exploratory Multivariate Analysis. The correlation among pollen morphological traits of Amaranthaceous taxa was determined based on Euclidean distance, and the taxa were grouped using a hierarchical clustering analysis method, and UPGMA (unweighted pair group method with arithmetic mean) was carried out with the PAST statistical tool version 3.0 software [30].

Principal component analysis (PCA) was performed to determine the most significant characters accounting for the greatest proportion of the variability. Thus, the pollen and seed morphological characters investigated were first separately subjected to PCA analysis via PAST software. The eigenvalues were plotted in a two-dimensional scatter plot to show variance percentage along the two principal component axis [31].

3. Results

3.1. Micromorphological Pollen Structure of Amaranthaceous Taxa. Amaranthaceous pollen visualization was done using scanning microscopy and using pollen characters like pores,

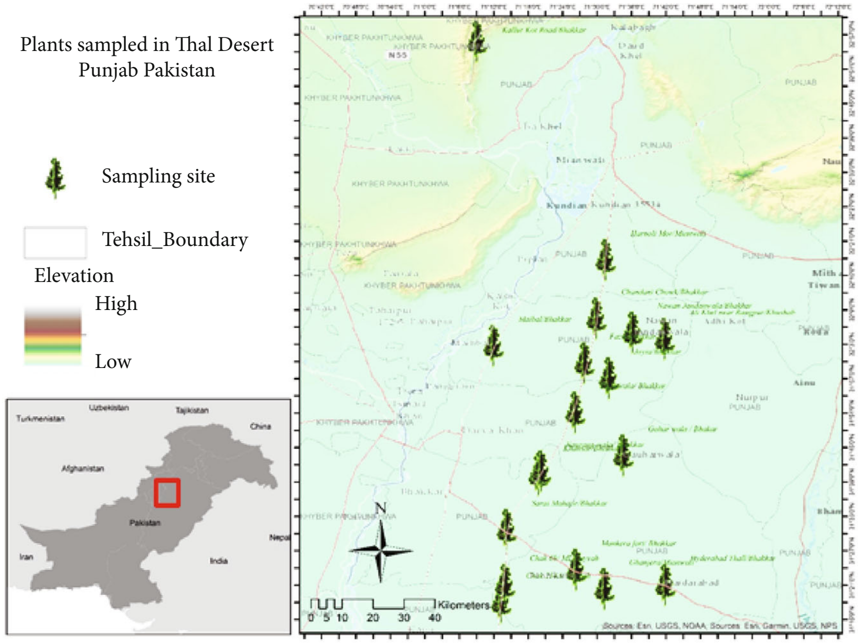


Figure 1: Map of study area: Thal Desert.

Table 1: Checklist of Amaranthaceous taxa of Thal Desert with life form, vouchering data, localities, and geographical coordinates.

S. no.	Amaranthaceous taxa	Voucher specimen no.	Accession no.	Life form	Locality/district	GPS coordinates
1.	*Achyranthes aspera* L.	SN-40	131967	Herb	Rangpur to Noorpur Thal road/ Khushab	32° 01′23.31″N 71°49′22.34″E
2.	*Aerva javanica* (Burm.f.) Juss. ex Schult.	SN-58	131916	Herb	Chak 47DB Rangpur/Khushab	32° 03′17.33″N 71°43′30.63″E
3.	*Aerva lanata* (L.) Juss.	SN-29	131953	Herb	Gohar Wala/Bhakkar	31° 43′04.49″N 71°34′31.31″E
4.	*Amaranthus graecizans* L.	SN-47	131982	Herb	Joyia/Bhakkar	31° 56′33.15″N 71°32′01.75″E
5.	*Amaranthus retroflexus* L.	SN-94	131985	Herb	Chak 234A/ Layyah	31° 17′29.54″N 71°13′14.15″E
6.	*Amaranthus viridis* L.	SN-34	131950	Herb	Dullewala/ Bhakkar	31° 50′33.75″N 71°26′09.14″E
7.	*Bassia indica* (Wight) A.J.Scot	SN-5	131917	Herb	Sarai Mahajir/Bhakkar	31° 30′07.17″N 71°14′12.96″E
8.	*Chenopodium album* L.	SN-26	131911	Herb	Chak 69/ ML/Layyah	31° 20′31.74″N 71°13′54.25″E
9.	*Chenopodium ficifolium* Sm.	SN-109	131947	Herb	Chandani Chwok/Bhakkar	32° 06′59.17″N 71°29′49.90″E
10.	*Chenopodium murale* L.	SN-95	131989	Herb	Maibal/Bhakkar	32° 02′13.36″N 71°11′53.96″E
11.	*Digera muricata* (L.) Mart.	SN-53	131921	Herb	Chak 234A/Layyah	31° 17′29.54″N 71°13′14.15″E
12.	*Haloxylon stocksii* (Boiss.) Benth. & Hook. f.	SN-19	131964	Shrub	Near Mankera Fort/Bhakkar	31° 23′08.65″N 71°26′19.11″E
13.	*Salsola tragus* L.	SN-89	131966	Herb	Maibal/Bhakkar	32° 02′13.36″N 71°11′53.96″E

Table 2: Pollen morphological characters among desert-inhabited Amaranthaceous species.

Sr. no.	Amaranthaceous taxa	Size	Amb/polar view	Operculum	Shape	Aperture character	Aperture group
1.	*Achyranthes aspera* L.	Small	Circular	Present	Prolate spheroidal	Periporate	Convex-shaped circular
2.	*Aerva javanica* (Burm.f.) Juss. ex Schult.	Small	Spheroidal	Present	Prolate spheroidal	Periporate	Collapsed-shaped circular
3.	*Aerva lanata* (L.) Juss.	Small	Spheroidal	Present	Oblate spheroidal	Periporate	Deeply collapsed circular
4.	*Amaranthus graecizans* L.	Small	Rounded	Present	Prolate spheroidal	Periporate	Convex-shaped circular
5.	*Amaranthus retroflexus* L.	Small to medium	Circular	Absent	Prolate spheroidal	Periporate	Collapsed-shaped circular
6.	*Amaranthus viridis* L.	Small	Rounded	Present	Prolate spheroidal	Periporate	Collapsed shaped circular
7.	*Bassia indica* (Wight) A.J.Scot	Medium	Circular	Absent	Oblate spheroidal	Periporate	Convex-shaped circular
8.	*Chenopodium album* L.	Small	Circular	Present	Prolate spheroidal	Periporate	Deeply collapsed circular
9.	*Chenopodium ficifolium* Sm.	Small	Spheroidal	Absent	Prolate spheroidal	Periporate	Collapsed-shaped circular
10.	*Chenopodium murale* L.	Small	Rounded	Absent	Prolate spheroidal	Periporate	Deeply collapsed circular
11.	*Digera muricata* (L.) Mart.	Small to medium	Spheroidal	Present	Oblate spheroidal	Periporate	Convex-shaped circular
12.	*Haloxylon stocksii* (Boiss.) Benth. & Hook. f.	Small	Spheroidal	Present	Prolate spheroidal	Periporate	Collapse-shaped circular
13.	*Salsola tragus* L.	Medium	Rounded	Present	Oblate spheroidal	Periporate	Deeply collapsed circular
14.	*Suaeda fruticosa* Forssk. ex J.F.Gmel.	Small to medium	Circular	Absent	Prolate spheroidal	Periporate	Convex-shaped circular

shape, size, and mesoporia as key characters. From this study of the palynology from the family Amaranthaceae for 14 species, nine genera are observed by scanning electron microscopy for observation of qualitative and quantitative traits (Tables 2–4). Chenopodiaceae and Amaranthaceae constitute a stenopalynologic group which contains similar pollen grains that were classified following [32]. A pollen-type definition based on different pollen characters, especially the pore numbers and pore structure, may help in inferring more precise environmental information from Amaranthaceous pollen, one of the most dominant components of pollen assemblages in arid regions. The means and standard deviations of pollen character measurements from LM and SEM are presented in Table 4. A summary of pollen morphological results and important findings for each genus is also provided in the table. The pollen morphological structures are illustrated in Figures 2–4.

3.1.1. Pollen Size. Pollen size was small in nine species, small to medium in three species, and medium in the rest studied in two species. Perveen and Qaiser [33] used pollen size as diagnostic character to differentiate between pollen types. However, Pan [34] reported that it can be influenced by humidity in various habitats.

3.1.2. Pollen Shape. Prolate spheroidal served as the dominant shape in *Achyranthes aspera*, *Aerva javanica*, *Amaranthus graecizans*, *Amaranthus retroflexus*, *Amaranthus viridis*, *Chenopodium album*, *Chenopodium ficifolium*, *Chenopodium murale*, *Haloxylon stocksii*, and *Suaeda fruticosa* while oblate spheroidal in *Aerva lanata*, *Bassia indica*, *Digera muricata*, and *Salsola tragus* (Table 2).

3.1.3. Pore Number. Pore number was 12-16 in *A. aspera*, 25-27 in *A. javanica*, 9-13 in *A. lanata* and *S. tragus*, 22-28 in *A. graecizans*, 16-19 in *A. retroflexus*, 33-35 in *A. viridis*, 20-24 in *B. indica*, 14-17 in *C. album* and *S. fruticosa*, 8-12 in *C. ficifolium*, 23-26 in *C. murale* and *H. stocksii*, and 32-36 in *D. muricata*. El Ghazali [12] mentioned that pore size, pore number, and spinule shape are considered valuable characteristics in Amaranthaceous taxa.

3.1.4. Operculum and Sculpturing Elements. The pollen grains of the Amaranthaceae may be operculate or nonoperculate. The presence/absence of opercula may occur even within the same genus [35]. Opercula were present in nine taxa and absent in the rest five species.

Smooth sparsely granulate as in *A. aspera*, scabrate-spinulose in *A. javanica*, microspinulose perforate in *C. album*, microechinate scabrate to metareticulate in *D.*

Table 3: Qualitative pollen micromorphological features of desert-inhabited Amaranthaceous taxa.

Sr. no.	Amaranthaceous taxa	Mesoporia	Ektexiniuos bodies	Pollen Surface	Exine Sculpture	Tectum
1.	*Achyranthes aspera* L.	Broadly flat	Present	Psilate-scabrate	Smooth, sparsely granulate	Nonpunctate
2.	*Aerva javanica* (Burm.f.) Juss. ex Schult.	Narrow and highly vaulted	Present	Scabrate	Scabrate-spinulose	Nonpunctate
3.	*Aerva lanata* (L.) Juss.	Narrow and highly vaulted	Absent	Scabrate	Pantoaperturate, microechinate perforate	Nonpunctate
4.	*Amaranthus graecizans* L.	Moderately and broadly vaulted	Present	Psilate	Granulate-spinulose, perforate	Nonpunctate
5.	*Amaranthus retroflexus* L.	Moderately and broadly vaulted	Present	Scabrate	Microechinate perforate	Nonpunctate
6.	*Amaranthus viridis* L.	Moderately and broadly vaulted	Present	Scabrate	Echinate, evenly granulate, perforate	Nonpunctate
7.	*Bassia indica* (Wight) A.J.Scot	Narrow and highly vaulted	Absent	Scabrate	Microechinate perforate	Punctate
8.	*Chenopodium album* L.	Broadly flat	Absent	Scabrate	Scabrate-microspinulose perforate	Nonpunctate
9.	*Chenopodium ficifolium* Sm.	Broadly flat	Present	Psilate-scabrate	Microechinate perforate	Nonpunctate
10.	*Chenopodium murale* L.	Broadly flat	Present	Scabrate	Microechinate perforate	Nonpunctate
11.	*Digera muricata* (L.) Mart.	Moderately and broadly vaulted	Absent	Scabrate	Microechinate scabrate, metareticulate	Punctate
12.	*Haloxylon stocksii* (Boiss.) Benth. & Hook. f.	Broadly flat	Present	Scabrate	Densely microechinate perforate	Punctate
13.	*Salsola tragus* L.	Narrow and highly vaulted	Present	Scabrate	Granulate, nanospinules	Nonpunctate
14.	*Suaeda fruticosa* Forssk. ex J.F.Gmel.	Moderately and broadly vaulted	Present	Scabrate	Granulate-spinulose perforate	Nonpunctate

muricata, granulate, nanospinulate in *S. tragus*, granulate-spinulose perforate in *S. fruticosa*, granulate-perforate echinate in *A. graecizans* and *A. viridis* and microechinate perforate in the rest six species (Figures 1–3, Table 3). The term metareticulate sculpture used for *D. muricata* was proposed by [36] to describe pantoporate pollen with a reticulum-like structure of vaulted mesopori and sunken pores with width to the height ratio of mesopori less than one.

3.1.5. Tectate Grains and Ektexinious Bodies. High resolution scanning electron microscopy revealed punctate tectum [37]. Punctate was in *B. indica*, *D. muricata*, and *H. stocksii* and not punctate in the rest 11 taxa.

The pores are covered by ektexinous bodies of different number and shape; they may be closely adjoined to each other or separated from each other. The sculpture of pore membranes was described as having hook-shaped stellately arranged ektexinous bodies, with ektexinous bodies arranged in a mosaic-like pattern [38]. It is absent in four species and present in the rest of the 10 taxa. [36] reported that differences in ektexinious body shapes in Chenopodiaceae are less variable than in Amaranthaceae, but their number and the number of microspines are characteristic for some species.

3.1.6. Aperture Specification and Mesoporia. The pollen in this study can be divided into three groups based on the character of the aperture, i.e., convex-shaped circular group, collapsed-shaped circular group, and deeply collapsed circular group.

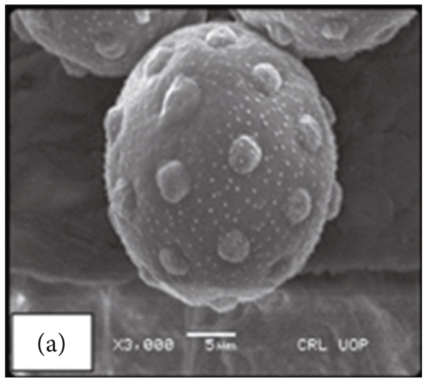
The mesoporia is an area of a pollen grain surface delimited by lines between the apices of adjacent colpi or the margins of adjacent pores also named mesh. The mesoporia refers to the tectate areas surrounding two sunken pores [26]. [39] introduced the concepts of metareticulate pollen, mesoporia, conjunction points, and structural elements to describe the ornamentation of the amaranthoid pollen. The pollen in this study can be divided into three groups based on the mesoporia, i.e., mesoporia broadly flat, mesoporia moderately and broadly vaulted, and mesoporia narrow and highly vaulted.

3.1.7. Quantitative Attributes. Pollen diameter variations in polar view ranged from 12.2 μm in *C. album* to 26.3 μm in *A. retroflexus*, while equatorial diameter measured minimum for *C. album* (11.5 μm) and maximum for *B. indica* (26.5 μm) as illustrated in Figure 5. Polar to equatorial ratio (P/E) index was determined highest for *S. fruticosa* (1.12) and lowest for *D. muricata* (0.94) as shown in Figure 6. The pore diameter ranged from 0.7 μm found in *A.*

Table 4: Pollen morphometric characters measurements among Amaranthaceous pollen.

S. no.	Amaranthaceous taxa	P/E ratio	ET ($x \pm$ SE μm)	PVD ($x \pm$ SE μm)	EVD ($x \pm$ SE μm)	PN	PD ($x \pm$ SE μm)	PDT ($x \pm$ SE μm)	IPD ($x \pm$ SE μm)	Fertility (%)	Sterility (%)
1.	*Achyranthes aspera* L.	1.05	1.04 ± 0.07	15.6 ± 0.13	14.8 ± 0.2	12-16	1.23 ± 0.21	3.1 ± 0.13	3.27 ± 0.62	89.4	10.5
2.	*Aerva javanica* (Burm.f.) Juss. ex Schult.	1.09	0.86 ± 0.34	19.4 ± 0.09	17.7 ± 0.37	25-27	0.85 ± 0.11	2.8 ± 1.19	1.42 ± 0.14	97.6	2.3
3.	*Aerva lanata* (L.) Juss.	0.98	1.25 ± 0.16	16.9 ± 0.23	17.2 ± 0.17	10-13	1.12 ± 0.53	4.45 ± 0.32	2.73 ± 0.33	93.2	6.7
4.	*Amaranthus graecizans* L.	1.07	0.78 ± 0.08	13.5 ± 0.11	12.6 ± 0.21	22-28	1.58 ± 0.24	2.61 ± 0.49	4.15 ± 1.24	95.6	4.3
5.	*Amaranthus retroflexus* L.	1.10	1.14 ± 0.19	26.3 ± 0.47	23.9 ± 0.86	16-19	0.70 ± 0.19	5.37 ± 0.23	3.43 ± 1.04	93.8	6.1
6.	*Amaranthus viridis* L.	1.04	1.30 ± 0.26	14.4 ± 0.18	13.8 ± 0.08	33-35	1.34 ± 0.12	4.76 ± 1.26	2.12 ± 0.46	88.4	11.5
7.	*Bassia indica* (Wight) A.J.Scot	0.96	1.74 ± 0.37	25.1 ± 0.63	26.1 ± 0.36	20-24	0.92 ± 0.36	3.54 ± 0.87	1.85 ± 0.74	98.3	1.6
8.	*Chenopodium album* L.	1.06	1.20 ± 0.11	12.2 ± 0.19	11.5 ± 0.13	14-17	1.35 ± 0.22	2.56 ± 0.61	2.84 ± 0.79	92.5	7.4
9.	*Chenopodium ficifolium* Sm.	1.05	0.97 ± 0.08	17.3 ± 0.39	16.4 ± 0.27	8-12	1.16 ± 0.09	5.13 ± 1.83	4.62 ± 0.93	90.8	9.1
10.	*Chenopodium murale* L.	1.03	1.47 ± 0.64	15.1 ± 0.07	14.6 ± 0.16	24-26	0.80 ± 0.17	4.32 ± 0.41	2.45 ± 0.32	94.2	5.7
11.	*Digera muricata* (L.) Mart.	0.94	1.20 ± 0.28	23.8 ± 0.2	25.2 ± 0.11	32-36	1.10 ± 0.44	3.68 ± 0.29	1.78 ± 0.18	89.3	10.6
12.	*Haloxylon stocksii* (Boiss.) Benth. & Hook. f.	1.06	1.85 ± 0.48	20.4 ± 0.26	19.2 ± 0.33	23-26	1.43 ± 0.32	2.34 ± 0.16	4.94 ± 1.41	92.1	7.8
13.	*Salsola tragus* L.	0.97	2.15 ± 0.96	25.4 ± 1.03	25.9 ± 0.68	9-13	1.64 ± 0.28	4.72 ± 1.06	3.78 ± 0.87	97.5	2.4
14.	*Suaeda fruticosa* Forssk. ex J.F.Gmel.	1.12	1.72 ± 0.36	25.4 ± 0.42	22.5 ± 0.56	14-17	1.31 ± 0.15	3.84 ± 0.93	2.93 ± 0.54	94.7	5.2

Keywords: P=polar; E=equatorial; ET=exine thickness; PVD=polar view diameter; EVD=equatorial view diameter; PN=pore number; PD=pore diameter; PDT=polar density; IPD=interporal distance; x=mean; SE=standard error ; μm=micrometer.



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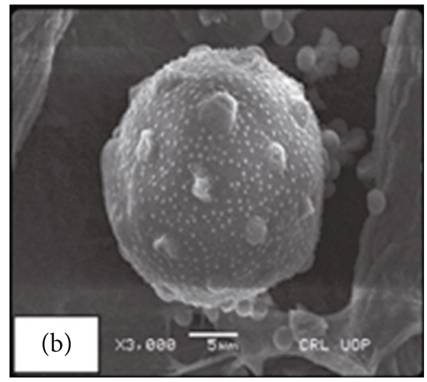

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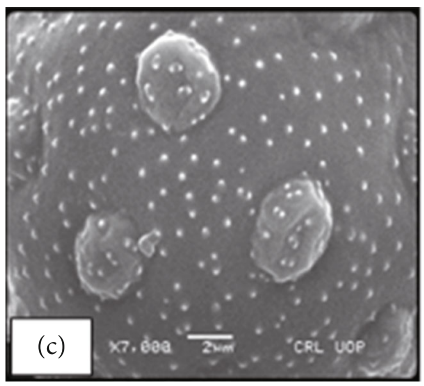

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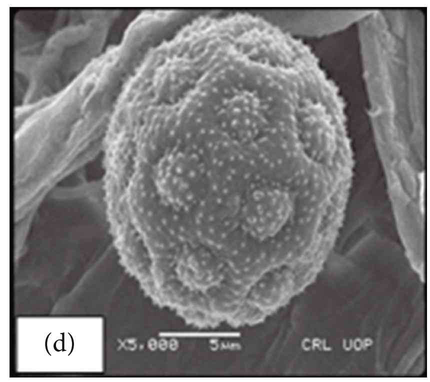

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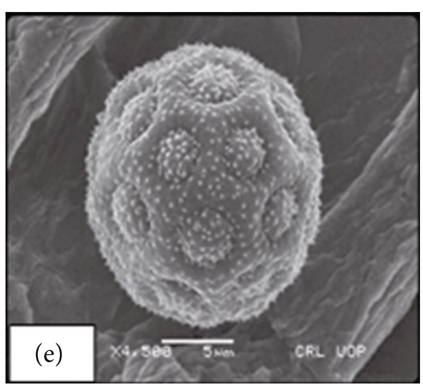

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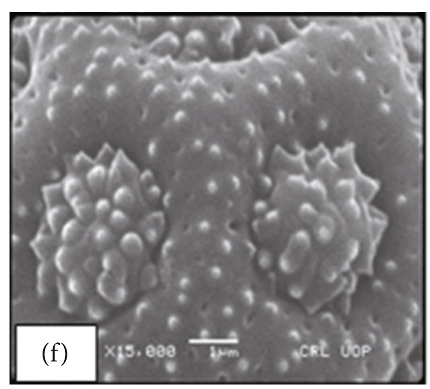

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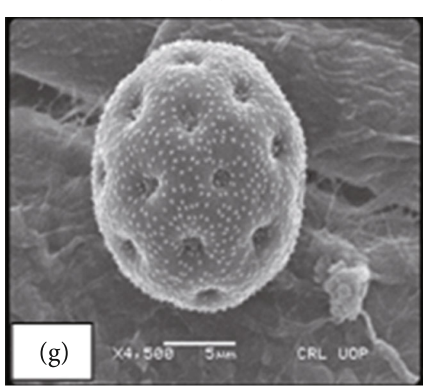

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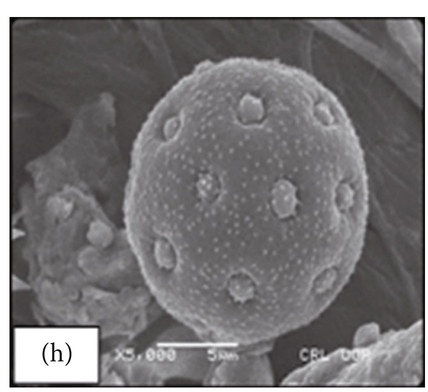

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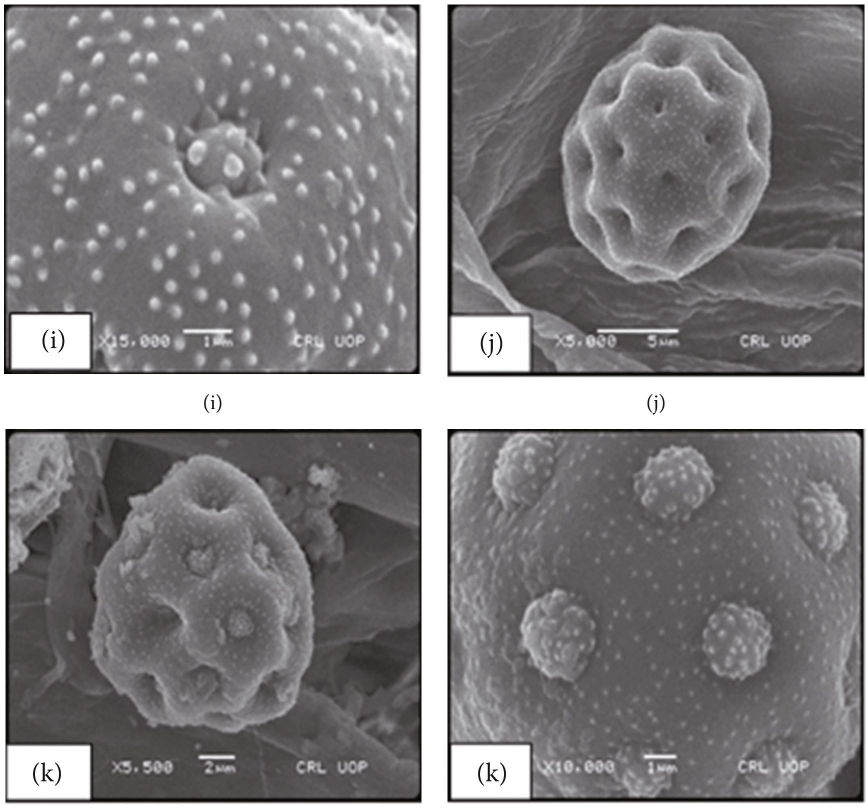


(i) (j)

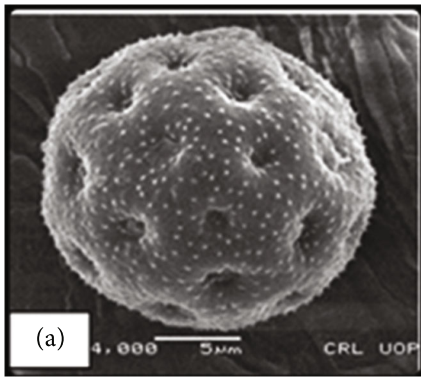
(k) (l)

Figure 2: Scanning electron photomicrographs of pollen grains of Amaranthaceae. (a–c) *Achyranthes aspera*: sparsely granulate nonpunctate (scale bar = 5 μm, 2 μm); (d–f) *Aerva javanica*: scabrate spiniulose and ektexiniuos bodies (scale bar = 5 μm, 1 μm); (g–i) *Aerva lanata*: microechinate perforate, nonpunctate (scale bar = 5 μm, 1 μm); (j–l) *Amaranthus graecizans*: granulate-spinulose exine (scale bar = 5 μm, 2 μm, and 1 μm).

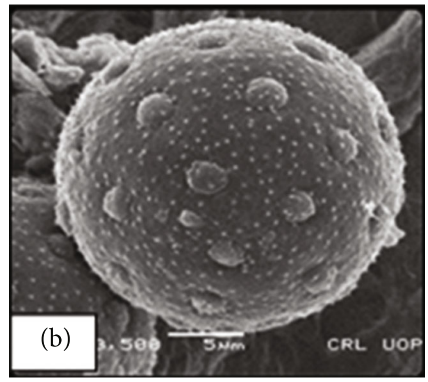
retroflexus to 1.64 μm found in *S. tragus* as mentioned in Figure 7. Pore density was also calculated and found to be maximum for *A. retroflexus* 5.37 μm and minimum for *H. stocksii* 2.34 μm (Figure 8). Interporal distance was calculated largest for *C. ficifolium* (4.94 μm) and shortest for *A. javanica* (1.42 μm) (Figure 9). The exine thickness of the pollen was noted to be minimum (0.78 μm) in *A. graecizans* and maximum (2.15 μm) in *S. tragus* (Figure 10).

3.2. UPGMA Dendrogram and PCA Clustering. The dendrogram distributed the Amaranthaceous species into two major clusters (Figure 11). Cluster I includes species *A. retroflexus*, *S. fruticosa*, *B. indica*, *D. muricata*, and *S. tragus* which are entirely distinct from other species. Cluster II is divided into two further clades: *H. stocksii* and *A. javanica* in clade 1, while clade 2 is further divided into subcluster I with *A. graecizans* and *C. album* while subcluster II with *A. lanata*, *C. ficifolium*, *A. viridis, C. murale*, and *A. aspera*. The highest similarities were observed among *A. viridis* and *C. murale* in subcluster II due to the nonpunctate tectum surface and *B. indica* and *D. muricata* in cluster I with convex-shaped circular group in aperture group and punctate tectum.

Principal component analysis (PCA) is one of the most important statistical tool used for factor calculations among groups to represent the variations. Typically, it is visualized by two-dimensional projections of sample data with principal axis [40]. In the present work, PCA was performed to examine pollen variability among 14 desert-inhabited Amaranthaceous taxa using variables like polar diameter, equatorial distance, P/E index, pore diameter, pore density, exine thickness, and interporal distance. Variable loadings for the seven components have been illustrated in Table 5. A total of 71.23% of the accumulative variance was summarized in our present study. Eigenvalues were found higher than 1 in PC1, PC2, and PC3 and hence are considered as significant in the PCA analysis. Variable loading analysis (Table 5) illustrated that PC1 holds about 39.105% of total data variation and delimited pollen of Amaranthaceous taxa. Interporal distance followed by pore density and pollen diameter was the most relevant variable in PC1. *A. graecizans* and *C. ficifolium* with higher values of pollen diameter and pore density were located on the positive side of the first axis whereas *A. lanata*, *A. viridis*, *C. album*, and *C. murale* on the negative side of the first axis (Figure 12). PCA second axis explained 23.70% variability separating pollen among Amaranthaceae species. The most significant variables in PC2 were pore diameter followed by exine thickness. *A. retroflexus*, *B. indica*, *D. muricata*, *S. fruticosa*, and *S. tragus* were found on the positive side while *A. aspera* was placed on the negative side of the second axis.



(a)



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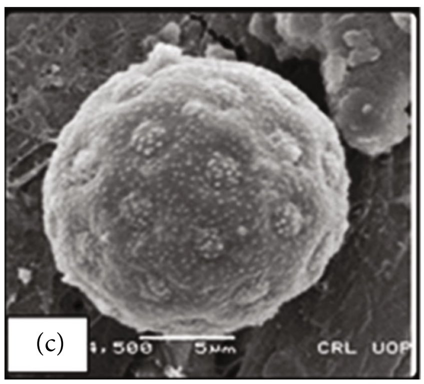

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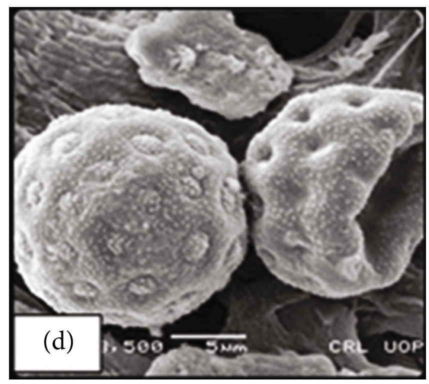

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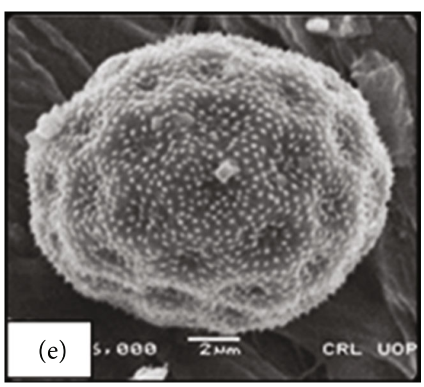

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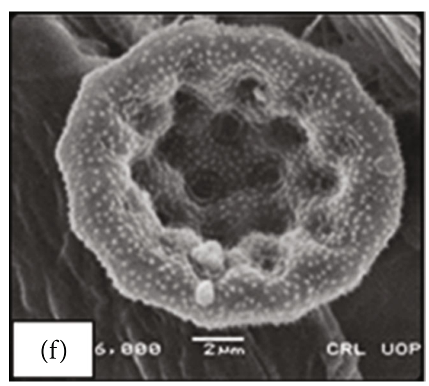

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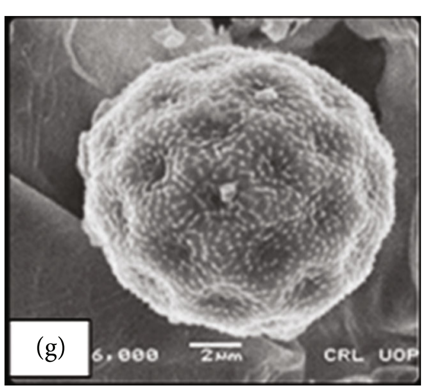

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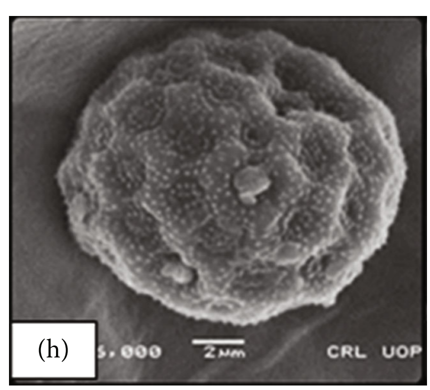
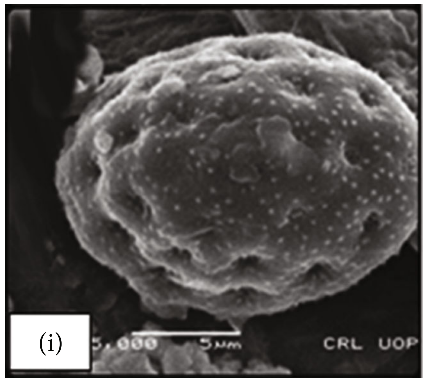
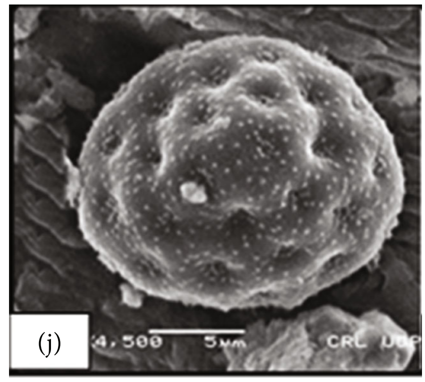


(h)

Figure 3: Continued.



(i)



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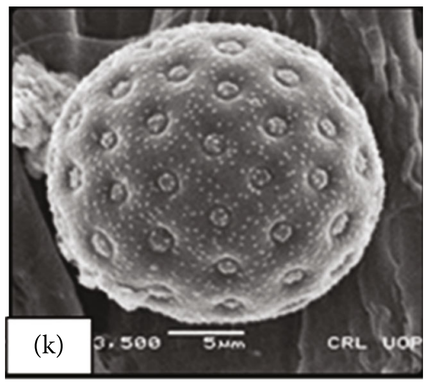

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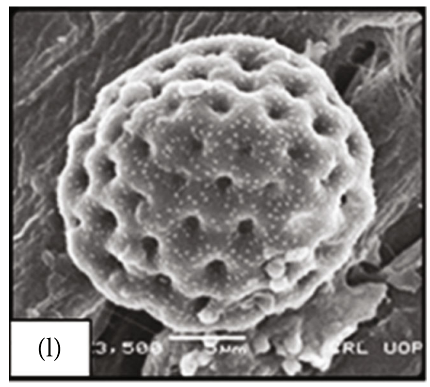


(l)

Figure 3: Scanning electron photomicrographs of pollen grains of Amaranthaceae. (a, b) *Achyranthes retroflexus*: microechinate-perforate exine (scale bar = 5 μm); (c, d) *Amaranthus viridis*: granulate perforate exine (scale bar = 5 μm); (e, f) *Bassia indica*: microechinate perforate, mesoporia narrow (scale bar = 2 μm); (g, h) *Chenopodium album*: scabrate microspinulose exine (scale bar = 2 μm); (i, j) *Chenopodium ficifolium*: nonpunctate, broad flat mesoporia (scale bar = 5 μm); (k, l) *Chenopodium murale*: microechinate-perforate sculpturing (scale bar = 5 μm).

3.3. Taxonomic Key for Amaranthaceous Pollen Identification

(1) +Operculum absent, broadly vaulted mesoporia, nonpunctate…………….…....*A. retroflexus*.

- Narrow vaulted mesoporia, punctate tectum, ektexiniuos bodies absent……….…*B. indica*.

(2) +Broadly vaulted mesoporia, ektexiniuos bodies present, nonpunctate tectum………………………………… ……………..…………………….....*C. ficifolium*.

- Ektexiniuos bodies present, broadly vaulted mesoporia, nonpunctate tectum, microechinate perforate……………… …………………………………………………..*C. murale*.

(3) +Moderately broad vaulted mesoporia, granulate-spinulose perforate, nonpunctate tectum, ektexiniuos bodies present…………………………..…… …………………..…...*S. fruticosa*.

(4) +Operculum present, sparsely granulate, nonpunctate tectum……………..….....*A. aspera*.

- Highly vaulted mesoporia, scabrate-spinulose tectum… ………………………...*A. javanica*.

(5) +Narrow vaulted mesoporia, pantoaperturate, nonpunctate tectum……….......*A. lanata*.

- Ektexiniuos bodies present, granulate-spinulose, perforate tectum……........*A. graecizans*.

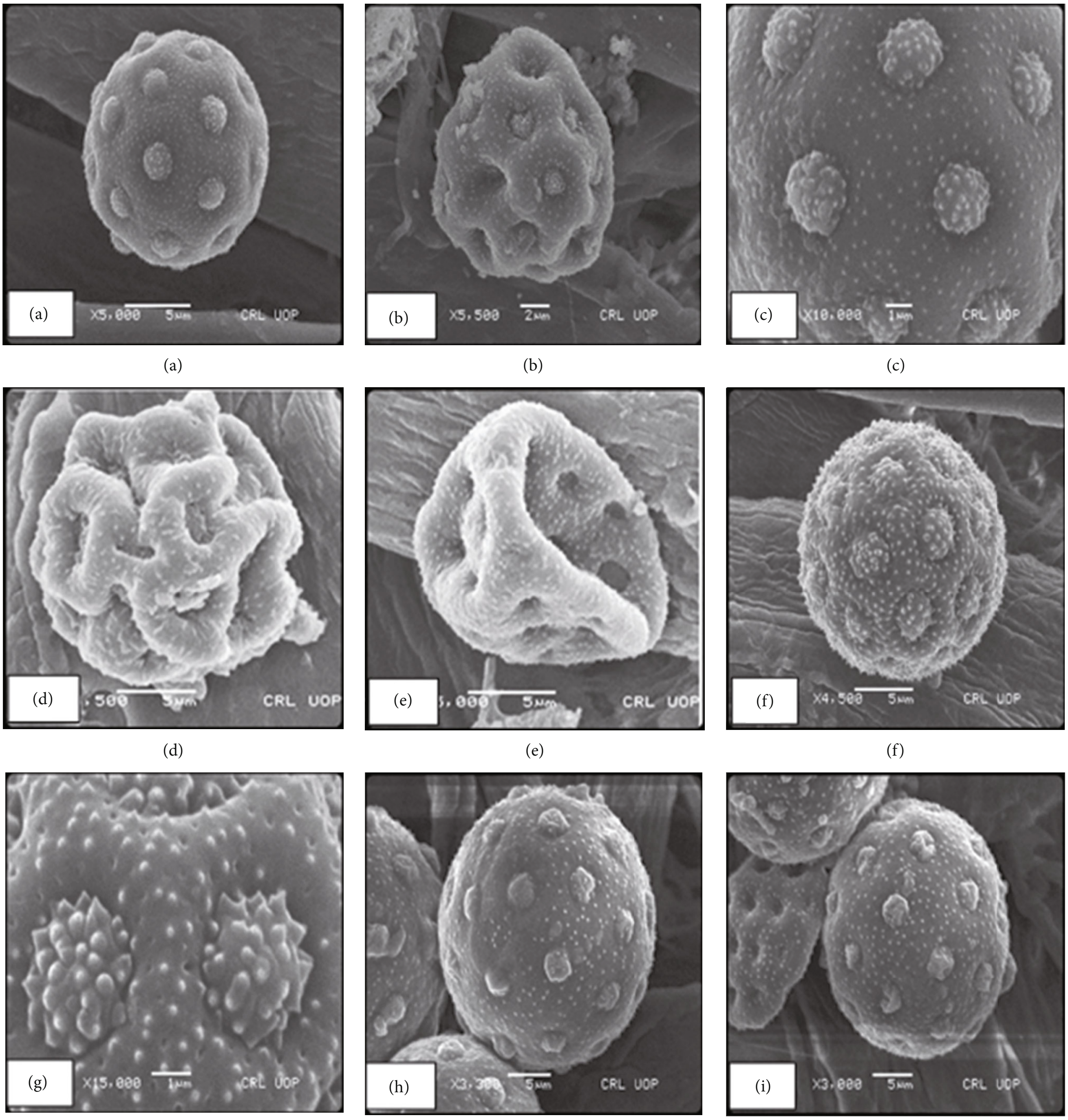
(6) +Moderately and broadly vaulted mesoporia, granulate-perforate, nonpunctate tectum………………… ………………………………….………………… …....*A. viridis*.

- Broadly flat mesoporia, echinate, granulate-perforate, ektexiniuos bodies absent…………………………………… ……………………………………………*C. album*.

(7) +Microechinate scabrate, metareticulate tectum, ektexiniuos bodies absent…………………………… ……………………………………...…..……*D. muricata*.

- Densely microechinate perforate, ektexiniuos bodies present, punctate tectum... ....…*H. stocksii*.

(8) +Granulate, nanospinules tectum, narrow and highly vaulted mesoporia……….*S. tragus*.



(a) (b) (c) (d) (e) (f) (g) (h) (i)

Figure 4: Scanning electron photomicrographs of pollen grains of Amaranthaceae. (a–c) *Digera muricata*: metareticulate exine (scale bar = 5 μm, 2 μm, and 1 μm); (d, e) *Haloxylon stocksii*: dense microechinate exine (scale bar = 5 μm); (f, g) *Salsola tragus*: granulate nanospinules, mesoporia narrow (scale bar = 5 μm, 1 μm); (h, i) *Suaeda fruticosa*: granulate-spinulose perforate exine (scale bar = 5 μm).

4. Discussion

Pollen grains of *Achyranthes aspera* were monad, radial symmetry, spheroidal in shape, small in size with diameter 12.48 ± 1.25 μm, apolar, 32 apertures, aperture diameter 3.2 μm, and polypantoporate aperture, and aperture areas have membrane covering the aperture making it look more convex and circular-like shape covered with sparse granules, mesoporia broadly flat, without ridge, and exine sculpturing smooth with sparsely distributed granulates. The character of this type is aperture areas with a membrane covering the aperture, making it look more convex and circular-like shape covered with sparse granules, mesoporia broadly flat, and without ridge [41]. Periporate; exine l μm, thick, tectate, tectum granulate; pores 47, annulus thick pore diameter 3 μm, circular, interporiun 2 μm, wide; pore membrane

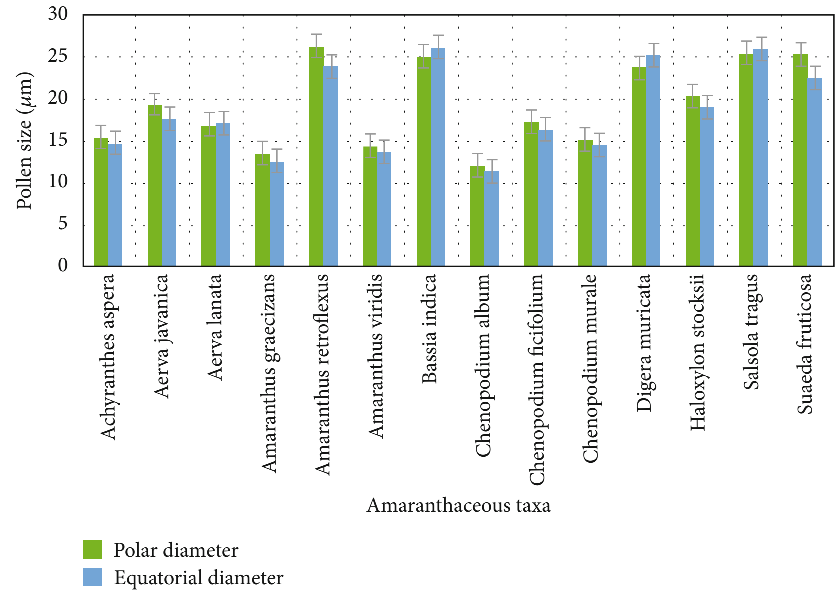


Figure 5: Mean pollen size variations among Amaranthaceous taxa.

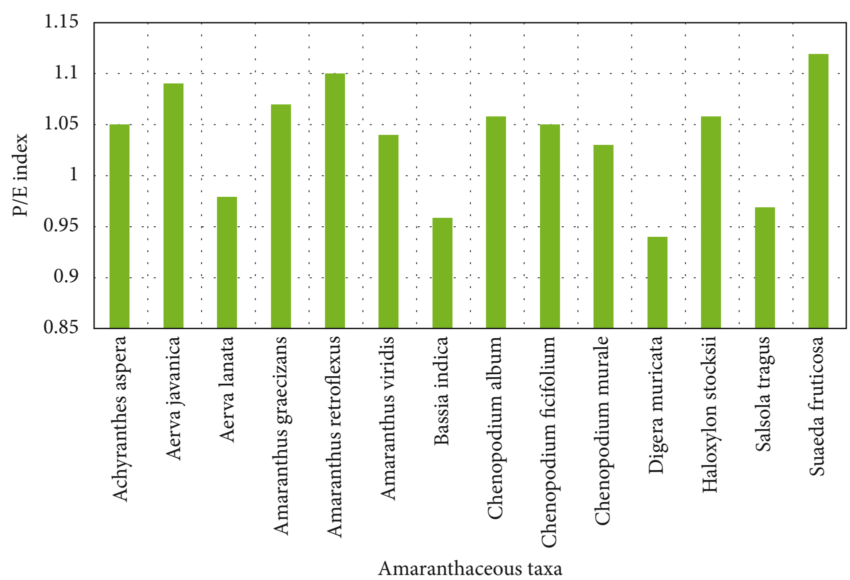


Figure 6: Polar to equatorial distance (P/E ratio) among Amaranthaceous taxa.

granular, circular; grains spheroidal [42]. Prabhakar and Ramakrishna [43] examined scabrate-type sculptural elements for *Achyranthes aspera*. The pollen of Amaranthaceae family was mostly uniform in morphological features which confirm its stenopalynous condition. According to [7], the pollen of *Achyranthes aspera* is small with large number of pores. Hence, our results corroborated with the result description of [7]. Saensouk and Saensouk [41] explained that exine sculpturing smooth with sparsely distributed granulates in *A. aspera* was contradicted with our results which shows microechinate-perforate sculptured elements. The pollen description for *Amaranthus viridis* observed by us highly corroborated with the pollen morphological results of [44] as prolate to spheroidal in shape with radial symmetry, periporate-type grains. Gasma et al. [45] briefly visualized scabrate-type ornamentation showing dissimilarity with the current findings revealed to be microechinate-perforate ornamentation.

Singh and Chaturvedi [46] analyzed rough exine type and scabrate-ornamented grains for *Aerva* species. In spite of the fact that the term metareticulate gained acceptance by many authors and is widely adopted, it is still not followed throughout, and descriptive phrases were used instead. Nowicke and Skvarla [14] described the pollen grains of *Aerva* as with sunken pores and convex mesoporial exines [12]. Bayoumy et al. [47] described punctate-type tectum in *Aerva javanica* as consistent with our findings. The spectrum of allergenic pollen in Karachi including *Aerva*

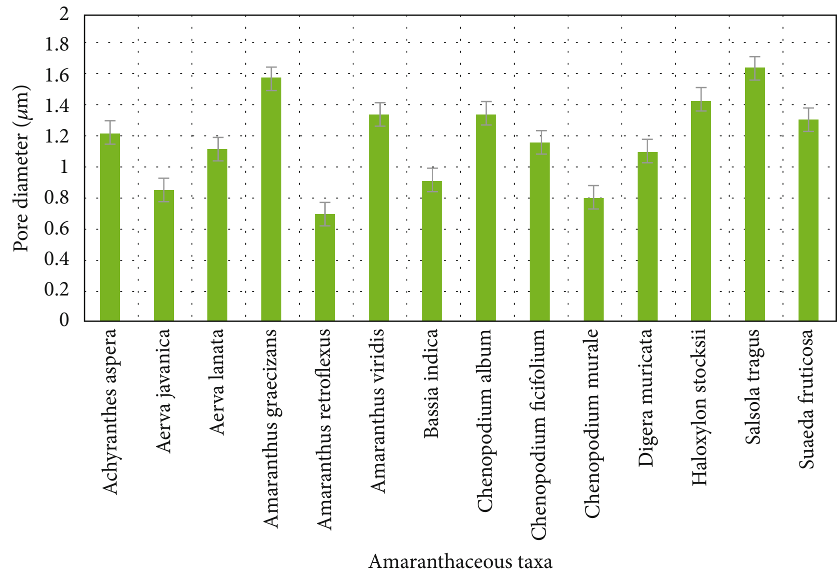


Figure 7: Average pore diameter size variations among Amaranthaceous taxa.

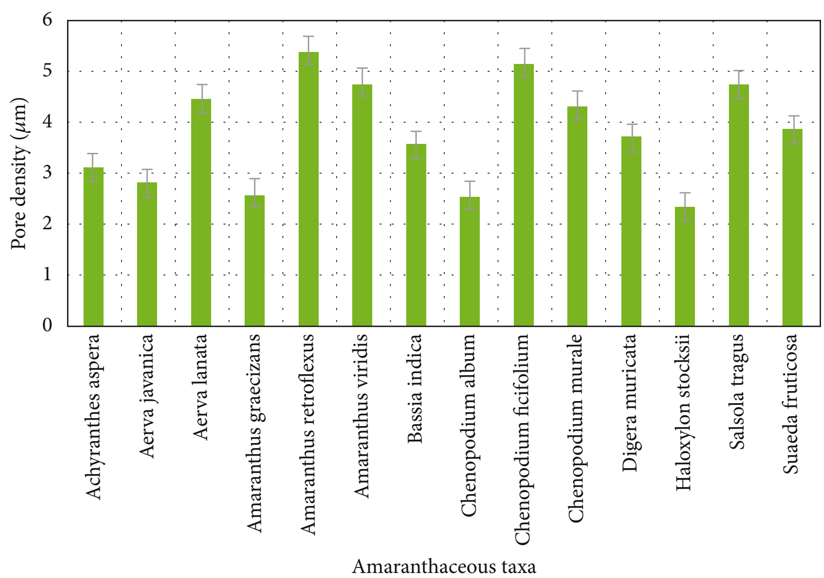


Figure 8: Mean pore density measured among Amaranthaceous taxa.

javanica a major allergen revealed also scabrate ornamentation [48]. Perveen and Qaiser [49] elaborate finely scabrate densely type pollen and exine thicker for *Aerva* species. [50] earlier observed pantoporate, psilate, and spheroidal grains for *Aerva lanata* from "Pollen Atlas of Santiniketan, West Bengal."

Pollen grains of *Amaranthus graecizans* have the thinnest exine, less than 70 pores, and greatest interpore distances [22]. Pollen grain was apolar, spheroidal, and polypantoporate in the genus *Amaranthus*. Grains were small in size, with spinulose tectum in *Amaranthus graecizans* from Southern Marshes (Iraq) of dicot wetland plants [51]. The pores, which are moderate in diameter, are superficial or protruded enriched by granules or echinae in different densities. Exine, thin tectate with granulate or echinate surfaces [52]. Zhigila et al. [53] provided evidence that *Amaranthus* species are multipalynous and that the genus is anemophilous. The pollen grains are generally small, rounded, smooth, thin-walled, and dry but vary among species within this genus.

The currently existing morphological characteristics of pollen give grounds to classify it to *Amaranthus* type with pore type II typical of that type of pollen [36]. Our data largely confirm the already published ones. The pollen in *Amaranthus retroflexus* populations has typical spherical shape covered by numerous perforations. It is characterized by small size and a big number of pores [54]. Terzieva and Grozeva [55] studied the pollen of seven species, including *Amaranthus retroflexus*. The characteristics published by them about the pollen of *Amaranthus retroflexus* were similar to those found by

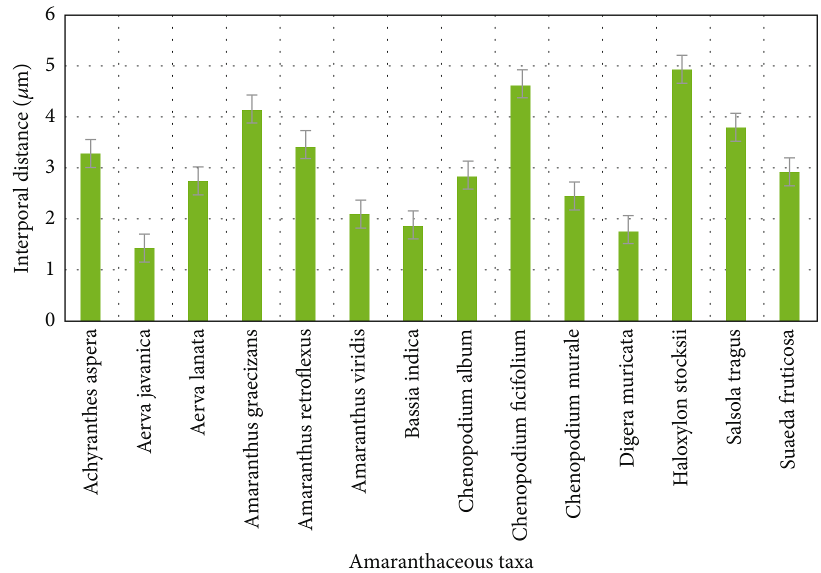


Figure 9: Mean interporal distance variations among Amaranthaceous taxa.

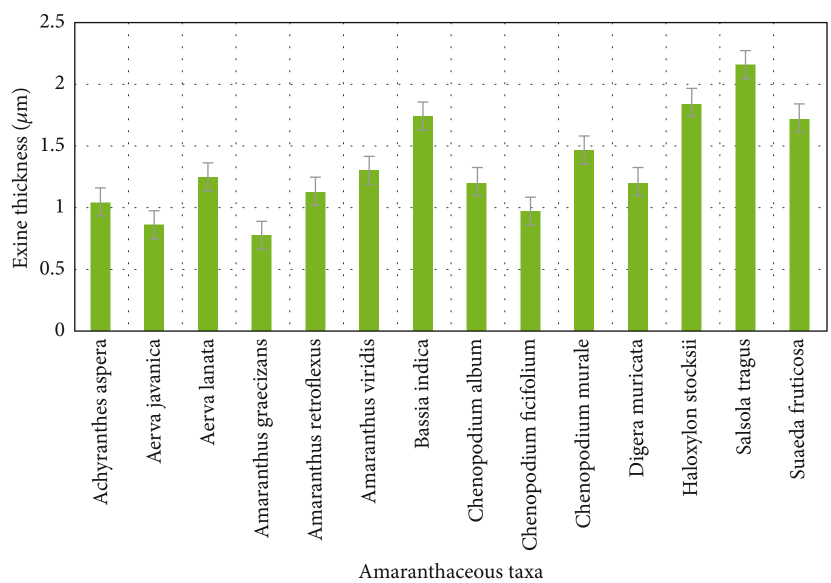


Figure 10: Mean exine thickness variations among Amaranthaceous taxa.

us. Borsch and Barthlott [36], stepping on profound studies of pollen in Amaranthaceae, distinguished 11 different types of pores and 17 pollen types. The pore characteristics established in the present study of *Amaranthus retroflexus* correlate to type II (pores are 1-2 μm in diameter and sharply set off against the tectum) and the pollen type described by the author as *Amaranthus* type (spheroidal, punctate tectum with numerous distributed microspines).

Ghosh and Mandal [50] described periporate, spheroidal, and psilate surface nature grains in *Amaranthus viridis*. While our findings contradict examined echinate, evenly granulate, perforate sculptural elements. Pollen grains of plant taxa *Amaranthus viridis* are pantoporate (stenopalynous plant), and they cannot be differentiated at species level, except for *Alternanthera*, which has pollen of distinctive character [56]. Previously, [45] also mentioned scabrate sculpture grains with spheroidal shape from diverse ecozones of Nigeria. Pollen morphotypes have been elaborated previously by [33, 57] in which they reported that exine sculpturing of the Amaranthaceae family was scabrate to psilate, while in our study *Amaranthus viridis* have microechinate ultrasculpturing.

Pollen shape was subprolate and densely scabrate ornamentation as in *Bassia eriophora* while sparsely scabrate peculiarities were analyzed for *Bassia arabica* from Egypt [47]. Study of pollen grains by SEM indicated the presence of differences represented in density and distribution of spinules on the pollen surface, number of apertures, pollen exine punctate or smooth, and number of mesoporial spinules [58]. The absence of ektexinous elements on the pores

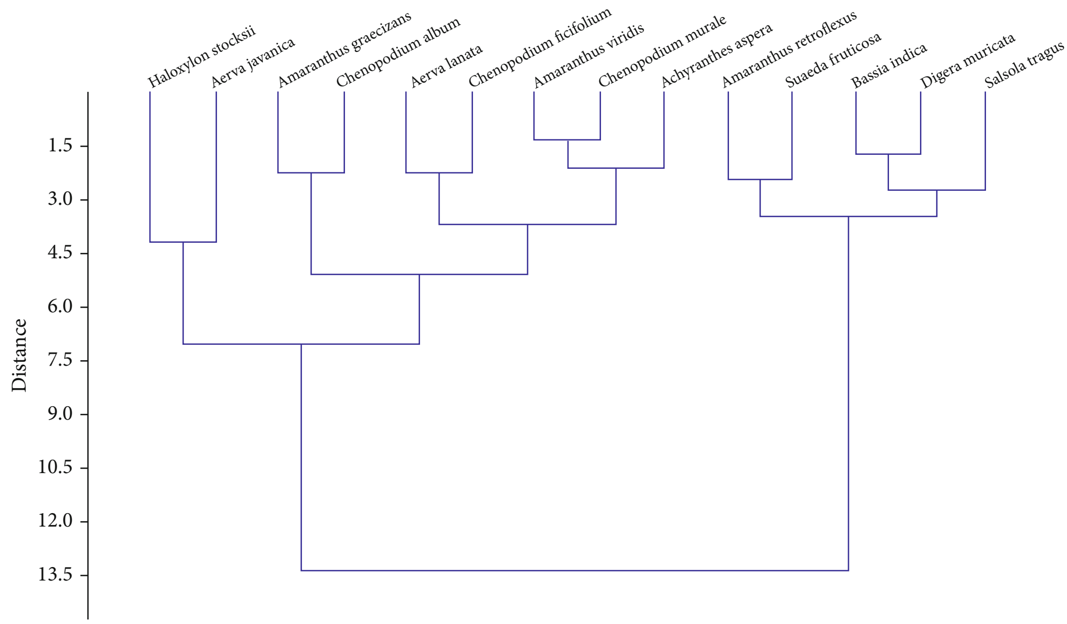


Figure 11: Dendrogram clustering showing relationship among Amaranthaceous taxa.

Table 5: Cumulative variance and eigenvectors of principal component analysis (PCA) using quantitative palynological characters.

PC	Eigen Values	% variance
PC1	2.73	39.105
PC2	1.65	23.707
PC3	1.08	15.444
PC4	0.84	12.018
PC5	0.40	5.727
PC6	0.27	3.994
PC7	0.0002	0.004

Keyword: PC=principal component.

(nonoperculate) was encountered in *Bassia* genus [12], while our work also show similar observation.

Micrographs of pollen grains in *Chenopodium album* revealed perforate tectum at the proximal face in *Chenopodium album* subsp. *striatum* and perforate ornamentation [59]. *C. album* pollen was seen to be apolar, radially symmetrical, polipantoporate, with circular pores, while scanning electron microscope defined numerous uniformly distributed spinules seen on the exine, both on the surface and also on the operculum. Numerous foreign particles attached to the surface of the grains were also observed [60]. *Chenopodium album* pollen is the smallest with a small size of 12.2 μm, whereas [61] measured the medium size of pollen of *Chenopodium album* in the Magnolia to be 30.9 μm. Nazish et al. [9] studied halophytic *Chenopodium ficifolium* to have pollen grains with pantoporate aperture, spheroidal shape, apolar, and microechinate scabrate sculptured elements. Perveen and Qaiser [33] studied the apolar-type pollen of Chenopodiaceae, while current findings revealed broadly flat mesoporia and microechinate-perforate peculiarities. The figure shows no correlation between pore number and pollen size of the species examined. Therefore, previous study indicating that there are two hole types on pollen surface in Chenopodiaceae, foveat and perforate, confirms this point of view that the *Chenopodium ficifolium* has tendency to have larger pollen grains.

Grains observed peripolporate, radial symmetrical, faveat tectum at the proximal face with scabrate ornamentation of exine in *Chenopodium murale* [59] whereas our examination revealed that microechinate-perforate exine structure was dissimilar to earlier studies. In another study [62], winter weeds honeybee flora performed optical microscopy of *Chenopodium murale* grains as polyporate, suboblate, and psilate. However, this research analyzed via SEM nonpunctate scabrate-type exine. *Digera muricata* pollen grains were monads, white, spheroidal, pantoporate, multiporate, dry, and fall as single grains [63]. Reticulate, circular, lobate, and thin granular exine grains were illustrated for *Digera muricata* [64]. Shinwari [42] described variations of tectum as metareticulate were consistent with our current findings. In the *Haloxylon* type, the pollen grains have pore membranes with dense microechini and larger pore diameter. The new pollen classification of the Chenopodiaceae opens up the possibility of linking this pollen type to the desert vegetation types such as a temperate dwarf semiarboreal desert [65]. Chenopodiaceae pollen inventory from Pakistan provided by [33] mentioned *Haloxylon stocksii* as densely scabrate-type grain elements, contradicting the present examination of punctate and densely microechinate-perforate pollen. Perveen et al. [48] also reported the scabrate surface grains in *Haloxylon stocksii* similar to the current observation. Jalilzadeh et al. [66] explained that the micromorphology of *Haloxylon ammodendron* had a high density of microechinate in exine ultrasculpture and was somehow in accordance to present study.

The pollen morphology in genus *Salsola* was discussed only briefly in the previous publication of [67]. General

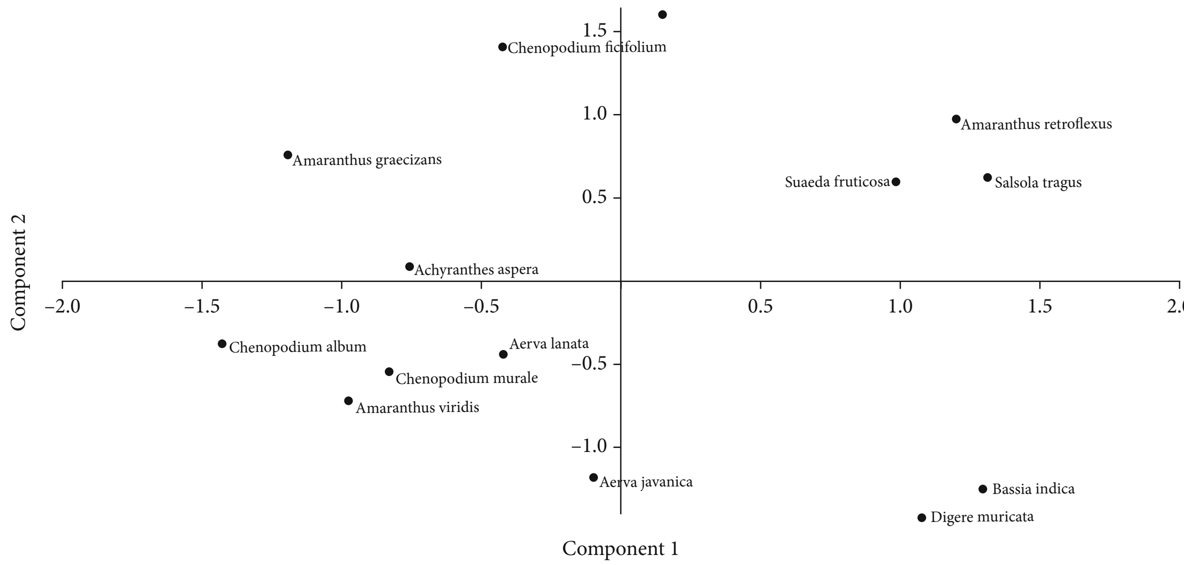


Figure 12: Principal component analysis (PCA) performed with the pollen quantitative data of Amaranthaceous species.

description of pollen grain of *Salsola tragus* is given in the classical work of [68]. The palynological data provided by [69] showed that the five studied populations of *Salsola tragus* had pantoporate, spheroidal pollen with spinulose tectum. Our data showed that *Salsola tragus* had greater diameter and polar axis and bigger pore area and greater distance between them. Pollen grains of 12 species of the genus *Salsola* were mentioned above, as pantoporate, the pore number vary within the limits, small, very rarely of medium size, angular-rounded in outline and the surface of the pollen grain is wavy [70].

Nazish et al. [9] examined microechinate exine surface of *Suaeda fruticosa* while microscopic palynomorph characters observed in this study illustrate granulate spinulose perforate stratification of exine with nonpunctate tectum. Our ongoing study among different genera of Amaranthaceae including *Suaeda* showed intergeneric and even interspecific differences in pollen traits. The smooth surface of grains and density of spinules were similar in *Suaeda* species to be included in one separate section [71]. A study of 28 taxa of the genus *Suaeda* from the old world by Dehghani and Akhani [72] showed apparently little difference in pollen characters between the studied species; however, a statistical study of the pollen characters was not undertaken. The pollen morphology of the new species was described as *Suaeda iranshahrii* from Southern Iran revealing periporate type and similar pollen diameter to other known species of the genus [73].

5. Conclusion

This is the foremost study that deals with the pollen micromorphology of selected Amaranthaceous taxa growing in the Thal Desert. Pollen ultrastructure of Amaranthaceous species provides significant taxonomic information about the microstructural traits. Morphological features considered in this research were a very advantageous tool at the taxonomic level to accurately verify the species. The identified pollen types were periporate and mostly of small size, and aperture group of convex- and collapsed-shaped was observed. Mesoporia of broadly flat, moderately broad, and narrow orientation was examined. Pore density was measured maximum for *Amaranthus retroflexus* 5.37 μm and minimum for *Haloxylon stocksii* 2.34 μm. Interporal distance was noted highest for *Chenopodium ficifolium* 4.94 μm and lowest for *Aerva javanica* 1.42 μm. Taxonomic key was constructed, and statistical analysis revealed taxonomic relationship. Furthermore, it is concluded that scanning electron microscopic character identification provides key diagnostic description of desert Amaranthaceous species that proved to be significant in classification.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no potential conflict of interest regarding the publication of this research work.

Authors' Contributions

S.M. and M.A. were responsible for the conceptualization; S.S and R.A were responsible for the methodology; Y.G. and S.Z. were responsible for the software; T.M., A.Y., and K.K. were responsible for the validation; O.K. was responsible for the investigation; A.D. was responsible for the resources; D.N.C. was responsible for the data curation; S.M. was responsible for the writing and preparation of the original draft; M.Z., M. A., and S.M. were responsible for the writing (review and editing); M.F.R. was responsible for the visualization; M.A. and M.Z were responsible for the supervision; M.Z. was responsible for the project administration; and A.T.A. was responsible for the funding acquisition.

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