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EXPLORATION OF SALINITY EFFECT ON PALYNO-MORPHOLOGICAL CHARACTERISTICS OF PLANT SPECIES COLLECTED FROM SALT RANGE

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Keywords: Exine sculpture; Pollen size; P/E ratio; Pollen viability.

Abstract

This study aims to explore the pollen morphology of 19 plant species from the Khewra Salt Range by using light microscopy (LM) and scanning electron microscopy (SEM). Plant specimens were collected from various sites in the Khewra Salt Range, identified, and deposited in the Herbarium of Pakistan. Pollen morphology was studied using LM and SEM. Quantitative characteristics, including polar and equatorial diameter, exine thickness, colpus and spines length and width were studied using LM. The studied pollen taxa exhibited extensive variation in size and sculpture, with potential taxonomic implications. Pollen shape of most of the studied species was sub-spheroidal but pollen sizes ranged from 0.98 µm to 14.3µm. Symmetry and polarity of pollen grains were observed, contributing to plant species identification. The P/E ratio varied from *Dichanthium annulatum* (0.56) to *Medicago polymorpha* (1.15). Scanning electron micrographs revealed diverse exine sculpturing patterns, such as scabrate and echinate. At the same time, *Nerium oleander* (0.7-2.1µm) showed high exine thickness and large size colpus with a length of 7.5µm and width of 8.26µm. The largest spines were observed in *Vicia sativa* (3.7µm long and 1.38 µm wide). The taxonomic key based on palyno-morphological characters offers a tool for quick and precise identification. Pollen viability values were determined. Highest pollen viability was noted for *Capparis decidua* (97%) that show the species adaptation in Salt range. Palynological features can aid in resolving taxonomic problems and contribute to further karyological and taxonomic evaluations. This study contributes valuable insights into the palynological diversity of plant species of salt range, emphasizing the importance of pollen morphology in understanding and conserving salt-affected ecosystems.

Introduction

Palyno-micromorphological is a useful tool in the phylogenetic and taxonomic classification of plant families (Khan *et al*., 2018; Nazish *et al*., 2019). The comprehensive analysis of pollen grains provides parameters to predict the evolution of problematic and related taxa and to conduct the systematic study of taxa (Ullah *et al.*, 2018). Palynology is associated with plant taxonomy to improve the taxonomic position of the taxa. The study of pollen characters is a preliminary step in accurately identifying and classifying complicated taxa. Palynological studies have proven helpful in solving problems related to plant taxonomy and Paleobotany (Hayat *et al*., 2023).

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Light (LM) and Scanning electron microscopy (SEM) techniques are significant for the characterization and identification of pollen taxa at species and genus level (Sarwar, 2011). Light microscopy is of great interest for taxonomists to investigate several taxonomic features (Khan *et al*., 2017). SEM provides ultrastructural details regarding qualitative variations (Belhadj *et al*., 2007). Palynologists explained the taxonomic importance of pollen taxa using SEM by showing variations in exine sculpturing (Andersen and Bertelsen, 1972; Faegri *et al*., 1989). The microscopic study is beneficial for differentiation among different types of pollens; therefore, it becomes a vital research technique (Arora and Modi, 2008). These microscopic techniques are invaluable regarding detailed observation of the surface morphology of different plant parts (Nazish *et al*., 2019).

Taxonomists utilize SEM and LM to study several taxonomic characters with great interest (Ullah *et al*., 2018). The family Amaranthaceae is considered as rich family in Pakistan in terms of rich diversity in pollen features possess by its members. Different scientists have studied palyno-morphological characteristics of the family Amaranthaceae utilizing LM and SEM worldwide (Talebi *et al*., 2016; Hussain *et al*., 2018; Nazish *et al*., 2019). The biometry and pollen morphology of 17 Amaranthaceae taxa have been examined by Angelini *et al*. (2014) They reported perforations and echinae on radially symmetrical and spheroidal pollens. Nazish and Althobaiti (2022) studied pollen morphology of Poaceae species from Salt Range using LM and SEM and reported annulus and monoporate pollen apertures in most of the species. The grass distribution in any region depends on the soil chemical and physical nature. Grasses act as soil stabilizers and make the soil fertile and productive (Ahmad *et al*., 2009; Khan *et al*., 2017).

Plants indicate the ecological conditions of any geographic region and are indicators of various soil features (Sarir *et al*., 1984). Poaceae taxa have adaptations to adapt to Salt Range habitat that reduces the inimical effects of salinity. These adaptations include root lignification, presence of hairs and glands on leaf surface to secrete salt, epidermal succulence and reduce leaf area (Monteverdi *et al*., 2008; Farooq *et al*., 2015). Landi *et al*. (2022) contributed new data in the taxonomy and palynology of the Boraginaceae family using microscopic techniques. They declared it as a eurypalynous family and depicted similarities in the exine ornamentation and aperture of the Heliotropiaceae species. Semerdjieva and Yankova-Tsvetkova (2017) conducted a study on pollen morphology of Zygophyllaceae species and reported spheroidal, oblate and colporate pollens. Pollen characters have proven invaluable in solving inter-relationship problems among taxa and their status assessment in classification (Ahmad *et al*., 2011).

Soil salinization is measured as a major key factor in land degradation (Milić *et al*., 2013). Salinity affects more than 7% of the total land area of the world and one-third of irrigated land as it is one of the common constraints in irrigated agriculture (Flowers and Muscolo, 2015). The saline soil mostly occurs under arid and semi-arid regions as 1 billion ha among the total 6.5 billion ha of arid and semi-arid regions is salt affected (Wiebe *et al*., 2005; Redden *et al*., 2015). Large regions in different countries are facing the issue of salinization including Mexico, Pakistan, Egypt, India, and South America (Khan and Qaiser, 2006). Pakistan ranks eighth in the case of saline-affected regions (Corbishley and Pearce, 2007). In Pakistan, 6 million ha of soil is salt-affected and 2.7 million ha of this salt-affected soil lies in Punjab (Alam, 2015).

The Khewra salt mine is the largest one among the 5 salt mines of Pakistan in the Salt Range of Pakistan. It is Pakistan's oldest and largest salt mine. The study area has a distinctive topography with a rich plant species diversity. In Pakistan, sparse vegetation is present in salt ranges needs to be explored and identified. In solving taxonomic problems, the investigation of the morphology of pollen grains is gaining vigorous extensive attention as it possesses distinctive taxonomic importance (Tellería and Daners, 2003; Inyama *et al*., 2015). In plant taxonomy, pollen morphology has proved to be a vital tool in plant identification found in different climates by providing valuable confirmation of the additional features of closely related plant species (Aftab and Perveen, 2006; Arora and Modi, 2008). There is no documented information on the pollen morphology of taxa in the Khewra salt mines of Pakistan. Pollen examination of selected plant species was conducted from Khewra and allied areas for the first time. LM and SEM were used to observe the several morphological characteristics of pollen grains such as pollen dimensions, size, type, polarity, symmetry, exine sculpturing, colpi, pore and spines detail study, and pollen viability. The pollen morphological characters investigation showed interesting findings. To identify different Salt range taxa quickly and precisely, a taxonomic key was prepared based on palyno-morphological characters. The pollen fertility was also estimated for the selected pollen taxa. The objectives of the current study are to describe the detailed pollen morphology of Salt Range taxa based on scanning and light microscopy to distinguish their taxonomic levels that will improve the paleoenvironmental analyses in future studies.

Materials and methods

Collection and identification of Plant species

Nineteen plant species belonging to 12 families were collected from different sites in different flowering seasons from the Khewra Salt Range from August 2021 to February 2022 for the current study (Fig. 1). In Pakistan, the Khewra salt mine is a subdivision of the Jhelum district in the province of Punjab. It lies 102 km away from the Jhelum. It is located at 32° 38' N and 73° 00' E. The underground part of the salt mine spread over an area of 110 km^2. It is the Pakistan's first and the world's second-largest salt mine. It lies in the mountains of Salt range of Punjab. Five specimens of single plant species were collected from each locality randomly (Table 1). The collected plant specimens were identified using Flora of Pakistan.

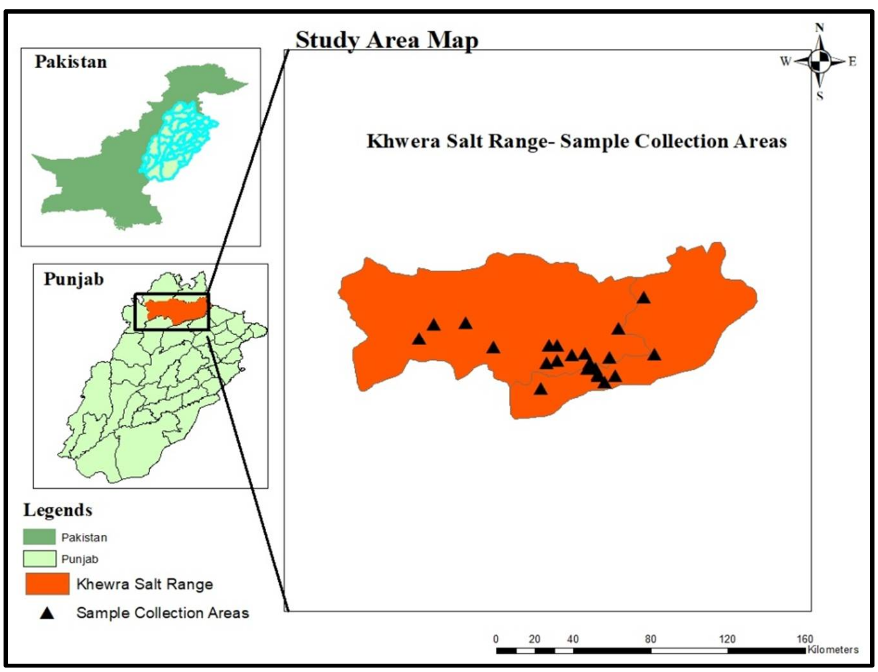

Fig. 1. Map of the study area.

The voucher numbers were allotted to specimens. To obtain the taxonomic validation, the botanical names of collected plant species were confirmed with the aid of the International Plant Name Index (IPNI) (www.ipni.org). Collected plant samples were meticulously processed, including shade-drying, pressing, cataloging, and identification. Herbarium entries were carefully labeled and archived for future reference. Comprehensive records, from botanical names to voucher specimen numbers, were diligently maintained, encompassing growth characteristics and medicinal properties, and kept in the Botanical Garden for the Public via deposition number QAU-IBD-BOT-5693400244. The collection of plant material abides by the relevant international, national, and institutional guidelines and legislation.

Table 1. Taxon sampling and their herbarium deposition.

Taxon	Accession number	Collectors	Locality	Altitudes (m)
Capparis decidua (Forssk.) Edgew.	ISL.129969	Moona Nazish	Khewra	181.01
Calotropis procera (Aiton) W.T.Aiton	ISL.129965	Moona Nazish and Asif Kamal	Kattas	836.33
Carthamus oxyacantha M.Bieb.	ISL.129957	Moona Nazish	Dandot	678.26
Chenopodium album L.	ISL.129974	Moona Nazish	Wara Buland Khan	208.00
Cymbopogon jwarancusa (Jones ex Roxb.) Schult.	ISL.129949	Moona Nazish	Tobar	648.27
Dichanthium annulatum (Forssk.) Stapf	ISL.129919	Moona Nazish and Asif Kamal	Kaslian	207.41
Dysphania ambrosioides (L.) Mosyakin & Clemants	ISL.129952	Moona Nazish	Khewra salt mines	181.03
Dysphania botrys (L.) Mosyakin & Clemants	ISL.129920	Moona Nazish	Sodian gujar	223.00
Echinochloa crus-galli (L.) P.Beauv.	ISL.129923	Moona Nazish	Khewra	181.17
Heliotropium europaeum L.	ISL.129932	Moona Nazish and Asif Kamal	Bhelowal	186.00
Lepidium didymum L.		Moona Nazish	Lilla	228.41
Medicago polymorpha L.	ISL.129942	Moona Nazish	Tobar	648.31
Nerium oleander L.	ISL.129924	Moona Nazish	Kattas	836.37
Peganum harmala L.	ISL.129929	Moona Nazish	Khewra Salt mines	181.09
Phyla nodiflora (L.) Greene	ISL.129977	Moona Nazish	Kaslian	207.38
Solanum surattense Burm. f.	ISL.129936	Moona Nazish	Pind Dadan Khan	206.00
Tribulus terrestris L.	ISL.129961	Moona Nazish and Asif Kamal	Kussak	334.00
Trichodesma indicum (L.) Sm.	ISL.129960	Moona Nazish	Pidh	254.66
Vicia sativa L.	ISL.129938	Moona Nazish and Asif Kamal	Lilla	228.01

Light microscopy (LM)

The flowers were collected carefully during collection in the field for the study of pollen morphology under light microscopy. In the laboratory, polleniferous material was catalyzed using the following procedure (Erdtman, 1960). The dissecting needles were used to remove the autolyzed anthers from filaments of stamen and then placed on a glass slide and crushed to release pollen grains. Then, pollen grains were mounted in glycerin jelly using the Wodehouse method (Ronald, 2000). The fertility of pollen grains was determined using the technique (Khan and Stace, 1999). The percentage of stained grains was calculated after staining using a mixture of 1% acetocarmine and neutral glycerin in equal amounts. Based on the readings of at least 20 grains, several morphological characters were measured (Fig. 2).

Fig. 2. Pictorial presentation of studied Salt Range plant species. (a) *Peganum harmala* (b) *Capparis decidua* (c) *Calotropis procera* (d) *Trichodesma indicum* (e) *Cymbopogon jwarancusa* (f) *Nerium oleander* (g) *Lepidium didymium* (h) *Tribulus terrestris* (i) *Carthamus oxyacantha* (j) *Medicago polymorpha* (k) *Vicia sativa* (l) *Solanum surattense*.

Detailed quantitative and qualitative pollen parameters were studied using a light microscope (Model: MX5300H, Meiji Techno, Japan). Various parameters were measured using 40× magnification. Pollen micrographs were taken at different resolutions using Leica Dialux Light microscope (Model 1000, Mannheim, Germany). The digital camera fitted on a light microscope was used to take micro-graphs of these mounted pollen grains. The oil emulsion is used during photography with different object lenses.

Scanning electron microscopy (SEM)

The mature flowers were used to separate the anthers (Ali *et al.*, 2021). The anthers were transferred into Eppendorf tubes after crushing on a glass slide with a few drops of acetic acid. A micropipette was used to take pollen samples from Eppendorf tubes and place them on metallic stubs. Gold palladium (2.3 nm) was used to coat the pollen grains and then examined under SEM (Model JEOL JSM-5910, Peabody, USA).

Statistical analysis

The pollen data was analyzed quantitatively with the help of software SPSS (16.0). The standard error and mean were calculated for all the quantitative characters. The P/E ratio was calculated for each species following the formula of Nazish *et al*. (2019) for the determination of pollen shape.

$$P/E = \frac{P \times 100}{E} \qquad (1)$$

Whereas P and E are the polar and equatorial diameters of the same pollen.

The pollen viability was determined using the technique of Nazish and Althobaiti (2022).

$$\text{Pollen viability} = \frac{F \times 100}{F + S} \qquad (2)$$

Whereas F and S are the number of fertile and sterile pollen grains.

The measured quantitative pollen morphological traits were polar and equatorial diameter, exine thickness, colpus, and spine length and width.

Results and Discussion

The pollens of Salt Range taxa growing in Khewra and surrounding areas were examined. The palynological characteristics of 19 plant species belonging to 12 families (Capparaceae, Apocynaceae, Asteraceae, Amaranthaceae, Poaceae, Boraginaceae, Brassicaceae, Fabaceae, Nitrariaceae, Verbenaceae, Solanaceae, Zygophyllaceae) were investigated quantitatively as well as qualitatively (Tables 2 and 3). The studied pollen taxa showed an extensive distinction in sculpture and size that exhibited potential taxonomic significance (Figs. 3-4). The largest grains were *Nerium oleander* 11.8 µm and *Heliotropium europaeum* 10.2 µm. The minimum size for pollen grain was found for *Dichanthium annulatum* 0.98 µm (Fig. 5). The equatorial diameter varied from 0.84 µm in *Medicago polymorpha* to 14.3 µm in *Nerium oleander*. The remaining taxa have intermediate-size pollens. The polar and equatorial relationship (P/E ratio) was also examined for all the plant species (Figs 6-7). *Dichanthium annulatum* is characterized by a low

P/E ratio (0.56), while *Medicago polymorpha* has high P/E ratio (1.15). The highest exine thickness was found in *Nerium oleander* (0.7-2.1 µm) while *Peganum harmala* is characterized by a thin exine (0.3-0.8 µm). In *Chenopodium album, Cymbopogon jwarancusa, Dichanthium annulatum, Dysphania ambrosioides, Dysphania botrys, Heliotropium europaeum, Nerium oleander,* and *Trichodesma indicum* the exine sculpturing is scabrate and Psilate in *Calotropis procera, Echinochloa crus-galli, Heliotropium europaeum, Peganum harmala, Solanum surattense,* and *Phyla nodiflora.* The perforate pollen types were examined in *Heliotropium europaeum* and *Carthamus oxyacantha* with echinate exine sculpturing.

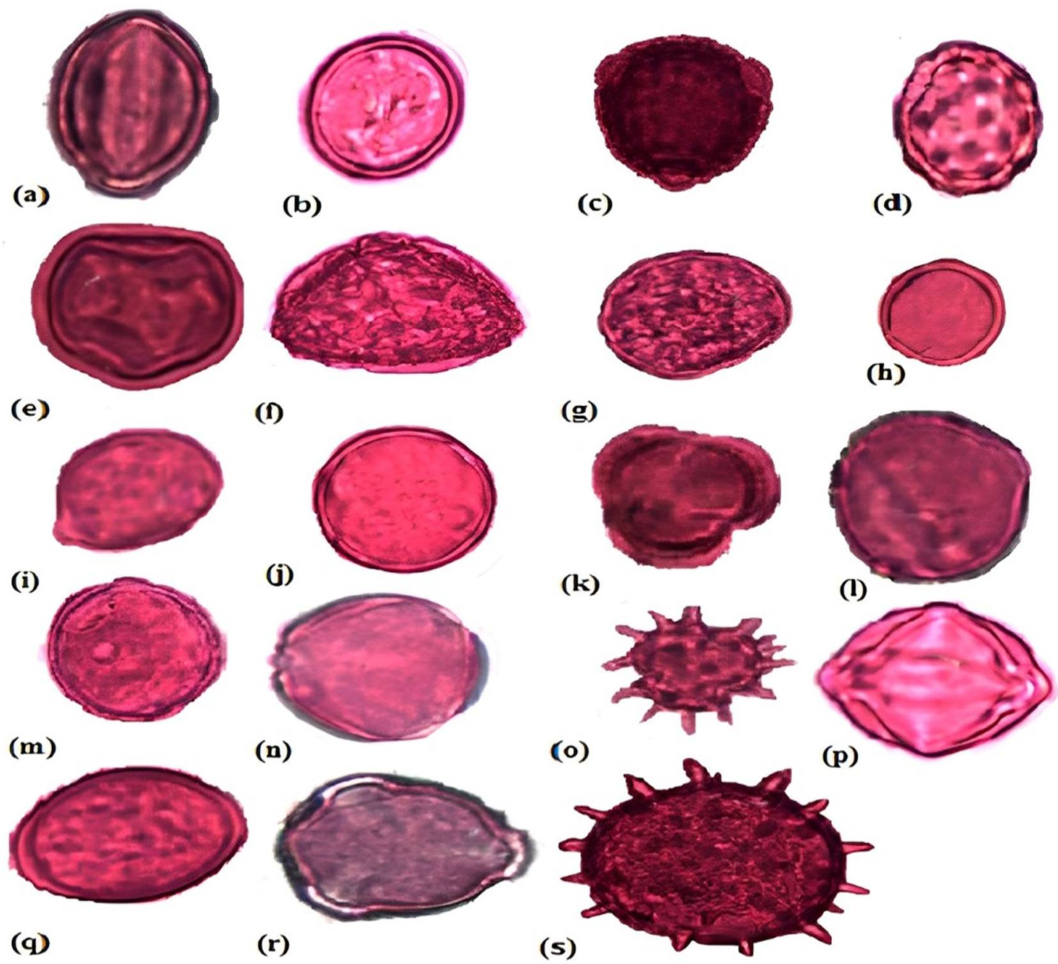

Fig. 3. Light microscope photomicrographs of pollens of studied taxa. PV = Polar view, EV = Equatorial view. (a) *Capparis decidua* (EV) (b) *Calotropis procera* (PV) (c) *Carthamus oxyacantha (*PV) (d) *Chenopodium album* (PV) (e) *Cymbopogon jwarancusa* (PV) (f) *Dichanthium annulatum* (PV) (g) *Dysphania ambrosioides* (PV) (h) *Dysphania botrys* (PV) (i) *Echinochloa crus-galli* (PV) (j) *Heliotropium europaeum* (PV) (k) *Lepidium didymium* (PV) (l) *Medicago polymorpha* (PV) (m) *Nerium oleander* (PV) (n) *Peganum harmala* (PV) (o) *Phyla nodiflora* (PV) (p) *Solanum surattense* (PV) (q) *Tribulus terrestris* (EV) (r) *Trichodesma indicum* (PV) (s) *Vicia sativa* (PV).

The symmetry and polarity of some pollen taxa were also observed. The pollen grains of *Chenopodium album, Dysphania ambrosioides, Dysphania botrys,* and *Heliotropium europaeum* are isopolar while *Vicia sativa* has heteropolar grains. The apolar pollen grain was observed in *Echinochloa crus-galli*. The spines are small in *Tribulus terrestris* (Fig. 4). The spine length varies from 0.9-1.2 µm in *Tribulus terrestris* to 3.3-4 in *Vicia sativa.* The colpi is larger in *Nerium oleander* (6.5-8.1 µm) while the colpi is smaller in *Solanum surattense* (1.1-2.1 µm). The variation in spine morphology is shown in Table 2.

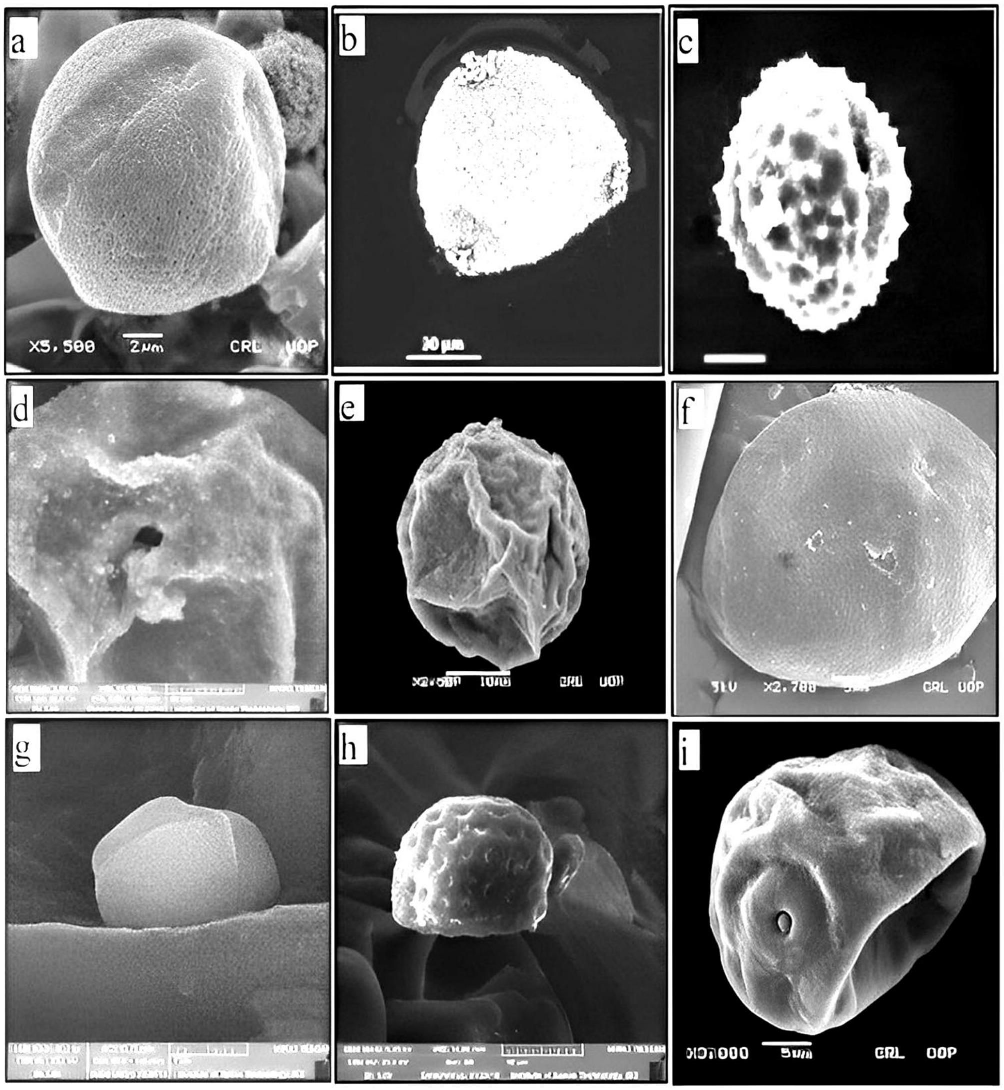

Fig. 4. SEM pollen micrographs of plant species. a) *Capparis decidua* b) *Calotropis procera* c) *Carthamus oxyacantha* d) *Chenopodium album* e) *Cymbopogon jwarancusa* f) *Dichanthium annulatum* g) *Dysphania ambrosioides* h) *Dysphania botrys* i) *Echinochloa crus-galli*.

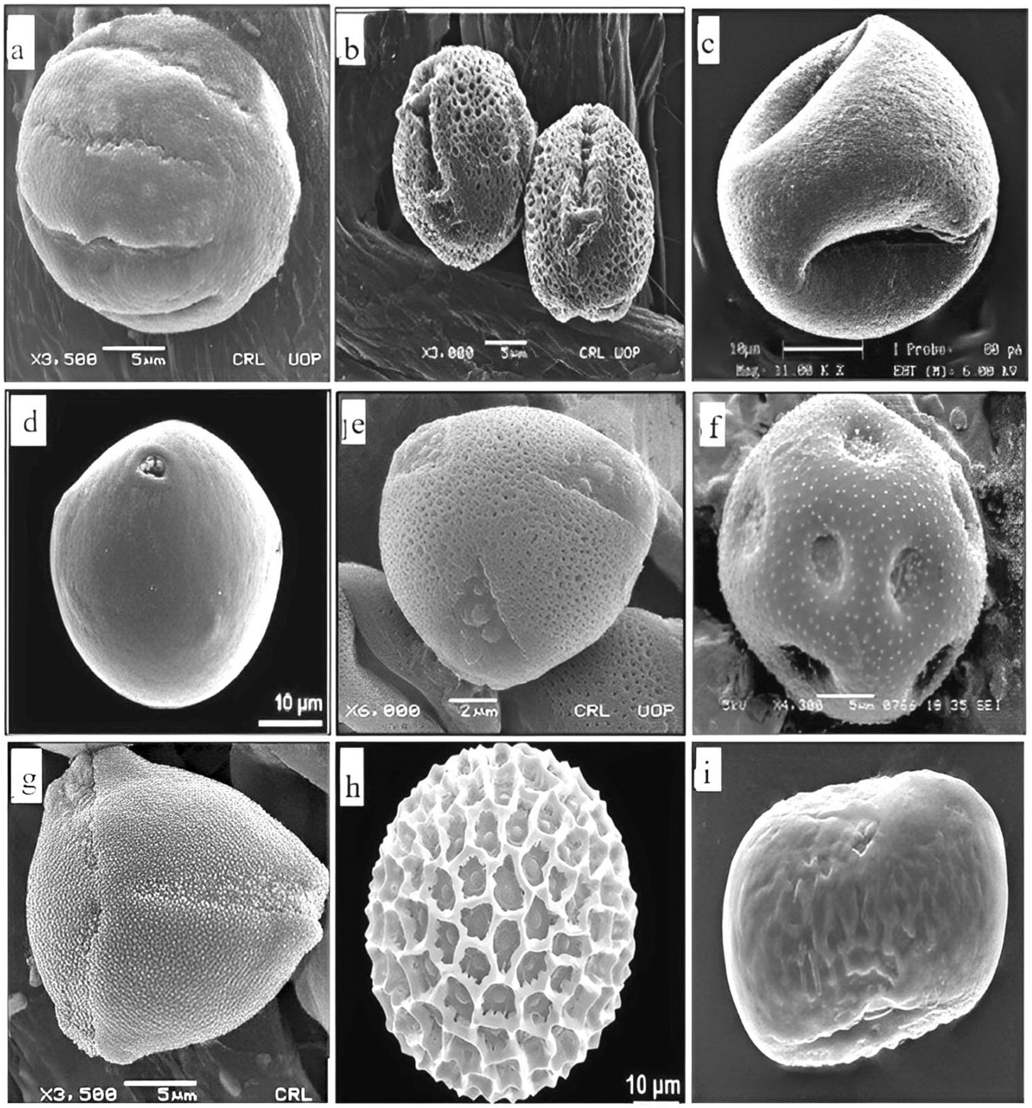

Fig. 5. SEM pollen micrographs of plant species. a) *Heliotropium europaeum* b) *Lepidium didymium* c) *Medicago polymorpha* d) *Nerium oleander* e) *Peganum harmala* f) *Phyla nodiflora* g) *Solanum surattense* h) *Tribulus terrestris* i) *Vicia sativa.*

Pollen viability provides information about pollen fertility and incompatibility. It supports the plant's distribution in their habitat. In this study, the pollen viability data confirm the stability of studied taxa in saline soil. The highest pollen viability was found for *Solanum surattense* (98%) *and Capparis decidua* (97%). The lowest pollen viability was found for *Carthamus oxyacantha* (76%) (Tables 3-4).

Table 2. Quantitative pollen characters of plant species from Khewra Salt Range.

Species name	Dimensions (µm)		P/E ratio	No. of Colpi	Colpi		No. of spines	Spines		Exine thickness (µm)
	Polar Diameter (µm) Min (Mean ± Std Error) Max	Equatorial diameter (µm) Min (Mean ± Std Error) Max			Length (µm) Min (Mean ± Std Error) Max	Width (µm) Min (Mean ± Std Error) Max		Length (µm) Min (Mean ± Std Error) Max	Width (µm) Min (Mean ± Std Error) Max	
Capparis decidua	6(7.66±0.71)10	6(7.46±0.69)10.10	1.02	3	3(4.76±0.58)6	5(6.62±0.72)9	-	-	-	0.6-0.9
Calotropis procera	5(6.68±0.41)7.4	5.1(6.22±0.49)7.4	1.07	3	1.8(2.02±0.86)2.3	1.3(1.52±0.86)1.8	-	-	-	0.4-1.1
Carthamus oxyacantha	6.1(9.32±1.08)11.2	6(9.94±1.94)16.9	0.93	3	2.1(2.42±0.08)2.6	2.7(2.82±.037)2.9	32-34	1.1(1.20±0.44)1.3	0.3(0.38±0.37)0.5	0.8-1.2
Chenopodium album	6(8.44±1.10)11.20	6.50(9.12±0.92)12	0.92	-	-	-	-	-	-	0.2-1.0
Cymbopogon jwarancusa	5.9(8.1±1.06)11.9	6(9.26±1.42)14	0.87	-	-	-	-	-	-	0.6-1.3
Dichanthium annulatum	0.6(0.98±0.1)1.2	0.8(1.72±0.27)2.5	0.56	3	2.6(2.84±0.81)3.1	2.9(3.02±0.05)3.2	-	-	-	0.7-1.3
Dysphania ambrosioides	6(7.06±0.35)8.2	5.5(6.8±0.43)8	1.03	-	-	-	-	-	-	0.6-1.5
Dysphania botrys	5.6(6.9±0.46)8	8.9(10.9±1.03)14.9	0.63	-	-	-	-	-	-	0.6-1.4
Echinochloa crus-galli	5(7.42±1.71)14	4.9(7.4±1.45)13	1.00	-	-	-	-	-	-	0.5-1.1
Heliotropium europaeum	7.2(10.2±0.77)11.2	6.4(12.4±1.98)17	0.82	3	1.4(1.52±0.03)1.6	1.5(1.74±0.06)1.9	-	-	-	0.6-1.1
Lepidium didymum	6.9(7.86±0.25)8.4	6.1(7.38±0.50)8.4	1.06	3	1.3(1.48±0.05)1.6	1.3(1.38±0.03)1.5	-	-	-	0.7-1.1
Medicago polymorpha	6.9(9.44±0.71)11.1	0.5(0.84±0.08)1.0	1.15	1	2(2.08±0.03)2.2	1.8(1.94±0.05)2.1	-	-	-	0.5-1.0
Nerium oleander	10(11.8±0.72)14	4.5(14.3±2.85)21	0.82	3	6.5(7.5±0.29)8.1	7.2(8.26±0.33)9	-	-	-	0.7-2.1
Peganum harmala	6.9(7.7±0.23)8.2	6.2(7.9±0.47)8.9	0.97	3	1.3(1.94±0.18)2.4	1.1(1.48±0.14)1.8	-	-	-	0.3-0.8
Phyla nodiflora	5.9(7.7±0.54)8.9	6.5(9.0±0.97)12.5	0.84	-	-	-	19-24	1.2(1.54±0.12)1.9	0.4(0.7±0.13)1.1	0.7-1.2
Solanum surat tense	8.9(9.26±0.19)10	9.1(9.9±0.31)10.9	0.93	4	1.1(1.46±0.18)2.1	1.8(2.3±0.20)3	-	-	-	0.4-1.6
Tribulus terrestris	4(5.44±0.64)7.3	5(6.0±0.43)7.1	0.90	-	-	-	33-36	0.9(1.06±0.05)1.2	0.3(0.44±0.05)0.6	0.5-1.5
Trichodesma indicum	5.1(5.72±0.23)6.2	4(5.0±0.41)6	1.14	3	3.9(4.7±0.2)5	2.1(2.74±0.31)3.8	-	-	-	0.6-0.9
Vicia sativa	1.6(5.14±1.23)9	4.3(6.88±1.0)9.3	0.74	-	-	-	23-29	3.3(3.7±0.13)4	1.1(1.38±0.18)2.1	0.3-1.1

Table 3. Qualitative pollen characters of plant species from Khewra Salt Range.

Plant Species name	Pollen size	Pollen type	Pollen Shapes	Exine sculpturing	Colpi/ Pore	Spines	Pollen description
Capparis decidua	Medium	Bicolporate, Tricolporate	Subspheroidal	Reticulate, sparsely granulated, Striate	Present	Absent	Broad colpi and colpus membrane sparsely granulated, finely reticulate lumina tectum
Calotropis procera	Small	Trizonocolporate	Subspheroidal	Psilate	Present	Absent	Long colpi, circular small pores
Carthamus oxyacantha	Large	Echinate, Tricolporate	Subspheroidal	Echinate perforate	Present	Present	Echinate with small perforations between spines, densely packed tectum
Chenopodium album	Medium	Polipantoporate	Subspheroidal	Scabrate	Present	Absent	Isopolar, circular pores, scabrate surface with distributed spinules on tectum
Cymbopogon jwarancusa	Large	Monoporate	Subspheroidal	Aerolate, Scabrate	Present	Absent	Thicker sexine than nexine, aerolate sculpturing
Dichanthium annulatum	Large	Tricolporate, Monoporate	Oblate	Scabrate, Areolate	Present	Absent	Sexine thicker than nexine, reticulate tectum
Dysphania ambrosioides	Medium	Perioporate	Subspheroidal	Granulate, Scabrate	Present	Absent	Isopolar, exine thicker than intine, scabrate surface with spinules, circular pores, irregular nexine
Dysphania botrys	Medium	Polipantoporate	Oblate	Scabrate	Present	Absent	Isopolar, circular pores, scabrate surface with conical spinules on tectum
Echinochloa crus-galli	Small	Monoporate	Subspheroidal	Psilate	Present	Absent	Apolar, finely reticuloid to granular surface, circular to ovoidal pore
Heliotropium europaeum	Large	Tricolporate	Subspheroidal	Psilate, Perforate, Scabrate	Present	Absent	Isopolar, broad colpi with three long and narrow alternating pseudocolpi, colpus membrane slightly perforated
Lepidium didymum	Medium	Tricolporate	Subspheroidal	Reticulate	Present	Absent	Long and broad colpi with thicker sexine than nexine, colpus membrane obscurely polygonal reticulate
Medicago polymorpha	Large	Monocolpate	Subspheroidal	Foveolate	Present	Absent	Long and broad colpi and colpus membrane finely granulate
Nerium oleander	Large	Tetraporate, Triporate	Subspheroidal	Scabrate, Granulate, Psilate	Present	Absent	Circular pore bordered by an annulus and thick exine with indistinct stratification and coarsely scabrate
Peganum harmala	Medium	Tricolporate	Subspheroidal	Reticulate, Rugulate, Psilate	Present	Absent	Long and broad colpi and colpus membrane coarsely reticulate
Phyla nodiflora	Medium	Tricolporate	Subspheroidal	Psilate, Reticulate	Present	Present	Long narrow colpi with thicker sexine than nexine and sub-psilate tectum
Solanum surattense	Medium	Tricolporate, Tetracolporate	Subspheroidal	Psilate	Present	Absent	Broad and short colpi at base and narrow at tip
Tribulus terrestris	Small	Echinate, Pantoporate	Subspheroidal	Coarsely reticulate	Present	Present	Sculpturing echinate with slightly expressed muri, disposed pores, compact outer nexine with lamellar inner nexine
Trichodesma indicum	Medium	Tricolporate	Subspheroidal	Scabrate	Present	Absent	Long and broad colpi and sexine is thicker than exine
Vicia sativa	Large	Tricolporate	Oblate	Reticulate, Heterobrochate	Present	Present	Heteropolar with bilateral symmetry, broader spines at the base and tip is pointed.

Table 4. Pollen viability study of Salt Range taxa.

Botanical name	No. of viable pollen	No. of non-viable pollen	Viability (%)
Capparis decidua	67	2	97
Calotropis procera	75	8	90
Carthamus oxyacantha	53	16	76
Chenopodium album	87	11	88
Cymbopogon jwarancusa	102	13	88
Dichanthium annulatum	86	4	95
Dysphania ambrosioides	98	26	79
Dysphania botrys	109	5	95
Echinochloa crus-galli	116	5	96
Heliotropium europaeum	96	18	84
Lepidium didymum	108	6	94
Medicago polymorpha	95	12	88
Nerium oleander	82	6	93
Peganum harmala	135	16	89
Phyla nodiflora	73	14	84
Solanum surattense	91	2	98
Tribulus terrestris	110	9	92
Trichodesma indicum	66	3	95
Vicia sativa	79	5	94

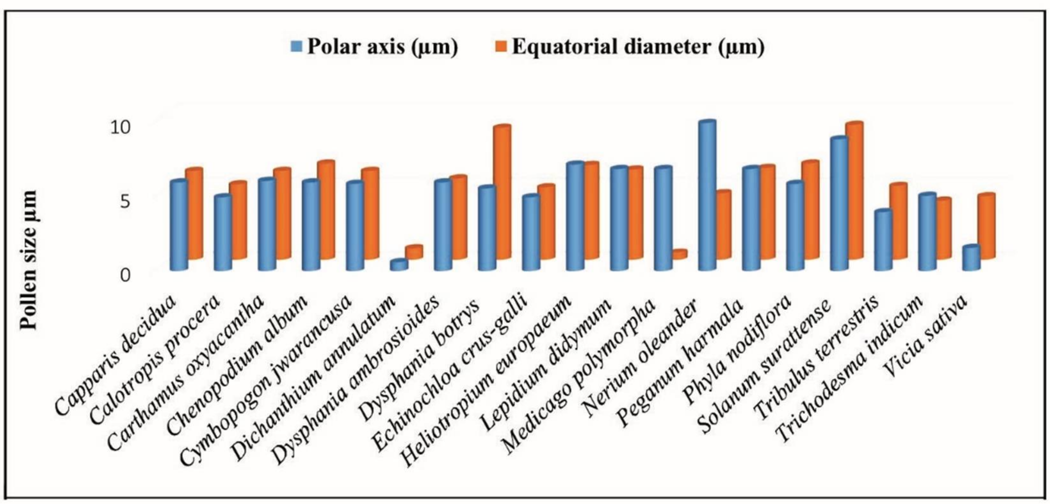

Fig. 6. Polar and Equatorial diameter of pollens of Salt Range taxa.

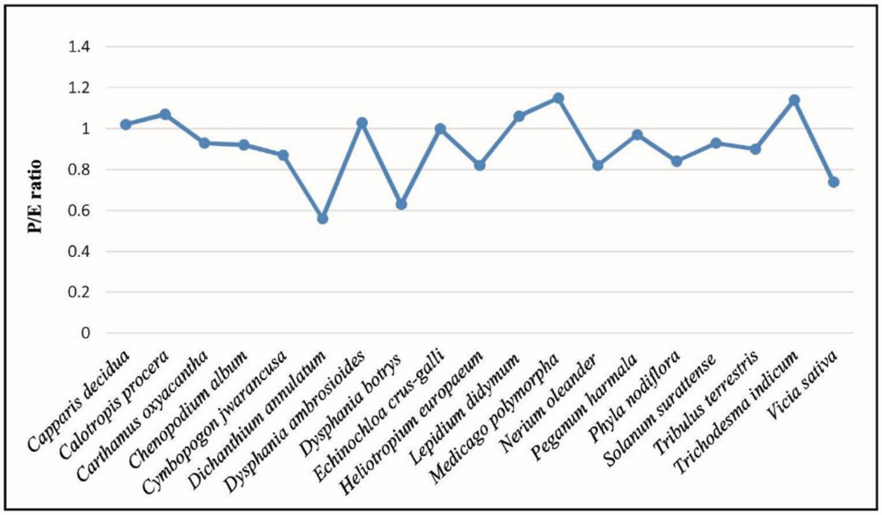

Fig. 7. P/E ratio of plants pollens present in Khewra Salt Range.

Taxonomic key based on pollen morphological characters

1. Medium size, Prolate, tricolporate with broad colpi, colpus membrane sparsely granulated *Capparis decidua*
2. Small size, prolate, long colpi, circular small pores *Calotropis procera*
3. Large size, oblate-spheroidal, tricolporate pollen grain with long and broad colpi, small perforations between spines *Carthamus oxyacantha*
4. Medium size, spheroidal, polipantoporate pollen grain, surface scabrate with spinules *Chenopodium album*
5. Large size, prolate, monoporate, aerolate exine sculpturing *Cymbopogon jwarancusa*
6. Large size, spheroidal, tricolporate, and monoporate pollen grain, Surface reticulate *Dichanthium annulatum*
7. Medium size, spheroidal, perioporate pollen grain, circular pores, surface scabrate with spinules *Dysphania ambrosioides*
8. Medium size, spheroidal, polipantoporate pollen grain, circular pores, surface scabrate with conical spinules *Dysphania botrys*
9. Small size, prolate and spheroidal, monoporate pollen grain, surface reticuloid to granular *Echinochloa crus-galli*
10. Large size, prolate, tricolporate with broad colpi, colpus membrane slightly perforated *Heliotropium europaeum*
11. Medium size, subprolate, tricolporate with long and broad colpi, colpus membrane obscurely reticulate *Lepidium didymum*
12. Large size, subprolate, monocolpate pollen grain with long and broad colpi, colpus membrane finely granulate *Medicago polymorpha*
13. Large size, sub-spheroidal, tri and tetraporate pollen grain, pore exine coarsely scabrate *Nerium oleander*

14.	Medium size, prolate, tricolporate pollen grain with long and broad colpi, colpus membrane coarsely reticulate	*Peganum harmala*
15.	Medium size, spheroidal, tricolporate with long and narrow colpi, sub-psilate tectum	*Phyla nodiflora*
16.	Medium size, prolate and spheroidal, Tri and tetracolporate with broad and short colpi at the base and narrow at tip	*Solanum surattense*
17.	Small size, spheroidal, pantoporate, echinate sculpturing with slightly expressed muri	*Tribulus terrestris*
18.	Medium size, sub-spheroidal and prolate, tricolporate pollen grain with long and broad colpi	*Trichodesma indicum*
19.	Large size, spheroidal, tricolporate, spines broad at base and tip is pointed	*Vicia sativa*

The size of pollen grains plays a significant role in pollen description. Based on size, pollen grains can be categorized as small size (10-25 µm) to medium size (25-50 µm) pollen grains and large size (50-100 µm) to extra-large size (100-200 µm) pollen grains (Almosawi, 2024). Most of the pollen grains were medium and isopolar. The current study presented a large amplitude in palynological characters as the size of pollens of selected taxa ranges from small to large. The size of pollen grains is vital in enhancing insect pollination (Ramamoorthy, 1991; Bank *et al.*, 2000). In the investigated 19 plant species various pollen types were observed i.e., bicolporate, tricolporate, trizonocolporate, echinate, polipantoporate, monoporate, perioporate, monocolpate, tetraporate, triporate, tetracolporate and pantoporate. The pollen grains of selected Salt Range taxa are sub-spheroidal and oblate and the size and number of colpus significantly differ among them. The current findings showed a large degree of variations in polar and equatorial shape of pollens and exine sculpturing patterns which were different in all studied pollens of Salt range taxa belonging to different families.

The diversity in palynological studies is one of the large pieces of evidence regarding exine sculpturing (Ashfaq *et al.*, 2018). A variation in exine sculpturing depicts the potential taxonomic value. The pattern of exine sculpturing is of great significance from a phylogenetic and evolutionary point of view (Walker and Doyle, 1975). It is obvious from the current results that Salt range taxa belonging to different families from similar areas possessed a great variety in exine sculpturing patterns. Different types of exine sculpturing patterns were observed such as scabrate in the Salt range taxa belonging to the family Amaranthaceae, Boraginaceae, Apocynacaeae, and Poaceae while the studied plant species belonging to other families such as Asteraceae showed echinate sculpturing. Present findings show that the tricolporate was the main pollen type in the studied species of Salt Range. The tricolporate was primitive and basic pollen type (Takhtajan, 1959; Doyle, 1969; Muller, 1970).

In plant systematics, the spine morphological traits are valuable taxonomic tools but variation among them is assumed to be large within a population (Mauseth, 2006; Hunt *et al*., 2006; Řepka and Gebauer, 2012; Gebauer *et al*., 2016). Spines are lifeless parts of the plant body but have various significant functions (Gibson and Nobel, 1986). The distinctive spine diversity was observed that exhibits the potential taxonomic significance of taxa in the Salt Range. Palynology with karyology and molecular study is very useful to solve taxonomic problems of taxa particularly at genus level (Joujeh *et al*., 2019). Palynological, phylogenetic and karyological study on *Centaurea* species to solve the taxonomic issues of this genus. The evolutionary divergence within a related taxa can be represented by variations in chromosome number. The plants adapted to arid regions could have a reduction in chromosome numbers (Uysal *et al*., 2015). Cytological features are considered as pivotal tool for delimitation of species in plant systematics (Taşar *et al.*, 2018). The highest pollen viability values (%) elucidates the adaptation of studied plant species in the Salt Range. Nazish and Althobaiti (2022) also reported *Echinochloa crus-galli* with high pollen viability value in Salt Range. The role of palynological study in plant taxonomy has evidenced valuable in solving disputed and critical taxonomic issues, therefore, the studied palynological features in the current study would be more useful for further karyological and taxonomic evaluation of salt-tolerant floral diversity.

Conclusion

Palyno-morphological characteristics are potential taxonomic tools in plant systematics. This project described the significance of pollen polarity, symmetry, exine sculpturing, and spine morphology that is useful to differentiate pollen types of different plant species of Salt Range. The findings elucidated the LM and SEM significance in the identification and differentiation of agricultural weeds and the taxa colonizing the surrounding hills of Khewra Salt mine. The differences and similarities in palyno-morphological characteristics of examined taxa can help mark out the different plant families at several taxonomic levels. Pollen key based on studied characters has proved useful to the correct taxa identification at species and genus level. This is the first reported study on the pollen morphology of taxa colonizing the Salt Range of Khewra. The palyno-morphological characteristics and pollen viability contribute to the taxonomic position of the Salt Range taxa and acts as a beneficial tool in the identification of problematic Salt Range taxa in the future.

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