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Palyno-morphological characterization of selected allergenic taxa of family Poaceae from Islamabad-Pakistan using microscopic techniques

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Abstract

Statistics around the world show an increased risk of high allergies associated with plants. The role of allergenic pollen is now well known. The most serious allergenic diseases are asthma and rhinitis, which affect 5–30% of the population of industrialized countries. This work focuses on the exploration of allergenic grass species of Islamabad, Pakistan and use microscopic techniques to correctly identify them. This study contains 11 species of grasses which showed variations in pollen morphological features. The dominant pollen shape was prolate, prolate-spheroidal and sub oblate. The largest polar diameter was calculated for *Echinochloa crus-galli* (56.00 µm) while the largest equatorial diameter was found in *Cenchrus ciliaris* (45.00 µm). Exine ornamentation varied from Gemmate-verrucate, Scabrate-reugulate, Verrucate-reticulate, Gemmate-Scabrate, Fine-Gemmate, Gemmate-rugulate, Gemmate-reticulate, Gemmate-scabrate, Scabrate-verrucate, and Scabrate. Maximum exine thickness was found in *Eleusine indica* (4.90 µm) while in *C. ciliaris* (1.50 µm) the minimum thickness of exine was measured. In *E. crus-galli* (1.54 µm), the largest *P* to *E* value was measured and the smallest value was noticed in *Setaria verticillata* (0.86 µm). The study was performed with an aim to identify the allergenic plant species in Islamabad-Pakistan using pollen morphological studies. This study provides significant information, to arrange the pollen types within their defined classes and to discuss various pollen features for developing an allergenic pollen atlas.

KEYWORDS

allergenic plants, LM, Poaceae, pollen morphology, SEM

1 | INTRODUCTION

Atmosphere contains mixture of gases and living organisms including fungal spores, bacteria, and airborne pollen (Secondo et al., 2021). Climatic changes may cause various problems. It may also cause changes in the reproductive life cycle of plants including pollen production which leads to different types of respiratory diseases (Rojo

et al., 2021). Pollen of grass family members releases proteins and many other secondary and primary phytochemicals in their atmosphere in different amount and different rates depending upon the environmental conditions. Due to these differences in quantity and rates the allergic reactions is caused by pollen grains (Basarkar & Saoji, 2007). Pollen concentration in the atmosphere depends partly on pollen dispersal of local flora and partly on the atmospheric

transport of pollen from adjacent and distant regions. Allergenic plants are usually very common, widespread species that produce pollen in high quantity and they are mostly wind pollinated (Behrendt & Becker, 2001; Culley, Weller, & Sakai, 2002). The most important allergenic pollen in the world belongs to members of the families of Betulaceae, Poaceae, Asteraceae, Oleaceae, and Urticaceae. Pollen allergens are water soluble, stable proteins or glycoproteins of molecular weight between 5 and 80 kDa (Grote et al., 2001; Grote, Vrtala, Niederberger, Valenta, & Reichelt, 2000; Knox & Suphioglu, 1996). Sequence comparison showed that they belong to only 29 of 2,615 protein families found in seed plants including pathogenesis-related proteins, profilins, expansins, and calcium-binding proteins (Chapman, Pomés, Breiteneder, & Ferreira, 2007; Radauer & Breiteneder, 2006). As a consequence, many allergens show remarkable sequence similarities which cause a phenomenon known as cross-reactivity. Cross-reactivity leads to the development of allergic reactions to a source without previous exposure, due to the similarity of the IgE epitopes of the proteins (Wopfner et al., 2005). Grass pollen group 1 allergens also show extensive cross-reactivity between grass species when they share more than 50% sequence identity (Chapman et al., 2007). A single pollen type usually contains several allergens. In grass pollen, 11 groups of allergens have been described (Andersson & Lidholm, 2003; Petersen et al., 2006).

Like other cells of plants pollen also contains proteins either in their cytoplasm or in the outer wall. It is also known that only a small number of the proteins can cause allergenic reactions (Seiberler, Scheiner, Kraft, Lonsdale, & Valenta, 1994). Staff, Schäppi, and Taylor (1999), Taylor, Staff, Singh, and Knox (1994), and Knox and Suphioglu (1996) reported the presence of localized allergens in the cytosol and starch granules of *Lolium perenne* pollen. Knox, Heslop-Harrison, and Reed (1970) assumed the presence of pollen allergens to be stored in the intine or exine of the pollen. So the study of pollen morphology using SEM will be helpful to study the fine structure of pollen walls that will help in detection of allergy causing plant species.

There are thousands of different species of Poaceae which has great ecological value, as they cover different area of the earth with most habitats around the world, in addition to crops, all Poaceae species have hanging anthers and most are wind-pollinated and produced a huge amount of the identical pollen grains in the air. Pollen is an important source of airborne allergens. It is generally believed that wind-pollinated plants are the leading cause of allergies. Therefore, the identification of allergy causing plants using palyno-morphological studies is receiving great attention (D'Amato et al., 2007).

Pollen morphology is one of the modern approaches used to identify plant species correctly at species and generic level. The most important features of pollen grains include the exine sculpturing, shape, and size using light microscope (LM) and scanning electron microscope (SEM). SEM can help us to study the pollen features in more detail. Grass pollen grains are spherical to sub lamellar and have a distinctive thickening of the outer and inner walls covers the pores (Erdtman, 1969). In late spring and early summer, grass pollen is present in the air that may induce allergies. After correct identification of these species pollen grains are used to make antiallergic vaccines

using various immunological approaches like induction of tolerance and low dose antigen sensitization approaches.

Pollen morphology of allergenic plant species of family Poaceae in Islamabad-Pakistan was never investigated before. Therefore, microscopic techniques for the identification of these species were used. The present research illustrated the key characteristics of Poaceae pollen as a prominent aeroallergen which may causes allergenicity and specifically on studies of regional variations in the pollen of family Poaceae. This is the first reported study aimed to explore the allergenic flora of Pakistan. For the identification of allergenic flora it is necessary to taxonomically evaluate their pollen by using LM and SEM techniques to study palyno-morphological characters.

2 | MATERIAL AND METHODS

2.1 | Plants collection, identification, and preservation

For the collection of plant materials, different field trips have been organized throughout the year covering almost every flowering season. Plant samples were collected from the major sites of Islamabad, that is, Margalla hills, Lakeview Park, Bari Imam, Malpur village, Shadra hills, Abpara, and different area of Quaid-e-Azam University, Islamabad, Pakistan. Each study trip was well planned and carried out efficiently. The collected plants species were identified and described with the assistance of expert and professionals taxonomist and also compared their morphological characters with the herbarium specimen of Herbarium of Pakistan ISL. Field data, that is, locality, coordinates of the area, habitat, flowering time, and flower color were recorded in the field notebook. After correct identification the plants are poisoned and preserved on the standard size sheets of herbarium and submitted to the herbarium (Table 1; Plate 1).

2.2 | Pollen analysis using light microscope

To prepare the pollen slides, flowers from plants were collected in the field. The anthers were separated and stored in a glass tube in a solution of glacial acetic acid and (NaClO₃) sodium chlorate in (1:2) ratios. The anthers were smashed with the glass rod to create homogeneous mixture and then filtered. Centrifuge the mixture at 1,000 rpm for 5 min, discard the supernatant, and suspend the precipitate in glacial acetic acid. Slides are made and stained with glycerol jelly mixed with 1% safranin to stain the slides and observe them under a microscope, and then a few drops of the mixture were taken and placed on the slides, which was put on a small piece of simple fuchsin-stained jelly. Place the slide on the heated plate, while carefully placing the coverslip on it to keep out air bubbles. The glass cover has sealed edges for long-term storage of the glass (Jiménez, Bernal, Del Nozal, Martin, & Mayo, 2007). Pollen grains are measured, analyzed, and micrographed under a LM at various resolutions of Leica Dialux 20. Based on five

TABLE 1 Plant sampling, voucher specimen number, and their distribution

S. no	Taxa	English name	Collection site	Collection date	Coordinates	Voucher specimen	Flowering period	Cultivation status/ distribution in Pakistan	Distribution in World	References
1	<i>B. reptans</i> (L.) C.A.	Creeping-panic grass	Lake View Park Islamabad	July, 15, 2020	33.714532°N 73.131368°E	IZH-PO-33	July–November	Sind, Baluchistan, Punjab, and KPK	India, South East Asian subcontinents, China, Philippines, Indonesia, Australia, and Canada	Mukaromah and Imron (2019); Ali et al. (2019); Saand et al. (2019)
2	<i>C. jwarancusa</i> (Jones) Schult	Lemon grass	Margalla Hills National Park	June 10, 2020	33.742172°N, 73.081796°E	IZH-PO-67	May–August	Sind, Baluchistan, Punjab, KPK, and Gilgit	Westwards Iraq, Socotra, Nepal, and Northwest India.	Bhattarai and Ghimire (2006); Raina, Srivastava, Aggarwal, Syamasundar, and Khanuja (2003); Enright, Miller, and Akhter (2005)
3	<i>C. ciliaris</i> L.	Buffel grass	QAU Islamabad	July 26, 2020	33.748171°N, 73.138588°E	IZH-PO-87	July–August	Islamabad, Sind, Baluchistan, Punjab, and KPK	Africa, Australia, The United States, Mexico, South America, and Western Asia	Nawazish, Hameed, and Naurin (2006); Arshad, Ashraf, Ahmad, and Zaman (2007)
4	<i>D. annulatum</i> (Forsk.) Stapf	Marvel grass	Bhra kahu Islamabad	August 8, 2020	33.746271°N, 73.175041°E	IZH-PO-94	July–August	Islamabad, Sind, Baluchistan, Punjab, KPK, and Kashmir	Kenya, Senegal, Middle East Indonesia, Southern Africa, Tropical America, and Australia	Sultan, Rahim, Yaqoob, Nawaz, and Hameed (2008); Simbaya, Chibinga, and Salem (2020)
5	<i>D. bipinnata</i> (L.) Stapf	Halfa grass	Margalla Hills National Park	August 10, 2020	33.742172°N, 73.081796°E	IZH-PO-54	July–November	Islamabad, Sind, Baluchistan, Punjab, KPK, and Kashmir	Middle East to Indo-China, North and tropical Africa	Javadi, Arjum, and Bajwa (2005); Asrar et al. (2017); Fakhireh et al. (2012)
6	<i>E. crus-galli</i> (L.) P.Beauv.	cockspur grass	QAU Islamabad	August 15, 2020	33.748171°N, 73.138588°E	IZH-PO-32	June–October	Sind, Baluchistan, Punjab, Gilgit, and Kashmir	Europe, Africa, The United Kingdom, Tropical America, and Australia	Hamayun, Hussain, Afzal, and Ahmad (2005); Gitsopoulos and Froud-Williams (2004)
7	<i>E. indica</i> (L.) Gaertn.	Yard-grass	QAU Islamabad	August 15, 2020	33.748171°N, 73.138588°E	IZH-PO-98	July–August	Islamabad, Swat, Quetta, Rawalpindi, and Kashmir	Mexico, Koyama, Taiwan, Neighb, and Australia	Irshad, Ahmad, Pervez, and Inoue (2015); Gallardo-Cruz, Pérez-García, and Meave (2009)
8	<i>E. coracana</i> (L.) Gaertn.	Finger millet	Margalla Hills National Park	June 22, 2020	33.742172°N, 73.081796°E	IZH-PO-58	July–October	Islamabad, Rawalpindi, Kashmir, and Sindh	Pakistan, Kenya, Uganda, Ethiopia, Tanzania, Zambia, Zimbabwe, Malawi, China, and Taiwan	Dida, Wanyera, Dunn, Bennetzen, and Devos (2008); De Wet, Rao, Brink, and Mengesha (1984)
9	<i>I. cylindrica</i> (L.) Raeusch.	Cogongrass	Bhra kahu Islamabad	August 22, 2020	33.746271°N, 73.175040°E	IZH-PO-12	July–August	Islamabad, Swat, Quetta, Rawalpindi, and Kashmir	Mexico, Koyama, Taiwan, and Neighb	Widyastuti et al. (2020); Butt et al. (2018); Harant and Lang (2020)
10	<i>S. spontaneum</i> L.	Kans grass (wild sugarcane)	QAU Islamabad	April 25, 2020	33.748171°N, 73.138589°E	IZH-PO-80	November–February	Islamabad, Sind, Punjab, N.W.F.P., Gilgit, and Kashmir	India, Pakistan, Sri Lanka, and Taiwan.	Ahmad et al. (2020); Sobhakumari (2020); Mahesh et al. (2020)
11	<i>S. verticillata</i> (L.) P.Beauv.	Pearl millet	Margalla Hills National Park	July 17, 2020	33.742172°N, 73.081796°E	IZH-PO-61	June–December	Sind, Baluchistan, Punjab, KPK, Gilgit, and Kashmir	North America, India, Iran, Taiwan, Pakistan, and India	Ahmad et al. (2011a, 2011b); Weber and Kashyap (2016); Mollaei, Darbandi, Aval, and Chauhan (2020)

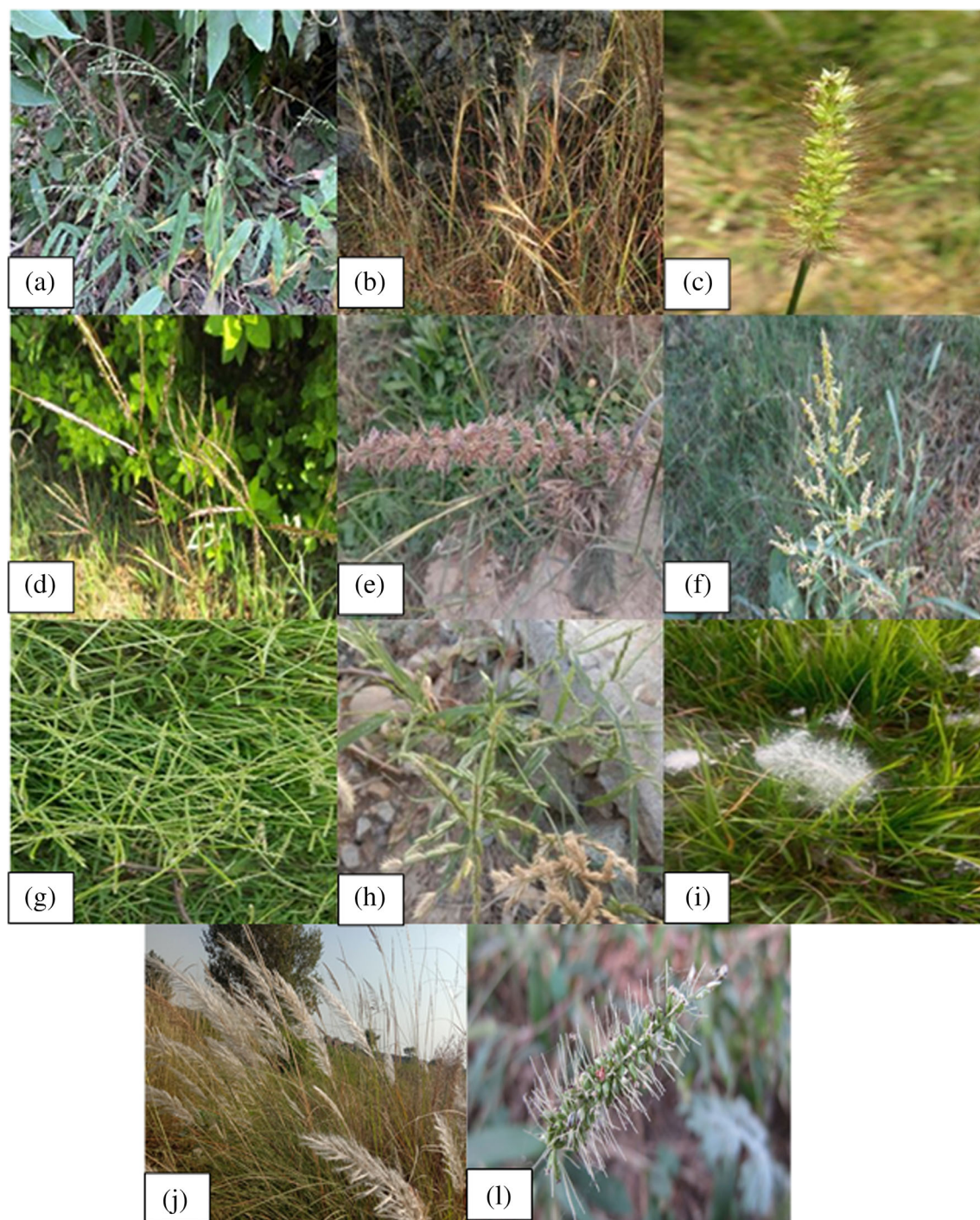


PLATE 1 Species collected of family poaceae, that is, (a) *B. reptans*, (b) *C. jwarancusa*, (c) *C. ciliaris*, (d) *D. annulatum*, (e) *D. bipinnata*, (f) *E. crus-galli*, (g) *E. indica*, (h) *E. coracana*, (i) *I. cylindrica*, (j) *S. spontaneum*, and (k) *S. verticillata* [Color figure can be viewed at wileyonlinelibrary.com]

grain readings, some morphological features were measured using this method (Nazish et al., 2019).

To find the average, five pollen readings were made for various (polar and equatorial) diameters, thickness of exine, length and width of colpi, and length of aperture. The shape of pollen was based on the ratio of the polar to equatorial diameter. The shape and size of pollen grain were determined by using the terminologies of Punt, Hoen, Blackmore, Nilsson, and Le Thomas (2007).

2.3 | Pollen analysis using scanning electron microscopy

The pollens are prepared using a SEM to obtain high quality images and methods can be used to distinguish closely related taxa (Ashfaq et al., 2018). Take electron micrographs with a SEM, separate pollen from the anthers of flowers, grind a few drops with 45% acetic acid, attach to a palladium gold-plated metal hub, and finally

observe the characteristics of the pollen below Jeol JSM SEM-15 kV T200.

2.4 | Statistical analysis

Quantitative data such as equatorial and polar diameter, length, and width of colpi, and thickness of exine were analyzed using SPSS 16 software. The fertility and sterility of pollen grains were determined by the method used of Nazish et al. (2019). Quantitative data was expressed as minimum – maximum (mean $\pm$ standard error).

2.5 | Estimation of sterility and fertility percentage

The pollen sterility and fertility percentage were determined by the formula used by Khan et al. (2021).

$$\text{Fertility} = \text{Fertility} / (\text{Fertility} + \text{Sterility}) \times (100).$$

$$\text{Sterility} = \text{Sterility} / (\text{Sterility} + \text{Fertility}) \times (100).$$

3 | RESULTS

The micromorphological characteristics of pollen grains, such as size and shape of the pollen, sculpturing of exine, and the presence and number of colpi and pores, are very important tools for the accurate identification of species at family and generic level. Qualitative and quantitative micromorphological characteristics of pollen grains were measured by using SEM and LM. In this study, 11 types of taxa were studied in which five species have prolate shapes (*Dichanthium annulatum*, *Desmostachya bipinnata*, *Echinochloa crus-galli*, and *Eleusine indica*), and five species have prolate-spheroidal (*Cenchrus ciliaris* and *Brachiaria raptens*), so the pollen shape turned out to be slightly different in *Eleusine*

coracana, *Imperata cylindrica*, and *Saccharum spontaneum*, and *Cymbopogon jwarancusa* are sub-oblate. The aperture condition of the all 11 taxa is monoporate. In the present investigation, the pollen morphological changes are observed in the species of family Poaceae in pollen form. For accurate identification and classification the morphological features are very important. SEM is used to investigate the major allergenic plant taxa of Islamabad, Pakistan (Tables 2 and 3; Plate 2).

3.1 | Size and shape of pollen

In the present studies the predominant shape and size of Poaceae pollen were prolate in (*D. annulatum*, *D. bipinnata*, *E. crus-galli*, *E. indica*, and *Setaria verticillata*) while prolate-spheroidal pollen were found in the (*Brachiaria reptans*, *C. ciliaris*, *E. coracana*, *I. cylindrica*, *S. spontaneum*, whereas *C. jwarancusa* has sub oblate shape. The largest mean pollen diameter on the polar axis was observed at *E. crus-galli* (56.00 μm) and the smallest was at *S. verticillata* (21.70 μm). Similarly the largest pollen diameter was observed on the equatorial axis in *C. ciliaris* (45.00 μm) and the smallest diameter was observed in *D. bipinnata* (18.50 μm). Size of the pollen was calculated on the base of Polar diameter to equatorial diameter ratio, among the species of family poaceae, variations were found in size. The highest *P* to *E* value was observed in *E. crus-galli* (1.54), and the lowest value was observed in *S. verticillata* (0.86; Figures 1 and 2).

3.2 | Exine ornamentation

In the present research work all the pollens of family poaceae are psilate, and the exine ornamentation was observed Gemmate-verrucate (*B. raptens*, *D. bipinnata*), Scabrate-reugulate (*C. ciliaris*), Verrucate-reticulate (*C. jwarancusa*), Gemmate-Scabrate (*D. annulatum*), Fine Gemmate (*Echinochloa crus-galli*), Gemmate-rugulate (*E. coracana*), Gemmate-reticulate (*E. indica*), Gemmate-scabrate (*I. cylindrica*),

TABLE 2 Qualitative palynological features of allergenic grass species through SEM

S. no	Taxa	Exine sculpturing	Aperture condition	Pore orientation
1	<i>B. reptans</i> (L.) C.A.	Gemmate-verrucate	–	–
2	<i>C. jwarancusa</i> (Jones) Schult	Scabrate-reugulate	Monoporate	Prominent
3	<i>C. ciliaris</i> L.	Verrucate-reticulate	Monoporate	Sunken
4	<i>D. annulatum</i> (Forssk.) Stapf	Gemmate-verrucate	Monoporate	Sunken
5	<i>D. bipinnata</i> (L.) Stapf	Gemmate-scabrate	Monoporate	Prominent
6	<i>E. crus-galli</i> (L.) P.Beauv.	Fine gemmate	Monoporate	Sunken
7	<i>E. indica</i> (L.) Gaertn.	Gemmate-rugulate	Monoporate	Sunken
8	<i>E. coracana</i> (L.) Gaertn.	Gemmate-reticulate	–	–
9	<i>I. cylindrica</i> (L.) Raeusch.	Gemmate-scabrate	Monoporate	Sunken
10	<i>S. spontaneum</i> L.	Scabrate-verrucate	–	–
11	<i>S. verticillata</i> (L.) P.Beauv.	Scabrate	Monoporate	Prominent

TABLE 3 Quantitative data of the pollen of allergenic species family Poaceae

S. no	Taxa	P/E ratio		Size in exine Mean (Min–Max) SE (µm)	Polar diameter	Equatorial diameter	Pore length	Pore width	Pollen fertility (%)	Pollen sterility (%)
		Mean	SE							
1	<i>B. reptans</i> (L.) C.A. Schult	1.08	± 0.37	2.81(2.00–3.75)	35.00(32.50–37.50) ± 0.88	32.40(30.00–34.50) ± 0.81	3.90(2.00–5.25) ± 0.68283	6.05(5.00–7.00) ± 0.35707	73.68	26.31
2	<i>C. jwarancusa</i> (Jones)	0.87	± 0.21	4.30(3.75–5.00)	25.50(22.50–28.75) ± 1.15	29.15(27.50–30.00) ± 0.47	2.00(1.50–2.50) ± 0.176	1.75(1.25–2.25) ± 0.176	81.08	18.91
3	<i>C. ciliaris</i> L.	1.11	± 0.32	1.50(0.75–2.50)	50.00(45.00–55.00) ± 1.76	45.00(42.50–47.50) ± 0.88	2.47(2.00–3.00) ± 0.21	1.68(1.25–2.00) ± 0.136	71.42	28.57
4	<i>D. annulatum</i> (Forssk.) Stapf	1.59	± 0.30	2.90(2.00–3.75)	38.25(32.50–42.50) ± 1.79	24.00(17.50–30.00) ± 2.21	2.75(2.25–3.25) ± 0.17	2.00(1.50–2.50) ± 0.17	76.19	23.8
5	<i>D. bipinnata</i> (L.) Stapf	1.35	± 0.17	1.75(1.24–2.25)	25.00(22.50–27.50) ± 0.88	18.50(15.00–21.25) ± 1.07	3.1(2.50–3.75) ± 0.231	4.15(3.75–5.30) ± 0.291	80	20
6	<i>E. crus-galli</i> (L.) P.Beauv.	1.54	± 0.58	2.80(1.25–4.50)	56.00(50.00–62.50) ± 2.31	36.25(32.50–40.00) ± 1.25	1.6(0.7–2.50) ± 0.30	2.80(1.25–3.75) ± 0.44300	82.5	17.5
7	<i>E. indica</i> (L.) Gaertn.	1.27	± 0.18	4.90(2.50–7.00)	28.75(25.00–32.50) ± 1.25	22.50(20.00–25.00) ± 0.88	3.85(2.50–4.75) ± 0.4279	4.05(2.50–5.00) ± 0.44300	75	25
8	<i>E. coracana</i> (L.) Gaertn.	1.21	± 0.56	3.10(2.00–5.00)	26.25(23.75–28.75) ± 0.88	21.60(20.00–23.75) ± 0.68	4.470(2.50–5.60) ± 0.525	6.8500(5.75–8.75) ± 0.36236	81.48	22.22
9	<i>I. cylindrica</i> (L.) Raesch.	1.17	± 0.51	3.20(2.00–5.00)	29.40(27.50–31.25) ± 0.62	25.00(22.50–27.50) ± 0.88	2.700(2.00–3.25) ± 0.21506	4.7500(2.50–7.25) ± 0.8513	80.6	19.35
10	<i>S. spontaneum</i> L.	1.23	± 0.21	1.80(1.25–2.50)	45.00(42.50–47.50) ± 0.88	36.50(32.50–40.00) ± 1.33	4.00(3.00–4.75) ± 0.30619	3.100(2.50–3.75) ± 0.2384	81.39	18.6
11	<i>S. verticillata</i> (L.) P.Beauv.	0.86	± 0.21	2.70(2.00–3.25)	21.70(20.00–22.75) ± 0.49	25.00(22.50–27.50) ± 0.88	3.500(3.00–4.00) ± 0.17678	2.700(2.00–3.25)0.2506	72.72	27.27

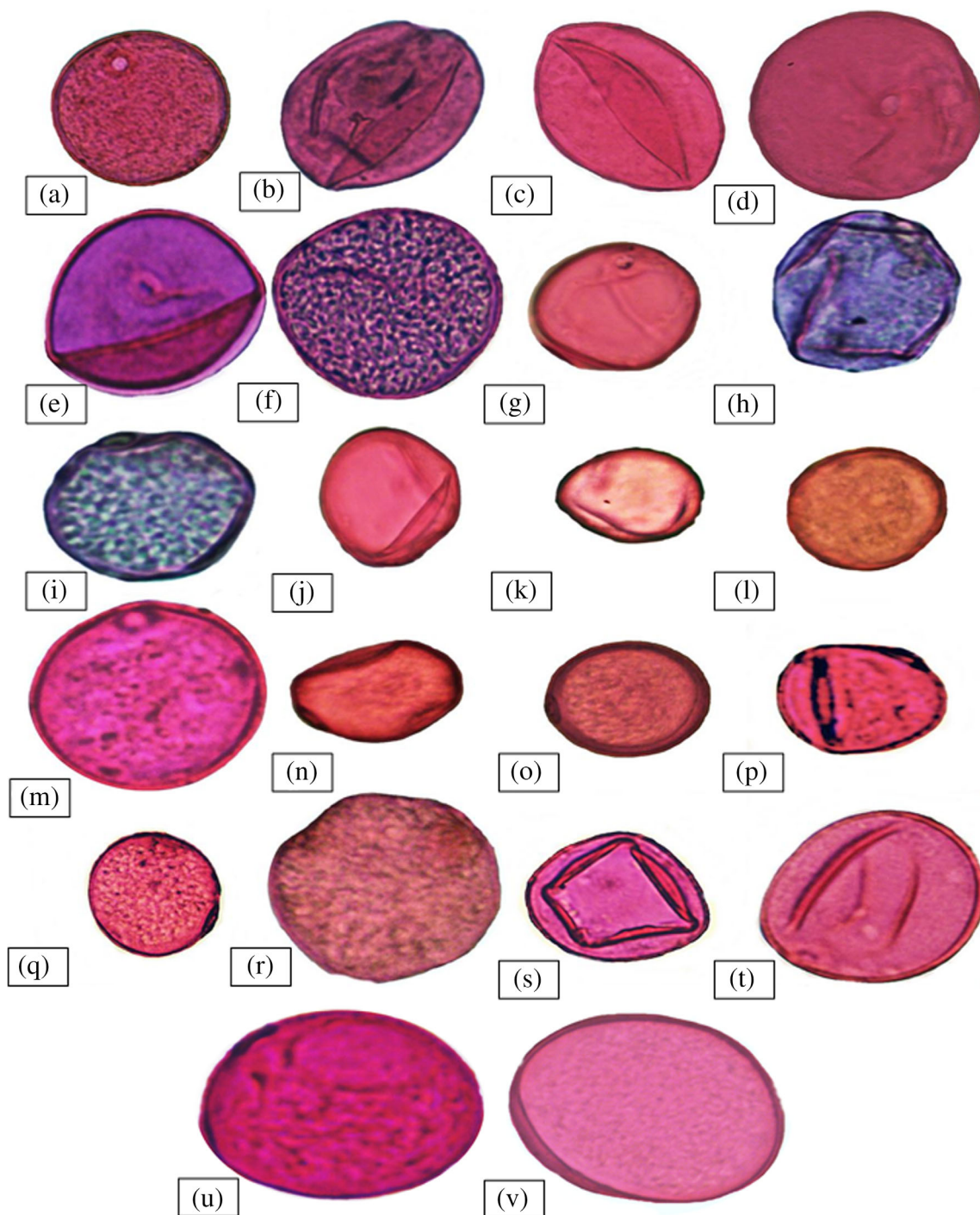


PLATE 2 Light microscopic pollen micrographs showing polar view (PV) and equatorial view (EV); (a) *B. reptans* (PV), (b) *B. reptans* (EV), (c) *C. ciliaris* (EV), (d) *C. ciliaris* (PV), (e) *C. jwarancusa* (EV), (f) *C. jwarancusa* (PV), (g) *D. annulatum* (PV), (h) *D. bipinnata* (EV), (i) *D. bipinnata* (PV), (j) *D. annulatum* (EV), (k) *E. crus-galli* (EV), (l) *E. crus-galli* (PV), (m) *E. coracana* (PV), (n) *E. coracana* (EV), (o) *E. indica* (PV), (p) *E. coracana* (EV), (q) *I. cylindrica* (PV), (r) *I. cylindrica* (EV), (s) *S. spontaneum* (EV), (t) *S. spontaneum* (PV), (u) *S. verticillata* (EV), and (v) *S. verticillata* (PV) [Color figure can be viewed at wileyonlinelibrary.com]

Scabrate-verrucate (*S. verticillata*), and Scabrate (*S. spontaneum*). Thickness of exine ornamentation is the most distinguishing character of the species. In this study the maximum thickness of exine ranges from *E. indica* (4.90 μm), whereas the minimum exine thickness was observed in *C. ciliaris* (1.50 μm ; Plates 3–5; Figure 3).

3.3 | Pore orientation

The main feature of the pollen of family poaceae is pore orientations. In the present research, prominent pore orientation was observed in (*C. ciliaris*, *D. annulatum*, and *S. spontaneum*), while sunken pore

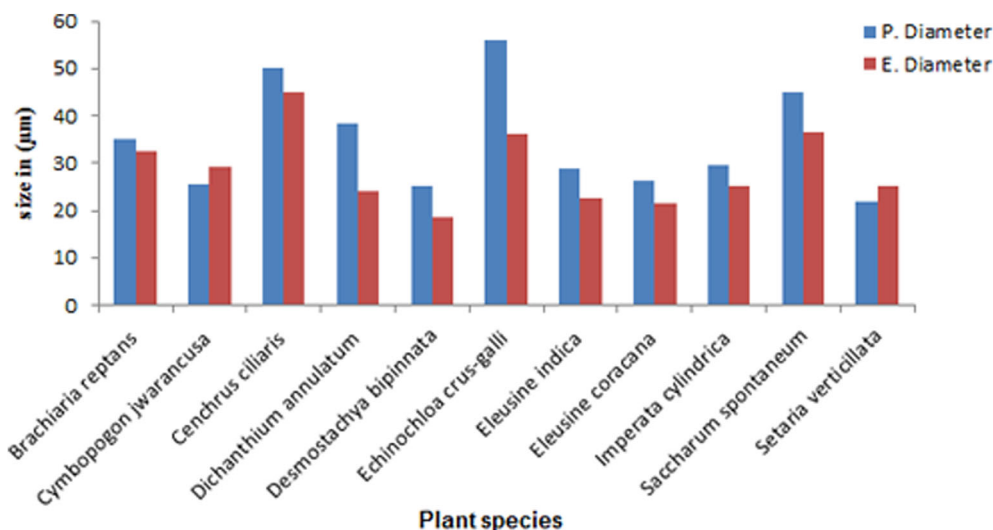


FIGURE 1 Variations in pollen size of allergenic taxa of Poaceae [Color figure can be viewed at wileyonlinelibrary.com]

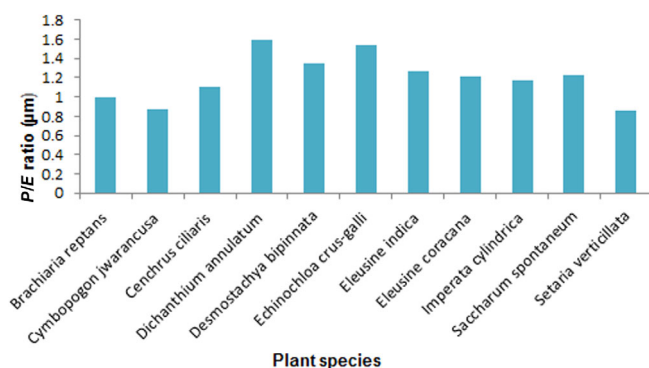


FIGURE 2 Represent P/E ratio of allergenic taxa of Poaceae [Color figure can be viewed at wileyonlinelibrary.com]

orientation was observed in (*C. jwarancusa*, *D. bipinnata*, *Echinochloa crus-galli*, *E. coracana*, and *I. cylindrica*). In the plant *B. reptans* and *S. verticillata* no pore orientation has been observed. The pollen pore of *E. coracana* had the largest mean diameter (4.470 µm) and smallest value length of the pore is found in *E. crus-galli* (1.6 µm) where the other species had mean pore size ranging from 25.00 to 50.00 µm. Similarly the pollen pore of *E. coracana* had the largest mean width of the pore is 6.85 µm while the smallest width of the pore is found in the *C. ciliaris* is 1.68 µm.

3.4 | %age of fertility and sterility

For the development of anthers, fertility/sterility is essential biological process such as flowering, fruiting, and seeds production. The production of more pollen is directly proportional to the pollen allergy. The percentage of sterility and fertility of pollen were measured for each species. The highest number of fertility was obtained in *E. crus-galli* (82.5%) whereas the lowest was observed in *C. ciliaris* (71.42%). The largest number of sterile pollen was found in *C. ciliaris* (28.57%) while the smallest sterile pollen was measured in *E. crus-galli* (17.5%; Figure 4).

4 | DISCUSSION

The present investigation of morphological features of Poaceae pollen is based on LM and SEM from various locations in Islamabad, Pakistan. The morphological characteristics of pollen play a very important role in the field of taxonomy and classification, and most of the taxonomist rely heavily on these characteristics for correct identification at different taxonomic ranks (Khan et al., 2019). In this study, we investigated that palyno-morphological feature of family Poaceae which plays significant role in classification and identification of the Poaceae taxa. In the present study SEM analysis has been used to describe the wall pattern of pollen grains as exine is responsible for causing allergic reactions (Guimarães et al., 2018). Due to its taxonomic application, palynology also plays a very important role in other interdisciplinary disciplines such as pharmacology, aeropalynology, and melissopalynology (Butt et al., 2018). According to Ullah et al. (2018), the pollen micro-morphological features are important in the evolutionary and phylogenetic studies of plant species. In a recent study, 11 species of the Poaceae family belonging to 10 different genera were studied. Current studies have shown that pollen grains are monad, monoporate, and psilate. Our this result is Similar to Guimarães et al. (2018), who found that Poaceae pollen is usually a single-pore particle with annealed and closed pores, and the species cannot be separated by using simple LM. In the current investigation small size pollen was observed in *E. indica* and *S. verticillata* and pollen having large size was observed in *E. crus-galli* and *C. ciliaris*. The present work is similar to the previous work done by Chaudhari, Arshad, Amjad, and Fatima (2013).

In *B. reptans* the pollen grains are medium in size, monad, prolate spheroidal, psilate circular, and radially symmetrical in polar view and the polar diameter is 35 ± 0.88 µm, while equatorial diameter is 32.40 ± 0.81 µm. Exine thickness is 2.81 ± 0.37 µm, while exine sculpturing is verrucate-reticulate but this result is dissimilar to previous work done by Shaheen et al. (2013). The P to E ratio is 1.08, length of pore is 3.90 ± 0.68283 µm, while width of the pore is 6.05 ± 0.35707 µm, while this results is similar to the results of Shaheen et al. (2013).

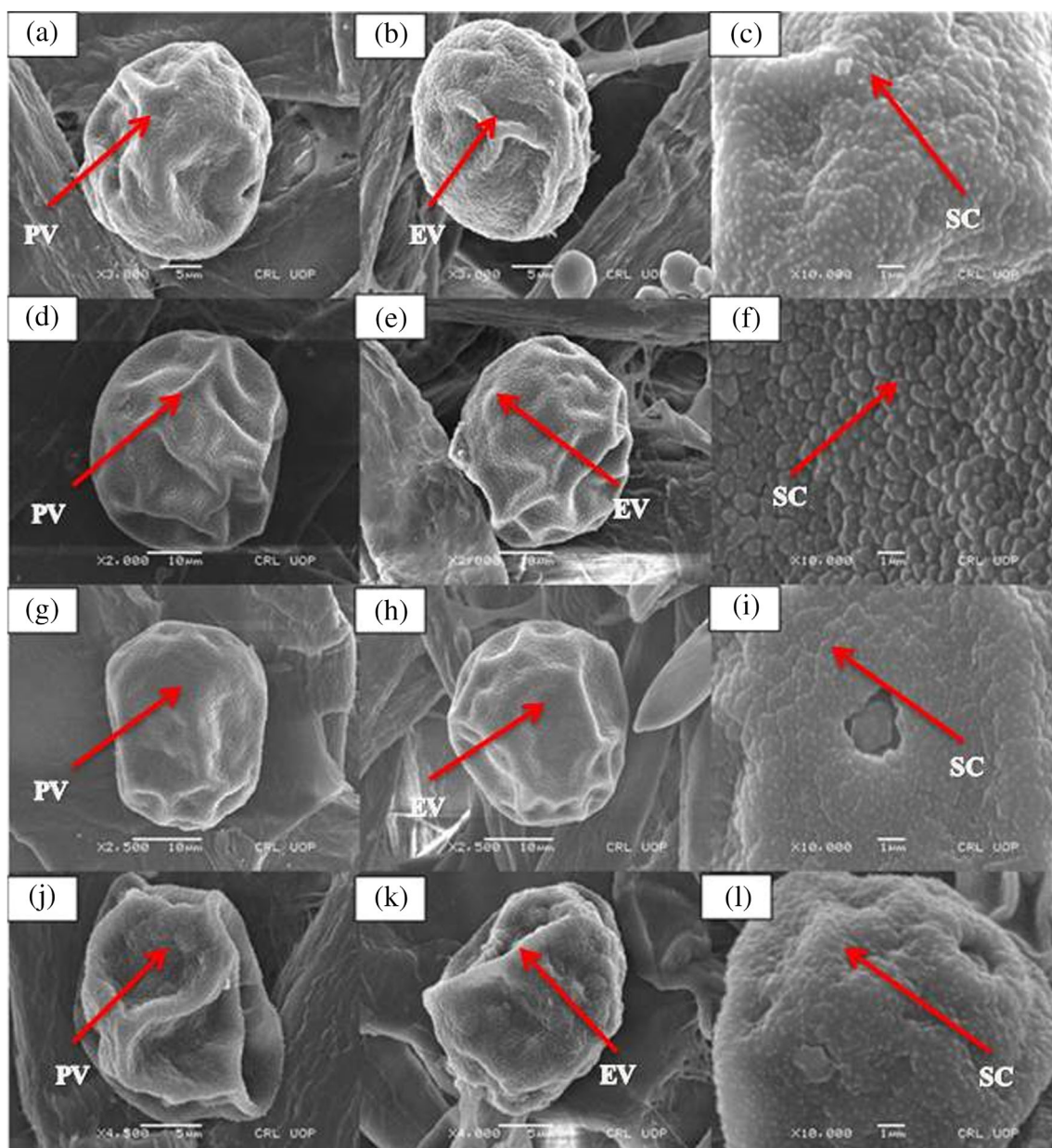


PLATE 3 Represent SEM micrograph of family Poaceae; (PV) represent polar view and (EV) represent equatorial view while (SC) represent exine sculpturing with arrows; *B. reptans* (a) polar view, (b) equatorial view, (c) exine sculpturing; *C. jwarancusa* (d) polar view, (e) equatorial view, (f) exine sculpturing; *C. ciliaris* (g) polar view, (h) equatorial view, (i) exine sculpturing; *D. annulatum* (j) polar view, (k) equatorial view, (l) exine sculpturing [Color figure can be viewed at wileyonlinelibrary.com]

In *C. ciliaris*, pollen is medium to large, monad, prolate spheroidal, psilate circular oval, and bilaterally symmetrical in polar view. Polar diameter is $50 \pm 1.76 \mu\text{m}$. Equatorial diameter is $45.0 \pm 0.88 \mu\text{m}$, similar work has been done by Morgado et al. (2015). *P* to *E* ratio is 1.11, length of pore is $2.47 \pm 0.21 \mu\text{m}$, while width of the pore is $1.68 \pm 0.136 \mu\text{m}$. Exine thickness is $1.50 \pm 0.32 \mu\text{m}$ and exine sculpturing is scabrate-reugulate, but this result is deviated from the results of Shaheen et al. (2011).

The pollen grains of *C. jwarancusa* are small to medium in size, monad and suboblate, psilate, elliptic, and radially symmetrical in polar view. Polar diameter is $21.4 \pm 0.414 \mu\text{m}$ and equatorial diameter is

$15.6 \pm 0.505 \mu\text{m}$ while polar and equatorial ratio is 1.37. Length and width of pore is $2.00 \pm 0.176 \mu\text{m}$ and $1.75 \pm 0.176 \mu\text{m}$. Thickness of exine is $2.12 \pm 0.113 \mu\text{m}$. Sculpturing of exine is verrucate-reticulate but this result is dissimilar to Khan et al. (2019). Perveen, Noori, Khan, and Qureshi (2015) conducted a study on the pollen morphology of allergenic pollen grains which are present in air of Karachi city of - Pakistan. The results of their study showed that the most abundant pollen that causes allergenic reactions belongs to family Poaceae.

In *D. bipinnata*, pollen grains are small to medium, prolate, monad and psilate, and radially symmetrical in polar view (Khan, Abidin, Mumtaz, Jamsheed, & Ullah, 2017). Polar diameter is $25 \pm 0.88 \mu\text{m}$

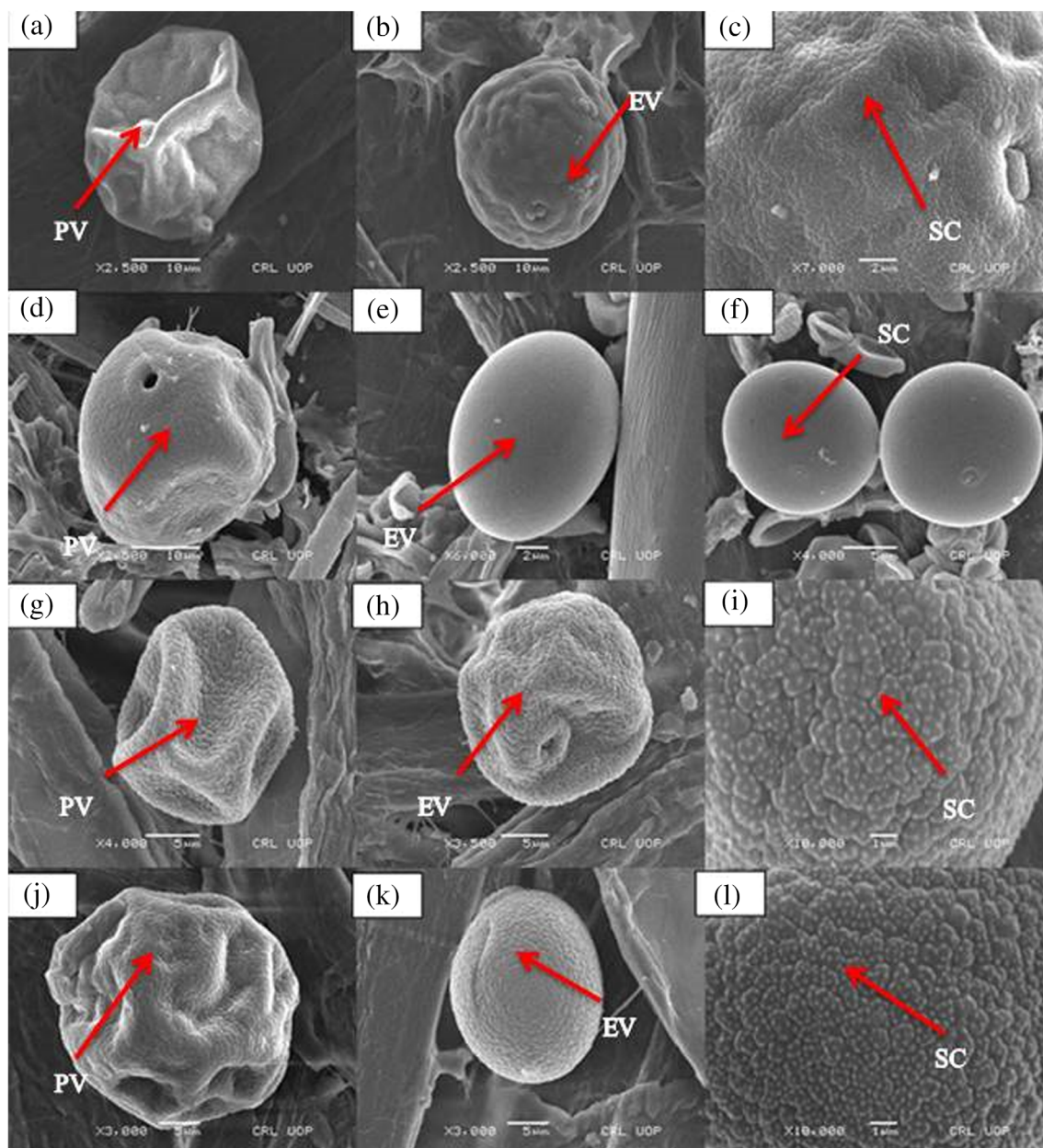


PLATE 4 Represent SEM micrograph of family Poaceae; (PV) represent polar view and (EV) represent equatorial view while (SC) represent exine sculpturing with arrows; *D. bipinnata* (a) polar view, (b) equatorial view, (c) exine sculpturing; *E. crus-galli* (d) polar view, (e) equatorial view, (f) exine sculpturing; *E. indica* (g) polar view, (h) equatorial view, (i) exine sculpturing; *E. coracana* (j) polar view, (k) equatorial view, (l) exine sculpturing [Color figure can be viewed at wileyonlinelibrary.com]

while equatorial diameter is $18.50 \pm 1.07 \mu\text{m}$, and similar work has been done by Morgado et al. (2015). While the *P* to *E* ratio is 1.37, the pore length and width is $3.1 \pm 0.231 \mu\text{m}$ and $4.15 \pm 0.291 \mu\text{m}$, respectively. Size in exine is $1.75 \pm 0.17 \mu\text{m}$ having gemmate-verrucate exine sculpturing. But this result is dissimilar to the work of Liu, Zhao, and Hao (2014).

In *D. annulatum*, pollen grains are small to medium, monad, prolate, psilate circular, and radially symmetrical in polar view. Polar diameter is $38.25 \pm 1.79 \mu\text{m}$ while equatorial diameter $24 \pm 2.21 \mu\text{m}$, but this result is dissimilar to the results of Sardar, Tarade, and Singhal (2013). *P* to *E* ratio is 1.59, length and width of the pore are $2.75 \pm 0.17 \mu\text{m}$ and

$2.00 \pm 0.17 \mu\text{m}$, respectively; but this result is dissimilar to the results of Mishra, Singh, and Oommachan (2002). Exine thickness is $2.90 \pm 0.30 \mu\text{m}$ while exine sculpturing is gemmate-scabrate.

In *E. crus-galli* the pollen grains are medium to large in size, psilate, monad, prolate, circular, and radially symmetrical in polar view. This result is similar to the previous work done by Ahlawat, Dahiya, and Chaudhary (2014) and Morim and Lughadha (2015). Polar diameter is $56 \pm 2.31 \mu\text{m}$ and equatorial diameter is $36.25 \pm 1.25 \mu\text{m}$ (Joly, Barillé, Barreau, Mancheron, & Visset, 2007). *P* to *E* ratio is 1.54. Length and width the pore are $1.6 \pm 0.30 \mu\text{m}$ and $2.80 \pm 0.44300 \mu\text{m}$, respectively. Exine thickness is $2.80 \pm 0.58 \mu\text{m}$ and exine sculpturing is fine

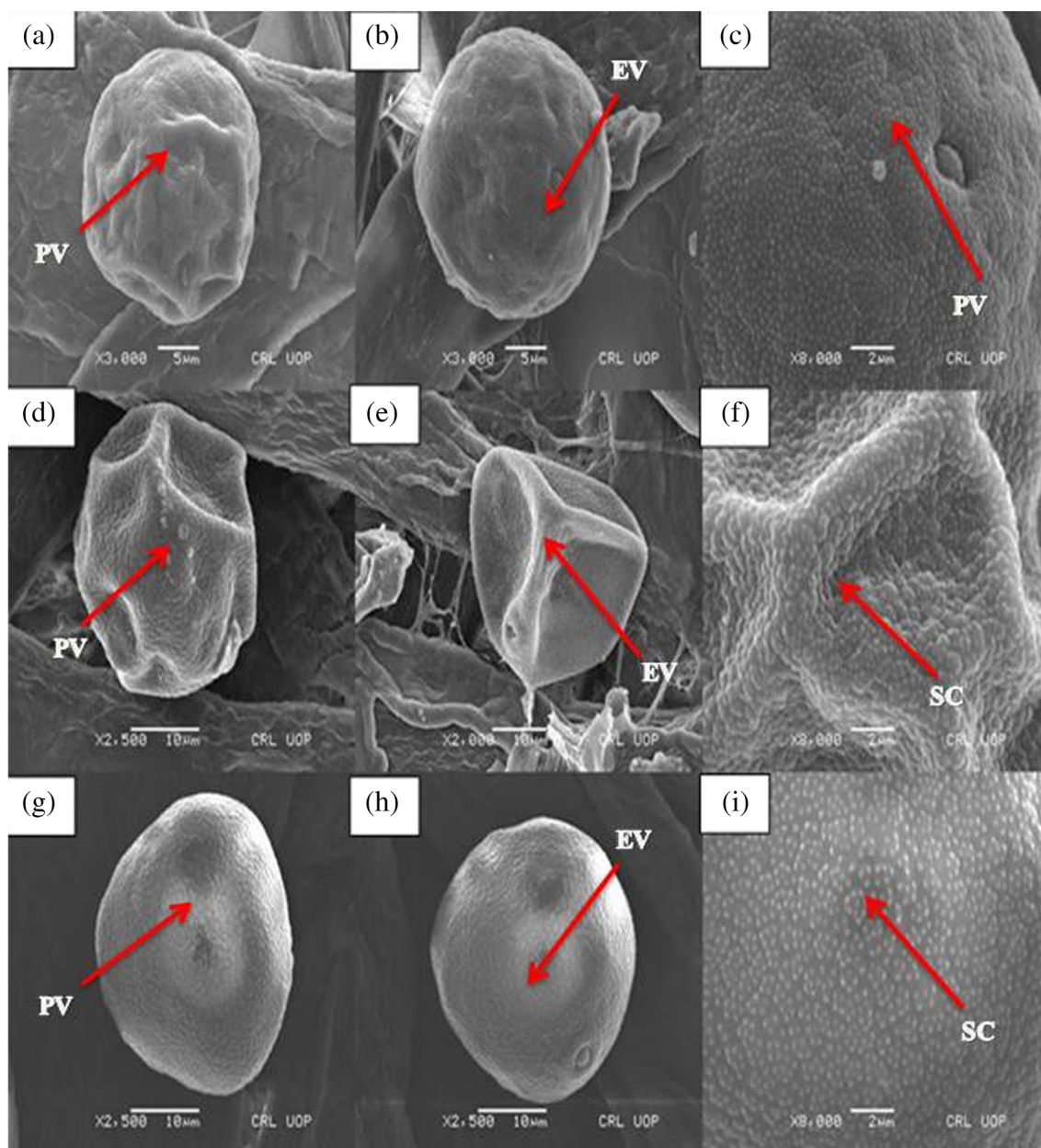


PLATE 5 Represent SEM micrograph of family Poaceae; (PV) represent polar view and (EV) represent equatorial view while (SC) represent exine sculpturing with arrows; *I. cylindrica* (a) polar view, (b) equatorial view, (c) exine sculpturing; *S. spontaneum* (d) polar view, (e) equatorial view, (f) exine sculpturing; *S. verticillata* (g) polar view, (h) equatorial view, (i) exine sculpturing [Color figure can be viewed at wileyonlinelibrary.com]

gemmate. But this result is dissimilar to the previous work done by El-Amier (2015).

The *E. coracana* pollen grains are small to medium, prolate spheroidal monad, psilate, circular, and radially symmetrical in polar view. Polar and equatorial diameters are $26.25 \pm 0.88 \mu\text{m}$ and $21.60 \pm 0.68 \mu\text{m}$, respectively. *P* to *E* ratio is 1.21 and the same work has been done by Liu et al. (2014). Length and width of the pore are $4.470 \pm 0.525 \mu\text{m}$ and $6.8500 \pm 0.36236 \mu\text{m}$, respectively. The exine thickness of the *E. coracana* is $3.10 \pm 0.56 \mu\text{m}$, while exine sculpturing is gemmate-rugulate.

In *E. indica* the pollen grains are small, monad prolate, psilate, and circular in polar view. Polar and equatorial diameters are 28.75 ± 1.25

and $22.50 \pm 0.88 \mu\text{m}$, respectively. *P/E* ratio of *E. indica* is 1.27 while length and width of the pore is 3.85 ± 0.4279 and 4.05 ± 0.443 , respectively. Exine thickness is $4.90 \pm 0.18 \mu\text{m}$. Exine sculpturing is gemmate-reticulate, but this result is not similar to the previous work done by Muhammad, Ali, and Uddin (2019).

The pollen grains of *I. cylindrica* is small to medium, monad, prolate spheroidal psilate, circular, and radially symmetrical in polar view. Polar and equatorial diameters are $29.40 \pm 0.62 \mu\text{m}$ and $25 \pm 0.88 \mu\text{m}$, and polar to equatorial ratio (*P/E*) ratio is 1.17. Length and width of the pore are $2.700 \pm 0.21506 \mu\text{m}$ and $4.7500 \pm 0.8513 \mu\text{m}$, respectively. Exine thickness is $3.20 \pm 0.51 \mu\text{m}$ while exine sculpturing of *I. cylindrica* is gemmate-scabrate, but this result is dissimilar to the

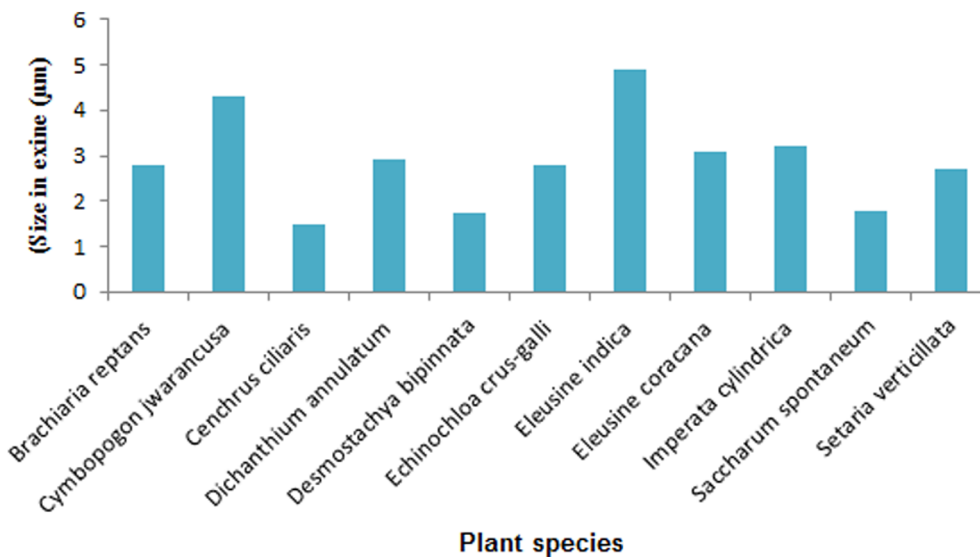


FIGURE 3 Graphical illustration of exine thickness variations [Color figure can be viewed at wileyonlinelibrary.com]

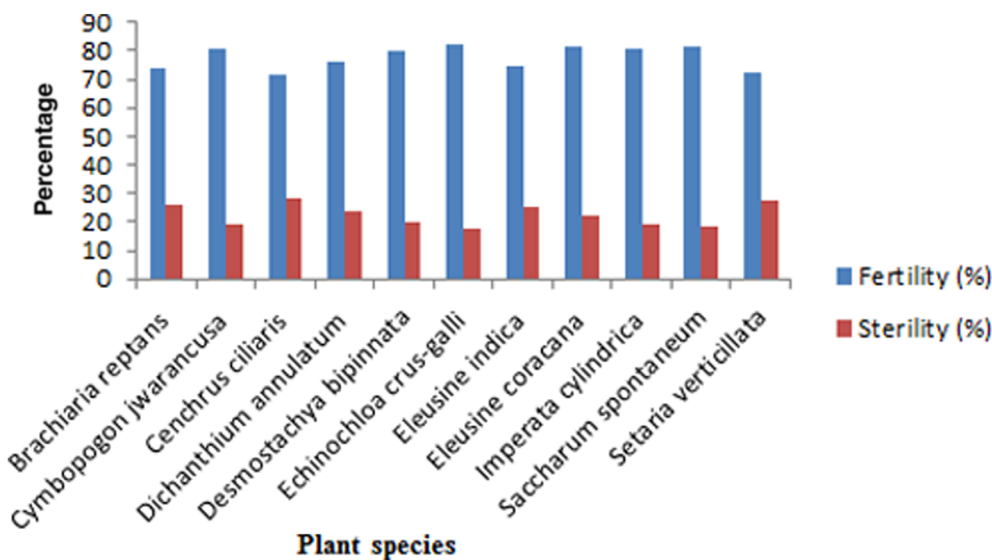


FIGURE 4 Represent fertility and sterility percentage of different species of family Poaceae [Color figure can be viewed at wileyonlinelibrary.com]

results of (Meo & Khan, 2004). *S. verticillata* pollen grains are small, monad prolate, psilate, and circular in polar view. Polar and equatorial diameters are $21.70 \pm 0.49 \mu\text{m}$ and $25.00 \pm 0.88 \mu\text{m}$, respectively. *P* to *E* ratio is 0.86. Length and width of the pore are $3.500 \pm 0.17678 \mu\text{m}$ and $2.700 \pm 0.2506 \mu\text{m}$. Thickness of exine is $1.70 \pm 0.21 \mu\text{m}$, while exine sculpturing is scabrate-verrucate. This result is dissimilar to the work of Ahmad et al. (2020).

Saccharum spontaneum pollen grains are medium, monad prolate, spheroidal psilate, circular, and radially symmetrical in polar view. Polar and equatorial diameters are $45 \pm 0.88 \mu\text{m}$ and $36.50 \pm 1.33 \mu\text{m}$, respectively. Polar to equatorial (*P/E*) ratio is 1.23. Length of pore is $4.00 \pm 0.30619 \mu\text{m}$ and width is $3.100 \pm 0.2384 \mu\text{m}$. Exine thickness is 1.80 ± 0.21 while sculpturing of exine is scabrate. For analyzing large data sets containing species from different locations around the world our current result is dissimilar with the previous work performed by Clark, Zhang, and Wendel (1995) and Baiakhmetov, Nowak, Gudkova, and

Nobis (2020). By using LM and SEM computer-assisted images analyze to identify different grass species by different structure, size, shape, and different surface ornamentation of most allergenic grass species from the research area. Based on SEM and LM it has been cleared that most of the Poaceae pollens are monoporate, psilate with sunken pore orientation and this results are similar to Khan et al. (2017). Cabauatan and Ramos (2012) conducted the study on the allergenicity of *S. spontaneum*, *I. cylindrical* through protein file analysis. The result of their study reveals that the pollen grains are responsible for causing allergic reactions.

Studies conducted by D'Amato et al. (2007) in Europe reported that 400 herbaceous grasses which pollinates through wind are present in Europe. In their study three grasses are found to be allergy causing, that is, *Phleum pratense*, *Dactylis glomerata*, and *Alopecurus pratensis*. *Secale cereale*, which has unusually high rate of pollen grains production, is another strong cause of allergens (Esch, Hartsell, Crenshaw, & Jacobson, 2001). Although, with very little exclusion, all

pollen grains of grasses exhibit a very high degree of cross reactivity (Aalberse, 1992; Martin, Mansfield, & Nelson, 1985).

5 | CONCLUSIONS

The present study investigates the micro-morphological attributes of allergenic taxa of family Poaceae from Islamabad, Pakistan. Both quantitative and qualitative characters of pollen were studied using LM and SEM. The key findings include *P/E* ratio which is highest in *E. crus-galli* (1.54 μm). Accordingly, the maximum exine thickness, polar diameter, and equatorial diameter was observed in *E. indica* (4.90 μm), *E. crus-galli* (56.00 μm), and *C. ciliaris* (45.00 μm) and maximum pore size was observed in *E. coracana* (4.470 μm). Similarly, the pollen qualitative characters of pollen indicate that poaceae species are distinguished by varying pore orientation from sunken to prominent pollen having prolate, prolate spheroidal, and suboblate in shapes. In addition, Poaceae pollen has numerous kinds of exine ornamentation. These various types of exine sculpturing are very important taxonomic characters and may be helpful in correct identification of these allergenic grass species. It has been found that the identification of pollen having allergenic potential plays a role in determining major or minor nectar pollen sources and is useful for establishing pollen literature. Such records are necessary for implementing floral management strategies in Pakistan for patients suffering from allergies to avoid staying near the identified allergenic flora during flowering season.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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