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RESEARCH ARTICLE

Heteromorphy in pollen grains of the tropical and subtropical selected Fabaceae species

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Abstract Pollen grains have long fascinated biologists who used their significant intra and inter-specific diversity as a marker to infer profiles of past and present vegetation and environment. Our study addresses the question of the diversity in pollen morphology at the intra and inter-specific level: how different are pollen grains of the same species sampled from the tropical and subtropical regions of China and Pakistan. Such differences are expected and are well known to palynologists, but at the same time technically challenging to quantify. We used both light microscopy (LM) and scanning electron microscopy (SEM), to explore the intra and inter-specific pollen variability and its taxonomic relevance in selected Fabaceae taxa. Pollen features were described in terms of size, shape, apertures and exine sculpturing and were then subjected to correlation and principal component analysis (PCA). A high morphological disparity and phenotypic plasticity were found in Leguminaceae species. A weak intra-specific variation was found in the exine sculpturing while it was high when considering the polar axis and equatorial diameter, colpus length and width, shape and P/E ratio. Variation in environmental factors explained a significant portion of the naturally occurring variation in pollen size. Relatively large size pollen was found in the warmer subtropical region, which leads to the conclusion that most of the pollen traits of Fabaceae species are plastic in nature. This intra-specific variability in pollen futures could indicate that plants are trying to adapt their pollen morphology to environmental conditions such as maximum temperature, and humidity.

Keywords Fabaceae · Pollen morphology · Intra and inter-specific variability · Tropical and subtropical environment

Introduction

Family Fabaceae is among the largest families of angiosperms after Asteraceae and Orchidaceae comprises almost 770 genera and 19,500 species and

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is widely distributed in tropical and sub-tropical regions (LPGW 2017; Soares et al. 2024). It has been divided into 3 sub families namely Caesalpinioideae Mimosoideae and Faboideae or Papilionoideae previously (Lewis et al. 2005). As of LPGW (2017), the Fabaceae family had major changes in its classification, the advances in molecular biology allowed the recognition of monophyletic groups, which permitted a more natural and homogeneous grouping of taxa, increasing the number of three traditional subfamilies to six subfamilies (Papilionoideae, Caesalpinioideae, Cercidoideae, Detarioideae, Dialioideae and Duparquetioideae). Based on APG IV, the Mimosoideae is now considered as a clade, within the Subfamily Caesalpinioideae. The Mimosoid clade includes almost 50 genera and 3000 species (Duan et al. 2021), subfamily Papilionoideae with 503 genera and almost 14,000 species (Lewis et al. 2005). Caesalpinioideae is the second largest subfamily of legumes (Leguminosae) with ca. 4680 species and 163 genera (Bruneau et al. 2024). Coupled with the addition of the Mimosoideae clade, the subfamily Caesalpinioideae is the one that has had the greatest number of modifications with the taxonomic research carried out, especially at the genus level.

Pollen grains exhibit changes in their ultrastructure, morphology, biochemistry, and physiology under the effect of changes in environmental conditions (Vasilevskaya 2022). Gottardini et al. 2004). Recently, pollen morphological characters have been used for the assessment of environmental quality, both in the past and in the present (Vasilevskaya 2022). Pollen grains in their fresh form can provide important information about the phenology of plants, gene flow ecophysiology and population dynamics (Savolainen et al. 2007). Many journals and books regarding palynology have described significant inter and intraspecific variability of the shape, size and other features of pollen grains. Even a slight variation in the size of a grain has significant consequences on the number of grains produced if the same amount of resources is used for the production of pollen. While pollen production may be strongly constrained by some environmental influences (Mousavi et al. 2024), the adaptive impact of inter and intraspecific variability in the number and size of pollen tradeoffs and morphological characters of pollen has rarely been investigated in this context (Ejsmond et al. 2011). The production of pollen grains is closely associated with temperature through which the optimization of the size and number of pollen grains is produced. In contrast, temperature does not considerably affect the shape of pollen grains (Hedhly et al. 2005). The relationship between these environmental pressures and the adaptive significance of inter- and intraspecific variation in pollen morphology is still not fully understood. Further research is needed to explore how these variations contribute to reproductive success, plant fitness, and ecosystem dynamics, particularly in the context of changing environmental conditions and their impact on pollen production and function.

Morphological research of pollen grains is an emerging field that draws the attention of many researchers of various fields due to their vast applications and also in solving complex taxonomic problems of interrelationships at different taxonomic levels (Ali et al. 2021; Hameed et al. 2021; Nabila et al. 2022; Khan et al. 2024; Butt et al. 2024) by using multiple microscopic techniques. It has become an integrated part of the multidisciplinary and collaborative approach to plant systematics and evolution (Zhang et al. 2017; De Almeida et al. 2024). The SEM and LM have an important role in investigating the microscopic characters of plant materials such as pollen and its taxonomic relevance in the Fabaceae taxa (Talip et al. 2024).

The morphological characters of pollen grains, ultra-structure and ultra-sculpture of Papilionoideae and Caesalpinioideae taxa are investigated in search of morphological traits of taxonomic importance by using light microscopy (LM), scanning (SEM) as well as transmission electron microscopy (TEM) (Antonio-Domingues et al. 2022). Although most of the research has documented the pollen morphological traits of the sub-family Papilionoideae (Bahadur et al. 2023; Wali and Akhtar 2017; Bano et al. 2019) subfamily Mimosoideae (Perveen and Qaiser 1998a, b, c; Khan et al. 2021; Bahadur et al. 2022a, b, c, d; Barduzzi et al. 2024) and sub-family Caesalpinioideae (Antonio-Domingues et al. 2018; Ullah et al. 2022; Mas'udah et al. 2024) and selected members of Fabaceae (Pranav Raj et al. 2024) to evaluate their taxonomic potential, correctly identify, discriminate and define species boundaries at various taxonomic levels (subfamily, tribe, genus and species). However, no specific study was yet documented regarding the extent of intra and inter-specific variability in pollen morphology of tropical and subtropical Fabaceae species. Hence, further research is necessary to collect a wide range of Fabaceae species from various tropical and subtropical regions. This should include both common and rare species to capture the full spectrum of morphological diversity.

Although, most of the research has been conducted on pollen morphological aspects of family Fabaceae from different geographical regions of the world. But no comprehensive record for the unique pollen morphology of the sub families i.e. Caesalpinioideae and Papilionoideae from tropical and sub-tropical regions was reported in detail. Therefore, more research is required to fill this research gap. Keeping in view this, the current research aims to document the common species diversity of Fabaceae from the tropical (Hainan) and sub-tropical (Punjab, Pakistan) regions and to explore the intra and inter-specific variability in some Fabaceae species by using multiple microscopic techniques. Understanding this variability is crucial for the conservation, ecological insight, and taxonomic clarification of the family Fabaceae.

Material and methods

Collection, identification and study area

Various field trips were arranged in subtropical regions of Hainan Island south China and tropical regions of Pakistan in the flowering season of the year 2021 (Table 1). Plants were first collected and compared with the Herbarium specimens for identification and then verified with the help of available literature, The World Flora Online (www.worldfloraonline.org). The samples from the tropical region were deposited to the Herbarium College of Forestry, Hainan University Haikou China, while the subtropical species were deposited to the Herbarium Department of Botany, University of Kotli Azad Jammu and Kashmir.

Hainan Island is located 18° 09′–20° 10′ North, 108° 03′–111° 03′ in South China (Fang and Bailey 1998). Hainan is one of the largest Island of the Indo-Burma Biodiversity Hotspot and preserved the extensive tropical forest in China (Chen et al. 2014). Species diversity in the island has mostly narrow distribution and few individuals and have more chances of becoming endangered and extinct (Frankham 1997; Francisco-Ortega et al. 2000). The island is characterized by a tropical monsoon climate with a wet season starting from May to October while the dry season is from November to April. The mean annual temperature is about 22 °C and the annual mean precipitations are ranging from 2350 to 2651 mm (Long et al. 2011, 2012; Wang et al. 2016). Overall, we investigated the 19 bioclimatic variables for Hainan Island (Fig. 1).

Punjab is a province located in the central-eastern region of Pakistan experiences a continental climate, characterized by extreme variations in temperature and seasonal weather patterns. It is the second largest province of Pakistan among the four provinces (Balochistan, Khyber Pakhtunkhwa, Punjab, and Sind) and is geographically located in between 27.70–33.80° N and 69.38–75.38° E. Punjab's climate can be classified as semi-arid or arid for the most part, with limited spatiotemporal variability in climate indicators such as relative humidity, precipitation, and maximum, mean, and minimum temperatures (Amin et al. 2018). The monsoons, Punjab's ultimate hydrometeorological reserve, are responsible for over half (59%) of the state's yearly precipitation. The temperature rises in the spring, from March to May, and stays that way until the summer (Jahangir et al. 2016; Syed et al. 2021). In addition, although weather patterns have been erratic since the early 1970s, the monsoons are predicted to reach Punjab by the end of May. Here we analyzed the 19 bioclimatic variable for the subtropical region of Punjab province Pakistan (Fig. 2).

Bioclimatic variables are derived from the monthly temperature and rainfall values in order to produce more biologically meaningful variables. These variables are mostly used in species distribution modeling and related ecological modeling techniques. The bioclimatic variables represent annual trends (e.g., mean annual temperature, annual precipitation) seasonality (e.g., annual range in temperature and precipitation) and extreme or limiting environmental factors (e.g., temperature of the coldest and warmest month, and precipitation of the wet and dry quarters). A quarter is a period of 3 months (1/4 of the year). The bioclimatic variables shown in Figs. 1 and 2 are coded as follows:

BIO1: Annual mean temperature, BIO2: Mean diurnal range (Mean of monthly (max temp–min temp)), BIO3: Isothermality (BIO2/BIO7) (×100), BIO4: Temperature seasonality (standard deviation ×100), BIO5: Max temperature of warmest month,

Table 1 Selected Fabaceae species sampled from tropical and subtropical regions

Species	A	B	C	D
Tropical				
Acacia auriculiformis A.Cunn. ex Benth.	Sanya	18.26415	109. 52084	119
Acacia confusa Merr	Wanning	18.69396	110.2317	118
Albizia lebbekoides (DC.) Benth.	Wanning	18.69396	110.2317	118
Alysicarpus ovalifolius (Schum.) Leonard	Qiong Zhong	19.25343	109.38799	445
Arachis duranensis Krapov. & W.C.Greg.	Haikou	20.062363	110.3186713	3
Bauhinia purpureae L.	Haikou	20.062363	110.3186713	3
Bauhinia variegata L.	Haikou	20.062363	110.3186713	3
Caesalpinia pulcherrima (L.) Sw.	Sanya	18.26415	109. 52084	119
Calliandra haematocephala Hassk.	Sanya	18.26415	109. 52085	119
Canavalia rosea (Sw.) DC.	Changjiang	19.2197	108.9978	33
Cassia javanica L.	Changjiang	19.33905	108.21	33
Centrosema pubescens Benth.	Wanning	18.69396	110.2317	118
Crotalaria pallida Aiton	Wanning	18.69396	110.2317	118
Delonix regia (Bojer ex Hook.) Raf.	Haikou	20.062363	110.3186713	3
Leptodesmia microphylla (Thunb.) H.Ohashi & K.Ohashi	Wanning	18.69396	110.2317	118
Grona triflora (L.) H.Ohashi & K.Ohashi	Changjiang	19.2197	108.9978	33
Entada phaseoloides (L.) Merr.	Wanning	18.69396	110.2317	118
Erythrina crista-galli L.	Changjiang	19.2197	108.9978	33
Indigofera colutea (Burm.f.) Mer	Changjiang	19.2197	108.9978	33
Leucaena leucocephala (Lam.) de Wit	Qionghai	19.2584	110.4746	3
Mimosa diplotricha Sauvalle	Wanning	18.69396	110.2317	118
Mimosa pudica L.	Changjiang	19.2197	108.9978	33
Rhynchosia minima (L.) DC.	Changjiang	19.2197	108.9978	33
Saraca asoca (Roxb.) Willd.	Haikou	20.062363	110.3186713	3
Senna surattensis (Burm.f.) H.S.Irwin & Barneby	Haikou	20.062363	110.3186713	3
Sesbania cannabina (Retz.) Pers.	Sanya	18.2263	109.5007	275
Zornia gibbosa Span.	Haikou	20.062363	110.3186713	3
Subtropical				
Acacia auriculiformis A.Cunn. ex Benth.	Wador	30.0489	70.6455	124
Acacia confusa Merr	Shah Kot Sheikhupura	31.5709	73.4853	190
Albizia lebbekoides (DC.) Benth.	Kot Shahan Gujranwala	32.2289	74.1812	88
Alysicarpus ovalifolius (Schum.) Leonard	Bhera, Sargodha	32.4770	72.9136	196
Arachis duranensis Krapov. & W.C.Greg.	Chakwal	32.9328	72.8630	498
Bauhinia purpureae L.	Head Marala Sialkot	32.6724	74.4644	250
Bauhinia variegata L.	Islamabad	33.6844	73.0479	507
Caesalpinia pulcherrima (L.) Sw.	Pabbi Forest	32.8336	73.8387	302
Calliandra haematocephala Hassk.	Ghazi Ghat	30.0937	70.8863	116
Cassia javanica L.	Pabbi Forest	32.8336	73.8387	302
Crotalaria pallida Aiton	Wador	30.0489	70.6455	124
Delonix regia (Bojer ex Hook.) Raf.	Sukheki Hafizaabad	31.8602	73.5064	207
Leptodesmia microphylla (Thunb.) H.Ohashi & K.Ohashi	Ghazi Ghat	30.0937	70.8863	116
Grona triflora (L.) H.Ohashi & K.Ohashi	Chicherwali Gujranwala	32.1620	74.2183	226
Entada phaseoloides (L.) Merr.	Chicherwali Gujranwala	32.1620	74.2183	226
Erythrina crista-galli L.	Chicherwali Gujranwala	32.1620	74.2183	226
Indigofera colutea (Burm.f.) Mer	Rahwali Gujranwala	32.2479	74.1680	227

Table 1 (continued)

Species	A	B	C	D
Leucaena leucocephala (Lam.) de Wit	Islamabad	33.6844	73.0479	507
Mimosa pudica L.	Gujrat	32.5731	74.1005	137
Rhynchosia minima (L.) DC.	DC Garden, DG Khan	30.0489	70.6455	123
Saraca asoca (Roxb.) Willd.	Peshawar	34.0151	71.5249	331
Senna bicapsularis (L.) Roxb.	Alipur, Kot Sultan	30.77501	70.936	210
Senna obtusifolia (L.) H.S.Irwin & Barneby	Rawalpindi	33.5651	73.0169	508
Senna surattensis (Burm.f.) H.S.Irwin & Barneby	Bhera, Sargodha	32.4770	72.9136	196
Sesbania cannabina (Retz.) Pers.	Chicherwali Gujranwala	32.1620	74.2183	226

(A) locality, (B) latitude (°), (C) longitude (°), (D) above sea level in meter (m)

BIO6: Min temperature of coldest month, BIO7 , Temperature annual range (BIO5-BIO6), BIO8 : Mean temperature of wettest quarter, BIO9: Mean temperature of driest quarter, BIO10: Mean temperature of warmest quarter, BIO11: Mean temperature of coldest quarter, BIO12: Annual precipitation, BIO13: Precipitation of wettest month, BIO14: Precipitation of driest month, BIO15: Precipitation seasonality (Coefficient of Variation), BIO16: Precipitation of wettest quarter, BIO17 = Precipitation of driest quarter, BIO18: Precipitation of warmest quarter, BIO19, Precipitation of coldest quarter, This scheme follows that of ANUCLIM, except that for temperature seasonality the standard deviation was used because a coefficient of variation does not make sense with temperatures between −1 and 1).

Scanning electron microscopy

For the SEM analysis, we followed the previously published protocol with little modification (Ali et al. 2021; Butt et al. 2021). The mature flowers were selected followed by anthers separation, then crushed on a glass slide by adding a few drops of ethanol. The pollen was then transferred and stored in small Eppendorf tubes for further SEM analysis. The Micropipette was used to take the pollen samples from the tubes and put them on stubs with the help of double-sided adhesive tape, sputtered with Gold for 30 s first and then with Platinum for 30 s by using Leica Microsystem GmbH made in Austria, a high vacuum coater ACE600. The prepared stub was then put in SEM (Model Thermo Scientific, Model: verios g 4uc) installed in the analytical and testing center of Hainan University. The pollen micrographs were then taken following previous study (Bahadur et al. 2022c, d, a, b). Similarly, the species sampled from tropical regions (Pakistan) were analyzed through scanning electron microscopy. The pollen grains were placed directly on stubs by applying double-sided adhesive tape. With the help of gold-palladium, the samples were sputtered and then observed under a microscope (Model JEOL JSM-5910). For the SEM micrographs, Polaroid P/N 665 film was used. For the pollen terminology, we mostly followed the previously published literature (Halbritter et al. 2018; Punt et al. 2007).

Light microscopy

For the light microscopic analysis, we mostly followed the previously published study with little modification (Ullah et al. 2021; Butt et al. 2024). A mature flower was selected and the anthers were separated from it. The anthers were put on a glass slide and a few drops of acetic acid were added and crushed with a needle. The debris was removed carefully and then covered it with a cover slip. The prepared samples were then observed under a light microscope and various micrographs were taken at the magnification of 40X.

Statistical analysis

For the statistical analysis, we used the quantitative data of the pollen including the polar axis, equatorial diameter, colpus length and width and P/E ratio by using Image J software. Ten grains of a single species were measured and then calculated the mean values. Based on the mean values of quantitative traits, the multivariate Pearson correlation and principal

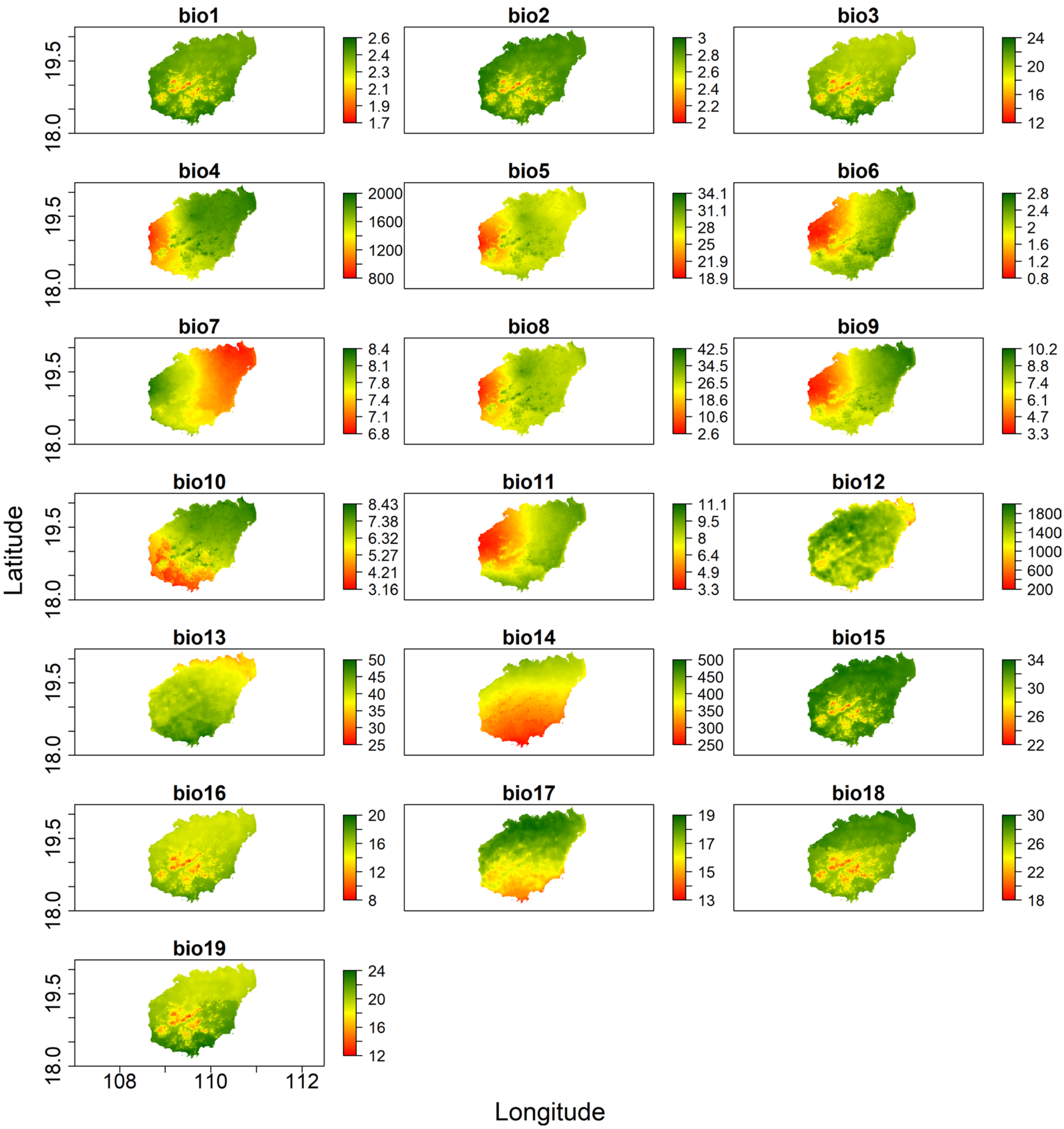


Fig. 1 The 19 bioclimatic variables of Hainan Island

component analysis (PCA) were performed by using R.4.4.1 to show the association and differences between the species (Figs. 3, 4).

To analyze the environmental data of the study area, we first collect global data from the updated version of WorldClim 2.1. To achieve this, we used different packages in R.4.4.1 such as *ggplot2, raster, tidyr, sf,* and *dplyr*. To extract the bioclimatic data of study area, we used the function "*crop*".

For the PCA, we used different packages such as (*FactoMiner, factoextra* and *ggplot2*). The biplot-PCA variables were constructed using the function *fviz_pca_var*. Likewise, for PCA-individuals we used *fviz_pca_ind* and for PCA-Biplot the *fviz_pca_biplot* functions were used.

Results

The pollen morphological study of selected Fabaceae species sampled from the tropical and subtropical regions of Hainan Island China and Pakistan has been examined. We used scanning electron microscopy (SEM), a powerful morphometric approach to explore the intra and inter-specific variability in selected taxa of the Fabaceae. We summarized the pollen morphology of specimens studied and facilitated the intra and inter-specific comparison of pollen features. The pollen quantitative results were illustrated in (Figs. 3, 4) and were presented in (Tables 2, 3, 4) while the qualitative features were presented in (Tables 5, 6). Likewise, the pollen SEM micrographs were illustrated in (Figs. 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18) and the LM micrographs were presented in (Figs. 18, 19).

Subfamily papilionoideae

The selected taxa of Papilionoideae were analyzed by using SEM techniques. The pollen SEM images are illustrated in (Figs. 3, 4, 5, 6, 7, 8, 9). The pollen description of each species is given below in detail.

Arachis duranensis: The pollen dispersal units of *A. duranensis* were monad, heteropolar, elliptical, operculate, lolangate and tricolpate. Pollen size 20.21 × 20 µm, subspheroidal, P/E ratio 1.02 µm, colpus surface reticulate, rarely scabrate and verrucate, colpus size 19.92 × 6.17 µm. Exine was micro-reticulate and homobrochate. The thin reticulas psilate. The lumena were filled with 1–4 granulate patterns. The other individual of this species was sampled from the subtropical region of Pakistan Bhera, Sargodha with an altitude of 196 m above sea level and only differences were found in sculpturing as perforate, and size was 26.1 × 22.1 µm, radial symmetry, and P/E ratio 1.1. The surface of the colpi is finely reticulate, size 7.5 × 2.1 µm (Fig. 3a–c).

Alysicarpus ovalifolius: The pollen dispersal unit of *A. ovalifolius* (syn: *A. vaginalis*) was monad, isopolar, lolangate, elliptical and pollen type 3-zonocoplate. Pollen size 32.64 × 33.99 µm, sub-spheroidal, radial symmetry, P/E ratio 0.97. Colpus sculpturing was mega to micro-gemmate and scabrate, colpus size 16.55 × 5.1 µm (Fig. 8e–f). Exine sculpturing fossulate and areolate. The other individual of this species was sampled from the subtropical region of Pakistan Bhera, Sargodha with an altitude of 196 m above sea level, and showed differences in sculpturing such as finely rugulate or fossulate and size was 28.1 × 27.7 µm, radial symmetry, P/E ratio 1. Sculpturing of colpi is micro-rugulate, size 8.2 × 1.6 µm.

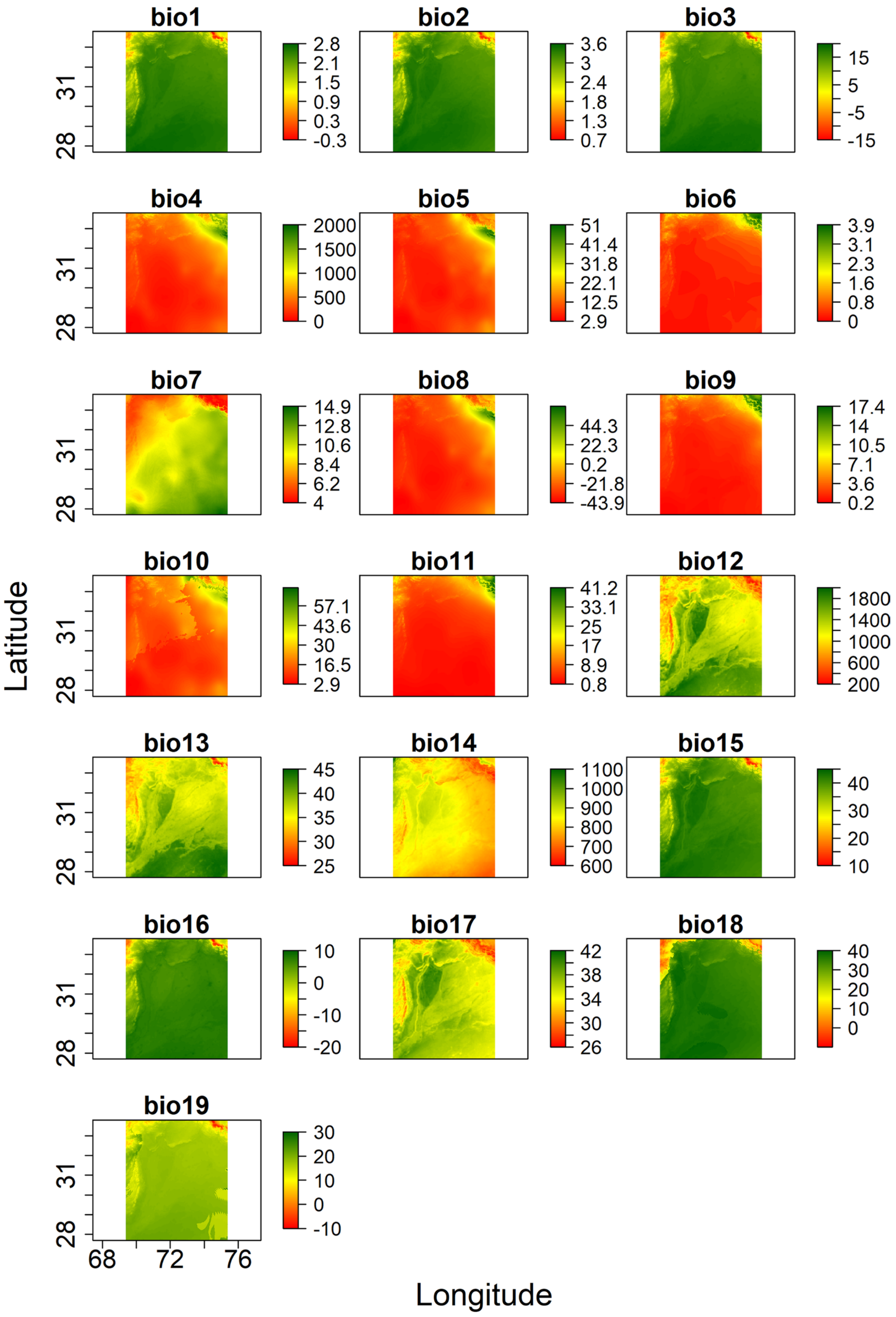
Centrosema pubescens: The pollen dispersal unit of *C. pubescens* was monad, lolangate, polarity isopolar, pollen type tri-colpate. Pollen size 17.3 × 17.78 µm, sub-spheroidal, radial symmetry, P/E ratio 0.98 µm. Colpus surface scabrate, colpus size 17.1 × 3.24 µm. Exine micro-reticulate to perforate while the polar area perforate to scabrate (Fig. 9a–c).

Canavalia rosea: The pollen dispersal unit of *C. rosea* was monad, tricolporate. Pollen size 19.17 × 17.29 µm, sub-spheroidal, P/E ratio 1.11 µm. Colpus surface scabrate and verrucate, colpus size 10.21 × 3.38 µm. Pollen radial symmetric, exine reticulate and heterobrochate, reticula surface microscabrate, lumen filled with microgranulate structure. A bridge and mergo are present (Fig. 5g–i).

Crotalaria pallida: The pollen dispersal unit of *C. pallida*, tricolporate. The size of the pollen was 19 × 14.4 µm, sub-spheroidal, bilateral symmetry, P/E ratio 1.32 µm. Colpus surface scabrate and verrucate. Exine reticulate, homobrochate, scabrate, reticula flat and psilate, bridge present and mergo absent (Figs. 6f–g, 7a–c). The second sample from the subtropical region of Pakistan Wador, Punjab with an altitude of 124 m above sea level showed differences in sculpturing as it is micro reticulate and size was 24.6 × 16.6 µm, radial symmetry, P/E ratio 1.4. Surface of colpi is scabrate to psilate, size 7.5 × 2.9 µm (Fig. 3d–f).

Dalbergia odorifera: The pollen dispersal unit of *D. odorifera* was monad, isopolar, lolangate, elliptical, tricolporate. Pollen size 19.11 × 53.29 µm, Peroblate, radial symmetry, P/E ratio 0.36 µm. Colpus brevicolpus and surface verrucate, scabrate and gemmate, colpus size 7.9 × 4.66 µm. Exine psilate rarely scabrate and perforate (Fig. 8b–d).

Leptodesmia microphylla: The pollen dispersal unit of *L. microphylla* (syn: *Desmodium microphyllum*) was monad, the pollen type tricolporate. 17.2 × 15.74 µm, sub-spheroidal, symmetry bilateral, P/E ratio 1.1 µm. Colpus scabrate rarely perforate, colpus


bio1
2.8
2.1
1.5
0.9
0.3
-0.3
bio2
3.6
3
2.4
1.8
1.3
0.7
bio3
15
5
-5
-15
bio4
2000
1500
1000
500
0
bio5
51
41.4
31.8
22.1
12.5
2.9
bio6
3.9
3.1
2.3
1.6
0.8
0
bio7
14.9
12.8
10.6
8.4
6.2
4
bio8
44.3
22.3
0.2
-21.8
-43.9
bio9
17.4
14
10.5
7.1
3.6
0.2
bio10
57.1
43.6
30
16.5
2.9
bio11
41.2
33.1
25
17
8.9
0.8
bio12
1800
1400
1000
600
200
bio13
45
40
35
30
25
bio14
1100
1000
900
800
700
600
bio15
40
30
20
10
bio16
10
0
-10
-20
bio17
42
38
34
30
26
bio18
40
30
20
10
0
bio19
30
20
10
0
-10
31
28
68
72
76
Latitude
Longitude

◂**Fig. 2** The 19 bioclimatic variables of the subtropical region of Punjab Pakistan

size 13.8 × 2.51 µm. Exine reticulate, muri thin and microscabrate, lumen filled with microscabrate pattern (Fig. 7d–f). The second sample from the subtropical region of Pakistan Ghazi Ghat, Punjab with an altitude of 116 m above sea level showed differences in exine sculpturing as it is mega-reticulate, muri thick with a large lumen filled with scabrate structure, size was 22.4 × 17.3 µm, radial symmetry, P/E ratio 1.2. The surface of the colpi is densely scabrate, size 9.4 × 2.1 µm (Fig. 3g–i).

Grona triflora: The pollen dispersal unit of *G. triflora* (syn: *Desmodium triflorum*) was monad, and the pollen type was tri-colporate. The pollen size was noted as 22.2 × 20.6 µm, prolate, radial symmetry, P/E ratio 1.08 µm, colpus size 14.39 × 2.82 µm. Exine Scabrate, verrucate and rarely perforate. Polar area densely verrucate, mergo present (Fig. 6a–c). The second sample from the subtropical region of Pakistan Chicherwali, Gujranwala with an altitude of 226 m above sea level showed variation in sculpturing as it is reticulate and psilate, size was 32.5 × 27.4 µm, radial symmetry, P/E ratio 1.1. Surface of colpi is psilate, size 11.1 × 3.4 µm (Fig. 4a–c).

Erythrina crista-galli: The pollen dispersal unit of *E. crista-galli* was monad, isopolar, elliptical, lolangate, tricolporate. Pollen size 34.37 × 21.33 µm, prolate, radial symmetry, P/E ratio 1.15 µm. Colpus brevicolpus and surface scabrate and verrucate, colpus size 12.62 × 6.65 µm. Exine mega-reticulate and hetrobrochate, reticula thin psilate and microscabrate surface, lumen filled with micro and macro-scabrate pattern. Polar area reticulate and prominent mergo present (Fig. 8j–k). The second sample from the subtropical region of Pakistan Chicherwali, Gujranwala with an altitude of 226 m above sea level showed intraspecific variability in sculpturing as it was micro reticulate, and size was 22.1 × 19.2 µm, radial symmetry, P/E ratio 1.1. Colpus surface is scabrate, size 10.5 × 4.2 µm (Fig. 4d–f).

Indigofera colutea: The pollen dispersal unit of *I. colutea* was monad, tricolporate. Pollen size 16.92 × 14.46 µm, prolate, P/E ratio 1.18 µm. Colpus surface verrucate and scabrate, colpus size 11.91 × 2.67 µm. Exine micro-reticulate, perforate and granulate (Fig. 9d). The second sample from the subtropical region of Pakistan Rahwali Gujranwala with an altitude of 227 m above sea level showed variations in exine sculpturing as it is scabrate to rarely perforate, and size was 20.5 × 23.5 µm, P/E ratio 0.9. The colpus surface was perforate, size 5.3 × 2.2 µm, redail symmetry (Fig. 4g–i).

Indigofera hirsuta: The pollen dispersal unit of *I. hirsuta* was monad, bilateral symmetry, tricolporate. Pollen Colpus sunken, brevicolpus. Exine micro-reticulate and fossulate. The polar area of the pollen was observed as micro-reticulate and perforate (Fig. 8d).

Macroptilium atropurpureum: The pollen dispersal unit of *M. atropurpureum* was monad, tricolporate, hetero-aperturate. Pollen size 23.91 × 22.35 µm, sub-spheroidal, both bilateral and radial symmetry, P/E ratio 1.07 µm (Fig. 9e–g). Colpus surface rugulate, verrucate and scabrate, colpus size 15.49 × 4.92 µm. Exine reticulate, scabrate, perforate, and rarely gemmate and verrucate, polar area psilate (Figs. 6h–i and g–i).

Macroptilium lathyroides: The pollen unit of *M. lathyroides* was monad, isopolar, elliptical, lolangate, tricolporate, and brevicolpus. Colpus surface scabrate to psilate. Exine psilate rarely scabrate, polar area psilate.

Rhynchosia minima: The pollen dispersal unit of *R. minima* was monad, pollen tricolporate. Pollen size 25.19 × 17.44 µm, prolate, bilateral symmetry, P/E ratio 1.45 µm. Colpus size 18.89 × 1.3 µm. Exine mega-reticulate and homobrochate, reticula flat and psilate, (Fig. 6d–e). The second sample from the subtropical region of Pakistan DC Garden, Multan with an altitude of 123 m above sea level showed differences in sculpturing as it is reticulate with psilate lumen, and size was 23.4 × 26.3 µm, radial symmetry, P/E ratio 0.9. Colpus surface is psilate, size 11.2 × 3.1 µm (Fig. 5a–c).

Sesbania canabina: The pollen unit of *S. canabina* was monad, tricolporate. 17.03 × 17.48 µm, subspheroidal, radial symmetry, P/E ratio 0.98 µm. Colpus surface verrucate, colpus size 7.07 × 2.19 µm. Exine reticulate rarely verrucate, reticula thin and sculpturing psilate to regulate. Polar area psilate to perforate (Fig. 7h–i). The second sample from the subtropical region of Pakistan Chicherwali Gujranwala with an altitude of 226 m above sea level showed variation in ornamentation as it is verrucate to slightly perforate, and size was 36.8 ×

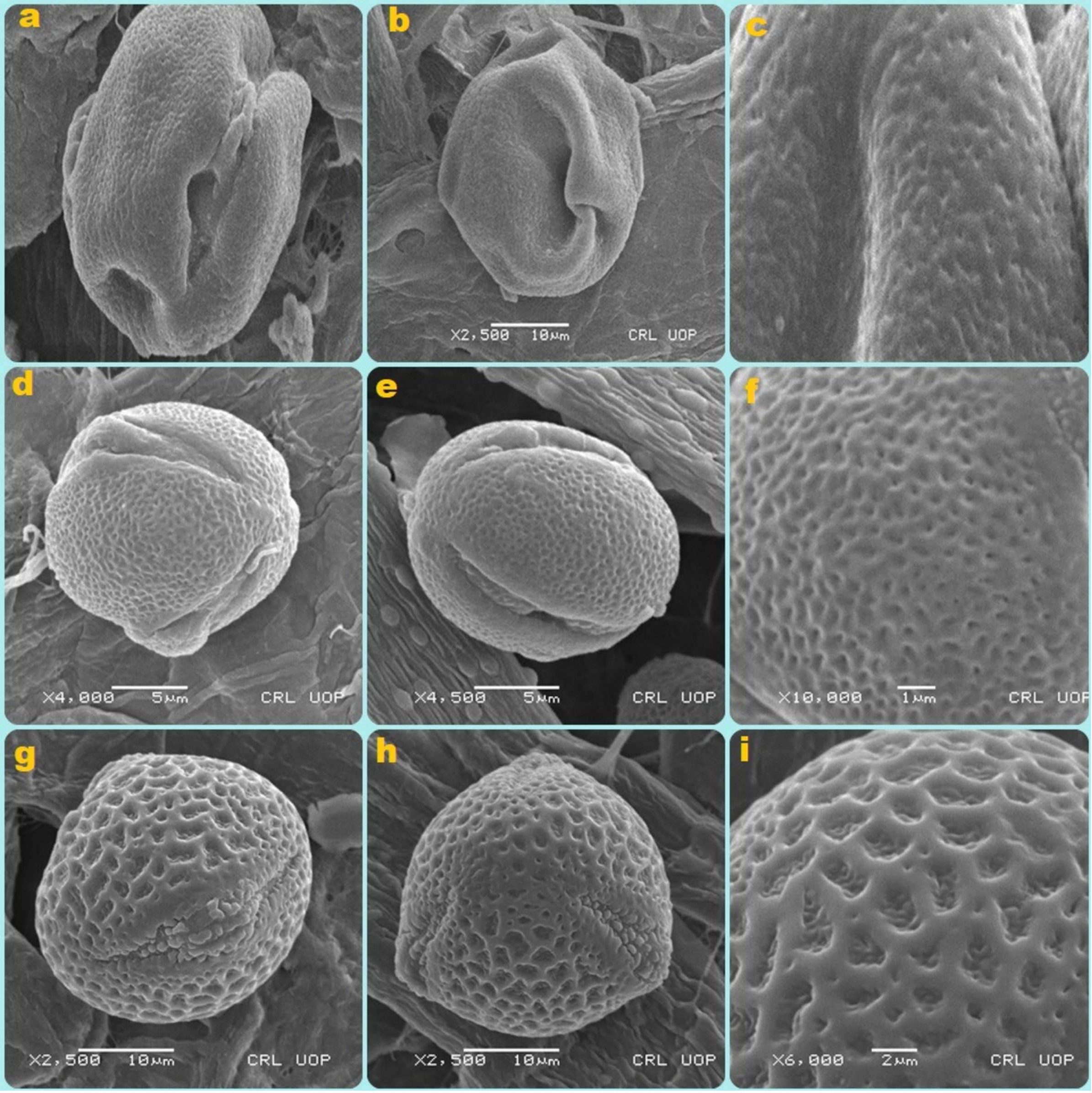


Fig. 3 Subfamily Papilionoideae; *Arachis duranensis* (**a**–**c** subtropical), **a** equatorial view, **b** dehydrated pollen, **c** close view of exine. *Crotallaria pallida* (**d**–**f** subtropical), **d** colporate pollen, **e** oblique equatorial view, **f** close view of exine. *Leptodesmia microphylla* (**g**–**i** subtropical) **g** equatorial view, **h** polar view, **i** close view of exine

27.4 µm, radial symmetry, P/E ratio 1.3. The Colpus surface is perforate, size 9.4 × 4.5 µm (Fig. 5d–f).

Mimosoid clade of the subfamily *Ceasalpinioideae*

The Mimosoid clade were analyzed by using scanning electron microscopic techniques. The SEM micrographs of the pollen are illustrated in (Fig. 10–11). The details of pollen traits for each species are given below.

Acacia confusa: The pollen dispersal unit of *A. confusa* is 12-grain polyads, rhomboidal in polar view, bilaterally symmetrical, hetero-polar and inaperturate. Exine mega-reticulate and sometimes scabrate, reticula thick, flattened and rarely scabrate, lumina filled with micro-scabrate structure

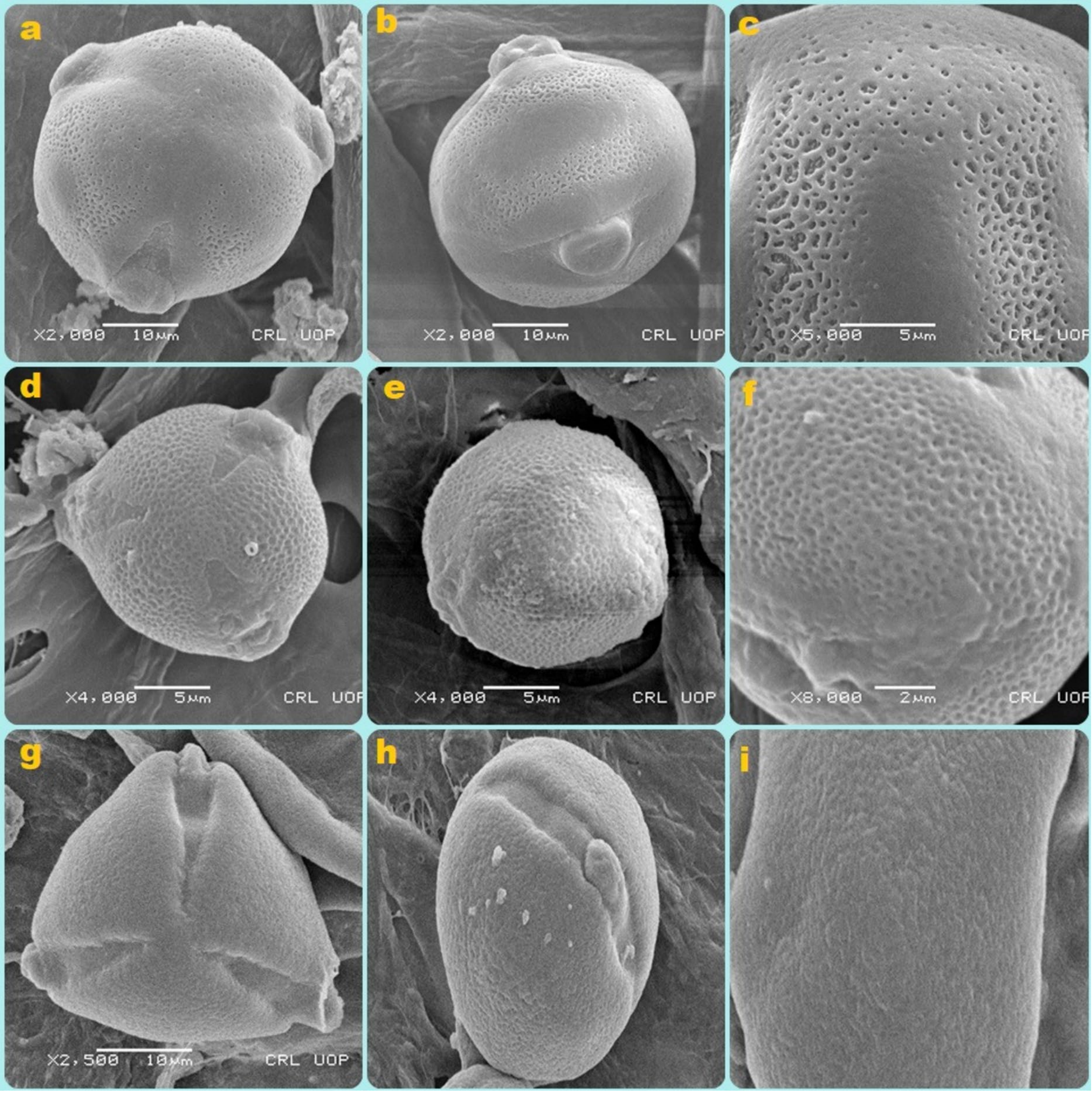


Fig. 4 Subfamily Papilionoideae: *Grona triflora* (**a**–**c** subtropical), **a** tricolporate **b** equatorial view, **c** mesocolpium and reticulate exine, *Erythrina crista-galli* (**d**–**f** subtropical), **d** tricolporate **e** polar view of the pollen, **f** close view of the exine. *Indigofera colutea* (**g**–**i** subtropical), **g** tricolporate pollen, **h** aperture, **i** close view of exine

Table 2 Eigenvalues of the correlation matrix

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8	Dim.9	Dim.10
Variance	4.519	2.199	1.385	0.659	0.575	0.281	0.202	0.126	0.036	0.018
% of var.	45.194	21.986	13.849	6.587	5.748	2.811	2.025	1.257	0.358	0.185
Cumulative % of var.	45.194	67.18	81.03	87.617	93.365	96.176	98.201	99.457	99.815	100

Table 3 Extracted eigenvectors of the pollen traits

Variables	Dim.1	ctr	cos2	Dim.2	ctr	cos2	Dim.3	ctr	cos2
PA.1	0.66	9.62	0.44	0.65	19.42	0.43	0.14	1.40	0.02
ED.1	0.38	3.13	0.14	0.89	36.08	0.79	0.01	0.00	0.00
P/e.1	0.36	2.81	0.13	−0.70	22.33	0.49	0.41	11.86	0.16
CL.1	0.77	13.26	0.60	−0.12	0.64	0.01	−0.45	14.77	0.21
CW.1	0.55	6.71	0.30	0.16	1.15	0.03	−0.67	32.56	0.45
PA.2	0.88	17.19	0.78	0.01	0.01	0.00	0.38	10.23	0.14
ED.2	0.67	9.98	0.45	0.34	5.13	0.11	0.50	17.90	0.25
P/E.2	0.66	9.73	0.44	−0.32	4.65	0.10	0.24	4.22	0.06
CL.2	0.80	14.28	0.65	−0.32	4.59	0.10	−0.26	4.76	0.07
CW.2	0.78	13.29	0.60	−0.36	6.02	0.13	−0.18	2.31	0.03

Table 4 Quantitative features in selected taxa of the tropical and subtropical Fabaceae

Species	Polar axis (µm)		Equatorial diameter (µm)		Colpi length (µm)		Colpi width (µm)	
	Tropical	Sub-tropical	Tropical	Sub-tropical	Tropical	Sub-tropical	Tropical	Sub-tropical
Acacia auriculiformis	32	53.3	23	55.4	–	–	–	–
Acacia confusa	23.19	46.1	13.88	49.9	–	–	–	–
Arachis duranensis	20.21	26.1	20	22.1	19.92	7.5	6.17	2.1
Albizia lebbekoides	71.81	70.8	81.01	89.05	–	–	–	–
Alysicarpus ovalifolius	32.64	28.1	33.99	27.7	16.55	8.2	5.1	1.6
Bauhinia purpureae	69.5	50.6	75.1	51.4	49.95	18.4	4.52	2.3
Calliandra haematocephala	82	32.2	93	25.4	–	–	–	–
Cassia javanica	36.81	43.3	20.68	33.3	34.47	19.2	3.29	8.9
Caesalpinia pulcherrima	19	24.6	14.4	16.6	17.69	7.5	5.18	2.9
Centrosema pubescens	17.3	–	17.78	–	17.1	–	3.24	–
Crotalaria pallida	17.78	16.52	16.2	16.32	15.86	12.31	2.55	2.29
Canavalia rosea	19.17	–	17.29	–	10.21	–	3.48	–
Leptodesmia microphylla	17.2	22.4	15.74	17.3	13.8	9.4	2.51	2.1
Dalbergia odorifera	19.11	–	53.29	–	7.9	–	4.66	–
Delonix regia	64.91	63.15	39.34	48.6	51.61	13.17	7.24	15.42
Grona triflora	22.2	32.5	20.6	27.4	14.39	11.1	2.82	3.4
Erythrina crista-galli	24.37	22.1	21.33	19.2	12.62	10.5	6.65	4.2
Entada phaseoloides	34.61	31.4	26.76	22.4	21.31	11.2	6.72	3.9
Indigofera colutea	16.92	20.5	14.46	23.5	11.91	5.3	2.67	2.2
Leucaena leucocephala	21.48	79	21.35	83.6	9.27	6.4	3.4	4.8
Macroptilium atropurpureum	23.91	–	22.35	–	15.49	–	4.92	–
Mimosa bimucronata	19.1	–	22.39	–	–	–	–	–
Mimosa pudica	7.8	27.8	7.75	16.6	0	7.4	0	3.2
Rhynchosia minima	25.19	23.4	17.44	26.3	18.89	11.2	1.3	3.1
Senna bicapsularis	44.75	36.2	34.25	29.1	38.75	19.2	3.52	4.1
Sesbania canabina	17.03	36.8	17.48	27.4	7.07	9.4	2.19	4.5
Saraca dives	21.81	0	11.39	0	17.67	0	1.18	0
Senna surattensis	50.11	33.66	49.55	24.5	32.61	21.2	6.03	1.9

The mean values for each pollen trait were provided in µm

Table 5 The polar to equatorial axis ratio (P/E) and shape of the Fabaceae species

Species	Tropical		Subtropical	
	P/E ratio	Shape	P/E ratio	Shape
Acacia auriculiformis	1.4	Prolate	1	Subspheroidal
Acacia confusa	1.68	Prolate	0.9	Subspheroidal
Alysicarpus ovalifolius	0.97	Subspheroidal	1	Subspheroidal
Arachis duranensis	1.02	Subspheroidal	1.1	Subspheroidal
Bauhinia purpureae	0.93	Subspheroidal	1	Subspheroidal
Caesalpinia pulcherrima	0.89	Subspheroidal	1.4	Prolate
Calliandra haematocephala	0.88	Subspheroidal	1.4	Prolate
Cassia javanica	1.78	Prolate	1.3	Subspheroidal
Crotalaria pallida	1.32	Subspheroidal	1.4	Prolate
Delonix regia	1.65	Prolate	1.2	Subspheroidal
Leptodesmia microphylla	1.1	Subspheroidal	1.2	Subspheroidal
Grona triflora	1.08	Subspheroidal	1.1	Subspheroidal
Entada phaseoloides	1.3	Subspheroidal	1.4	Prolate
Erythrina crista-galli	1.15	Subspheroidal	1.1	Subspheroidal
Indigofera colutea	1.18	Subspheroidal	0.9	Subspheroidal
Leucaena leucocephala	1.01	Subspheroidal	0.9	Subspheroidal
Mimosa pudica	1.01	Subspheroidal	1.7	Prolate
Rhynchosia minima	1.45	Prolate	0.9	Subspheroidal
Saraca dives	1.92	Prolate	1.10	Subspheroidal
Senna bicapsularis	1.31	Subspheroidal	1.2	Subspheroidal
Senna obtusifolia	0.94	Subspheroidal	1.1	Subspheroidal
Senna surattensis	1.02	Subspheroidal	1.4	Subspheroidal
Sesbania canabina	0.98	Subspheroidal	1.3	Subspheroidal

(Fig. 10a–b). The pollen size was noted as 23.19 × 13.88 µm, prolate, radial symmetry, P/E ratio 1.68 µm and individual grain diameter 5.745 µm. The second sample from the subtropical region of Pakistan Shah Kot, Multan with an altitude of 190 m above sea level showed a difference in sculpturing as it is perforate, and size was 46.1 × 49.9 µm, radial symmetry, P/E ratio 0.9.

Mimosa pudica: The pollen dispersal unit of *M. pudica* was tetrad polyad, inaperturate, tetrahedral, bilaterally symmetrical, hetero-polar. The uniplanar tetrad with the proximal sides of two individual grains is directly connected while the rest of the two grains are separated. Exine areolate and psilate rarely perforate. The areolae were flat and compactly arranged. Pollen size 7.8 × 7.75 µm, sub-spheroidal, radial symmetry, P/E ratio 1.01, individual grain diameter 3.85 µm, verruca diameter 0.64 µm (Fig. 10f–g). The second individual sampled from the subtropical region of Pakistan Gujraat with an altitude of 137 m above sea level showed variability in exine sculpturing such as the areolae were oriented and loosely arranged. The size was 27.8 × 16.6 µm, radial symmetry and P/E ratio 1.7. Colpus surface is psilate, size 7.4 × 3.2 µm (Fig. 11a–c).

Mimosa diplotricha: The pollen dispersal unit of *M. diplotricha* was tetrad polyad, tetrahedral, bilaterally symmetrical, hetero-polar and inaperturate. The uniplanar tetrad with the proximal sides of two individual grains was linked which separated the rest of the two units. Exine areolate, scabrate, verrucate, rarely perforate. Ubisch bodies were observed on the exine surface. Pollen size 19.1 × 22.39 µm, sub-spheroidal, radial symmetry, P/E ratio 0.86 µm, individual grain diameter 14.28 µm (Fig. 10h–i).

Leucaena leucocephala: The pollen dispersal unit of *L. leucocephala* was monad, polar view triangular, bilaterally symmetrical, heteropolar and tricolpate. Colpus surface scabrate to regulate. Exine perforate and rarely scabrate. The pollen size was 21.48 × 21.35 µm, sub-spheroidal, radial symmetry, P/E ratio 1.01 µm. Colpus size was 9.27 × 3.4 µm, pore

Table 6 Intra- and inter-specific heteromorphy in pollen of Fabaceae species

Species	Tropical (Pollen diagnostic traits)	Sub-tropical (pollen diagnostic traits)
Albizia lebbekoides	Psilate	Psilate to rugulate
Alysicarpus ovalifolius	Fossulate and areolate	Finely rugulate or fossulate
Arachis duranensis	Micro-reticulate, homobrochate	Perforate
Bauhinia purpureae	Bireticulate, perforate, rarely scabrate, rugulate	Reticulate
Bauhinia variegata	Striate, perforate, striae thin	Striate, striae very thick
Caesalpinia pulcherrima	Reticulate to perforate, scabrate at polar, mergo prominent	Reticulate, gemmate at polar, mergo absent
Calliandra haematocephala	8-grains polyads, exine scabrate, verrucate, rugulate	8-grain polyad, exine fossulate
Cassia javanica	Micro-reticulat to perforate	Microreticulate to rugulate
Centrosema pubescens	Microreticulate to perforate, scabrate at polar area	Reticulate
Crotalaria pallida	Reticulate, scabrate	Microreticulate
Delonix regia	Mega-reticulate, operculum absent	Megareticulate, muri psilate, operculum present
Leptodesmia microphylla	Reticulate, scabrate, muri thin	Megareticulate, muri thick
Grona triflora	Scabrate, rarely perforate	Reticulate, perforate at polar area
Entada phaseoloides	Perforate rarely scabrate to psilate	Psilate
Erythrina crista-galli	Megareticulate, lumena filled with scabrate structures	Reticulate, microreticulate
Indigofera colutea	Microreticulate, perforate and scabrate	Scabrate rarely perforate
Leucaena leucocephala	Perforate to scabrate	Perforate
Mimosa pudica	Areolate, areolae flattened, compactly arranged	Areolate, areolae oriented loosely arranged
Rhynchosia minima	Muri very thick, lumen filled with dense scabrate structure	Muri thin, lumen filled with less scabrate structure
Saraca asoca	Psilate to perforate rarely scabrate	Perforate to scabrate
Senna surattensis	Colpus tip acute	Colpus surface obtuse
Sesbania cannabina	Perforate to verrucate	Perforate to micro-reticulate, rarely scabrate

diameter was 4.39 µm (Fig. 11g–i). The second individual sampled from the subtropical region of Pakistan Islamabad with an altitude of 507 m above sea level showed variability in sculpturing as it is scabrate and rarely perforate, and size was 34.5 × 33.4 µm, P/E ratio 0.9. Colpus surface is scabrate, size 6.4 × 4.8 µm (Fig. 11d–f).

Albizia lebback: The pollen grain of *A. lebback* was shed in a 12-grain polyad, spheroidal in polar view. The 4 grains in tetragonal form were arranged in the center while the rest were arranged in a peripheral position. Exine ornamentation was found as psilate to regulate (Fig. 3a–c). The size of the pollen was noted as 87.54 × 77.97 µm, sub-spheroidal, redial symmetry, P/E ratio 1.13 µm while the individual grain diameter was 24.35 µm. The second sample was from the subtropical region of Pakistan Islamabad with an altitude of 570 m above sea level and the only differences were found in sculpturing as it is perforate to psilate, and size was 87.2 × 76.9 µm, P/E ratio 1.1 (Fig. 11j–l).

Albizia lebbekoides: Pollen shed in 12-grains polyad, inaperturate, elliptical in polar view. The tetragonal 4 grains were grouped in the center surrounded by peripheral ones. The exine pattern was psilate. The pollen size was 71.81 × 81.01 µm, sub-spheroidal, radial symmetry, P/E ratio 0.89 µm, and individual grain diameter was 25.13 µm. The second sample was collected from the tropical region of Pakistan Kot Shahan Gujranwala with an altitude of 88 m above sea level and the only differences were found in sculpturing as it is psilate to rugulate, and size was 37.8 × 89.05 µm, radial symmetry, P/E ratio 0.4.

Calliandra haematocephala: Pollen shed in 8-grains polyad, pyramidal in polar view. The central 2 large size grains were surrounded by 6 peripheral grains. Pollen size 88 × 93 µm, sub-spheroidal, radial symmetry, P/E ratio 0.88 µm. Exine

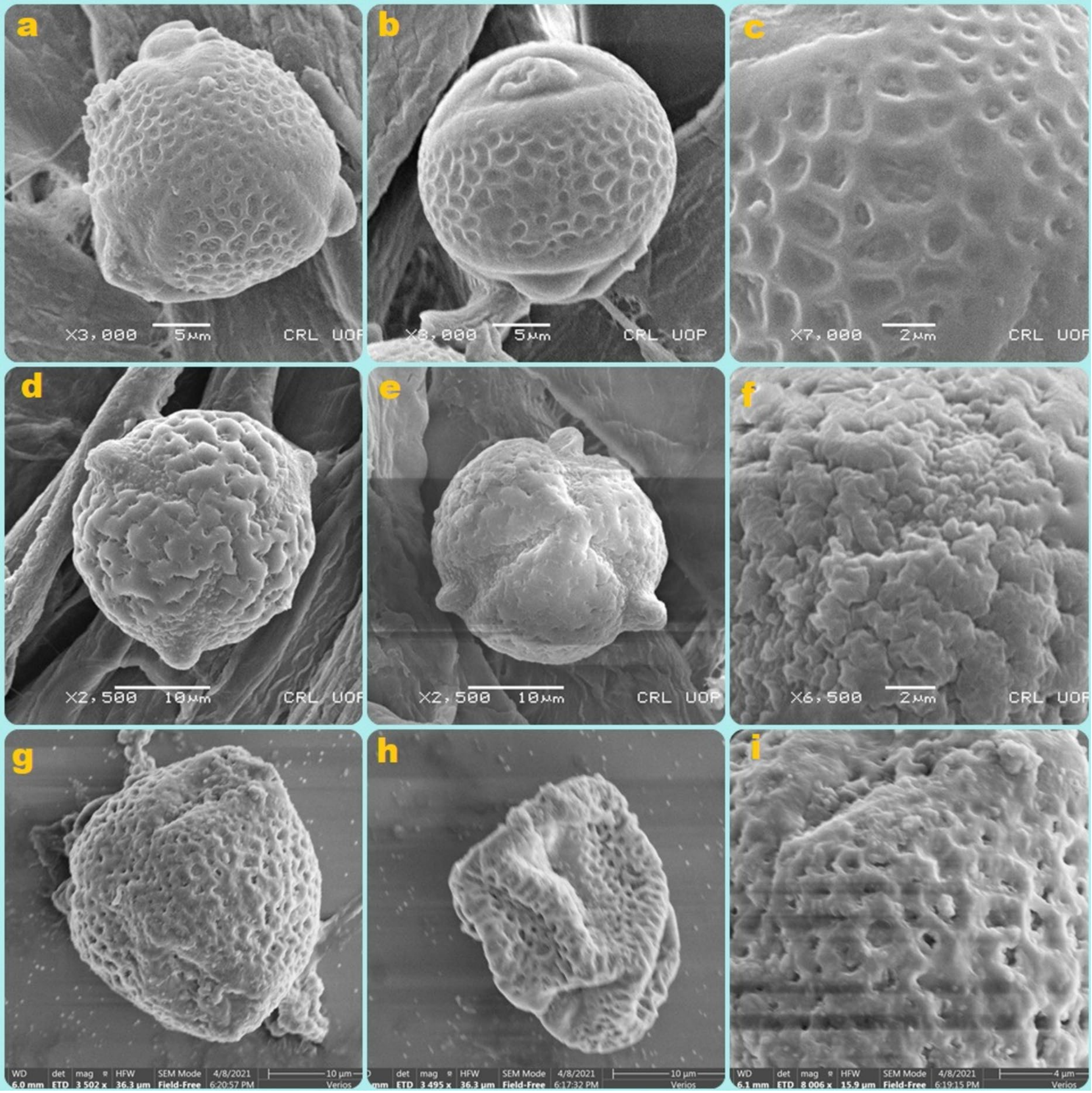


Fig. 5 Subfamily Papilionoideae. *Rhynchosia minima* (a-c subtropical), **a** polar view, **b** equatorial view, **c** close view of exine. *Sesbania cannabina* (**d**–**f** subtropical), **d** tricolporate, **e** polar view, **f** close view of exine. *Canavalia rosea* (**g**–**i** tropical), **g** oblique equatorial view, **h** dehydrated pollen, **i** close view of exine

verrucate, aerolate and rarely reticulate towards the margins. The apical portion of the pollen was acute having a constricted base and undulated surface. The second individual of this species from the subtropical region of Pakistan Gujranwala with an altitude of 216 m above sea level showed variability in exine ornamentation as it exhibits rugulate to the fossulate surface, and size was 32.2 × 25.4 µm, radial symmetry, P/E ratio 1.4.

Entada phaseoloides: The pollen dispersal unit of *E. phaseoloides* was monad, oval in polar and elliptical in equatorial view, bilateral symmetry, heteropolar, and tri-zono-colpate. Colpus surface granulate to verrucate rarely psilate. The tectal ornamentation

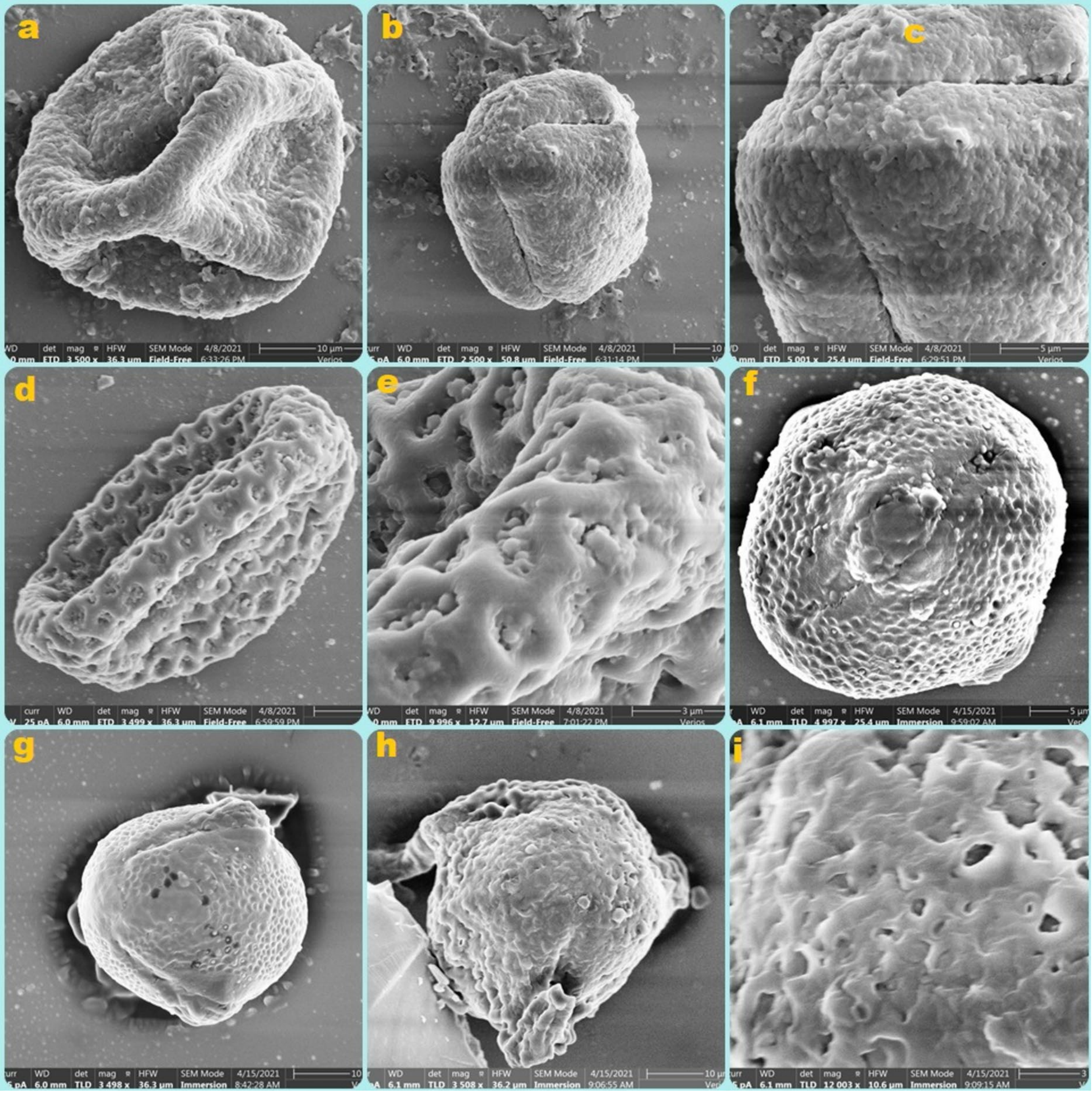


Fig. 6 Scanning electron microscopy (SEM) pollen micrographs of Papilionoideae taxa: *Grona triflora* (**a**–**c** tropical), **a** dehydrated pollen, **b** equatorial view, **c** close view of polar area. *Rhynchosia minima* (d-e tropical), **d** oblique equatorial view, **e** close view of exine. *Crotalaria pallida* (**f**–**g** tropical), **f** colporate pollen, **g** oblique equatorial view. *Macroptilium atropurpureum* (**h**–**i** tropical). **h** germination tube of pollen, **i** close view of exine

was observed as perforate, to scabrate. The size of the pollen was found as 34.61 × 26.76 µm, sub-spheroidal, radial symmetry, P/E ratio 1.3 µm. The colpi size was 21.31 × 6.72 µm, polar area diameter was 5.45 µm and mesocolpium diameter was reported as 25.33 µm (Fig. 10c–e). The other individual sampled from the tropical region of Pakistan Chicherwali Gujranwala with an altitude of 226 m above sea level showed variability in sculpturing as finely reticulate, and size was 31.4 × 22.4 µm, radial symmetry, P/E ratio 1.4. The Colpus surface is scabrate, size 11.2 × 3.9 µm.

Acacia auricifoliformis: The pollen shed as 16 grains polyads, inaperturate. Pollen size 32 × 23 µm,

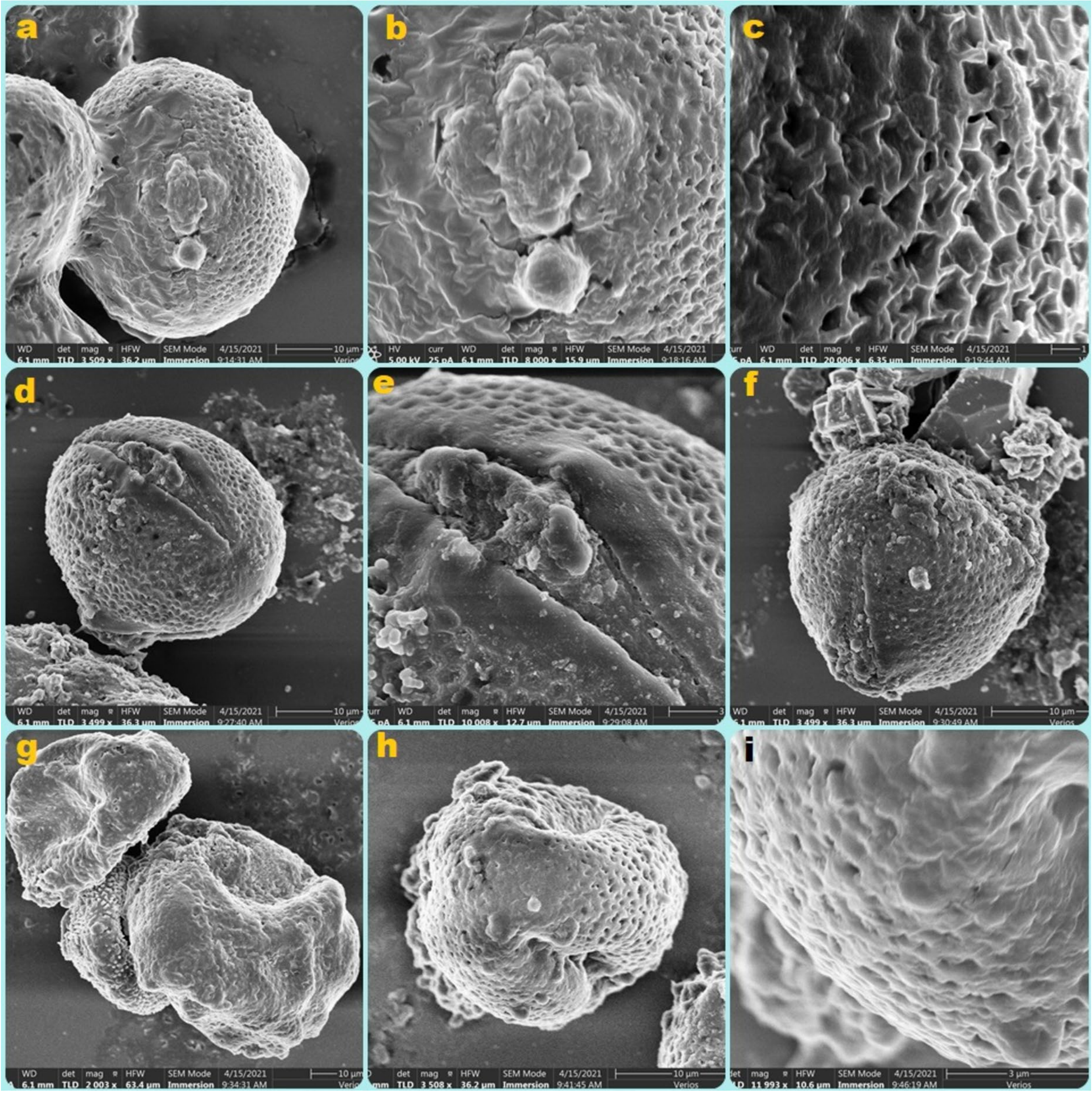
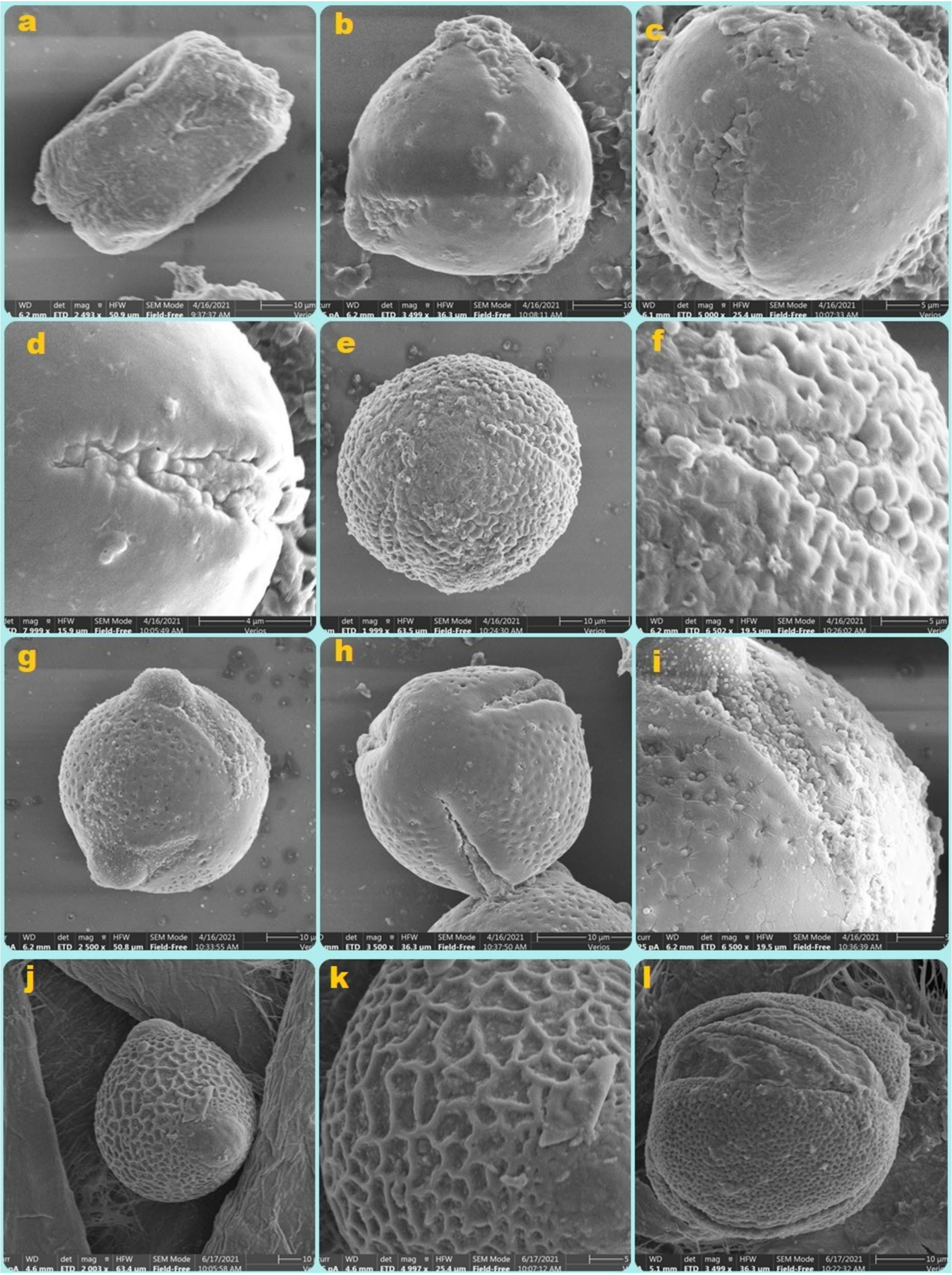


Fig. 7 *Crotalaria pallida* (**a**-**c** Tropical Changjiang), **a** attached pollen, **b** close view of aperture. *Leptodesmia microphylla* (Wanning **d**–**f**), **d** equatorial view, **e** close view of aperture, **f** polar view. *Indigofera hirsuta* (g tropical), **g** dehydrated pollen. *Sesbania cannabina* (**h**–**i** tropical), **h** polar view, **i** close view

prolate, radial symmetry, P/E ratio 1.4 µm. The central 8-grains were surrounded by eight peripheral ones. The exine pattern was noted as psilate. The mean diameter of the polyads was 32 µm. The other individual was sampled from the tropical region of Pakistan Wador Multan with an altitude of 124 m above sea level that showed differences in exine sculpturing such as verrucate, and size was 53.3 × 55.4 µm, radial symmetry and P/E ratio 1.

Subfamily *Ceasalpinioideae*

The pollen micro-morphology of Caesalpinioideae taxa was analyzed by using light and scanning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10 μm
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10 μm

◂**Fig. 8** Subfamily Papilionoideae; *Arachis duranensis* (**a** Wanning), **a** equatorial view. *Dalbergia odorifera* (**b-d** tropical), **b** polar view, **c** polar area **d** close view of aperture. *Alysicarpus ovalifolius* (**e**–**f** tropical), **e** polar view **f** gemmate structure. *Macroptilium atropurpureum* (**g**–**i** tropical), **g** equatorial view, **h** oblique equatorial view, **i** surface view of colpus. *Erythrina crista-galli* (**j**–**k** Haikou), **j** equatorial view, **k** close view of exine. *Arachis duranensis* (l Haikou) equatorial view

electron microscopy (SEM). The obtained results are illustrated in (Figs. 12, 13, 14, 15, 16).

Bauhinia purpurea: Pollen grains shed in a monad, isopolar, prolate-spheroidal, radial symmetry, P/E ratio 1.06, size 69.5 × 75.1 µm, pollen type tricolporate, colpi size 49.95 × 4.52 µm, or a 1a-longate. Tectum bireticulate and perforate rarely scabrate to rugulate at the polar area (Fig. 16a–b). The other individual of this species from the subtropical region of Pakistan Head Marala Sialkot with an altitude of 250 m above sea level showed differences in exine sculpturing such as striate and the pollen size was larger 50.6 × 51.4 µm, radial symmetry, P/E ratio 1. Colpi size 18.4 × 2.3 µm with scabrate surface.

Bauhinia variegata: Pollen grains shed in a monad, isopolar, sub-spheroidal, bilateral symmetry, P/E ratio 1.06, size 60.41 × 57.35 µm, pollen type tri-colporate, colpi size 45.57 × 20.05 µm, or a 1a-longate, colpi surrounded by granular colpi margin. Tectum striate, striae thin and psilate (Fig. 16c–d). The other individual of *B. variegata* was sampled from Kotli, Azad Kashmir with an altitude of 699 m above sea level and showed differences in exine ornamentation that was striate, striae psilate and very thick. The pollen size was larger 37.1 × 40.3 µm, radial symmetry, P/E ratio 0.9. Colpi size 36.39 × 4.6 µm with psilate surface (Fig. 15a–c).

Cassia javanica: Pollen grains shed in a monad, heteropolar, prolate, bilateral symmetry, P/E ratio 1.78, size 36.81 × 20.68 µm, pollen type tri-zono-colporate, colpi size 34.47.5 × 3.29 µm. Tectum micro-reticulate to perforate (Fig. 13d–e). The other individual of this species was sampled from the tropical region of Pakistan Pabbi Forest with an altitude of 302 m above sea level that showed variability in exine sculpturing such as perforate and the pollen size was larger 43.3 × 33.3 µm, radial symmetry and P/E ratio 1.3. Colpi size 19.2 × 8.9 µm with perforate surface (Fig. 12a–c).

Caesalpinia pulcherrima: The pollen grains of *C. pulcherrima* shed in a monad, heteropolar, sub-spheroidal, radial symmetry, P/E ratio 0.96, size 64 × 66.11 µm. Pollen type tri-zono-colporate, colpi 47.01 × 24.22 µm, lolongate, colpi surface sculpturing as densely gemmate and verrucate, mergo surface psilate, rarely perforate and micro-scabrate (Fig. 13f–g). The other individual from the tropical region of Pakistan Pabbi Forest with an altitude of 302 m above sea level showed plasticity in exine ornamentation such as micro-reticulate, polar area gemmate and the large size pollen size was 64.15 × 67.1 µm, radial symmetry, P/E ratio 1.4. Colpi size 23.3 × 10.2 µm with gemmate surface (Fig. 15d–f).

Delonix regia: Pollen grains shed in a monad, heteropolar, sub-spheroidal, bilateral symmetry, P/E ratio 1.31, size 63.15 µm × 48.5 µm, pollen type tri-colporate, colpi 51.61 µm × 7.24 µm. Tectum mega reticulate and heterobrocheate. Each polygonal lumina was ornamented with several baculas, scabrate and psilate structures. The reticula were thick and psilate (Fig. 13h–i). The other individual was sampled from the tropical region of Pakistan Sukheki Hafizaabad with an altitude of 207 m above sea level and the only differences in exine sculpturing was found as reticulate with each lumen containing scabrate structures and the pollen size was larger 63.15 × 48.6 µm, radial symmetry, P/E ratio 1.2. Colpi size 13.17 × 15.42 µm with psilate surface (Fig. 15g–i).

Saraca asoca: Pollen grains shed in a monad, heteropolar, prolate, bilateral symmetry, P/E ratio 1.92, size 21.81 × 11.39 µm, pollen type tri-colporate, colpi was 17.67 × 1.18 µm and sculptured as psilate. Exine psilate rarely perforate to scabrate (Fig. 14a–c). However, the other individual from the subtropical region shows intra-specific morphological disparity such as their exine was perforate to scabrate (Fig. 15j–l). The pollen size was 34.1 × 37.52 µm, radial symmetry, P/E ratio 1.10, the shape was sub-spheroidal, and the colpi was 17.7 × 7.70 µm.

Senna surattensis: Pollen grains shed in a monad, hetero-polar, sub-spheroidal, bilateral symmetry, P/E ratio 1.02, size 50.11 µm × 49.55 µm, pollen type tri-colporate, colpi was 37.01 × 26.72 µm, lolongate and sculptured as scabrate to psilate. Tectum finely reticulate and perforate. A clear mergo was also observed having scabrate to the somewhat psilate surface (Fig. 14g–i). The second individual of this species was sampled from the tropical region of Pakistan Bhera, Sargodha with an altitude of 196 m above sea level and the only differences in exine sculpturing

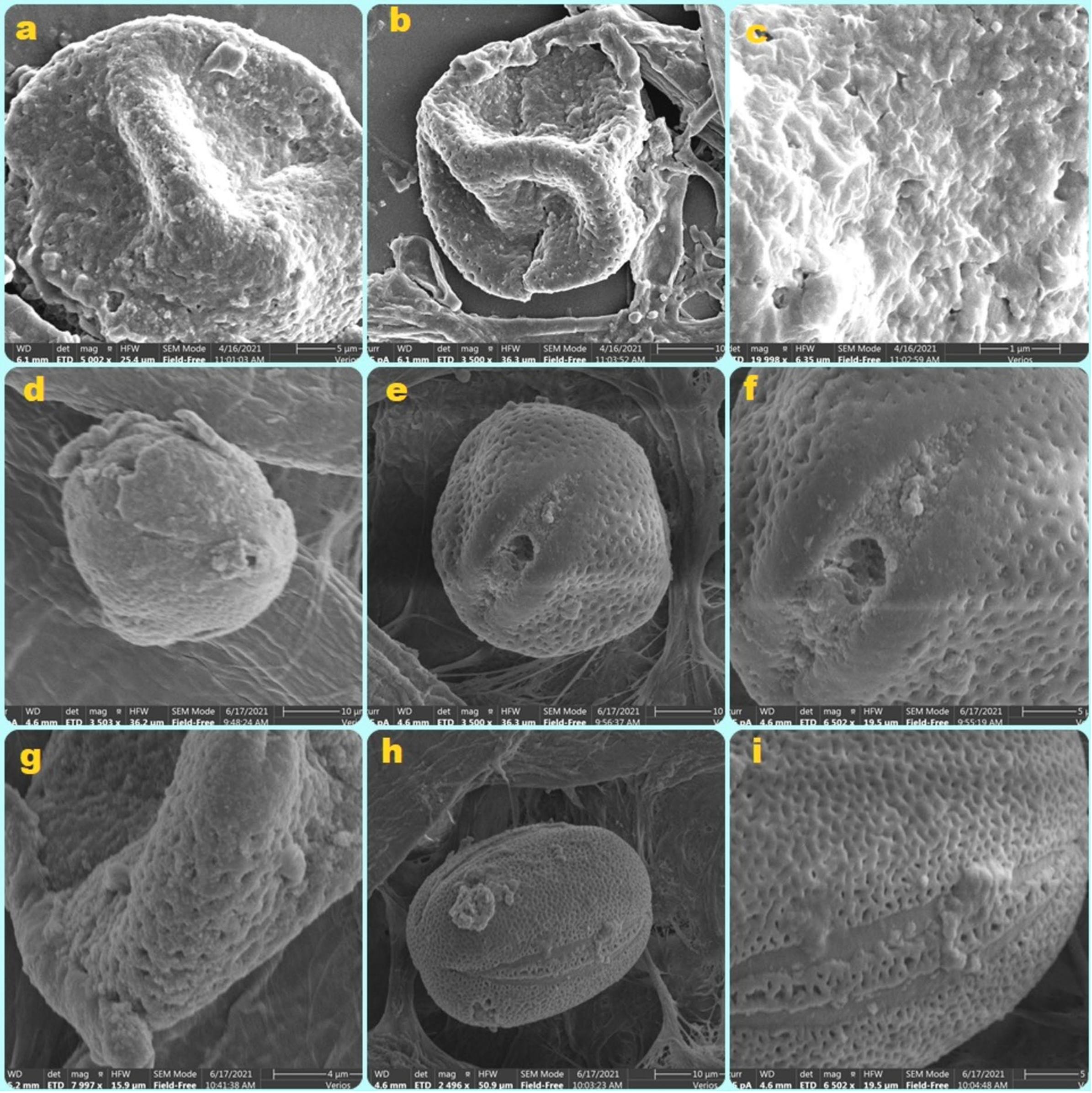


Fig. 9 Subfamily Papilionoideae; *Centrosema pubescens* (**a**–**c**), **a**–**b** dehydrated pollen, **c** close view of exine. *Indigofera hirsuta* **d** equatorial view, *Macroptilium atropurpureum* (**e**–**g** Sanya), **e** equatorial view, **f** close view of aperture, **g** close view of exine. *Arachis duranensis* (**h**–**i** Wanning) **h** equatorial view, **i** close view of exine

were found as scabrate and the pollen size was larger 33.66 × 24.5 µm, radial symmetry, P/E ratio 1.4. Colpi size 21.2 × 1.9 µm with psilate surface (Fig. 11h–i).

Senna bicapsularis: Pollen grains shed in a monad, isopolar, sub-spheroidal, bilateral symmetry, P/E ratio 1.31 µm, size 44.75 × 34.25 µm, pollen type tri-colporate, colpi was 37.01 × 26.72 µm, lolongate and sculptured as psilate rarely scabrate. The exine pattern was found perforate. Furthermore, the connection between the margin of an aperture called a bridge was also reported (Fig. 14d–f). The *S. bicapsularis* can be delimited from the *S. surattensis* by the presence of clear mergo in *S. surattensis* and absent in *S. bicapsularis*. The other individual was sampled from the tropical region of Pakistan Alipur,

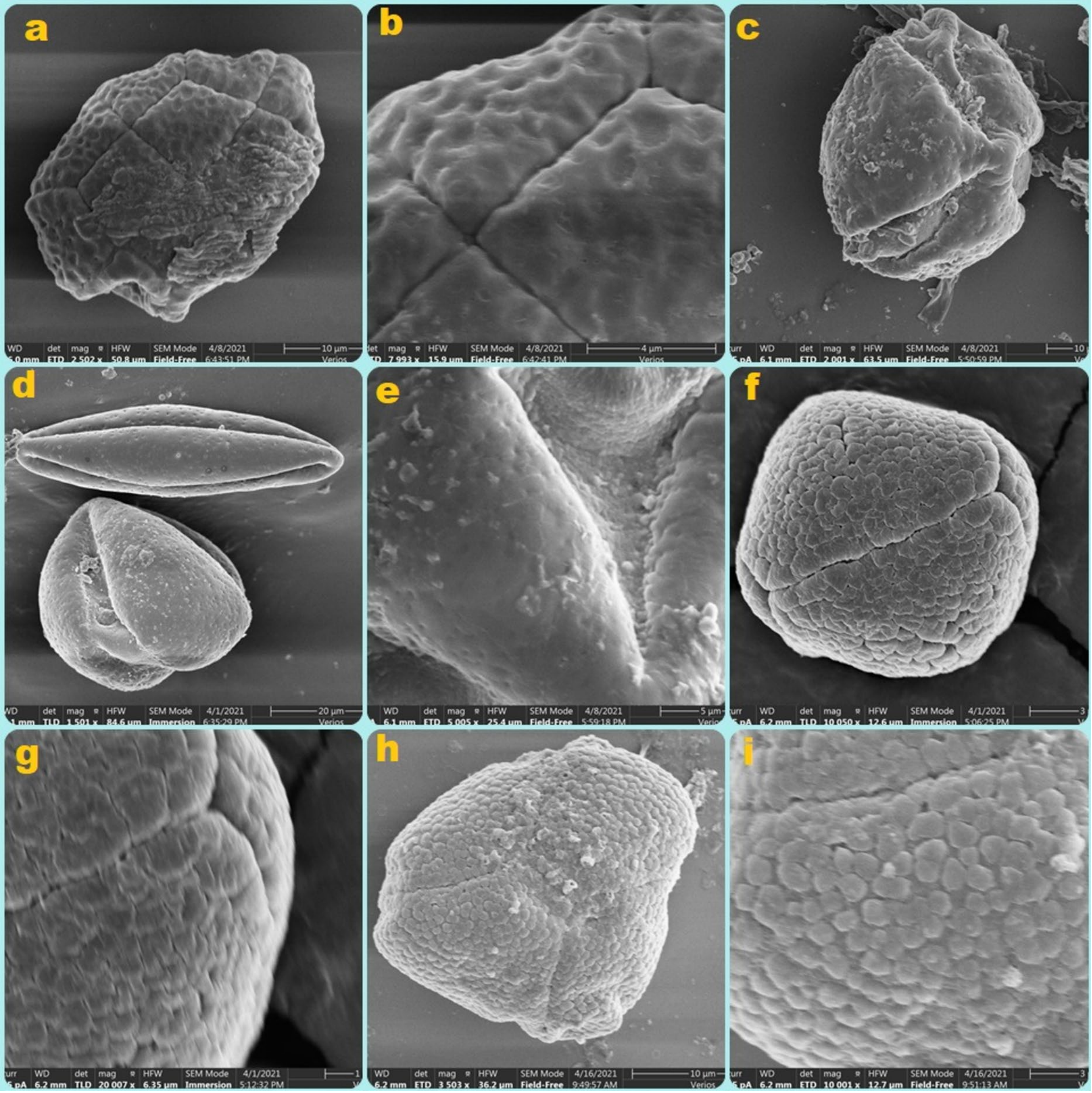


Fig. 10 Mimosoid clade: *Acacia confusa* (**a**–**b** tropical), **a** polyad pollen **b** close view of exine. *Entada phaseoloides* (**c**–**e** tropical), **c** shows mesocolpium, **d** dehydrated pollen, **e** close view of aperture. *Mimosa pudica* (**f**–**g** tropical Wanning), **f** tetrad pollen, **g** close view of polar area. *Mimosa diplotricha* (**h**–**i** tropical Hainan) polyad pollen, **i** close view of the pollen

Kot Sultan with an altitude of 210 m above sea level and the only differences in exine pattern were found as perforate and the pollen size was larger 36.2 × 29.1 µm, radial symmetry, P/E ratio 1.2. Colpi size 19.2 × 4.1 µm with perforate surface (Fig. 12d–e).

Senna obtusifolia: Pollen unit monad, tricolpate, radial symmetry, apocolpium region small. Exine sculpturing was perforate to regulate. The colpus surface membrane was psilate to rarely scabrate having an acute tip. P/E ratio was 1.1. pollen size 36.5 × 39.15 µm and colpi size 14.3 × 2.7 µm (Fig. 12f–g).

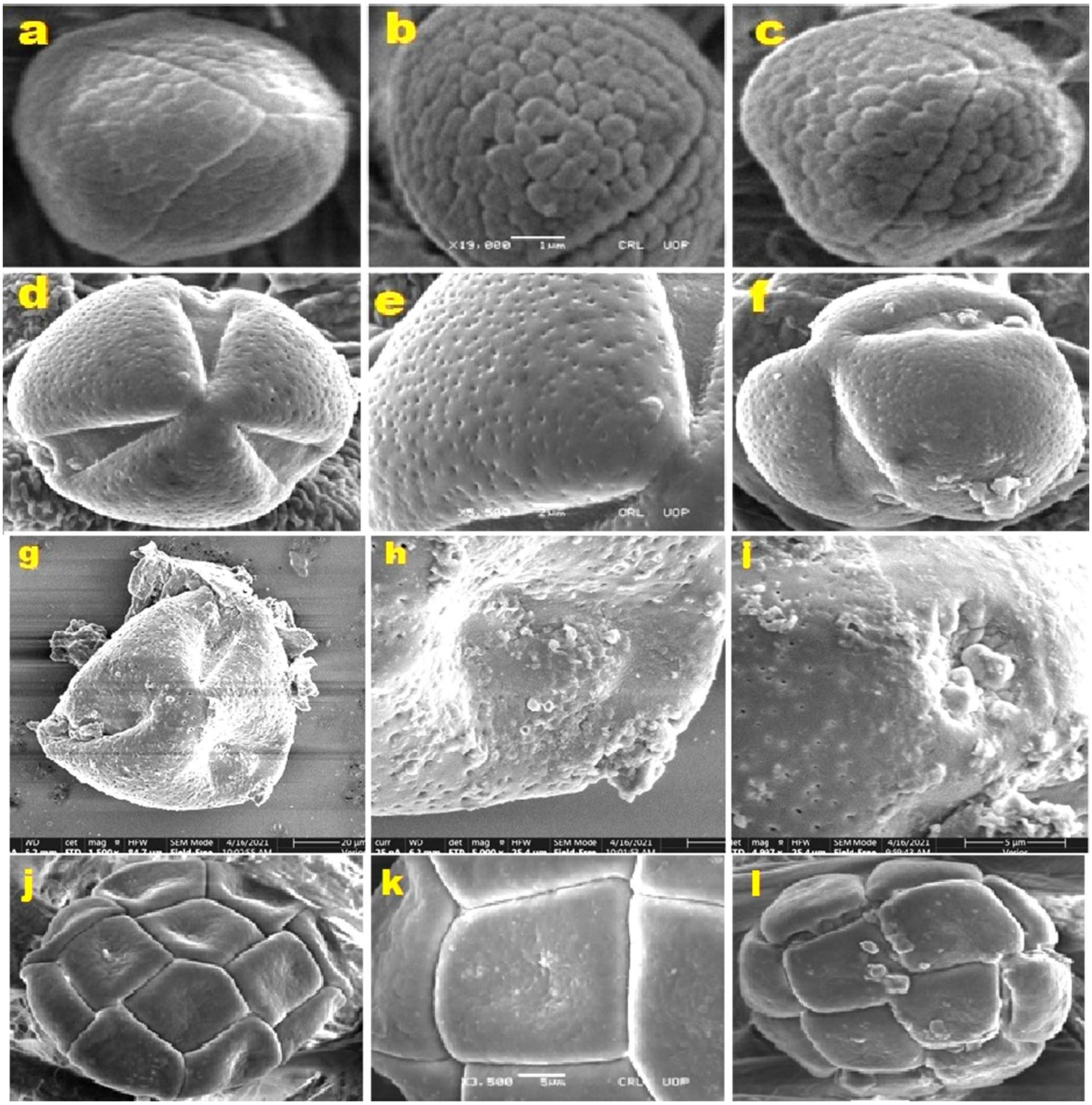


Fig. 11 Mimosoid clade: *Mimosa pudica* (**a**-**c** subtropical), **a** polyad pollen, **b** close view of pollen, **c** equatorial view. *Leucaena leucocephala* (**d**-**f** subtropical), **d** polar view, **e** close view of exine, **f** oblique equatorial view. *Leucaena leucocephala* (**g**-**i** tropical), **g** polar view, **h** close view of aperture surface, **i**) apocolpium region. *Albizia lebbeck* (**j**-**l** subtropical), **j** polyad pollen, **k** close view exine **l** general view of polyad

Pattern of pollen features in the tropical and subtropical *Fabaceae* species

The multivariate PCA was performed to show the variation, dimensionality and independence between the Fabaceae taxa sampled from the tropical regions of Hainan Island, south China and subtropical regions of Pakistan. The first two PCs accounted for 67.2% of the total variance. The biplot vectors showed the pollen's quantitative traits. The PC1 was accounted for 45.2% of the total variance and primarily dominated by colpi

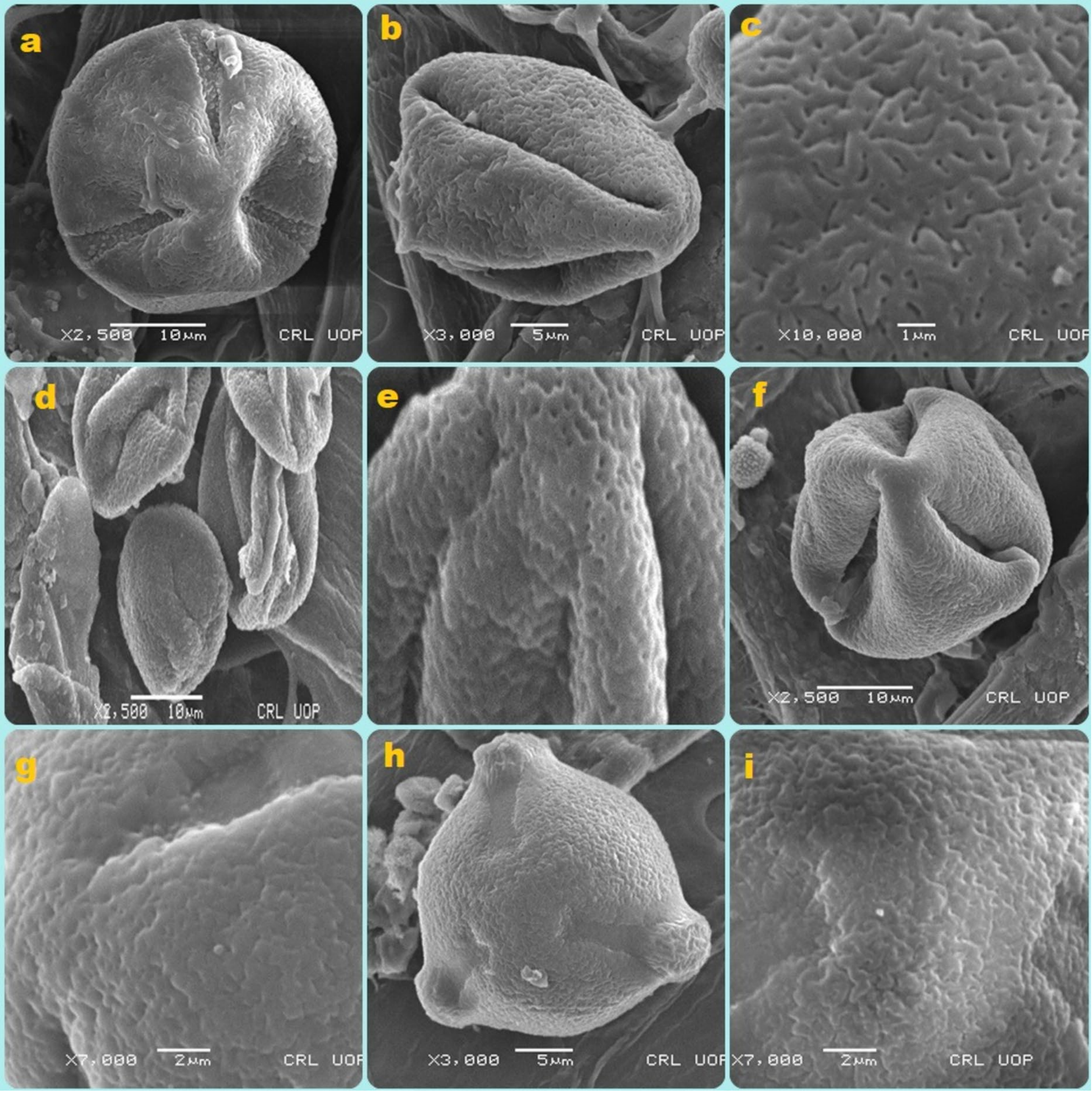


Fig. 12 Scanning electron microscopy (SEM) pollen micrographs of Caesalpinioideae taxa: *Cassia javanica* (**a**-**c** subtropical), **a** polar view, **b** equatorial view **c** close view of exine. *Senna bicapsularis* (**d**-**e** subtropical), **d** dehydrated pollen, **e** close view of exine. *Senna obtusifolia* (**f**-**g** subtropical), **f** polar view, **g** close view of exine. *Senna surattensis* (**h**-**i** subtropical), **g** polar view, **i** close view of exine

length and width, the polar axis of the pollen of tropical Pakistan. Likewise, the second axis was accounted for 22% of the total variance and mostly dominated by the polar axis and equatorial diameter of the pollen of subtropical species. However, in Dim2, the colpus length and width and P/Eratio in both regions were negatively associated with one another. This confirmed the phenotypic plasticity in pollen quantitative traits of the selected Fabaceae species. The loadings of each principal component were provided in detail (Fig. 19, Tables 2, 3).

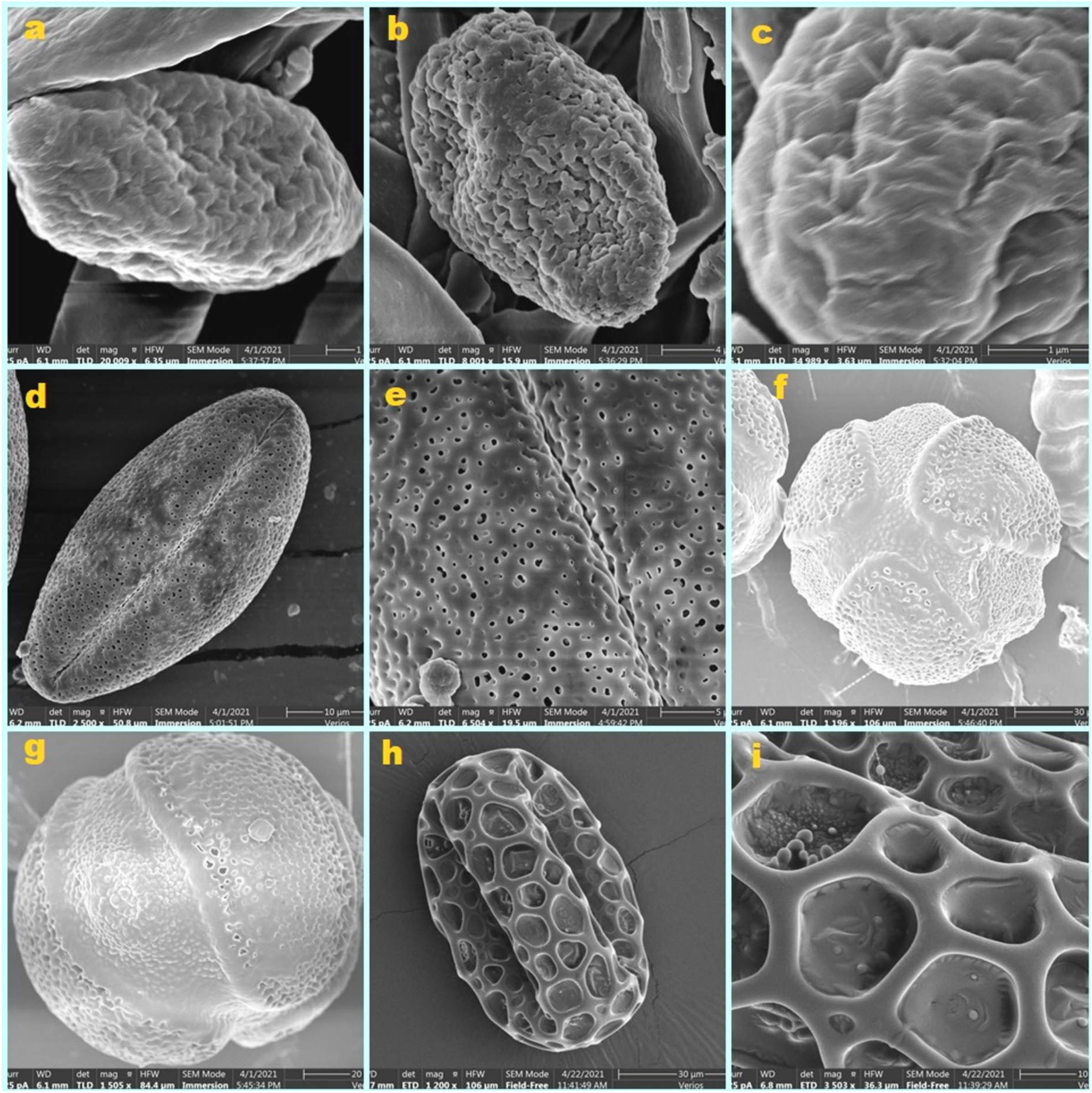


Fig. 13 *Bauhinea purpurea* (**a**-**c** tropical), **a**-**b** general view of the pollen, **c** close view of exine. *Cassia javanica* (**d**-**e** tropical), **d** oblique equatorial view, **e** sunken aperture. *Caesalpinia pulcherrima* (**f**-**g** tropical), **f** polar view, **g** close view of colporate pollen. *Delonix regia* (**h**-**i** tropical) **h** equatorial view **i** close view of exine

Discussion

In this study we investigated the pollen morphology of selected tropical and subtropical Fabaceae species to better understand their structural diversity and taxonomic significance. Fabaceae, being one of the largest plant families, exhibits a wide range of morphological traits that can provide insight into evolutionary adaptations and phylogenetic relationships. The variation in pollen grain size, shape, and surface ornamentation observed across species highlights potential diagnostic features that can contribute to species identification and classification within the family. These findings may also have wide implications for understanding plant

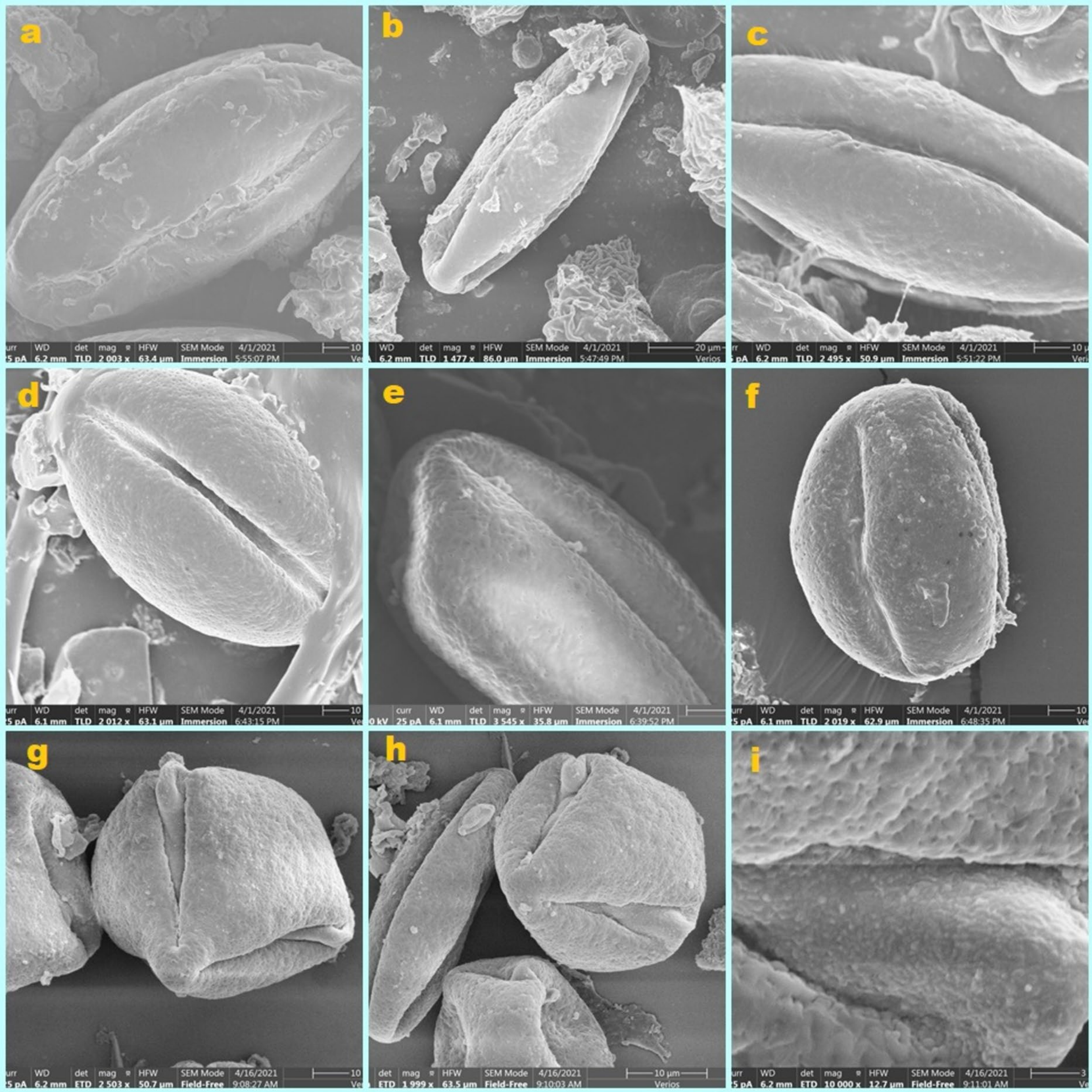


Fig. 14 *Saraca asoca* (**a**-**c** tropical), **a** oblique equatorial view, **b** dehydrated pollen, **c** close view of exine. *Senna bicapsularis* (**d**-**f** tropical), **d** sunken aperture, **e** close view of exine, **f** equatorial view. *Senna surattensis* (**g**-**i** tropical) **g** general view, **h** shows both hydrated and dehydrated pollen, **i** close view of colpus surface membrane

reproduction strategies and ecological interactions in tropical and subtropical environments.

Papilionoideae

We investigated the pollen morphology of selected Papilionoideae species of the tropical and subtropical regions to observe the intra and inter-specific variability. We found a remarkable intraspecific variability in size and shape while less in qualitative traits. Most of the Papilionoideae species had reticulate microreticulate and psilate exine sculpturing. In line with this, the eurypalynous Papilionoideae in the subtropical monsoonal climate of Karachi, Pakistan are investigated by Perveen and Qaiser (1998a, b, c) where the maximum temperature was

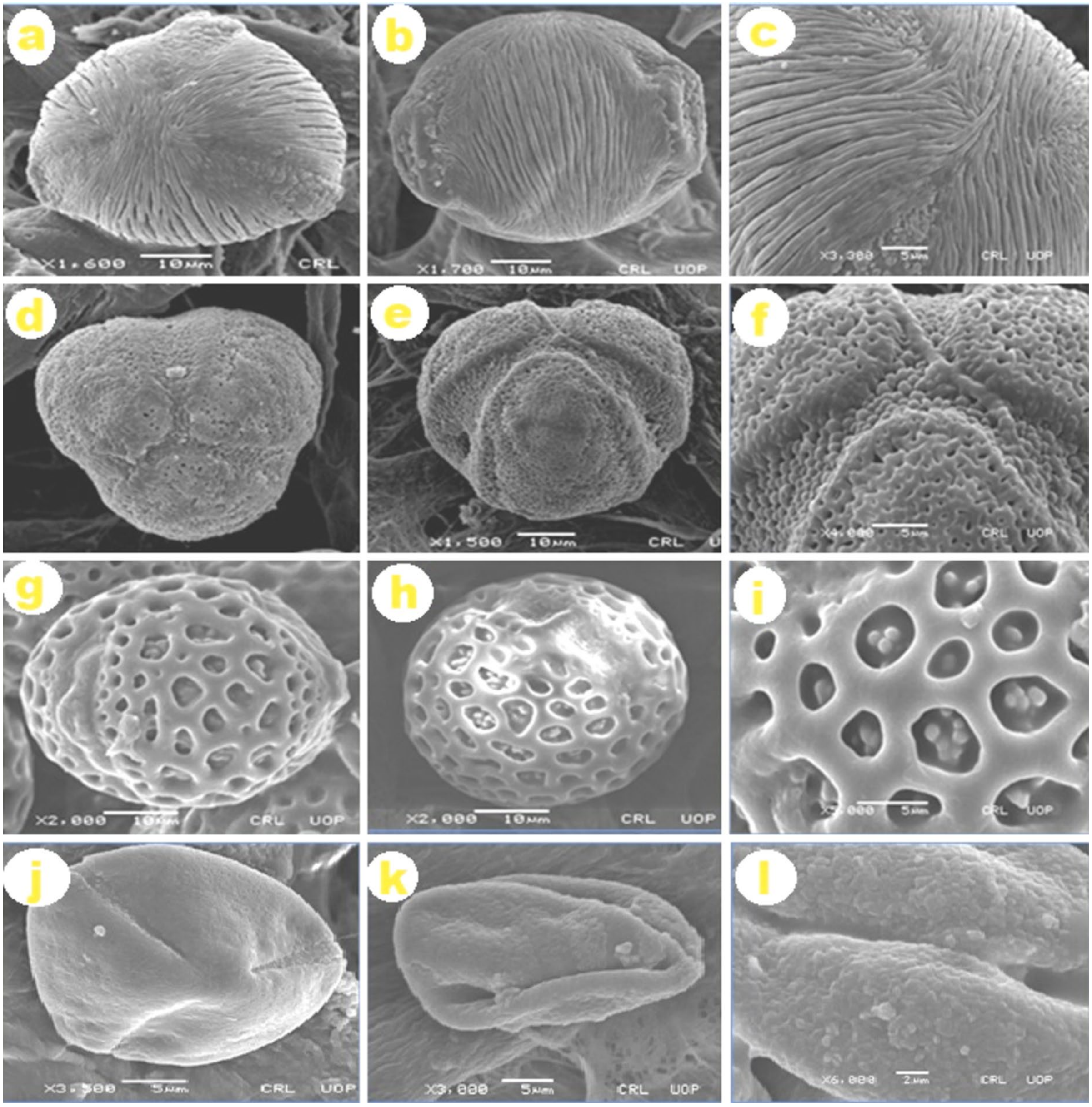


Fig. 15 *Bauhinia variegata* (**a**-**c** subtropical). **a** polar view, **b** equatorial view, **c** close view of polar area. *Caesalpinia pulcherrima* (**d**-**f** subtropical), **d** polar view, **e** equatorial view, **f** close view of polar area. *Delonix regia* (**g**-**i**), **g** equatorial view, **h** oblique equatorial view, **i** close view of exine. *Saraca asoca* (**j**-**l** subtropical), **j** polar view, **k** dehydrate pollen, **l** close view of aperture

recorded as 45.64 °C and the average precipitation was 100.25 mm. They reported that pollen of this subfamily was mostly tricolprate and rarely colpate. The shape was commonly prolate or prolate-spheriodal. The ora was mainly la-longate or circular rarely lolongate. The surface of the colpus was sculptured as psilate and scabrate. The exine sculpturing was observed as regulate, striate and foveolate. Based on the pollen morphology, the delimitation of genera is difficult due to overlapping in most of the features. However, shape and exine were found as useful taxonomic characters.

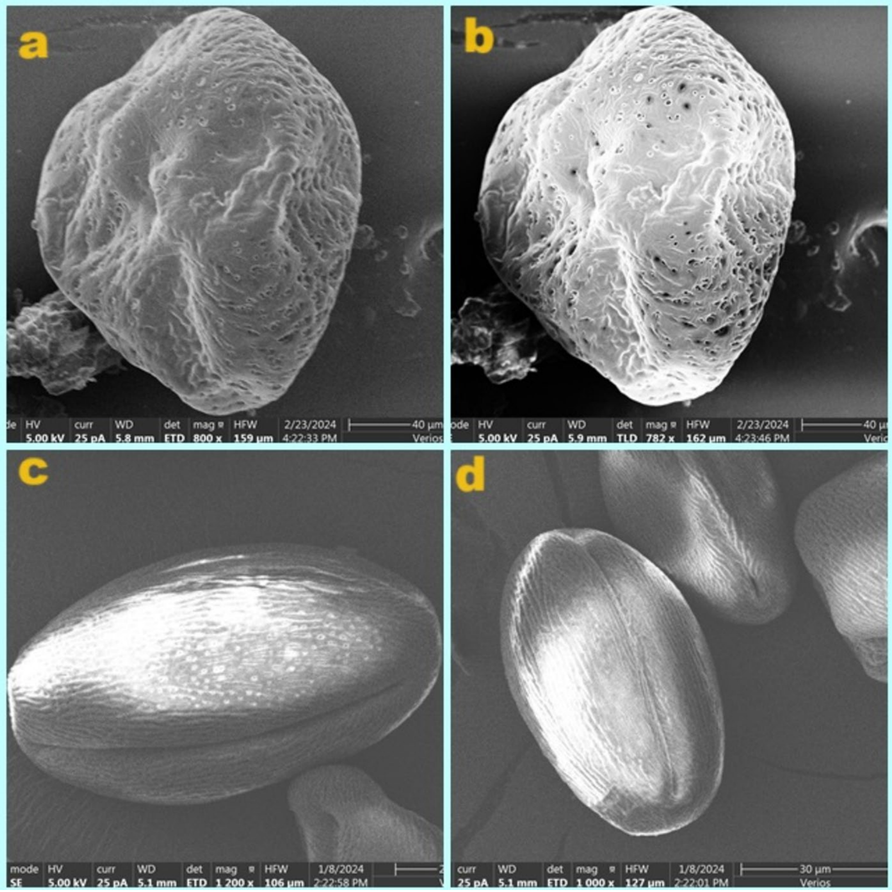
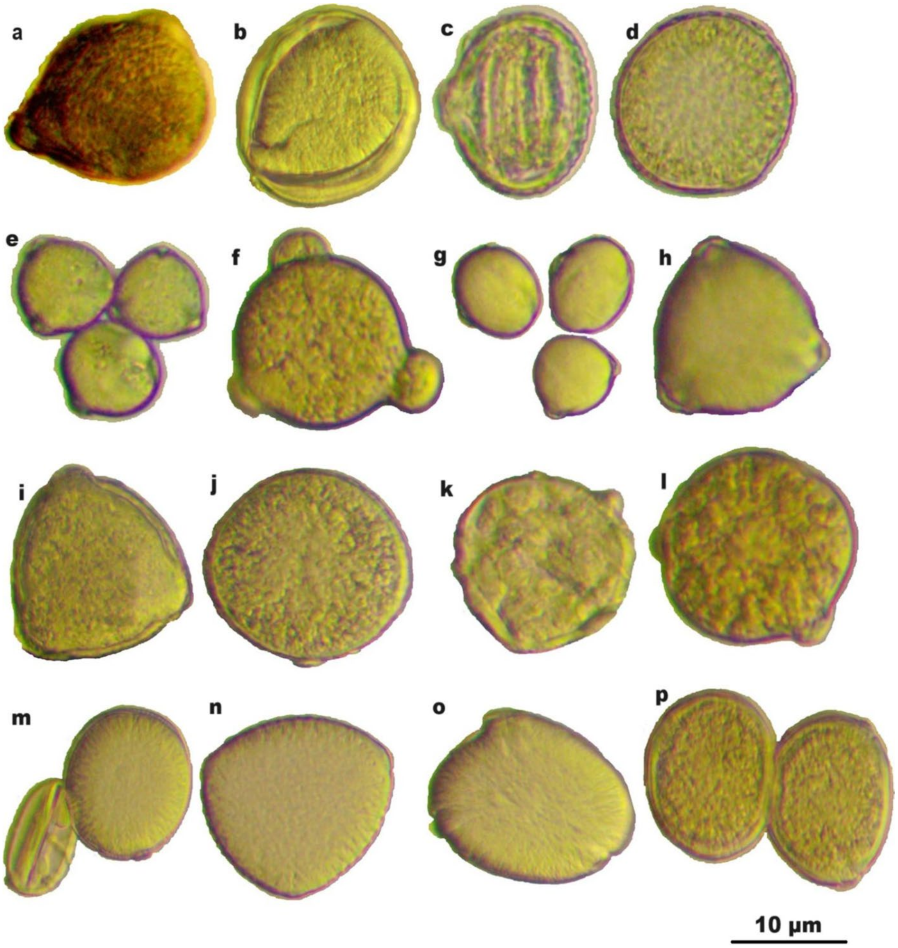


Fig. 16 *Bauhinia purpureae* (**a**-**b** Haikou) showing perforate and bireticulate exine. *Bauhinia variegata* (**c**-**d** Haikou) showing striate and perforate pollen

In *Aeschynomene indica* we found reticulate pattern while only the size and spheroidal pollen in *A. indica* were reported by Gandhi et al. (2014) in a dry climate Pondicherry region of south India where the temperature extends between 20–30 °C and the relative humidity was ranged from 70 to 90%. Similarly, in the tropical monsoon climate of Maharashtra India, Patil et al. (2012) only used LM techniques and reported scabrate exine pattern in *Alysicarpus vaginalis* that were not in line with our findings. The reason for the disagreement with their study was the use of microscopic techniques such as light microscopy. In *Arachis duranensis* we observed the micro-reticulate and homobrochate tectum ornamented with granulate structure while Chandran and Pandya (2003) investigated the pollen morphology of *Arachis* sampled from the subtropical climate zone of Gujrat India with an average temperature in summer was 41 °C and highest relative humidity was 80.09 mm (August) and analyzed the subprolate, tri-lobate and reticulate tectum in *A. duranensis* that were partially similar to our study and show infra-specific variability. This plasticity in pollen morphology may be due to different climatic conditions in two different habitats. The reticulate tectum was seen in *A. hypogaea* sampled from the semi-arid tropical region of India. Based on this intra and inter-specific plasticity of the *Arachis* pollen, this genus can be treated as stenopalynous (Chaturvedi et al. 1990).

By using only light microscopy, the reticulate exine in *Centrosema pubescens* was documented by Naik et al. (2016) in the tropical region of Andaman Island, India with an average temperature of 23 °C but we explore an additional supra-structural scabrate pattern in *C. pubescens* sampled from Hainan Island. They also define the micro-reticulate exine for *Canavalia rosea* and we explore an additional pollen trait called mergo, which are distant exine area that delimit colpi from the exine. The reticulate and micro-reticulate tectum was observed in *Crotalaria*

Fig. 17 Light microscopic micrographs of *Canavalia rosea* **a** showing the equatorial view, *Grona triflora* **b** showing thick sexine. *Rhynchosia minima* (**c**, **d**), **c** equatorial view, **d** polar view. *Crotalaria pallida* **e** showing tri-colporate pollen. *Macroptilium atropurpureum* **f** polar view. *Crotalaria pallida* (**g**–**h**, Chang Jiang), **g** showing oblique equatorial view, **h** polar view. *Leptodesmia microphylla* (**i**, Wanning) polar view, *Indigofera hirsuta* **j** polar view, *Sesbania canabina* **k** polar view. *Macroptilium atropurpureum* (**l**) polar view. *Arachis duranensis* (**m**, Sanya) showed both polar and equatorial views, *Erythrina crista-galli* **n** polar view. *Arachis duranensis* (**o**, Haikou) polar view, *Dalbergia odorifera* **p** showing polar view. Scale bar (**a**–**p**) = 10 μm



pallida sampled from the tropical region of Hainan Island. Furthermore, the perforate ornamentation in the mesocolpium and psilate in the apocolpium area were found in the previous study (Lin and Huang 1999) that was dissimilar to our study.

In present study, psilate, perforate and scabrate pattern was reported for *Dalbergia odorifera* that were consistent with the findings of Bahadur et al. (2023). Moreover, the perforate tectum was found in *Desmodium miscolobium* sampled from the tropical regions of the Pau-de-Fruta, Brazil, where the average annual temperature is 19.3 °C, and average precipitation is about 1279 mm (Luz et al. (2013). Similarly, the rugulate and reticulate tectum in *Desmodium* was observed by Huaicheng et al. (2018) and recommended that pollen features are useful at inter-specific level. Both *Desmodium triflorum* and *D. heterophyllum* are morphologically related and taxonomically complex taxa (Rahman and Rahman 2013). This was similar to Chen and Huang (1993) findings, who also put these taxa in the same group and analyzed the semi-angular and psilate tectum. We revised the previous findings and observed the perforate and verrucate pattern for *D. triflorum*. The reticulate and rarely rugulate tectum was noted in the previous study in the subtropical region of Taiwan, where the average annual temperature is 21 °C, and the average precipitation is 2590 mm (Chen and Huang 1993), which was approximately similar to our findings. This similarity in pollen features may be due to the same environmental conditions. Overall, the *Desmodieae* tribe is a complex and morphologically diverse taxonomic group in Fabaceae and needs further taxonomic research to strengthen their systematics.

Reticulate sculpturing was found in *Erythrina* but showed variations in several traits such as granulate (Hemsley and Fergusion 1985). The *Erythrina* pollen shares morphological similarities and can be

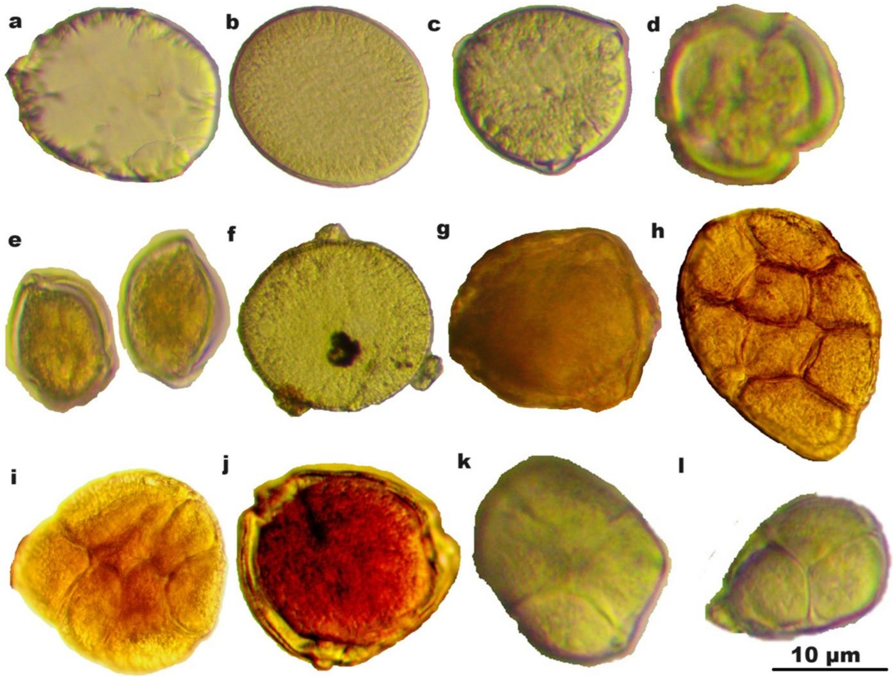
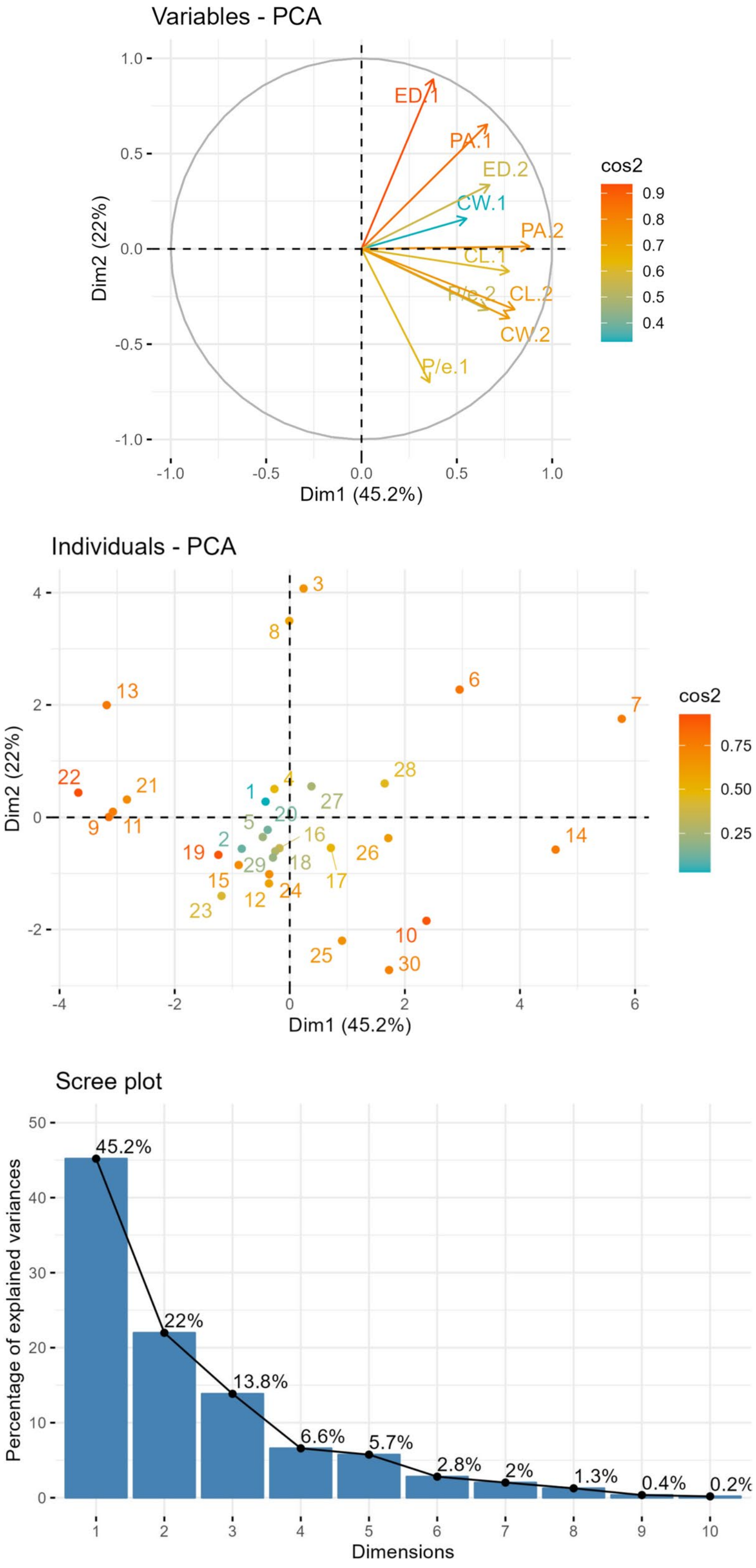


Fig. 18 *Macroptilium lathyroides* **a** very thin exine, *Alysicarpus ovalifolius* **b** oblique equatorial view. *Macroptilium atropurpureum* (**c**, Haikou) polar view. *Centrosema pubescens* **d** polar view. *Senna surattensis* **e**, thick exine. *Caesalpinia pulcherrima* **f**, tricolporate pollen. *Delonix regia* **g**, tetra-colporate pollen. *Calliandra haematocephala* (**h**–**i**), polyad pollen. *Entada phaseoloides* **j** tricolporate pollen having thick exine. *Mimosa pudica* (**k**-**l**), **k** tetrad, **l** triad pollen. Scale bars (**a**–**l**) = 10 µm

expected to exhibit possible evolutionary changes. Although pollen size and shape vary widely and both traits have low taxonomic potential, apertures and tectal patterns are taxonomically important in defining the species boundaries of *Indigofera* sampled from the temperate Sino-Himalayan region of China (Zhao et al. 2016). They reported a microperforate tectum for *I. colutea* while we found micro-reticulate, perforate and scabrate patterns for this species sampled from Hainan Island. To compare the pollen features of *Indigofera hirsuta* with previous work, the shape and sizes of the pollen show remarkable differences and these may be due to the species selection from different habitats having different environmental conditions. Therefore, species sampling should be considered crucial for the *Indigofera* pollen research (Schrire and Sims 1997). The reticulate, perforate and scabrate, rarely verrucate and gemmate tectum was found in *Macroptilium atropurpureum* whereas the psilate and rarely punctate tectum was observed by Torres-Colín et al. (2010), which were not in line with our results. In *M. lathyroides* we found psilate and sparsely granulate features while the punctate, favolate, or semi-tectate, micro-reticulate exine was analyzed by Torres-Colín et al. (2010) which were not similar to our results.

The pollen morphology of *Rhynchosia minima* from the semi-arid region of Caatinga, Brazil, with a mean annual rainfall is 800 mm, and the mean temperature between 28 °C and 30 °C while the maximum temperature in the severe deforestation areas reached 36 °C had been investigated by Buril et al. (2011), and reported the reticulate and heterobrochate pollen. The micro-reticulate tectum, lumen filled with granules and rugulae was observed by Oliveira et al. (2019). Hence, the present study validates this information. The overlapping macro-morphological features make the correct identification of *Sesbania* taxa difficult (Chanda et al. 2019). To solve this problem, this study will help to discriminate the closely related species of *Sesbania*. We observed the reticulate and verrucate pattern in *S. canabina*. In line with this, Pritha et al. (2015) in subtropical areas of West Bengal, where the maximum temperature ranges between 38 °C and 45 °C and the minimum is around 20 °C, while the average rainfall is 1750 mm, observed the reticulate tectum in *Sesbania*. Keeping in view this intra and interspecific association, it is suggested that the subfamily Papilionoideae is taxonomically multifaceted and recommended as stenopalynous (Luz et al. 2013).

Fig. 19 Principal component analysis (PCA) of some tropical and subtropical Fabaceae species based on pollen traits. The first graph showing biplot-PCA of the variables, the second graph showing individual PCA, while the third graph showing scree plot of all the principal components



Subfamily *Ceasalpinioideae*

In tropical *Ceasalpinioideae* taxa the pollen grains were mostly striate, regulate, perforate and reticulate. In line with this, the subtropical species of this subfamily were mostly reticulate, rugulate, fossulate, foveolate and sometimes staite have been investigated by Perveen and Qaiser, (1998a, b, c). The pollen features, of this subfamily, are significant at the generic and specific levels. The taxonomic history of *Bauhinia* is usually complex and has been the subject of a wide range of taxonomic research in which it has been recognized as a large genus of the Ceasalipinioidea. We found straite-reticulate, gemmate and verrucate exine pattern in the tropical *Bauhinia purpureae*. The same species were recently investigated by Maw et al. (2020) in the tropical region of Mandalay, Myanmar with an average temperature of 34 °C and humidity of 78%, and found granulate, gemmate and striate-reticulate, hence approximately supporting our findings. The reason behind the similarities in the pollen structure, in due to similarities in the habitat conditions. The straite exine in *B. purpureae* of West Bengal was reported by Sarkar et al. (2019), where the maximum temperature varies from 26 to 32 °C while the striate-reticulate pattern was observed by Tidke et al. (2012). Comparing their findings with our own, we found differences in pollen size and exine sculpturing such as striate-reticulate pattern. To know about the inter-specific variation, the *Bauhinia variagata* of the humid sub-tropical region of Islamabad Pakistan where the average temperature in June is 31 °C, the striate tectum having psilate reticulum was analyzed by Ullah et al. (2022) while the straite reticulate exine pattern of *Bauhinia variagata* in the tropical wet region of Minas Gerais, Brazil with an average temperature of 27 °C was analyzed by Antonio-Domingues et al. (2018). Hence, these intra and inter-specific variations validate the eurypalynous status of the genus *Bauhinia*.

The *Caesalpinia pulcherrima* sampled from the tropical region of Hainan Island, the tectum was micro-reticulate to perforate and raised colpal margins with a prominent mergo while the reticulate exine pattern was observed by Antonio-Domingues et al. (2018) in the tropical region of Patos de Minas, Brazil where the average temperature was 21.8 °C. Similarly, the reticulate rugulate structure was reported by Ullah et al. (2022) in the subtropical region of Peshawar, Pakistan with an average temperature of 32.3 °C. Hence a slight intra-specific variability was found in the pollen morphology of *C. pulcherrima* such as the presence of mergo, raised colpal margin and polar area diameter and pollen size. Moreover, in *C. pulcherrima*, the apertures were joined at the polar area while it was closed in *C. echinata* hence these findings confirm the inter-specific variation between the species of the same genus (Antonio-Domingues et al. 2018).

In *Cassia javanica* the reticulate tectum was observed by Ganga Kailas et al. (2014) in the tropical region Karimnagar, India with an average temperature of 27.5 °C and average precipitation was 249 mm which corroborates our findings. This similarity may be due to the occurrence of *C. javanica* in similar environmental conditions in the tropics. Moreover, in *C. fistula* the psilate, reticulate and rugulate tectum have been reported previously in the subtropics that indicate interspecific variation between the *Cassia* species (Ullah et al. 2022). Moreover, the reticulate exine was reported by Quamar et al. (2017). In line with this, the reticulate to rugulate exine structure was observed in the previous study except for the rugulate sculpturing (Ullah et al. 2022). In *Leucaena leucocephala* the subpsilate exine was reported by Aftab and Perveen (2006), which is not in line with our study. We found sub-spheroidal pollen while Sufyan et al. (2018) observed prolate pollen.

In Hainan Island, the *Saraca asoca* had a reticulate-striate exine pattern that supports the study of Ullah et al. (2022). Likewise, Yang et al. (2001) indicated the reticulate tectum while Sarwar et al. (2015) noted the psilate, coarsely rugulate and rugulate-perforate tectum that independently evolved from micro-reticulate patterns. The macro-rugulate surface of *Saraca indica* may evolve from reticulate (Sil et al. 2019). Furthermore, they sampled *S. indica* from two different altitudinal gradients and found intra-specific variability at the micro-level, such as thick exine in high and thin exine in lower altitudes. The diagnostic pollen traits of the *Saraca* species that define species boundaries within the genus were reticulate straite exine in *S. dives*, reticulate in *S. asoca*, macro-rugulate in *S. declinata*, micro-rugulate in *S. indica* and granulate in *S. thaipingensis*. These findings confirmed the inter-specific variation between the *Saraca* species of Shibpur west Bengal India having a semi-arid climate with an average temperature

33 °C (Sil et al. 2019). From the above concept, it was concluded that high variability in the exine pattern of *Saraca dives* evolved from the reticulate pattern. Intra and inter-specific variability in the size and shape of *Saraca* pollen may also carry such influences. Harmomegathic stress is produced due to the loss of water causing a decrease in size and changes in pollen shape to avoid dehydration (Blackmore and Barnes 1986). Among the *Saraca* species, *S. asoca* has relatively large-sized pollen as the abundance of this species can be found near forests having higher rainfall (Sil et al. 2019). Hence it was concluded that latitudinal gradient, temperature and humidity are crucial in the evolution of the shape and size of pollen due to harmomegathy (Kriebel et al. 2017).

In *Senna surattensis*, plasticity in pollen features was observed when compared with the study of Ullah et al. (2022), who found perforated and clavate tectum. This variation may be due to different habitats i.e. tropical and subtropical regions of Hainan Island and Pakistan having different environmental conditions. Similarly, the *Senna bicapsularis* of Hainan have a perforate and finely reticulate pattern which validates the information of Soares et al. (2021) for *S. bicapsularis* sampled from the tropical region of Cerrado forest Brazil with an average annual temperature range from 22 to 27 °C and average precipitation between 80 to 200 cm. Moreover, in the *S. corymbosa* the exine was psilate while perforate and tactate in *S. occidantalis*. This confirms the inter-specific variability in *Senna* (Ullah et al. 2022).

Mimosoid clade

In the present study, we found both polyad and monad-type pollen in Mimosoid clade. The polyad type pollrn delimits this subfamily from the Papilionoideae. The polyad features in *Acacia* were first time reported by Kunth in 1818 (Sorsa 1969). Later, after the detailed investigations the polyad and monad types have been reported in Mimosoideae by different researchers. However, a complete range of variations within polyads such as tetrads, octads and several other polyads types were reported by Mohl (1835). The pollen morphology of eurypalynous Mimosoideae from the sub-tropical regions of Sindh, Pakistan was investigated by Perveen and Qaiser (1998a), and reported that the pollen was mainly polyads or monads. The monad type grains were tricolporate, exine mostly foveolate or foveolate-rugulate. These features were found significant at the generic and tribal levels. Similarly, the foveolate or psilate exine pattern was found in most of the subtropical Mimosoideae taxa such as *Leucaena*, *Mimosa* and *Albizia* species (Khan et al. 2021).

Among the Mimosoid clade species, *Mimosa* is one of the most studied genera concerning pollen morphology. This genus contains about 540 species occupying in different habitats (Simon et al. 2011), about 38% of which have been analyzed to date (Erdtman 1945; Santos-Silva et al. 2013). These findings showed that the *Mimosa* group has rich pollen morphological diversity such as grains per polyads (4, 8, 12, 16), size and shape of the polyads, verifying the genus as eurypalynous. The tubercules were found on the exine surface which was dissimilar to our findings except for the tetrad pollen in *Mimosa pudica* (Caulton et al. 2019). The tetrahedral and inaperturate pollen with a mean diameter of 9µm for *Mimosa pudica* in the tropical forest of Singapore has also been analyzed by Rao and Lee (1970). Similarly, the pollen sampled from the tropical *Mimosa diplotricha* of the Hainan flower had a 4-grains polyads and the exine sculpturing was areolate and a space was found between areola. The same species has a tuberculate/verrucate exine and no space was found between the areola, sampled from the tropical region of Chaing Mai, Thailand, with an average annual temperature of about 25.1 °C and an annual rainfall was about 1108 mm (Khongkarat et al. 2022). The aforementioned features confirmed the intra-specific variability in *M. diplotricha* and showed that the areolate type evolved from tuberculate-type pollen in which circular bodies or areolae were compactly arranged. From the taxonomic perspective, the shape and number of grains in polyad was a significant taxonomic feature in the identification of *Mimosa* taxa (Santos-Silva et al. 2013). The present study also verified this information that shapes and grains in polyad were useful taxonomic traits for the delimitation of *Mimosa* taxa.

The pollen of *Acacia confusa* shed as 12-grain polyad, aperture condition was inaperturate. The megareticulate and scabrate tectum, has thick flattened and scabrate reticula, and a lumen filled with micro-scabrate structures. The size of the pollen was 23.19 × 13.88 µm and the individual grain diameter was 5.74 µm. No previous literature was found on this species. In the *Acacia auricifoliformis*, we found

16-grains polyads and exine psilate. In line with this, other species of *Acacia* also showed 16–32 grains of polyad with or without distinct apertures (Erdtman 1952; Brown et al. 2008; Khan et al. 2019; 2021). The colporate apertures were present but the colpate type was not found in Mimosoideae (Guinet 1981; Al-Watban et al. 2013).

In *Albizia lebback* we found 16-grain polyads but in line with this, 16-grain polyads were reported by Agashe and Caulton, (2009) which corroborated with our results. The colpi and exine ornamentation of *Albizia* are smooth or sub smooth which delimits the *Calliandra, Cylindrokelupha*, *Zygia* which was tuberculate (Zhi-Min 1994). Similarly, the pollen morphology of *Albizia lebbekoides* was mostly similar to *Albizia lebback* and *A. procera*. However, variation was found in the size and exine sculpturing that can help to delimit the species of *Albizia* (Khan et al. 2021). In *Calliandra haematocephala*, each flattened polyad is composed of two median pollen grains and six peripheral ones. One of the peripheral grains is modified slightly and has a somewhat eccentric and pronounced projection. This special foot grain lies at the proximal end of each polyad and the two-foot grains of the polyad pair are directed toward one another supporting the previous study of Nevling and Elias (1971). In *Calliandra haematocephala* we found 8-grain polyads, exine verrucate to aerolate while the bi-tetrads and polyads have been analyzed by Van Campo and Guinet (1961). The bi-tetrad in *Calliandra* was investigated by Chen. (1973) and suggest that these features were rarely found in Angiosperms.

Other species of the *Calliandra* in the tropical climate of Bahia Brazil, characterized by high temperature and high humidity have polyads pollen having rugulate exine pattern (De Assis Ribeiro dos Santos and De Oliveira Romão 2008). The polyads were widely assumed to confer selective vegetation in reproduction, as they could provide an effective way of pollen dispersal by entomophily (Kenrick and Knox 1982). However, an alternative hypothesis indicated that the structure of polyads may protect the pollen from dehydration in dry habitats (Wyatt et al. 2000).

The *Leucaena leucocephala* from the tropical region of Hainan Island have a perforate and scabrate tectum while this species from the arid climate of Karachi Pakistan was investigated by Aftab and Perveen. (2006) and reported the sub-psilate exine that were not in line with our findings. This phenotypic plasticity is due to the differences in habitat environment such as differences in altitudes, temperature and humidity.

In *Entada phaseoloides* the tectum was perforate to scabrate and rarely psilate. Contrary to this, the pertectate sexine sculpturing was reported by Sorsa (1969), this confirmed that psilate features were primitive and the perforate to scabrate patterns were the evolved ones. Moreover to compare with other species, the tri-colporate, subprolate, size 30 × 35 µm, and reticulate exine were found in *Entada spiralis*. Furthermore, the pollen shape of *Entada scandens* and other pollen traits of *E. phaseoloides* were found in the findings of Erdtman. (1952). Hence, these features showed an interspecific variability between the species of *Entada*.

Plasticity in pollen morphology

Phenotypic plasticity is the ability of genotypes to produce different phenotypes when exposed to different climatic conditions (Gómez et al. 2020). In this study, we analyzed intra and interspecific pollen plasticity in Leguminosae taxa in the tropical and subtropical regions. Environmental conditions were very different in these two sites. Literature about the effects of environmental conditions on pollen morphology is very rare and even not reported yet at the family level such as Fabaceae. We found plasticity in the pollen size and shape of the Fabaceae species. In this regard, Ejsmond et al. (2011) documented that the size and number of pollen were mostly associated with environmental temperature while Lau and Stephenson (1994) also indicated that pollen size may vary with growing climatic conditions. Other research suggested that pollen size may vary like seed size along with growing climatic conditions (Schlichting 1986). In addition, the habitat conditions that reduce pollen production can negatively affect male function by decreasing the amount of pollen that is available for a plant to transfer to conspecifics (Devlin et al. 1992). Hence, it can be hypothesized that adverse growing conditions can depress fitness through male function by reducing equality such as performances of the pollen, mostly under the condition of pollen competition (Schlichting 1986). In line with this, the pollen phenotypic plasticity in *Berbaris microphylla* was investigated by Radice et al. (2018) in different climatic conditions of Argentina and reported that Pollen grains sampled from Moreno had a temperate rainy climate with an annual temperature of 16 °C had a polar diameter of ~60 µm were significantly different from Ushuaia pollen (57.11 µm) where the climate is tundra type and the annual temperature in the warmest month is slightly below 10 °C. This intra-specific variability in pollen futures could indicate that plants are trying to adapt their pollen morphology to environmental conditions such as maximum temperature, and humidity. The high temperature and high humidity could affect the pollen's vigor and vitality (Miaonan et al. 2000).

Variation in the quantitative traits of the pollen grains makes it difficult for morphological-based identification of Fabaceae taxa. Individuals sampled from the tropical and subtropical regions of the same species showed a considerable variation specifically in the size and shape which leads to the conclusion that most of the pollen traits of Fabaceae species are plastic in nature. Our results are in agreement with Sil et al. (2019), who found intra-specific plasticity in pollen features of *Saraca* species. The plasticity in shape size and exine sculpturing may be regulated by harmomegathic stress produced due to changing humidity dependent on temperature.

The evolution of pollen morphology within Fabaceae is complex and has been shaped by various factors including pollination strategies, ecological niches, and evolutionary history. Generally, the pollen grains in Fabaceae typically exhibit some common characteristics such as being tricolpate or tricolporate, meaning they have three furrows or pores, which is a common trait among angiosperms. Within the family Fabaceae, a significant considerable heteromorphy was seen in pollen, reflecting the diverse evolutionary pathways and adaptations of different lineages within the family. This variability can range from subtle differences in ornamentation to more significant differences in size and shape. For example, tetrads and polyads pollen have evolved from monads at least 39 times (Harder and Johnson 2008). The evolution of inaperturate pollen grains in primitive angiosperms appears to be the most significant evolutionary trend. From such inaperturate pollen, there was a second major radiation of uniquely angiospermous monosulcate-derived aperture types, much as occurred previously in the initial radiation of anasulcate pollen through a successive or simultaneous microsporogenesis (Walker 1974; Banks et al. 2010). Subsequently, from the most common type of colpate pollen, i.e., tricolpate pollen, a major radiation of tricolpate-derived aperture types took place, which today characterizes the pollen of most dicotyledonous angiosperms such as Fabaceae, and with the evolution of tricolpate pollen came the tremendous diversity of angiosperm pollen in general. In addition, the colpate type of pollen aperture evolved from the inaperturate. In conclusion, the evolution of pollen morphology in the Fabaceae is a complex process shaped by a combination of ecological (such as temperature, humidity, precipitation), reproductive, and evolutionary factors. Analyzing these morphological adaptations provides insights into the evolutionary history, reproductive biology, and ecological interactions of legume species.

Conclusion

High morphological diversity and phenotypic plasticity of tropical and subtropical pollen at intra and inter-specific levels were found in Leguminaceae subfamilies, such as reticulate microreticulate and psilate in Papilionoidea, striate, perforate and reticulate in Ceasalpinoideae while polyad, psilate, perforate, and rugulate in Mimosoid clade. A weak intra-specific variation was found in the exine sculpturing while it was high when considering the polar axis and equatorial diameter, colpus length and width, shape and P/E ratio. Using scanning electron microscopy, a powerful morphometric tool can largely improve the potential to distinguish the genera within the same pollen morphotypes, especially the tricolporate type which occurred in most of the species. Individuals sampled from the tropical and subtropical regions of the same species showed a considerable variation specifically in size and shape which leads to the conclusion that most of pollen traits of Fabaceae species are plastic in nature. The plasticity in shape size and exine sculpturing may be regulated by harmomegathic stress produced due to changing humidity dependent on temperature. Hence, this intra-specific variability in pollen futures could indicate that plants are trying to adapt their pollen morphology to environmental conditions such as maximum temperature, and humidity. Furthermore, diverse sampling and advanced imaging techniques are essential for examining both quantitative and qualitative features of pollen for taxonomic considerations. Focusing on diagnostic pollen traits, investigating environmental influences, and understanding pollinator interactions that shape pollen morphology. Additionally, integrating molecular research will strengthen the correlation between morphological traits and genetic diversity within and between species, providing insights into evolutionary processes. By implementing these recommendations, researchers can deepen their understanding of the complex patterns of variability in pollen morphology within the Fabaceae family, contributing valuable knowledge to both taxonomy and ecology.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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