Folia Hort. 35(2) (2023): 479–498

DOI: 10.2478/fhort-2023-0034

FOLIA HORTICULTURAE

Published by the Polish Society
for Horticultural Science since 1989

ORIGINAL ARTICLE | Open access | https://sciendo.com/journal/FHORT

Exploring intraspecific pollen morphology variation in Apocynaceae: A roadmap for horticultural innovation

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ABSTRACT

This study aimed to examine the pollen and pollinia morpho-structure of 18 horticultural Apocynaceous species. Advanced light and scanning electron microscopy (LM and SEM) were used to elaborate on and examine the systematic importance of pollen and pollinia micromorphology. Pollen grains were first acetolysed, which was followed by visualisation of their sculpturing features. The quantified data were subjected to statistical tools to elucidate dendrogram clustering and principal component analysis to reveal pollen/pollinia morphotypes. The size of pollen is variable, ranging from 113.45 µm in *Cascabela thevetia* to 23.4 µm in *Alstonia scholaris*. The study revealed tetrad, tetraporate, and tricolporate grains. Sculpturing (exine ornamentation) varies from reticulate perforate to reticulate. Pollinum shape was observed to be narrow oblong, obovate, orbicular, and reniform. Reticulate-psilate sculptural features were prominent among pollinia surfaces. Based on examination, it was ascertained that the minimum exine thickness in *Periploca aphylla* was 4.9 µm, whereas the corresponding number in *Cryptolepis dubia* was 1.35 µm. Taxonomic identification keys were constructed separately based on pollen/pollinia characters to identify the Apocynaceous taxa. In the presented study, seven pollen shapes were observed: from oblate to per prolate. The findings confirm that morphopollinic traits differ amongst genera of Apocynaceous species. However, these features can be used to distinguish the Apocynaceous taxa. The results show that structural characteristics of pollen and pollinia can help accurately identify Apocynaceous species.

Keywords: cultivars, exine, morpho-palynology, pollinia, SEM

INTRODUCTION

Apocynaceae is one of the important angiosperm families founded in 1789 as 'Apocineae' by Jussieu (El Gazzar et al., 2018). It comprises 375 genera and 5100 species present mainly in subtropical and tropical regions of the world (Endress et al., 2007). Naz et al. (2019) have reported that Pakistan has 19

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genera, comprised of 13 cultivated genera (20 species) and 6 endemic genera (6 species). Brown in 1810 separated Asclepiadaceae from Apocynaceae based on pollinium and classified them into three subfamilies (Secamonoideae, Asclepidoideae and Periplocoideae). Schlechter (1905) segregated Periplocaceae based on tetrads pollens from Asclepiadaceae but Endress and Bruyns (2000) classified the family Apocynaceae into five subfamilies, namely those of Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae and Asclepiadoideae. From a global perspective, Asclepiadoideae are comprised of 250 genera and more than 2,500 species; however, in Pakistan, 23 genera and 41 species are presently reported (El-Fiki et al., 2019).

Apocynaceous plants are perennial shrubs, trees or herbs that occasionally grow as annuals, with five joined petals on flowers, astringent milky latex, tufted seeds, and fruits that are typically in pairs (Shah and Ahmad, 2014). The leaves are exstipulate, lanceolate, alternate, simple, opposite decussate, ovate, obovate, oblong, linear, whorled, elliptic or oblong; sessile; or petiolate with an entire or undulate border and an acute apex while occasionally reduced or turned into spines (El-Fiki et al., 2019). Mesophytes and semi-succulent taxa have cylindrical stems, whereas succulent taxa have angled stems. Flowers have traits such as being actinomorphic, hypogynous, hermaphrodite, sympetalous and possessing pentamerous (sometimes tetramerous) whorls with a range of sizes. Further characteristics are a corolla with five joined petals, imbricate lobes and a calyx with five united imbricate sepals; the corona arises from the base of the petals or staminal filaments, and an androecium with five free stamens. The fruit of a parietal placenta consists of two carpels, two free ovaries, two styles that fuse just beneath the capitate stigma, two seeds that generally contain a distal tuft of silky hairs, and two dehiscent follicles containing multiple seeds (Morais et al., 2021).

In Pakistan, it is mostly present in subtropical regions of Mianwali, Khushab (Sakesar), Chakwal, Talagang, Jehlum, Sindh, Rawalpindi, Islamabad etc. in the form of twining shrubs, lianas, perennial herbs, scramblers, vines and also leafless succulent stems present in the order Gentianales. In Apocynaceae, some plants have medicinal values such as diuretic, antidiabetic, anti-inflammatory, stomachic and laxative, as well as utility in the treatment of respiratory disorders (Bahadur et al., 2018). To treat scorpion sting, snake bite and skin disorders, *Nerium oleander* is mainly used (Ashfaq et al., 2019). Phytochemical analysis reveals that *Calotropis procera*, *Dregea volubilis*, *Pergularia daemia*, *Carissa spinarum*, *Cryptolepis dubia* etc. have a significant place, both in traditional and modern medicine (Khan et al., 2022).

Pollen and seed germination tests can be used to identify salt-tolerant crops and pollen grain shapes can be utilised to identify interspecific and intraspecific relationships. These investigations were conducted to ascertain the links, both intraspecific and interspecific, between fruit tree species, which may be significant for processes related to fruit development, fertilisation and pollination (Khaleghi et al., 2019). Breeders and academics have already documented numerous cultivars and genotypes with undesirable pollen. Some cultivars/genotypes, for example, have sterile pollen or pollen with a low germination percentage. Pollen form and ultrastructure have been linked to growing habitat and pollination biology (Ćalić et al., 2013).

Modern taxonomists are particularly interested in palynological characterisation to distinguish closely related taxa and identification and assessment of interactions among species at several taxonomic levels rely heavily on pollen morphology (Majeed et al., 2023). Palynological studies can help us comprehend plant evolution and phylogeny. Scanning electron microscopy (SEM) is a contemporary technique for studying the micromorphological structure in depth (Majeed et al., 2022). We employ a scanning electron microscope with high-resolution power to examine the surface of pollen grains (Khan et al., 2022; Nabila et al., 2022). SEM is presently known to be one of the most innovative techniques for taxonomic research. Many palynologists are currently using pollen features to precisely and accurately distinguish closely related taxa (Bahadur et al., 2018; Abid et al., 2023).

The study aims to investigate intraspecific variations in pollen morphology within the Apocynaceae plant family. It seeks to provide a comprehensive understanding of the pollen traits that differ among closely related species, offering insights into their genetic diversity and potential applications in horticultural innovation. By creating a roadmap for this exploration, the study intends to contribute to the development of new horticultural techniques and strategies for the Apocynaceae family.

MATERIALS AND METHODS

Taxon sampling, identification, preservation and herbarium deposition

During the summer, several field trips were organised across the country. Several elements of vegetation were in full bloom. As shown in Table 1, diverse Apocynaceous samples were obtained from Islamabad, and various areas in Punjab such as Namal, Daud Khail, Sawans, Sakesar, Talagang, Chakwal, Rawalpindi and Sindh. The sites of plant collection are represented in the form of a map (Figure 1). Field area coordinates were collected with the help of the global positioning system (GPS) devices Garmin eTrex (Garmin eTrex 10 GPS, Garmin Oregon 750) and eTrex Venture (Garmin eTrex Venture HC GPS Receiver). Photographs of each plant were taken with the use of a Panasonic ZS 20 digital camera DMC-ZS20K as shown in Figures 2–4. Plant species taken from different regions were identified with the assistance of taxonomists and specimens were compared with already deposited specimens forming part of the collection of the Islamabad (ISL) Herbarium,

Table 1. Checklist of Apocynaceous plants sampling and herbarium accession.

Sr. No.	Apocynaceous taxa	Flowering period	Voucher No.	Localities	Coordinates	Collector's name	Accession No.	Altitude (feet)	District/province
1.	*Allamanda cathartica* L.	June to September	AC-01	Green town	32.576547° N, 71.575377° E	Rizwan & Salman	ISL-133416	688	Mianwali/ Punjab
2.	*Alstonia scholaris* (L.) R. Br.	November to January	AS-16	Cantt.	31.513099° N, 74.373667° E	Rizwan	ISL-133415	712	Lahore/Punjab
3.	*Asclepias curassavica* L.	June to October	AC-32	Rawalpindi	33.567410° N, 73.085568° E	Rizwan	ISL-133428	1,680	Rawalpindi/ Punjab
4.	*Calotropis procera* (Aiton) Dryand.	October to December	CP-06	Mirpur	25.514458° N, 69.023728° E	Rizwan & Prem	ISL-133431	60	Mirpur/Sindh
5.	*Carissa macrocarpa* (Eckl.) A. DC.	October to November	CM-15	Chakwal	32.939325° N, 72.864004° E	Rizwan	ISL-133422	1,965	Chakwal/Punjab
6.	*Carissa spinarum* L.	July to August	CS-10	Rumli	33.752956° N, 73.136338° E	Rizwan & Prem	ISL-133426	2,346	Islamabad
7.	*Cascabela thevetia* (L.) Lippold	June to October	CT-11	ICT	33.745615° N, 73.137888° E	Rizwan & Prem	ISL-133418	2,031	Islamabad
8.	*Catharanthus roseus* (L.) G. Don	July to August	CR-03	QAU	33.745623° N, 73132074° E	Rizwan	ISL-133419	2,031	Islamabad
9.	*Cryptolepis dubia* (Burm.f.) M.R.Almeida	June to August	CD-13	Sakesar	32.541652° N, 71.934362° E	Rizwan	ISL-133430	4,960	Khushab/Punjab
10.	*Leptadenia pyrotechnica* (Forssk.) Decne.	July to September	LP-09	Namal	32.668914° N, 71.614760° E	Rizwan & Salman	ISL-133425	1,129	Mianwali/ Punjab
11.	*Nerium oleander* L.	July to September	NO-12	Talagang	32.913338° N, 72.426670° E	Rizwan	ISL-133424	1,666	Talagang/Punjab
12.	*Oxystelma esculentum* (L. f.) Sm	July to January	OE-23	Mirpur	25.504108° N, 69.037589° E	Rizwan & Jamil	ISL-133427	63	Mirpur/Sindh
13.	*Pergularia daemia* (Forssk.) Chiov.	October to November	PD-14	Jehlum	32.951902° N, 73.689400° E	Rizwan	ISL-133429	768	Jehlum/Punjab
14.	*Periploca aphylla* Decne.	June to September	PA-05	Sakesar	32.558860° N, 71.913981° E	Rizwan	ISL-133414	5,092	Khushab/Punjab
15.	*Plumeria rubra* L.	July to October	PR-08	Mirpur	25.359219° N, 69.143117° E	Rizwan & Jamil	ISL-133421	59	Mirpur/Sindh
16.	*Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult.	August to September	TD-04	GHS	32.577587° N, 71.537370° E	Rizwan	133423	650	Mianwali/ Punjab
17.	*Vincetoxicum spirale* (Forssk.) D.Z.Li	June to August	VS-07	Daud Khail	32.888711° N, 71.614760° E	Rizwan	133420	692	Mianwali/ Punjab
18.	*Dregea volubilis* (L.f.) Benth. ex Hook.f	July to August	WV-02	QAU	33.746286° N, 73.138156° E	Rizwan	133417	2,031	Islamabad

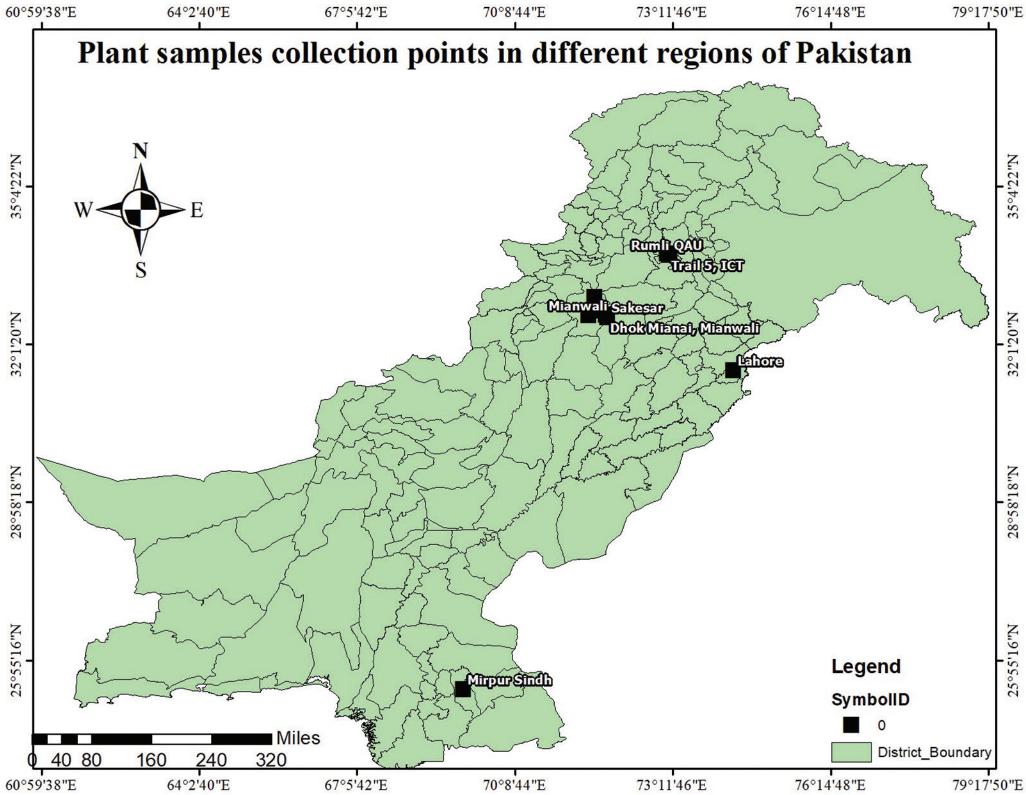

Figure 1. Map showing the sampling localities of Apocynaceous species.

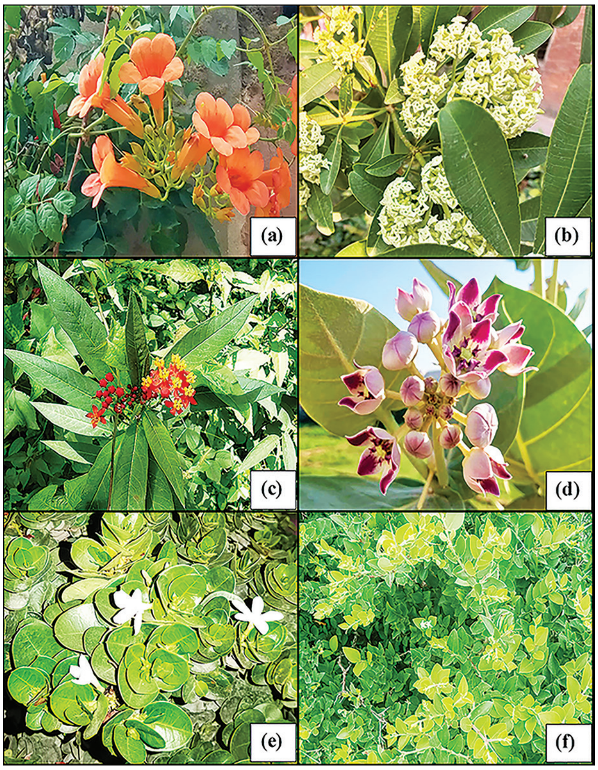

Figure 2. Field pictorial view: (A) *Allamanda cathartica*; (B) *Alstonia scholaris*; (C) *Asclepias curassavica*; (D) *Calotropis procera*; (E) *Carissa macrocarpa*; (F) *Carissa spinarum.*

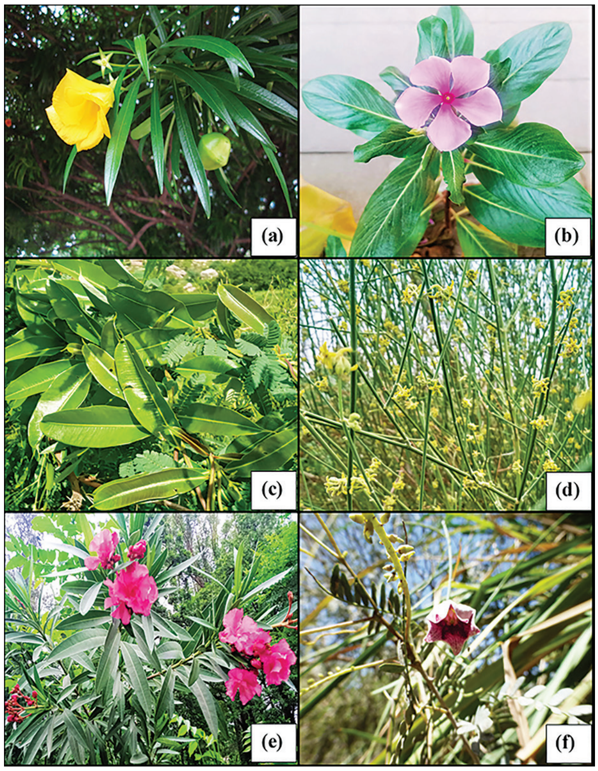

Figure 3. Field pictorial view: (A) *Cascabela thevetia*; (B) *Catharanthus roseus*; (C) *Cryptolepis dubia*; (D) *Leptadenia pyrotechnica*; (E) *Nerium oleander*; (F) *Oxystelma esculentum.*

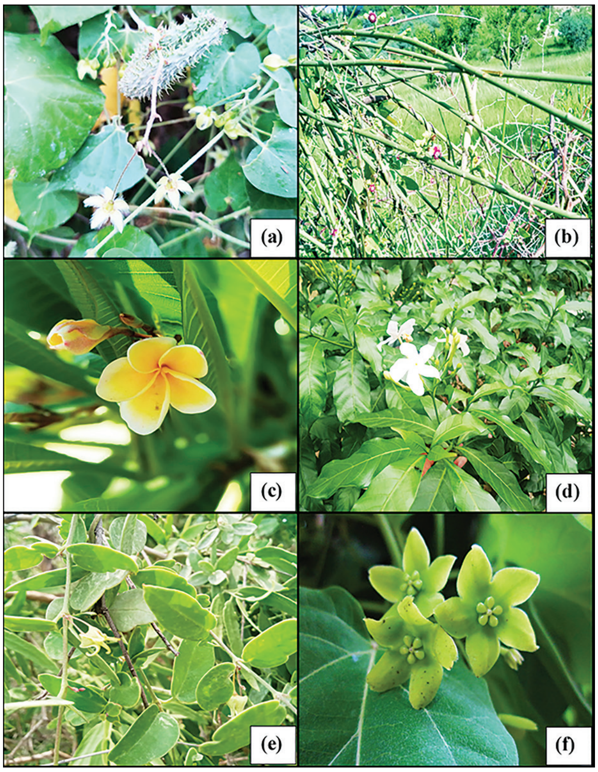

Figure 4. Field pictorial view: (A) *Pergularia daemia*; (B) *Periploca aphylla*; (C) *Plumeria rubra*; (D) *Tabernaemontana divaricata*; (E) *Vincetoxicum spirale*; (F) *Dregea volubilis.*

Pakistan. The confirmation of samples was compared from given literature from the flora of Pakistan (http://legacy.tropicos.org/Project/Pakistan), International Plant Name Index (https://www.ipni.org/) and World Flora Online (http://www.worldfloraonline.org/). After being preserved with ethanol and mercuric chloride solution, the identified specimens were pressed with blotter sheets and shade-dried. The poisoned and shade-dried specimens were mounted on herbarium sheets, documented and deposited in the ISL Herbarium (De Vogel, 1987).

Light microscopy

Pollen grains' micromorphological characteristics were investigated using a Meiji light microscope (MX 5200H, Japan). The slide is prepared by applying Blackmore's (1983) and Erdtman's (1986) acetolysis procedure with marginal modifications, which is a widely used technique among palynologists worldwide. Anthers were gathered from dried blossomed flowers, typically when they were in the anthesis stage. Polleniferous material such as anthers was removed from flowers and placed in a glass slide using clean forceps. Two to three drops of acetic acid were deposited over the anthers, and this was followed by smashing them with a glass rod. Pollen grains were discharged from the anthers in 2–3 min. While pollen grains were stuck to the slide, the remaining debris was removed. In the case of pollinia, the methodology is slightly different; here we use a tweezer to pick up pollinia and place it over a glass slide without any crushing, because pollinia are fragile and they can easily break up. The pollen was then stained using one to two drops of glycerine jelly, and a cover slip was placed over the glass slide to preserve it over time. Slides that had been prepared were examined both quantitatively and qualitatively.

SEM

SEM (Model JEOL-5910, Japan) photomicrographs were used to investigate sculptured components of pollen grain. The pollen was released after one drop of acetic acid (45%) was dropped over the separated anthers from blossomed flowers and they were placed on a glass slide. A rounded stub was adhered to the glass using double-sided adhesive tape so that it could take up pollen grains from the slide. After that, the stub was coated in gold palladium. Specimens were coated in gold using a sputtering apparatus. To capture micrographs of exine sculpture, these stubs were installed in an SEM machine (Mir et al., 2019; Naz et al., 2019). The description of the pollen grains was taken from the glossary of Punt et al. (2007).

P/E index

The ratio between the polar diameter and equatorial diameter is determined by the following formula:

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

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

Pollen fertility and sterility (%)

The percentage of fertility and sterility were calculated by using the following formulae (Umber et al., 2022), as mentioned in Table 2.

$$\text{Fertility} = F/F + S \times 100$$

where fertile pollen is represented by F and sterile pollens by S on ocular.

$$\text{Sterility} = S/S + F \times 100$$

where sterile pollen is represented by S and fertile pollens by F on ocular.

Taxonomic key based on palynological characters

To construct a taxonomic key for accurately identifying species within the Apocynaceous taxa, the distinct floral palynological features discussed were further scrutinised and differentiated.

SPSS statistical analysis

For statistical analysis of pollen grains, we use the SPSS 16.0 software, USA. Data were utilised to statistically calculate the equatorial diameter, polar diameter, exine thickness, P/E ratio, colpi width, colpi length and their

Table 2. Pollen ferity and sterility count among Apocynaceous species.

Sr. No.	Apocynaceous taxa	Fertile pollen/pollinia	Sterile pollen/pollinia	Fertility%	Sterility%
1.	*Allamanda cathartica* L.	26	12	68.42	31.57
2.	*Alstonia scholaris* (L.) R.Br.	18	06	75.00	25.00
3.	*Asclepias curassavica* L.	05	01	83.33	16.66
4.	*Calotropis procera* (Aiton) Dryand.	08	04	66.66	33.33
5.	*Carissa macrocarpa* (Eckl.) A. DC.	23	09	71.87	28.12
6.	*Carissa spinarum* L.	14	04	77.77	22.22
7.	*Cascabela thevetia* (L.) Lippold	21	08	72.41	27.58
8.	*Catharanthus roseus* (L.) G. Don	14	06	70.00	30.00
9.	*Cryptolepis dubia* (Burm.f.) M.R. Almeida	12	02	85.71	14.28
10.	*Leptadenia pyrotechnica* (Forssk.) Decne.	08	02	80.00	20.00
11.	*Nerium oleander* L.	09	02	81.81	18.18
12.	*Oxystelma esculentum* (L. f.) Sm	10	03	76.92	23.07
13.	*Pergularia daemia* (Forssk.) Chiov.	05	02	71.42	28.57
14.	*Periploca aphylla* Decne.	11	04	73.33	26.66
15.	*Plumeria rubra* L.	11	03	78.57	21.42
16.	*Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult.	16	05	76.19	23.80
17.	*Vincetoxicum spirale* (Forssk.) D.Z. Li	06	02	75.00	25.00
18.	*Dregea volubilis* (L.f.) Benth. ex Hook.f	07	03	70.00	30.00

mean, maximum, minimum and standard error values. For each parameter, the mean (minimum–maximum) standard error (SE) was statistically analysed.

Statistical multivariate analysis

We conducted the study and applied the unweighted pair group method with arithmetic mean (UPGMA) with the help of paleontological statistics (PAST) statistical tool version 3.0 software to create a dendrogram based on Euclidean distances to determine the closer relationship between the species. Using principal component analysis (PCA), the most important features that accounted for the largest share of the variability were identified (Osman et al., 2014).

RESULTS

The pollen grains of different plants species belong to different genera, such as *Allamanda*, *Alstonia*, *Asclepias*, *Calotropis*, *Carissa*, *Cascabela*, *Catharanthus*, *Cryptolepi*, *Dregea*, *Leptadenia*, *Nerium*, *Oxystelma*, *Pergularia*, *Periploca*, *Plumeria*, *Tabernaemontana* and *Vincetoxicum*. The light microscopic results are shown in Figures 5 and 6, whereas the SEM results in Figures 7–11. The palynological traits of 18 Apocynaceae species that were examined have been summarised in Tables 3 and 4. The major taxonomical characteristics for identifying a species are the pollen size, pollen type, equatorial view, polar view, dispersal unit, aperture ornamentation, exine sculpturing, P/E ratio, exine thickness, polar diameter, equatorial diameter, length of colpi, width of colpi and mesocolpium (Tables 5 and 6).

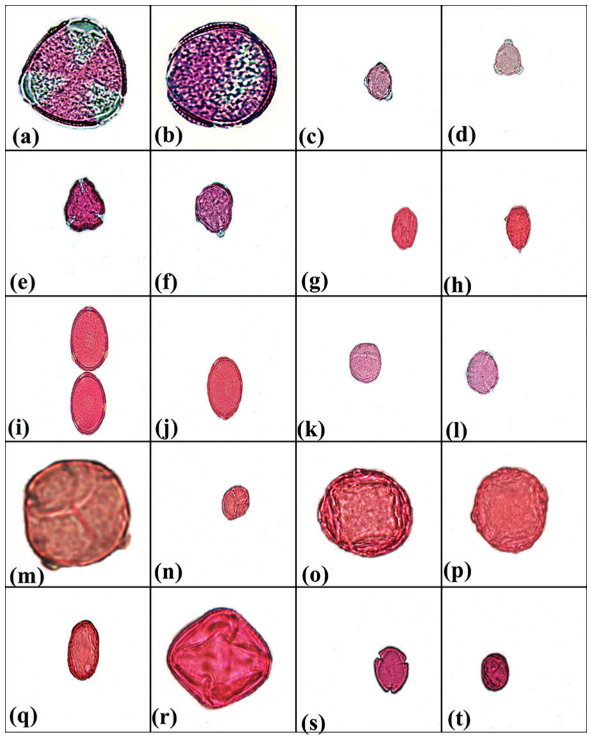

Figure 5. Light microphotographs of Apocynaceous pollen showing polar and equatorial view taken at 40× magnification, scale bar 10 µm (A,B) *Allamanda cathartica*; (C,D) *Alstonia scholaris*; (E,F) *Carissa macrocarpa*; (G,H) *Carissa spinarum*; (I,J) *Cascabela thevetia*; (K,L) *Catharanthus roseus*; (M,N) *Cryptolepis dubia*; (O,P) *Nerium oleander*; (Q,R) *Plumeria alba*; (S,T) *Tabernaemontana divaricata.*

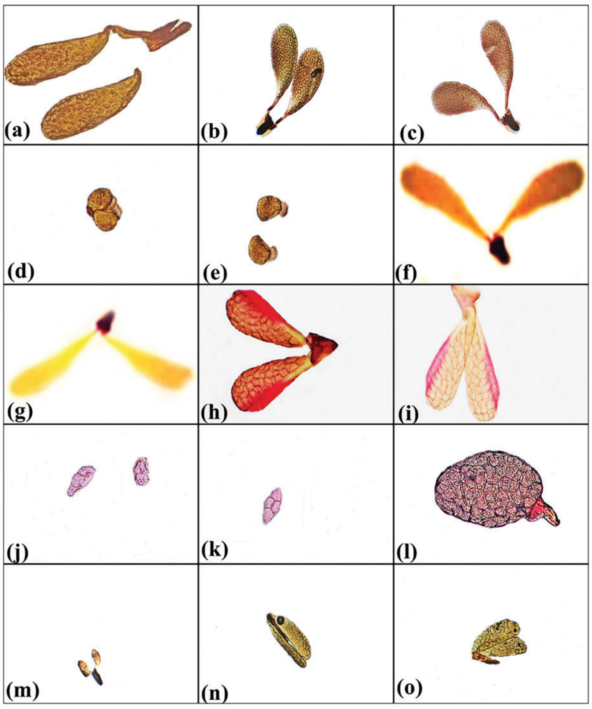

Figure 6. Light microphotographs of Apocynaceous pollen showing polar and equatorial view taken at 40× magnification, scale bar 10 μm (A) *Asclepias curassavica*; (B,C) *Calotropis procera*; (D,E) *Leptadenia pyrotechnica*; (F,G) *Oxystelma esculentum*; (H,I) *Pergularia daemia*; (J,K) *Periploca aphylla*; (L,M) *Vincetoxicum spirale*; (N,O) *Dregea volubilis.*

Pollen morphological description

Allamanda cathartica L.

Allamanda cathartica pollen grains were medium-sized, tricolporate, elliptic and triangular (convex), and shed as monad. Reticulate exine sculpturing has been observed, and polar and equatorial diameters were observed to be 30.1 ± 0.89 μm and 30.25 ± 0.95 μm, respectively. The length of the colpi was measured as 13.65 ± 0.70 μm and the width of the colpi as 13.65 ± 0.70 μm. Exine thickness was taken as 1.95 ± 0.26 μm. Mesocolpium was 21.60 ± 0.74 μm. The P/E ratio was calculated as 0.99. The numbers of fertile and sterile pollen were 26 and 12, respectively (Figures 5A, 5B and 7A–7C).

Alstonia scholaris (L.) R.Br.

Alstonia scholaris pollen grains were small, tricolporate, sub-spheroidal, lobate and isopolar, and shed as a monad. Psilate to perforate exine sculpturing has been observed; polar and equatorial diameters were observed to be 20.75 ± 0.51 μm and 23.40 ± 0.30 μm, respectively. The length of the colpi was measured as 5.30 ± 0.34 μm and the width of the colpi as 4.80 ± 0.72 μm. Exine thickness was taken as 3.10 ± 0.18 μm. Mesocolpium was 21.95 ± 0.62 μm. The P/E ratio was calculated as 0.86. The numbers of fertile and sterile pollen were 18 and 6, respectively (Figures 5C, 5D and 7D–7F).

Asclepias curassavica L.

Asclepias curassavica pollinia were large, brown, pendent, narrowly oblong and characterised by the absence of sterile margins; further, translator attachment to pollinium was basal and corpuscular arm was absent, while translator attachment to corpusculum was sub-apical and shed as a pair of pollinium sacs; and the length and width of pollinium sac were observed to be 209.60 ± 1.62 μm and 82.70 ± 0.44 μm, respectively. The length of the translator was taken as 90.05 ± 2.85 μm and the breadth was 23.25 ± 0.35 μm. The length and width of the corpusculum were calculated as 89.25 ± 1.64 μm and 33.25 ± 2.800 μm, respectively. The number of fertile pollinia was five and that of sterile pollen was one (Figure 6A).

Calotropis procera (Aiton) Dryand

Calotropis procera pollinia were large, canary yellow, pendent, obovate and characterised by the absence of sterile margins; further, translator attachment to pollinium was basal and the corpuscular arm was absent, while translator attachment to corpusculum was sub-apical and shed as a pair of pollinium sacs; and the length and breadth of pollinium sac were observed to be 315.81 ± 0.48 μm and 129.44 ± 0.55 μm, respectively. The length of the translator was taken as 47.25 ± 0.27 μm and the breadth as 12.87 ± 0.48 μm. The length and breadth of the corpusculum were calculated as 90.93 ± 0.61 μm and 56.68 ± 0.20 μm, respectively. The number of fertile pollinia was eight and that of sterile pollen was four (Figures 6B, 6C and 7G–7I).

Carissa macrocarpa (Eckl.) A. DC.

Carissa macrocarpa pollen grains were medium, tricolporate, oblate sub-spheroidal, circular and isopolar, and shed as a monad. Perforate to regulate exine sculpturing has been observed, and polar and equatorial diameters were observed to be 36.80 ± 0.89 μm and 36.95 ± 0.46 μm, respectively. The length of the colpi was measured as 3.00 ± 0.34 μm and the width of the colpi as 5.50 ± 0.48 μm. Exine thickness was taken as 3.20 ± 0.14 μm. Mesocolpium was 35.85 ± 0.07 μm. The P/E ratio was calculated as 0.99. The numbers of fertile and sterile pollen were 23 and 9, respectively (Figures 5E, 5F and 7J–7L).

Carissa spinarum L.

Carissa spinarum pollen grains were medium, tricolporate, oblate sub-spheroidal, lobate and isopolar, and shed as monad. Perforate and psilate to regulate exine sculpturing have been observed, and polar and equatorial diameters were observed to be 24.30 ± 0.55 μm and 26.35 ± 0.46 μm, respectively. The length of the colpi was measured as 2.8 ± 0.16 μm and the width of the colpi as 2.25 ± 0.18 μm. Exine thickness was taken as 3.35 ± 0.32 μm. Mesocolpium was 20.55 ± 0.93 μm. The P/E ratio was calculated as 0.92. The numbers of fertile and sterile pollen were 14 and 4, respectively (Figures 5G, 5H and 8A–8C).

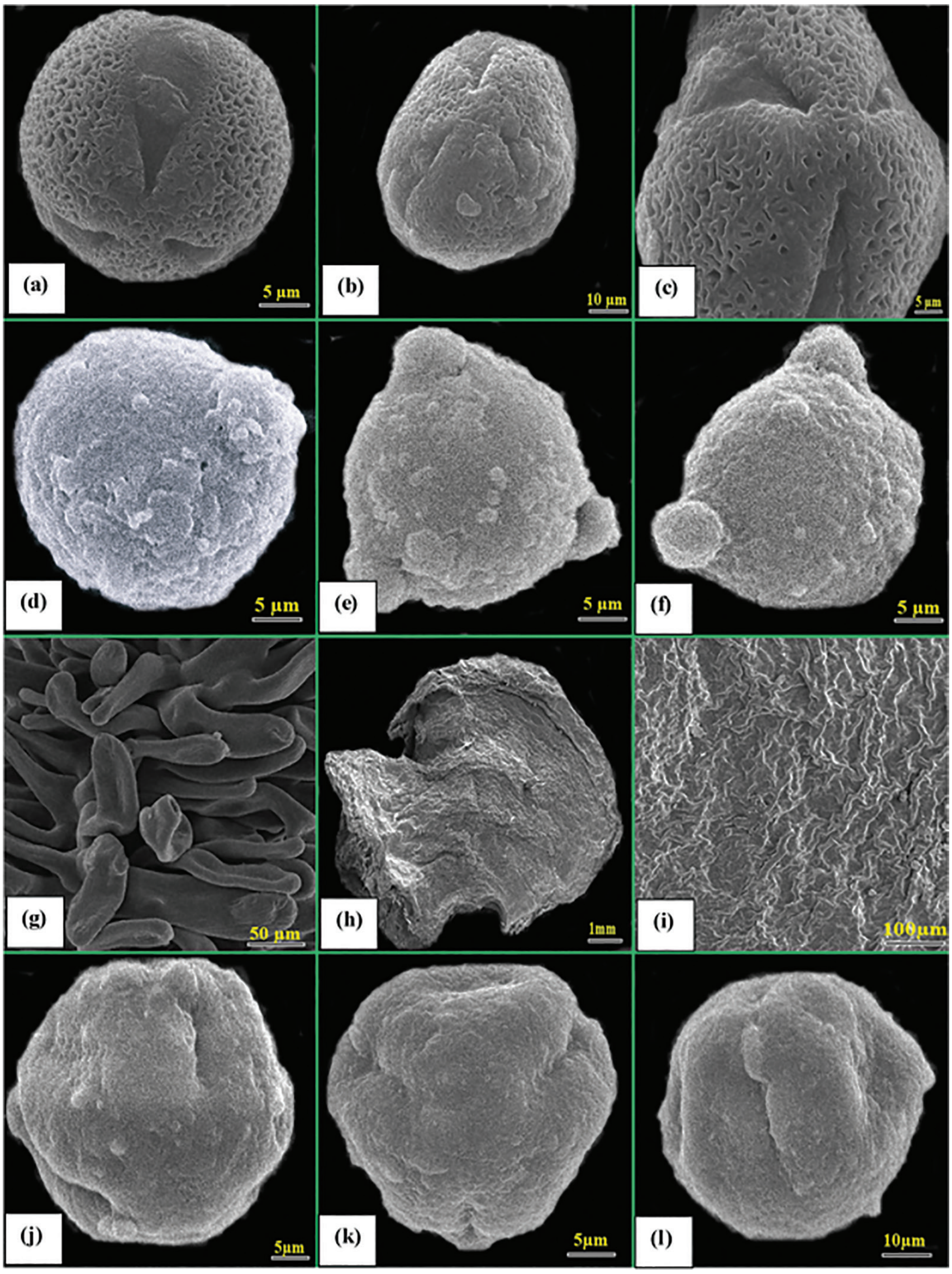

Figure 7. Scanning electron photomicrographs of Apocynaceae pollen (A–C) *Allamanda cathartica*; (D–F) *Alstonia scholaris*; (G–I) *Calotropis procera*; (J–L) *Carissa macrocarpa.*

Cascabela thevetia (L.) Lippold

Cascabela thevetia pollen grains were very large, tricolporate, prolate spheroidal and lobate, and shed as a monad. Micro-reticulate to perforate exine sculpturing has been observed; and polar and equatorial diameters were observed to be 113.45 ± 0.80 µm and 109.20 ± 0.40 µm, respectively. The length of the colpi was measured as 5.20 ± 0.13 µm and the width of the colpi as 15.20 ± 0.50 µm. Exine thickness was taken as 2.8 ± 0.28 µm. Mesocolpium was 18.33 ± 0.55 µm. The P/E ratio was calculated as 1.02. The numbers of fertile and sterile pollen were 21 and 8, respectively (Figures 5I, 5J and 8D–8F).

Catharanthus roseus (L.) G. Don

Catharanthus roseus pollen grains were large, tricolporate, oblate spheroidal and irregular, and shed as a monad. Perforate to scabrate exine sculpturing has been observed; and polar and equatorial diameters were observed to be 56.45 ± 0.89 µm and 57.30 ± 0.38 µm, respectively. The length of the colpi was measured as 13.60 ± 0.48 µm and the width of the colpi as

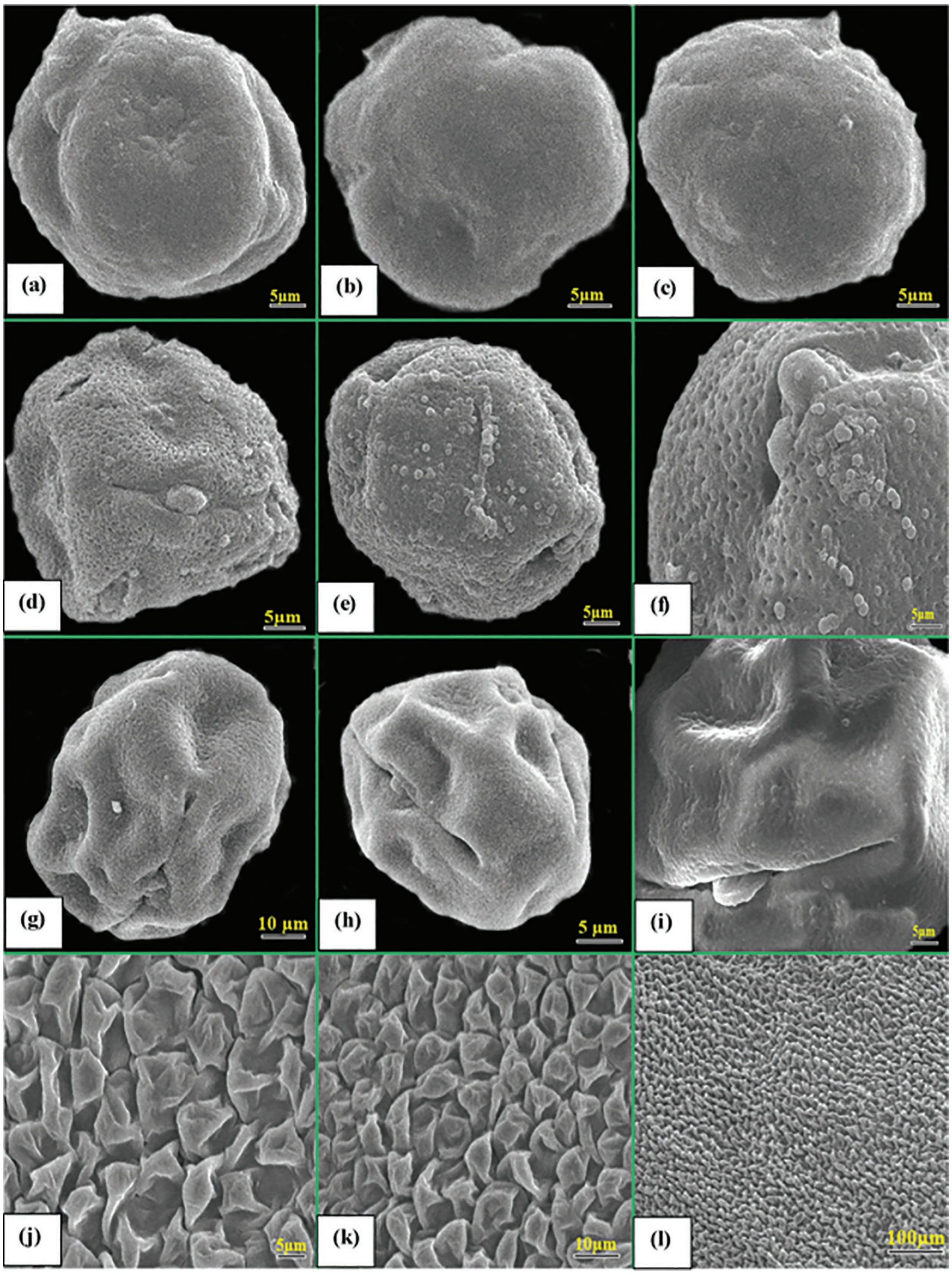

Figure 8. Scanning electron photomicrographs of Apocynaceae pollen (A–C) *Carissa spinarum*; (D–F) *Cascabela thevetia*; (G–I) *Catharantheus roseus*; (J–L) *Cryptolepis dubia.*

6.50 ± 0.395 μm. Exine thickness was taken as 4.85 ± 0.37 μm. Mesocolpium was 44.25 ± 0.379 μm. The P/E ratio was calculated as 0.98. The numbers of fertile and sterile pollen were 14 and 6, respectively (Figures 5K, 5L and 8G–8I).

Cryptolepis dubia (Burm.f.) M.R. Almeida

C. dubia pollen grains were small to medium, tetrad and rhomboidal, and shed as a tetrad. Reticulate exine sculpturing has been observed, and polar and equatorial diameters were observed to be 20.30 ± 0.16 μm and 20.30 ± 0.16μm, respectively. Colpi was absent. Exine thickness was taken as 1.35 ± 0.43 μm. The P/E ratio was calculated as 0.98. The numbers of fertile and sterile pollen were 12 and 2, respectively (Figures 5M, 5N and 8J–8L).

Leptadenia pyrotechnica (Forssk.) Decne.

Leptadenia pyrotechnica pollinia were medium, brown, transverse, orbicular and characterised by the presence of sterile margins; further, the orientation of sterile margins was apical, translator attachment to pollinium was basal and the corpuscular arm was absent while translator attachment to corpusculum was apical and shed as a pair of pollinium sacs; and the length and breadth of pollinium sac were observed to be 46.30 ± 0.82 μm

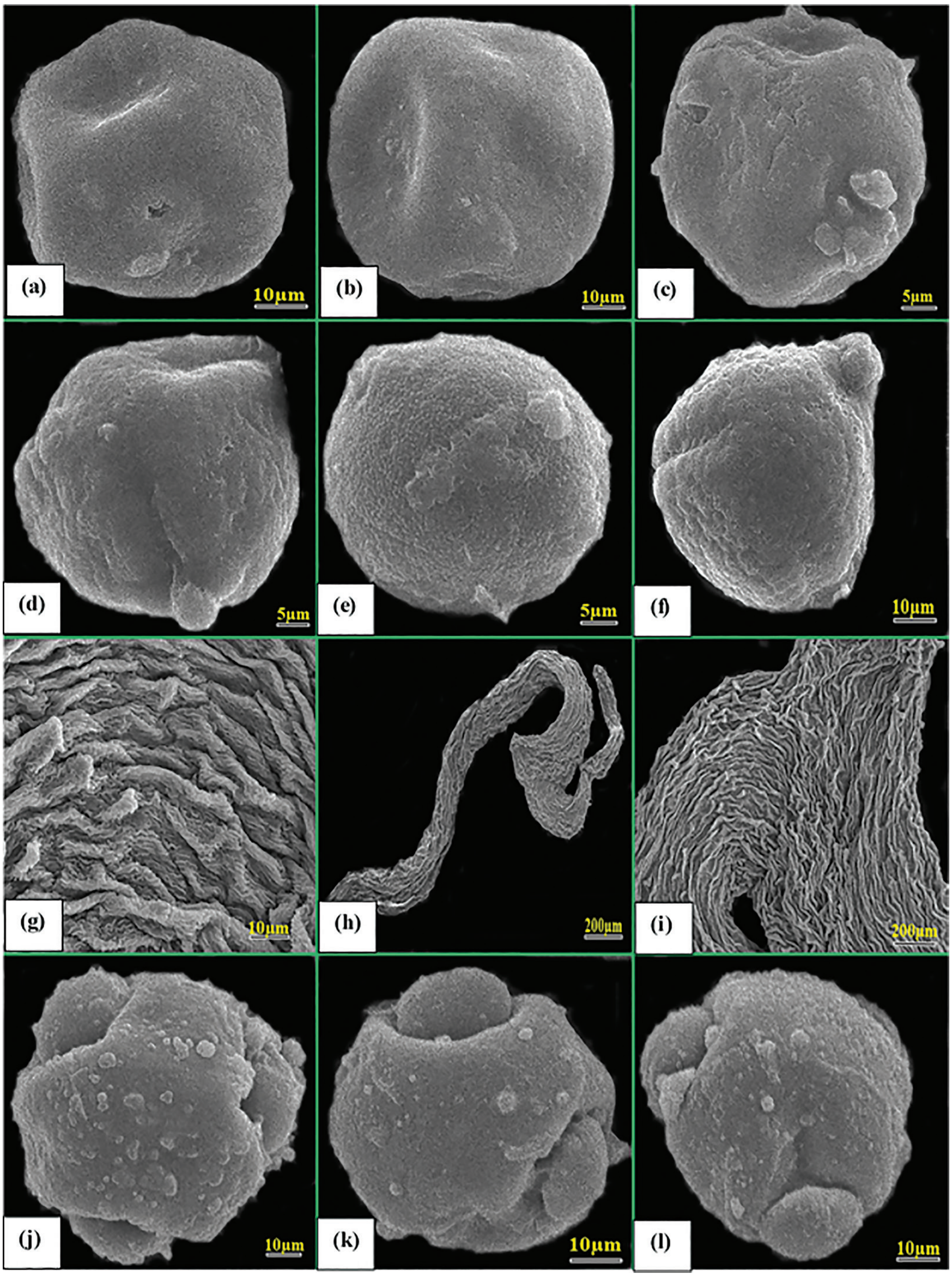

Figure 9. Scanning electron photomicrographs of Apocynaceae pollen (A–C) *Nerium oleander*; (D–F) *Pergularia daemia*; (G–I) *Periploca aphylla*; (J–L) *Rhazya stricta.*

and 33.35 ± 0.19 μm, respectively. The length of the translator was taken as 7.25 ± 0.66 μm and the breadth as 7.75 ± 0.26. The length and breadth of the corpusculum were calculated as 16.80 ± 0.02 μm and 13.40 ± 0.35 μm, respectively. The number of fertile pollinia was eight while that of sterile pollen was two (Figures 6D and 6E).

Nerium oleander L.

Nerium oleander pollen grains were medium, tricolporate, oblate spheroidal and circular, and shed as a monad. Psilate exine sculpturing has been observed; and polar and equatorial diameters were observed to be 33.65 ± 0.69 μm and 34.25 ± 0.70 μm, respectively. The length of the colpi was measured as 3.10 ± 0.52 μm and the width of the colpi as 5.25 ± 0.34 μm. Exine thickness was taken as 3.20 ± 0.20 μm. Mesocolpium was 36.70 ± 0.89 μm. The P/E ratio was calculated as 0.98. The numbers of fertile and sterile pollen were nine and two, respectively (Figures 5O, 5P and 9A–9C).

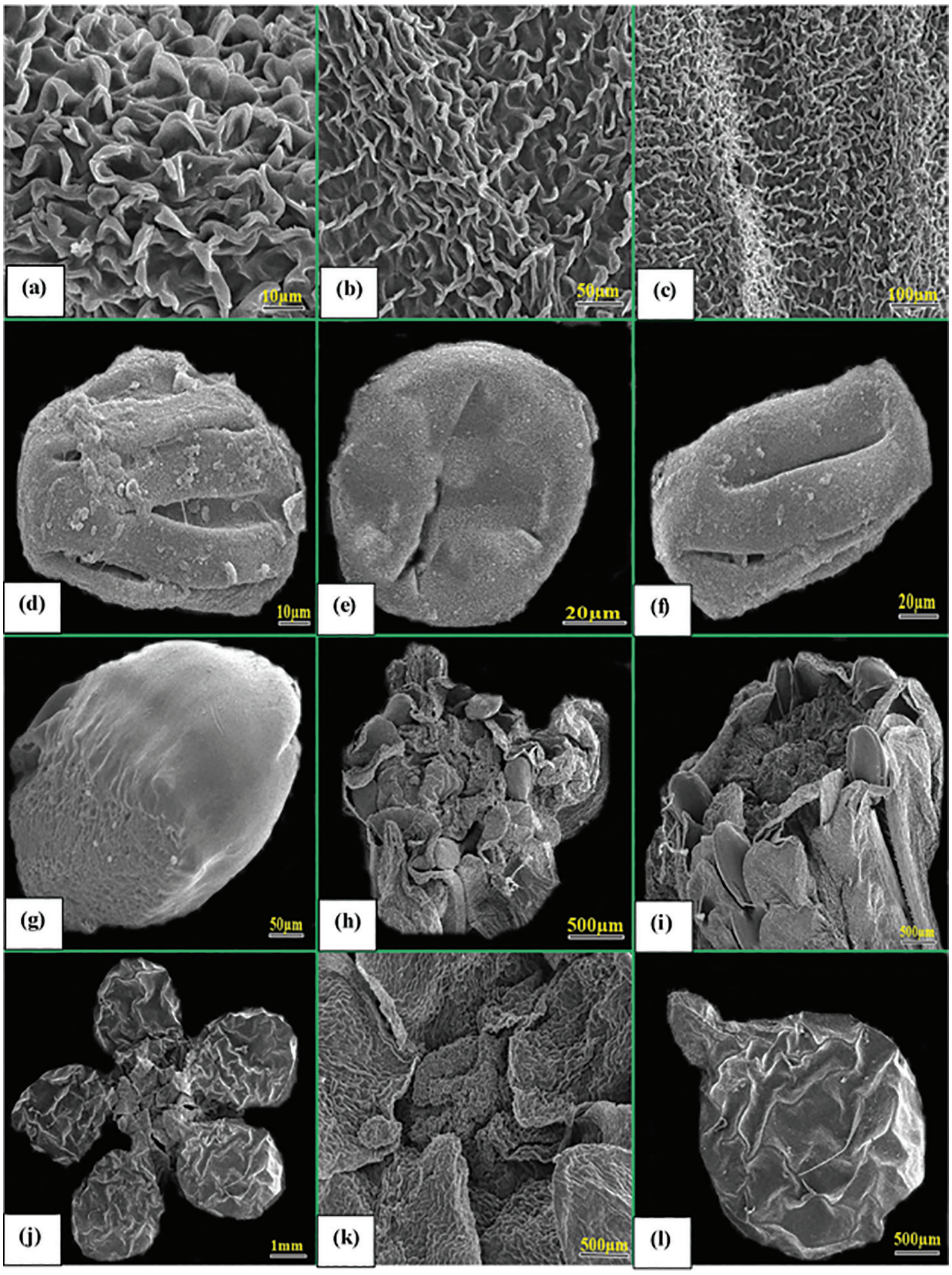

Figure 10. Scanning electron photomicrographs of Apocynaceae pollen (A–C) *Tabernaemontana divaricata*; (D–F) *Vinca major*; (G–I) *Vincetoxicum spirale*; (J–L) *Dregea volubilis.*

Oxystelma esculentum (L. f.) Sm

Oxystelma esculentum pollinia were large, yellow, pendent, narrowly oblong and characterised by the absence of sterile margins; further, translator attachment to pollinium was basal and corpuscular arm was absent while translator attachment to corpusculum was apical and shed as a pair of pollinium sacs; and the length and breadth of pollinium sac were observed to be 179.55 ± 0.69 µm and 37.62 ± 0.25 µm, respectively. The length of the translator was taken as 24.7 ± 0.82 µm and the breadth as 8.75 ± 0.62 µm. The length and breadth of the corpusculum were calculated as 74.63 ± 0.82 µm and 34.40 ± 1.12 µm, respectively. The number of fertile pollinia was 10 and that of sterile pollinia was 3 (Figure 6F, 6G and 9D–9L).

Pergularia daemia (Forssk.) Chiov.

Pergularia daemia pollinia were large, pinkish brown, pendent, obovate and characterised by the presence of pseudo-sterile margins; further, the orientation of margin was parallel, translator attachment to pollinium was basal and the corpuscular arm was absent while translator attachment to corpusculum was apical and shed as a pair of pollinium sacs; and the length and breadth of pollinium sac were observed to be 168.45 ± 1.37 µm and 61.30 ± 0.90 µm, respectively, with the translator

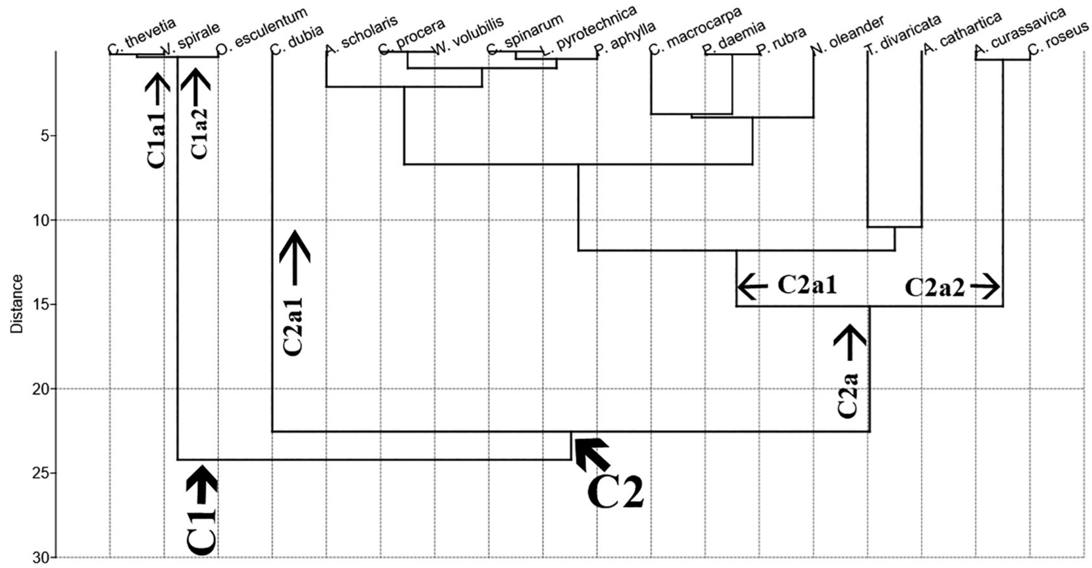

Figure 11. Dendrogram clustering showing the relationship among different Apocynaceous taxa.

being absent. The length and breadth of the corpusculum were calculated as 51.65 ± 0.49 µm and 41.40 ± 0.23 µm, respectively. The number of fertile pollinia was five and that of sterile pollinia was two (Figures 6H, 6I and 9D–9F).

Periploca aphylla Decne

Periploca aphylla pollen was small to large, the shape of the pollen was tetrad, the polar view was rhomboidal while the equatorial view was oblong and pollen shed as a tetrad. Psilate exine sculpturing has been observed; and polar and equatorial diameters were observed to be 52.00 ± 0.72 µm and 24.16 ± 1.02 µm, respectively. Colpi was absent, and exine thickness was taken as 4.90 ± 0.20 µm. The P/E ratio was calculated as 3.53. The numbers of fertile and sterile pollen were, respectively, 11 and 4 (Figures 6J, 6K and 9G–9I).

Plumeria rubra L.

Plumeria rubra pollen grains were medium in size, tricolpate, prolate sub-spheroidal, circular and isopolar, and shed as a monad. Perforate to regulate exine sculpturing has been observed; and polar and equatorial diameters were observed to be 36.80 ± 0.89 µm and 36.95 ± 0.46 µm, respectively. The length of the colpi was measured as 3.00 ± 0.34 µm and the width of the colpi as 5.50 ± 0.48µm. Exine thickness was taken as 3.20 ± 0.14 µm. Mesocolpium was 35.85 ± 0.07. The P/E ratio was calculated as 0.99. The numbers of fertile and sterile pollen were 23 and 9, respectively (Figures 5Q, 5R and 9J–9L).

Tabernaemontana divaricata (L.) R.Br. ex Roem. & Schult

Tabernaemontana divaricata pollen grains were medium-sized, tricolporate, oblate spheroidal, circular and isopolar, and shed as a monad; perforate exine sculpturing has been observed, and polar and equatorial diameter were observed to be 28.85 ± 0.42 µm and 29.80 ± 0.86 µm, respectively. The length of the colpi was measured as 8.45 ± 0.50 µm and the width of the

Taxonomic identification key of Apocynaceous pollen

1+ Pollen tetraporate, oblate-spheroidal, psilate ornamentation .... *N. oleander*
– Pollen tetrad, tricolporate 2
2 + Tetrad, oblong, psilate sculpturing .... *P. aphylla*
2 – Tetrad, rhomboidal, reticulate sculpturing .... *C. dubia*
3 + Tricolporate, oblate spheroidal, perforate-regulate exine .... *C. macrocarpa*
3 – Tricolporate, lobate, psilate-regulate sculpturing .... *C. spinarum*
4 + Oblate-spheroidal, tricolporate, perforate-scabrate .... *C. roseus*
4 – Colporate, perforate exine sculpturing .... *T. divaricata*
5 + Sub-spheroidal, reticulate exine, tricolporate .... *A. cathartica*
5 – Lobate, psilate-perforate exine .... *A. scholaris*
6 + Prolate-spheroidal, tricolporate, psilate to scabrate exine .... *P. alba*
6 – Colporus, micro-reticulate exine, lobate .... *C. thevetia*

Table 3. Qualitative pollen micromorphological characters of Apocynaceous taxa.

Sr. No.	Apocynaceous taxa	Size	Pollen type	Pollen shape	Polar view (Amb)	Dispersal unit	Aperture orientation	Exine sculpturing
1.	*Allamanda cathartica* L.	Medium	Tricolporate	Sub-spheroidal	Triangular (convex)	Monad	Colporate	Reticulate
2.	*Alstonia scholaris* (L.) R. Br.	Small	Tricolporate	Sub-spheroidal	Lobate & isoplar	Monad	Scabrate	Psilate to perforate
3.	*Carissa macrocarpa* (Eckl.) A. DC.	Medium	Tricolporate	Oblate spheroidal	Circular & isoplar	Monad	Colporate	Perforate to regulate
4.	*Carissa spinarum* L.	Medium	Tricolporate	Oblate spheroidal	Lobate & isopolar	Monad	Colporate	Perforate, psilate to regulate
5.	*Cascabela thevetia* (L.) Lippold	Very large	Tricolporate	Prolate spheroidal	Lobate	Monad	Colporus	Microreticulate to perforate
6.	*Catharanthus roseus* (L.) G. Don	Large	Tricolporate	Oblate spheroidal	Irregular	Monad	Colporate	Perforate to scabrate
7.	*Cryptolepis dubia* (Burm.f.) M.R. Almeida	Small to medium	Tetrad	Rhombodial	Rhombodial	Tetrad		Reticulate
8.	*Nerium oleander* L.	Medium	Tetraporate	Oblate spheroidal	Circular	Monad	Porate	Psilate
9.	*Periploca aphylla* Decne.	Small to large	Tetrad	Oblong	Rhomboidal	Tetrad	Porate	Psilate
10.	*Plumeria rubra* L.	Medium	Tricolporate	Prolate spheroidal	Lobate & isoploar	Monad	Colporate	Psilate to scabrate
11.	*Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult.	Medium	Tricolpate	Oblate spheroidal	Circular & isopolar	Monad	Colpoate	Perforate

Table 4. Qualitative traits of morpho-structure of pollinia.

Sr. No.	Apocynaceous taxa	Pollinium orientation	Pollinium shape	Sterile margins	Orientation of sterile margins	Translator attachment to pollinium	Corpuscular arm	Translator attachment to corpusculum	Colour of pollinia	Exine sculpturing
1.	*Asclepias curassavica* L.	Pendent	Narrowly oblong	Absent	Absent	Basal	Absent	Sub-apical	Brown	Psilate & Perforate
2.	*Calotropis procera* (Aiton) Dryand.	Pendent	Obovate	Absent	Absent	Basal	Absent	Sub-apical	Canary yellow	Psilate
3.	*Leptadenia pyrotechnica* (Forssk.) Decne.	Transverse	Orbicular	Present	Apical	Basal	Absent	Apical	Brown	Psilate
4.	*Oxystelma esculentum* (L. f.) Sm	Pendent	Narrowly oblong	Absent	Absent	Basal	Absent	Apical	Yellow	Perforate
5.	*Pergularia daemia* (Forssk.) Chiov.	Pendent	Obovate	Pseudo-sterile	Parallel	Basal	Absent	Apical	Pinkish Brown	Psilate, perforate
6.	*Vincetoxicum spirale* (Forssk.) D.Z.Li	Pendent	Narrowly oblong	Absent	Absent	Basal	Present	Apical	Golden	Psilate, perforate
7.	*Dregea volubilis* (L.f.) Benth. ex Hook.f	Erect	Reniform	Absent	Absent	Basal	Absent	Apical	Sulphur yellow	Perforate

Table 5. Quantitative measurement of pollinia.

Sr. No.	Apocynaceous taxa	Length of pollinium Sac mean (min–max) SE (µm)	Breadth of pollinium Sac mean (min–max) SE (µm)	Length of translator mean (min–max) SE (µm)	Breadth of translator mean (min–max) SE (µm)	Length of corpusculum mean (min–max) SE (µm)	Breadth of corpusculum mean (min–max) SE (µm)
1.	*Asclepias curassavica* L.	209.60 (200.25–215.75) ± 1.62	82.70 (73.75–88.00) ± 0.44	90.05 (83.50–100.50) ± 2.85	23.25 (22.25–24.25) ± 0.35	89.25 (83.75–93.50) ± 1.64	33.25 (23.75–41.00) ± 2.800
2.	*Calotropis procera* (Aiton) Dryand.	315.81 (306.50–323.25) ± 0.48	129.44 (128.25–130.50) ± 0.55	47.25 (41.25–51.25) ± 0.27	12.87 (11.50–13.75) ± 0.48	90.93 (85.25–95.50) ± 0.61	56.68 (51.00–65.75) ± 0.20
3.	*Leptadenia pyrotechnica* (Forssk.) Decne.	46.30 (35.25–55.75) ± 0.82	33.35 (26.50–41.25) ± 0.19	7.25 (5.25–9.00) ± 0.66	7.75 (5.75–12.75) ± 0.26	16.80 (10.25–22.75) ± 0.02	13.40 (12.75–14.75) ± 0.35
4.	*Oxystelma esculentum* (L. f.) Sm	179.55 (81.25–98.32) ± 0.69	37.62 (40.23–35.02) ± 0.25	24.7 (23.15 + 26.25) ± 0.82	8.75 (8.3–9.2) ± 0.62	74.63 (78.36–70.92) ± 0.82	34.40 (32.26–36.55) ± 1.12
5.	*Pergularia daemia* (Forssk.) Chiov.	168.45 (159.00–178.00) ± 1.37	61.30 (55.25–71.50) ± 0.90	Absent	Absent	51.65 (46.00–54.00) ± 0.49	41.40 (32.00–60.75) ± 0.23
6.	*Vincetoxicum spirale* (Forssk.) D.Z.Li	74.55 (73.25–77.00) ± 0.73	47.65 (42.75–57.75) ± 0.70	21.55 (18.25–25.25) ± 1.36	21.45 (17.75–25.50) ± 0.33	75.85 (71.25–82.75) ± 0.14	37.40 (31.25–42.75) ± 0.82
7.	*Dregea volubilis* (L.f.) Benth. ex Hook.f	98.15 (58.25–117.25) ± 1.90	38.35 (32.00–43.25) ± 0.01	14.85 (12.25–18.00) ± 1.01	7.95 (7.25–8.75) ± 0.25	258.60 (244–270.50) ± 0.35	33.95 (17.75–56.25) ± 7.85

Table 6. Quantitative findings of Apocynaceous pollen.

Sr. No.	Apocynaceous taxa	P/E ratio	Exine thickness mean (min–max) SE (µm)	Polar diameter mean (min–max) SE (µm)	Equatorial diameter mean (min–max) SE (µm)	Length of colpi mean (min–max) SE (µm)	Width of colpi mean (min–max) SE (µm)	Mesocolpium mean (min–max) SE (µm)
1.	*Allamanda cathartica* L.	0.99	1.95 (1.25–2.75) ± 0.26	30.1 (27.25–32.75) ± 0.89	30.25 (28.00–33.75) ± 0.95	13.65 (12.25–16.25) ± 0.70	6.05 (4.75–7.25) ± 0.48	21.60 (21.75–23.75) ± 0.74
2.	*Alstonia scholaris* (L.) R. Br.	0.86	3.10 (3.75–2.75) ± 0.18	20.75 (15.25–23.25) ± 0.51	23.40 (20.25–27.75) ± 0.30	5.30 (4.50–6.50) ± 0.34	4.80 (2.00–6.25) ± 0.72	21.95 (20.50–23.75) ± 0.62
3.	*Carissa macrocarpa* (Eckl.) A. DC.	0.99	3.20 (2.75–3.50) ± 0.14	36.80 (34.50–39.50) ± 0.89	36.95 (35.75–38.25) ± 0.46	3.00 (2.25–4.00) ± 0.34	5.50 (4.50–7.25) ± 0.48	35.85 (32.75–38.50) ± 0.07
4.	*Carissa spinarum* L.	0.92	3.35 (2.75–4.50) ± 0.32	24.30 (23.25–26.25) ± 0.55	26.35 (25.25–27.75) ± 0.46	2.8 (2.25–3.25) ± 0.16	2.25 (2.00–3.00) ± 0.18	20.55 (18.50–23.75) ± 0.93
5.	*Cascabela thevetia* (L.) Lippold	1.02	2.8 (3.20–4.10) ± 0.28	113.45 (109.4–114.2) ± 0.8	109.20 (107.7–110.2) ± 0.40	5.20 (4.70–5.57) ± 0.13	15.20 (15.30–15.20) ± 0.50	18.33 (17.50–19.25) ± 0.55
6.	*Catharanthus roseus* (L.) G. Don	0.98	4.85 (4.50–5.25) ± 0.37	56.45 (55.75–57.75) ± 0.89	57.30 (55.25–61.25) ± 0.38	13.60 (13.00–14.25) ± 0.48	6.50 (6.00–7.00) ± 0.395	44.25 (44.25–47.25) ± 0.379
7.	*Cryptolepis dubia* (Burm.f.) M.R. Almeida	0.74	1.35 (0.25–2.50) ± 0.43	20.30 (19.75–20.75) ± 0.16	27.10 (25.25–29.50) ± 0.78	Absent	Absent	Absent
8.	*Nerium oleander* L.	0.98	3.20 (2.75–3.75) ± 0.20	33.65 (32.00–35.75) ± 0.69	34.25 (33.25–37.00) ± 0.70	3.10 (1.25–4.25) ± 0.52	5.25 (4.00–6.00) ± 0.34	36.70 (33.75–38.50) ± 0.89
9.	*Periploca aphylla* Decne.	3.53	4.90 (4.50–5.50) ± 0.20	52.00 (49.50–55.25) ± 0.72	24.16 (21.25–26.00) ± 1.02	Absent	Absent	Absent
10.	*Plumeria rubra* L.	1.08	3.10 (2.75–3.50) ± 0.12	36.85 (31.00–40.25) ± 0.63	33.95 (29.50–37.00) ± 0.32	0.45 (0.25–0.75) ± 0.09	10.50 (10.25–10.75) ± 0.11	38.55 (38.00–39.50) ± 0.25
11.	*Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult.	0.96	1.65 (0.75–2.25) ± 0.28	28.85 (28.25–30.50) ± 0.42	29.80 (27.75–32.75) ± 0.86	8.45 (2.75–10.75) ± 0.50	8.45 (2.75–10.75) ± 0.50	14.100 (13.25–15.25) ± 0.35

colpi as 8.45 ± 0.50 μm. Exine thickness was taken as 1.65 ± 0.28 μm. Mesocolpium was 14.100 ± 0.35 μm. The P/E ratio was calculated as 0.96. The numbers of fertile and sterile pollen were, respectively, 16 and 5 (Figures 5S, 5T and 10A–10C).

Vincetoxicum spirale (Forssk.) D.Z. Li

Vincetoxicum spirale pollinia were canary hollow, pendulous, corpusculum ovate and characterised by oval pollinial sac (Figures 6L, 6M and 10D–F).

Dregea volubilis (L.f.) Benth. ex Hook.f

Dregea volubilis pollinia were large, sulphur yellow, erect, reniform and characterised by the absence of sterile margins; further, translator attachment to pollinium was basal and corpuscular arm was absent while translator attachment to corpusculum was apical and shed as a pair of pollinium sacs; and the length and breadth of pollinium sac were observed to be 98.15 ± 1.90 μm and 38.35 ± 0.01 μm, respectively. The length of the translator was taken as 14.85 ± 1.01 μm and the breadth as 7.95 ± 0.25 μm. The length and width of the corpusculum were calculated as 258.60 ± 0.35 μm and 33.95 ± 7.85 μm, respectively. The number of fertile pollinia was seven and that of sterile pollen was three (Figures 6N, 6O and 10G–10I).

Dendrogram and PCA clustering tool analysis

Cluster analysis based on Euclidean distance was performed based on analytical pollen data of selected Apocynaceous species. Two major associations were formed, named Association C1 and Association C2. Association C1 consists of three species. These species have similarities based on colpi length and width and exine thickness. Association C2 consists of 15 species and these associations have similarities based on mesocolpium distance, polar and equatorial distance, and the P/E ratio, as shown in Figure 11. PCA analysis was used on the pollen quantitative mean data of Apocynaceae species to determine the variance among different variables in the context of eigenvariables. PCA illustration showed that the length of colpi on the PC1 axis of *A. curassavica* and *D. volubilis* was characterised by a higher exine thickness, whereas on PC2 a higher value in terms of mesocolpium distance was observed with reference to *V. spirale* (Table 7 and Figure 12).

Table 7. PCA % variance loadings for the Apocynaceous pollen.

PC	Eigenvalue	% variance
1	2.35994	39.332
2	1.53998	25.666
3	1.0319	17.198
4	0.82796	13.799
5	0.239496	3.9916
6	0.000728	0.012128

PCA, principal component analysis.

DISCUSSION

The study on intraspecific pollen morphology variation in Apocynaceae is of significant importance as it provides a roadmap for horticultural innovation. Understanding these variations can aid in breeding

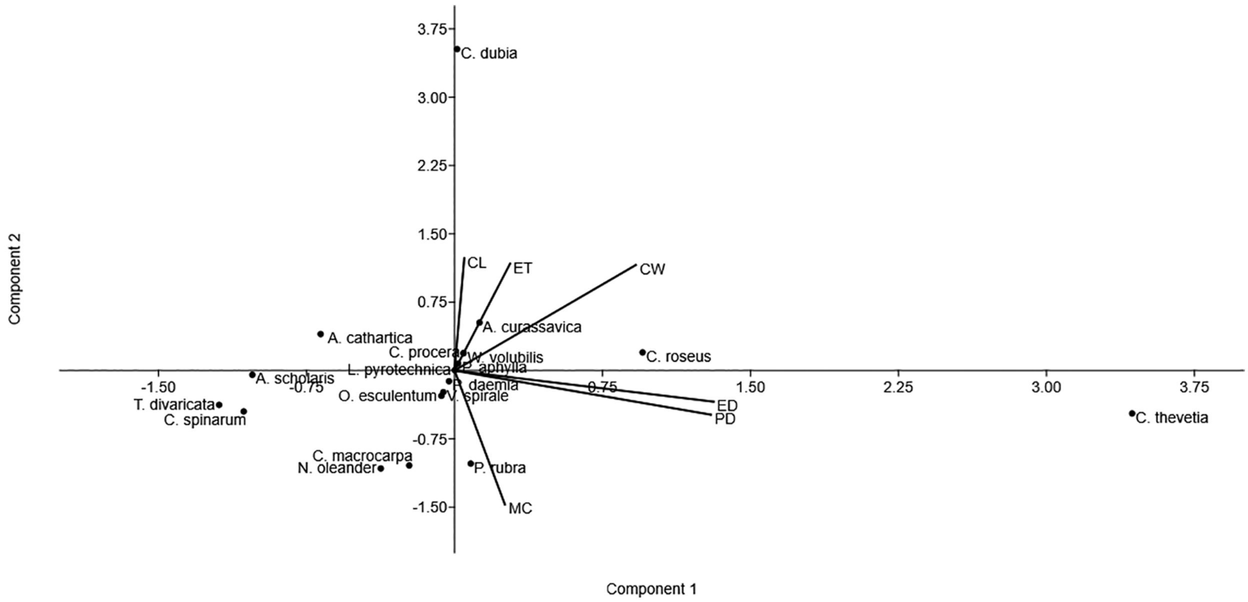

Figure 12. PCA performed with the pollen quantitative data from Apocynaceous taxa. PCA, principal component analysis; CL, colpi length; CW, colpi width; ED, equatorial diameter; ET, exine thickness; MC, mesocolpium; PC, polar diameter.

programmes, helping to develop more resilient and productive cultivars. This research can also contribute to conservation efforts, as it aids in the preservation of genetic diversity within the family. Ultimately, the study's findings have far-reaching implications for both horticulture and biodiversity conservation.

In the present study, the palynological traits of 18 Apocynaceae species were studied quantitatively and subjectively with the use of microscopy. The systematic study of palynomorphological features in flowering plants at higher taxonomic levels has considerably benefitted from the use of both a light and a scanning electron microscope (Al-Hakimi et al., 2015). Based on the traits of pollen that were studied, a taxonomy key was developed for fast and precise identification. Apocynaceae identification at the species level is too difficult because of its increased diversity and variable pollen and pollinia forms. The primary objective of this work was to morphologically evaluate the Apocynaceae family using pollen and pollinia to identify traits that would be helpful for taxonomic classification.

Previous studies that attempted to provide important and useful palynomorphological data on this family were unsuccessful in combining a comprehensive approach to pollens and pollinia in a single investigation. In this study, we present palynological studies on the Apocynaceae, which would help taxonomists correctly identify several highly significant plants. When examining pollen, microscopy is crucial for comprehending the taxonomic significance of plant species within families (Raza et al., 2020).

Colpi, mesocolpium, translator, corpusculum, exine sculpturing, aperture ornamentation, exine thickness, pollen type, size, polar and equatorial views, pollen sac length and width, pollinium ornamentation, pollinium shape and many other features were examined using SEM. These palynological characteristics are essential for correctly classifying different species in plant taxonomy (Ur Rahman et al., 2019).

The pollen grains and pollinia of the family Apocynaceae exhibited a considerable degree of morphological variety. Pollens ranged in size from small to quite large, and tricolpate to tetracolporate. Pollen shapes ranged from sub-spheroidal, to oblate spheroidal to rhomboidal, while exine sculptures ranged from psilate, through scabrate, to rugulate. Polar views were lobate, isopolar to irregular. On the other hand, in pollinia, the orientation was pendent to transverse, the shape was obovate to narrowly oblong, sterile margin was mostly absent and translator attachment to corpusculum was sub-apical to apical. The pollen of *A. cathartica* was described as sub-oblate, prolate, isopolar, having radial symmetry and having a granulate tectum in earlier research. In contrast, *A. scholaris* pollens had sub-circular to obtusely triangular apertures in polar view and were medium-sized, oblate spheroidal, colporate and characterised by scabrate exine sculpturing (Meena et al., 2011). This is not the case for the pollen grains of *A. scholaris*, which were found to be small, tricolporate, sub-spheroidal, lobate and isopolar, and to have psilate to perforate exine sculpturing. In contrast, the pollen grains of *A. cathartica* were found to be medium in size, tricolporate, elliptic and triangular (convex), and to have reticulate exine sculpturing.

In the present research, *C. macrocarpa* and *C. spinarum* pollen grains were medium, tricolporate, oblate sub-spheroidal, circular, lobate and isopolar, and shed as a monad. Exine sculpturing was perforated, and psilate to regulate has been observed. In *C. thevetia*, pollen grains were very large, tricolporate, prolate or sub-spheroidal, and lobate, and shed as a monad. Exine sculpturing with the characteristics of microreticulate to perforate has been observed (Naz et al., 2019). *C. roseus* and *C. dubia* pollen grains were large, tricolporate, oblate spheroidal and irregular; exine sculpturing was perforate to scabrate and tetrads; and rhomboidal, reticulate exine sculpturing has been detected, which represents a variation from the findings in Zafar et al.'s study (2022). Pollen grains in *N. oleander* and *T. divaricata* were medium, tricolporate, oblate spheroidal, and circular, while exine sculpturing was psilate and perforate, respectively. Pollen grains in *P. rubra* were medium in size, tricolpate, prolate sub-spheroidal, round and isopolar, and exine sculpturing was observed to be a perforate in regulation; these findings demonstrate a good consistency with those of Mallick's study (2020). *P. aphylla* pollen ranged in size from small to large, the shape of the pollen was tetrad, the polar view was rhomboidal while the equatorial view was oblong, pollen shed as tetrad and psilate exine sculpturing was detected; however, these interpretations vary (Swarupanandan et al., 1995).

In *A. curassavica*, pollinia were large, brown, pendent and narrowly oblong, sterile margins were absent, translator attachment to pollinium was basal, corpuscular arm was absent, translator attachment to corpusculum was sub-apical and psilate perforate exine sculpturing was present. In *C. procera*, pollinia were large, canary yellow, pendent and obovate, sterile margins were absent, translator attachment to pollinium was basal, corpuscular arm was absent while translator attachment to corpusculum was sub-apical, and the pollen type was polyads. On the other hand, in *L. pyrotechnica*, pollinia were medium, brown, transverse and orbicular, sterile margins were present, the orientation of sterile margins was apical, translator attachment to pollinium was basal and corpuscular arm was absent while translator attachment to corpusculum was apical; these outcomes confirm the interpretations derived in the study of Shah and Ahmad (2014). In *O. esculentum* and *P. daemia*, pollinia were large, yellow, pendent and narrowly oblong, sterile margins were absent, translator attachment to pollinium was basal, the corpuscular arm was absent, translator attachment to corpusculum was apical and pinkish brown, obovate, pseudo-sterile margins were present and the corpuscular arm was absent while translator attachment to corpusculum was apical, respectively. In *D. volubilis*, pollinia were large, sulphur yellow, erect and reniform, sterile margins were absent, translator attachment to pollinium was basal and corpusculum arm was absent while translator attachment to corpusculum was apical. These results were highly distinguishable from those of Pal and Mondal (2019). In *V. spirale*, pollinia were canary yellow, pendulous and corpusculum ovate, and oval pollinial sac confirms the findings of Yaseen and Perveen (2014).

The morphopalynological study linked to horticultural exploration harnesses the knowledge that contributed to the development of more resilient and productive horticultural practices within the Apocynaceae family.

CONCLUSION

Comparative studies of pollen structures among Apocynaceous taxa reveal that the taxonomy of exine wall characteristics has raised scholarly interest in pollen biology. This research focusses on the qualitative and quantitative micromorphological microscopic features of 18 Apocynaceae pollen. The microscopic authentication of closely related pollen characters of species is identified based on pollen taxonomy. The maximum mesocolpium distance was noted in *C. roseus* (44.25 µm) and the minimum in *T. divaricata* (14.1 µm). Exine sculptured tectum was examined as reticulate regulated and perforated. This work shows that palynology is very dependable in reconstructing historical vegetation and decrypting taxonomic links based on microscopic visualisation of pollen micromorphology and serves for accurate species identification. This study's findings on intraspecific pollen morphology variation in Apocynaceae provide valuable insights for horticultural innovation, enabling targeted breeding and cultivation strategies for improved plant species.

ACKNOWLEDGEMENT

The authors extend their appreciation to the Researchers Supporting Project number (RSP2024R483), King Saud University, Riyadh, Saudi Arabia.

FUNDING

Researchers Supporting Project number: RSP2024R483.

CONFLICT OF INTEREST

The authors declare that no conflict of interest exists.

AUTHOR CONTRIBUTION STATEMENT

A.M. – conceptualisation. S.S. – methodology. J.R. – software. P.K. – validation. M.R.K. – formal analysis. M.R.K. – investigation. M.R.K. – writing original draft preparation. S. M. and M.Z. – writing review and editing. M.F.R. – visualisation. M.Z. and M.A. – supervision.

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Received: August 12, 2023; Accepted: November 9, 2023