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Taxonomic implications of the palyno-morphological study of *Catharanthus roseus* (L.) G. Don cultivars from Lucknow, India

Mohammad Firoze Quamar[1, 2*], Nagendra Prasad[1, 2], Mohammad Javed[3] and Salman Khan[4]

[1, 2, 3, 4]Birbal Sahni Institute of Palaeosciences, 53 University Road, Lucknow–226007, India.
[1, 2]Academy of Scientific and Innovative Research, Ghaziabad–201002, India.
E-mail: [1*]mohdfiroze_quamar@bsip.res.in, [2]nagendrap303@gmail.com,
[3]javedmohammad1996@gmail.com, [4]salmangeo@bsip.res.in
[*]Corresponding author

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ABSTRACT

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The study of pollen morphology and understanding of the pollen morphological features of a variety of plant taxa is of immense help in plant taxonomy and systematics. In the present study, 8 cultivars of *Catharanthus roseus* (L.) G. Don (*Apocynaceae*) were observed under the light microscopic (LM), confocal laser scanning microscopic (CLSM) and field emission scanning electron microscopic (FESEM) techniques from Lucknow, India. The observed pollen morphological characters, their similarities and variations among the studied cultivars, were documented, analyzed and illustrated as well in taxonomic perspective. Pollen grains of the *Catharanthus roseus* cultivars are essentially stenopalynous and a monad, subtriangular, isopolar, radially symmetrical, prolate, medium-sized, tri (tetra) colporate with perforated sexine, whereas prevalent variations in the pollen size (P/E values), aperture diameter, nature and arrangement, as well as exine thickness are apparent. Principal component analysis (PCA) was performed to show the relationship among the 8 studied cultivars of *Catharanthus roseus*, which showed that polar axis, equatorial diameter, colpi length, colpi width, os length, os width, os shape and exine thickness were found significant palyno-morphological attributes. Moreover, a taxonomic key was also developed to show the variations in pollen morphological features and also to delimit the 8 cultivars of *Catharanthus roseus* investigated for quick and correct identification. The study underlines the taxonomic significance of pollen morphology in correct identification of the studied *Catharanthus roseus* cultivars.

Keywords: Floral materials, advanced microscopy techniques, systematics, Uttar Pradesh, India.

INTRODUCTION

Pollen morphological studies provide high resolution pollen morphological attributes, which can be useful in plant taxonomy, because many pollen traits (characters) are influenced by the strong selective forces involved in various reproductive processes, including pollination, dispersal and germination (Erdtman 1952, Moore et al. 1991, Nowicke & Skvarla 1979). Birks and Birks (1980) were of the view that palynological research into the history of Earth's vegetation is underpinned by the classification of fossil palynomorphs (pollen and spores) into taxonomic groups. Pollen morphology has assumed great significance in plant taxonomy, and the advancements in microscopy have led to the effective use of new pollen morphological parameters to taxonomic purposes (Erdtman 1969, Mao et al. 2012, Gosling et al. 2013, Quamar et al. 2017, Nazish et al. 2019, Ullah et al. 2019, 2021, Sampson 2000, Shepherd et al. 2021, Liao et al. 2021, Garg et al. 2023). Moreover, the very study is also useful in determining the degrees of kinship and similarity among phylogenetically related plant groups (Walker & Doyle 1975, Punt 1976, Clark et al. 1980; Doyle 2005, Bahadur et al. 2022, Khan et al. 2018, Gonçalves-Esteves 2021, Quamar et al. 2022a).

To the best of our knowledge, no comprehensive study on the pollen morphology and ultrastructure of *Catharanthus roseus* cultivars, using advanced microscopy techniques (LM, CLSM and FESEM), from India were attempted yet. However, Quamar et al. (2017, 2022a, b, 2023) have studied the pollen morphology of the various taxa from Lucknow, and central India, respectively; but the fine resolution study for understanding the ultrastructure of the taxa in question, using the FESEM was not taken up in the earlier studies except Quamar et al. 2022a. Also, the pollen morphological studies have implications in the fossil pollen records for the reconstruction of past vegetation dynamics and the hydroclimatic changes, as well as to know the early history of the family (Muller 1981, Tellerı´a et al. 2010). Further research is, therefore, needed to fill this gap. The significance of pollen morphological traits as an aid to the taxonomy of various plants groups is also evident in the contribution of APG II (2003) and APG III (2009). Keeping in view of the importance of LM, CLSM and FESEM techniques in pollen micro-morphological studies for the correct identification of *Catharanthus roseus* cultivars, the present study was undertaken and primarily aimed 1) to observe, document, analyze and illustrate, for the first time in India, the detailed pollen micro-morphology and ultrastructure of the *Catharanthus roseus* cultivars, using the LM, CLSM and FESEM techniques, 2) to strengthen the taxonomic study of the members of the family *Apocynaceae*, 3) to categorize the pollen characters of taxonomic significance through numerical analysis, and 4) to use pollen micro-morphological features observed under the LM, CLSM and FESEM for the correct identification and discrimination of the studied cultivars of the family *Apocynaceae* (a eurypalynous family).

AN OVERVIEW OF *CATHARANTHUS ROSEUS* (L.) G. DON WITH REGARDS TO MEDICINAL VALUES

Catharanthus roseus, formerly known as *Vinca rosea* L., and the most common name is Madagascar periwinkle, is a species of flowering plants (angiosperms) of the order *Gentianales* and the family *Apocynaceae* A.L. de Jussieu (dogbane family). The name *Catharanthus* G. Don comes from the Greek, meaning “pure flower”. There are eight species of *Catharanthus* G. Don (e.g. *Catharanthus roseus* (L.) G. Don, *Catharanthus ovalis* Markgr., *Catharanthus pussilus* (Murray) G. Don, *Catharanthus longifolius* (Pichon) Pichon, *Catharanthus lanceus* (Bojer ex A.DC.) Pichon, *Catharanthus coriaceus* Markgr., *Catharanthus scitulus* (Pichon) Pichon, *Catharanthus trichophyllus* (Baker) Pichon), out of which seven are native and endemic to Madagascar island, whereas *Catharanthus pussilus* (Murray) G. Don is native to India and Sri Lanka. It is widely grown and/or cultivated as a garden and medicinal plant in the tropical and sub-tropical regions of the world. Owing to its various and attractive flower colours with an excellent ability to tolerate dry and nutrient- deficit conditions, it is considered as a widespread ornamental plant (Nejat et al. 2015). More specifically, it prefers full sunlight (although, able to live in partially shaded environments as well) and well-drained soil. It also prefers light soils, rich in humus, to grow. It avoids saline, alkaline soil or water-logged condition to grow. It makes a great ground cover owing to its speedy and vine-like growth, and behaves as if it grows like a weed. Its thick glossy leaves aid in this ground cover. Its flowering period is throughout the year in tropical conditions, and from spring to late autumn in warm temperate climates. Its leaves are almost oppositely arranged and flowers are usually solitary in the leaf axils. Each flower has a calyx with five long, narrow lobes and a corolla with a tubular throat and five lobes (Mabberley 1987, 2017, Schmelzer et al. 2008, https://en.wikipedia.org/wiki/ *Catharanthus*, searched on 25.10.2022). It is visited by a wide variety of insects, such as butterflies, moths, bees and even beetles; hence, it has entomophilous mode of pollination. It was earlier considered as an autogamous species (Kulkarni et al. 2005), although Darwin and Dalpino long ago reported that selfing within individual periwinkle flowers is not automatic and that pollination typically occurs through nectar-seeking insects (Knuth 1909, Rendle 1971). But, Kulkarni (1999) and Sreevalli et al. (2000) have confirmed these observations, and further suggested that geitonogamy and phenotype assertive mating for flower colour brought about by butterflies give a false impression that periwinkle is autogamous, when flower colour is used as a marker trait to determine the breeding system. It is perennial, and an evergreen herb or sub-shrub, found distributed in India, Pakistan Bangladesh, Malaysia, Saudi Arabia, Australia, Italy, and United States. In India, it is distributed throughout tropical and sub-tropical parts, especially in Uttar Pradesh, Madhya Pradesh, Chhattisgarh, Bihar, West Bengal, Maharashtra, Gujarat, Rajasthan, Tamil Nadu, Karnataka, Andhra Pradesh, Assam, and northern India. *Catharanthus roseus* is highly valued for its anticancer (vincristine and vinblastin) and antihypertension (ajmalicine) alkaloids present in its leaves and roots, respectively (Chopra et al. 1956, Balaji 2014, Makki et al. 2019). Owing to the presence of alkaloids, which possess potent anticancerous activity to cure blood cancer, the plant is popularly known as "plant/flower that cures cancer". Moreover, it is also traditionally used in treatment of diseases, such as diabetes, diarrhoea, scurvy, chronic wounds, ulcer, helminthes infection, loss of memory, hypertension/high blood pressure, rheumatism, asthma, malaria, respiratory allergy and wasp sting (Chopra et al. 1956, Ghosh et al. 2007, Balaji 2014, Makki et al. 2019). The plant is also the source of rosinidin, a pink anthocyanin pigment found in the flowers of *Catharanthus roseus*, and lochnericine, a major alkaloid in roots (https://en.wikipedia.org/wiki/ *Catharanthus*, searched on 25 October 2022).

MATERIALS AND METHODS

Fresh floral materials of *Catharanthus roseus* cultivars were collected in July 2022 from the lawns of the Birbal Sahni Institute of Palaeosciences (BSIP) (Lucknow) and The Citadel Apartments at Chinhat, Lucknow (Figure 1). All precautionary measures were taken while collecting the floral materials to avoid mixing and contamination. Also, each cultivar was placed and/ or planted at a quite larger distance (~100 m apart) at each site, ultimately eliminating the chances of cross-pollination, even accidental. Moreover, each floral material was taken to a separate vial (while collection) and then initiated the processing for understanding their pollen morphologies. The details of the floral materials collected, their varied colours, habit, mode of pollination, and their respective reference laboratory numbers of living pollen slides, as well as their common names and synonyms are given in Table 1.

Protocols for sample preparation and microscopy: The pollen grains were prepared for the LM study following the standard methods described by Erdtman (1943, 1954), which involves 1) soaking up of the fresh floral material(s) in glacial acetic acid in a sterilised centrifuge tube, 2) gently crushing the same using a glass rod, 3) sieving the material with 150 mesh (~105 m)-size-sieves, 4) transferring the sieved material to a 50 ml sterilised centrifuge tube having distilled water, 5)

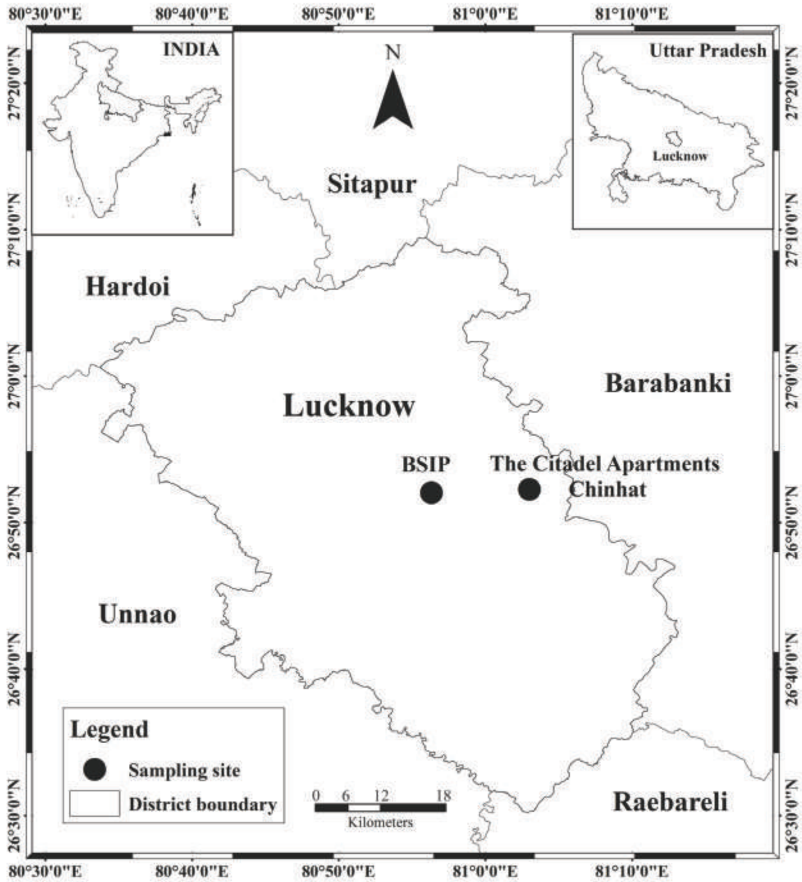

Figure 1. Geographical map of the Lucknow District (Uttar Pradesh), India, showing the location of the study sites, i.e. 1. Birbal Sahni Institute of Palaeosciences (BSIP), Lucknow and 2. The Citadel Apartments: Chinhat, Lucknow. **Inset left:** Geographical map of India showing the location of Uttar Pradesh. **Inset right:** Geographical map of Uttar Pradesh showing the location of Lucknow District. Source: The Figure has been made using ArcGIS 10.8.2.

centrifugation and decantation to remove water, 6) treatment of the material with glacial acetic acid to dehydrate through centrifugation and decantation, 7) acetolysis (mixture of acetic anhydride and concentrated sulphuric acid in the ratio of 9:1) in hot water bath for 5 min (Erdtman 1943), which follows centrifugation and decantation, 8) treatment of the material with 50% glycerine solution to make it homogeneous, and 9) mounting of the material in glycerine jelly (on glass slide) and sealed with wax for permanent record.

The prepared reference pollen slides (Figures 2, 3) with details of the concerned cultivar of *Catharanthus roseus* were deposited at the BSIP Sporothek, Lucknow. Published literatures (Chatterjee et al. 2014) aided in the identification of the pollen grains of *Catharanthus roseus*. The terminology used for describing the morphological features of the pollen grains of *Catharanthus roseus* cultivars is based on Erdtman (1952), Faegri and Iversen (1964), Walker and Doyle (1975) and Hesse et al. (2009).

Light microscopy (LM): Olympus BX50 microscope with attached DP 26 BSW Software for photography was used for LM studies at the Quaternary Palynology Laboratory, BSIP, Lucknow. Measurements of pollen in polar and/or equatorial views were recorded during the analysis. Amb, symmetry and mode of pollen grains arrangement were also observed under the same microscopic technique. Furthermore, shape, size/ overall dimension, aperture (colpi and pores [ora

Table 1. Showing common names (in remote rural and urban areas in India) and Reference laboratory numbers of living pollen slide of 8 studied cultivars of *Catharanthus roseus* (L.) G. Don. **Abbr.:** CR 1 to CR 8 = *Catharanthus roseus* cultivars (*Apocynaceae* Juss., Dogbane family). The following are common to all the cultivers. **Synonyms:** *Vinca rosea* L., *Pervinca rosea* (L.) Gaterau, *Lochnera rosea* (L.) Rchb. ex Spach, *Ammocallis rosea* (L.) Small; **Habit, physiognomy and flowering periodicity:** Perennial herb or sub-shrub, evergreen (flowering: throughout the year; **Mode of pollination:** Entomophily (e.g. butterflies, moths, bees and even beetles).

Name of the taxa investigated	Common names	Reference laboratory numbes of living pollen slide
1. *Catharanthus roseus*, CR 1	Madagascar periwinkle	11820
2. *Catharanthus roseus*, CR 2	Pink periwinkle	11821
3. *Catharanthus roseus*, CR 3	Cape periwinkle	11822
4. *Catharanthus roseus*, CR 4	Rose periwinkle	11823
5. *Catharanthus roseus*, CR 5	Old maid	11824
6. *Catharanthus roseus*, CR 6	Graveyard plant	11825
7. *Catharanthus roseus*, CR 7	Bright eyes	11826
8. *Catharanthus roseus*, CR 8	Sadabahar and Baaramaasi	11827

Table 2. Salient morphological features of the 8 studied cultivars of *Catharanthus roseus* (L.) G. Don pollen grains. Pollen of all the cultivers are monad, prolate in shape (Walker and Doyle 1975) and of medium size. **Abbr.:** P/E = Ratio of the measurements of the polar axis and equatorial diameter; Prolate (Erdtman 1954).

Name of the taxa investigated	(P/E) (µm)	Aperture (number, nature, diameter and arrangement)	Exine thickness and ornamentation
1. *Catharanthus roseus*, CR 1: Flower pure white in colour and dark yellow at the base	28/19	Tri (Tetra) colporate, colpus length and width (26.5 × 1.5 µm), variable, os diameter (1.2 × 2.6 µm), lalongate, variable	Exine 1 µm thick, perforated sexine
2. *Catharanthus roseus*, CR 2: Flower light pink in colour and dark pink at the base	24/15	Tricolporate, colpus length and width (22.5 × 1.5 µm), variable, os diameter 1.5 × 2.5 µm, lalongate, variable	Exine 1 µm thick, perforated sexine
3. *Catharanthus roseus*, CR 3: Flower dark pink in colour and light yellow at the base	26/17	Tricolporate, colpus length and width (24.5 × 1.5 µm), variable, os (1.5 × 2.5 µm), lalongate, variable	Exine 0.5 µm thick, perforated sexine
4. *Catharanthus roseus*, CR 4: Flower dark pink shade in colour	30/18	Tricolporate, colpus length and width (28.5 × 1 µm), variable, os (2.0 × 2.5 µm), lalongate, variable	Exine 0.5 µm thick, perforated sexine
5. *Catharanthus roseus*, CR 5: Flower pure pink in colour and white shade at the base	25/18	Tricolporate, colpus length and width (23.5 × 1.5 µm), variable, os (1.5 × 2.0 µm), lalongate, variable	Exine 1 µm thick, perforated sexine
6. *Catharanthus roseus*, CR 6: Flower dark pink colour having white patches at petals, and light yellow colour at the base	30/17	Tricolporate, colpus length and width (28.5 × 1 µm), variable, os (2.0 × 2.5 µm), lalongate, variable	Exine 0.5 µm thick, perforated sexine
7. *Catharanthus roseus*, CR 7: Flower light purple colour having white patches at petals, and dark purple colour at the base	27/17	Tricolporate, colpus length and width (25 × 1.5 µm), variable, os (2.5 × 2.0 µm), lolongate, variable	Exine 1 µm thick, perforated sexine
8. *Catharanthus roseus*, CR 8: Flower light pure purple colour	26/18	Tricolporate colpus length and width (23.5 × 1 µm), variable, os (2.0 × 3.0 µm), lalongate, variable	Exine 0.5 µm thick, perforated sexine

Table 3. The PCA analysis, based on the quantitative traits of the *Catharanthus roseus* (L.) G. Don cultivars.

Number of taxa investigated	Polar axis × Equatorial diameter (µm)	Colpi length × width (µm)	Os length × width (µm)	Exine thickness (µm)
1. *Catharanthus roseus*, CR 1	28.0 × 19.0	26.5 × 1.5	2.6 × 1.2	1.0
2. *Catharanthus roseus*, CR 2	24.0 × 15.0	22.5 × 1.5	2.5 × 1.5	1.0
3. *Catharanthus roseus*, CR 3	26.0 × 17.0	24.5 × 1.5	2.5 × 1.5	0.5
4. *Catharanthus roseus*, CR 4	30.0 × 18.0	28.5 × 1.0	2.5 × 2.5	0.5
5. *Catharanthus roseus*, CR 5	25.0 × 18.0	23.5 × 1.5	2.0 × 1.5	1.0
6. *Catharanthus roseus*, CR 6	30.0 × 17.0	28.5 × 1.0	2.5 × 2.0	0.5
7. *Catharanthus roseus*, CR 7	27.0 × 17.0	25.0 × 1.5	2.0 × 2.5	1.0
8. *Catharanthus roseus*, CR 8	26.0 × 18.0	23.5 × 1.0	3.0 × 2.0	0.5

Table 4. The eigenvectors and cumulative variance of PCA use the pollen quantitative traits of the *Catharanthus roseus* cultivars.

Number of taxa investigated	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
1. *Catharanthus roseus*, CR 1	0.54	1.35	0.97	-1.12	1.05	0.09	0.85
2. *Catharanthus roseus*, CR 2	-1.39	-1.47	0.83	-0.45	-0.20	-0.48	0.94
3. *Catharanthus roseus*, CR 3	-0.40	-0.29	-1.96	-0.11	0.01	1.43	0.00
4. *Catharanthus roseus*, CR 4	1.38	-0.20	-0.80	0.22	-0.09	-1.35	1.28
5. *Catharanthus roseus*, CR 5	-0.80	1.00	-0.44	-0.94	-1.29	-0.59	-1.18
6. *Catharanthus roseus*, CR 6	1.32	-1.00	0.67	0.09	0.48	-0.83	-1.42
7. *Catharanthus roseus*, CR 7	-0.09	-0.28	0.32	0.17	2.35	-0.27	-0.57
8. *Catharanthus roseus*, CR 8	-0.57	0.90	0.41	2.14	0.15	-0.46	0.10

Table 5. Eigenvalues generated which contribute most to the variance are provided below.

PC	Eigenvalue	% variance
1	10.4145	85.88
2	1.1641	9.5994
3	0.307702	2.5374

in this case]) number, nature and diameter, as well as and pollen wall architecture (exine thickness and pattern) were considered for description.

Confocal laser scanning microscopy (CLSM): CSLM study, as a matter of fact, distinguishes internal wall structures and ill-defined morphological features of the pollen grains that cannot be detected by the LM study, thus, providing high-resolution images and 3D characterization of the pollen grains; also the very technique is rapid (i.e. less time-consuming) and non-destructive (i.e. the chances of getting the material used for the CLSM study spoiled remain bleak and remote (Shute et al. 1996, Salih et al. 1997, Feist-Burkhardt & Pross 1999, Hochuli & Feist-Burkhardt 2004, Vitha et al. 2009; Quamar et al. 2017, 2022a, b). Furthermore, the same pollen slides, prepared for conducting the LM study, were utilized for capturing the images of the 8 studied pollen grains of *Catharanthus roseus* cultivars through the CLSM. The CLSM study was carried out at the CLSM and Raman Spectroscopy Laboratory of the BSIP, Lucknow.

Field emission scanning electron microscopy (FESEM): The acetolyzed pollen samples were used for the FESEM study in order to have the ultrastructure (high resolution features) of the pollen grains of the 8 studied *Catharanthus roseus* cultivars. The acetolyzed sample was dehydrated in a series of alcohol and was mounted on a smooth glass piece fixed on aluminium stubs. The samples were coated with Palladium-Platinum for observation in SEM (Farooqui et al. 2019, Quamar et al. 2023, Javed et al. 2024, and references cited therein). The FESEM study was carried out at the FESEM Laboratory of the BSIP, Lucknow.

Statistical analysis: Principal Component Analysis (PCA) was performed using multivariate analysis tool in the PAST 4.11 software (version 3.24), considering the different quantitative pollen morphological traits, observed under LM,

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Figure 2. 1. Flower of *Catharanthus roseus*, CR1. **2–3.** LM microphotographs. **2.** Equatorial view. **3.** Polar view showing apertures, exine and surface features. **4–5.** CLSM microphotographs. **4.** Equatorial view. **5.** Polar view showing apertures, exine and surface features with colour depth. **6–8.** FESEM microphotographs. **6, 8.** Equatorial view. **7.** Polar view showing apertures, surface features and exine. **9.** Flower of *Catharanthus roseus*, CR2. **10–11.** LM microphotographs, Equatorial view. **12–13.** CLSM images. **12.** Equatorial view. **13.** Polar view showing apertures, exine and surface features with colour depth. **14-16.** FESEM images. **14.** Equatorial view. **15.** Polar view. **16.** Enlarged view showing apertures, surface features and exine. **17.** Flower of *Catharanthus roseus*, CR3. **18–19.** LM microphotographs. **18.** Polar view. **19.** Equatorial view, showing surface features and exine. **20–21.** CLSM images, Equatorial view showing apertures, exine and surface features with colour depth. **22–23.** FESEM images. **22.** Polar view. **23.** Enlarged view, showing apertures, surface features and exine. **24.** Flower of *Catharanthus roseus*, CR4. **25–26.** LM microphotographs, Equatorial view, showing apertures, exine and surface features. **27–28.** CLSM images. **27.** Equatorial view. **28.** Polar view, showing apertures and exine. **29–30.** FESEM images; **29.** Equatorial view. **30.** Polar view, showing apertures, surface features and exine.

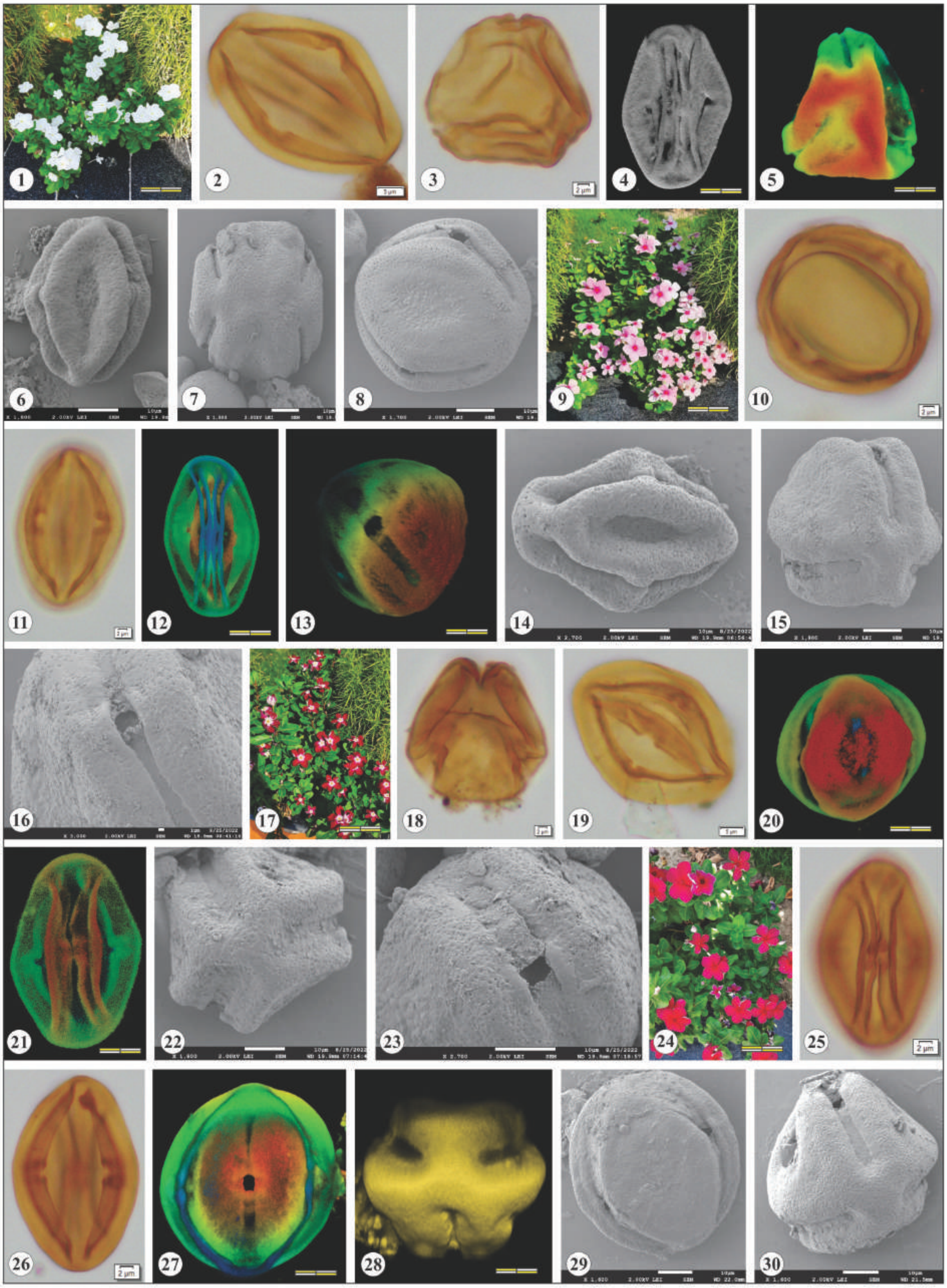
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Figure 2

CLSM and FESEM, to explore the differences and relationships among the 8 studied *Catharanthus roseus* cultivars (Table 2). We used eight variables for the PCA, which include polar axis, equatorial diameter, colpi length, colpi width, os length, os width, os shape and exine thickness, to generate the PCA scatter plot (Tables 2, 3, Figure 4).

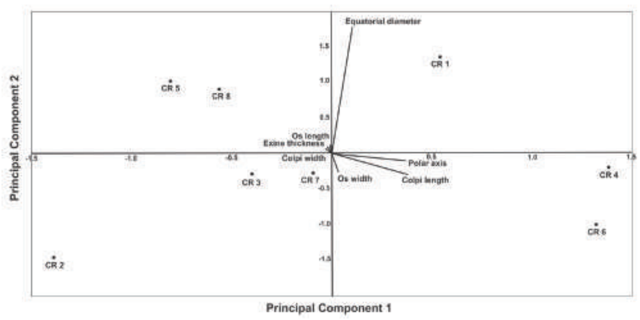

Figure 4. Principal component analysis with 7 metric variables of the 8 studied cultivars of *Catharanthus roseus* pollen grains (Please see Table 3 for the abbreviations used in the PCA analysis).

OBSERVATION AND RESULTS

Description of pollen morphology

Palyno-morphological study using advanced microscopy techniques, such as LM, CLSM and FESEM, improve the precise fossil pollen identification through significantly improving the taxonomic resolution. The detailed pollen morphological features considered and used in the present study include amb, polarity, symmetry, nature of pollen grain arrangement, size, shape, number, nature and arrangement of apertures (colpi and pores), as well as the exine ornamentation. Pollen grains of the *Catharanthus roseus* cultivars are stenopalynous and are, in general, essentially a monad (nature of pollen grains arrangement), subtriangular (amb, polar view), isopolar (polarity), and radially symmetrical (symmetry), prolate (shape), medium size (size), tricolporate (aperture-colpi [sing. colpus] and ora [sing. os] number) and perforated sexine (exine ornamentation); however, widespread variations in the pollen size (P/E values), aperture diameter as well as its nature and arrangement, and exine thickness are evident (Figures 5, 6), which have been separately discussed, as observed under the LM, CLSM and FESEM, as follows:

1. *Catharanthus roseus*, CR 1: Flower pure white in colour and dark yellow at the base; pollen grain tri (tetra) colporate, colpus length and width (26.5 × 1.5 µm), os (endoaperture) diameter (1.2 × 2.6 µm), lalongate, prolate, exine thickness 1 µm, perforated sexine with tiny perforations and a very high perforation density.
2. *Catharanthus roseus*, CR 2: Flower light pink in colour and dark pink at the base; pollen grain tricolporate, colpus length and width (22.5 × 1.5 µm), os (endoaperture) diameter (1.5 × 2.5 µm), lalongate, prolate, exine thickness 1 µm, perforated sexine with tiny perforations and a very high perforation density.
3. *Catharanthus roseus*, CR 3: Flower dark pink in colour and light yellow at the base; pollen grain tricolporate, prolate, colpus length and

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Figure 3. 1. Flower of *Catharanthus roseus*, CR5. **2**–**3.** LM microphotographs, Equatorial view, showing apertures, exine and surface features, **4–5.** CLSM images. **4.** Equatorial view. **5.** Polar view, showing apertures, exine and surface features with colour depth. **6**–**8.** FESEM images; **6.** Equatorial view. **7–8.** Polar view, showing apertures, surface features and exine. **9.** Flower of *Catharanthus roseus*, CR6. **10**–**11.** LM microphotographs. **10.** Equatorial view. **11.** Polar view, showing apertures, exine and surface features. **12–13.** CLSM images. **12.** Equatorial view. **13.** Polar view, showing apertures, exine and surface features with colour depth. **14–16.** FESEM images; **14–15.** Equatorial view. **16.** Polar view, showing apertures, surface features and exine. **17.** Flower of *Catharanthus roseus*, CR7. **18–19.** LM microphotographs, **18.** Equatorial view, showing apertures, exine and surface features, **20–21.** CLSM images. **20.** Polar view. **21.** Equatorial view, showing apertures, exine and surface features with colour depth. **22–23.** FESEM images. **22.** Equatorial view. **23.** Polar view, showing enlarged apertures, surface features and exine. **24.** Flower of *Catharanthus roseus*, CR8. **25–26.** LM microphotographs; Equatorial view, showing apertures, exine and surface features. **27–28.** CLSM images; **27.** Equatorial view. **28.** Polar view, showing enlarged apertures, exine and surface features with colour depth. **29–30.** FESEM images. **29.** Equatorial view. **30.** Enlarged view, showing apertures, surface features and exine.

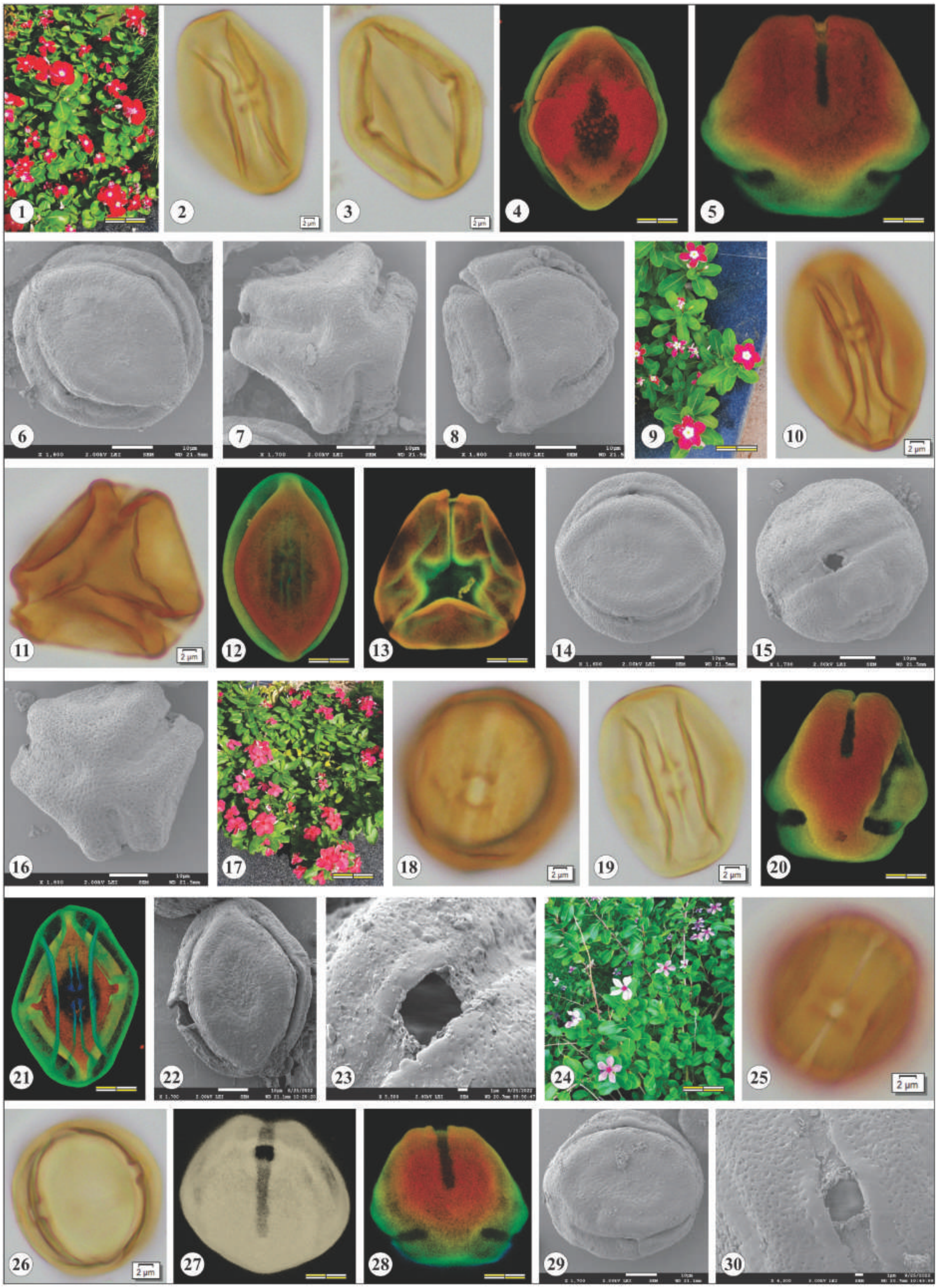

Figure 3

width (24.5 × 1.5 µm), os (endoaperture) diameter (1.5 × 2.5 µm), lalongate, variable, exine thickness 0.5 µm, perforated sexine with tiny perforations and a very high perforation density.

4. *Catharanthus roseus*, CR 4: Flower dark pink shade in colour, pollen grain tricolporate, colpus length and width (28.5 × 1 µm), os (endoaperture) diameter (2.0 × 2.5 µm), lalongate, prolate, exine thickness 0.5 µm, perforated sexine with tiny perforations and a very high perforation density.
5. *Catharanthus roseus*, CR 5: Flower pure pink in colour and white shade at the base; pollen grain tricolporate, colpus length and width (23.5 × 1.5 µm), os (endoaperture) diameter (1.5 × 2.0 µm), lalongate, prolate, exine thickness 1 µm, perforated sexine with tiny perforations and a very high perforation density.
6. *Catharanthus roseus*. CR 6: Flower dark pink colour having white patches at petals, and light yellow colour at the base; pollen grain tricolporate, colpus length and width (28.5 × 1 µm),), os (endoaperture) diameter (2.0 × 2.5 µm), lalongate, prolate, exine thickness 0.5 µm, perforated sexine with tiny perforations and a very high perforation density.
7. *Catharanthus roseus*, CR 7: Flower light purple colour having white patches at petals, and dark purple colour at the base; pollen grain tricolporate, colpus length and width (25 × 1.5 µm), os (endoaperture) diameter (2.5 × 2.0 µm), lolongate, prolate, exine thickness 1 µm, perforated sexine with tiny perforations and a very high perforation density.
8. *Catharanthus roseus*, CR 8: Flower light pure purple colour, pollen grain tricolporate, colpus length and width (23.5 × 1 µm), os (endoaperture) diameter (2.0 × 3.0 µm), lalongate, prolate; exine thickness 0.5 µm, perforated sexine with tiny perforations and a very high perforation density.

The salient features of the present study are summarized in Table 2, and the representative LM, CLSM and FESEM microphotographs are shown in Figures 2 and 3.

Statistical analysis: The PCA results, illustrated in Figure 4, are described through component 1 (PC1) and component 2 (PC2). Moreover, the PCA results highlight the significance of similarities between various palyno-morphological traits along with PCA biplot with all the pollen traits of the 8 analyzed pollen of the *Catharanthus roseus* cultivars (Bahadur et al. 2022). The extracted variance revealed through a variance-covariance matrix and coordinates in a distance-based biplot. The obtained results of the PCA explained 95% of the variance with principal component 1 and 2, which significantly highlights

Pollen Identification Key/Taxonomic Key

1. Pollen grains prolate, tri- (tetra-) colporate, exine thickness 1 µm, lalongate os and perforated sexine
   a. P/E (µm) 28/19, colpus diameter (26.5 × 1.5 µm), os diameter (1.2 × 2.6 µm)…………......*Catharanthus roseus*, CR 1
   b. P/E (µm) 24/15, colpus diameter (27.5 × 1.5 µm), os diameter (1.5 × 2.5 µm)…………......*Catharanthus roseus*. CR 2
   c. P/E (µm) 25/18, colpus diameter (23.5 × 1.5 µm), os diameter (1.5 × 2.0 µm)……….....….*Catharanthus roseus*, CR 5
1. Pollen grains prolate, tricolporate, exine thickness 0.5 µm, lalongate os and perforated sexine
   a. P/E (µm) 26/17, colpus diamater (24.5 × 1.5 µm), os diameter (1.5 × 2.5 µm)…...………....*Catharanthus roseus*. CR 3
   b. P/E (µm) 30/18, colpus diameter (28.5 × 1 µm), os diameter (2.0 × 2.5 µm)…...…………...*Catharanthus roseus*, CR 4
   c. P/E (µm) 30/17, colpus diameter (28.5 × 1 µm), os diameter (2.0 × 2.5 µm)……….....…..…*Catharanthus roseus*, CR 6
   d. P/E (µm) 26/18, colpus diameter (23.5 × 1 µm), os diameter (2.0 × 3.0 µm)…...……..……*Catharanthus roseus*, CR 8
2. Pollen grains prolate, tricolporate, exine thickness 1 µm, lolongate os and perforated sexine
   a. P/E (µm) 27/17, colpus diameter (25 × 1.5 µm), os diameter (2.5 × 2.0 µm)……………...*Catharanthus roseus*, CR 7

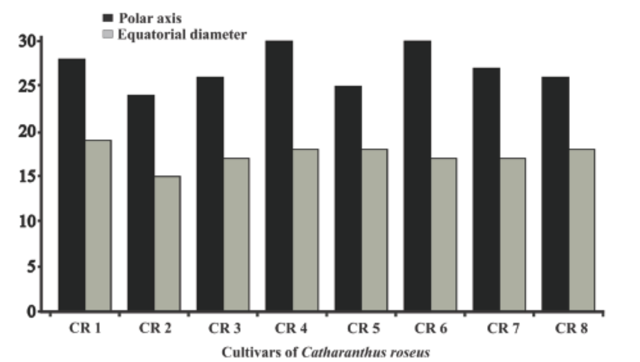

Figure 5. Variations in the values of the Polar axis and the Equatorial diameter (m) of the 8 studied cultivars of *Catharanthus roseus* pollen grains.

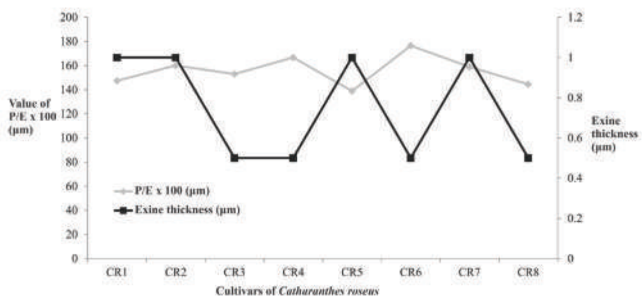

Figure 6. Variations in the size (values of P/E x 100 (m), as well as the exine thickness (m) of the 8 studied cultivars of *Catharanthes roseus* pollen grains.

the diversity among the pollen grains of the 8 analyzed *Catharanthus roseus* cultivars (Tables 2, 3; Figure 6).

DISCUSSION

Morphology of pollen is an important taxonomic tool in the classification and identification of plants (Erdtman 1969, Sampson 2000, Ullah et al. 2019, 2021, Quamar et al. 2017, 2022a, b, Bahadur et al. 2022) and is widely used to clarify taxonomic issues among angiosperms (Christensen 1986, Souza-Souza et al. 2016). Moreover, the constant pollen morphological features, as well as the exine ornamentation make a particular pollen grain a highly recognizable object by which parent genus or even species may be recognized (Moore & Webb 1978, Perveen 2000). This study showed significant variation among the pollen morphological traits (characters/attributes) of the 8 cultivars of *Catharanthus roseus* of the family *Apocynaceae* (dogbane family) investigated, which are drived and affected by different local competitive and ecological environments, and also likely to affect fertilization success (Wodehouse 1959, Erdtman 1966, Dajoz et al. 1991, Pire & Dematteis 2007). These variations greatly aid in the assessment of the taxonomic significance of *Catharanthus roseus* cultivars.

In the present study, P/E (µm) values, colpi and ora (aperture) diameter and their arrangement, and exine thickness serve as the diagnostic features regarding the taxonomic point of view. Amb (subtriangular), symmetry (radially symmetrical), pollen grain arrangement (monad), shape (prolate), size (medium-sized grains), varied aperture (colpi length and width; ora) number, diameter and arrangement (os-lalongate and lolongate), and exine architecture (exine thickness and ornamentation; exine thickness vary from 0.5–1 µm, perforated sexine, respectively) are the constant pollen morpholophical attributes, however. These constant attributes of the very pollen played a pivotal role in strengthening the taxonomy of the 8 studied cultivars of *Catharanthus roseus*. The pollen morphological data are compared with the available other pollen study to evaluate the taxonomic value of pollen traits in the 8 studied cultivars of *Catharanthus roseus*, which showed variation in the P/E values and shape (15.96 ± 17.22 m/15.12 ± 16.80 m), prolate spheroidal (Chatterjee et al. 2014). However, comparing the pollen size/shape data, as well as the exine ornamentation of the present study with that of the previous studies is difficult and impossible also in the light of the application of different preparation techniques, such as acetolyzed vs. critical point dried (CPD) pollen grains, as the preparation technique can affect pollen shape by modifying colpus membranes (Demissew & Harley 1992, Harley et al. 2004, Moon et al. 2008a, b). The observed variations in pollen shape may be common in monocots owing to a monosulcate aperture, however (Goldblatt & Le Thomas 1992, Kim et al. 2021). Furthermore, despite the effects of different preparation treatments (acetolysis [Dontas-Queiroz & de Luz 2016] or CPD [Goldblatt & Le Thomas 1992, Mitić et al. 2013] on pollen shape, the surface ornamentation remain congruent with the general pollen exine features [Goldblatt & Le Thomas 1992, Pinar & Dönmez 2000, Dontas-Queiroz & de Luz 2016]) as we see in the case of present study.

The PCA allows in ascertaining whether these data on the pollen morphological traits of the 8 studied pollen grains of the *Catharanthus roseus* cultivars allow any grouping of the different cultivars. The results obtained are plotted in a 2–dimensional plot between Principal Component 1 (PC1) and Principal Component 2 (PC2). PCA graph is prepared to understand the variability among 8 species and the characters that account for that variability. The Eigen values represent the total number of variables which is often used to ascertain the number of factors to retain. In our study, two Principal Components limited more than 1 Eigen values and also explain 95.47 % of the total variance of the analyzed data (Tables 4, 5). The PC 1 accounts for the 85.88% variance and PC 2 accounts for the 9.59% variance. PCA biplot reveals that CR 2, CR 3, CR 5, CR 7 and CR 8 correlate along exine thickness and os length; CR 1, CR 4 and CR 6 have clustered along polar axis, colpi length, os width and equatorial diameter, describing the importance of these traits for grouping of the respective taxa.

The present study also suggests that the 8 studied cultivars of *Catharanthus roseus* may be considered advanced, owing to their tricolporate nature of the pollen grains (Walker & Doyle 1975, Pal et al. 1993, Nadot et al. 2000, Perveen 2000, Yildiz et al. 2009).

CONCLUSIONS

The pollen morphological variations and similarities, observed, documented, analyzed and illustrated using the LM, CLSM and FESEM techniques, in the pollen characters of the 8 studied *Catharanthus roseus* cultivars provide clue of the importance of palynology in separating and delineating the same. Moreover, the findings highlight the taxonomic significance of the pollen morphological attributes in correct identification and differentiation of the 8 studied *Catharanthus roseus* cultivars. The developed taxonomic key, based on the examined pollen morphological attributes, has proved helpful in the high resolution identification and also in delimitation of the 8 studied *Catharanthus roseus* cultivars. The PCA analysis suggests that polar axis, equatorial diameter, colpi length, colpi width, os length, os width, os shape and exine thickness were significant pollen morphological characters having taxonomic implications. The study provides a baseline information for further studies.

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REFERENCES

APG II (Angiosperm Phylogeny Group) 2003. An update of the Angiosperm Phylogeny Group Classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnaean Society 141(4): 399–436.

APG III (Angiosperm Phylogeny Group) 2009. An update of the Angiosperm Phylogeny Group Classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnaean Society 161(2): 105–121.

Bahadur S., Taj S., Long W. & Ahmad M. 2022. Pollen morphology and its implication in the taxonomy of some selected taxa of the bi and tri-ovulate *Euphorbiaceae* of the Hainan Island by using multiple microscopic techniques. Microscopy Research and Technique. https://doi.org/10.1002/jemt.24062

Balaji D.H. 2014. Versatile therapeutic effects of *Vinca rosea* Linn. International Journal of Pharmaceutical Science and Health 1: 59–76.

Birks H.J.B. & Birks H.H. 1980. Quaternary Palaeoecology. London (reprinted 2004 by Blackburn Press, New Jersey). Edward Arnold.

Chatterjee R., Sarkar S. & Rao G.N. 2014. Pollen morphology and palynotaxonomical studies of common garden plants of *Apocynaceae*, in Kolkata, West Bengal, India. International Journal of Environment 3(4): 109–116.

Chopra R.N., Nayar S.L. & Chopra I.C. 1956. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi.

Christensen P.B. 1986. Pollen morphological studies in the *Malvaceae*. Grana 25: 95–117.

Clark W.D., Brown G.K. & Mayes R.A. 1980. Pollen morphology of *Haplopappus* and related genera (*Compositae-Astereae*). American Journal of Botany 67: 1391–1393.

Dajoz I., Till-Bottraud I. & Gouyon P.H. 1991. Evolution of pollen morphology. Science 253(5015): 66–68. https://doi.org/10.1126/science.253.5015.66

Demissew S. & Harley M.M. 1992. Trichome, seed surface and pollen characters in *Stachys* (*Labiatae*) in Tropical Africa. In: Harley R.M. & Reynolds T. (Editors) Advances in *Labiatae* science. Royal Botanic Gardens, Kew: 149–166.

Dontas-Queiroz M.V & de Luz C.F.P. 2016. Palynotaxonomy of *Iridaceae* Juss. from Goias and Tocantins states, Brazil. Brazilian Journal of Botany 39(2): 689–707.

Doyle J.A. 2005. Early evolution of angiosperm pollen as inferred from molecular and morphological phylogenetic analyses. Grana 44: 227–251.

Erdtman G. 1943. An Introduction to Pollen Analysis. Chronica Botanica Company, Waltham, Mass., U.S.A.

Erdtman G. 1952. Pollen morphology and plant taxonomy of Angiosperms. Waltham, Mass., U.S.A

Erdtman G. 1954. An Introduction to Pollen Analysis. Chronica Botanica Company, Waltham, Mass., U.S.A.

Erdtman G. 1966. Pollen morphology and plant taxonomy. Hafner Publishing Company, New York.

Erdtman G. 1969. Handbook on palynology: Morphology, taxonomy, ecology. An introduction to the study of pollen grains and spores. Hafner Publishing Company, New York.

Faegri K. & Iversen J. 1964. Text Book of Pollen Analysis. Munksgaard, Copenhagen.

Farooqui A., Tripathi S., Garg A., Shukla A.N., Murthy S., Prasad V. & Sinha G.P. 2019. Palaeotropical lineage of Indian water Primrose (*Ludwigia* L., *Onagraceae*) using pollen morphometric analysis. Review of Palaeobotany and Palynology 269: 64–77.

Garg A., Singh P. & Quamar M.F. 2023. Pollen morphology of family *Thymelaeaceae* Juss. in India and its taxonomic qimplications. Flora 303: 152291. https://doi.org/10.1016/j.flora.2023.152291

Harley R.M., Atkins S., Budantsev A.L., Cantino P.D., Conn B.J., Grayer R., Harley M.M., De Kok R., Krestovskaja T., Morales R., Paton A.J., Ryding O. & Upson T. 2004. *Labiatae*. In: Kadereit J.W. (Editor) The families and genera of vascular plants VII. Flowering plants dicotyledons: *Lamiales* (except *Acanthaceae* including *Avicenniaceae*). Springer, Berlin 167–275.

Hesse M., Halbritter H., Zetter R., Weber M., Buchner R., Frosch-Radivo A. & Ulrich S. 2009. Pollen Terminology: An illustrated Handbook. Springer. Vienna.

Hochuli P. & Feist-Burkhardt S. 2004. A boreal early cradle of Angiosperms? Angiosperm like pollen from the Middle Triassic of the Barents Sea (Norway). Journal of Micropalaeontology 23: 97–104. https:// doi.org/10.1144/jm.23.2.97 https://en.wikipedia.org/wiki/Catharanthus; searched on 25.10.2022

Javed M., Prasad N., Farooqui A., Quamar M.F. & Singh M. 2024. Taxonomic insights into medicinal plants pollen, using advanced microscopy techniques, from the Western Ghats, India. Grana 63(4). https://doi/10.1080/00173134.2024.2391515.

Khan R, Abidin U., Zain S., Ahmad M., Zafar M, Liu J. & Amina H. 2018. Palyno-morphological characteristics of gymnosperm flora of Pakistan and its taxonomic implications with LM and SEM methods. Microscopy Research and Technique 81(1): 74–87.

Kim H., Choi B., Lee C., McAdam S.A., Paik J.H. & Jang T.S. 2021. Micromorphological differentiation of Korean *Disporum* species using light and scanning electron microscopy. Microscopy Research and Technique 84(11): 2614–2624.

Knuth R. 1909. Handbook of flower pollination, vol. III Oxford: Clarendon Press.

Kulkarni R.N. 1999. Evidence for phenotypic assortative mating for flower colour in periwinkle. Plant Breed 118:.561–564.

Kulkarni R.N., Sreevalli Y. & Baskaran K. 2005. Allelic differences at two loci govern different mechanisms of intra-flower self-pollination in self-pollinating strains of periwinkle. Journal of Heredity 96 (1): 71–77.

Liao M., Ullah F., Deng H.N., Zhang J.L., Xu B. &.Gao X.F. 2021. Pollen morphology of the genus *Sophora* (*Fabaceae*) and its taxonomic implications. Microscopy Research and Technique 1–19. DOI: 10.1002/jemt.24034

Mabberley D.J. 1987. The Plant Book. Cambridge: Cambridge University Press.

Mabberley D.J. 2017. Mabberley's Plant-book: A Portable Dictionary of Plants, their Classification and Uses (4th ed.). Cambridge: Cambridge University Press. doi:10.1017/9781316335581

Makki R.M., Saeedi A.A., Khan T.K., Ali H.M. & Ramadan A.M. 2019. Single nucleotide polymorphism analysis in plastomes of eight *Catharanthus roseus* cultivars. Biotechnology & Biotechnological Equipment 33(1): 419–428.

Mao L., Batten D.J., Fujiki T., Li Z., Dai L. & Weng C. 2012. Key to mangrove pollen and spores of southern China: an aid to palynological interpretation of Quaternary deposits in the South China Sea. Review of Palaeobotany and Palynology 176–177: 41–67.

Mitić B., Halbritter H., Šoštarić R. &.Nikolić T. 2013. Pollen morphology of the genus *Iris* L. (*Iridaceae*) from Croatia and surrounding area: taxonomic and phylogenetic implications. Plant Systematics and Evolution 299(1): 271–288.

Moon H.K., Vinckier S., Walker J.B., Smets E & Huysmans S 2008a. A search for phylogenetically informative pollen characters in the subtribe *Salviinae* (*Mentheae*: *Lamiaceae*). International Journal of Plant Sciences 169: 455–471.

Moon H.K., Vinckier S., Smets E. & Huysmans S. 2008b. Comparative pollen morphology and ultrastructure of *Mentheae* subtribe *Nepetinae* (*Lamiaceae*). Review of Palaeobotany and Palynology 149: 174–198. doi:10.1016/j.revpalbo.2007.12.001

Moore P.D. & Webb J.A. 1978. An Illustrated Guide to Pollen Analysis. Hodder and Stoughton, London.

Moore P.D., Webb J.A. &.Collinson M.E. 1991. Pollen analysis. 2nd ed. London: Blackwell; p. 216.

Muller J. 1981. Fossil pollen records of extant angiosperms. The Botanical Review 47(1): 1–142.

Nadot S., Ballard Jr. H.E., Creach J.B. &Dajoz I. 2000. The evolution of pollen heteromorphism in Viola: a phylogenetic approach. Plant Systematics and Evolution 223: 155–171. https://doi. org/10.1007/bf00985276

Nazish M., Zafar M., Ahmad M., Sultana S., Ullah R., Alqahtan A.S., Ullah F., Ahmad S., Ashfaq S. &.Ullah F. 2019. Palyno-morphological investigations of halophytic taxa of *Amaranthaceae* through SEM from salt range of Northern Punjab, Pakistan. Microscopy Research and Technique 82(3):.304–316.

Nejat N., Valdiani A., Cahill D., Tan Y.H., Maziah M. &.Abiri R. 2015. Ornamental exterior versus therapeutic interior of Madagascar periwinkle (*Catharanthus roseus*): the two faces of a versatile herb. The Scientific World Journal. doi: 10.1155/2015/982412

Nowicke J.W. &. Skvarla J.J. 1979. Pollen morphology: The potential influence in higher order systematics. Annals of the Missouri Botanical Garden 66(4): 633–700.

Pal J., Datta B., Mandal S. & Bhattacharya G.N. 1993. Cyto-palynological investigation in *Cassia fistula* L. Environmental Ecology 11:435–438.

Perveen A. 2000. Pollen characters and their evolutionary significance with special reference to the flora of Karachi. Turkish Journal of Biology 24: 365–377.

Pınar N.M. & Oybak Dönmez E. 2000. Pollen morphology of Turkish *Iris* L. (*Iridaceae*) with reference to evolutionary trends at the infra-generic level. Israel Journal of Plant Sciences 48: 129–141.

Pire S.M. & Dematteis M. 2007. Pollen aperture heteromorphism in *Centaurium pulchellum* (*Gentianaceae*). Grana 46(1): 1–12. https://doi. org/10.1080/00173130601101245

Punt W. 1976. Evolutionary trends in the pollen grains of *Dichapetalaceae*. In: Ferguson IK, Muller J (Editors) The evolutionary significance of the exine. Academic Press, London. 139–146.

Quamar M.F., Ali S.N., Morthekai P. & Singh V.K. 2017. Confocal (CLSM) and light (LM) photomicrographs of different plant pollen taxa from Lucknow, India: Implications of pollen morphology for systematics, phylogeny and preservation. Review of Palaeobotany and Palynology 247: 105–119. https://doi.org/10.1016/j.revpalbo.2017.09.005

Quamar M.F., Singh P., Garg A., Tripathi S., Farooqui A., Shukla A.N. & Prasad N. 2022a. Pollen characters and their evolutionary and taxonomic significance: Using light and confocal laser scanning microscope to study diverse plant pollen taxa from central India. Palynology. DOI: 10.1080/01916122.2022.2070294

Quamar M.F., Garg A., Farooqui A., Prasad N., Khan S. & Shukla A.N. 2022b. A new pollen aperture in *Schleichera oleosa* (Lour.) Oken from Madhya Pradesh, central India. International Journal of Plant and Environment 8(2): 133–136.

Quamar M.F., Farooqui A., Prasad N. & Khan S. 2023. Pollen morphology of *Hibiscus rosa-sinensis* L. hybrids (*Malvaceae*) from Lucknow, India: A combined LM, CLSM and FESEM study with implications in taxonomy and systematic. International Journal of Plant and Environemnt.

Rendle A.B. 1971. The classification of flowering plants II Cambridge: Cambridge University Press.

Salih A., Jones A.S., Bass D. & Cox G. 1997. Confocal imaging of exine as a tool for grass pollen analysis. Grana 36: 215–224. https://doi. org/10.1080/00173139709362610

Sampson F.B. 2000. Pollen diversity in some modern Magnolids. Internatrional Journal of Plant Science 161: 193–210. DOI: 10.1086/317573.

Schmelzer G.H., Gurib-Fakim A. &.Schmelzer G.H. (Eds.) 2008. Plant Resource of Tropical Africa 11 (1). Medicinal Plants 1. PROTA Foundation, Wageningen, Netherlands/ Backhuys Publishers, Leiden, Netherlands/CTA, Wageningen, Netherlands, 791.

Shepherd S., Sharifi-Rad J., Harun N., Khan M.A., Ali M., Khalid N. & Khan F. 2021. Morphological and palynological assessment of some taxa of genus *Echinocloa* through light and scanning electron microscopy, Microscopy Research and Technique 84: 1–7.

Shute C., Hemsley A.R. & Strother P. 1996. Reassessment of dyads contained in a Late Silurian Rhynophytoid sporangium. Special Papers in Palaeontology 55: 137–145.

Souza-Souza R.M.B., Loeuille B., Mendonça C.B.F., Esteves R.L. &.Gonçalves-Esteves V. 2016. Pollen morphology of the genus *Paralychnophora* (*Vernonieae–Asteraceae*). Palynology 40(2): 280–288.

Sreevalli Y., Baskaran K., Kulkarni R.N. & Kumar S. 2000. Further evidence for the absence of automatic and intra-flower self-pollination in periwinkle. Current Science 79: 1648–1649.

Ullah F., Shah S.N., Zaman W., Zafar M., Ahmad M., Ayaz A., Sohail A. & Saqib S. 2019. Using palynomorphological characteristics for the identification of species of *Alsinoideae* (*Caryophyllaceae*): a systematic approach. Grana 58(3): 174–184, https://doi.org/10.1080/00173134.2019.1569719

Ullah F., Ahmad M., Zafar M., Parveen B., Ashfaq S., Bahadur S., Safdar Qura-tul-ain, Safdar L.B., Alam F. &.Luqman M. 2021. Pollen morphology and its potential in some selected taxa of *Caesalpiniaceae* observed under light microscopy and scanning electron microscopy. Microscopy Research and Technique: 1–11.

Vitha S., Bryant V.M., Zwa A. & Holzenburgh A. 2009. Confocal imaging of pollen. Microscopy and Microanalysis 15: 622–623. https://doi.org/10.1017/s1431927609094367

Walker J.W. & Doyle J.A. 1975. The bases of Angiosperm Phylogeny: palynology. Annals of the Missouri Botanical Garden 62: 664–723.

Wodehouse R.P. 1959. Pollen grains, their structure, identification, and significance in science and medicine. Hafner Pub. Co. New York.

Yildiz K., Gucel S. & Dadandi M.Y. 2009. A palynological investigation of endemic taxa from Northern Cyperus. Pakistan Journal of Botany 41(3): 991–1007.