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RESEARCH ARTICLE

# Palynological diversity in the Rosaceae flora of Northern Khyber Pakhtunkhwa, Pakistan

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**Abstract** The pollen morphology of the Rosaceae was investigated using light microscopy, despite its systematic implications. The main aim of this study was to verify the taxonomic significance of the pollen traits studied in 25 species and collected from different localities in Swat district, northern Khyber Pakhtunkhwa, Pakistan. In the present study, we have assessed both the qualitative and quantitative characteristics, including pollen form, size, and surface features. The pollen types were categorized into tetracolpate, tricolpate, and tricolporate in all the studied species. Significant variations were recorded in exine thickness, polar and equatorial diameters, colpi sizes, pore sizes, and polar to equatorial ratio (P/E ratio).

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The highest pollen fertility (94.79%) was recorded in *Prunus avium*, while the lowest (51.89%) was recorded in *Rosa webbiana.* Hence, ordination and clustering analyses were performed to determine relationships among the studied species. In addition, ordination and clustering analyses extend the knowledge of their pollen morphology, investigating whether pollen traits can help in the phylogenetic optimization of the taxa studied.

**Keywords** Diversity · Microscopy · Pollen attributes · Rose family · Swat district · Systematics

## Introduction

Rosaceae is a medium sized family in angiosperms and currently comprises approximately 100 genera and 3000 species (Potter et al. 2007; Pang et al. 2011). Many species of Rosaceae are economically important as fruit and ornamental plants (Pang et al. 2011). Despite Rosaceae encompassing a diverse array of flowering plants, hold significant ecological importance in the world and Pakistan too, particularly in the northern region of Khyber Pakhtunkhwa (Ibrar et al. 2015). The northern region in Khyber Pakhtunkhwa has boasts a unique and captivating flora, characterized by a variety of economically cash crops such as wild and cultivated apples, peaches, pears, plums, apricots and pomegranates etc. which contribute to the national economy of Pakistan (Ali et al. 2023). Besides these cash crops, norther region is a rich hot spot of biodiversity and medicinal flora as well (Sher & Khan 2007). Among the economically important wild flora, most of the plant's species belong to Rosaceae (Ali et al. 2023). Therefore, the northern region of Khyber Pakhtunkhwa is also a significant hotspot for a variety of endemic plant species (Ali et al. 2024). The Himalayan strawberry *Fragaria vesca*, *Spiraea canescens, Duchesnea indica* and *Rubus ellipticus* and some wild species of *Rose*, including *Rosa brunonii* and *R. webbiana* exist in the study area abundantly.

An abundant amount of Rosaceae taxa in the northern region of Khyber Pakhtunkhwa exists including wild edible fruits, including wild rose species, apples, peaches, plums etc. which significantly contribute to the region's food security and economic well-being (Singh et al. 2023). In addition, they play a vital role in maintaining ecological balance by preventing soil erosion and providing habitat for diverse fauna (Mutlu et al. 2023).

Pollen grains are the male reproductive unit of flowering plants, carries essential genetic information for successful reproduction. Palynology explores the intricate morphology and characteristics of pollen grains, providing valuable information about plant taxonomy, evolution, and ecology (Bahadur et al. 2022; Nabila et al. 2022; Ahmad et al. 2020). Pollen analysis is very important for understanding the relationship between Rosaceae plants. With the help of palynological analysis scientists can identify the specific Rosaceae species present in the anther of flowers (Amet et al. 2023). This knowledge is essential for promoting sustainable conservation practices. Additionally, palynological research of fossil pollen grains can reveal past vegetation patterns and shed light on the evolutionary history of the Rosaceae family in the region (Obigba 2022). The pollen of Rosaceae plants exhibits unique characteristics. Typically, Rosaceae pollen grains are tricolpate, possess three furrows (colpi) on their surface (Erdtman 1954). However, variations in size, ornamentation, and exine structure exist among different genera of Rosaceae (Fatemi et al. 2012). These variations provide valuable taxonomic markers for identifying specific plant species through pollen analysis (Nabila et al. 2022; Ahmad et al. 2020).

Few extensive investigations have been conducted on the pollen morphology of Rosaceae. Palynological study of this family is important due to stenopalynous types of pollen grains. Using light microscopy, most of the pollen grains were examined to be radially symmetrical monads, tricolporate, and sometimes tetracolporate (Ullah et al. 2022). Exine ornamentations are striate-verrucate, endoapertures elliptic or spheroidal, and perforations are extended on large tectal striae

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(Wrońska-Pilarek & Jagodziński 2011; Ahmad et al. 2020). Exine sculpturing of pollen grains are the important feature due to which taxa can be distinguished from each other. The apertures of the pollen grains consist of the equatorial endoaperture and colpus. The important characteristics of exine sculpture were examined to be the number, size, and area of perforations, as well as the number of grooves and striae (Jin-Rong et al. 2011).

The palynological study of Rosaceae is very helpful for the identification of plant taxa. Most of the morphological features of pollen, including polar diameter, equatorial diameter, spine length, spine width, spine number, pore length, width, colpi length, width, p/e ratio, and exine thickness, were examined in terms of qualitative and quantitative features that help in solving systematic problems. The study of pollen morphology plays an important role in the study of plant systematics and taxonomy (Xiong et al. 2019; Nabila et al. 2022; Ahmad et al. 2020)). Pollen grains analyzed through microscopic study varied in size and shape (Li et al. 2023). (Khan 2006) observed that palynological features are very important for describing the taxonomic characters of taxa in plant taxonomy.

## Materials and methods

### Study area

The current research work focusing on Swat district, located in the Malakand Division of Khyber Pakhtunkhwa (K.P) province, Pakistan. Geographically, it lies between 34.57° and 35.45° north latitudes and 72.08° and 72.50° east longitudes. The district is characterized by its mountainous terrain and fertile valleys. The elevation of district Swat ranges from 600 to 2900 m above sea level. It is bordered by Shangla district to the east, Buner district to the south, and Chitral district to the north. Covering a total area of 5337 square kilometers, Swat experiences a variety of climatic conditions, ranging from humid subtropical in the lower areas to alpine in the higher elevations. The hottest month, July, sees temperatures with highs of 35°C and lows of 19°C, while the coldest month, January, experiences highs of 8°C and lows of − 5 °C (Fig. 1).

### Samples collection

The present research work was carried out during the spring and summer seasons of 2023. Extensive fieldwork was conducted from March to August in different localities of Swat district, Khyber Pakhtunkhwa for the collection of Rosaceae species. Plant specimens were collected from various geographical locations, including Mingora, Saidu Sharif, Ghoda, Kuza Samai, Odigram, Drush Khela, Shah Deraii, Mankyal, Barikot, Sarsainai, Charbagh, Marghzar, Miandam, Maho Dandh, Jarogho, Jambil, Behar, Guligram, Qamber, Chail, Kanjo, Bahrain, and Madyan. We have totally collected and analyzed 25 plant species of Rosaceae (Table 1). The field photos of plants in natural habitat were taken and provided (Figs. 2, 3). Subsequently, the plants were collected, pressed, and properly dried. These specimens were then shifted to the Herbarium of Plant Systematics and Biodiversity, Quaid-i-Azam University, Islamabad. Identification was carried out with the field experts, flora of Pakistan (http://legacy.tropicos.org/project/Pakistan), International plant Name Index (https://www.ipni.org/), World Flora Online (https://wfoplantlist.org/), and the plant list with literature (https://www.gbif.org/dataset/d9a4eedb-e985-4456-ad46-3df8472e00e8) for further confirmation compared with the prototypes at the Herbarium. The latitude and longitude of study has been mentioned inside the map (Fig. 1).

### Microscopic analysis

To observe pollen grains, they were isolated from the anthers of mature flowers through a needle, three to five anthers were carefully removed from the collected specimens and placed on a glass slide. One to two drops of acetic acid were added, and the anthers were slightly crushed to release the pollen grains. After 20 to 30 s, the remaining debris was removed though needle. The pollen grains were stained with one to two drops of glycerin jelly, and a glass coverslip was placed over the slide to hold the pollen in place and prevent contamination. The corners of the coverslip were sealed with transparent nail polish. Each slide was labeled with the species name and voucher number and stored vertically in a wooden box (Majeed et al. 2023; Nabila et al. 2022; Ahmad et al. 2019).

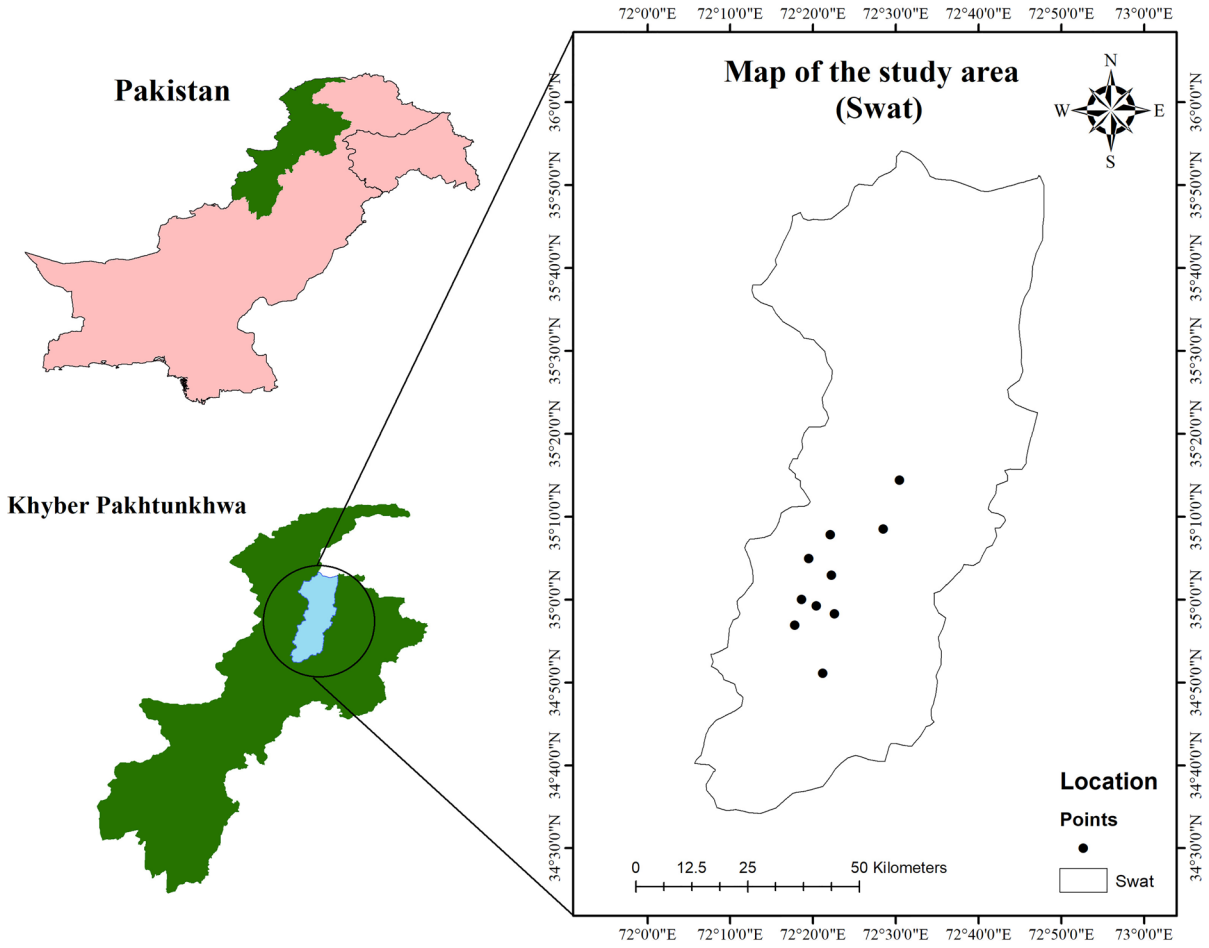



**Fig. 1** Map of the study area

The prepared slides were examined under a light microscope to study various qualitative and quantitative palynological characteristics at magnifications of 10× and 40×. For each slide, ten pollen (both in polar and equatorial views) were analyzed. Qualitative characteristics included pollen shape, size, type, aperture, and exine sculpturing. Quantitative measurements, such as polar diameter, equatorial diameter, colpi dimensions, mesocolpium, exine thickness, and P/E ratio, were taken using an eyepiece scale at 40× magnification (Ullah et al. 2024; Ahmad et al. 2019).

Data analysis

For each slide, ten pollen grains were counted to calculate the arithmetic mean. The mean value and standard error (SE) of each quantitative measurement were computed using SPSS 16.0 statistical software. Measurements taken at 40× magnification were converted to micrometers by multiplying by 2.5 according to the ocular meter calibration. Values are presented as mean ± SE in tabular form, as shown in Table 4.

The percentages of fertile and sterile pollen were calculated using the following equations:

Fertility = $(F/(F + S)) \times 100$

Sterility = $(S/(S + F)) \times 100$. where F represents fertile pollen and S represents sterile pollen while treating pollen grains with glycerin jelly if they are stained and look good, they should be considered fertile pollen, and vice versa. Using the technique of (Ahmad et al. 2010) the rate of fertility was

**Table 1** Detail Table on Current Collected Species of Rosaceous taxa

| Sr. No | Taxa | Common Name | Habit | Status | Locality | Coordinates | Collector Name | Date of collection | Accession No |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *Pyrus pashia* Buch. -Ham. ex D.Don | Himalayan pear | Tree | Wild | Ghoda | 34°58′31.78″N 72° 13′42.97″E | Abdullah | 16/02/2022 | ISL-132097 |
| 2 | *P. calleryana* Decne | Callery pear | Tree | Wild | Kuza Samai | 34°55′03.34″N 72° 13′20.97″E | Abdullah | 27/03/2022 | ISL-132098 |
| 3 | *P. communis* L | Common pear | Tree | Cultivated | Manai | 34°58′21.23″N 72° 12′38.67″E | Abdullah | 21/03/2022 | ISL-132099 |
| 4 | *P. persica* (L.) Batsch | Peach | Tree | Cultivated | Odigram | 34°45′15.30″N 72° 17′27.50″E | Abdullah | 16/02/2022 | ISL-132102 |
| 5 | *P. domestica* L | European plum | Tree | Cultivated | Odigram | 34°44′58.90″N 72° 17′10.74″E | Abdullah | 19/02/2022 | ISL-132103 |
| 6 | *Prunus armeniaca* L | Apricot | Tree | Cultivated | Drush khela | 35°00′09.57″N 72° 26′44.27″E | Abdullah | 13/02/2022 | ISL-132104 |
| 7 | *P. dulcis* (Mill.) D.A.Webb | Almond | Tree | Cultivated | Shah Deraii | 34°53′01.34″N 72° 14′04.16″E | Abdullah | 20/03/2022 | ISL-132105 |
| 8 | *P. avium* (L.) L | Wild cherry | Tree | Cultivated | Mankyal | 35°20′52.22″N 72° 36′32.33″E | Abdullah | 26/03/2022 | ISL-132107 |
| 9 | *R. indica* L | Cyme rose | Shrub | Cultivated | Barikot | 34°40′04.24″N 72° 12′21.05″E | Abdullah | 16/02/2022 | ISL-132112 |
| 10 | *R. banksiae* R.Br | Banks' rose | Shrub | Cultivated | Sarsainai | 34°48′57.34″N 72° 16′36.05″E | Abdullah | 10/03/2022 | ISL-132113 |
| 11 | *Rosa*×*damascena* Herrm | Damask rose | Shrub | Wild | Charbagh | 34°50′32.98″N 72° 31′58.46″E | Abdullah | 29/03/2022 | ISL-132115 |
| 12 | *R. brunonii* Lindl | Himalayan musk rose | Shrub | Wild | Marghzar | 34°40′04.88″N 72° 20′36.42″E | Abdullah | 02/05/2022 | ISL-132117 |
| 13 | *R. canina* L | Dog rose | Shrub | Wild | Miandam | 35°03′13.90″N 72° 33′54.05″E | Abdullah | 13/05/2022 | ISL-132118 |
| 14 | *R. webbiana* Wall. ex Royle | Wild rose | Shrub | Wild | Maho dandh | 35°42′37.19"N 72°39′29.44"E | Abdullah | 19/07/2022 | ISL-133374 |
| 15 | *Rubus macilentus* Jacquem. ex Cambess | Lean raspberry | Shrub | Wild | Jarogho | 35°06′06.11″N 72° 12′48.63″E | Abdullah | 29/03/2022 | ISL-132120 |
| 16 | *R. vestitus* Weihe | European blackberry | Shrub | Wild | Odigram | 34°45′02.80″N 72° 17′17.21″E | Abdullah | 26/03/2022 | ISL-132121 |
| 17 | *R. ellipticus Sm* | Yellow Himalayan Raspberry | Shrub | Wild | Jambil | 34°41′29.68″N 72° 28′29.11″E | Abdullah | 17/03/2022 | ISL-132119 |
| 18 | *R. niveus Thunb* | Mysore raspberry | Shrub | Wild | Jambil | 34°40′37.90″N 72° 28′51.25″E | Abdullah | 07/05/2022 | ISL-132122 |
| 19 | *F. vesca* L | Wild strawberry | Herb | Wild | Madyan | 34°40′04.24″N 72° 12′21.05″E | Abdullah | 01/04/2022 | ISL-132125 |

**Table 1** (continued)

| Sr. No | Taxa | Common Name | Habit | Status | Locality | Coordinates | Collector Name | Date of collection | Accession No |
|---|---|---|---|---|---|---|---|---|---|
| 20 | *S. canescens* D.Don | Grey stem spiraea | Shrub | Wild | Behar | 34°54′46.65″N 72° 31′41.24″E | Abdullah | 02/05/2022 | ISL-132129 |
| 21 | *C. nummularius* Fisch. & C.A.Mey | Nummular | Shrub | Wild | Guligram | 34°43′28.29″N 72° 19′57.88″E | Abdullah | 03/04/2022 | ISL-132130 |
| 22 | *C. oblonga* Mill | Quince | Tree | Wild | Qamber | 34°46′19.43″N 72° 19′53.86″E | Abdullah | 16/02/2022 | ISL-132133 |
| 23 | *Duchesnea indica* (Jacks.) Focke | Mock strawberry | Herb | Wild | Chail | 35°08′27.41″N 72° 34′3078″E | Abdullah | 18/02/2022 | ISL-132134 |
| 24 | *M. pumila* Mill | Paradise apple | Tree | Cultivated | Kanjo | 34°49′17.12″N 72° 20′54.15″E | Abdullah | 26/03/2022 | ISL-132136 |
| 25 | *F. ulmaria* (L.) Maxim | Meadows sweet | shrub | Wild | Ghoda | 34°58′17.03"N 72°12′34.66"E | Abdullah | 18/07/2022 | ISL-133370 |

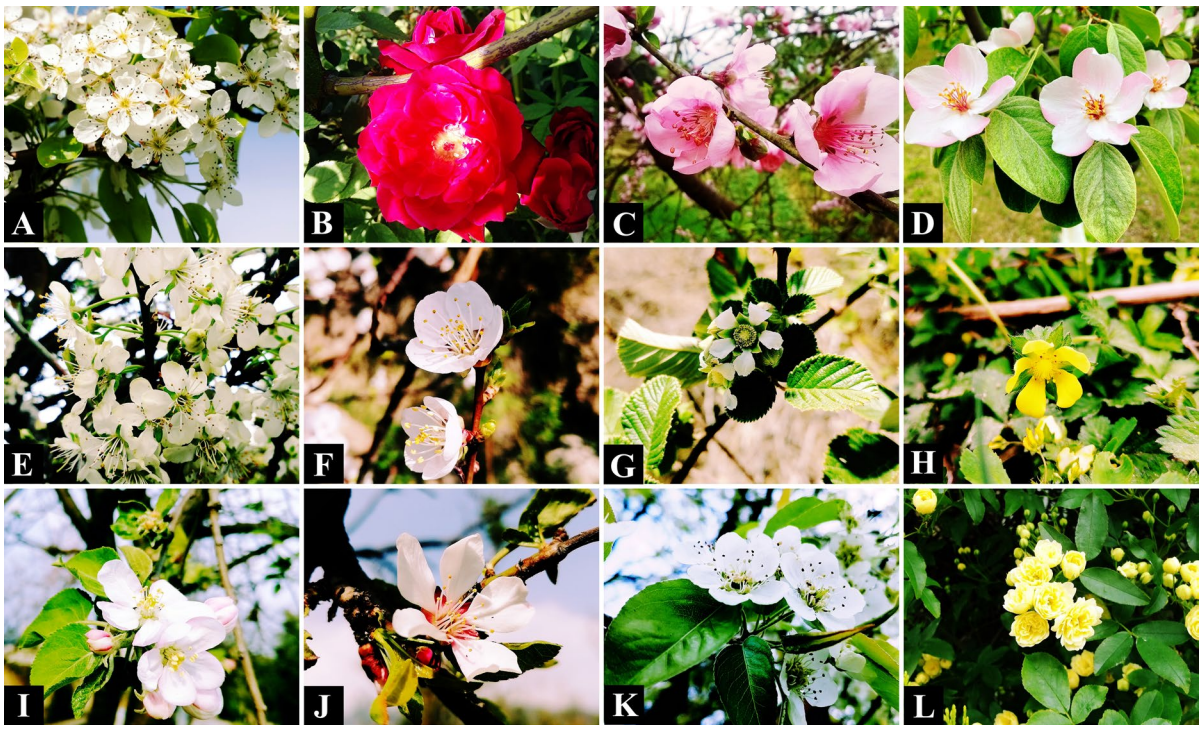



**Fig. 2** Floral field photographs of **a** *P. pashia,* **b** *R. indica,* **c** *P. persica,* **d** *C. oblonga,* **e** *P. domestica,* **f** *P. armeniaca,* **g** *R. ellipticus,* **h** *D. indica,* **i** *M. pumila,* **j** *Prunus dulcis,* **k** *P. calleryana,* and **l** *R. banksiae.*

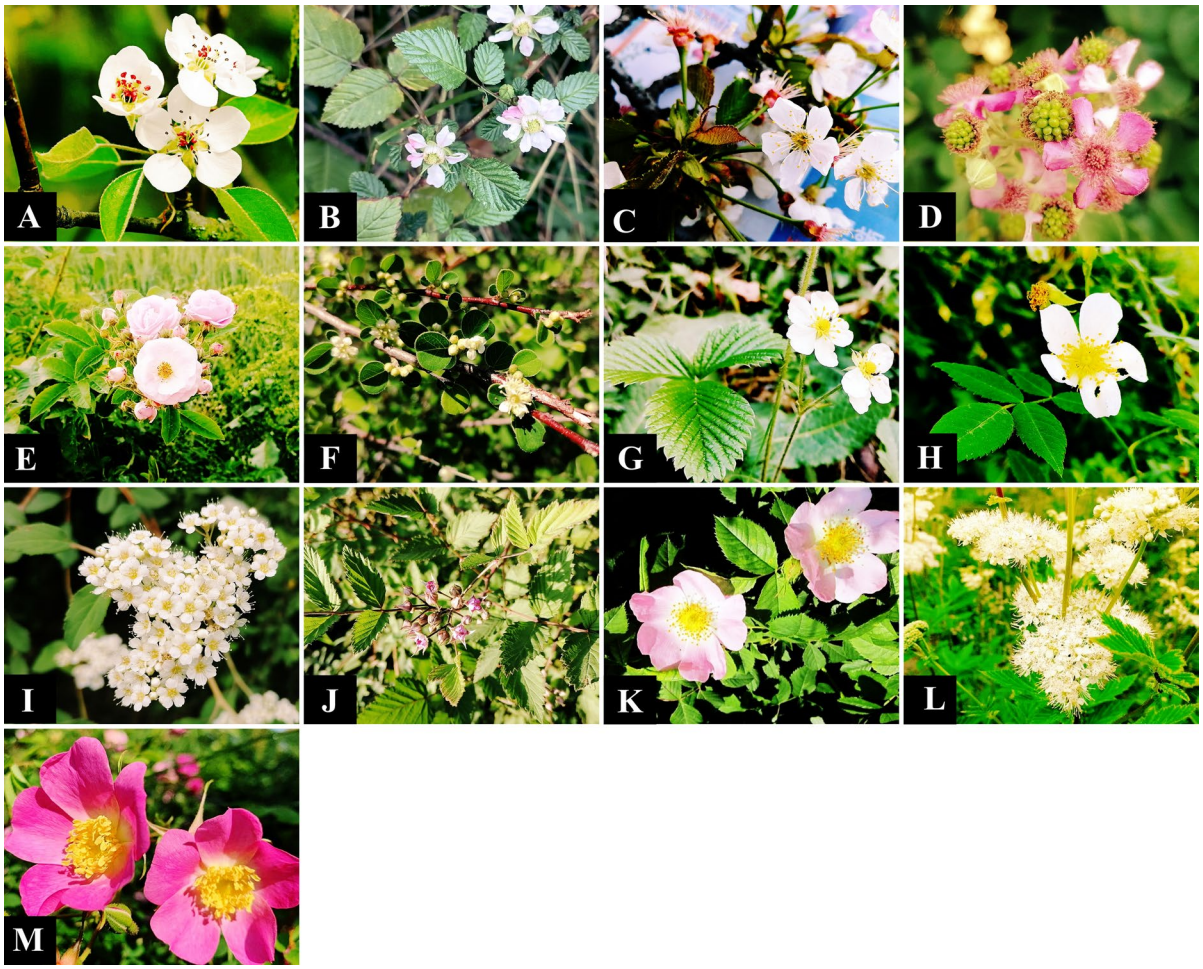



**Fig. 3** Floral field photographs of **a** *P. communis,* **b** *Rubus macilentus,* **c** *P. avium,* **d** *R. vestitus,* **e** *Rosa damascene,* **f** *C. nummularius*, **g** *F. vesca,* **h** *R. brunonii,* **i** *S. canescens,* **j** *R. niveus,* **k** *R. canina,* **l** *F. ulmaria,* **m** *R. webbiana*

determined. Pollen grains having a high fertility rate show the high stability of plants in a study area.

## Results

### Morphological description of pollen

In the present study, we presented the pollen morphology of 25 species belonging to 11 genera, including *Cotoneaster, Cydonia, Duchesnea, Filipendula, Malus, Prunus, Pyrus, Fragaria, Rosa, Rubus,* and *Spiraea* by using light microscopy. A description of the pollen morphology in all studied species showing numerous variations in both qualitative and quantitative features (Figs. 4, 5 and 6) and (Tables 2, 3, 4 and 5). In addition, the present study provides the percentage of assessed plant species is 52% shrubs species followed by 40% trees species and 8% herbs in Rosaceae.

### Qualitative parameters

The qualitative micromorphological features of rosaceous pollen grains were pragmatic under a light microscope (LM). In the current palynological findings, a diverse variation has been observed in the profile of pollen grains in size of pollen, pollen type, pollen class polar and equatorial view. Most of the pollen shapes in polar position were triangular;

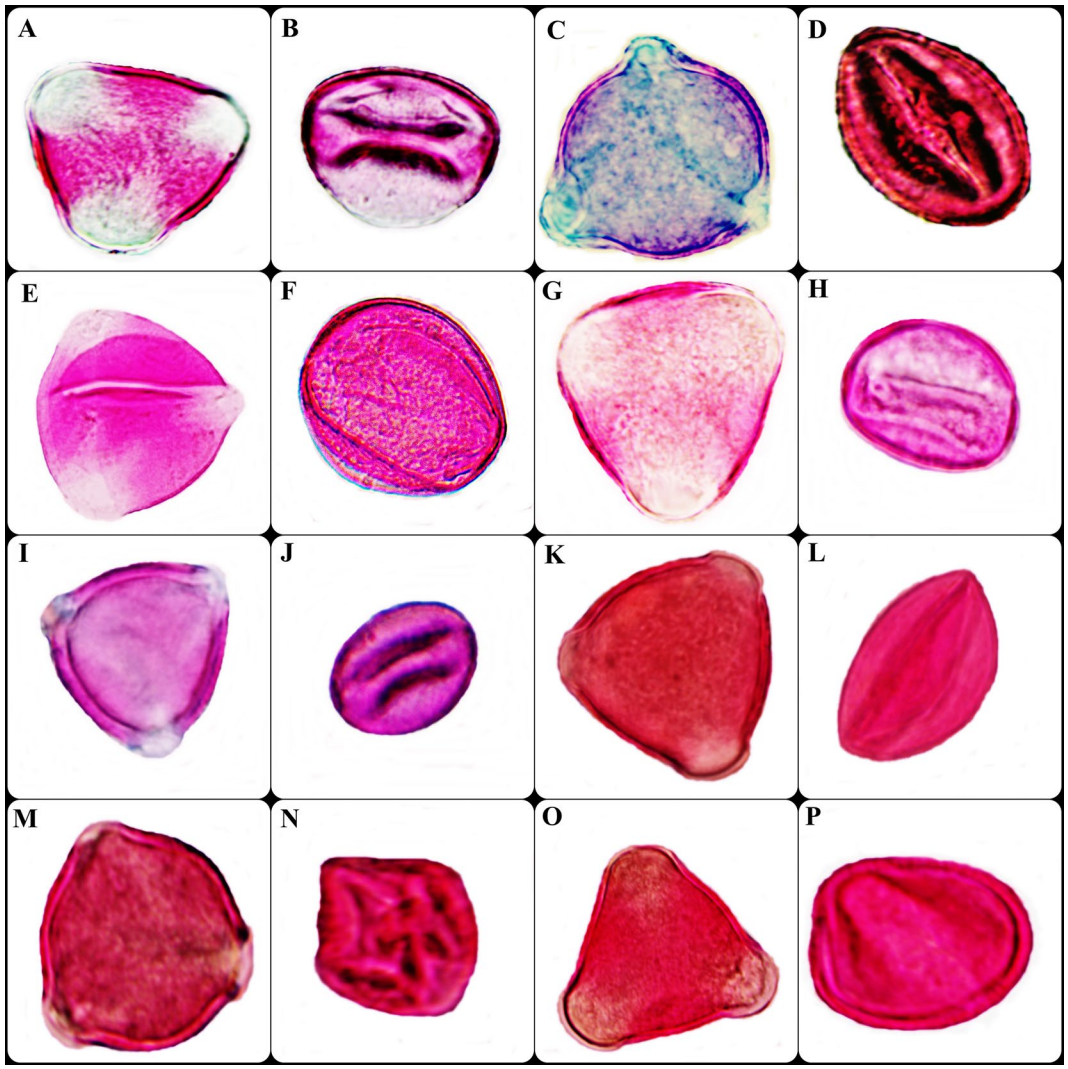



**Fig. 4** LM pollen microphotographs of *Pyrus pashia* **A** Polar view, **B** Equatorial view, *R. indica,* **C** Polar view, **D** Equatorial view, *P. persica,* **E** Polar view and **F** Equatorial view, *C. oblonga* **G** Polar view and **H** Equatorial view, *P. domestica* **I** Polar view and **J** Equatorial view, *Prunus armeniaca* **K** Polar view and **L** Equatorial view, *R. ellipticus* **M** Polar view and **N** Equatorial view, *Duchesnea indica* **O** Polar view and **P** Equatorial view

however, quadrangular and circular shapes of pollen in polar position were also examined in some species. Immense variations in types of pollen were not observed in the family Rosaceae; only three types of pollen among the 25 pollens were observed: tricolpate, tricolporate, and tetracolpate. In the pollen type, 15 out of 25 Rosaceous taxa were tricolpate pollen grains, while 9 out of 25 Rosaceous taxa have tricolporate pollens, and only 1 taxa *Cotoneaster nummularius, has tetracolpate* pollens. Almost all Rosaceous pollen in the current research were isopolar and monads having 3 colpi except for *Cotoneaster* which exhibit 4. The colpi ornamentations were noted to vary from perforate, psilate, reticulate, and microreticulate. Exine ornamentations of Rosaceous taxa via light microscope are almost reticulated, with a few species, namely *Prunus domestica, Rubus macilentus,* and *F. vesca,* having microreticulates, and only one species, *Rosa indica* exine ornamentation, is psilate type, as illustrated in Table 3. Most of the pollen size in the current palynological study of the family Rosaceae was noted as smaller; however,

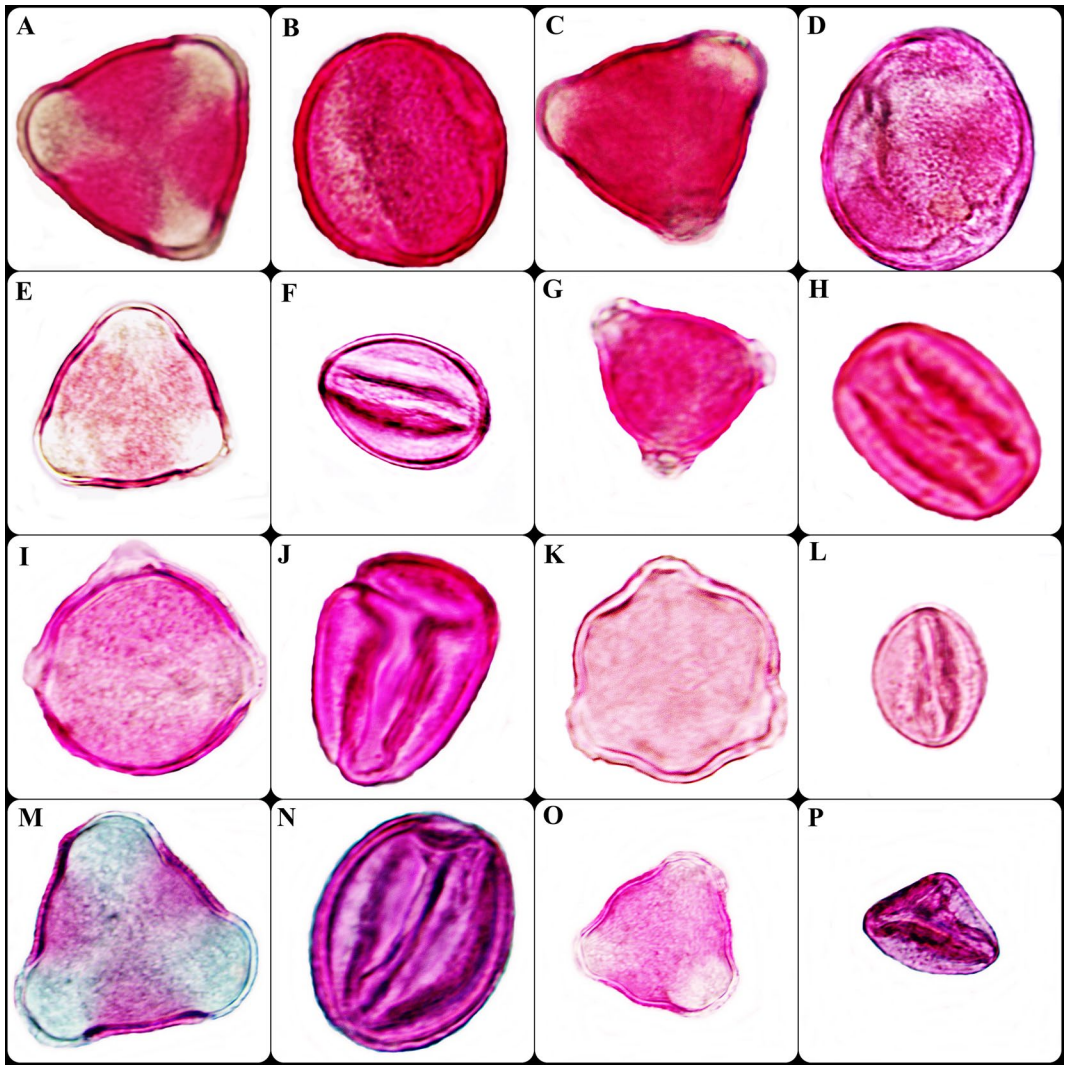



**Fig. 5** LM pollen microphotographs of *M. pumila* **A** Polar view and **B** Equatorial view, *P. dulcis* **C** Polar view and **D** Equatorial view, *P. calleryana* **E** Polar view and **F** Equatorial view, *R. banksiae* **G** Polar view and **H** Equatorial view, *P. communis* **I** Polar view and **J** Equatorial view, *Rubus macilentus* **K** Polar view and **L** Equatorial view, *P. avium* **M** Polar view and **N** Equatorial view, *R. vestitus* **O** Polar view and **P** Equatorial view,

only in six species have large pollen been reported. Diversity in the shape of the equatorial view, such as oblate, sub-oblate, prolate, sub-prolate, and circular, in palynological data of the Rosaceae, was also recorded among the 25 species, only three, *Malus pumila, Filipendula ulmaria,* and *R. webbiana,* were noted as circular.

Among the 25 Rosaceous taxa studied, the pollen grains of five species, *Pyrus pashia*, *Duchesnea indica*, *Rosa banksiae*, *Pyrus communis*, and *R. brunonii* were classified as prolate spheroidal based on their P/E ratio. Prolate class pollen was observed only in 8 species, including *Rosa indica, P. domestica, Pyrus calleryana, P. avium, C. nummularius, Rosa canina, F. ulmaria,* and *R. webbiana.* Sub-prolate class pollen shapes have been observed in 12 species of Rosaceae, as shown in Tables 2 and 3.

## Quantitative parameters

In the current pollen morphological characters of rosaceous taxa, a diverse variation has been observed

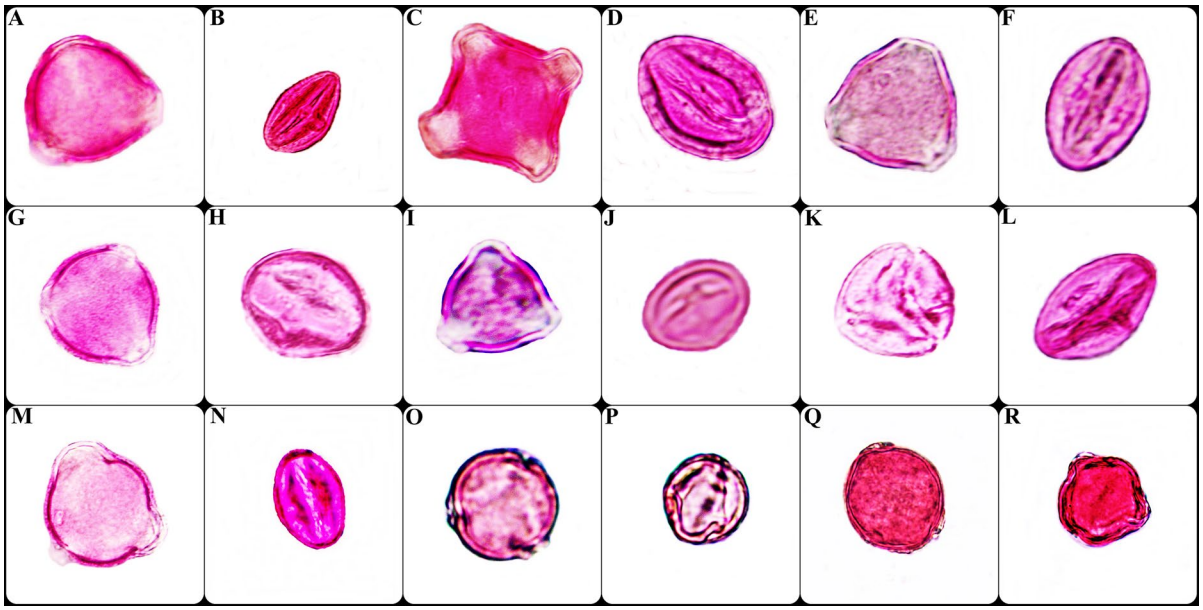



**Fig. 6** LM pollen microphotographs of *R. damascena* **A** Polar view **B** Equatorial view, *C. nummularius* **C** Polar view **D** Equatorial view, *F. vesca* **E** Polar view and **F** Equatorial view. *R. brunonii* **G** Polar view and **H** Equatorial view, *S. canescens* **I** Polar view and **J** Equatorial view, *R. niveus* **K** Polar view and **L** Equatorial view, *R. canina* **M** Polar view and **N** Equatorial view, *F. ulmaria* **O** Polar view and **P** Equatorial view, *R. webbiana* **Q** Polar view and **R** Equatorial view

in polar diameter, P/E ratio, length of colpi, length of pore, equatorial thickness, width of colpi, width of pore, mesocolpium, and exine thickness.

Pollen diameter and P/E index

The minimum polar diameter was observed in the pollen of *S. canescens* ($10.4 \pm 0.93$ µm), while the maximum polar diameter was noted in *Prunus armeniaca* ($44.2 \pm 0.72$ µm), as shown in Fig. 7. Similarly, minimum equatorial diameter was also noted in *S. canescens* ($7.95 \pm 0.48$ µm), while maximum equatorial diameter was observed in *Prunus armeniaca* ($38.3 \pm 0.87$ µm), as demonstrated in Fig. 7. In *P. communis* (1.05 µm), the lowest P/E ratio in the quantitative data of the Rosaceae was examined. while the highest P/E ratio was noted in *F. ulmaria* (1.9 µm), as displayed in Table 4.

Measurement of the colpi and pore

The immense variations in the quantitative data in length and width of pollen colpi were also found. The lowest possible length of Pollen colpi was observed in *Rubus niveus* ($3.30 \pm 0.14$ µm), while the largest length of Pollen colpi was seen in *R. webbiana* ($13.7 \pm 0.93$ µm), as shown in Fig. 8. Similarly, the minimum width of pollen colpi was noted in *S. canescens* ($3.5 \pm 0.25$ µm), while the maximum width of pollen colpi in *Rubus vestitus* ($9.65 \pm 0.35$ µm), as displayed in Fig. 8. Nine species out of 25 have tricolporate pollen, so variations in quantitative data in the length and width of pollen pores were also observed in some species of the family Rosaceae. The smallest length of pore was observed in *R. canina* ($0.45 \pm 0.93$ µm), while the largest length of pore was noted in *F. ulmaria* ($1.75 \pm 0.12$ µm), as illustrated in Table 4. Similarly, a minimum width of pore was seen in *R. indica* ($0.4 \pm 0.10$ µm), while a maximum width of pore was observed in *C. nummularius* ($1.75 \pm 0.16$ µm), as shown in Table 4.

Exine thickness and fertility percentage

The quantitative variations in exine thickness were also noted in Rosaceous pollen; the minimum exine thickness was calculated in *S. canescens* ($3.20 \pm 0.16$ µm), while the maximum was calculated in *Prunus armeniaca* ($6.40 \pm 0.38$ µm), as noted in Fig. 9. Maximum fertile pollen was calculated in *P.*

**Table 2** Qualitative features of Rosaceous taxa

| Sr. No | Rosaceous taxa | Size of pollen | Pollen type | Pollen class | Polar view | Equatorial view |
|---|---|---|---|---|---|---|
| 1 | *Pyrus pashia* Buch | Medium | Tricolpate | Prolate spheroidal | Triangular | Sub oblate |
| 2 | *R. indica* L | Large | Tricolporate | Prolate | Slightly circular | Prolate |
| 3 | *P. persica* (L.) | Medium | Tricolpate | Sub prolate | Slightly circular | Sub prolate |
| 4 | *C. oblonga* Mill | Large | Tricolpate | Sub prolate | Triangular | Sub oblate |
| 5 | *P. domestica* L | Small | Tricolporate | Prolate | Triangular | Oblate |
| 6 | *Prunus armeniaca* L | Large | Tricolpate | Sub prolate | Triangular | Prolate |
| 7 | *R. ellipticus* Sm | Large | Tricolporate | Sub prolate | Slightly circular | Sub prolate |
| 8 | *D. indica* Jacks | Medium | Tricolpate | Prolate spheroidal | Triangular | Sub prolate |
| 9 | *M. pumila* Mill | Large | Tricolpate | Sub prolate | Triangular | Circular |
| 10 | *P. dulcis* (Mill.) | Medium | Tricolpate | Sub prolate | Triangular | Sub prolate |
| 11 | *P. calleryana* Decne | Small | Tricolpate | Prolate | Triangular | Oblate |
| 12 | *R. banksiae* R.Br | Small | Tricolporate | Prolate spheroidal | Triangular | Prolate |
| 13 | *P. communis* L | Small | Tricolporate | Prolate spheroidal | Circular | Sub prolate |
| 14 | *Rubus macilentus* Jacq | Small | Tricolporate | Sub prolate | Slightly circular | Sub prolate |
| 15 | *P. avium* (L.) L | Large | Tricolpate | Prolate | Triangular | Sub prolate |
| 16 | *R. vestitus* Weihe | Small | Tricolpate | Sub prolate | Triangular | Sub oblate |
| 17 | *Rosa*×*damascena* Hem | Small | Tricolpate | Sub prolate | Slightly circular | Prolate |
| 18 | *C. nummularius* | Medium | Tetracolpate | Prolate | Quadrangular | Sub oblate |
| 19 | *F. vesca* L | Medium | Tricolpate | Sub prolate | Slightly triangular | Prolate |
| 20 | *R. brunonii* Lindl | Medium | Tricolpate | Prolate spheroidal | Slightly circular | Sub oblate |
| 21 | *S. canescens* D.Don | Small | Tricolpate | Sub prolate | Triangular | Sub oblate |
| 22 | *R. niveus* Thunb | Small | Tricolporate | Sub prolate | Circular | Prolate |
| 23 | *R. canina* L | Medium | Tricolporate | Prolate | Circular | Prolate |
| 24 | *F. ulmaria* (L.) | Medium | Tricolporate | Prolate | Circular | Circular |
| 25 | *R. webbiana* Wall | Small | Tricolpate | Prolate | Circular | Circular |

*avium at* 94.79%, while the minimum *R. webbiana at* 51.89%, as shown in Table 5.

### Cluster analysis

The palyno-taxonomic characters of 25 species were clustered together by multivariate cluster analysis. The quantitative characters of pollen were used to find the affinities. As a result of Euclidian distance, the dendrogram shows two major clusters as shown in Fig. 10.

In the first cluster, *Prunus armeniaca* and *Spiracea canescens* show a diverse position from all other taxa in the present study. *R. webbiana* and *F. ulmaria* are closely related to each other, but they have a distant position from all other taxa. *Rosa Damasciena* and *R. vestitus* are closely related with each other in taxonomic features. In addition, *P. avium* and *M. pumila* are closely related to each other, but they are sisters linked to *Prunus delcis. Cydonia oblonga* has a distinct position in the first cluster among all other taxa. *R. ellipticus* and *P. domestica* are closely linked to each other. *P. calleryana* and *Prunus persica* are closely linked to each other, but they have a sister linked to *D. indica* and *R. indica*.

In the second cluster, *R. canina* and *Cotoneaster nummularis* are closely linked to each other. *F. vesca* and *Rubus macilentus* are closely linked to each other, but they have a sister linked to *R. niveus* and *R. brunonii. P. communis* and *Rosa banksiae* are closely related to each other. *P. pashia* has a distinct position in the second cluster in the present investigation.

## Discussion

Rosaceous taxa showed considerable diversity with respect to qualitative and quantitative pollen

**Table 3** Qualitative palynological traits of Rosaceous taxa

| Sr. No | Plant Taxa | Polarity | Dispersal unit | No of colpi | Pore in copi | Colpi ornamentations | Exine Ornamentations (LM) |
|---|---|---|---|---|---|---|---|
| 1 | *Pyrus pashia* Buch | Isopolar | Monads | Three | Absent | Perforate | Reticulate |
| 2 | *R. indica* L | Isopolar | Monads | Three | Absent | Psilate | Psilate |
| 3 | *P. persica* (L.) | Isopolar | Monads | Three | Present | Perforate | Reticulate |
| 4 | *C. oblonga* Mill | Isopolar | Monads | Three | Absent | Psilate | Reticulate |
| 5 | *P. domestica* L | Isopolar | Monads | Three | Present | Reticulate | Micro reticulate |
| 6 | *Prunus armeniaca* L | Isopolar | Monads | Three | Absent | Perforate | Reticulate |
| 7 | *R. ellipticus* Sm | Isopolar | Monads | Three | Present | Perforate | Reticulate |
| 8 | *D. indica* Jacks | Isopolar | Monads | Three | Absent | Psilate | Reticulate |
| 9 | *M. pumila* Mill | Isopolar | Monads | Three | Absent | Reticulate | Reticulate |
| 10 | *P. dulcis* (Mill.) | Isopolar | Monads | Three | Absent | Psilate | Reticulate |
| 11 | *P. calleryana* Decne | Isopolar | Monads | Three | Absent | Reticulate | Reticulate |
| 12 | *R. banksiae* R.Br | Isopolar | Monads | Three | Present | Perforate | Reticulate |
| 13 | *P. communis* L | Isopolar | Monads | Three | Present | Perforate | Reticulate |
| 14 | *Rubus macilentus* Jacq | Isopolar | Monads | Three | Present | Perforate | Micro reticulate |
| 15 | *P. avium* (L.) L | Isopolar | Monads | Three | Absent | Microreticulate | Reticulate |
| 16 | *R. vestitus* Weihe | Isopolar | Monads | Three | Absent | Psilate | Reticulate |
| 17 | *Rosa*×*damascena* Hem | Isopolar | Monads | Three | Absent | Nanoreticulate | Reticulate |
| 18 | *C. nummularius* | Isopolar | Monads | Four | Absent | Psilate | Reticulate |
| 19 | *F. vesca* L | Isopolar | Monads | Three | Absent | Microreticulate | Micro reticulates |
| 20 | *R. brunonii* Lindl | Isopolar | Monads | Three | Absent | Reticulate | Reticulate |
| 21 | *S. canescens* D.Don | Isopolar | Monads | Three | Absent | Psilate | Reticulate |
| 22 | *R. niveus* Thunb | Isopolar | Monads | Three | Present | Perforate | Reticulate |
| 23 | *R. canina* L | Isopolar | Monads | Three | Present | Perforate | Reticulate |
| 24 | *F. ulmaria* (L.) | Isopolar | Monads | Three | Present | Perforate | Reticulate |
| 25 | *R. webbiana* Wall | Isopolar | Monads | Three | Absent | Reticulate | Reticulate |

micro-morphological data using light microscopy. However, all studied species shared similar physical surface outlines, such as reticulate sculpturing. The qualitative characters used in the present study include size of the pollen, pollen type, pollen class, polar and equatorial views, polarity, dispersal unit, number of colpi, pore in copi, colpi ornamentations, and equatorial ornamentations of the pollen. The quantitative features of pollen include polar diameter, equatorial diameter, ratio between the polar and equatorial values, length of colpi, width of colpi, length of pollen pore, width of pollen pore, mesocolpium, exine thickness, and percentage of fertile and sterile pollen (Nabila et al. 2022; Ahmad et al. 2020). The basic criteria for correctly identifying species are various parameters of palynological characters; the shape of the pollen, polar and equatorial diameter, length, and width of colpi, etc. (Ullah, 2022; Wang et al. 2023). The study results highlight the crucial role of Rosaceae diversity and contributing to conservation efforts in the study area. For instance, *P. avium* exhibited the highest pollen fertility at 94.79%, while *R. webbiana* showed the lowest at 51.89%, highlighting the variability in reproductive potential among the species.

The variations in pollen morphology were observed in the studied taxa, except for the number of apertures in the form of colpi and pore. Although Rosaceous taxa is mainly represented by tricolporate pollen (Fernández and Alli 2023; Li, et al. 2023), tetrad as well as hexacolporate pollen were also found in Rosaceous taxa. In this study, tricolporate pollen grains in the family Rosaceae are consistent with the observations of (Lee, et al. 2011; Chung, et al. 2010).

**Table 4** Quantitative pollen traits of selected Rosaceous taxa

| S. No | Plants species | P.D (μm) (Mean ± SE) μm | E.D (μm) | P/E ratio | L.C (μm) | W.C (μm) | L.P (μm) | W.P (μm) | M.C (μm) | E.T (μm) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *P. pashia* Buch | 26.6 ± 2.47 | 24.7 ± 1.87 | 1.07 | 6.15 ± 0.55 | 7.75 ± 0.26 | – | – | 17.6 ± 0.92 | 3.5 ± 0.25 |
| 2 | *R. indica* L | 34.8 ± 2.47 | 22.6 ± 0.76 | 1.53 | 5.75 ± 0.26 | 5.55 ± 0.14 | 0.6 ± 0.12 | 0.4 ± 0.10 | 14.4 ± 0.62 | 4.65 ± 0.20 |
| 3 | *P. persica* (L.) | 34.6 ± 2.17 | 26.7 ± 2.13 | 1.29 | 6.50 ± 0.39 | 6.50 ± 0.35 | – | – | 17.5 ± 1.14 | 4.55 ± 0.64 |
| 4 | *C. oblonga* Mill | 40.9 ± 0.95 | 35.0 ± 0.75 | 1.16 | 7.90 ± 0.64 | 9.55 ± 0.46 | – | – | 17.4 ± 0.44 | 4.50 ± 0.11 |
| 5 | *P. domestica* L | 24.5 ± 2.18 | 17.4 ± 0.20 | 1.40 | 3.95 ± 0.21 | 5.70 ± 0.42 | 0.95 ± 0.18 | 0.65 ± 0.16 | 13.1 ± 0.12 | 5.40 ± 0.10 |
| 6 | *Prunus armeniaca* L | 44.2 ± 0.72 | 38.3 ± 0.87 | 1.15 | 4.55 ± 0.93 | 5.2 ± 0.53 | – | – | 15.3 ± 0.66 | 6.40 ± 0.38 |
| 7 | *R. ellipticus* Sm | 23.9 ± 1.40 | 18.9 ± 0.71 | 1.26 | 7.65 ± 0.30 | 7.3 ± 0.30 | 1.25 ± 0.17 | 1.50 ± 0.27 | 11.6 ± 0.26 | 5.60 ± 0.18 |
| 8 | *D. indica* Jacks | 32.2 ± 1.04 | 29.3 ± 1.39 | 1.09 | 4.55 ± 0.93 | 5.55 ± 0.14 | – | – | 17.8 ± 0.63 | 4.95 ± 0.31 |
| 9 | *M. pumila* Mill | 35.9 ± 1.72 | 29.5 ± 1.17 | 1.21 | 10.2 ± 0.2 | 8.4 ± 0.4 | – | – | 14.6 ± 0.28 | 5.30 ± 0.28 |
| 10 | *P. dulcis* (Mill.) | 31.3 ± 1.42 | 23.8 ± 0.50 | 1.31 | 7.2 ± 0.30 | 8.9 ± 0.33 | – | – | 14.0 ± 0.62 | 5.25 ± 0.36 |
| 11 | P. calleryana Decne | 35.6 ± 1.14 | 24.8 ± 1.27 | 1.43 | 5.75 ± 0.26 | 7.2 ± 0.50 | – | – | 12.6 ± 0.26 | 4.5 ± 0.32 |
| 12 | *R. banksiae* R.Br | 22.9 ± 0.43 | 20.6 ± 0.45 | 1.11 | 4.45 ± 0.33 | 6.1 ± 0.38 | 0.85 ± 0.25 | 1.5 ± 0.25 | 10.7 ± 0.35 | 4.05 ± 0.40 |
| 13 | *P. communis* L | 20.4 ± 0.37 | 19.4 ± 0.55 | 1.05 | 5.40 ± 0.24 | 6.25 ± 0.35 | 1.4 ± 0.18 | 0.75 ± 0.17 | 11.5 ± 0.32 | 3.9 ± 0.31 |
| 14 | *Rubus macilentus* Jacq | 25.4 ± 0.67 | 19.9 ± 0.41 | 1.27 | 5.55 ± 0.42 | 5.85 ± 0.29 | 0.65 ± 0.12 | 1.45 ± 0.18 | 12.4 ± 0.18 | 4.45 ± 0.26 |
| 15 | *P. avium* (L.) L | 34.6 ± 1.20 | 25.2 ± 0.96 | 1.37 | 10.3 ± 0.39 | 8.55 ± 0.42 | – | – | 10.9 ± 0.60 | 5.40 ± 0.18 |
| 16 | *R. vestitus* Weihe | 25.8 ± 0.64 | 19.6 ± 0.9 | 1.31 | 9.0 ± 0.35 | 9.65 ± 0.43 | – | – | 11.9 ± 0.49 | 4.50 ± 0.28 |
| 17 | *Rosa × damascena* Hem | 21.1 ± 0.50 | 17.1 ± 0.44 | 1.24 | 9.85 ± 0.23 | 8.95 ± 0.46 | – | – | 11.4 ± 0.33 | 4.70 ± 0.16 |
| 18 | *C. nummularius* | 25.5 ± 0.52 | 17.8 ± 0.66 | 1.43 | 9.05 ± 0.31 | 7.0 ± 0.48 | 1.05 ± 0.09 | 1.75 ± 0.13 | 12.5 ± 0.45 | 3.80 ± 0.45 |
| 19 | *F. vesca* L | 21.1 ± 0.34 | 17.7 ± 0.35 | 1.19 | 6.65 ± 0.53 | 4.9 ± 0.26 | – | – | 11.1 ± 0.43 | 4.85 ± 0.23 |
| 20 | *R. brunonii* Lindl | 18.1 ± 0.26 | 16.2 ± 0.33 | 1.11 | 4.40 ± 0.26 | 5.2 ± 0.26 | – | – | 9.20 ± 0.41 | 4.40 ± 0.18 |
| 21 | *S. canescens* D.Don | 10.4 ± 0.93 | 7.95 ± 0.48 | 1.30 | 3.85 ± 0.30 | 3.5 ± 0.25 | – | – | 8.05 ± 0.14 | 3.20 ± 0.16 |
| 22 | *R. niveus* Thunb | 21.9 ± 0.52 | 18.3 ± 0.68 | 1.19 | 3.30 ± 0.14 | 3.8 ± 0.37 | 0.65 ± 0.15 | 1.20 ± 0.18 | 12.6 ± 0.47 | 3.75 ± 0.35 |
| 23 | *R. canina* L | 18.6 ± 0.34 | 13.4 ± 0.46 | 1.38 | 6.9 ± 0.29 | 5.9 ± 0.29 | 0.45 ± 0.93 | 1.65 ± 0.23 | 12.4 ± 0.21 | 3.90 ± 0.15 |
| 24 | *F. ulmaria* (L.) | 16.1 ± 0.35 | 8.45 ± 0.21 | 1.90 | 12.2 ± 0.28 | 7.45 ± 0.54 | 1.75 ± 0.17 | 0.9 ± 0.16 | 4.45 ± 0.28 | 9.4 ± 0.38 |
| 25 | *R. webbiana* Wall | 35.1 ± 1.50 | 21.2 ± 0.37 | 1.65 | 13.7 ± 0.93 | 8.85 ± 0.40 | – | – | 31.4 ± 0.67 | 31.4 ± 0.67 |

**Keynotes*** PD = (polar diameter), ED = (Equatorial diameter), LC = (Length of colpi), WC = (Width of colpi), LP = (Length of pore), WP = (Width of pore), MC = (Meso-colpium), ET = (Exine thickness),—mean (Absent)

**Table 5** Percentage of fertility and sterility of Rosaceous pollens

| Sr.No | Plant Taxa | Number of fertile pollens | Number of sterile pollens | Fertility (%) | Sterility (%) |
|---|---|---|---|---|---|
| 1 | *Pyrus pashia* Buch | 72 | 13 | 84.70 | 15.29 |
| 2 | *R. indica* L | 78 | 18 | 81.25 | 18.75 |
| 3 | *P. persica* (L.) | 67 | 21 | 76.13 | 23.86 |
| 4 | *C. oblonga* Mill | 56 | 23 | 70.88 | 29.11 |
| 5 | *P. domestica* L | 57 | 09 | 86.36 | 13.63 |
| 6 | *Prunus armeniaca* L | 44 | 07 | 86.27 | 13.72 |
| 7 | *R. ellipticus* Sm | 81 | 05 | 94.18 | 5.8 |
| 8 | *D. indica* Jacks | 77 | 11 | 87.5 | 12.5 |
| 9 | *M. pumila* Mill | 63 | 16 | 79.74 | 20.25 |
| 10 | *P. dulcis* (Mill.) | 52 | 18 | 74.28 | 25.71 |
| 11 | *P. calleryana* Decne | 53 | 21 | 71.62 | 28.37 |
| 12 | *R. banksiae* R.Br | 74 | 15 | 83.14 | 16.85 |
| 13 | *P. communis* L | 68 | 28 | 70.83 | 29.16 |
| 14 | *Rubus macilentus* Jacq | 83 | 23 | 78.30 | 21.69 |
| 15 | *P. avium* (L.) L | 91 | 05 | 94.79 | 5.20 |
| 16 | *R. vestitus* Weihe | 84 | 11 | 88.42 | 11.57 |
| 17 | *Rosa*×*damascena* Hem | 56 | 15 | 78.87 | 21.12 |
| 18 | *C. nummularius* | 66 | 12 | 84.61 | 15.38 |
| 19 | *F. vesca* L | 77 | 19 | 80.20 | 19.79 |
| 20 | *R. brunonii* Lindl | 45 | 22 | 67.16 | 32.83 |
| 21 | *S. canescens* D.Don | 41 | 21 | 66.12 | 33.87 |
| 22 | *R. niveus* Thunb | 86 | 17 | 83.49 | 16.50 |
| 23 | *R. canina* L | 73 | 15 | 82.95 | 17.04 |
| 24 | *F. ulmaria* (L.) | 65 | 56 | 53.71 | 46.28 |
| 25 | *R. webbiana* Wall | 41 | 38 | 51.89 | 48.10 |

The current study clarifies the taxonomic implications of pollen morphology for taxa identification and delimitation of Rosaceous taxa. The majority of examined species show similar morphological features but some variations in the size of polar and equatorial views, pores, apertures, pollen outline, colpi length and width, P/E ratio, and exine sculpture (Ullah et al. 2022). In the present research work, *P. pashia* Buch is medium-sized, tricolpated, prolate spheroidal, triangular, and suboblate. *P. calleryana* and *P. communis* are small, tricolporated, prolate spheroidal, triangular-circular, and oblate to subprolate. While pollen grains of *Pyrus* sp. recorded from India showed tricolporate and zonaperturate ornamentations. The pollen morphology of *R. indica, R. banksiae, and R. damascene. R. webbiana and R. canina* showed sizes of small-medium tricolpated, prolate-subprolate, circular triangular in polar view, and prolate-circular in equatorial views (Wrońska-Pilarek & Jagodziński 2011). Singh et al. (2020) extensively examined the pollen morphology of *Rosa* species in the Western Himalaya, revealing that the pollen grains are predominantly spheroidal to sub-spheroidal in shape; surface textures range from smooth to sculptured, with diverse exine ornamentations observed. The pollen morphology of *Rubus* species reveals a range of spheroidal to oblate pollen grains with diverse exine ornamentation, including tectate and sculptured surfaces. Pollen grains of *Sanguisorba* species are predominantly tricolpate and exhibit a range of shapes from sub-spherical to ellipsoidal, with varying exine textures, including reticulate and verrucate patterns (Lee et al. 2011). The pollen grains of *P. avium* were tricolpated, prolate, triangular, and subprolate, while the pollen of *P. duclis* were medium, tricolpated, subprolate in the equatorial view, and triangular in the polar view (Ghosh & Saha 2017).

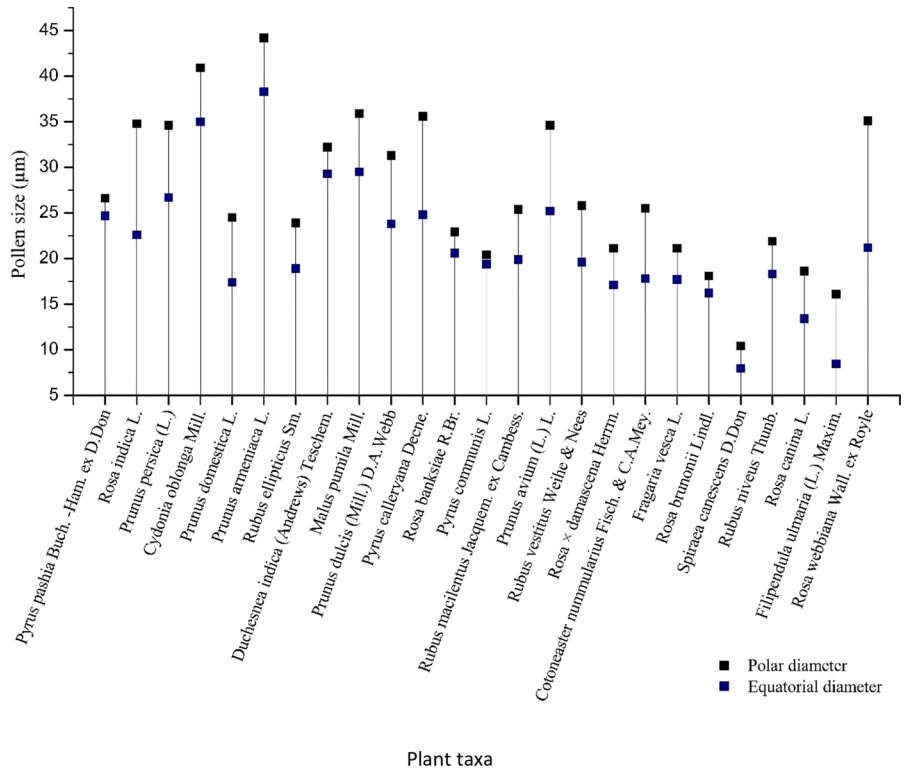



**Fig. 7** Comparison between polar and equatorial diameters of pollens

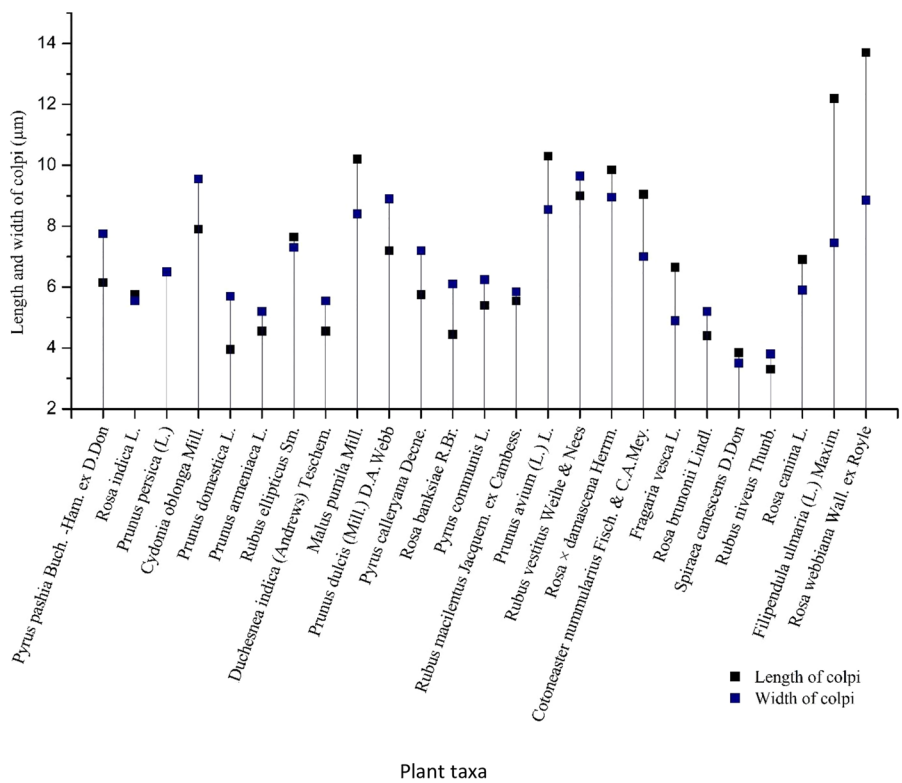



**Fig. 8** Comparison between length and width of colpi

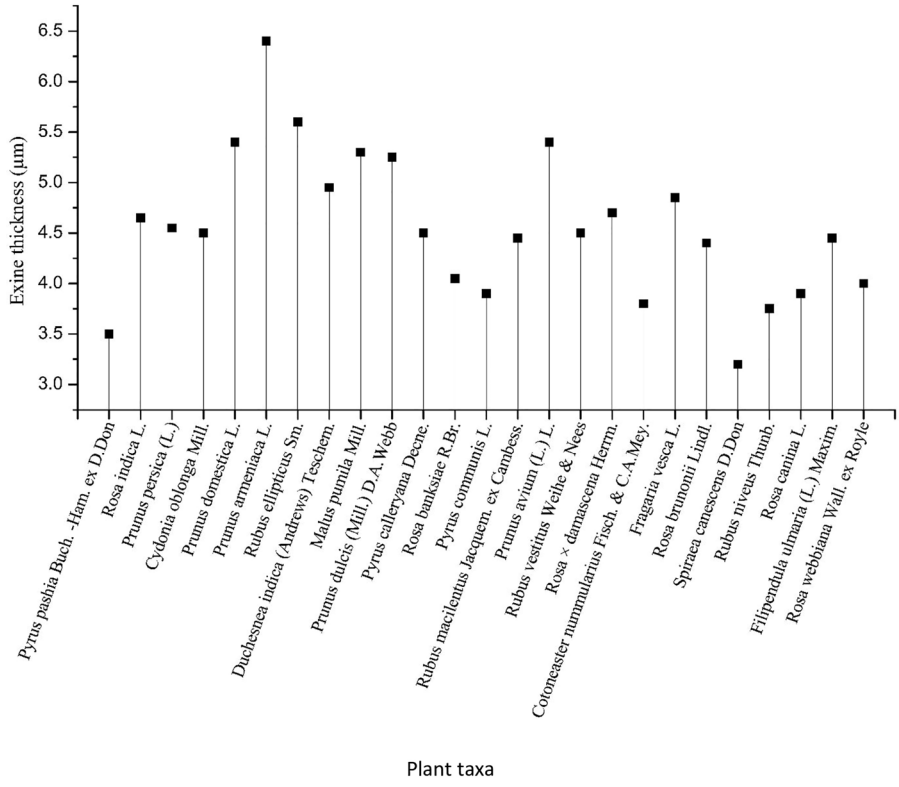



**Fig. 9** Graphical representation of exine thickness

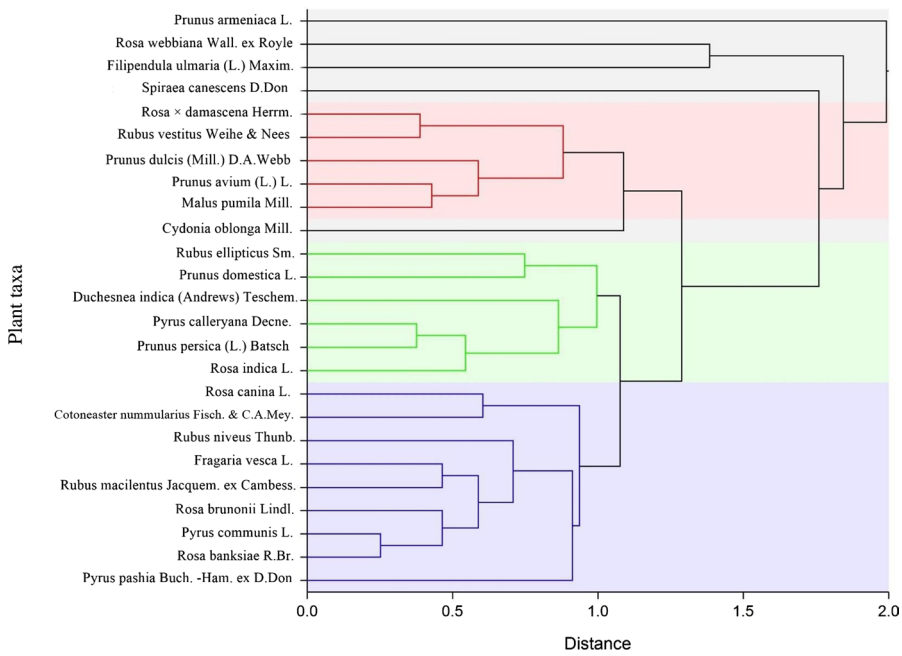



**Fig. 10** Dendrogram, based on micro-morphological features

Pollen morphological characters are the diagnostic features used in the identification of Rosaceae taxa and these characters recognize in their certain genera at the various taxonomic level (Naruhashi and Toyoshima 1979). Some previous literatures indicated that the pollen size in some genera of

Rosaceae is correlated with chromosome number (Fischer et al. 1943). Pollen exine ornamentation is described with many terms in Rosaceae and it is the most important distinguishing character in rosaceous taxa (Erdtman 1952; Shi et al. 2013; Song et al. 2016). Pollen trait was seemed to be significant reliable characters to use for the classification of *Rosa* at sectional level (Ueda and Tomita 1989). The pollen apertures exhibited by tricolpate type, pollen shape is sub-spherical to ellipsoidal and exine textures is reticulate and verrucate patterns were observed in the species of *Sanguisorba* (Lee et al. 2011). The pollen morphology was described in Turkish *Crataegus* in which the pollen is monad; frequent aperture form is tricolporate, trisyncolporate and tetracolporate (Oybak- Dönmez 2008). The results of the present correlated with characters assessed in *Rubus* and their pollen shape is spheroidal to oblate and exine ornamentation was observed as tectate type (Lechowicz et al. 2020). Our finding agree with the pollen features were observed in *Rosa* species occurring in Western Himalaya and revealed that the pollen grains shape is spheroidal to sub-spheroidal and surface texture is smooth (Singh et al. 2020). Similar study was conducted on the pollen morphology of the 24 species in Rosaceae and showed diversity in size, shape, exine characters (Ghosh and Saha 2017).

While the study offers valuable insights into the palynological diversity of Rosaceae in Swat, it also has several limitations. Additionally, the study focused on a limited number of villages in Swat, which may not represent the entire diversity of Rosaceae flora in northern Khyber Pakhtunkhwa. Future research should consider expanding the geographical scope and temporal range of the study to provide a more comprehensive understanding of taxonomic study of Rosaceae.

Furthermore, the study relied on light microscopy for pollen analysis, while effective, may not capture the full range of micromorphological traits. Advanced techniques such as scanning electron microscopy (SEM) could provide more detailed insights into pollen surface features and their systematics implications (Xiong et al. 2019). Integrating molecular techniques to analyze genetic diversity within Rosaceae species could also enhance our understanding of their evolutionary relationships and potential adaptation to local environmental conditions (Potter et al. 2007).

The primary goal of the current study was to investigate the micromorphological features in the family Rosaceae under a light microscope (LM), with a special emphasis on diversity and variations in rosaceous pollen. Taxonomic study using a light microscope (LM) for accurate taxonomic group identification and delimitation thus plays a crucial role in the field of taxonomy and offers the taxonomist valuable potential to distinguish and segregate any taxonomic group or level up to a certain extent using palynological micro-morphological features.

## Conclusion

In the current research palynological investigations of 25 Rosaceae taxa were examined using microscopic techniques. In the present work, pollen morphology via qualitative and quantitative traits of 25 species belonging to different genera in Rosaceae was investigated. Palynological characters, i.e. pollen size, shape, apertures and ornamentations etc. were used as a tool for the correct identification of taxa. The pollen size was examined in the range of small-large, pollen type tricolpate-tricolporate and pollen class prolate-subprolate. Our results demonstrate that there is high diversity and variations in palynological features among the Rosaceae taxa. This study highlights the taxonomic and systematic implications of Rosaceae. However, further investigations are needed to examine the palynological diversity of Rosaceae from a detailed evolutionary point of view. It has been investigated that the palynological research alone is insufficient to elucidate the taxonomic relationships within Rosaceae at sub-generic level, but they can provide some useful information for further taxonomic treatment at specific levels.

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**Author contributions** M.A; helped in the write up of original manuscript. M. A; Supervision Z.U; Editing and reviewing. A.K; Methodology and field survey S.A; Experiments H.S; Softwares A.U; Write up. M.A.F; Field survey O.E; Reiewing R.1; Editing A.O; Reviewing M.A; Revieing H.R; Ediring M.S.E; Experiments.

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**Declarations**

**Conflict of interest** The authors of the manuscript have no conflict of interest to declare it.

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