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Petal micromorphology of some *Rosa* species in Iran

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Abstract

The micromorphological characteristics of the upper and lower epidermis of the petals of 15 *Rosa* species in Iran were investigated for the first time. The study used light microscopy (LM) and scanning electron microscopy (SEM) to compare epidermal patterns of petals among different species. Quantitative and qualitative characteristics related to the petals, including cell density, flower color, cell type, and stomata and trichomes on both surfaces, were compared between the *Rosa* and *Hulthemia* subgenera species in Iran. No evidence of hairs and stomata was found on either surface of the epidermis. The arrangement of the adaxial surface in all species consists of conical cells called papillae with different degrees of projection and interconnected and a cuticle layer covers their surface. The abaxial surface pattern is often seen as flat cells with parallel lines or irregular folds. The cluster analysis using Past software showed that, although the petal epidermis is a consistent feature within the species, this trait is unsuitable for classifying *Rosa* species. In addition, in the present study it was found that, the difference in epidermal pattern in some species such as *R. beggeriana* and *R. moschata* was found to be useful to identify them from each other.

Keywords: Electron microscopy, light microscopy, petal epidermis, *Rosaceae*, variation

ریزریخت‌شناسی گلبرگ در برخی از گونه‌های نسترن وحشی در ایران*

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خلاصه

مطالعه حاضر، نخستین بررسی در مورد ویژگی‌های ریزریخت‌شناسی اپیدرم پشتی و رویی گلبرگ‌های ۱۵ گونه نسترن وحشی (گل‌سرخیان) را در ایران ارایه می‌دهد. ساختارهای سطح اپیدرم با استفاده از میکروسکوپ نوری و الکترونی روبشی با هدف مقایسه الگوهای اپیدرمی گلبرگ‌ها بین گونه‌های جنس مذکور مورد مطالعه قرار گرفت. ویژگی‌های کمّی و کیفی مرتبط با گلبرگ‌ها از جمله تراکم سلولی، رنگ گل، نوع سلول و وجود روزنه‌ها و کرک‌ها در هر دو سطح پشتی و رویی، برای مقایسه و بررسی بین گونه‌های هر دو زیرجنس *Rosa* و *Hulthemia* در ایران در نظر گرفته شد. در نتیجه بررسی حاضر، هیچ شواهدی از کرک و روزنه روی هر دو سطح اپیدرم در گونه‌های مطالعه حاضر وجود نداشت، به طوری که آرایش سطح رویی گلبرگ در همه گونه‌ها از سلول‌های مخروطی موسوم به پاپیلا با درجات متفاوت برجستگی و به هم پیوسته با یک لایه پوشش کوتیکولی تشکیل گردید. الگوی سطح پشتی بیشتر به صورت سلول‌های مسطح با خطوط موازی یا دارای چینه‌های نامنظم مشاهده گردید. همچنین، نتایج به دست آمده منتج از آنالیز خوشه‌بندی با استفاده از نرم‌افزار Past در بررسی حاضر نشان داد که اگرچه اپیدرم گلبرگ یک ویژگی ثابت در درون گونه بود، اما این صفت برای طبقه‌بندی گونه‌های جنس مورد مطالعه مناسب نبود. به علاوه در این تحقیق، تفاوت در الگوی اپیدرم در برخی گونه‌ها مانند *R. beggeriana* و *R. moschata* برای شناسایی آن‌ها از یکدیگر مفید شناخته شد.

واژه‌های کلیدی: اپیدرم گلبرگ، تنوع صفات، گل‌سرخیان، میکروسکوپ الکترونی (SEM)، میکروسکوپ نوری (LM)

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Introduction

Wild roses are the ancestors of all garden roses. Many garden roses have been created through selection, mutation, and hybridization (Eugster & Marki-Fischer 1991). The genus *Rosa* Linnaeus (1753: 491) (*Rosaceae*) is widespread throughout the Northern Hemisphere, Central and Southwest Asia being the main distribution centers (Ku & Robertson 2003, Wissemann & Ritz 2005). Biogeographic studies indicate that, Asia has been a critical genetic reservoir in the evolution of the *Rosa* species (Fougère-Danezan *et al.* 2015). There are ca. 200 species of roses in the world (Ku & Robertson 2003). In the Flora Iranica (Zielinski 1982), 19 *Rosa* species, 16 hybrids and eight doubtful records were reported, of which there are 13 species, eight hybrids, and three doubtful records in the Flora of Iran (Khatamsaz 1992). A new species, namely, *R. abrica* Khatamsaz & Koobaz (Koobaz *et al.* 2011), two new records, *R. freitagii* Zielinski (1982) (Sharghi *et al.* 2014), and *R. kokanica* (Regel 1878) Regel ex Juzepczuk (1941) (Arjmandi *et al.* 2016), and a new natural rose hybrid, *R. x binaloudensis* Vaezi, Arjmandi & Sharghi (Vaezi *et al.* 2019) are new additions to flora of Iran. Of the four subgenera introduced for the genus *Rosa*, i.e., *R.* subgen. *Hulthemia* (Dumortier 1824) Focke (1888), *R.* subgen. *Platyrhodon* (Hurst 1928) Rehder (1940), *R.* subgen. *Hesperhodos* Cockerell (1913) and *R.* subgen. *Rosa*., only two subgenera, *Rosa* and *Hulthemia*, exist in Iran. The subgenus *Rosa* includes all Iranian *Rosa* species except *Rosa persica* Gmelin (1791), which belongs to the monotypic subgenus *Hulthemia* (Zielinski 1982, Khatamsaz 1992, Vaezi *et al.* 2019). One of the most important problems regarding this genus is the presence of high hybridization and the effective role of humans in the cultivated samples, which leads to diversity in this genus (Fatemi *et al.* 2012). However, polyploidy and cross-pollination have resulted in the creation of a wide range of hybrids within the *Caninae* section (Koobaz *et al.* 2019).

The classification of plants based on flower characteristics has been of interest to botanists. This feature is given great importance in the first classifications presented. Augustus Quirinus Rivinus's (1652–1723) botanical system proposed classifying plants according to flower structure and corolla shape (Müller-Wille 2013). Today, it is possible to study the details of the structures, including the petal's epidermis and to use this feature for classification. Examining the structure of the epidermis has a high taxonomic value (Riahi *et al.* 2024). This is made possible by the availability of accurate tools for study at the microstructural level, particularly the scanning electron microscope. Petal surface micromorphology can be an essential marker for characterizing species (Sharma *et al.* 2005), although petal morphology constantly changes from flower opening to senescence (Han *et al.* 2019).

Studies on *Rosaceae* petals have been limited to a small number of species in the genus *Sibbaldia* L. (Tahir *et al.* 2010), *Rubus* L. (Sharifnia & Behzadi Shakib 2012), *Spiraea* L. (Omer *et al.* 2017), and *Cotoneaster* Medik. (Niaki *et al.* 2020). Petals of the tribe *Spiraeae* members have also been studied recently (Song *et al.* 2020). The first attempts at the classification of genus *Rosa* turn back to the 16th. century when the rose was treated as a wild species and classified according to flower color (Wissemann 2000). The complicated evolutionary history of wild species combined with a long history of cultivation, hybridization, and introgression contribute to most taxonomic confusion in the genus *Rosa* (Koopman *et al.* 2008, Vozarova *et al.* 2021). Despite many taxonomic studies that have been carried out on the genus *Rosa* (Joly & Bruneau 2006, Millan *et al*. 1996, Wissemann & Ritz 2005), the identification and classification of this genus have remained unresolved due to the similarity of morphological features and hybridization (Ritz & Wissemann 2003, Ritz *et al.* 2005, Schanzer & Kutlunina 2010, Gao *et al.* 2019).

To understand the function of the petal, the study of the flower epidermis is critically essential. It is also the primary point of contact with the abiotic and biotic environment and provides visual, tactile, and olfactory cues to pollinating animals (Whitney *et al.* 2011). Studies on the epidermal micromorphology of petals of

Rosa species are relatively limited. SEM analysis suggests that, *Rosa* species' papillae differ in shape and size (Zuraw *et al.* 2015). A comparison of the petal profiles of four *Rosa* species [i.e., *Rosa canina* Linnaeus (1753), *R. damascene* Miller (1768), *R. gallica* Linnaeus (1753), and *R. rugosa* Thunberg (1784)] showed that, the thickest petals with the densest cell arrangement occur in *R. damascena* (Zuraw *et al.* 2015). A comprehensive study that deals explicitly with rose petals has yet to be reported which is however, considered as an important factor here.

This study aims to provide a detailed description of the micromorphological features of the lower (abaxial) and upper (adaxial) surfaces of the petals from 15 wild species of the genus *Rosa* in Iran. The goal is to evaluate the use of these characters in determining species relationships and taxonomic delimitation of species. The study covers all two subgenera and four sections [Sect. *Pimpinellifoliae* Candolle ex Seringe (1818), Sect. *Cinnamomeae* Candolle ex Seringe (1825), Sect. *Caninae* Candolle ex Seringe (1825), and Sect. *Synstylae* Candolle (1813].

Materials and Methods

- Petal sampling

In this study, 25 individuals from 15 different taxa of *Rosa* were examined in various habitats in Iran. At least three flowers for each individual were sampled for the study to ensure the accuracy of the obtained results. The data used included all species belonging to two subgenera (*Rosa* and *Hulthemia*) of Iran, except for the recently reported species i.e., *R. freitagii*, *R. kokanica*, *R. x binaloudensis*, and *R. abrica*. The 15 taxa used in this study are listed in table 1. Flowers were selected for sampling at the final stage of flowering time (a mature flower). Samples available at the Central Herbarium of Tehran University (TUH) were used for this purpose. Field studies were also carried out at the end of the spring season to collect flowering specimens from various areas of Iran. The focus was to find out flowering samples of specific species that were essential for the study but were unavailable among the herbarium specimens. Pictures from field studies of some rose species are shown in figure 1. The collected specimens were identified with the help of specimens available in the herbarium of the University of Tehran, Flora Iranica (Zielinski 1982) and Flora of Iran (Khatamsaz 1992). The distribution map of the species selected for this study is displayed in figure 2.

- Light Microscopy (LM)

The thin layer of the epidermis of 15 species of Iranian rose was separated from the abaxial and adaxial side of the petals using colorless nail polish (Golden Rose Quick Dry Top Coat, Turkey). The sample's surface was first cleaned with a brush and then some nail polish was gently applied to the petal's surface with a nail polish brush. After 3–5 min., tweezers separated the dried layer from the sample's surface. At this stage, the epidermal layer and the nail polish were separated from the sample surface. This layer was placed on a microscope slide to examine the epidermal characteristics, including stomata, trichomes, and the shape of the epidermal cells on the adaxial and abaxial petal surfaces. They were examined under a light microscope (model Olympus LM). To ensure the accuracy and reliability of the data collected, an average of 3–5 slides were examined for each species. For accuracy, at least 20 photographs were taken under the microscope (40x lens) for each sample. Before examination, the photographs were processed using Photoshop Cs6 software to increase the resolution of the images. All samples were examined and photographed using an Olympus x40/0.65 lens. The scale was also calculated using the microscope's field of view (FOV). To observe the original shape of the papilla, the petals were immersed in distilled water for 15 min. Then, the epidermal layer was removed with a sharp blade and immediately examined under a light microscope.

Fig. 1. Diversity of flowers in the Iranian *Rosa* species: A. *R. persica*, B. *R. beggeriana*, C. *R. webbiana*, D. *R. canina*, E. *R. elymaitica*, F. *R. iberica*, G. *R. orientalis*, H. *R. pulverulenta*, I. Pollinator insects.

Table 1. List of the *Rosa* species used in micromorphological study along with related data

Taxon	Locality and voucher information (TUH*)
Subgen. *Hulthemia*	
R. persica	Esfahan Prov.: Shahreza-Semirom Hwy, 45 km from Semirom to Shahreza, 2288 m, Jun. 2019, Seyedipour 49088
	Tehran Prov.: Damavand, Cheshmeh-sabzi Road, 2136 m, Jul. 2022, Seyedipour 49089
Subgen. *Rosa*	
Sect. *Pimpinellifoliae*	
R. foetida	Kurdestan Prov.: 5 km after Marivan to Sanandaj, Zarivar Lake, May 2007, Attar, Zamani & Fatemi 37169
R. hemisphaerica	Semnan Prov.: After Shah-kuh to Derazno, Jun. 2007, Ghahreman & Attar 37399
	Alborz Prov.: Karaj to Chalus, near Shahrestanak, Jun. 2008, Attar, Zamani & Fatemi 38172
R. pimpinellifolia	E. Azarbaijan Prov.: Kalibar, Qale-Babak, next to forest, Jun. 2008, Attar, Zamani & Fatemi 38164
Sect. *Cinnamoneae*	
R. beggeriana	
	Mazandaran Prov.: Minak, Pol-e Zanguleh-Baladeh Road, 2405 m, Jul. 2020, Seyedipour 49091
R. webbiana	Lorestan Prov.: Azna, Oshtorankuh,1769 m, Jun. 2022, Seyedipour 49083

Table 1 (contd)

Sect. *Caninae*	
R. boissieri	Alborz Prov.: Gachsar, behind Gachsar hotel, Jun. 1965, Mobayen 9317
	Tehran Prov.: Lavasan, Bujan-Rahatabad Road, 2109 m, Jun. 2019, Seyedipour 49093
R. canina	Esfahan Prov.: Semirom, Semirom-Shahreza Hwy, 2597 m, Jun. 2020, Seyedipour 49082
	Semnan: Shahmirzad, Jun. 2008, Attar, Zamani & Fatemi 38175
R. elymaitica	Charmahal-Bakhtiari Prov.: Naghan Chahar-Tagh, Jun. 1997, Gahreman & Mozaffarian 20063
	Lorestan Prov.: Khorramabad-Borujerd Hwy, 1891 m, Sept. 2022, Seyedipour 49086
R. iberica	E. Azarbaijan Prov.: Kalibar, Jun. 2007, Attar, Zamani & Fatemi 37152
	Mazandaran Prov.: Veresk, 1546 m, Jun. 2019, Seyedipour 49094
R. orientalis	Hamedan Prov.: Ganjnameh-Tuyserkan Road, 2550 m, Jun. 2021, Seyedipour 49090
	Kordestan Prov.: Hawraman, Kuh-e Salan, 2900 m, May 2021, Advay 49085
R. pulverulenta	Alborz, Hasanakdar, 2143 m, Jun. 2019, Mahdigholi, Seyedipour & Ordane 49081
R. villosa	E. Azarbaijan Prov.: Hasht-rud to Maraqe, 16 km to Maraqe, Khalife-kandi, 1829 m, Jun. 2008, Attar, Zamani & Fatemi 38155
Sect. *Synstylae*	
R. moschata	Esfahan Prov., Nov. 1988, Erther 9316
R. damascena	Esfahan Prov., May 2008, Attar, Zamani & Maleki 38070
	Esfahan Prov.: Kashan, after Ghamsar toward Qohrud, 1650 m, May 2004, American-Iranian Botanical Delegation 33727

* TUH: Central Herbarium of Tehran University (Tehran, Iran)

- Scanning Electron Microscopy (SEM)

Sampled mature petals from species were collected from field studies along with herbarium samples for SEM photography. The voucher specimen data of these collected samples is listed in table 1. The specimens were photographed using a VEGA3 TESCAN scanning electron microscope at 20 kV acceleration voltage and 10–15 mm working distance. The small fragments (3–5 mm) of mature petal specimens, without any treatment, were fixed to the specimen stubs and covered with a thin layer of gold before being inserted into the chamber of the SEM microscope.

- Data analysis

Cell density, flower color, epidermal cell type, stomata, and trichome characteristics were assessed using light and scanning electron microscopy. The number of cells per square millimeter was counted using ImageJ software to measure cell density. For each species, one-millimeter squares were randomly selected in different areas of the microscopic photographs. Counts were recorded for each square millimeter. Ten counts were taken for each species and the mean values were recorded in table 2. The species-cell density graph for comparison data was created using Microsoft Excel software. Multivariate statistical analyses were done using Past Ver. 4.03 software to analyze morphological data. The terminology used to distinguish the type of epidermal cells was derived from Kay *et al.* (1981), Ojeda *et al.* (2009), and Song *et al.* (2020).

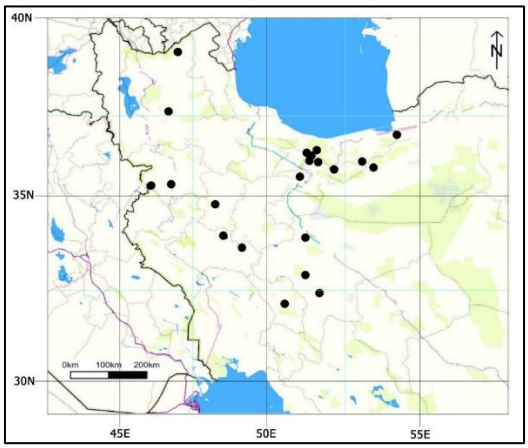

Fig. 2. Distribution map of the studied *Rosa* species in Iran.

Results

- Petal micromorphology

The roses in this study were five-petalled and came in various colors, from yellow, white, and pink to white-pink (Table 2, Fig. 1). The type of surface epidermis in each species was confirmed by examining at least two samples from different geographical regions. Comparisons were then made between species. In addition, the correlation of electron microscope photographs with light microscope photographs gives us confidence in the accuracy of the resulting data. The microscopic images obtained (LM & SEM) are presented as follows (Figs 3–6):

- Epidermal cell type

Micromorphological studies of petals of *Rosa* species have shown that, the adaxial epidermis consisted of a series of interconnected raised papillate cells (Figs 2 & 4). In contrast, the lower epidermis showed different structure covered by a waxy outer layer containing variety of arrangements (Figs 4 & 6). Petal Epidermis cells in *Rosa* species can be categorized into following levels: 1. Perimeter shape: Epidermal cell pattern (papillose and tabular), 2. Projection: the amount of projection from the cell surface (conical and flat cells), and 3. Cell surface micromorphology: micromorphology of the cellular surface (e.g., rugose, striate, and smooth).

- Adaxial epidermis

The epidermal cells of the upper surface of rose petals generally follow a specific pattern. In all the studied *Rosa* species, the outer wall of these cells can be seen as conical projections with varying degrees of protrusion, known as papillary modifications. A broad cuticle layer covered the surface of the papillae. The cuticles were often extended in more or less parallel lines from the base of the cone to the apex often denser and more rugose at the apex of the papilla. The cuticular layers gave the cells an elastic property and were stretched when substances accumulate in the cells. In this study, three main groups of epidermal cells were identified: Type I (PCR) refers to papillose conical cells with rugose at the apex. All studied *Rosa* species except *R. beggeriana* Schrenk (1841) and *R. moschata* Herrmann (1762) belong to this group. In terms of the feature of the striated conical at the base, the members of this group are divided into two categories. All group members, except *R. foetida* Herrmann (1762), and *R. hemisphaerica* Herrmann (1762: 18), were placed in a group striated at the base of the conical (Table 2), Type II (PCSm) refers to the presence of papillose conical cells that had smooth surfaces without any striations. *R. beggeriana* is the only species considered in this group (Figs 3E, 3F & 5G), and Type III (PrbCR) is characterized by papillose cells with a distinct and raised border with a conical shape. This unique type of epidermal cells was seen in the petal surface of *R. moschata* (Figs 3M, 3N & 5R).

- Abaxial epidermis

The lower epidermal layer had different patterns of cuticle covering the surface. These patterns can vary from species to species. They were often seen as flat cells with parallel lines or irregular folds (Figs 4–5). In some areas, the cuticular arrangement was raised at the cell boundary

and had a honeycomb or circular to oval shape. In some other parts, the boundary between the cells was not clear and were generally covered with dense cuticles, making it impossible to see the shape of the epidermal cells. Spherical or circular shapes, which were located next to the cuticular lines, are probably places for the accumulation of secretory substances under the petals. Although there were some signs of accumulation of material in the layers of the cuticular lines, their appearance was flattened, indicating that, stretching may had occurred due to the accumulation of material, which caused them smooth appearance. Observations also showed that, secretion and accumulation occurred on both the dorsal and upper surfaces.

The outer surface of the epidermis can be divided into several groups. All Abaxial cells were flat and had no distinct protrusions. The first type was a group in which flat cells were packed together in a dense honeycomb pattern. Striated cuticular layers were seen inside these nests. In some parts, the cuticle was raised at the junction of the two sides (Figs 4K, 4L, 4M, 6B, 6D, 6F, 6L & 6O). The second type of cuticle was seen as interconnected lines that cover much of the surface. These lines were parallel, although these lines were not completely straightely arranged along each other covering the surface. (Figs 4E, 4F & 6K). The third group consisted of cuticular lines, with circular shapes scattered among them where towards the center of the circles, the cuticle became unstriated (Figs 4A, 4B, 4O, 4P, 6I & 6U). The fourth group, was those with cuticle consisted of relatively small and interconnected circles, which also had cuticular striate in the center (Figs 4C, 4D, 4G, 4H, 6G & 6J). The next and final group was different from the four other groups which was seen as a folded cuticle and compact and intertwined rugoses (Figs 4I, 4J & 6Q).

- Trichome and stomata

The surface of the petals was examined for the presence or absence of hairs and stomata. No hairs or stomata were observed on the epidermis of the back and upper surface of the petals of the studied *Rosa* species.

Table 2. Epidermal cell types and main petal characteristics in the studied *Rosa* species. Perimeter shape (P: papillose, T: tabular), projection (F: flat, C: conical), cell surface micromorphology (Sm: smooth, St: striate, G: granular)

Taxon	Abaxial cell type	Striation in conical projection base	Mean No. of conical projection (1 mm^2)	Color of flower
Rosa persica	PCR	+	920	Yellow with red blotch at the base
R. foetida	PCR	-	800	Yellow
R. hemisphaerica	PCR	-	750	Yellow
R. pimpinellifolia	PCR	+	320	Creamy white
R. beggeriana	PCSm	-	620	White
R. webbiana	PCR	+	320	White, pink
R. boissieri	PCR	+	290	White, pink
R. canina	PCR	+	330	Pink, white
R. elymaitica	PCR	+	580	Pink, rarely white
R. iberica	PCR	+	240	Pink, rarely white
R. orientalis	PCR	+	360	White, pink
R. pulverulenta	PCR	+	360	Pink
R. villosa	PCR	+	420	Pink, white
R. moschata	PrbCR	+	450	White
R. damascena	PCR	+	290	Pink

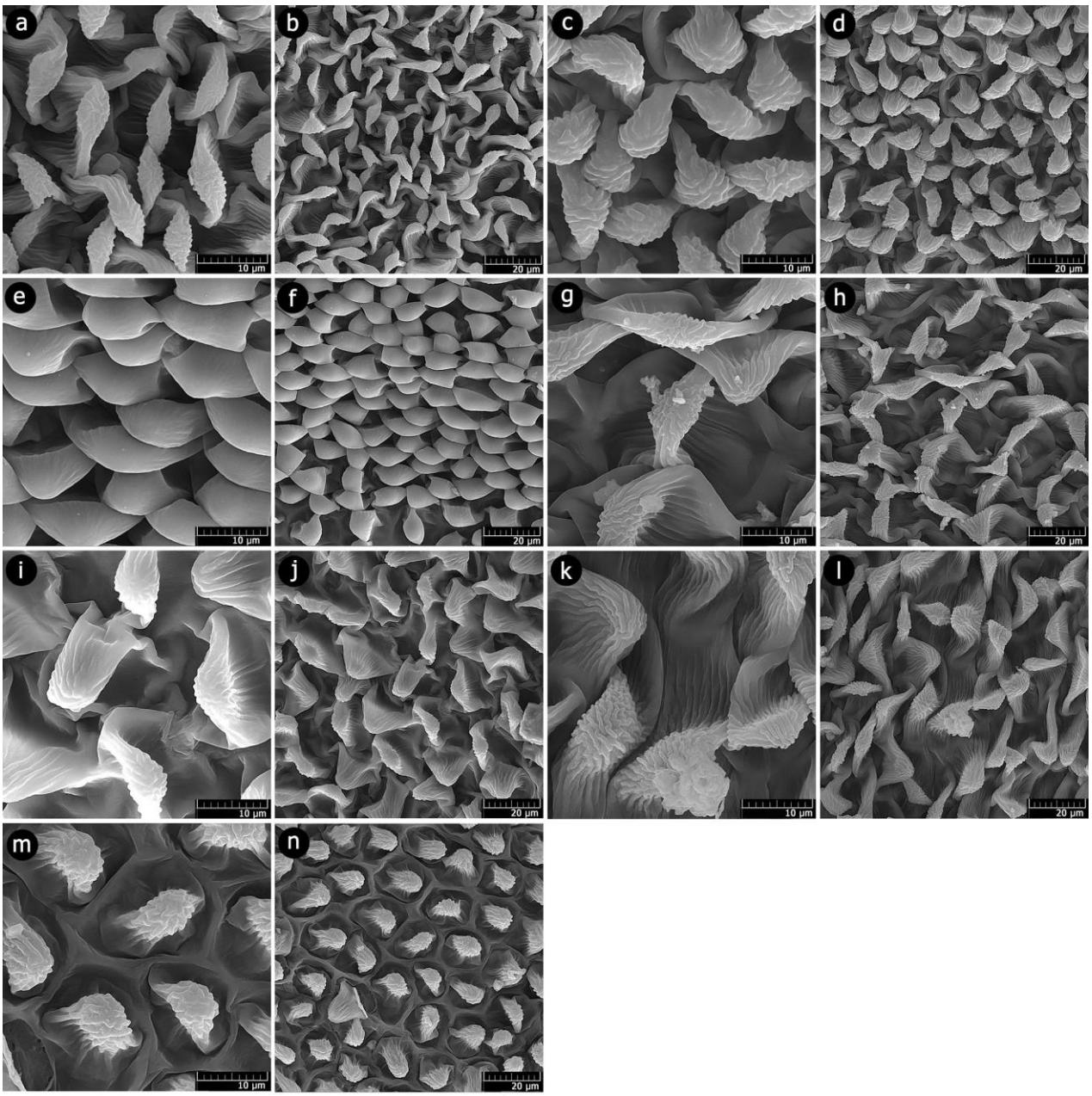

Fig. 3. SEM micrographs of the adaxial surface of petal epidermis with visible papillae in the studied *Rosa* species: A-B. *R. persica*, C-D. *R. foetida*, E-F. *R. beggeriana*, G-H. *R. canina*, I-J. *R. pulverulenata*, K-L. *R. villosa*, M-N. *R. moschata*.

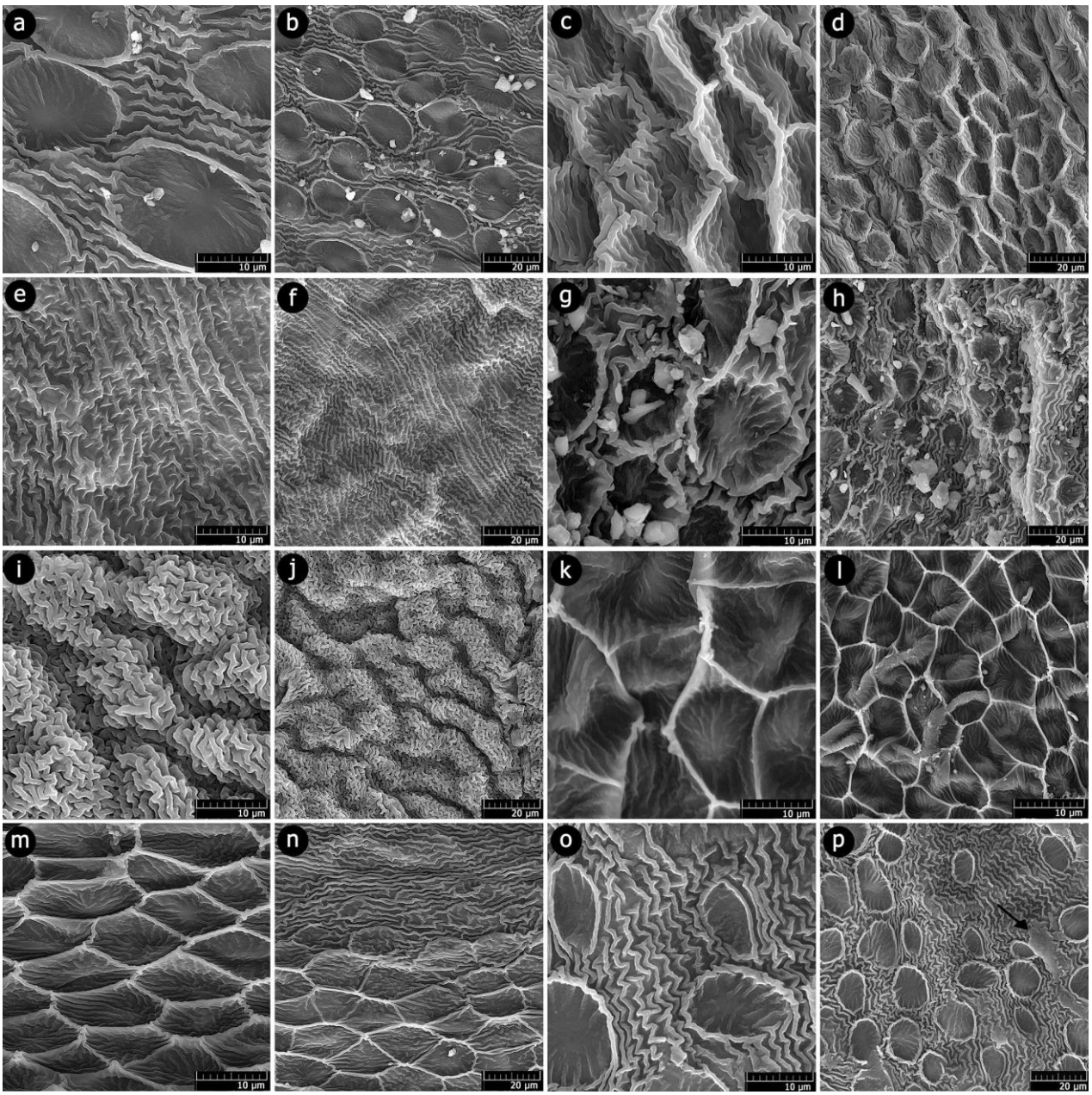

Fig. 4. SEM micrographs of the abaxial surface of petal epidermis with characteristic cuticular ornamentation in the studied *Rosa* species: A-B. *R. hemisphaerica*, C-D. *R. pimpinellifolia*, E-F. *R. webbiana*, G-H. *R. boissieri*, I-J. *R. elymaitica*, K-L. *R. iberica*, M-N. *R. orientalis*, O-P. *R. damascene* (P: Black arrow shows the epidermis with secretion stored under the cuticle).

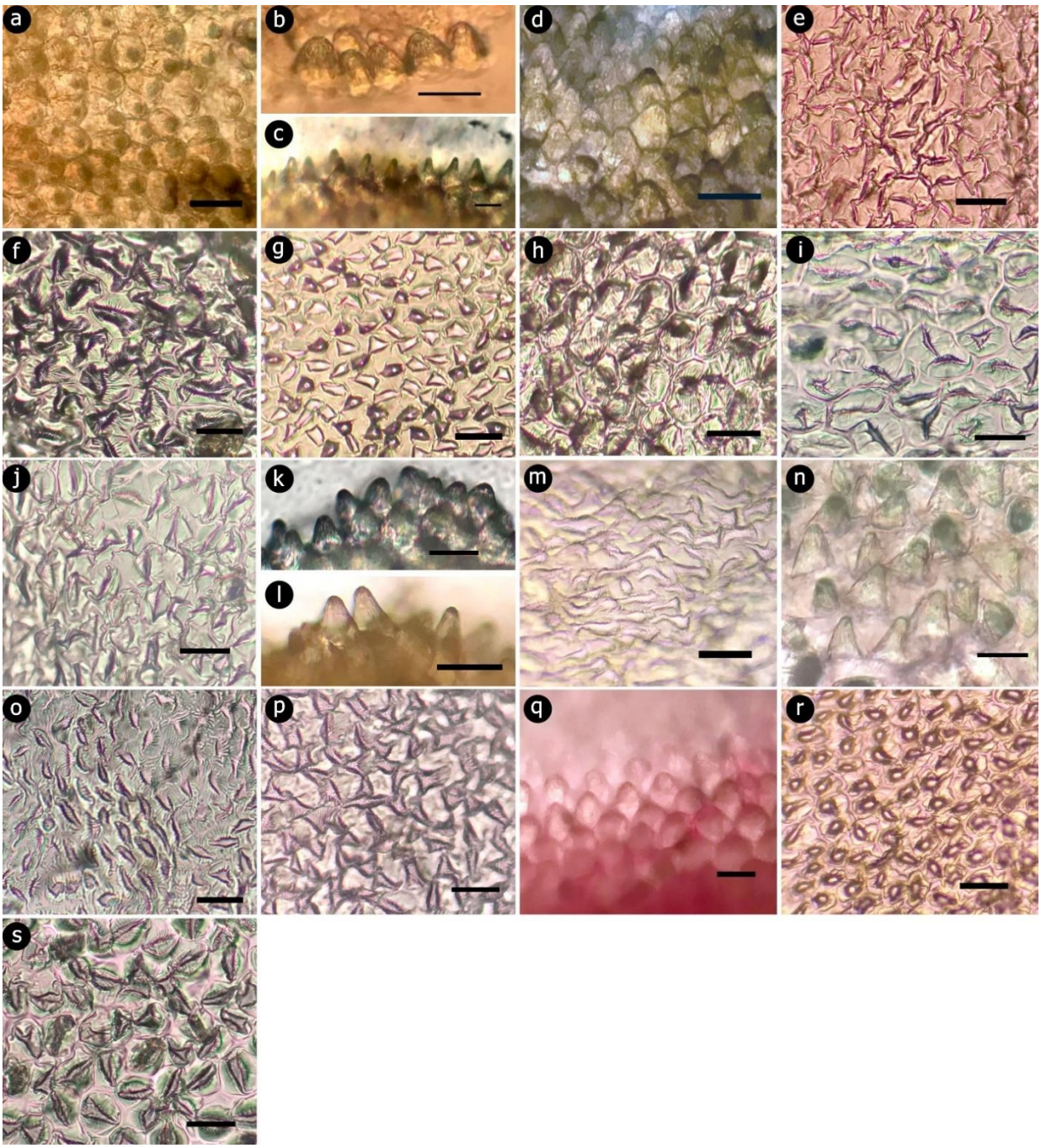

Fig. 5. LM micrographs of adaxial surface of petals in the studied *Rosa* species: A-B. *R. persica*, C-D. *R. foetida*, E. *R.hemisphaerica*, F. *R. pimpinellifolia*, G. *R. beggeriana*, H. *R. webbiana*, I. *R. boissieri*, J-K. *R. canina*, L-M. *R. elymaitica*, N. *R. iberica*, O. *R. orientalis*, P-Q. *R. pulverulenata*, R. *R. moschata*, S. *R. damascene* (Bar = 25 µm).

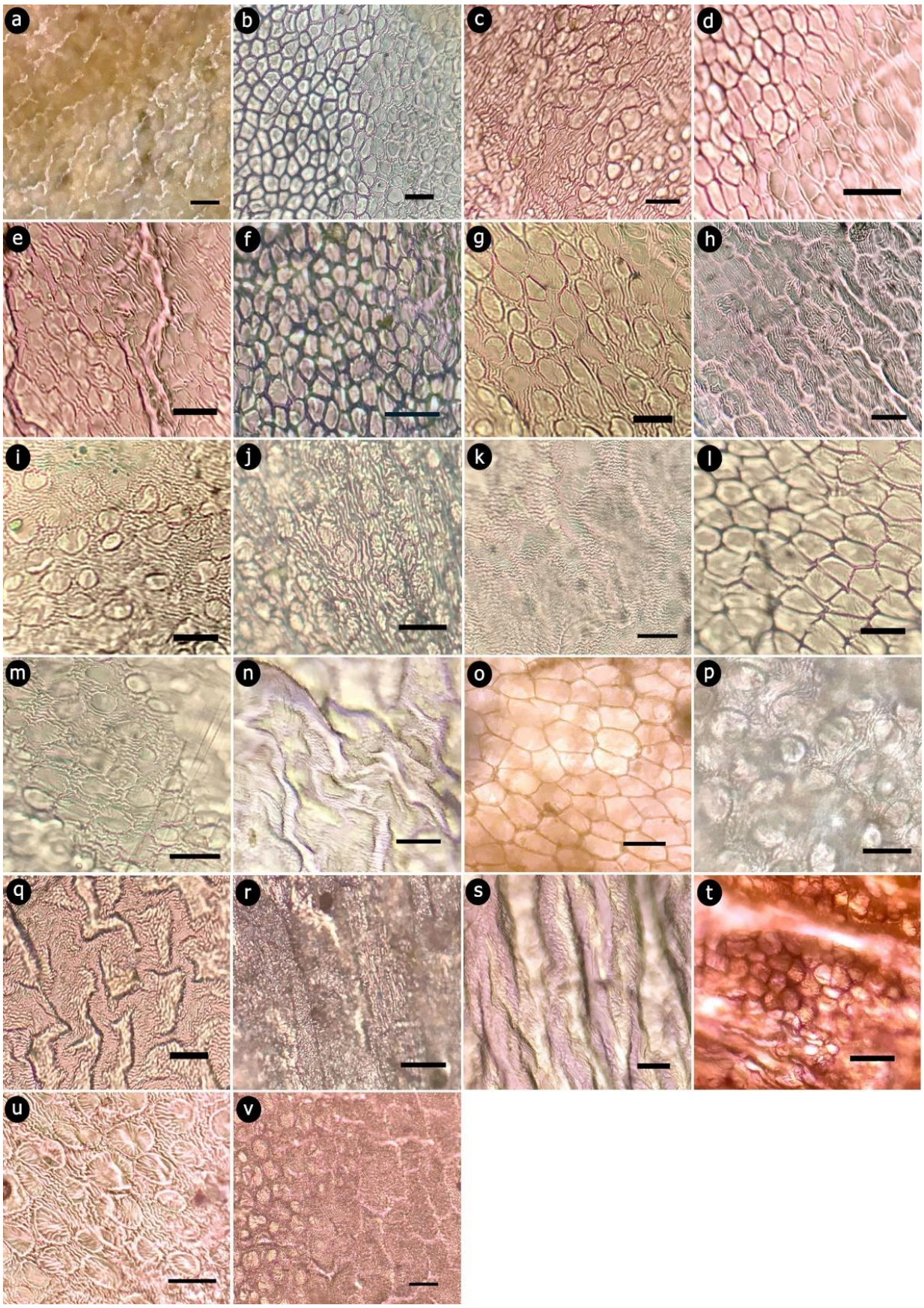

Fig. 6. LM micrographs of abaxial surface of petals in the studied *Rosa* species: A. *R. persica*, B-C. *R. foetida*, D-E-F. *R. hemisphaerica*, G. *R. pimpinellifolia*, H. *R. beggeriana*, I. *R. webbiana*, J. *R. boissieri*, K-L, M. *R. canina*, N. *R. elymaitica*, O-P. *R.iberica*, Q. *R. orientalis*, R. *R. pulverulenta*, S-T. *R. moschata*, U-V. *R. damascene* (Bar = 25 µm).

Discussion

Petal micromorphology can be an essential marker of *Rosa* species identity (Sharma *et al.* 2005). Knowing the epidermal cell types on the two petal surfaces made it easy to distinguish the adaxial-abaxial axis. The PCR type was typically found in the upper epidermis, except in a few cases of PCSm and PrbCR types. In contrast to all the studied *Rosa* species, the papillae of the upper surface of the petals of *R. beggeriana* were smooth and without cuticular folds (Fig. 3E-F). This feature can be used as a marker to identify *R. beggeriana* by studying the characteristics of the petal epidermis. In addition, in *R. moschata*, the papillae are arranged in a particular order, which is diagnostically essential for correctly recognizing this species.

Although the features mentioned for *R. beggeriana* and *R. moschata* distinguish them from other species, this feature is not distinctive in other studied *Rosa* species without any significant difference. The present study provides useful taxonomic information for species identification and delimitation based on petal morphological characteristics; however, this trait is not considered suitable for isolating subgenera and even sections in the genus *Rosa*.

Abaxial cells were all flat and the surface of the epidermis had various patterns of cuticles (Figs 4 & 6). Thus, several types of these patterns could be seen in one species. This condition was clearly shown in the dorsal epidermis of *R. damascene* and *R. orientalis* Dupont (1825) species (Figs 4N & 6V). The diversity of epidermal cells on the dorsal surface of the epidermis of *R. canina* was considerable, indicating this trait's instability in one species (Figs 6K-M). These observed petal epidermis patterns are entirely consistent with previous studies of rose species in petal micromorphology (Sharma *et al.* 2005, Zuraw *et al.* 2015).

In addition, the absence of petal surface stomata and trichomes was confirmed by previous studies (Stubbs & Francis 1971, Bergougnoux *et al.* 2007, Sulborska *et al.* 2012). Contrary to the present results, which did not support a proper grouping based on petal micromorphology within the genus *Rosa* provided the first comprehensive analysis of the tribe *Spiraea* of *Rosaceae* based on petal micromorphological data in the context of molecular phylogeny (Song *et al.* 2020). The research results indicate that, the petal epidermal characteristics strongly support the independent taxonomic position of the *Pentactina* Nakai genus. Therefore, it is recommended that, a comprehensive study on Rosoideae petals be conducted, along with the molecular study, to determine the appropriateness of the petal epidermis trait at higher levels for the members of this group.

Flower color appears to be significantly related to the density of papilla cells on the petal surface (Figs 7A-B). As shown in table 2, flowers with yellow petals, including *R. persica*, *R. foetida*, and *R. hemisphaerica* species, had a higher cell density than other species (750–920 cells in mm^2). Among them, *R. persica* has the highest cell density. The high density of epidermal cells occurred in response to climatic conditions. According to the environmental conditions of this species, which grows in Irano-Turanian regions (Khatamsaz 1992) and an almost dry climate, the flowers were small, with a petal length of 15–18 mm. The three species observed with pink petals, i.e., *R. iberica* Sennen & Elias (1928), *R. boissieri* Crépin (1869), and *R. damascena*, had the lowest cell density (240–290 cells in mm^2) and the largest papilla cell size. *R. iberica* is known to be the least dense species (Fig. 7B). The high density of papillae on the upper surface of the petals provides a natural defense mechanism against dry air (Niaki *et al.* 2020).

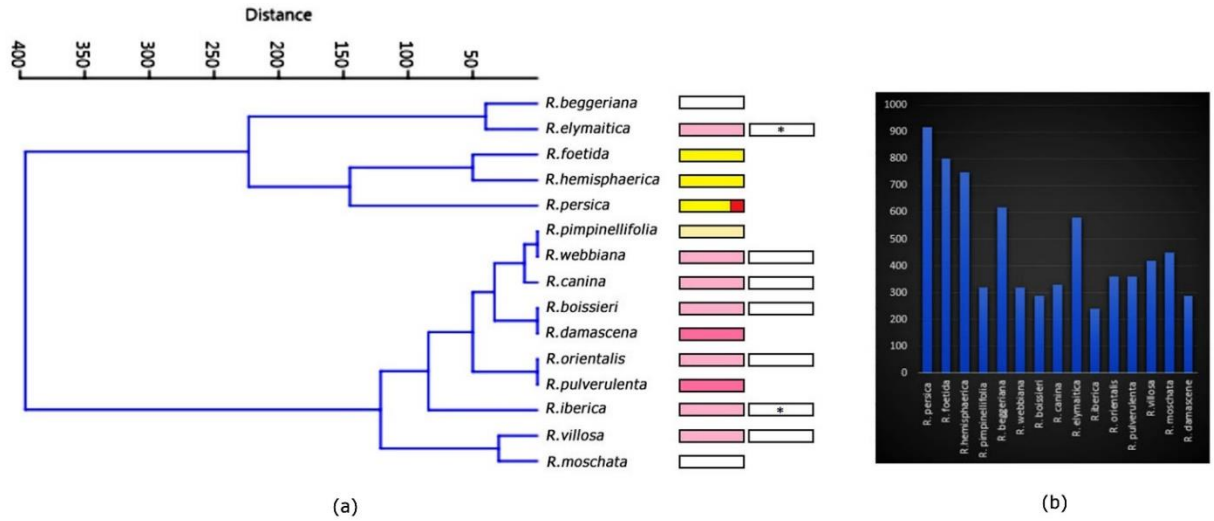

Fig. 7. Hierarchical clustering analysis from conical cell density on the adaxial surface of petals: A. The colored rectangles in the figure indicate the flower color for each species. Asterisks show the rare colors, B. Comparative cell density graph calculated for 15 species.

Petal traits appear consistent across different species within this genus are not influenced by ecological conditions (Niaki *et al.* 2020). Interspecific wild rose hybrids do not exhibit intermediate morphology between parental species, even in early generations. The intricate inheritance of morphological traits makes it challenging for accurate identification of wild rose hybrids based on their morphology, particularly in multiple hybridizations (Schanzer & Kutlunina 2010).

Cluster analysis of 15 *Rosa* species showed two major clusters. The first cluster comprises two sub-clusters, which include *R. beggeriana*, *R. elymaitica*, *R. foetida*, *R. hemisphaerica*, and *R. persica*. These species are part of the subgenus *Hulthemia* and the *Caninae*, *Cinnamomeae*, and *Pimpinellifoliae* sections within the subgenus *Rosa*. The second cluster consisted of a larger group comprising ten species from the *Canine* section, including *R. caninae*, *R. boissieri*, *R. orientalis*, *R. pulverulenta*, *R. iberica*, and *R. villosa*. It also included *R. pimpinellifoliae* from the *pimpinellifoliae* section and *R. webbiana* from the *Cinnamoneae* section. *Rosa moschata* from the *Synstylae* section is grouped with *R. villosa* in a separate sub-cluster and *R. damascena*, a hybrid species, is grouped with *R. boissieri* (*Caninae* section members were present in both clusters). Koobaz *et al.* (2019) studied a clustering analysis of the morphological traits of populations within the *caninae* section in Iran. The study showed various groupings for each species in different populations, linked to diverse ecological conditions and variations in morphological traits across different populations. The taxonomy of complex nature of the *Caninae* section arises from factors such as allopolyploid constitution, skewed maternal inheritance and ongoing hybridization, making it notably challenging to classify (Koobaz *et al.* 2019).

The petal epidermis characteristics of different *Rosa* species in the present study has yielded several significant findings. It was found that, the characteristics of the petal epidermis were similar across the different species belonging to various sections. Specifically, all species upper epidermis cells were found conical. This finding is consistent with study on 16 cotoneaster species, which also found little difference between them (Niaki *et al.* 2020). The present observations indicated that, flowers in the first cluster typically had yellow petals and the highest papilla density. In contrast, the larger cluster often contained species with pink flowers and the number of conical projections per unit area was lower. While the type of these cells was similar across different species, they varied in cell density based on different ecological conditions. It was also found that, the high density of epidermal cells is likely connected to the yellow color of the flower, attributed to the pigments that protect the plant against dry air and intense light.

Conclusion

Analyzing petal samples from 15 *Rosa* species has revealed an important finding. Based on data from the micromorphology of petal samples, the current study showed that, while petal epidermis is consistent within species, it could not be relied as an isolated characteristic for taxonomic classification within the *Rosa* genus. It is only a distinguishing feature in certain species, such as *R. beggeriana* and *R. moschata*, where it can be used for species identification. This emphasizes the necessity of adopting a more comprehensive approach to genus classification. The correlation between flower color, pigmentation, papilla density, and epidermal cell size plays a significant role in the ecological adaptation for reproductive success in the *Rosa* genus. To thoroughly and accurately explore the micromorphological diversity of petal surfaces and definitively determine species boundaries using this characteristic, examining other species within the *Rosa* genus and other sections not currently present in Iran is advisable.

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References

Arjmandi, A.A., Sharghi, H.R., Memariani, F. & Joharchi, M.R. 2016. *Rosa kokanica* (Rosaceae) in Binalood Mountains: A new record for the flora of Iran. Iranian Journal of Botany 22(1): 11–15. DOI: 10.22092/IJB.2016.106629.

Bergougnoux, V., Caissard, J.C., Jullien, F., Magnard, J.L., Scalliet, G., Cock, J.M. & Baudino, S. 2007. Both the adaxial and abaxial epidermal layers of the rose petal emit volatile scent compounds. Planta 226(4): 853–866. DOI: 10.1007/s00425-007-0531-1.

Eugster, C.H. & Märki-Fischer, E. 1991. The chemistry of rose pigments. Angewandte Chemie International Edition in English 30(6): 654–672. DOI: 10.1002/anie.199106541.

Fatemi, N., Attar, F., Zaeifi Zadeh, M., Hamzeh'ee, B. & Assareh, M.H. 2012. Morphological and micromorphological study within the taxonomical complex section Caninae, subsection Caninae *Rosa canina* L. Taxonomy and Bio-systematics 4(11): 33–45 (In Persian). DOI: 20.1001.1.20088906.1391.4.11.5.6.

Fougère-Danezan, M., Joly, S., Bruneau, A., Gao, X.F. & Zhang, L.B. 2015. Phylogeny and biogeography of wild roses with specific attention to polyploids. Annals of Botany 115(2): 275–291. DOI: 10.1093/aob/mcu245.

Gao, Y., Gao, X. & Harris, A. 2019. Species boundaries and parapatric speciation in the complex of alpine shrubs, *Rosa sericea* (Rosaceae), based on population genetics and ecological tolerances. Frontiers in Plant Science 10: 321. DOI: 10.3389/fpls.2019.00321.

Han, Y., Yong, X., Yu, J., Cheng, T., Wang, J., Yang, W. & Zhang, Q. 2019. Identification of candidate adaxial-abaxial-related genes regulating petal expansion during flower opening in *Rosa chinensis* "Old Blush". Frontiers in Plant Science 10: 1098. DOI: 10.3389/fpls.2019.01098.

Joly, S. & Bruneau, A. 2006. Incorporating allelic variation for reconstructing the evolutionary history of organisms from multiple genes: An example from *Rosa* in North America. Systematic Biology 55(4): 623–636. DOI: 10.1080/10635150600863109.

Kay, Q.O.N., Daoud, H.S. & Stirton, C.H. 1981. Pigment distribution, light reflection and cell structure in petals. Botanical Journal of the Linnean Society 83(1): 57–83. DOI: 10.1111/j.1095-8339.1981.tb00129.x.

Khatamsaz, M. 1992. *Rosa* L. Pp. 247–315. *In*: Flora of Iran 6. Research Institute of Forests and Rangelands, Tehran (In Persian).

Koobaz, P., Jafarkhani Kermani, M., Hosseini, Z., Jokar, A. & Khatamsaz, M. 2011. Biosystematic study of *Rosa* (Sect. Pimpinellifoliae) and described *R. abrica* (Rosaceae) as a new species from Iran. Rostaniha 12(1): 51–62 (In Persian). DOI: 10.22092/BOTANY.2011.101431.

Koobaz, P., Hosseini, Z.S., Khatamsaz, M. & Jafarkhani Kermani, M. 2019. Taxonomical study of section Caninae (*Rosa*) and their hybrids in Iran. Rostaniha 20(2): 85–97. DOI: 10.22092/BOTANY.2019.125378.1146.

Koopman, W.J., Wissemann, V., De Cock, K., Van Huylenbroeck, J., De Riek, J., Sabatino, G.J. & Smulders, M.J. 2008. AFLP markers as a tool to reconstruct complex relationships: a case study in *Rosa* (Rosaceae). American Journal of Botany 95(3): 353–366.

Ku, T.C. & Robertson, K.R. 2003. *Rosa* Linnaeus. Pp. 339–381. *In*: Wu, C.Y. & Raven, P.H. (eds), Flora of China, Vol. 9. Science Press/Missouri Botanical Garden Press, Beijing/St, Louis.

Millan, T., Osuna, F., Cobos, S., Torres, A. & Cubero, J. 1996. Using RAPDs to study phylogenetic relationships in *Rosa*. Theoretical and Applied Genetics 92: 273–277. DOI: 10.1007/BF00223385.

Müller-Wille, S. 2013. Systems and how Linnaeus looked at them in retrospect. Annals of Science 70(3): 305–317. DOI: 10.1080/00033790.2013.783109.

Niaki, N.A.R., Attar, F., Mirtadzadini, M. & Mahdigholi, K. 2020. Pollen and floral micro-morphological Studies of the genus *Cotoneaster* Medik. (Rosaceae) and its systematic importance. Caryologia 73(3): 133–151. DOI: 10.13128/caryologia-569.

Ojeda, I., Francisco-Ortega, J. & Cronk, Q.C. 2009. Evolution of petal epidermal micromorphology in Leguminosae and its use as a marker of petal identity. Annals of Botany 104(6): 1099. DOI: 10.1093/aob/mcp211.

Omer, S.A., Rajput, M.T.M. & Tahir, S.S. 2017. Micromorphological studies on petals of *Spiraea* L. species (Rosaceae) From Pakistan. Pakistan Journal of Botany 49(1): 283–287. http://www.pakbs.org/pjbot/PDFs/49(1)/37.pdf.

Riahi, M., Kahdouei, E., Tavakkoli, Z. & Ghahremaninejad, F. 2024. Comparative anatomical and morphological studies on leaves of some *Citrus* species and their systematic implications. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology 158(1): 51–58. DOI: 10.1080/11263504.2023.2287536.

Ritz, C.M. & Wissemann, V. 2003. Male correlated non-matroclinal character inheritance in reciprocal hybrids of *Rosa* section Caninae (DC) Ser. (Rosaceae). Plant Systematics and Evolution 241(3): 213–221. DOI: 10.1007/s00606-003-0058-2.

Ritz, C.M., Schmuths, H. & Wissemann, V. 2005. Evolution by reticulation: European dogroses originated by multiple hybridization across the genus *Rosa*. Journal of Heredity 96(1): 4–14. Doi.org/10.1093/jhered/esi011.

Schanzer, I.A. & Kutlunina, N.A. 2010. Interspecific hybridization in wild roses (*Rosa* L. sect. Caninae DC.). Biology Bulletin 37(5): 480–488. DOI: 10.1134/S1062359010050079.

Sharghi, H.R., Arjmandi, A.A., Memariani, F. & Joharchi, M.R. 2014. *Rosa freitagii* Ziel. (Rosaceae), a new record for the flora of Iran. Iranian Journal of Botany 20(2): 183–187. DOI: 10.22092/IJB.2014.11014.

Sharma, G., Sharma, M.L., Sharma, S.K. & Kang, H.P.S. 2005. Single-step surface replication of flower petals for SEM. Microscopy and Analysis 19(1): 21–23.

Sharifnia, F. & Behzadi Shakib, S. 2012. Epidermal petal patterns of 13 Iranian *Rubus* L. (Rosaceae) species. Annals of Biological Research 3(6): 2734–2740. http://scholarsresearchlibrary.com/ABR-vol3-iss6/ABR-2012-3-6-2734-2740.pdf.

Song, J.H., Roh, H.S. & Hong, S.P. 2020. Petal micromorphology and its systematic implications in Rosaceae tribe Spiraeeae. Brittonia 72(2): 111–122. DOI: 10.1007/s12228-020-09609-w.

Stubbs, J.M. & Francis, M.J.O. 1971. Electron microscopical studies of rose petal cells during flower maturation. Planta Medica 20(5): 211. DOI: 10.1055/s-0028-1099694.

Sulborska, A., Weryszko-Chmielewska, E. & Chwil, M. 2012. Micromorphology of *Rosa rugosa* Thunb. petal epidermis secreting fragrant substances. Acta Agrobotanica 65(4): 21–28. DOI: 10.5586/aa.2012.018.

Tahir, S.S. & Rajput, M.T.M. 2010. SEM studies of petal structure of corolla of the species *Sibbaldia* L. (Rosaceae). Pakistan Journal of Botany 42(3): 1443–1449. https://www.pakbs.org/pjbot/PDFs/42(3)/PJB42(3)1443.pdf.

Vaezi, J., Arjmandi, A.A. & Sharghi, H.R. 2019. Origin of *Rosa x binaloudensis* (Rosaceae), a new natural hybrid species from Iran. Phytotaxa 411(1): 23–38. DOI: 10.11646/phytotaxa.411.1.2.

Vozárová, R., Herklotz, V., Kovařík, A., Tynkevich, Y.O., Volkov, R.A., Ritz, C.M. & Lunerová, J. 2021. Ancient origin of two 5S rDNA families dominating in the genus *Rosa* and their behavior in the Canina-type meiosis. Frontiers in Plant Science 12: 643548. DOI: 10.3389/fpls.2021.643548.

Whitney, H.M., Bennett, K.V., Dorling, M., Sandbach, L., Prince, D., Chittka, L. & Glover, B.J. 2011. Why do so many petals have conical epidermal cells? Annals of Botany 108(4): 609–616. DOI: 10.1093/aob/mcr065.

Wissemann, V. & Ritz, C.M. 2005. The genus *Rosa* (Rosoideae, Rosaceae) revisited: molecular analysis of nrITS-1 and atpB-rbcL intergenic spacer (IGS) versus conventional taxonomy. Botanical Journal of the Linnean Society 147(3): 275–290. DOI: 10.1111/j.1095-8339.2005.00368.x.

Wissemann, V. 2000. Molekulargenetische und morphologisch-anatomische Untersuchungen zur Evolution und Genomzusammensetzung von Wildrosen der Sektion *Caninae* (DC.) Ser. Botanische Jahrbücher fur Systematik, Pflanzengeschichte und Pflanzengeographie 122: 357–429.

Zielinski, J. 1982. *Rosa* L. Pp. 13–31. *In*: Rechinger, K.H. (ed.), Flora Iranica 152. Akademische Druck und Verlagsanstalt, Graz.

Zuraw, B., Sulborska, A., Stawiarz, E. & Weryszko-Chmielewska, E. 2015. Flowering biology and pollen production of four species of the genus *Rosa* L. Acta Agrobotanica 68(3): 267–278. DOI: 10.5586/aa.2015.031.