IRANIAN JOURNAL OF BOTANY 30 (2), 2024
DOI: 10.22092/ijb.2024.367518.1500

LEAF MICROMORPHOLOGY OF THE GENUS SAXIFRAGA (SAXIFRAGACEAE) IN IRAN

K. Rezaee Chamanie, M.B. Faghir, M. Mahmoodi, M. Amini Rad & Sh. Kazempour Osaloo

Received 2024. 11.02; accepted for publication 2024.12.26

Rezaee Chamanie, K., Faghir, M.B., Mahmoodi, M., Amini Rad, M. & Kazempour Osaloo, Sh. 2024.12.30: Leaf micromorphology of the genus *Saxifraga* (Saxifragaceae) in Iran. - *Iran. J. Bot. 30 (2): 184-206.* Tehran.

In this study, the micromorphological characteristics of the leaf epidermis of 14 species (28 samples) of the genus *Saxifraga* (Saxifragaceae) were examined with a scanning electron microscope (SEM). Leaf samples were examined on both abaxial and adaxial surfaces. Based on the results, micromorphological traits such as the presence or absence of simple and glandular hairs, the presence or absence of verruca and granules on the hair surface, the position of hairs relative to the epidermis, the shape of epidermal cells, the anticlinal wall, the outer periclinal layer, epidermal surface pattern, wax ornamentation on the cuticle, the shape of the stomata and the distribution of wax on the cuticle, and stomata showed the most diversity. The result showed that micromorphological characters are taxonomically informative and can be used to identify species. Multivariate analysis was used to estimate the potential contribution of micromorphology data to inter- and intraspecific relationships using R software. The cluster and principal component analysis results showed that leaf morphological characters are useful for determining species boundaries.

Kosar Rezaee Chamanie, Marzieh Beygom Faghir (correspondence < kosar.rezaee84@gmail.com & faghirmb@guilan.ac.ir>), Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran.- Mohammad Mahmoodi & Mohammad Amini Rad, Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.- Shahrokh Kazempour Osaloo, Department of Plant Biology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.

Keywords: Epicuticular wax; trichome; *Saxifraga*; scanning electron microscopy; multivariate analysis.

مطالعه ریزریخت‌شناسی برگ جنس Saxifraga (Saxifragaceae) در ایران

کوثر رضایی چمنی: دکتری سیستماتیک گیاهی، گروه زیست‌شناسی، دانشکده علوم، دانشگاه گیلان، رشت، ایران

مرضیه بیگم فقیر: دانشیار گروه زیست‌شناسی، دانشکده علوم ، دانشگاه گیلان، رشت، ایران

محمد محمودی: موسسه تحقیقات جنگلها و مراتع کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران

محمد امینی راد: موسسه تحقیقات جنگلها و مراتع کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران

شاهرخ کاظم‌پور اوصالو: استاد گروه زیست‌شناسی گیاهی، دانشکده علوم زیست شناسی، دانشگاه تربیت مدرس، تهران، ایران

در این مطالعه، صفات ریزریخت‌شناسی اپیدرم برگ ۱۴ گونه (۲۸ نمونه هرباریومی) از جنس Saxifraga (Saxifragaceae) با میکروسکوپ الکترونی روبشی (SEM) مورد بررسی قرار گرفت. بر اساس نتایج حاضر، صفات ریزریخت‌شناسی مانند وجود یا عدم وجود کرک‌های ساده و غده‌دار، وجود یا عدم وجود زگیل و گرانول‌ها در سطح کرک، موقعیت کرک‌ها نسبت به اپیدرم، شکل سلول‌های اپیدرمی ، دیواره آنتی‌کلینال، بیرونی‌ترین لایه پری‌کلینال، الگوی سطح اپیدرم، تزیینات مومی روی کوتیکولی، شکل روزنه‌ها و توزیع موم روی کوتیکولی روی سطح روزنه‌ها بیشترین تنوع را نشان دادند. این صفات از دارای اهمیت تاکسونومیک هستند و می‌توان از آنها برای شناسایی گونه‌ها استفاده کرد. آنالیز چند متغیره

برای داده‌های ریزریخت‌شناسی در روابط بین و درون گونه‌ای با استفاده از نرم افزار R مورد استفاده قرار گرفت. نتایج آنالیز خوشه‌ای و تجزیه مؤلفه‌های اصلی نشان داد که صفات ریزریخت‌شناسی از لحاظ تاکسونومیک مهم هستند و می‌توان از این صفات برای شناسایی گونه‌ها و تعیین مرز گونه‌ها استفاده کرد.

INTRODUCTION

With more than 440 species distributed worldwide. *Saxifraga* Linnaeus (1753) is the largest genus within the Saxifragaceae s. str., (Ferguson & Webb 1970; Gornall 1987; Zhmylev 2004; Soltis 2007; APG III 2009; Deng & al. 2015; Tkach & al. 2015; Rezaee Chamanie & al. 2024). The term "*Saxifraga*" was originally coined by Dioscorides in the first century AD. It is derived from the Latin words "saxum," meaning rock or stone, and "frangere," meaning to split or cleave (Webb & Gornall 1989). Linnaeus (1753) originally described 31 species within *Saxifraga*. The genus is a prominent element of the herbaceous flora of temperate and alpine mountains in the northern hemisphere, particularly in the cold rocky regions of Europe, North America, and the Sino-Himalayan region, and has diversified into boreal and alpine zones (Webb & Gornall 1989; Pan & al. 2001; Soltis & al. 2001a; Zhmylev 2004; Akiyama 2012; Zhang, 2013; Tkach & al. 2015; Ebersbach & al. 2017a; Ebersbach & al. 2017b; Mabberley 2017). The mountainous regions of southern Europe are one of the versatile areas for *Saxifraga* (Ebersbach & al. 2017b; Tkach & al. 2015; Soltis & al. 1996). This genus is characterized by a wide variety of morphological characters, which has led to much taxonomic ambiguity. Haworth (1803) was the first to classify *Saxifraga* at the section level (49 species in six sections). Later, Haworth (1812), Sternberg (1810, 1822, and 1831), Tausch (1823), Seringe (1830), and Don (1822) continued research on this genus. Engler and Irmscher (1916-1919) identified several species of the genus and arranged them in different sections based on the details of the morphological characteristics of flowers. In addition, the two later authors identified more than a hundred new species and described leaf, flower (especially ovule), and seed morphological characters, and explained anatomical characters of hair and aspects of vegetative reproduction (axial branches and stems), (Engler and Irmscher 1916). This classification was modified by some subsequent authors. Gornall (1987), classified the genus into 15 sections, 19 subsections, and 34 series. Section Haworth is the largest, with about 179 species (Webb & Gornall 1989; Zhang & al. 2008), and has been divided into four subsections by Pan (1991). Phylogenetic studies have also been conducted to justify inconsistencies at different taxonomic levels, especially at the family and generic levels (Chase & al. 1993; Soltis & al. 1996& Soltis & Soltis 1997; Soltis & al. 2001a; Soltis & al. 2001b; Soltis 2007). These studies showed that *Saxifraga* is a polyphyletic genus and comprises at least 13 sections and 9 subsections (Soltis & al. 1993; Soltis & Soltis 1997; Tkach & al. 2015). *Saxifraga* includes 20 species in the flora of Turkey (Güner & al. 2012; Matthews 1972), 21 species in Flora Iranica (Schönbeck-Temsey 1967), and 10 species in the flora of Iran (Jamzad 1995). They occur mainly in northern, northwestern, and western Iran (except *S. sibirica* L., which occurs in eastern and western Iran). There are five endemics in the flora of Iran, including S. *iranica* Bornm, *S. ramsarica* Jamzad, *S. koelzii* Schonbeck-Temesy, *S. wendelbio* Schonbeck-Temesy and *S. mazanderinica* Schonbeck-Temesy (Schönbeck-Temsey 1967; Jamzad 1995; Aghaahmadi & al. 2014). Because of its taxonomic complexity, *Saxifraga* has received much attention due to the important studies on this genus including anatomical (Webb & Gornall 1989), cytological (Zhmylev 2004; Tkach & al. 2015), chemical properties (Webb & Gornall 1989; Liu & al. 2016; Matthäus & Otgonbayar 2016) and palynological studies (Ferguson & Webb 1970; Rabe & Soltis 1999; Zhang & al.2015a). However, the study of the micromorphological structure of the leaves of this genus has not yet been carried out. Therefore, we aimed to describe the micromorphological characteristics of the leaf epidermis of the genus *Saxifraga* and determine its taxonomic implications.

MATERIALS AND METHODS

Plant materials

In this study, dried leaves of 28 samples of 14 species (including 24 from Iran, 3 from Romania, and one from Georgia for comparison) were collected from the herbarium specimens of the Research Institute of Forests and Rangelands of Iran (TARI), the Guilan Agriculture and Natural Resources Research and Education Center (GILAN), the Central Herbarium of Tehran University (TUH), the Herbarium of Tabriz Faculty of Pharmacy (TBZ-FPH) and the Herbarium Ministries of Iranian Agriculture (IRAN), and samples from Romania were used (Table 1).

Flora Iranica (Schönbeck-Temsey 1967), Flora of Iran (Jamzad 1995), and Flora of Turkey (Matthews

1972) were the principal reference books for identification. Barthlott & al. (1998),) Kellermann (2011), Akin & al. (2013), and Kumar and Morgan (2015) were used for micromorphological terms. For the SEM observation, herbarium specimens were assembled on bottoms with double-sided cellophane tape and covered in a thin coating with 25 nm of gold-palladium at an accelerating voltage of 10-15 kV. The micrographs were prepared using a scanning electron microscope (EDAX AMETEK model Octane Prime). The morphological characteristics of leaves are reported in Tables 2-4. The micromorphological characteristics of wax, stomata, and trichomes were studied.

Tab. 1: The collection data of the studied taxa of the genus *Saxifraga*.

Species	Collection Data
1. *Micranthes stellaris* (L.) Galasso, Banfi &Soldano	Romania, Transsilvania., k. Linger. Alt: 1900m. 1257
2. *Saxifraga bryoides* L.	Romania, Transsilvania., Borzan Gurtler. Alt: 2280m. 1258.
3. *S. cymbalaria* L.	Iran, Guilan: Syahkal, Deylaman, bala–road. Alt: 1091 m. A.Moradi. 330 (GILAN).
3.1.	Iran, Mazandaran: Kelardasht, Abbas Abad, Khayrood forest. 38621/2(IRAN).
3.2.	Iran, East Azarbaijan: Kalibar, Yaralujeh, Gherkh–Bulagh. Alt: 2500m.727851/1 (IRAN)
3.3. *S. cymbalaria* ssp. *cymbalaria*	Iran, Mazandaran Prov: Kelar–dasht, Rudbarak. 38618/2 (IRAN).
3.4. *S. cymbalaria* ssp. *cymbalaria*	Iran, Guilan: Amarloo, Damash. Alt: 1750–1920m.38623/3 (IRAN).
3.5. *S. cymbalaria* subsp. *cymbalaria*	Iran, East Azerbaijan: Tabriz, Varzeqan, Molke Talesh, Andwan mountain. Alt: 1878m., Nazimia & Talebpour. 1081 (TBZ-FPH).
4. *S. exarata* Vill	Iran, Guilan: Chaboksar, Javaherdasht, Samamus Mt. Alt: 2700–3200 m. J.Noroozi. 3492 (TARI).
4.1	The republic of Georgia, kazbegi District, East of kazbegi peak, M. Khutsishvili (TBI). 49574 (IRAN).
5. *S. iranica* Bornm	Iran, Guilan. 2243 (GILAN).
5.1.	Iran, Tehran: Firuz kuh, Lazur, Mishineh–Marg MT. Amini-rad & Pahlevani. Alt: 3300–3400m. 107337 (TARI).
5.2.	Iran, Mazandaran: Baladeh road, Kalak Olia, Azad-kuh Mt. Ale: 3230– 3436 m, Amini Rad. 105943 (TARI).
6. *S. kotschyi* Boiss.	Iran, Weast Azarbaijan: Rezaiyeh, Targevar valley. W. of Anbi village. Alt1900m. Runemark and Foroughi.19782 (TARI)
6.1	Iran, Wast Azarbaijan: Zar Abad, Dibak, and Mamish Khan mt. Alt: 2800-3100 m. Leg: Amini Rad & Bahrami Shad. 78507 (TARI).
7. S. *koelzii* Schonb.-Tem.	Iran, Semnan: Shahrod, Shahkuh- e Olia. Alt: 2800–3250 m., Amini Rad & Bahrami Shad. 78508 (TARI).
8. *S. mazanderanica* Rech.f.	Iran, Mazandaran Prov., Tunekabon, among Jannat Rudbar and Eshkevar. Alt: 1100–2450m. Qahreman & Mozaffarian.9608 (TUH).
9. S. *paniculata* Mill**.**	Iran, Guilan: Talesh, sobatan. Leg: Aiuob moradi, Ahmad Aghaee. Aiuob Moradi. Alt: 1952m. 2063 (GILAN).
9.1.	Iran, East Azerbaijan: Tabriz, Varzeqan, Andwan mountain. Alt: 2. Nazimia and Talebpour. 1079 (TBZ-FPH).
9.2.	Iran, East Azerbaijan: Kalibar, Qala–e–Babak. 71353/1 (IRAN).
10. *S. pedemontana* ssp. *cymosa* Engl.	Romania.yezer. A.oanea
11. *S. ramsarica* Jamzad	Iran, Mazandaran: Ramsar, Jawaher Deh, Kooh Samamos. 69536 (IRAN).
12. *S. sibirica* L.	Iran, Guilan: Asalem to khalkhal–matash. Alt: 1900m, A.Moradi. 3258 (GILAN).
12.1	Iran, Ardabil: Alvāresi, Alt: 3500–4050m. Amini Rad& Eskandari.66473 (IRAN).
13. *S. tridactylites* L.	Iran, Guilan: Asalem to khalkhal, Almas Mountain. Mozaffarian. & Aiuob Moradi. Alt:2200m. 4549 (GILAN).
13.1.	Iran, Fars: 18 km on road from kazerun to Dalaki. Alt: 800m. Runemark & Mozaffarian.26760 (TARI).
14. *S. wendelboi* Schonb.-Tem.	Iran, Semnan: 50 kilometers north of Semnan, between Sheli and Hikoh. Alt: 2400–2700 m. Mozaffarian & Assadi. 40668 (TARI).
14.1	Mazandaran: Alt: 2500-300m. 38633/1 (IRAN).

Data analysis

Cluster analyses were performed using the unweighted paired group method with arithmetic mean (UPGMA); the similarity matrix was computed using the Euclidean coefficient. Squared Euclidean distances were used as the dissimilarity coefficient of the micromorphological data in cluster analysis (CA), (Sokal 1958; Badry & Elkordy 2020) and principal component analysis (PCA) to assess the relationship between species. Both PCA and CA analyses were accomplished in R software "version 4.1.2 "(2021) using the hclust function for cluster analysis (CA), and principal component analysis (PCA) was carried out with the vegan package, using a matrix of the characters shown in Table 5 (Team 2009; Team 2021). In this analysis, 36 micromorphological characters were analyzed with hierarchical cluster analysis carried out based on multistate (Table 2-5). The character coding was numerical, with qualitative states represented by numbers ranging from 0, 1, 2, 3, to 8. Each species was then analyzed for morphological characters and elevation range (Table 5). We used a similarity matrix for UPGMA cluster analysis. Each principal component describes a part of the variables as an eigenvalue in the similarity matrix (Sneath & Sokal 1973; Johnson & Wichern 2002). Species scores on two main axes were plotted as a two-dimensional scatter plot (PC1, PC2).

RESULTS

Micromorphological study

SEM analysis of the leaf micromorphological characteristics of the 14 *Saxifraga* species (including 10 species from Iran and 3 Romanian species of *Saxifraga* , were carefully examined, and their structural details are presented in Tables 2-4 and micrographs in Figs. 1-7.

Epidermis surface pattern, epidermal cell shapes or outline, the anticlinal and outer periclinal cell walls, epicuticular wax type, trichome, the position of the trichome on the epidermis surface; glandular trichomes and characters related to stomata (including stomata shape, outer stomatal rim, peristomatal rim, inner stomatal rim, wax distribution on the stomata rims, pore, and epidermal cells) are among the characteristics that were carefully examined.

Epidermis surface pattern

The surface pattern of the epidermis is formed based on the variation in the shape of epidermal cells, types of anticlinal and outer periclinal walls, and epicuticular wax ornamentation. In this study, nine types and five subtypes of epidermis surface patterns were identified as follows: Type I: Ruminate -irregular reticulate in *S. sibirica* (Figs. 1 A-A1); Type II: Sclariformis in *S. bryoides* (Figs. 1 B-B1); *S. paniculata* (Fig. 1 C); Type II subtype I: Ruminate- sclariformis in *S. paniculata* (Fig. 1 C1); Type III: Colliculate in *S. wendelboi* (Fig. 1 D**)**; *S. ramsarica* (Figs. 1 E-E1); Type IV: Areolate in *S. ramsarica* (Fig. 1 E2), it includes one subtype, Type IV subtype I: Ruminate-areolate in *S. kotschyi* (Fig. 1 G); Type V: Rugulate in. *kotschyi* (Fig. 1 G1); *S. iranica* (Fig. 2 A), including two subtypes: Type V subtype I: Ruminate- Rugulate in *S. iranica* (Fig. 2 A1), *S. exarata* (Fig. 2 B), Type V subtype II: Rugulate - undulate in *S. exarata (*Fig. 2 B1); Type VI: Tuberculate in *S. wendelboi* (Fig. 2 C). It includes one subtype. Type VI subtypes I: Ruminate-tuberculate in *S. koelzii* (Figs. 2 D-D1); Type VII: Rugose*: S. tridatylites* (Figs. 2 E-E1); Type VIII: Ruminate-foveate in *M. stellaris* (Figs. 3 A, A1); *S. Cymbalaria* (Figs. 3 B-B1); *S. pedemontana* subsp. *cymosa* (Figs. 3 C-C2); *S. mazandranica* (Fig. 3 D); Type IX: Ruminate -striate in *S. mazandranica* (Fig. 3 D1).

The result also showed epidermal surface pattern on both sides of the leaf was similar in seven species (*S. sibirica, S. bryoides, S. koelzii, S. tridatylites, S. cymbalaria, S. pedemontana* subsp. *cymosa* and *M. stellaris*), but different in the remaining seven species (*S. paniculata, S. wendelboi, S. ramsarica, S. kotschyi, S. iranica, S. exarata, S. mazandranica*).

Epidermal cell shapes

According to the results, epidermal cell shapes showed diversity in adaxial and abaxial surfaces of the leaves; four different types were identified (Table 2, column 1):

Type I Irregular: it was found on both the adaxial and abaxial surfaces of the leaves e.g. *S. sibirica* (Figs. 1 A-A1), *S. wendelboi* (Fig. 1 D, Fig. 2 C), *S. exarata* (Figs. 2 B-B1), *S. koelzii* (Figs. 2, D-D1), *S. tridactylis* (Figs. 2 E-E1), *S. cymbalaria* (Figs. 3 B-B1), *M. stellaris* (Figs. 3 A-A1). Type II: Irregular- rectangular, on the abaxial surface (e.g. *S. kotschyi* (Fig. 1 G). Type III: Regular-rectangular, on both surfaces (e.g. *S. bryoides* (Figs. 1 B-B1) and on the abaxial surface of e.g. *S. paniculata* (Fig. 1 C)*; S. iranica* (Fig. 2 A)*; S. mazanderanica* (Fig. 3 D); Type IV: Regular-rectangular - ovoid**,** on the abaxial surface of the leaves e.g. *S. ramsarica* (Fig. 1E).

According to the results, the shape of the epidermal cells on both leaf surfaces was similar in 8 species, and 6 species showed a different shape of the epidermal cells on the adaxial and abaxial side of the leaf surface.

The anticlinal and outer periclinal walls

Three types of anticlinal wall were identified: Type I: raised, on the leaf abaxial and adaxial surfaces of *S. sibirica* (Figs. 1 A-A1) *S. pedemontana* subsp. *cymosa* (Figs. 3 C-C2), on the abaxial surface of *S. bryoides*

(Fig. 1 B), *S. wendelboi* (Fig. 2 C), *S. tridactylites* (Fig. 2 E), on the adaxial surface of *S. iranica* (Fig. 2 A1);Type: II Depressed on the both leas surfaces of *S. paniculata* (Figs. 1 C-C1), *S. ramsarica* (Figs. 1 E-E1), on the adaxial surface of *S. bryoides* (Fig. 1 B1), *S. kotschyi* (Fig. 1 G1), *S. wendelboi* (Fig. 2 C1), *S. tridactylites* (Fig. 2 E1), and *S. mazanderanica* (Fig. 3 D2); Type III: Raised -Undulate, on the both surfaces (e.g. *S. cymbalaria* (Figs. 3 B-B1), *S. exarata* (Figs. 2 B-B1), *M. stellaris* (Figs. 3 A-A1), *S. koelzii* (Figs. 2 D-D1), and on the abaxial surface of e.g. , *S. kotschyi* (Fig. 1 G), *S. mazanderanica* (Fig. 3 D1).

Three types of outer periclinal cell walls were detected: Type I: Depressed on both surfaces *S. sibirica* (Figs. 1 A-A1), *S. exarata* (Figs. 2 B-B1), *M. stellaris* (Figs. 3 A-A1), *S. cymbalaria* (Figs. 3 B-B1), *S. pedemontana* subsp. *cymosa* (Figs. 3 C-C2), on the adaxial surface e.g. *S. kotschyi* (Fig. 1 G1), on the abaxial surface (e.g. *S . iranica* (Fig. 2 A), *S. wendelboi* (Fig. 1 D), *S. tridactylites* (Fig. 2 E), *S. mazanderanica* (Fig. 3 D), *S. bryoides* (Fig. 1 B); Type II: Raised, on both abaxial and adaxial surfaces e.g. *S. paniculata* (Figs. 1 C-C1), *S. ramsarica* (Figs. 1 E-E1), adaxial surface of *S. iranica* (Fig. 2 A1), *S. bryoides* (Fig. 1 B1), *S. wendelboi* (Fig. 2 C1), *S. tridactylites* (Fig. 2 E1), *S. mazanderanica* (Fig. 3 D2), and on the abaxial surface (e.g. *S. kotschyi* (Fig. 1 G), and Type III: Oblate - almost raised, on both surfaces e.g. *S. koelzii* (Figs. 2 D-D1), (Table 2 column 3).

Epicuticular wax

The epicuticular wax on both leaf surfaces of all studied species consists of films (including smooth layer, crust, and fissured) and crystalloids structures (including Granules, platelets, and rodlets) showed the following patterns among the studied species:
Type I: Crust-granule on both leaf surfaces (e.g. *S. kotschyi* (Figs. 1 G-G1), *S. tridactylites* (Figs. 2 E-E1), *S. mazanderanica* (Figs. 3 D-D1), and in the abaxial surface (e.g. *S. wendelboi* Fig. 1 D), Type I subtype I: Crust-scattered granule on both leaf surfaces (e.g. *S. bryoides* (Figs. 1 B- B1), Type II: Crust-fissured-granule on both leaf surfaces (e.g. *S. koelzii* (Figs. 2 D-D1), Type III: Crust-granule-platelets on both leaf surfaces (e.g. *S. sibirica* (Fig. 1 A-A1), *S. ramsarica* (Fig. 1 E-E1), *S. exarata* (Fig. 2 B-B1), *S. pedemontana* subsp. *cymosa* (Figs. 3 C -C2), in adaxialside of e.g. *S. paniculata* (Fig. 1 C1), Type III subtype I: Crust-granules, scattered platelets on both leaf surfaces (e.g. *S . iranica*: Figs. 2 A-A1), Type III subtype II: Crust-granules-thin and scattered platelets on both leaf surfaces (e.g. *M. stellaris* (Fig. 3 A-A1), *S. cymbalaria* (Figs. 3 B-B1), Type IV: Crust-granular-rodlets on the abaxial surface (e.g. *S. paniculata* (Fig. 1 C), Type V: Smooth layer on the adaxial surface of e.g. *S. wendelboi* (Fig. 2 C1), (Table 2 column 4).

Trichome

The leaf lamina of the studied species has simple and glandular trichomes (Table 3; columns1-3; Figs. 4 A-G1, Figs. 5 A-E2). All the species that have been investigated have linear- narrow triangular, flat, multicellular trichomes, with cells arranged in rows or forming striate patterns, except *S. wendelboi* which has wide triangular trichomes, especially on the abaxial surface of the leaves (Fig. 4 F).

The surface of the trichomes has either granule (e.g., in *S. exarata*, *S. bryoides*, and *M. stellaris* (Figs. 4 E-E1, Figs. 5 D-D1, C) or granule and warts *e.g.* in *S . iranica*, *S. ramsarica*, *S. koelzii*, *S. wendelboi* (Figs. 4 A-A1, B, D-D1, F-F1). The apex of the trichomes were obtuse (e.g. in *S . iranica, S. ramsarica, S. koelzii*, *S. exarata S. wendelboi* (Figs. 4 A-A1, B, D-D1, F-F1) and acute (e.g. *S. bryoides*, *M. stellaris*) (Figs. 5 D-D, C).

The position of the trichome on the epidermis surface varied. Five types were identified: Type I: Erect**,** on both surfaces of the leaves (Fig. 4 *S. koelzii*, *S. wendelboi*); Type II: Semi erect on both surfaces of the leaves (Fig. 4 *S. iranica, S. ramsarica*); Type III: Erect - semi erect on adaxial surface of the leaves (Fig. 5 *M. stellaris*); Type IV: Erect-appressed (Fig. 4 *S. exarata*); and Type V: Erect-flexuous on both surfaces of the leaves (Fig. 5 *S. bryoides*).
The species studied were founded on five types of glandular trichomes, which include the following: Type I: Multicellular trichome (5-6 cells) (Figs. 4 G, G1) in *S. tridactylites*; Type II: Multicellular with single-celled round head (Figs. 4 E, E1) in *S. exarata*; Type III: Multicellular with long stalk, single-celled round head (Figs. 5 B, B1) in *S. mazandranica*; Type IV: Multicellular with long stalk in *M. stellaris* (Fig. 5 C); Type V: Three-celled trichome (two large stalk cells and round head) (Figs. 5 E, E1, E2) in *S. pedemontana* subsp. *cymosa.*

Stomata

Stomata were visible on both surfaces of the leaves of the studied species. There were four types observed to determine the stomata shape: Type I: Elliptical on both surfaces (Fig. 6 *S. cymbalaria* (B-C), *S. tridactylites*, and Fig.7 *M. stellaris*), on the abaxial surface (Figs. 6 *S. sibirica*, *S. wendelboi*, and Fig.7 S. *mazanderanica*), and on the adaxial surface of the leaves (Fig. 7 *S. pedemontana* subsp. *cymosa*, *S. koelzii*); Type II: Round on both surfaces (Fig. 7 *S. kotschyi*), on the adaxial (Fig. 6 *S. koelzii*), on the adaxial (Fig. 6 *S. ramsarica*, *S. wendelboi*) ; Type III: Oval, on the adaxial (Fig. 7 *S. exarata*) and abaxial surfaces of the leaves (Fig. 6 *S. paniculata*, Fig. 7 S.

mazanderanica, *S. pedemontana* subsp. *cymosa*,); Type IV: Ovoid - oblong on both surfaces of the leaves (Fig. 7 *S. bryoides*).

The oute stomatal rim was identified in three types on both surfaces: Type I: Raised on both surfaces (Fig. 6 *S. tridactylites*, and Fig.7 S. *mazanderanica*, *M. stellaris*, *S. bryoides*, *S. pedemontana* subsp. *cymosa*) and on the adaxial surface of the leaves (Fig.6 *S. koelzii, S. wendelboi* Fig. 7 *S. exarata*, *S. kotschyi*); Type II: Depressed on the adaxial surface (Fig. 6 *S. ramsarica*), and on the abaxial surface of the leaves (Fig. 6 *S. cymbalaria*, *S. sibirica*, *S. koelzii*, *S. wendelboi*); Type III: Raised-overlapping on the abaxial surface of the leaves (Fig. 6 *S. paniculata*).

The results revealed seven types of prestomatal rim: Type I: Overlapping on both surfaces (Fig. 6 *S. tridactylites,* Fig.7 S. *mazanderanica, M. stellaris, S. bryoides*), on the abaxial surface (Fig. 6 *S. ramsarica*, Fig. 7 *S. exarata, S. kotschyi*); Type II: Depressed-overlapping on both surfaces (Fig. 6 *S. cymbalaria* (B, B1, C)); Type III: Overlapping - stout on both surfaces (Fig. 7 S. *mazanderanica*), on the abaxial surface (Fig. 6 *S. paniculata*), and on the adaxial surface (Fig.7 *S. pedemontana* subsp. *cymosa*); Type IV: Raised on the adaxial surface (Fig. 6 *S. ramsarica*, *S. koelzii*); Type V: depressed on both surfaces (Fig. 6 *S. wendelboi*); Type VI: Raised-overlapping on the abaxial surface (Fig. 6 *S. sibirica*).

Based on wax distribution on the stomata rims, pore, and epidermal cells, two types were identified: Type I: Pore free, epidermal cells and guard cell covered by wax on both surfaces (Fig. 6 *S. cymbalaria*, *S. koelzii*, *S. tridactylites*, and Fig 7 S. *mazanderanica*, *S. bryoides*), on the abaxial surface (Fig. 6 *S. paniculata*, *S. sibirica*, *S. wendelboi*, and Fig 7 *S. exarata*, *S. kotschyi*); on the adaxial surface (Fig.6 *S. ramsarica*); Type II: Pore and guard cell free, and epidermal covered by wax on both surfaces (Fig. 7 *M. stellaris*, *S. pedemontana* subsp. *cymosa*), on the adaxial surface (Fig. 6 *S. wendelboi*).

Based on inner stomatal rim variations, four types were identified: Type I: Smooth and sinuolate on both surfaces (Fig. 6 *S. tridactylites*), on the abaxial surface (Fig. 7 *M. stellaris*, *S. pedemontana* subsp. *cymosa*, *S. kotschyi*); Type II: Sinuolate on the abaxial surface (Fig. 6 *S. koelzii*); Type III: Sinuolate - erose on both surfaces (Fig. 6 *S. wendelboi*, and Fig. 7 S. *mazanderanica*, *S. bryoides*), on the adaxial surface (Fig. 6 *S. cymbalaria*, *S. koelzii*, *S. wendelboi* Fig. 7 *M. stellaris*, *S. pedemontana* subsp. *cymosa* B1), on the abaxial surface (Fig. 6 *S. paniculata*, *S. exarata*); and Type IV: Smooth and erose on the abaxial surface (Fig. 6 *S. cymbalaria* , *S. sibirica, S. ramsarica*) (Table 3).

Tab. 2: Leaf micromorphologcal characters of the studied species.

Species	**ECS Ad/Ab**	**AW Ad/Ab**	**OPW Ad/Ab**	**WTP/ Ad/Ab**	**WT/ Ad/Ab**
1– *M. stellaris*	Irr / Irr	Ra–Un / Ra–Un	Dep / Dep	Cr–Gr–thin–scat–Plat / Cr–Gr–thin–scat–Plat	Fil + Cry / Fil + Cry
2–*S. paniculata carteliginea*	Irr/ Re–Rect	Dep /Dep	Ra / Ra	Cr–Gr–Plat /Cr–Gr–Rod	Fil + Cry / Fil + Cry
3– S. *paniculata*	Irr/ Re–Rect	Dep /Dep	Ra / Ra	Cr–Gr–Plat /Cr–Gr–Rod	Fil + Cry / Fil + Cry
4–S. *paniculata*	Irr/ Re–Rect	Dep /Dep	Ra / Ra	Cr–Gr–Plat /Cr–Gr–Rod	Fil + Cry / Fil + Cry
5– *S. cymbalaria*	Irr / Irr	Ra-Un ⁄Ra–Un	Dep / Dep	Cr–Gr–thin–scat–Plat/ Cr–Gr–thin–scat–Plat	Fil + Cry / Fil + Cry
6– *S. cymbalaria*	Irr /Irr	Ra–Un ⁄Ra–Un	Dep / Dep	Cr–Gr–thin–scat–Plat/ Cr–Gr–thin–scat–Plat	Fil + Cry/ Fil + Cry
7–*S .cymbalaria*	Irr /Irr	Ra–Un ⁄Ra–Un	Dep / Dep	Cr–Gr–thin–scat–Plat/ Cr–Gr–thin–scat–Plat	Fil + Cry/ Fil + Cry
8–*S. cymbalaria* var. *cymbalaria*	Irr / Irr	Ra-Un ⁄Ra–Un	Dep / Dep	Cr–Gr–thin–scat–Plat/ Cr–Gr–thin–scat–Plat	Fil + Cry / Fil + Cry
9–*S. cymbalaria* var. *cymbalaria*	Irr /Irr	Ra–Un ⁄Ra–Un	Dep / Dep	Cr–Gr–thin–scat–Plat/ Cr–Gr–thin–scat–Plat	Fil + Cry/ Fil + Cry
10– *S. cymbalaria* var. *cymbalaria*	Irr /Irr	Ra–Un ⁄Ra–Un	Dep / Dep	Cr–Gr–thin–scat–Plat/ Cr–Gr–thin–scat–Plat	Fil + Cry/ Fil + Cry
11– *S. sibirica*	Irr / Irr	Ra ⁄Ra	Dep⁄Dep	Cr–Gr–Plat / Cr–Gr–Plat	Fil + Cry / Fil + Cry
12– *S. sibirica*	Irr / Irr	Ra ⁄Ra	Dep⁄Dep	Cr–Gr–Plat / Cr–Gr–Plat	Fil + Cry / Fil + Cry

13– *S. iranica*	Irr / Re–Rect	Ra/ Dep	Ra /Dep	Cr–Gr–scat–Plat / Cr–Gr–scat–Plat	Fil + Cry / Fil + Cry
14– *S.iranica*	Irr / Re–Rect	Ra/ Dep	Ra /Dep	Cr–Gr–scat–Plat / Cr–Gr–scat–Plat	Fil + Cry / Fil + Cry
15– *S. iranica*	Irr / Re–Rect	Ra/ Dep	Ra /Dep	Cr–Gr–scat–Plat / Cr–Gr–scat–Plat	Fil + Cry / Fil + Cry
16– *S. ramsarica*	Irr / Re–Rect–Ov	Dep /Dep	Ra/Ra	Cr–Gr–Plat /Cr–Gr–Plat	Fil + Cry / Fil + Cry
17– *S. kotschyi*	Irr / Irr–Rect	Dep / Ra–Un	Dep /Ra	Cr–Gr / Cr–Gr	Fil + Cry / Fil + Cry
18– *S. kotschyi*	Irr / Re–Rect	Ra /Dep–Un	Dep/ Ra	Cr–Gr / Cr–Gr	Fil + Cry / Fil + Cry
19– S. *koelzii*	Irr / Irr	Ra–Un /Ra–Un	Ob–alm–Ra / Ob–alm–Ra	Cr–Fiss–Gr /Cr–Fiss–Gr	Fil + Cry / Fil + Cry
20– *S. exarata*	Irr / Irr	Ra-Un / Ra–Un	Dep / Dep	Cr–Gr–Plat / Cr–Gr–Plat	Fil + Cry / Fil + Cry
21– *S. exarata* ssp. *moschata*	Irr / Irr	Ra-Un / Ra–Un	Dep / Dep	Cr–Gr–Plat / Cr–Gr–Plat	Fil + Cry / Fil + Cry
22– *S. wendelboi*	Irr / Irr	Dep / Ra	Ra/ Dep	Smoot / Cr–Gr	Fil + Cry / Fil + Cry
23– *S. wendelboi*	Irr / Irr	Dep / Ra	Ra/ Dep	Smoot / Cr–Gr	Fil + Cry / Fil + Cry
24– *S. tridactylites*	Irr/ Irr	Ra / Ra	Dep / Dep	Cr –Gr /Cr –Gr	Fil + Cry / Fil + Cry
25– *S. tridactylites*	Irr/Irr	Ra/Ra	Dep/ Dep	Cr-Gr / Cr –Gr	Fil + Cry / Fil + Cry
26– *S. mazanderanica*	Irr/ Re–Rect	Dep /Ra–Un	Ra/ Dep	Cr –Gr /Cr –Gr	Fil + Cry / Fil + Cry
27– *S. pedemontana* subsp. *cymosa*	Irr/ Irr	Ra /Ra	Dep / Dep	Cr –Gr–Plat/Cr –Gr–Plat	Fil + Cry / Fil + Cry
28– *S. bryoides*	Re–Rect / Re–Rect	Dep/Ra	Ra/ Dep	Cr–scat–Gr / Cr–scat–Gr	Fil + Cry / Fil + Cry

Tab. 3: Trichome micromorphological characters of the studied species.

Species	**T Ad/Ab**	**TSH Ad/Ab**	**Gr. V Ad/Ab**	**PT Ad/Ab**	**TT Ad/Ab**	**TPE Ad/Ab**	**G Ad/Ab**
1– *M. stellaris*	+/+	MStriR/ MStriR	Gr / Gr	Mar / Mar	Ac / Ac	Er–semi–Er / Er–semi–Er	Type 4/Type 4
2– *S. paniculata carteliginea*	–/–	–/–	–/–	–/–	–/–	–/–	–/–
3– S. *paniculata*	–/–	–/–	–/–	–/–	–/–	–/–	–/–
4– S. *paniculata*	–/–	–/–	–/–	–/–	–/–	–/–	–/–
5– *S. cymbalaria*	–/–	–/–	–/–	–/–	–/–	–/–	–/–
6– *S. cymbalaria*	–/–	–/–	–/–	–/–	–/–	–/–	–/–
7– *S .cymbalaria*	–/–	–/–	–/–	–/–	–/–	–/–	–/–
8– *S. cymbalaria* var. cymbalaria	–/–	–/–	–/–	–/–	–/–	–/–	–/–
9– *S. cymbalaria* var. cymbalaria	–/–	–/–	–/–	–/–	–/–	–/–	–/–
10– *S. cymbalaria* var. cymbalaria	–/–	–/–	–/–	–/–	–/–	–/–	–/–
11– *S. sibirica*	–/–	–/–	–/–	–/–	–/–	–/–	–/–
12– *S. sibirica*	–/–	–/–	–/–	–/–	–/–	–/–	–/–
13– *S. iranica*	+/+	MStriR/ MStriR	Gr+V / Gr+V	Mar / Mar	Ob / Ob	Se–Er/ Se–Er	–/–
14– *S.iranica*	+/+	MStriR/ MStriR	Gr+V / Gr+V	Mar / Mar	Ob / Ob	Se–Er/ Se–Er	–/–

15– *S. iranica*	+/+	MStriR/ MStriR	Gr+V / Gr+V	Mar / Mar	Ob / Ob	Se–Er/ Se–Er	–/–
16– *S. ramsarica*	+/+	MStriR/ MStriR	–/Gr+V	Mar / Mar	Ob / Ob	Se–Er/ Se–Er	–/–
17– *S. kotschyi*	–/–	–/–	–/–	–/–	–/–	–/–	–/–
18– *S. kotschyi*	–/–	–/–	–/–	–/–	–/–	–/–	–/–
19– S. *koelzii*	+/+	MStriR/ MStriR	Gr+V / Gr+V	Mar / Mar	Ob / Ob	Er/ Er	–/–
20– *S. exarata*	+/+	MStriR/ MStriR	Gr / Gr	Bl / Bl	Ob / Ob	Er–Ap / Er–Ap	Type 2 / Type 2
21– *S. exarata* ssp. moschata	+/+	MStriR/ MStriR	Gr / Gr	Bl / Bl	Ob / Ob	Er–Ap / Er–Ap	Type 2 / Type 2
22– *S. wendelboi*	+/+	Tri / Tri	Gr+V/ Gr+V	Mar / Mar	Ob / Ob	Er/ Er	–/–
23– *S. wendelboi*	+/+	Tri / Tri	Gr+V/ Gr+V	Mar / Mar	Ob / Ob	Er/ Er	–/–
24– *S. tridactylites*	–/–	–/–	–/–	–/–	–/–	–/–	Type 1/Type 1
25– *S. tridactylites*	–/–	–/–	–/–	–/–	–/–	–/–	Type 1/Type 1
26–*S. mazanderanica*	–/–	–/–	–/–	–/–	–/–	–/–	Type 3/ Type 3
27– *S. pedemontana* ssp. *cymosa*	–/–	–/–	–/–	–/–	–/–	–/–	Type 5
28– S. bryoides	+/+	MStriR/ MStriR	Gr / Gr	Mar / Mar	Ac / Ac	Er–Fl / Er–Fl	–/–

Tab. 4: Stoma micromorphological characters of the studied species.

Species	**S Ad/Ab**	**SSH Ad/Ab**	**OSR Ad/Ab**	**PSR Ad/Ab**	**AT Ad/Ab**	**WDS Ad/Ab**
1– *M. stellaris*	+/+	Elip / Elip	Ra Ra /	Over/ Over	Sinu–Er/ Smoot–Sinu	Type2 /Type2
2– *S. paniculata. carteliginea*	–/ +	–/ Ov	–/Ra–Over	–/ Over–St	–/ Sinu–Er	–/ Type 1
3– S. *paniculata*	–/ +	–/ Ov	–/Ra–Over	–/ Over–St	–/ Sinu–Er	–/ Type 1
4–S. *paniculata*	–/ +	–/ Ov	–/Ra–Over	–/ Over–St	–/ Sinu–Er	–/ Type 1
5– *S. cymbalaria*	+/+	Elip / Elip	Ra / Dep	Dep–Over / Dep–Over	Sinu–Er / Smoot–Er	Type 1/ Type 1
6– *S. cymbalaria*	+/+	Elip / Elip	Ra / Dep	Dep–Over / Dep–Over	Sinu–Er / Smoot–Er	Type 1/ Type 1
7–*S .cymbalaria*	+/+	Elip / Elip	Ra / Dep	Dep–Over / Dep–Over	Sinu–Er / Smoot–Er	Type 1/ Type 1
8–*S. cymbalaria* var. cymbalaria	+/+	Elip / Elip	Ra / Dep	Dep–Over / Dep–Over	Sinu–Er / Smoot–Er	Type 1/ Type 1
9–*S.cymbalaria* var. cymbalaria	+/+	Elip / Elip	Ra / Dep	Dep–Over / Dep–Over	Sinu–Er / Smoot–Er	Type 1/ Type 1
10– *S.cymbalaria* var. cymbalaria	+/+	Elip / Elip	Ra / Dep	Dep–Over / Dep–Over	Sinu–Er / Smoot–Er	Type 1/ Type 1
11– *S. sibirica*	–/ +	–/ Elip	–/ Dep	–/ Ra–Over	–/ Smoot–Er	–/ Type 1
12– *S. sibirica*	–/ +	–/ Elip	–/ Dep	–/ Ra–Over	–/ Smoot–Er	–/ Type 1
13– *S. iranica*	–/ –	–/ –	–/ –	–/ –	–/ –	–/ –
14– *S.iranica*	–/ –	–/ –	–/ –	–/ –	–/ –	–/ –
15– *S. iranica*	–/ –	–/ –	–/ –	–/ –	–/ –	–/ –
16– *S. ramsarica*	+/–	Ro /–	Dep /–	Ra/ Over	–/ Smoot–Er	Type 1 /–
17– *S. kotschyi*	+/+	Ro / Ro	Ra /–	–/ Over	–/ Smoot–Sinu	– / Type 1
18– *S. kotschyi*	+/+	Ro / Ro	Ra /–	–/ Over	–/ Smoot–Sinu	– / Type 1
19– S. *koelzii*	+/+	Ro / Elip	Ra / Dep	Ra / Over	Sinu–Er / Sinu	Type 1/ Type 1
20– *S. exarata*	–/ +	–/ Ov	Ra /–	–/ Over	–/ Sinu–Er	–/ Type 1

21– *S. exarata* ssp. moschata	–/ +	–/ Ov	Ra /–	–/ Over	–/ Sinu–Er	–/ Type 1
22– *S. wendelboi*	+/+	Elip /Ro	Ra / Dep	Dep / Dep	Sinu–Er / Sinu–Er	Type 2 / Type 1
23– *S. wendelboi*	+/+	Elip /Ro	Ra / Dep	Dep / Dep	Sinu–Er / Sinu–Er	Type 2 / Type 1
24– *S. tridactylites*	+/+	Elip / Elip	Ra / Ra	Over/ Over	Smoot–Sinu/ Smoot–Sinu	Type 1/ Type 1
25– *S. tridactylites*	+/+	Elip / Elip	Ra / Ra	Over/ Over	Smoot–Sinu/ Smoot–Sinu	Type 1/ Type 1
26–*S. mazanderanica*	+/+	Ov / Elip	Ra / Ra	Over–St / Over–St	Sinu–Er / Sinu–Er	Type 1/ Type 1
27– *S. pedemontana* ssp. *cymosa*	+/+	Ov/ Elip	Ra / Ra	Over- St / -	Sinu–Er/ Smoot–Sinu	Type2 ⁄Type2
28– *S. bryoides*	+/+	Ov–Ob/ Ov -Ob	Ra / Ra	Over/ Over	Sinu–Er/ Sinu–Er	Type 1/ Type 1

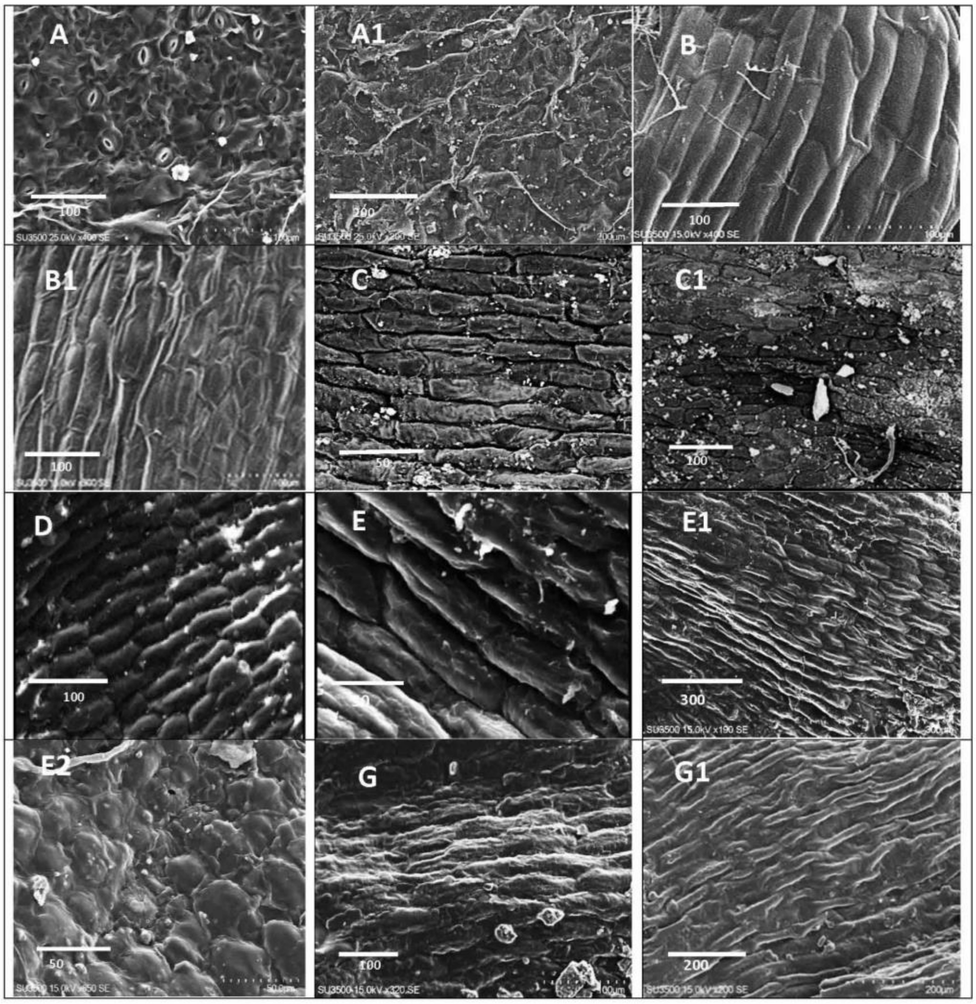

Fig. 1 SEM micrographs showing leaf epidermis surface patterns: Ruminate -irregular reticulate in *S. Sibirica* (A-A1)*;* Sclariformis in *S. bryoides* (B-B1); *S. Paniculata* (C)*;* Ruminate- sclariformis in *S. Paniculata* (C1); Colliculate in *S. wendelboi* (D); *S. ramsarica* (E-E1), Areolate in *S. ramsarica* (E2*)*; Ruminate-areolate in *S. kotschyi* (G); Rugulate in S. *kotschyi* (G1). A- G abaxial side; A1, B1, D, C1, E2 adaxial side.

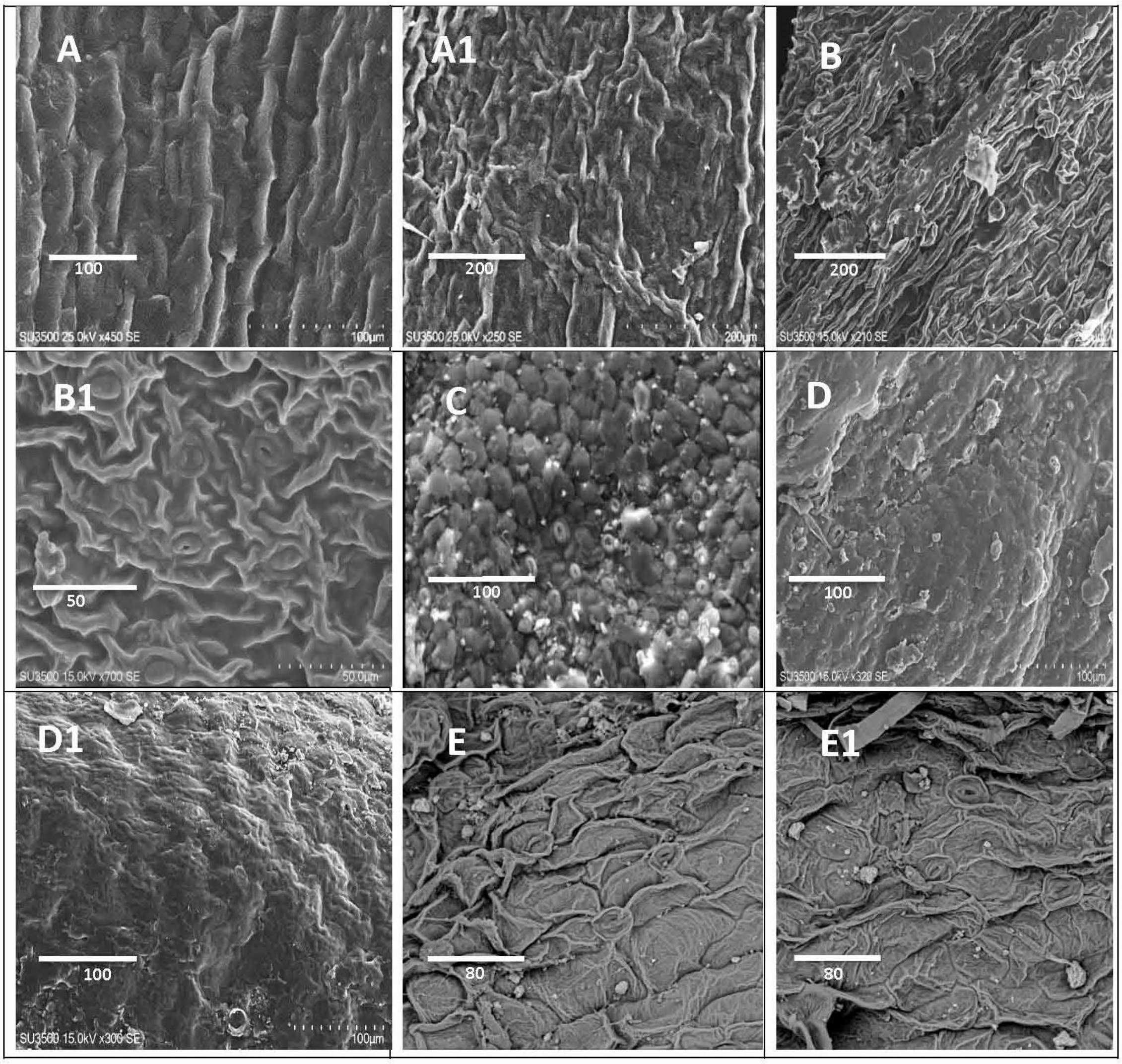

Fig 2. SEM micrographs showing leaf epidermis surface patterns. Ruminate- Rugulate in *saxifraga iranica* (A-A1); *S. exarata* (B), II Rugulate - undulate in *S. exarata* (B1); Tuberculate in *S. wendelboi* (C); Ruminate-tuberculate in *S. koelzii* (D-D1); Rugose in *S. tridatylites* (E-E1). A- E abaxial side, A1, B1, D1, E1 adaxial side.

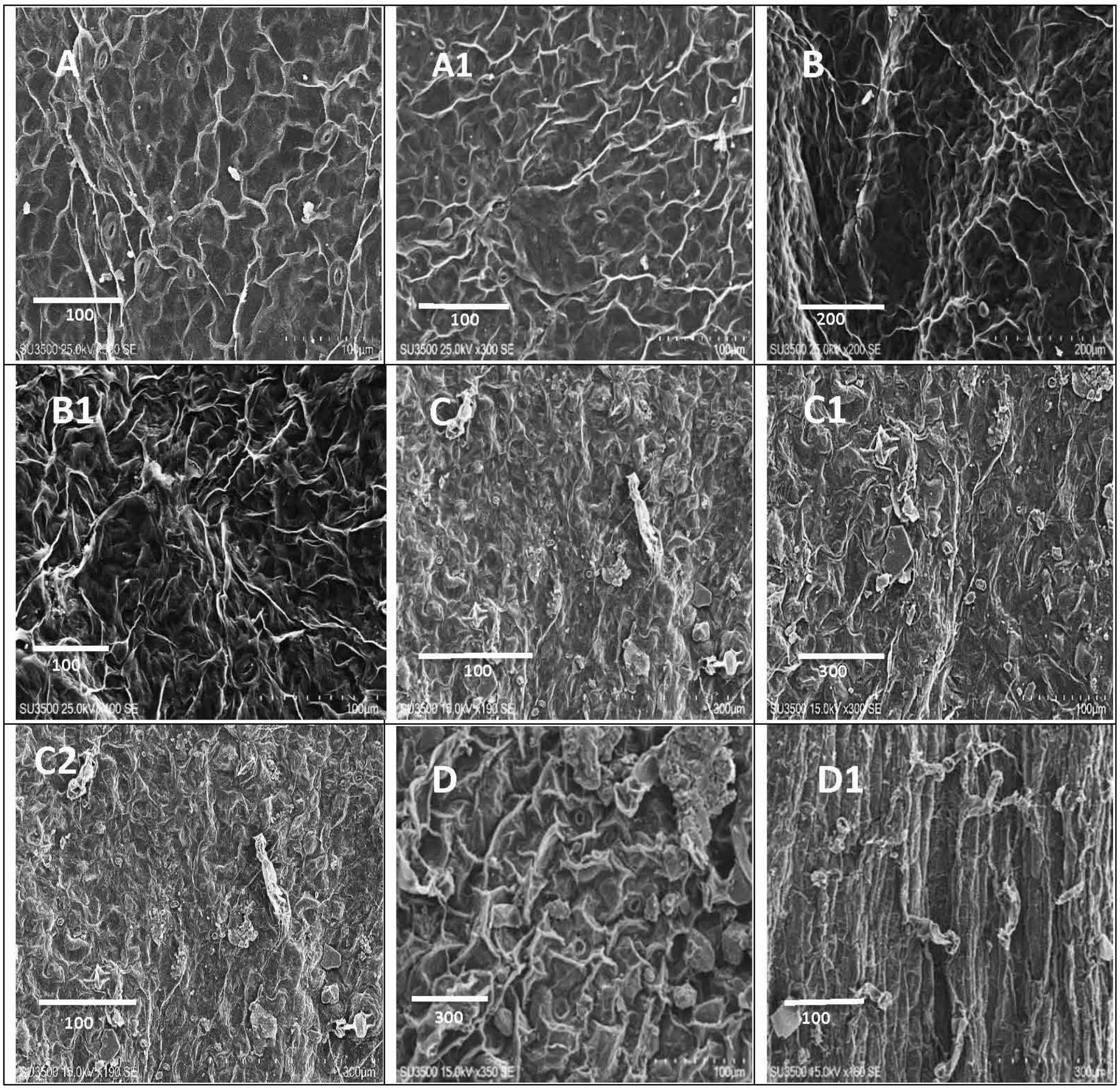

Fig. 3. SEM micrographs showing leaf epidermis. Ruminate-foveate in *Micranthes stellaris* (A-A1); *saxifraga cymbalaria* (B-B1)*; S. pedemontana* subsp. *cymosa* (C-C2); *S. mazandranica* (D); Ruminate -striate in *S. mazandranica* (D1).

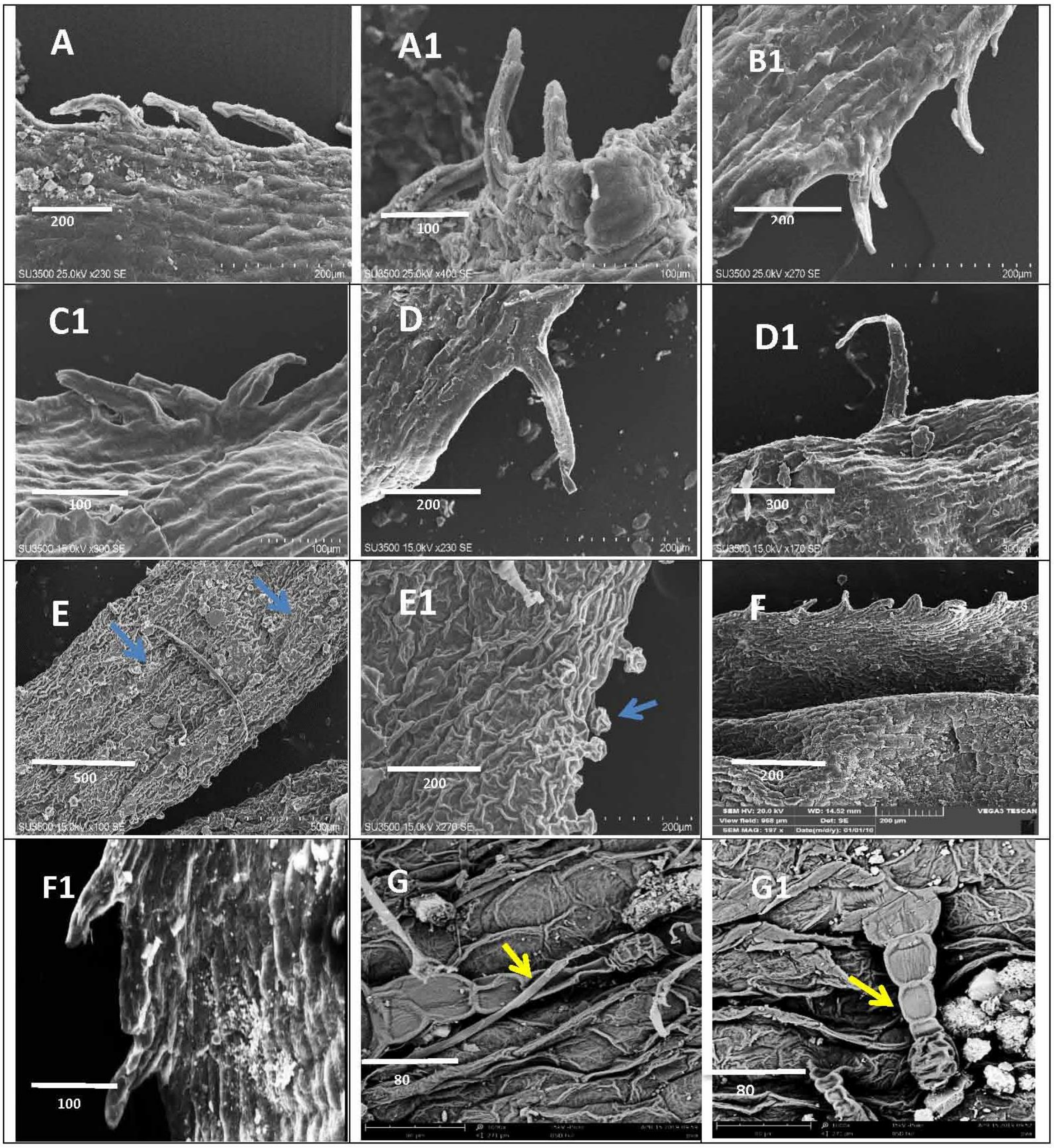

Fig. 4. SEM micrographs showing trichrome. *Saxifraga iranica* (A, A1)*; S. ramsarica* (B); *S. kotschyi* (C); *S. koelzii* (D, D1); *S. exarata* (E, E1); *S. wendelboi* (F, F1); *S. tridactylites* (G, G1). Blue arrow multicellular, single-celled trichome with a round head, yellow arrow multicellular trichome with 5-6 cells. A-G: abaxial side and A1, C1, D1, E1, F1, G1: adaxial side.

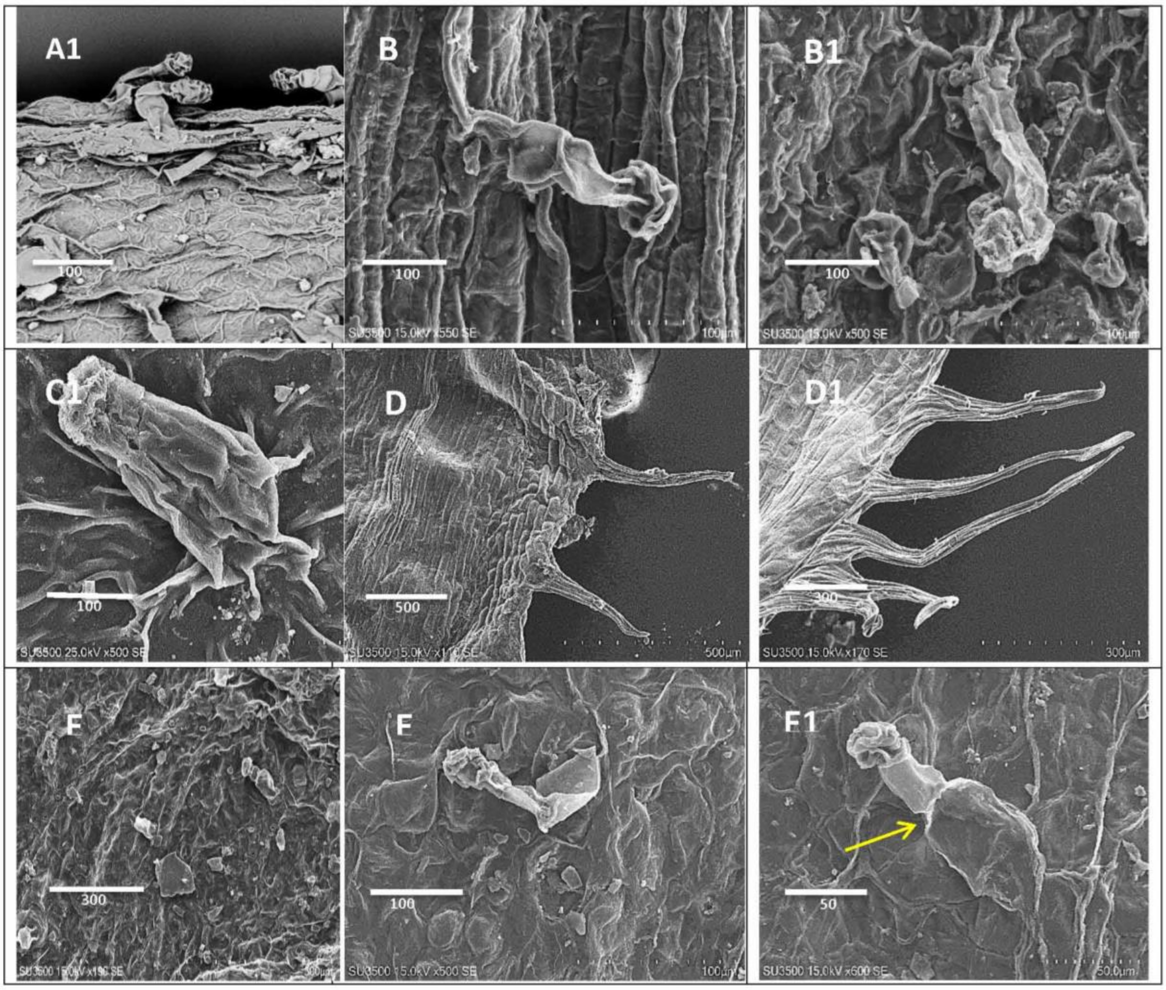

Fig. 5. SEM micrographs showing trichrome. *Saxifraga iranica* (A); *S. mazanderanica* (B, B1); *Micranthes stellaris* (C); *S. bryoides* (D, D1); *S. pedemontana* subsp. *cymosa* (E, E1, and E2). Yellow arrow, three-celled trichome (two large stalk cells and a round head). A- E: abaxial side and B1, C1, D1, E1, E2: adaxial side.

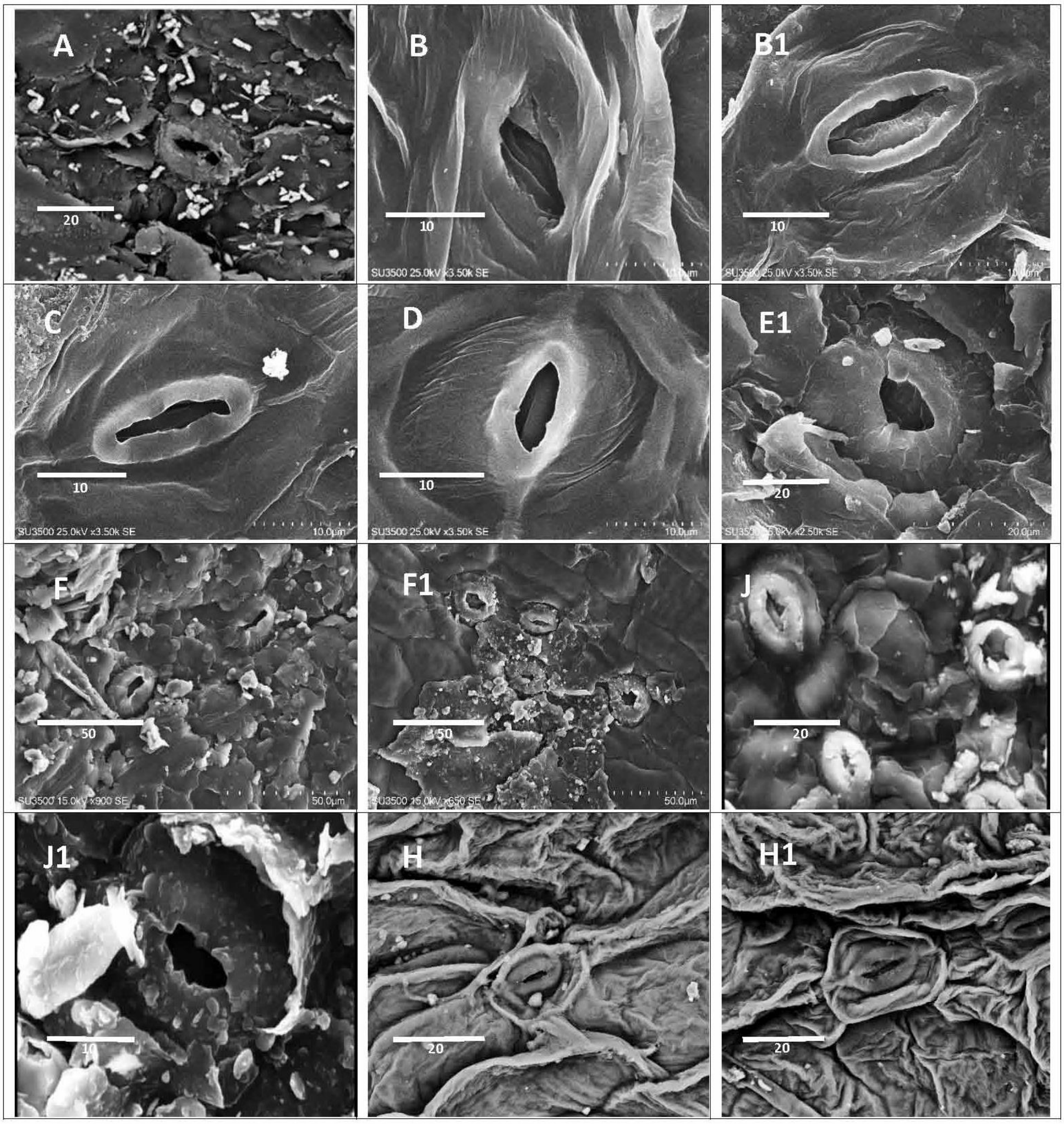

Fig. 6**.** SEM micrographs showing stomata. *Saxifraga paniculata.* (A); *S. cymbalaria* (B, B1, C); *S. sibirica* (D); *S. ramsarica* (E); *S. koelzii* (F, F1); *S. wendelboi* (J, J1); *S. tridactylites* (H, H1). A- H abaxial side and B1, F1, J1, H1 adaxial side.

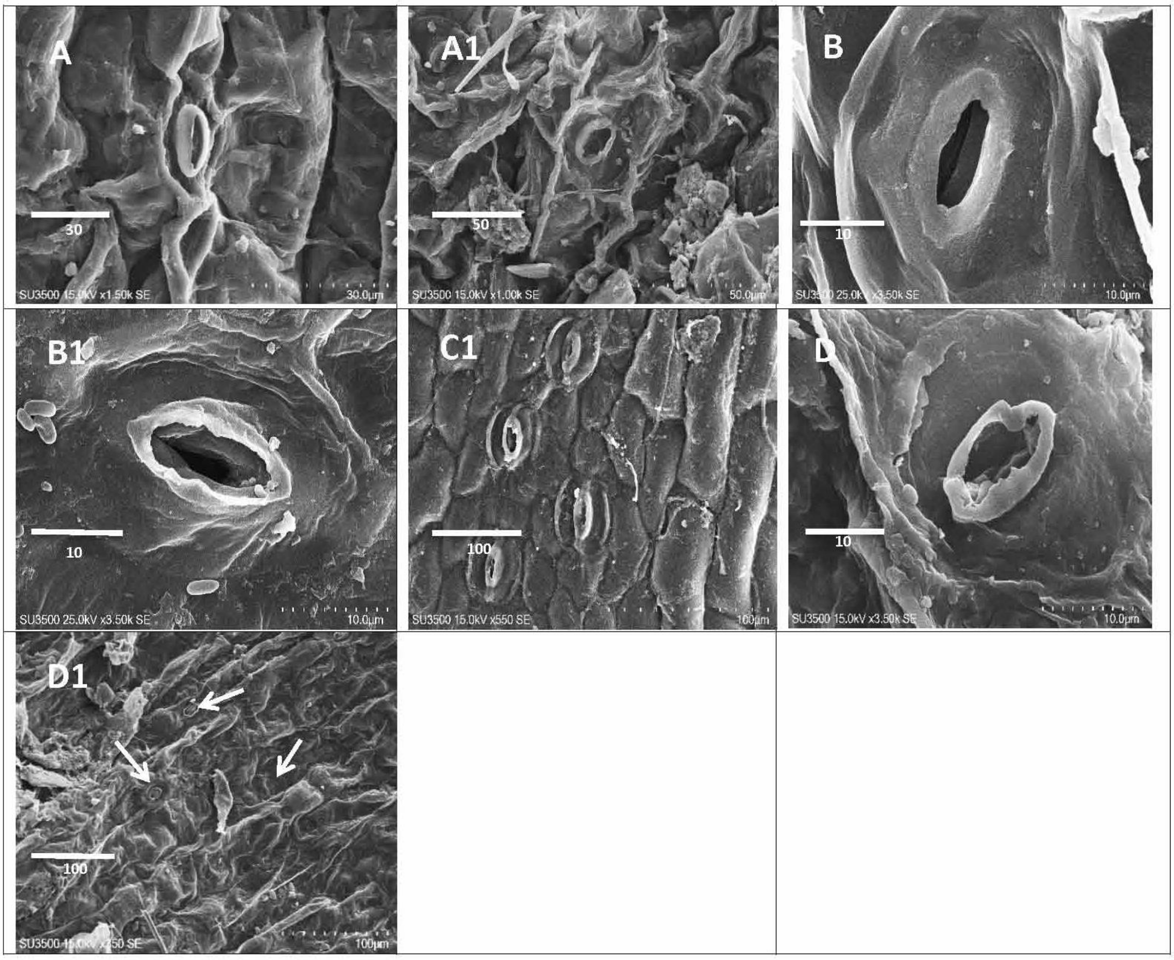

Fig. 7. SEM micrographs showing stomata: *Saxifraga mazanderanica* (A, A1); *Micranthes stellaris* (B, B1); *S. bryoides* (C); *S. pedemontana* subsp. *cymosa* (D, D1); *S. exarata* (E); *S. kotschyi* (F). A- F on the abaxial and A1, B1, C1, D1 on the adaxial side.

Numerical analysis

Cluster analysis (CA): On the UPGMA dendrogram (Fig. 4), the studied species were placed in two main clusters A and B, with Euclidean distance from 0 to 0.5. Cluster A includes two species*:* *S. iranica* and S. *ramsarica* (group 1 in PCA), and the main cluster B is subdivided into two clusters B1 and B2. Cluster B1 is further subdivided into two clusters: B1.1 (containing three taxa of *S. paniculata*) and B1.2 (containing two taxa of *S. sibirica*). Cluster B2 was also divided into two clusters B2.1 (containing five species) and B2.2 (five species). The two clusters were further subdivided. B2.1 was subdivided into sub-cluster C (containing *S. pedemontana* subsp. *cymosa* and *S. cymbalaria*) and D (*S. mazandaranica*, *S. kotschyi*, and *S. tridactylites*). While, B2. 2 clusters segregated into two clusters L *(S. exarata*) and M (S. *koelzii, S. wendelboi, M. stellaris*, and *S. bryoides*).

Principal component analysis (PCA)

The principal components analysis (PCA) result explained 52.82% of the total variation among 14 species of *saxifraga*. The first and second axes explain 32.41% and 21.41% of the variances, respectively. The most important variables of the first principal component are trichome absent or present on the adaxial (TD) and abaxial surfaces (TB), trichome shape of adaxial (TSHD) and abaxial surfaces (TSHB), position trichome on adaxial (PTD) and abaxial surfaces (PTB), trichome tip of adaxial (TTD) and adaxial surfaces (TTB), trichome position relative to the epidermis of adaxial (TPED) and abaxial surfaces (TPEB).

The most significant variables of the second principal component are as follows: epidermal cell shape of adaxial (ECSD), wax sculpturing type of adaxial (WTPD), and abaxial surfaces (WTPB), presence and absence of stomata of adaxial (SD) and abaxial surfaces (SB), stomatal shape of adaxial (SSHD) and abaxial surfaces (SSHB), wax distribution on the stomata of adaxial (WDSD) and abaxial surfaces (WTPB), outer stomatal edge of adaxial (OSRD), peristomatal edge of adaxial surface (PSRD), type of inner stomata (aperture) of adaxial surface (ATD).

The results led to the formation of four groups of species. Group 1 includes two species: *Saxifraga iranica* and *S. ramsarica*. Characters such as epidermal cell shape (ECSD) and outer periclinal wall of adaxial surface (OPWD) were involved in forming this group. Group 2 contains *S. sibirica* and *S. paniculata*, formed due to wax-type patterns of the adaxial (WTPD) and abaxial surfaces (WTPB).

Group 3 contains *S. cymbalaria*, *S. tridactylites* (26760), *S. tridactylites* (4549), *S. kotschyi*, *S. pedemontana* subsp. *cymosa*, and *S. mazandaranica*. This result is due to traits such as the anticlinal wall of adaxial (AWD), abaxial surfaces (AWB), outer stomata rim of adaxial surface (OSRD), peristomatal rim of adaxial surface (PSRD), peristomatal rim of abaxial surface (PSRB), stomata presence and absence of abaxial (SB), and stomata shape of abaxial surface (SSHB).

Group 4 includes *S. exarata*, *S. bryoides*, *M. stellaris*, *S. koelzii*, and *S. wendelbo* which are grouped according to characteristics such as hair shape of adaxial (TSHD) and abaxial surfaces (TSHB), the position of trichome on the adaxial (PTD) and abaxial surfaces (PTB), presence or absence of trichome on the adaxial surface (TD), trichome tip of adaxial (TTD) and abaxial surfaces (TTB), trichome position relative to the epidermis of adaxial (TPED) and abaxial surfaces (TPEB), gland and verruca on trichome of leaf adaxial (GTVD) and abaxial surfaces (GTVB).

Table 5: The Characters used in cluster and principal component analysis, with abbreviations.

Abbreviation	character	Abbreviation	character
1 - ECSD	**Epidermal cell shape on the adaxial surface:** Irregular: 0 Irregular-almost rectangular: 1 Irregular Rectangular: 2 Regular Rectangular: 3 Regular, Rectangular to Ovoid: 4 Rectangular: 5	**19- TTD**	**Trichome tip on the adaxial surface:** Absence: 0 Acute: 1 Obtuse: 2
2– ECSB	**Epidermal cell shape on the abaxial surface:** Irregular: 0 Irregular-almost rectangular: 1, Irregular Rectangular: 2 Regular Rectangular: 3 Regular, Rectangular to Ovoid: 4 Rectangular: 5	**20– TTB**	**Trichome tip on the abaxial surface:** Absence: 0 Acute: 1 Obtuse: 2
3– AWD	**The anticlinal wall on the adaxial surface:** Depressed: 0 Depressed–undulate: 1 Raised: 2, Raised–undulate: 3	**21– TPED**	**Trichome position on epidermis on the adaxial surface:** Absence: 0 Semi–erect: 1, Erect: 2, Erect–semi erect: 3 Erect–appressed: 4, Erect–flexuous: 5
4– OPWD	**The outer periclinal wall on the adaxial surface** Depressed: 0 Raised: 1 Oblate–almost raised: 2	**22– TPEB**	**Trichome position on epidermis on the abaxial surface** Absence: 0, Semi–erect: 1, Erect: 2, Erect–semi-erect: 3, Erect–appressed: 4, Erect–flexuous: 5
5– AWB	**The anticlinal wall on the abaxial surface** Depressed: 0 Depressed–Undulate: 1 Raised: 2	**23– GTD**	**Type of Granular trichome on the adaxial surface** Absence: 0 Three celled trichome:1 Multicellular trichome: 2

	Raised–Undulate: 3		Multicellular with single-celled round head:3 Multicellular with long stalk: 4 Multicellular, with a long stalk, single-celled round head: 5
6– OPWB	**The outer periclinal on the abaxial surface** Depressed: 0 Raised: 1 Oblate–almost raised: 2	**24– GTB**	**Type of Granule trichome on the abaxial surface** Absence: 0 Three celled trichome:1 Multicellular trichome: 2 Multicellular with single-celled round head:3 Multicellular with long stalk: 4 Multicellular with a long stalk, single-celled round head: 5
7– WTPD	**Wax sculpturing on the adaxial surface:** smooth layer: 0 Crust–granule: 1 Crust–scattered granule: 2 Crust–fissured–granule: 3 Crust–granule–platelets: 4 Crust–granules, scattered platelets: 5 Crust–granules–thin and scattered platelets: 6, Crust–granular–rodlets: 7	**25– PSD**	**Presence of Stomata on the adaxial surface** Absence: 0 Presence: 1
8– WTP B	**Wax sculpturing on the abaxial surface:** smooth layer: 0 Crust–granule: 1 Crust–scattered granule: 2 Crust–fissured–granule: 3 Crust–granule–platelets: 4 Crust–granules, scattered platelets: 5 Crust–granules–thin and scattered platelets: 6, Crust–granular–rodlets: 7	**26– PSD**	**Presence of Stomata on the adaxial surface** Absence: 0 Presence: 1
9– WTD	**Wax type on the adaxial surface** Film: 0 Film and crystalloid: 1	**27– SSHD**	**Stomata shape on the adaxial surface** Absence: 0 Elliptical: 1, Round: 2, Oval: 3, Ovoid to oblong: 4
10– WTB	**Wax type on the abaxial surface** Film: 0 Film and crystalloid: 1	**28– SSHB**	**Stomata shape on the abaxial surface** Absence: 0 Elliptical: 1, Round: 2, Oval: 3, Ovoid to oblong: 4
11– PrTD	**Present of Trichome on the adaxial surface** Absent: 0 Present:1	**29– WDSD**	Wax distribution on the stomata on the adaxial surfaces Absence: 0 Pore free, epidermal cells and guard cells covered by wax: 1 Pore and guard cell free and epidermal covered by wax: 2
12– PrTB	**Present of Trichome on the abaxial surface** Absent: 0 Present:1	**30– WDSB**	**Wax distribution on the stomata on the abaxial surfaces** Absence: 0

			Pore free, epidermal cells and guard cells covered by wax: 1 Pore and guard cell free and epidermal covered by wax: 2
13– TSHD	**Trichome shape on the adaxial surface;** Absent: 0 Multicellular, Striates and Rows: 1 Triangular: 2	**31– OSRD**	**Outer stomata rim on the adaxial surface** Absent: 0 Raised :1 Depressed: 2, Raised–overlapping: 3 Depressed–folded: 4
14– TSHB	**Trichome shape on the abaxial surface:** Absent: 0 Multicellular, Striates and Rows: 1 Triangular: 2	**32– OSRB**	**Outer stomata rim on the abaxial surface** Absent: 0 Raised :1 Depressed: 2, Raised–overlapping: 3 Depressed–folded: 4
15– Gr. VD	**Granular, verrucose on trichome on the adaxial surface:** Absent: 0 Smooth: 1 Verrucose: 2 Granular and verrucose: 3	**33– PSRD**	**Peri–stomatal rim on the adaxial surface:** Absent: 0 Depressed: 1, Depressed–overlapping: 2 Overlapping: 3, Overlapping and stout: 4 Raised: 5, Raised–overlapping: 6, Stout: 7
16– Gr. VB	**Granular, V: verrucose of trichome on the abaxial surface:** Absent: 0 Smooth: 1 Verruca: 2 Granular and verrucose: 3	**34– PSRB**	**Peri–stomatal rim on the abaxial surface:** Absent: 0 Depressed: 1, Depressed–overlapping: 2 Overlapping: 3, Overlapping and stout: 4 Raised: 5, Raised–overlapping: 6, Stout: 7
17–PTD	**Position Trichome on the adaxial surfaces** Absent: 0 Margin :1 Blade :2	**35– ATD**	**Aperture type on the adaxial surface:** Absence: 0 Sinuolate: 1, Sinuolate and erose: 2 Smooth and sinuolate: 3 Smooth and erose: 4
18–PTB	**Position Trichome on the abaxial surfaces** Absent: 0 Margin :1 Blade :2	**36– ATB**	**Aperture type on the abaxial surface:** Absence: 0 Sinuolate: 1, Sinuolate and erose: 2 Smooth and sinuolate: 3 Smooth and erose: 4

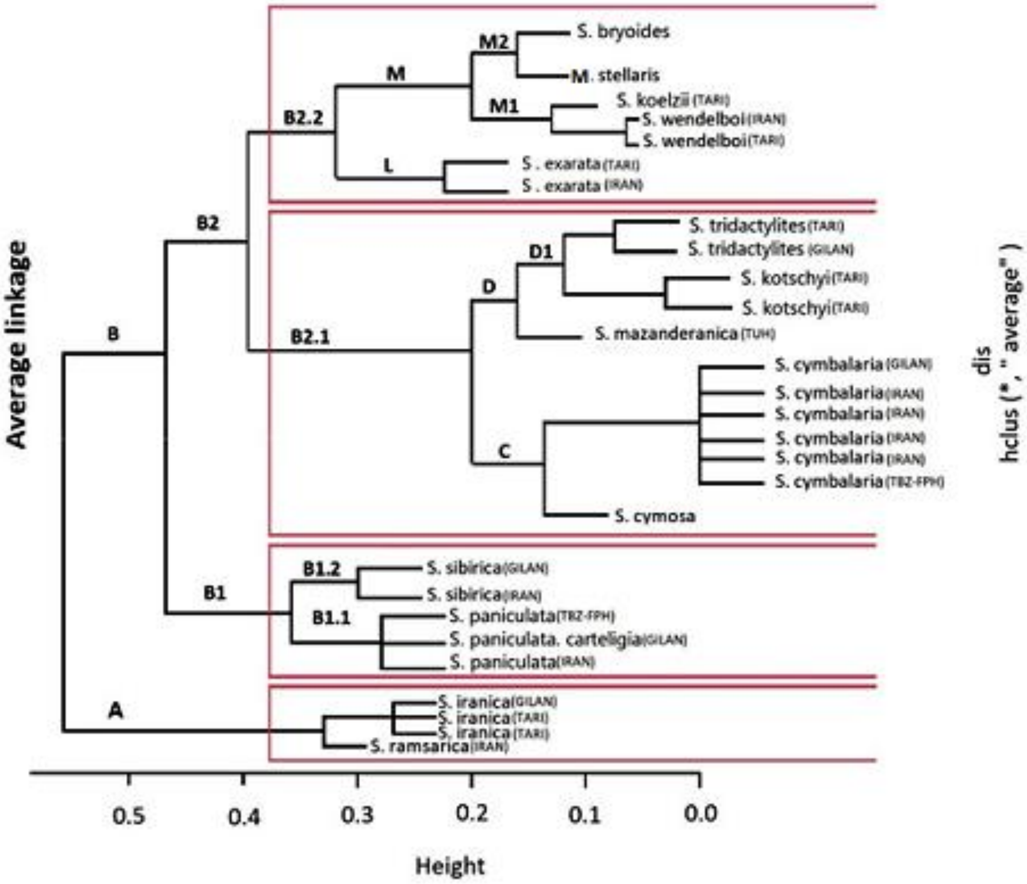

Fig. 8. UPGMA dendrogram derived from *saxifrage* leaf traits.

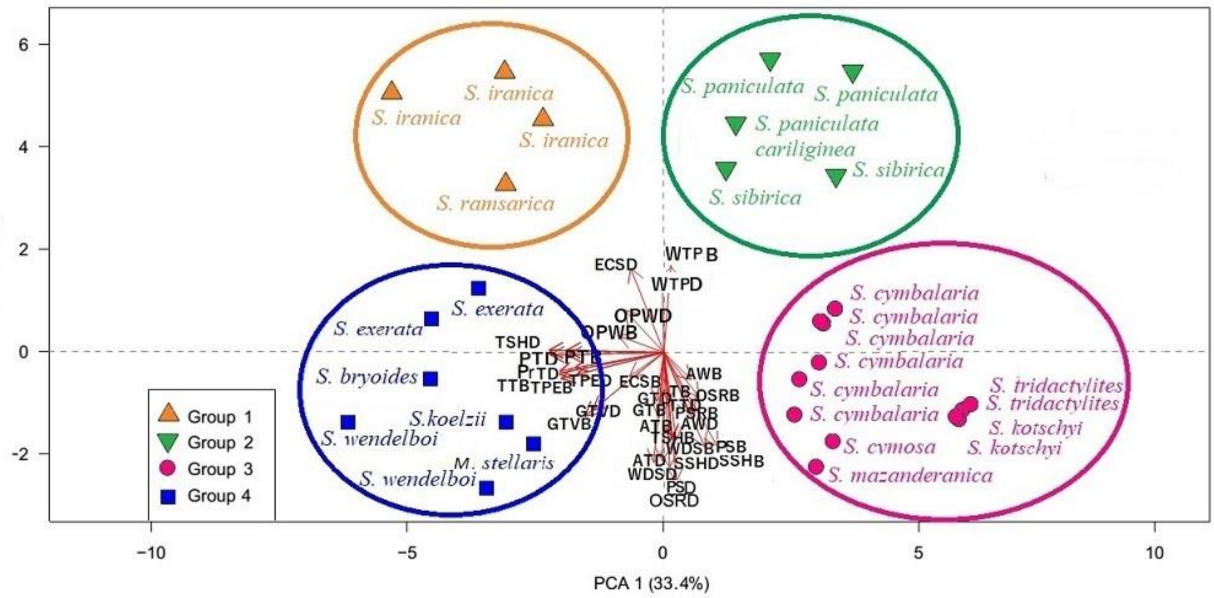

Fig. 9. PCA diagram based on the micromorphology traits of *saxifraga* species.

DISCUSSION

The results of our work revealed the remarkable diversity of micromorphological leaf characteristics in *Saxifraga* species in Iran. Based on the results, micromorphological traits such as the presence or absence of simple and glandular hairs, the presence or absence of verruca and granules on the hair surface, the position of hairs on the epidermis, epidermal surface pattern, the shape of epidermal cells, the anticlinal wall, the outer periclinal layer, epicuticular wax ornamentation, the distribution of wax on the stomata, and the shape of the stomata showed the greatest diversity. Some researchers, including Engler and Irmscher (1916-1919), Webb and Gornall (1989), have made significant contributions to the study of morphological characters of *Saxifraga*. Previous studies showed that morphological characters related to hair play an important role in the classification of *Saxifraga* species. For example, the presence or absence of single and glandular hairs, together with other characteristics such as leaf size and leaf margin (with cavities with calcareous deposits), forms the basis for distinguishing species such as *S. kotschyi*, *S. koelzii*, *S. cymbalaria*, S. *exarata*, and *S. tridactylites* (with single and glandular hairs) from *S. wendelboi*, *S. iranica, S. ramsarica, S. paniculata cartilaginea*, and *S. sibirica* (with single hairs) (Jamzad 1372*)*.

It was also found that the micromorphological characteristics of the leaf hair are of diagnostic importance for species separation (Faghir & al. 2018). Based on the results of this study, two types of hair surface were identified: 1) granular (in *M. stellaris, S. exarata, S. bryoides*) and 2) verrucate - granular (*S. wendelboi* and *S. koelzii*), confirming the diagnostic and taxonomic value of this character. In addition, based on previous studies, epidermal surface pattern (Belhadj & al. 2007; Lynch & al. 2006; Erden and Menemen 2023), wax ornamentation on the cuticle (Fehrenbach and Barthlott 1988, Neinhuis and Barthlott 1997, Neinhuis and Neinhuis Barthlott, Neinhuisth, 1997. et al. 1998, Wissemann 2000) and characters related to stomata (Akçin & al. 2013; Kumar and Murugan 2015) are the most prominent morphological characters of leaves that can be used to classify and identify species. Our results are in agreement with the previous findings.

The results of the multivariate analysis, particularly the UPGMA dendrogram, showed the differentiation of the studied species into two principal clusters A and B (Fig. 4). The species composition of the clusters corresponded to the groups of the principal component analysis (Figs. 6 and 7). In cluster A, *S. iranica and S. ramsarica* formed a group with similar micromorphological characteristics (such as the shape of the upper epidermal cells, scaly wax ornamentation, with granules and small plates, and the presence of hairs on the leaf margin). The union of two species is enhanced by common morphological characters such as stems with overlapping leaves, leaf margins without teeth, and stamens shorter than petals, (Jamzad 1374). Moreover, these two native Iranian species are located in a similar geographic area (from the Caucasus to the Alborz: an area between the Black Sea and the Caspian Sea and the Alborz Mountains, which form the boundary between the Euro-Siberian and Irano-Turanian regions (Zohary 1973; Aghaahmadi & al. 2014).

Cluster B is divided into two clusters B1 (B1.1-B1.2) and B2. In cluster B1, two species including S. *sibirica* and *S. paniculata* are found together. These two species have similar micromorphological characteristics such as the distribution of the wax pattern on the stomata and the type of wax sculpturing, which is supported by floral morphology such as superior or nearly almost superior ovary (Jamzad 1372) and their geographic distribution (in Euro-Siberian) (Aghaahmadi & al. 2014).

Cluster B2 contains five species, and is divided into two clusters: B2.1 (including subclusters C and D) and B2.2 (including subclusters clusters M and L). In cluster B2.1 (group C of PCA), *S. pedemontana* subsp. *cymosa* was united with *S. cymbalaria*, because of their shared micromorphology affinities (such as irregular shape of epidermal cells on adaxial and abaxial surfaces, prominent outert periclinal layer, absence of hairs and glandular hairs). The first species is a shrub, whose geographical distribution extends from the eastern and southern Carpathians to the northern Balkan Peninsula, and the second species is a perennial plant with a range from eastern Romania to northern and western Iran and Algeria (https://powo.science.kew.org.).

Two European species, *S. bryoides* and *M. stellaris*, were united in a small group because of common micromorphology character. This result is supported by their geographic distribution. These two species occur in European mountains, mainly in temperate, subalpine, and subarctic biotopes (https://powo.science.kew.org/). The two Iranian endemics, *S. wendelboi* and S. *koelzii* in sub-cluster M1 exhibit similar micromorphology character (especially leaf margin covered with ciliated hairs, oblong leaves, which is supported by their geographical distribution (i.e. both grow in Iranino-Turanian region). *Saxifraga wendelboi* occurs in N and C Iran and *S. koelzii* is found in C Iran, mainly on rocks and in limestone crevices (Schönbeck-Temsey 1967; Jamzad 1972).

ACKNOWLEDGMENTS

The authors are grateful to Dr. Ms. F. Attar, (Central Herbarium of Tehran University (TUH)) for accessing plant materials.

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