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Micromorphology of the epidermis and anatomical structure of the leaves of *Scorzonera hispanica* L.

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Abstract

In Poland *Scorzonera hispanica* L. is rare in the wild. This species is used as a vegetable and medicinal plant. Currently, attempts are being made to introduce this plant into cultivation in Poland. In this study, comparative analyses were conducted of the epidermis surface micromorphology and anatomical structure of the leaves of *S. hispanica* 'Maxima' and 'Meres'. The investigations were performed using fluorescence, light and scanning electron microscopy. The cuticle on the surface of epidermal cells is smooth or striated. In the epidermis, there are anomocytic stomata. The stomatal index in the epidermis of the studied cultivars is 9.3–11%. In the midrib of the leaf, there is an aerial cavity which occupies a substantial area. In this place, cracking and breaking of the leaf blade were observed. Over the aerial cavity under the adaxial epidermis, there is a single layer of collenchyma cells and 1–2 rows of parenchyma cells. Tangential collenchyma is also present between the abaxial epidermis and large vascular bundles located in the midrib and on both sides of the large vascular bundles in the lamina. This tissue strengthens the leaf margin. The mesophyll cells located in the abaxial epidermis of the midrib form protrusions surrounding the large vascular bundles. The leaves of *S. hispanica* represent the equifacial type.

Keywords: *Scorzonera hispanica*; leaf; epidermis; cuticle; stomata; micromorphology; anatomy; SEM

Introduction

The genus *Scorzonera* comprises 180 species [1]. It originates from Eurasia and Northern Africa [2]. The name of the genus *Scorzonera* is derived from Spanish words *escorzon* or *escuerzo* meaning a venomous snake or *scorza* and *nera*, i.e., black bark. This corresponds to the German name *Schwarzwurzel* – black root [3]. In Poland five species from the genus *Scorzonera* occur in the natural environment; these include *S. cana* O. Hoffm, *S. hispanica* L., *S. humilis* L., *S. purpurea* L., and *S. rosea* Waldst. & Kit [4,5]. *Scorzonera hispanica* grows in the wild in Central and Southern Europe, in the region of the Caucasus, and in southern Siberia. The species is rare in Poland and occurs primarily in mid-forest meadows and scrubs [2].

Scorzonera hispanica is a vegetable and medicinal plant [6,7]. In some European and African countries, it is cultivated and used as a root vegetable [8–10]. It produces a long, fragile root with a dark skin and creamy flesh [6]. Given their high nutritional value, young leaves are used in salads [6,10,11]. In the past, scorzonera roots were used as a coffee substitute [10].

Biologically active substances contained in the tissues of *S. hispanica*, e.g., inulin [12,13], sesquiterpenoids including bisabolane [14–16], lignans including syringaresinol [16,17], polyphenols such as flavonoids and phenolic acids [16], may play a role in the prevention and treatment of various diseases.

Inulin is a natural prebiotic stimulating the growth of beneficial intestinal flora [18–22]. It is used in diabetic diet [18]. It has anti-artheriosclerotic activity, i.e., reduction of blood cholesterol levels [22,23], and enhances calcium absorption [24,25]. Bisabolane derivatives were active against colon cancer cell lines and may be interesting as lead structures for the development of new anti-colon cancer agents [16]. Syringaresinol accelerates wound healing and exhibits anti-inflammatory and immunomodulatory activities through its effects on cellular and humoral immunity. It shows cytotoxic properties against tumour cells in colon cancer and myeloma [16,17,26]. Extracts from *S. hispanica*

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have diuretic and antipyretic activities [10]. In the past, plants from the genus *Scorzonera* were used as an antidote for snake venom [3].

The medicinal and nutritional properties of *S. hispanica* argue for the introduction of this species into cultivation in Poland. Despite its undeniable nutritional and pharmacological properties, *S. hispanica* is rarely grown in Europe due to troublesome harvesting of its fragile and even 40-cm long roots [6]. An advantage of the vegetable is its low soil requirements and low-temperature resistance of the roots, which allow the plants to be left in the ground over the wintertime [6,27]. This method of storage promotes the maintenance of a high quality of roots, which otherwise lose firmness and wither in traditional storage houses [10].

Our preliminary experiments showed that *S. hispanica* leaf blades tend to crack in the adaxial part and break after heavy rainfall. This reduces the assimilation capacity of damaged leaves and hence plant yields. Therefore, the aim of the study was to carry out comparative analyses of the micromorphology and anatomical structure of the leaves of two *S. hispanica* cultivars, with special emphasis on tissues located at the site of fracture. Furthermore, in this study the micromorphology of the epidermis surface of the leaf blade was determined because there is no literature on this subject.

Material and methods

Research material

The analyzed plants of two cultivars of *Scorzonera hispanica* L., i.e., 'Meres' and 'Maxima', originated from a field plantation in an experimental farm of the University of Life Sciences in Lublin (51°23′ N, 22°56′ E).

In August 2014 (in the fourth month of the first year of growing) fragments of leaf blades with the midrib from the basal (4 cm from the blade base) and medial parts as well as the marginal part were sampled. In the fragments collected from the basal (I) and medial parts (II) of the leaves, the tissues within (a) and near the midrib (b) were examined.

The observations and morphometric measurements of leaf tissues were performed using hand-made cross sections of fresh material and semi-thin sections of fragments of fixed leaf blades. The comparative analyses of the epidermis surface micromorphology and anatomical structure of the leaves were performed using fluorescence (FM), light (LM), and scanning electron microscopies (SEM).

Stereoscopic microscopy

Preliminary observations of the surface of the leaf epidermis and the distribution of tissues in the leaf blades were carried out under a stereoscopic microscope SMT 800 coupled with a Nicon Coolpix 4500 photographic camera.

Fluorescence microscopy

Slides of the leaf cross sections were placed in a drop of 0.01% auramine O fluorochrome and embedded in a 50% glycerol solution [28]. Observations of the slides were carried out under a Nikon Eclipse 90i fluorescent microscope equipped with an FITC filter (excitation light 465–495 nm) and a barrier filter (wavelength 515–555 nm).

Light microscopy

In order to prepare semi-thin sections, the sampled leaf segments were fixed in 4% glutaraldehyde and 0.1 M phosphate buffer, pH 7.0 for 6 hours at room temperature. The material was then washed in 0.1 M phosphate buffer with a pH of 7.0 at 4°C for 48 hours. After washing, the plant samples were post-fixed in a 1.5% solution of osmium tetroxide for 1.5 h at room temperature. After rinsing with distilled water, the leaf fragments were dehydrated in an ethanol series at the following concentrations: 15, 30, 50, 70, 90, 96%, and twice at 99.8%, for 15 minutes in each solution. The dehydrated plant samples were embedded in Spurr Low Viscosity resin and the medium were polymerized at a temperature of 60° C for 48 h.

Semi-thin 0.8–1.0 µm thick longitudinal sections were cut with a glass knife from the fixed material using a Reichert Ultracut S microtome. The leaf sections were stained with 1% toluidine blue and 1% azure II (1:1) at 60°C for 5 minutes. The presence of polysaccharides was determined using the periodic acid Schiff's (PAS) reaction [29].

Cross-sectional fragments prepared from fresh material were stained with toluidine blue (basic staining) and Sudan Red (detection of lipids) [30]. Comparative observations of the tissue structure and measurements of the cells of the leaves analyzed were performed using a Nikon Eclipse 400 light microscope.

Scanning electron microscopy

The micromorphology of the adaxial and abaxial epidermis surface of the leaves in the medial part was compared. The fixed fragments (the method specified in the description of light microscopy) were dehydrated in acetone with increasing concentration: 15, 30, 50, 70, 90, and 99.5% (anhydrous acetone was used twice), for 15 minutes in each solution. Next, the preparations were critical-point dried in liquid CO_2 and sputter-coated with gold using an EMITECH K550X sputter coater. Observations of the epidermis structure were carried out under a TESCAN VEGA II LMU scanning electron microscope.

Morphometric analysis included:

(***i***) leaf size: the length and width of the leaf blades;

(***ii***) micromorphology of the adaxial and abaxial epidermis in the medial part of the leaf blades: the length and width of stomata and apertures between the cuticular ledges, the number of stomata per 1 mm^2, the value of the stomatal index calculated according to Meidner and Mansfield [31], and the diameter of the stomatal complex;

(***iii***) anatomical characteristics of selected tissues in the basal and medial parts of the leaf blades: the thickness of the leaf blade, the height of the cells in the abaxial and adaxial epidermis, the thickness of the outer cell wall of the epidermis on both leaf surfaces, the thickness of the cells strand located over and under the aerial cavity, the diameter of the largest cells adjacent to the aerial cavity on the adaxial side, the height and width of the aerial cavity, the number of cells layers, the diameter of collenchyma cells, and the height of palisade cells. Measurements of each feature investigated were made in 16 replicates.

Statistical analysis

The significance of the differences in the morphological and anatomical traits of the leaf tissues investigated was statistically analyzed using the integrated statistical package Statistica 6.0. Univariate analyses of variance (ANOVA) were performed. Statistical inference was carried out at a significance level of $P = 0.05$.

Results

In the first year of growing, the *S. hispanica* cultivars 'Maxima' and 'Meres' produce a leaf rosette. The leaf blades of the former cultivar are larger (47.6 cm length / 5.4 cm width) than those of the latter one (45.0/4.9 cm; Fig. 1). The leaves have a lanceolate shape, a finely serrate margin, and a pointed apex (Fig. 2a–d).

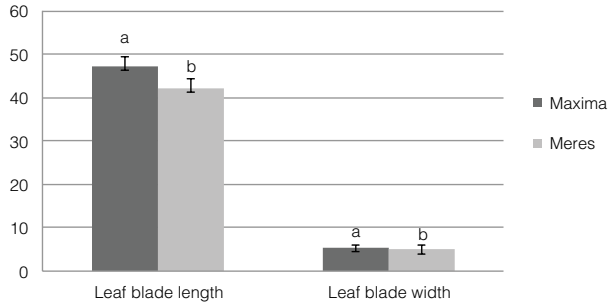

Fig. 1 Leaf blade length and width of the investigated cultivars of *Scorzonera hispanica*.

Micromorphology of the epidermal cell surface

The leaves of the cultivars analyzed represent the amphistomatic type. Both epidermis surfaces bear anomocytic stomata. The stomata are located at the level of the other epidermal cells. They have thick cuticular ledges. Four epidermal cells are adjacent to the guard cells (Fig. 2e–h, Fig. 3a–f).

The cuticle on the surface of the guard cells is striated in both cultivars. Such sculpture with a radial arrangement of striae around the stomata is visible on the surface of the cells adjacent to the guard cells. Other epidermal cells have a smooth or slightly striated cuticle (Fig. 2g,h, Fig. 3c–f).

The stomata in 'Maxima' are longer (on average 41 µm) and wider (on average 32 µm) than those in 'Meres' (on average 35 µm and 28 µm). Similarly, the size of the aperture between the cuticular ledges is greater in the former cultivar (24–28 µm length / 6–8 µm width) than in the latter one (15–17 µm / 5–6 µm). The stomata in the epidermis of both leaf surfaces exhibit higher density in 'Meres' than in 'Maxima'. In these cultivars, there were 14 and 12 stomata per 1-mm^2 area in the abaxial epidermis and 10 and 8 stomata per 1-mm^2 area in the adaxial epidermis, respectively. The stomata index is 9.3% and 9.8% in the adaxial epidermis of the cultivars analyzed, whereas in the abaxial epidermis it is 10.8% and 11.3%. The diameter of the stomatal complex in the adaxial epidermis of 'Maxima' and 'Meres' is 120–141 µm and 96–111 µm, respectively, while in the abaxial epidermis it is in the range of 125–156 µm and 97–126 µm (Tab. 1). Both leaf surfaces bear multi-branched protective trichomes (Fig. 2f, Fig. 3a).

Anatomical features

The leaves of the two cultivars have an almost triangular outline in the midrib area (cross section). There is an aerial cavity here (Fig. 4a–c). The thickness of the leaves in the midrib at the basal and medial parts of the lamina in 'Maxima' is 3.1 mm and 1.9 mm, respectively, while in 'Meres' it represents 84% and 61% of the values found in the former cultivar (Tab. 2). In turn, the lamina tissues located between the veins near the leaf base in 'Maxima' and 'Meres' have a thickness of 677 µm and 577 µm. These values are lower by 47% and 52%, respectively, in the medial part of the leaves (Tab. 3).

Adaxial side of the midrib

In the analyzed leaves, there is a thin layer of cells over the aerial cavity (Fig. 4a–d) with a thickness of 117–170 µm in 'Maxima' and 106–188 µm in 'Meres'. The strand of these cells comprises the epidermis, a single cell layer with a collenchymatous thickening of the walls, and 1–2 rows of parenchymal cells. The parenchymal cells are adjacent to the aerial cavity. They have a large diameter, which is 3- or 4-fold greater (70–105 µm in 'Maxima' and 70–109 µm in 'Meres') than that in the epidermal cells (Tab. 2, Fig. 4e,k).

Aerial cavity

In the basal part of the leaf blades, the aerial cavity occupies a substantial surface area (cross section). It has an irregular outline (Fig. 4a,c) and a height and width of 2.2 mm and 5.7 mm, respectively, in 'Maxima' and 1.9 mm and 2.2 mm in 'Meres'. In the medial part of the leaf, the lumen of the aerial cavity is smaller (Tab. 2, Fig. 4b) and extends from the base to 2/3 of the lamina length.

Abaxial side of the midrib

Cells located in the abaxial epidermis of the midrib form protrusions, which are 1.0–1.8-mm thick in 'Maxima' and 0.8–1.3-mm thick in 'Meres' and are oriented towards the aerial cavity. These tissues surround large vascular bundles (3–6; Fig. 4a,c,f). Sclerenchymatous fibres arranged in 2, 3, or 4 rows are visible in the bundles. Laticifers, which are responsible for the release of latex, are situated near the phloem in the midrib (Fig. 4g) and in the leaf blades (Fig. 5d). Between the bundles, there is a strand of cells with a thickness of 0.5–0.7 mm in 'Maxima' and 0.3–0.5 mm in 'Meres' (Tab. 2). It is formed by 7–10 layers which include the epidermis, 4–7 rows of the mesophyll, and a few layers of parenchymal cells with a large diameter (Fig. 4f,i).

Epidermal cells

The cells of both surfaces of the epidermis in the leaf fragments analyzed have a similar height in 'Maxima' (31–38 µm), but are more varied in 'Meres' (22–40 µm; Tab. 2, Tab. 3). The outer cell wall of the epidermis forms serrated protrusions corresponding to the cuticular striae observed in SEM (Fig. 5d,h). The cuticle of the epidermis surface emits

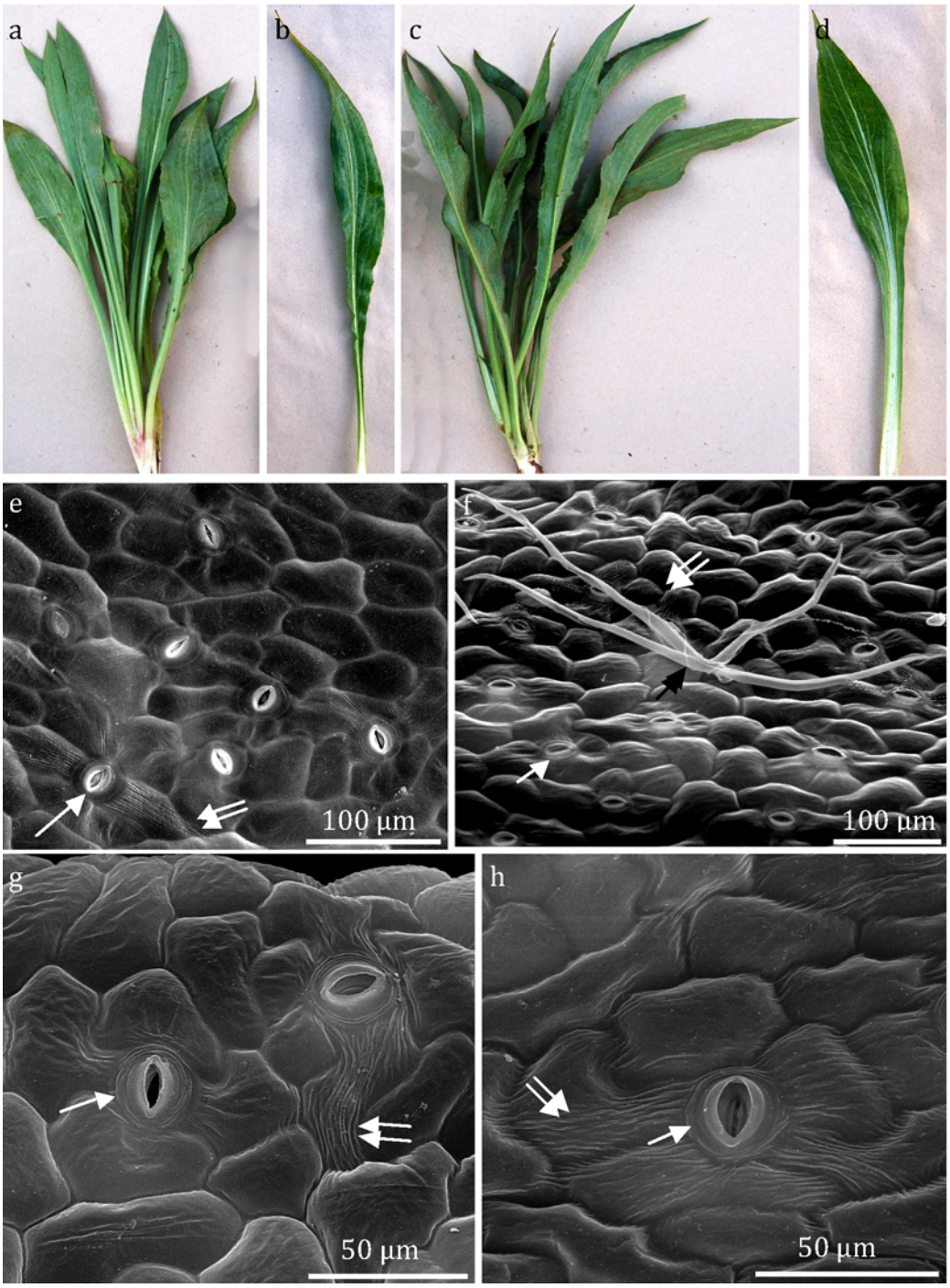

Fig. 2 Leaves (**a–d**) and fragments of the abaxial epidermis surface in the medial part of *S. hispanica* 'Maxima' (**a,b,e,g**) and 'Meres' (**c,d,f,h**). **e–h** SEM. Designations: stomata (arrows), striated cuticle (two arrows), protective trichomes (double-headed arrow).

light green fluorescence (Fig. 5a). Lipid compounds present in the outer cell wall of the epidermis stained orange-red with Sudan Red (Fig. 4f,j,k, Fig. 5b,c).

The outer periclinal cell wall of the epidermis is substantially thicker than the other walls (Fig. 4e,h–k, Fig. 5d–h). Its thickness in the cells of the epidermis covering the midrib and the intervein region of the lamina in the two cultivars analyzed is in the range of 6–10 µm and 3–8 µm, respectively (Tab. 2, Tab. 3). Such thickenings were observed in the cells of the epidermis covering the leaf margin (Fig. 4h, Fig. 5e).

The PAS reaction revealed the presence of polysaccharides in the cell walls (Fig. 4e,i, Fig. 5d–f,h).

Collenchyma

Tangential collenchyma is present in the leaves analyzed. Under the adaxial epidermis over the aerial cavity, a single layer of cells with a collenchymatous thickening of the cell wall is visible (Fig. 4e,k). The collenchyma cells in the short section between the abaxial epidermis and the large vascular bundles located in the midrib form 2–5 cell rows (Fig. 4f,g,j).

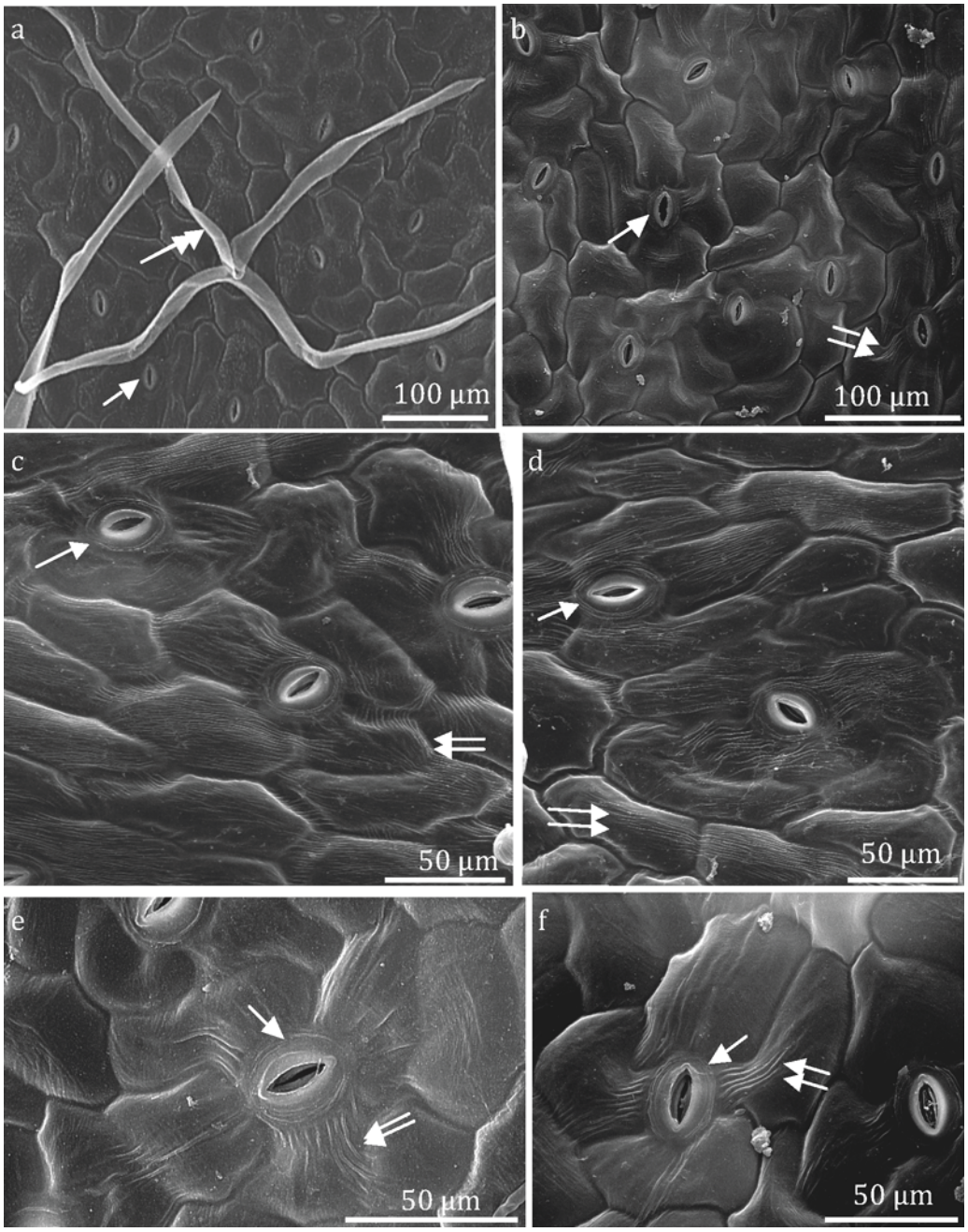

Fig. 3 Fragments of the adaxial epidermis surface in the medial part of *S. hispanica* 'Maxima' (**a,c,e**) and 'Meres' (**b,d,f**) leaves, SEM. Designations the same as for Fig. 2.

The thickness of this strand is 78–118 µm in 'Maxima' and 87–96 µm in 'Meres'. The height of the collenchyma in the leaves of 'Maxima' and 'Meres' is 24–30 µm and 22–25 µm, respectively (Tab. 2). The collenchyma is also situated on both sides of the large vascular bundles in the leaf blade (1–3 layers on the abaxial side and 2–4 below the adaxial epidermis; Fig. 5b–d). This tissue also strengthens the leaf margin and forms 2–4 cell layers (Fig. 4e).

Assimilation parenchyma

The leaves of *S. hispanica* represent the equifacial type. There are two layers of palisade parenchyma cells on both sides of the epidermis (Fig. 5f,g). These cells are higher (50–53 µm) in the leaves of the 'Maxima' cultivar than in 'Meres' (33–44 µm; Tab. 3). Between the palisade parenchyma layers, there are 2–3 layers of spongy parenchyma cells. The number of rows of parenchyma cells increases from the margin to the midrib (cross section).

The assimilation parenchyma cells located in the abaxial epidermis in the midrib form 4–7 layers (Fig. 4f,i). The strand of this parenchyma is interspersed with collenchyma cells situated in the vascular bundles (Fig. 4f,g).

Discussion

The leaves of the *S. hispanica* cultivars analyzed are characterized by a lanceolate shape, a serrate margin, and

Tab. 1 Characteristics of the epidermal surface in *S. hispanica* leaves.

Feature examined		Cultivar			
		'Maxima'		'Meres'	
		Epidermis			
		Adaxial	Abaxial	Adaxial	Abaxial
Length of stomata (μm)		39.87 ±1.82a	41.96 ±2.19a	33.92 ±1.69b	36.01 ±3.45b
Width of stomata (μm)		31.10 ±1.48a	32.58 ±1.22a	28.18 ±1.34b	28.00 ±2.65b
Length of aperture between cuticular ledges (μm)		27.76 ±3.99a	23.69 ±1.64a	16.91 ±1.82b	14.82 ±2.79b
Width of aperture between cuticular ledges (μm)		7.51 ±1.84a	6.11 ±1.05a	5.10 ±0.72b	5.95 ±0.86a
Number of stomata per 1 mm^2		7.93 ±1.30b	11.74 ±2.91b	10.09 ±2.76a	14.33 ±1.15a
Stomatal index (%)		9.81 ±1.24a	11.30 ±1.43a	9.34 ±1.27a	10.80 ±0.92a
Diameter of stomatal complex (μm)	Minimum	119.68 ±17.67a	125.00 ±21.74a	96.32 ±12.54b	96.96 ±12.98b
	Maximum	141.44 ±29.39a	155.52 ±13.72a	111.04 ±15.89b	126.00 ±20.57b

Data show the mean values ±*SE*. Means followed by the same letter do not differ significantly between cultivars in the same leaf fragments.

varied sizes. The morphological features of the leaves are consistent with those reported in the investigations of other taxa from the subtribe Scorzonerinae [32]. Various lamina (linear, lanceolate, ovate, and elliptic) and margin (entire, pinnatifit, or pinnatisect) shapes have been found in other species of the genus *Scorzonera* [33]. Leaf size, shape, and margin are important phenetic traits of plants [34].

The epidermis of the analyzed leaves bears multi-branched protective trichomes. It also exhibits diverse cuticular ornamentation, i.e., smooth or striated on the surface of the guard cells, striated on the cells adjacent to the guard cells, and smooth or striated on other epidermal cells. No data were found about the epidermal cuticle in the leaves of plants from the tribe Cichorieae. Cuticular striae have been found on the leaf surface in various species from the family Asteraceae representing the genera *Vernonia*, *Bidens*, and *Baccharis* [35,36]. A smooth cuticle was reported in *Mikania lanuginosa* leaves [37], whereas varied cuticular ornamentation (smooth, undulating, wrinkled, or striated) was observed in several species of the genus *Cyanus* [38]. As a chemically stable epidermal layer, the cuticle serves a very important function in plant water metabolism and constitutes a mechanical protective barrier against abiotic environmental factors, pathogenic microbial penetration, and pest infestation inside the organ [39–43].

The *S. hispanica* cultivars analyzed as well as the species *S. ahmet-duranii* and *S. semicana* described in the literature have amphistomatic leaves [44]. Anomocytic stomata were found in the leaf epidermis of the investigated cultivars and in other species from the genus *Scorzonera* [45,46]. Staurocytic, brachyparacytic, and amphianisocytic stomata have been observed in different taxa of this genus [47]. Stace [34] reports that the features of the leaf epidermis, e.g., the type of stomata, the pattern of cuticular ornamentation, and the type of trichomes, have taxonomic significance. According to Cutler and Bradham [48], they have a genetic background.

The stomata in the epidermis of the analyzed cultivars have varied sizes (41 μm length / 32 μm width – 'Maxima', 35/28 μm – 'Meres'). Similar sizes of stomata were found in the epidermis of several taxa from the genus *Scorzonera* [47]. The size of stomata has an impact on the efficiency of photosynthesis and is associated with important ecological and physiological specialization of leaves. It is positively correlated with the genome in a wide range of the major taxonomic groups [49].

The adaxial epidermis of the 'Maxima' and 'Meres' cultivars shows a lower number of stomata per 1 mm^2 surface area (8 and 10) than the abaxial epidermis (12 and 14). A similar number of stomata in the epidermis of several species from the genus *Scorzonera* growing in Pakistan were reported by Qureshi et al. [47]. According to these authors, the adaxial epidermis bears 4–12 stomata, while the abaxial epidermis 6–14. In other taxa from this genus occurring in Turkey, the number of stomata on both epidermis surfaces was reported to be considerably greater [44]. Their density is related to the adaptation of plants to the climatic conditions of different regions [50]. The stomatal index in the analyzed *Scorzonera* cultivars is higher in the abaxial than in the adaxial epidermis. A similar relationship in the stomatal index has been described in other species of this genus [45,46]. Its value depends on environmental factors and plant species [51]. These traits of the epidermis are stable and have great diagnostic importance for differentiation between closely related taxa [35,52,53].

The *S. hispanica* cultivars analyzed and other taxa from the genus *Scorzonera* described in the literature [45] produce equifacial leaves with a similar number of palisade parenchyma layers. The midrib of the leaves contains an aerial

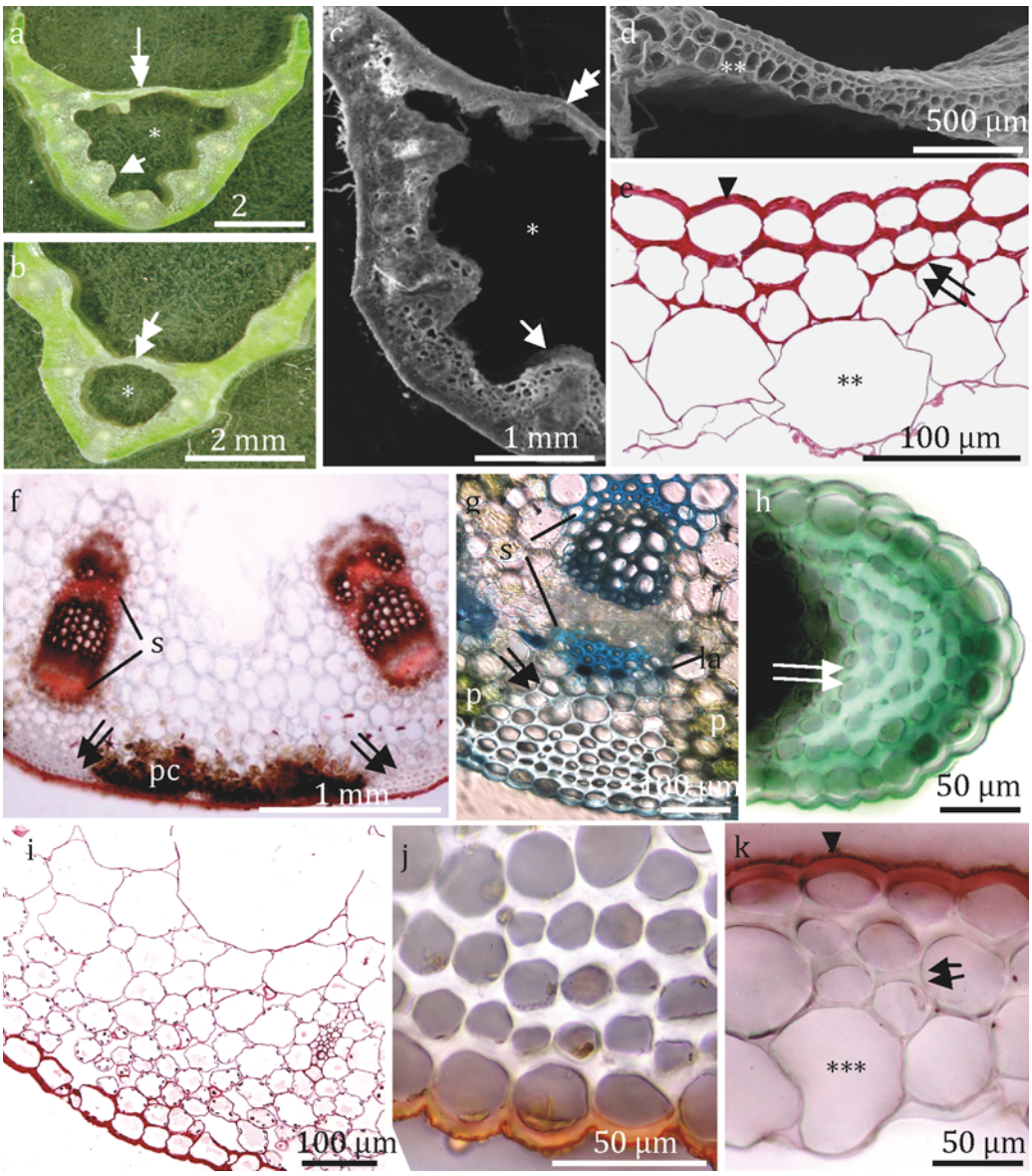

Fig. 4 Fragments of the cross sections in the basal (**a,c–k**) and in the medial (**b**) part of *S. hispanica* 'Maxima' (**a,b,e,f,h,i,k**) and 'Meres' (**c,d,g,j**) leaves. **a–c** Thin base of cells (double-headed arrow), visible tissue invaginations (arrow), irregular (**a,c**) or circular (**b**) aerial canal outline (asterisk). **d,e,k** Adaxial side of the midrib, visible 3–4 cell layers, a thick outer epidermal cell wall (arrowhead), a collenchymatous thickening of the cell wall (two arrows), large mesophyll cells (double asterisk). **f,g** Vascular bundles located within tissue invaginations, sclerenchymatous fibres (sl), collenchyma cells (two arrows), assimilation parenchyma (pc), laticifers (la). **h** Collenchyma cells (two arrows) reinforcing the lamina margin. **i** Epidermal cells with a thick outer wall, visible a stoma and mesophyll cells. **j** Tangential collenchyma cells between the abaxial epidermis and vascular bundle, visible a thick outer wall of epidermal cells with red stained lipid compounds. **a,b,e–k** LM; **c,d** SEM. Staining: **e,i** PAS reaction; **f,j,k** Sudan Red; **g,h** toluidine blue.

cavity extending from the leaf base to 2/3 of its length. It occupies a large area of the midrib in the basal part of the lamina. According to Makbul et al. [45], the presence of the aerial cavity in the leaves is associated with the *Scorzonera* species. Only eight of the 18 taxa of the genus exhibited an aerial cavity surrounded by parenchymal cells in the midrib, while this trait was not noted in the remaining taxa.

The leaves of the analyzed cultivars are strengthened by tangential collenchyma. It is situated in the adaxial epidermis over the aerial cavity (a single layer), between the abaxial epidermis and vascular bundles (2–5 rows) present in the midrib and on both sides of large vascular bundles (1–2 layers of cells below the adaxial epidermis and 2–3 rows under the abaxial epidermis) located in the blade leaf away from the aerial cavity as well as in the lamina margins (3–4 layers).

In the analyzed cultivars, a single collenchyma layer is located under the adaxial epidermis over the large aerial cavity as well as between the abaxial epidermis and large vascular bundles situated in the midrib. Makbul et al. [45]

Tab. 2 Characteristics of midrib tissues (a) in the basal (I) and (II) medial parts of *S. hispanica* leaves.

Feature examined		'Maxima'		'Meres'	
		I	II	I	II
Thickness of leaves in the midrib (mm)		3.11 ±0.16a	1.90 ±0.08a	2.61 ±4.13b	1.15 ±0.08b
Height of epidermal cells (μm)	adaxial	37.70 ±8.28a	32.64 ±2.88b	39.56 ±5.97a	35.84 ± 4.18a
	abaxial	32.77 ±2.27a	30.40 ±1.59a	28.08 ±1.48b	29.02 ±3.33a
Thickness of the outer wall of epidermal cells (μm)	adaxial	7.13 ±0.76b	6.08 ±1.28a	8.77 ±2.40a	5.60 ±1.03a
	abaxial	8.77 ±2.24a	9.92 ±1.59a	5.85 ±0.86a	7.31 ±0.84b
Thickness of the cell strand over		170.40 ±20.88b	116.48 ±16.30a	188.32 ±25.97a	105.92 ±21.96a
Diameter of the largest parenchymal cells adjacent to	aerial cavity (μm)	105.00 ±15.33a	69.60 ±13.67a	108.50 ±6.04a	70.24 ±12.02a
Height	of aerial cavity (mm)	2.17 ±0.13a	1.24 ±0.18a	1.90 ±0.21a	0.77 ±0.13b
Width		5.69 ±0.59a	1.68 ±0.24a	2.24 ±0.21b	0.95 ±0.13a
Thickness of the cell strand located (μm)	in main vascular bundle	1762.51 ±200.59a	1017.19 ±169.79a	1255.56 ±194.93b	763.81 ±137.41b
	between bundles	706.25 ±107.94a	473.44 ±81.33a	453.87 ±71.15b	294.16 ±56.90b
Number of layers	of collenchyma cells located next to main vascular bundle (μm)	3.31 ±0.60b	3.94 ±0.56a	4.38 ±0.5a	3.50 ±0.73a
Thickness of the strand		78.40 ±15.40b	118.06 ±17.45a	95.68 ±14.42a	87.36 ±14.81b
Height		23.66 ±1.34a	30.16 ±3.70a	21.84 ±1.88b	25.30 ±3.01b

Explanations as in Tab. 1.

Tab. 3 Characteristics of leaf blade tissues between veins (b) in the basal (I) and (II) medial parts of *S. hispanica* leaves (μm).

Feature examined		Cultivar			
		'Maxima'		'Meres'	
		I	II	I	II
Thickness of leaf blade		676.56 ±136.1a	361.75 ±28.45a	576.96 ±95.24b	327.62 ±25.81b
Height of epidermal cells	adaxial	34.24 ±4.72b	35.49 ±6.67a	25.86 ±3.12a	24.42 ±3.50b
	abaxial	31.73 ±2.73a	30.90 ±3.91a	27.45 ±1.36b	21.61 ±2.89b
Thickness of the outer wall of epidermal cells	adaxial	5.64 ±0.84b	4.76 ±0.90a	7.62 ±1.22a	5.82 ±0.91a
	abaxial	5.53 ±0.80a	7.31 ±1.20a	5.17 ±0.82a	2.77 ±0.66b
Height of palisade cells from the following side	adaxial	50.88 ±6.12a	50.75 ±6.00a	32.98 ±3.42b	36.32 ±5.22b
	abaxial	53.44 ±7.17a	49.76 ±6.54a	43.84 ±6.26b	37.76 ±2.88b

Explanations as in Tab. 1.

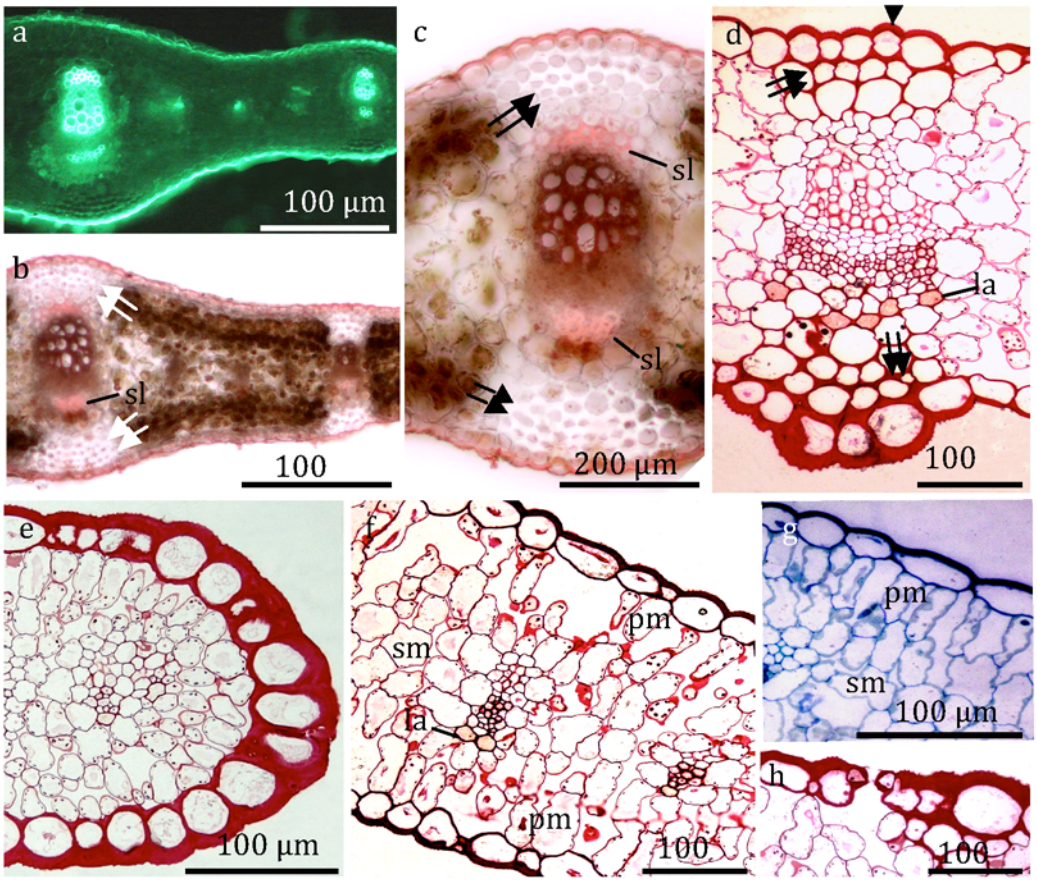

Fig. 5 Cross-sectional fragments of *S. hispanica* 'Maxima' (**b,c,e,f**) and 'Meres' (**a,d,g,h**) leaves in the medial part. **a** Fluorescent epidermal cells and vascular elements. **b,c** Orange-stained cutinised layer of the outer epidermal cell wall, collenchyma cells on both sides of vascular bundles (arrows), sclerenchymatous fibres (sl). **d** Thick outer epidermal cell wall (arrowhead), a collenchymatous thickening of the cell wall (two arrows), laticifers (la). **e** Lamina margin, visible a wall thickening of epidermal cells. **f,g** Palisade mesophyll cells (pm), spongy mesophyll cells (sm), laticifers (la). **h** Stoma, visible protrusions of the outer cell wall. **a** FM; **b–h** LM. Staining: **a** fluorochrome auramine O; **b,c** Sudan Red; **d–f,h** PAS reaction; **g** toluidine blue.

demonstrate that the collenchyma in the leaves of various *Scorzonera* species forms 2–12 layers and is present in the midrib between the main vascular bundle and the abaxial epidermis. Among the 8 taxa of this genus that produce leaves with an aerial cavity, only two were found to have a single-layered collenchyma under the adaxial epidermis. As reported by Leroux [54], in the leaves of many plant species this tissue is located in the large vascular bundles on the phloem side and along the leaf margin, which is consistent with the present results. The author distinguishes perivascular collenchyma (located in the phloem, xylem, or in the circumfascicular position) and peripheral collenchyma (a continuous layer or strands interspersed with mesophyll cells). These types of collenchyma (except for the circumfascicular type) were also observed in this study. Aydin et al. [55] show that the number of collenchyma layers in the stem of *Centaurea* species has taxonomic importance.

The collenchyma functions in peripheral parts of organs exposed to bending and torsion stress. Mechanical stimuli have a stimulating effect on its development expressed by the number of cell layers and the thickness of the cell walls and tissue layer [56]. Stem or leaf bending induced by gusts of wind during growth contributes to thickening and strengthening of collenchyma walls [57,58].

In the analyzed leaves, the thickest layer of this tissue can be found in the vascular bundles located in the midrib. The number of bundles in this location is 4–6. In turn, in other *Scorzonera* taxa the number ranges from 1 to 5 [46].

The micromorphological characteristics of epidermal cell surfaces described in the present study: cuticular ornamentation pattern, anomocytic stomata, their number per unit area and the stomatal index value, can be used in the identification of closely related species. This comparative anatomical study has shown that the single collenchyma layer located under the adaxial epidermis over the large aerial cavity probably has insufficient tensile strength; hence, during heavy rainfalls the leaves crack and break in this place. Also, the large area of the aerial cavity in the midrib in the basal part of the lamina greatly lowers the resistance of the leaf blades to mechanical damage. It is known that some nutrients, for example calcium and boron, affect cell wall reinforcement. Therefore, further studies on their effect on the resistance of *S. hispanica* leaves to mechanical stress are needed.

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Authors' contributions
The following declarations about authors' contributions to the research have been made: research designing: MC, MK, SC, PK; microscopic analysis: MC; photographs SC; conducting experiments: MK, PK; writing the manuscript: MC, MK; statistical analysis: SC, PK.

Competing interests
No competing interests have been declared.

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