

I. STRUCTURAL AND FUNCTIONAL DIVERSITY OF PLANT ORGANISMS

THE COMPARATIVE MORPHO-ANATOMICAL STUDY OF NEW CULTIVARS AND SOME SPECIES OF BLACKBERRY

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Abstract. The morphological and anatomical characteristics of sp. *Rubus fruticosus* L., *R. laciniatus* and new cultivars of blackberry (Thornless Evergreen, Thornfree, Arapaho, Chester), multiplied by *in vitro* micro-technologies in the Laboratory of Embryology and Biotechnology of the Botanical Garden (I) of Academy of Sciences of Moldova, planted in the greenhouse and in the field, were studied. The morpho-technological analysis was performed based on characteristics like: stem position and viability; period of maturation and fruit firmness; plant productivity and adaptability. The study was carried out on a complex of anatomical indices: cuticle type, size, degree of packing of cells, types of trichomes and their distribution, the presence and the way of distribution of calcium oxalate druses, the correlation index of thickness of the upper/lower epidermis, epidermis/mesophyll, leaf/mesophyll. The screening of results has highlighted the morphological and anatomical characteristics of the studied taxa and of those with adaptive characteristics that are suitable to the soil and the climate of the Republic of Moldova, which may provide support for the development of strategies of management of blackberry plantations in our country on more extensive areas.

Keywords: blackberry, species, varieties, morphology, anatomy.

INTRODUCTION

The fruit trees are a valuable fruit plant category, which provides fruits, rich in various natural chemical compounds, for balanced nutrition. Due to the presence of biologically active substances (vitamins, anthocyanins, flavonoids, tannins, organic acids, and alimentary fibers), fruits are required more frequently as a source of raw material for the production of pharmaceuticals, cosmetics and for zoo veterinary [6, 15, 16]. To satisfy the increasing demand, in recent years, breeders have created new cultivars of blackberry with new biological peculiarities (without thorns, erect stem), resistant to drought and frost and with commercially important qualities (transport and storage firmness, large sized fruits, high and special taste qualities, flavors etc.) [2, 6].

For this purpose, in the Laboratory of Embryology and Biotechnology of the Botanical Garden (Institute), biotechnological works were undertaken for determining the optimal conditions for *in vitro* propagation of planting material without viruses, of some new cultivars of blackberry, produced in the USA and in Europe [8], and a field collection was created from 11 new cultivars of blackberry [9].

The purpose of this paper is the morphological and anatomical comparative study of blackberry species and new cultivars derived from them, for elucidating the indices with diagnostic character for identification and those with adaptive role to the climatic conditions in the R. Moldova.

MATERIALS AND METHODS

Blackberry leaves, collected during flowering from the collection of the Botanical Garden (I) of ASM of, have served as botanical material (fresh and dried) for study: spontaneous species *Rubus fruticosus* var. *ulmifolius* Schott (s. Susleni, r. Orhei, R. Moldova) [12], sp. *R. laciniatus*, *Thornless Evergreen* cultivar (cultivated in Romania, Transylvania) and 3 cultivars of blackberry with American origins, *Thornfree* (patented in Moldova) and cultivars *Arapaho*, *Chester*, obtained from sp. *R. fruticosus* [15]. The studied leaves dried naturally, arranged in thin layers in well-ventilated rooms. The anatomical study was conducted according to the classical methods [8, 10, 13]. The microscopic examination was performed at the binocular optical microscope *Micros* (Austria)

with digital camera, coupled to the computer, on the cross sections obtained from fresh/dried leaves and superficially preparations, cleared with chlorhydrate or 3% NaOH [10] at the Department of pharmacognosy and pharmaceutical botany of the State University of Medicine and Pharmacy "Nicolae Testemitanu". The measurements of anatomical structures were performed on cross sections, at 4×, 10× and 40 × magnification. The results were statistically processed by the Statistical Programme 7.

RESULTS AND DISCUSSIONS

The Morpho-Technological Characteristics of Species and Cultivars of Blackberry

The morpho-technological analysis of sp. *R. fruticosus* and *R. laciniatus*, and blackberry cultivars was carried out based on the following indicators: biological features of the stem, technological indices of fruits, the period of maturation of fruits, productivity and adaptability (tab. 1).

The morphology of the studied taxa is similar, only with some differences: all of them are characterized by vigorous aspect of shrubs, exception – *Arapaho* cultivar. The stem position in space is different: upright for *Arapaho* cultivar, repent – specific for sp. *R.laciniatus*, *Thornless Evergreen* and *Thornfree* cultivars; semi-erect – *Chester* cultivar. This biological feature of the plant is an important indicator for the management, location and organization of blackberry plantations. A common feature of all the studied taxa is the presence of leaves in winter too, but *Thornless Evergreen* cultivar differs by even more obvious leaf persistence.

Table 1. The morpho-technological characteristics of species and varieties of blackberry

VARIETY	Biological characters	Morpho-technological characteristics of fruits	Period of maturation (month)	The level of firmness of fruit	Productivity kg/plant	Adaptability in the Republic of Moldova's conditions
<i>Thornless Evergreen</i>	vigorous; repent stem.	weight – 4-5g; shape – spherical; taste – sweetish flavor.	VII-IX	++	4-5	good growth and development; resistant to drought and frost.
<i>Chester</i>	vigorous; semi-erect stem.	weight – 5 g; shape – oval-spherical; taste – sweet-sour.	VIII-IX	+++	5-6	moderate growth and development; resistant to frost.
<i>Arapaho</i>	semi-vigorous; erect stem.	weight - 6-7g; shape - cylindrical; taste - sweet-sour.	VI-VII	+++	5-6	good growth and development; resistant to frost.
<i>ThornFree</i>	vigorous; semi-erect stem.	weight – 5g; shape – oval; taste – sweet-sour.	VI-VIII	++	4-5	good growth and development; resistant to drought and frost.
<i>Rubus fruticosus</i> (spontaneous)	vigorous; repent stem.	weight – 4g; shape – oval; taste – sweetish slightly astringent.	VII-VIII	+	0,5-1	good growth and development; resistant to drought and frost.

Note: the level of firmness of fruit: + - reduced; ++ - moderate; +++ - increased.

The biological and technological characteristics of the fruits are of the greatest interest to growers and consumers. The mass of one fruit ranges from 4 g to 7 g; 4 g – *Thornless Evergreen* cultivar sp. *R. fruticosus* (spontaneous); 5 g – *Chester* cultivar; 6-7 g – *Arapaho* cultivar. The studied taxa also differ in fruit shape: cylindrical – *Arapaho*, spherical – *Thornless Evergreen*, and *R. fruticosus*, oval – *Thornfree* and intermediate, oval-spherical – *Chester*. The fruits possess a broad range of tastes: from sweet (*Thornless Evergreen*) to sweet sour (*Chester* and *Arapaho*) and sweet sour, astringent taste (*Thornfree* cultivar and sp. *R. fruticosus*), which allow the consumer the opportunity to choose blackberry fruits according to preferences and needs.

The blackberry cultivars differ in the fruit maturation period: early – *Arapaho*, followed by *Thornless Evergreen* and *Thornfree* and sp. *R. fruticosus* (spontaneous), the most belated maturation period is characteristic of *Chester* cultivar. The duration of ripening is different, the longest being characteristic of *Thornless Evergreen* and *Chester* cultivars. This index, which characterized these new cultivars, could make fresh blackberry fruits available over a long period, starting from June and ending in September, but the maximum – still remains for August.

The studied taxa develop polydrupe fruits (an aggregate of multiple drupelets), characterized by juiciness, determined by the high degree of vacuolation of mesocarp cells [14]. Thus, drupelets can easily lose their integrity at mechanical manipulations performed during harvesting, sorting, packing, transporting [16]. The presence of sclereids and the endocarp thickness, the degree of compaction of the parenchyma cells and vacuolation ensure fruit quality. The firmness of fruits is determined by their integrity, which is ensured by the development of superficial structures such as the presence of a waxy epicuticular layer, the cuticle thickness and its depth among epidermal cells [1]. The analysis of these anatomical indicators shows that the fruits of *Chester* and *Arapaho* cultivars have the highest firmness (+++), followed by *Thornless Evergreen* and *Thornfree* (++) and those with reduced firmness from the spontaneous sp. *R. fruticosus*.

According to phenological observations made on blackberry plants from the collection created in the field, all taxa show good growth and development in the climatic conditions of our country, only on plants of the *Chester* cultivar, we noted yellowing of leaves during drought. To ensure plant vigor and high productivity, all cultivars require irrigation. Resistance to both drought and frost characterizes the studied taxa, but *Arapaho* and *Chester* cultivars are only frost resistant. According to morphological and technological characteristics, all analyzed cultivars can be recommended for cultivation in large areas of the Republic of Moldova, but need to be considered: the stem position in space, the degree of firmness of fruits, plant productivity and adaptability. Given the results of the comparative morpho-technological study, the most recommended blackberry cultivars would be *Arapaho* and *Chester*.

Anatomical Study

Sp. *R. fruticosus*. The analysis of superficial preparations and cross sections (fig. 1) of the leaf shows that the upper and lower epidermis are single-layered, composed of cells that are well wrapped, polygonal, with external walls slightly thickened. The epidermis is covered by a layer of cuticle, which easily penetrates between the epidermal cells. On the superficial preparation of the upper epidermis, cells with wavy walls were observed. Both epidermises develop unicellular trichomes in cluster and covered with thick membrane. Pubescence is more pronounced on the lower epidermis, trichomes being distributed both along the veins and leaf surface. We note the presence of anomocytic stomata on both epidermises, but, numerically, more on the lower one. Leaf mesophyll is differentiated, dorsal-ventral, the palisade tissue consists of two rows of cells, slightly elongated, well arranged under the upper epidermis. The spongy tissue consists of parenchymal, and lobed cells.

Collateral closed bundles, accompanied by sclerenchymatic sheath, cross the mesophyll. The presence of cells with druses of calcium oxalate, both along the veins and dispersed in the mesophyll, is obvious. In addition, mechanical, collenchyma tissue was observed under both epidermises, on cross sections, in the region of the ribs.

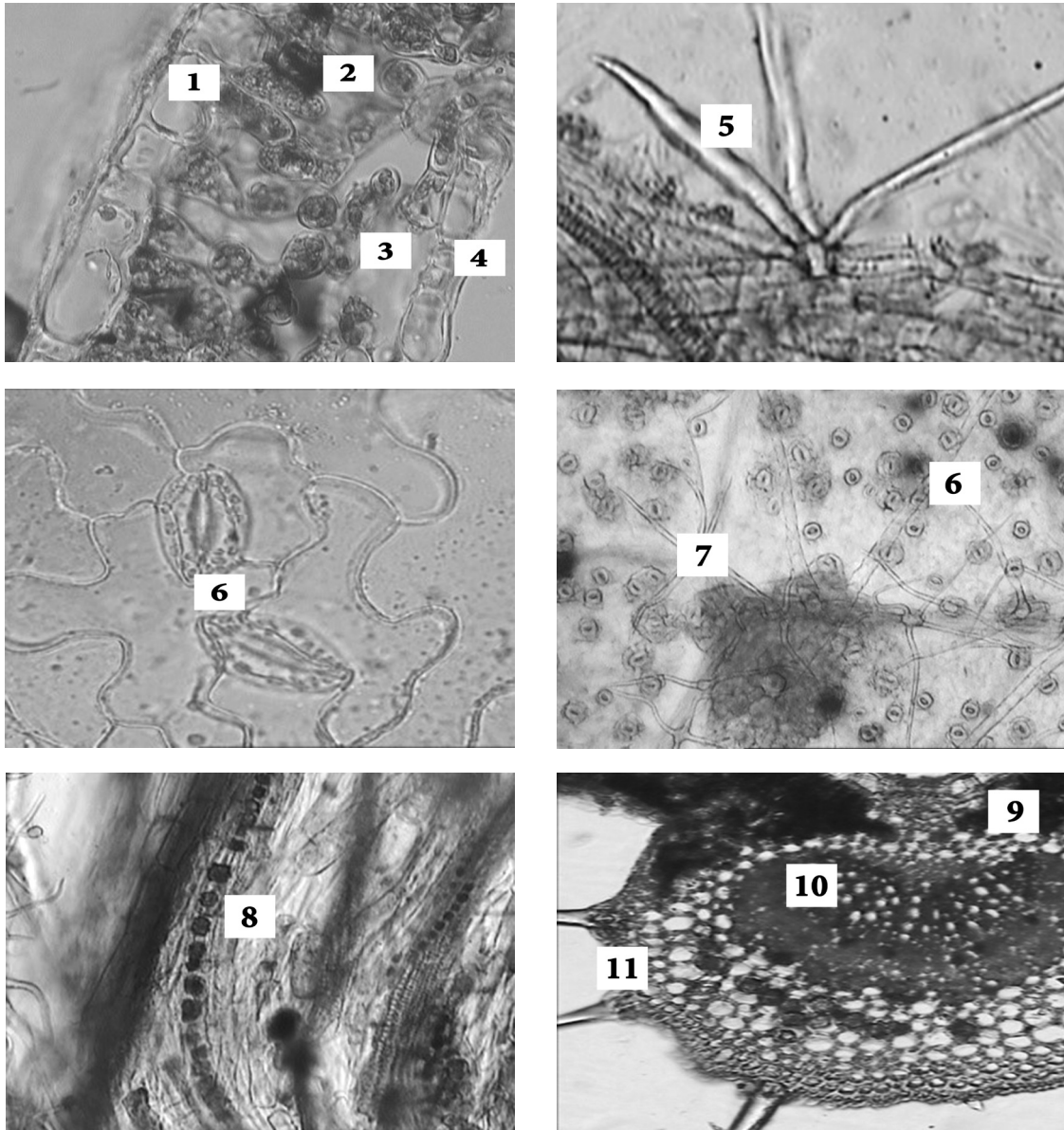


Fig.1. Leaf anatomy of sp. *R. fruticosus*: A (40×) – transversal section of leaf lamina; B (40×), D, E (10×) – superficial preparation of lower epidermis; C (40×) – superficial preparation of upper epidermis; G (40×) – transversal section trough main rib: 1 – upper epidermis, 2 – lower epidermis, 3 – palisade tissue, 4 – spongy tissue, 5 – unicellular trichomes in cluster, 6 – stomates, 7 – unicellular trichomes in cluster (view from above), 8 – druse of calcium oxalate along the vein, 9 – mechanical tissue, 10 – vascular bundle, 11– unicellular trichome.

***Arapaho* cultivar.** The leaves of this cultivar are characterized by the same anatomical structure as the sp. *R. fruticosus*, with only minor differences (fig. 2). Alike, the epidermis consists of compactly arranged cells, but its shape is slightly flattened. We mention the presence of trichomes, unicellular, but solitary. We

distinguish trichomes that are short, straight and thickened at the base and trichomes that are long, slightly curved, included into the basal socket. Cells with an abundance of big druses of calcium oxalate are dispersed in the leaf blade. Spongy tissue is more developed.

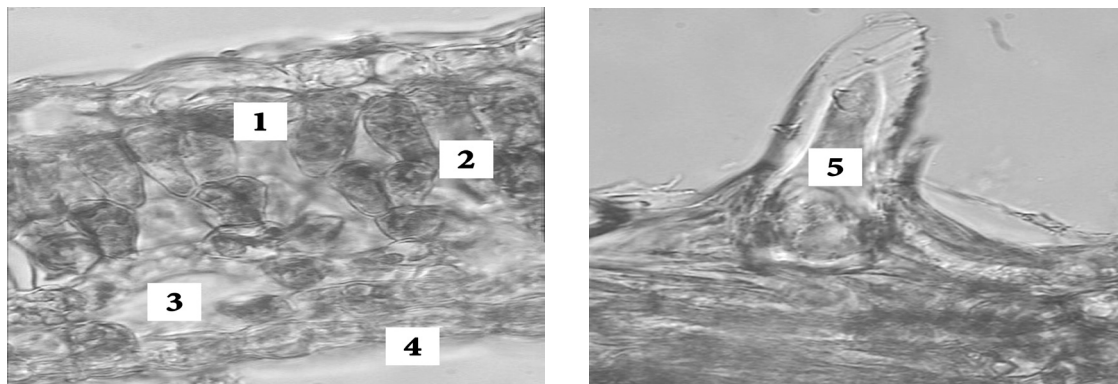


Fig.1. Leaf anatomy of *Arapaho* cultivar: A (40 \times) – transversal section of leaf lamina; B (40 \times) – unicellular trichome in longitudinal section: 1 – upper epidermis, 2 – palisade tissue, 3 – spongy tissue, 4 – lower epidermis, 5 – cellular wall.

***Chester* cultivar.** The microscopic analysis of multiple preparations (fig. 3) shows that the leaves of this cultivar develop the same anatomical type as sp. *R. fruticosus* with some specifications: cuticle does not penetrate between epidermal cells and epidermal cells have oval shape. The pubescence is not so evident, with unicellular, solitary trichomes, but long and rare, entwined and fixed in the basal socket and the calcium oxalate druses are arranged in the sheath of the vascular bundle. Palisade tissue cells are elongated, arranged in two rows with small intercellular spaces, spongy parenchyma cells are lobed with large intercellular spaces.

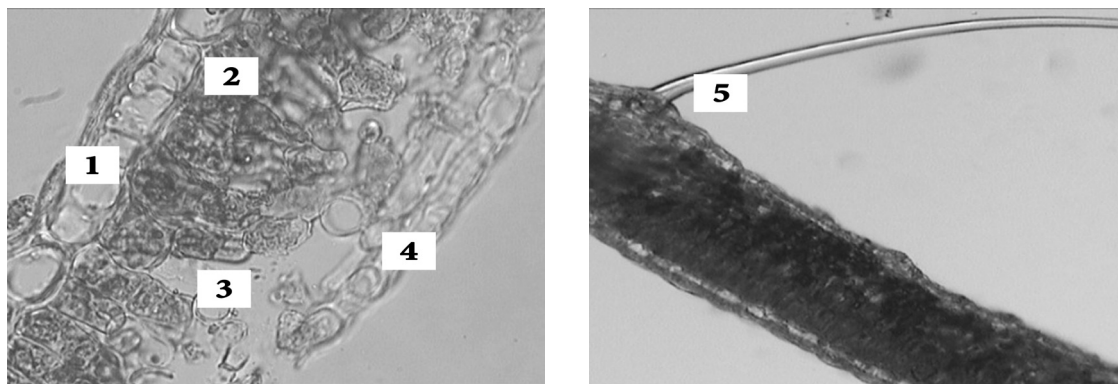


Fig. 3. Leaf anatomy of *Chester* cultivar: A (40 \times), B (10 \times) – transversal section of leaf lamina: 1 – upper epidermis, 2 – palisade tissue, 3 – spongy tissue, 4 – lower epidermis, 5 – unicellular solitary trichome.

Thornfree cultivar. The leaf anatomy of this cultivar (fig. 4) is characterized by the presence of trichomes: in clusters or solitary, long and unicellular, sharp at the peak, relatively large, with a dilated and rounded base, which is included in a multicellular socket with browning content, with more pronounced pubescence. Epidermal cells are isodiametric. There are also druses of calcium oxalate.

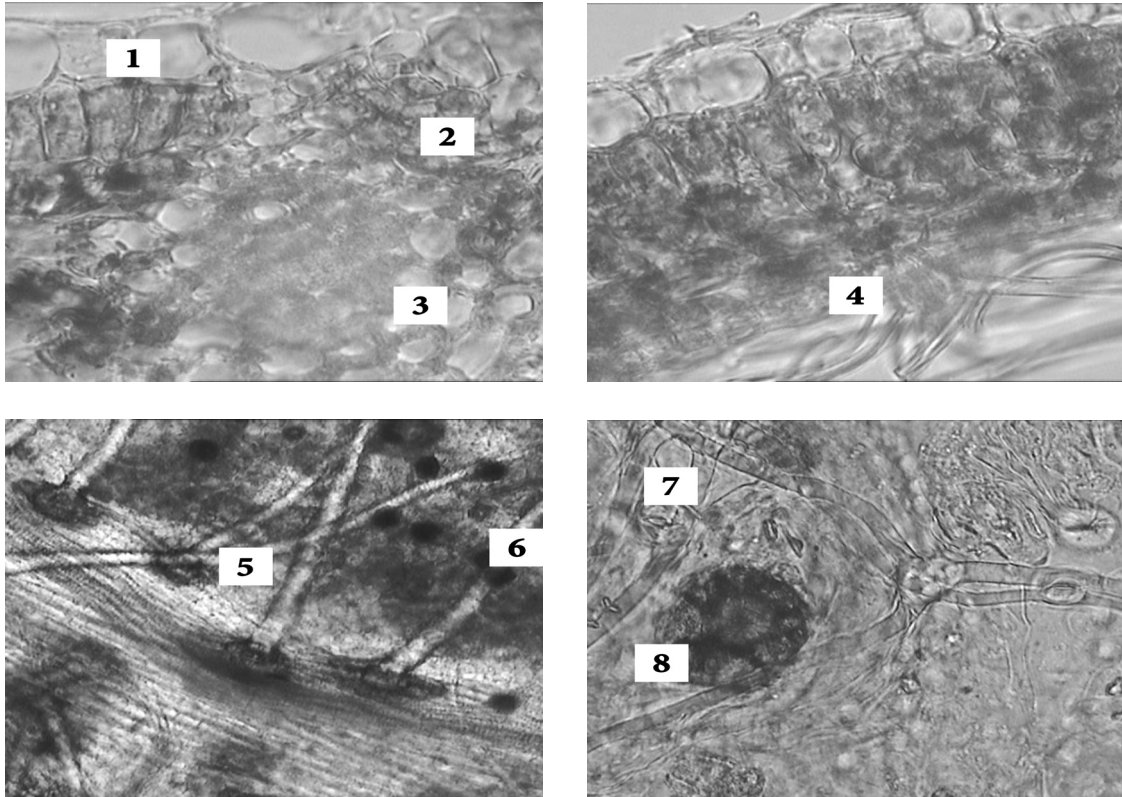


Fig. 4. Leaf anatomy of *Thornfree* cultivar: A, B (40×) – transversal section of leaf lamina; C, D (40×) – superficial preparation of lower epidermis: 1 – upper epidermis, 2 – palisade tissue, 3 – vascular bundle; 4 – trichomes arranged in cluster; 5 – unicellular, solitary trichomes; 6 – calcium oxalate druses, 7 – stomata; 8 – the base of one trichome with brownish content.

***Thornless Evergreen* cultivar (obtained from sp. *R. laciniatus*).** The anatomical structure of the leaf is of dorsal-ventral type, which is characterized by spongy tissue with large intercellular spaces. The cells of both upper and lower epidermises are slightly flattened and well packed. The stomata are present only on the lower epidermis. The upper epidermis consists of cells with regular contour with almost straight sidewalls on the upper epidermis of leaf and tortuous visible walls on the lower epidermis. Tector trichomes are distinguished; they are unicellular, solitary or anastomosed by 2 or 5, often located on veins. The venation is well pronounced on the lower epidermis of the leaf.

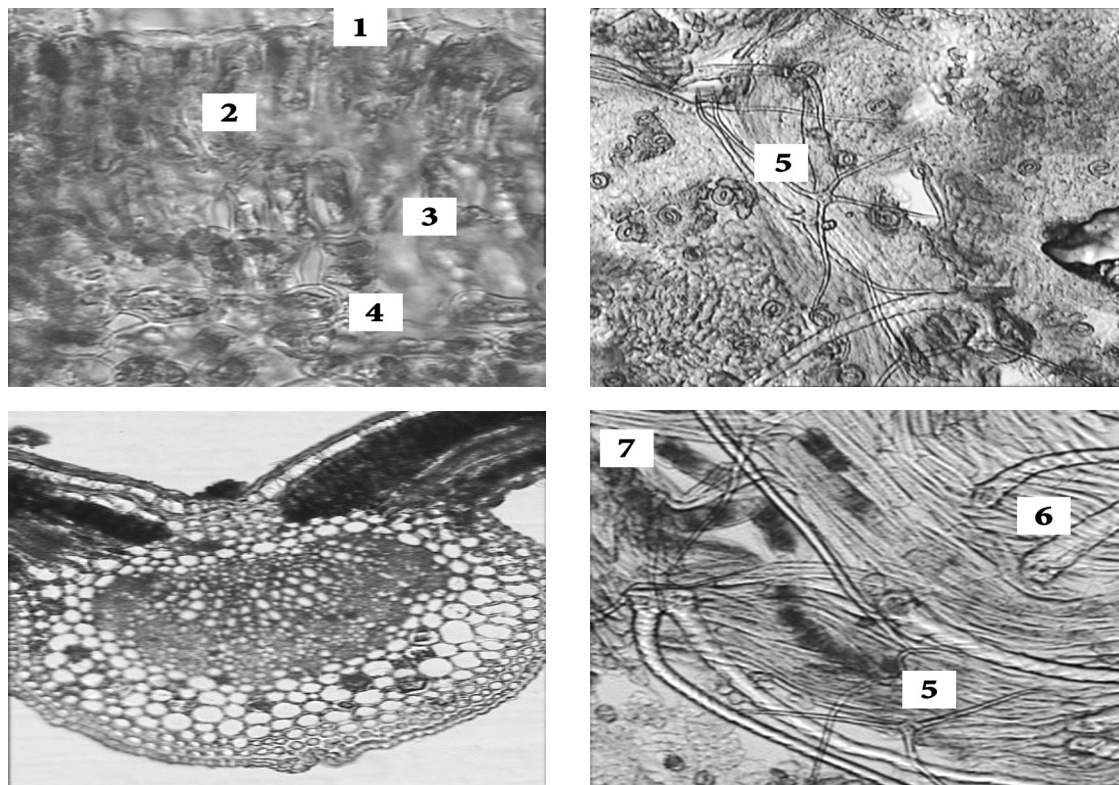


Fig. 5. Leaf anatomy of *Thornless Evergreen* cultivar: A(40 \times) – transversal section of leaf lamina; B, D (40 \times) – superficial preparation of the lower epidermis; C (40 \times) – section trough central vein: 1 – upper epidermis, 2 – palisade tissue, 3 – lacunar tissue; 4 – lower epidermis, 5 – anastomosed trichomes, 6 – unicellular and solitary trichomes; 7 – calcium oxalate druses.

Comparative Anatomical Study of the Leaves of Studied Taxa

The comparative anatomical study was conducted based on the following indicators: the thickness of the leaf, the epidermis (upper and lower), the mesophyll and their correlation; the cuticle type (cuticle penetration between the epidermal cells); the presence, type and distribution of trichomes; the presence and the distribution of stomata; the mesophyll type and anatomical characteristics, the type of vascular bundle, the presence and location of calcium oxalate druses.

According to the results of the analysis of different structural indicators (tab. 2), the lowest values of leaf thickness (114 μm) are in the sp. *R. fruticosus* (spontaneous). The comparative analysis of anatomical parameters of the cultivars of the sp. *R. fruticosus* and *R. laciniatus* shows that, for all cultivars, leaf thickness is greater in plants grown in open field than in the greenhouse. Comparatively, the leaves of *Arapaho* cultivar develop the greatest thickness (240.0 μm), followed by *Thornfree* (191.5 μm), then *Thornfree Evergreen* (170.0 μm) and the smallest – *Chester* cultivar (148.0 μm). A common characteristic of the leaves of the studied taxa is that the epidermis develops cuticle of external-internal type, except the leaves of *Chester* cultivar – with external cuticle type. The extent of the cuticle is an informative index with key role in plant adaptation to environmental conditions and in their resistance against excessive insolation, radiation, increased temperature, and lack of humidity [1, 3, 5], which suggests that the new studied dewberry cultivars have a well-developed protective potential, which provides resistance to unfavorable climatic conditions of the R. Moldova.

Table 2. Anatomical characteristics of the leaves of spontaneous species and new cultivars of blackberry

Species, cultivars	Thickness of leaf ($\mu\text{m}\pm\text{n}$)	Type of cuticle	Thickness of epiderma ($\mu\text{m}\pm\text{n}$)		Thickness of mesophyll ($\mu\text{m}\pm\text{n}$)	The correlation of thickness index		
			Upper	Lower		Upper / lower epidermis	Epidermis /leaf	Mezophyll /leaf
<i>R.fruticosus</i>	114.0 \pm 4.1	External-internal	20.95 \pm 1.51	8.53 \pm 1.19	83.74 \pm 4.23	2.45	3.87	0.73
<i>Arapaho</i> (open ground)	240.0 \pm 14.01	External-internal	44.3 \pm 2.73	35.4 \pm 2.03	168.2 \pm 11.0	1.2	3.0	0.67
<i>Arapaho</i> (greenhouse)	121.0 \pm 7.72	External-internal	29.7 \pm 4.39	21.14 \pm 2.62	71.4 \pm 3.0	1.33	2.32	0.58
<i>Chester</i> (open ground)	148.0 \pm 3.84	External	27.4 \pm 0.87	22.3 \pm 1.13	98.9 \pm 3.50	1.22	2.97	0.66
<i>Chester</i> (greenhouse)	130.0 \pm 6.23	External	26.6 \pm 1.73	22.63 \pm 1.34	77.5 \pm 3.80	1.17	2.64	0.59
<i>Thornfree</i> (open ground)	191.5 \pm 2.62	External-internal	34.3 \pm 1.35	29.1 \pm 0.87	125.0 \pm 2.41	1.17	3.01	0.65
<i>Thornfree</i> (greenhouse)	117.0 \pm 2.03	External-internal	21.4 \pm 1.16	11.88 \pm 0.41	80.0 \pm 5.20	1.8	3.51	0.68
<i>Thornless Evergreen</i> (open ground)	170.0 \pm 7.71	External-internal	35.0 \pm 1.6	27.7 \pm 0.7	107.0 \pm 7.43	1.26	2.7	0.62
<i>Thornless Evergreen</i> (greenhouse)	142.0 \pm 5.4	External-internal	25 \pm 1.5	18.95 \pm 1.5	96.0 \pm 4.54	1.31	3.23	0.67

Note: n – deviation

For all blackberry taxa studied, including the spontaneous sp. *R. fruticosus*, it is specific that the upper epidermis is thicker than the lower. The epidermis with the highest values (i. e., upper and lower epidermis – 44.3 and 35.4 μm) characterizes the leaves of *Arapaho* cultivar. In the lineage, we mention the values of the leaf epidermises of the cultivars *Thornless Evergreen* (35.0 and 27.7 μm), *Thornfree* (34.3 and 29.1 μm). The leaves of the spontaneous sp. *R. fruticosus* have the thinnest epidermises (20.9 and 8.5 μm). However, we must highlight the value of the correlation between the thickness of the epidermises, which is the highest (2.4) in the spontaneous species and twice as high as in the other cultivars *Arapaho*, *Chester*, *Thornfree*, and *Thornless Evergreen* (tab. 2). This is a criterion indicating the anatomical plasticity and flexibility of the epidermis as a first barrier to external factors. It is known that [3, 5, 11, 14] the thickness of the epidermis determines the protection of the leaf mesophyll, and the increased value of the correlation between the thickness of the upper/lower epidermises for sp. *R. fruticosus* is a measure of adaptability to adverse conditions, especially resistance to drought, cold and radiation, which have been very pronounced during the last two decades [1, 3]. For all analyzed cultivars, the upper epidermis is thicker in the plants grown in open ground than in the plants grown

in the greenhouse, which is an indicator of the adaptability of plants to environmental conditions and ensure a more effective protection for the leaf mesophyll from the action of unfavorable conditions in summer and winter.

The leaves of the cultivars of blackberry, cultivated in field, differ from the spontaneous sp. *R. fruticosus* in the thickness of leaf mesophyll, which is better developed than in the latter. Comparatively, the leaves of the field cultivars *Arapaho* (168.2 μm) *Thornfree* (125.0 μm) and *Thornless Evergreen* (107.0 μm) form a thicker mesophyll than *Chester* cultivar (98.9 μm). The values of epidermis and mesophyll thickness correlate with the values of the ratio of mesophyll thickness and leaf thickness. Thus, the highest value is characteristic of the leaves of sp. *R. fruticosus* (0.73), followed by the varieties (decreasingly): *Arapaho* (0.67), *Chester* (0.66) and *Thornfree* (0.65), which shows high photosynthetic assimilation efficiency. According to experimental data, obtained by a group of authors [7, 14], the better the mesophyll is developed, the greater the photosynthetic activity of the plant is. Thus, the varieties of blackberry have a more efficient capacity of assimilation, which is an important source for the development of reproductive organs, which, in turn, ensure the higher productivity of plants, expressed in larger fruits and higher yields (tab. 1).

The leaves of all the analyzed taxa are pubescent, especially on the lower epidermis and along the veins, constituting a protective cover of leaves against insolation and dehydration. A common anatomical feature of taxa is the presence of tector unicellular trichomes. The sp. *R. fruticosus* has trichomes that frequently form clusters, *Arapaho* cultivar – solitary trichomes, short with thick membrane, *Chester* cultivar – long, solitary and rare trichomes, *Thornfree* cultivar – trichomes that are solitary or in clusters, by 2 or 3, with dilated base in the socket of cells with brownish content, *Thornless Evergreen* cultivar – solitary trichomes anastomosed by 2 or 5, often on the veins. These morphological differences of trichomes represent a distinctive anatomical feature that helps to identify the taxa.

Another common anatomical criterion of the studied taxa is the development of anomocytic stomata on both epidermises, mainly on the lower epidermis. The only exception is *Thornless Evergreen* cultivar, with stomata only on the lower epidermis, which represent a distinctive anatomical feature.

The blackberry species and the cultivars derived from them are characterized by the development of salts and the formation of calcium oxalate druses, and their differences relate to the manner of distribution: in the sheath of vascular bundles and in mesophyll (sp. *R. fruticosus*, *Thornfree* cultivar), only in mesophyll (*Arapaho* cultivar) and only in vascular bundle (*Chester* cultivar). In addition to the diagnostic role of the calcium oxalate druses, they have also a physiological role, demonstrated in the last few decades [1, 4], giving them a role in increasing the protective potential of leaves under unfavorable conditions and, especially, responding to the action of stress conditions. Thus, we highlight sp. *R. fruticosus* and *Thornfree* cultivar with a more pronounced capacity of adaptation to the action of unfavorable growth conditions, as compared with other studied taxa of blackberry.

Proceeding from the qualitative and quantitative anatomical data, reported above, the comparative anatomical study of the leaves of taxa, carried out according to a complex of anatomical indicators, shows that all taxa develop a structural adaptive potential to the action of environmental conditions, supplanted by external structures: thick, external-internal cuticle, tector trichomes, the size and the degree of packing of the epidermal cells, and internal ones: the degree of mesophyll development, the presence and the distribution of calcium oxalate druses, the degree of development of mechanical tissue. These structures work synergistically and make up a protector compensator hysto-anatomic complex of leaf to external factors (lack of moisture, high temperatures, of soil and air, during summer and low – in winter). The development of pubescence (the number and the distribution of trichomes on the epidermis) and the calcium oxalate druses (number and location), depending on taxa and growing conditions (field or greenhouse), and reveal structural-adaptive plasticity of the leaf apparatus to the environmental conditions.

CONCLUSIONS

1. The morphological and technological analysis of the studied taxa shows that they differ in stem position in space, vitality, technological characteristics of the fruits (period of maturity, firmness, productivity and the adaptability to the climatic conditions of the R. Moldova).
2. The anatomical comparative study of taxa resulted in the anatomical characteristics of leaves of each taxon and revealed structures with diagnostic character to identify species and new blackberry cultivars (cuticle type, trichome type – simple, short or long, with or without socket and their distribution – solitary, anastomised by 2-3 or in clusters and the location on the epidermis, the distribution of calcium oxalate druses).
3. The anatomical comparative study of the new cultivars and some species of dewberry, grown in open ground and in greenhouse, revealed anatomical indicators, with adaptive character to unfavorable conditions, such as: the degree of pubescence, the degree of development of the epidermis and its derivatives, the correlation index of epidermal/leaf thickness, upper/lower epidermis, mesophyll/leaf, level of development and distribution of the calcium oxalate druses.
4. The morphological, technological and anatomical characteristics, of the new blackberry cultivars and 2 species of g. *Rubus*, may provide complex and conclusive support for the elaboration of strategies for the management of dewberry plantations on large areas in the R. Moldova.

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