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Micromorphological features of the leaf epidermis of the evening primrose cultivars of the VILAR biocollection

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Abstract. Novelty of the work. For the first time, a comparative study of the epidermis of the leaf plates of four cultivars of *Oenothera biennis* L. was carried out. **The aim of the work**. Study and comparison of morpho-anatomical signs of the epidermis of the leaf plate of the cultivars of evening primrose to reveal their potential ecological plasticity. **Materials and methods**. There were used leaves of plants of the second year of life in the phase of mass flowering. Micromorphological study of the epidermis of leaf plates included: determination of the shape of the main cells of the epidermis, the size and number of stomata per 1 mm^2, the type of stomatal apparatus, the presence and parameters of trichomes. The number of trichomes was calculated per 1 mm^2. **Results.** In the *Genoteros* cultivar, the stomata were smaller; their number on the lower epidermis was the lowest. Two types of trichomes were classified. At cultivar *Genoteros* unicellular covering trichomes were mainly located in the upper epidermis of the leaf. No covering trichomes were found in the *Svetlyachok* cultivar. The *Tverskoj* cultivar differs in the length of the hairs: from 774 to 790 microns. Papillary single-celled trichomes on the leaves of the studied cultivar of evening primrose are concentrated on both sides of the epidermis, mostly in the middle part of the leaf, less often - in the mesophyll space. **Conclusion.** Based on the obtained data, the authors established the potential ecological plasticity of the studied varieties of evening primrose for the subsequent study of the influence of stress factors on the described cultivars. The results obtained may be important taxonomic for the correct identification of problematic *Onagraceae* taxa and in further breeding work.

Key words: *Oenothera biennis* L., leaf plate, cultivars, epidermis, stomata, trichomes.

INTRODUCTION

The evening primrose (*Oenothera biennis* L.) is a biennial herb of the evening-primrose family (*Onagraceae* L.). It grows in the central part of the European part of the Russian Federation, in the Crimea, in the Caucasus, in the Ussuriysk Territory, in the Middle Urals, and the Far East (Brem, 2007; Totskaya & Gryaznov, 2019).

The evening primrose has been cultivated in VILAR since 1989. It is well adapted to the conditions of the Nonblack Earth region of the Russian Federation. The evening primrose fruit is a promising source of medicinal raw materials (Vandyshev et al., 2002; Totskaya & Gryaznov, 2019). Seed cultivation technology has been developed for the

Oenothera biennis L. in the Nonblack Earth region of the Russian Federation, along with investigation of the methods of fatty oil separation (Klimakhin et al., 2006; Zalepugin et al., 2006).

Based on the multi-year research results, it is possible to cultivate evening primrose as an oilseed crop in the Nonblack Earth region of the Russian Federation. There are about 50 cultivars of (*Oenothera biennis* L., *Oenothera fruticosa* L., *Oenothera macrocarpa* Nutt.) in the world for various uses (Totskaya & Gryaznov, 2019; UPOV).

The evening primrose is considered as infrequently distributed crop in Russian Federation. Nowadays, there exist four cultivars of the evening primrose (*Oenothera biennis* L.) of all-Russia scientific research institute of medicinal and aromatic plants (FGBNU VILAR, Russia, Moscow) selection (Klimakhin et al., 2014; Gryaznov et al., 2015; Gryaznov et al., 2016; Gryaznov et al., 2021).

Previous studies were conducted to analyze micromorphological peculiar properties of the aboveground and underground organs of *Oenothera biennis* L. in which have been determined the type of leaf space position, the shape of the cells, and the type of trichomes and stomata (Popov et al., 2009; Cheryatova, 2014). Previous researchers have not conducted a comparative study of samples of *Oenothera biennis* L. of different geographical origin. The existing data on the micromorphological features of the epidermis of the leaves of *Oenothera biennis* L. do not reflect the completeness of intraspecific differences. However, the metrological characteristic of the studied epidermal structures of *Oenothera biennis* L. cultivars has not been carried out earlier.

Foliar epidermal features were based on the micromorphology of trichomes types, epidermal cells and stomatal complex. Even though each feature has its own limited taxonomic value but collectively these characteristics may be systematically important especially for the discrimination and identification of complex and problematic taxa (Saba Gul et al., 2019).

Abiotic stress is one of the main limiting factors in crop cultivation worldwide (Asma Ayaz et al., 2021). In the course of breeding work, which consists in creating highly productive and resistant varieties, knowledge about the mechanisms of plant resistance to stress factors is important (Sunera Amna et al., 2020; Fiza Liaquat et al., 2021). The use of morphological features associated with the manifestation of resistance to adverse environmental factors will be important in the selection process. These morphological features include stomata (size, number); leaves (area, shape, growth, orientation, aging, pubescence, wax content in the cuticle) and many other aspects (Lonbani & Arzani, 2011).

It was also noted that among the vegetative organs of plants, the leaf is the most sensitive to changes in abiotic environmental factors. The characteristics of the anatomical structure of the leaf plate may indicate the potential adaptability of the plant to adverse environmental factors (Kuznetsova, 2015).

Therefore, the leaf blades were chosen as the object of study.

This work aims to investigate the leaf plate epidermis anatomical features of the evening primrose cultivars selected by the FGBNU VILAR.

Research objectives:

1. Perform an anatomical analysis of the leaf plate's epidermis of the cultivars;
2. To determine the micromorphological differences of the leaf plates epidermis of the cultivars;
3. To determine the potential ecological plasticity of the studied varieties

MATERIALS AND METHODS

The object of the study was the two-year-old cultivars of the evening primrose plants of the VILAR FGBNU: *Tverskoj*, *Genoteros*, *Svetlyachok*, *Fonarik*.

Seeds of the original population of the cultivar *Genoteros* were obtained from the USA, *Tverskoj* - from the Tver region of Russia, *Fonarik* - from the UK, *Svetlyachok* - from the Czech Republic.

As a result of long-term observations, it was found that even with a successful introduction of the plant, the *Oenothera biennis* L. continue to retain some morphological features inherent in their natural habitats (Mishurov et al., 2005; Totskaya & Gryaznov, 2019; Totskaya & Gryaznov, 2020).

The *Tverskoj* cultivar is based on the native population. The all cultivars of the evening primrose were obtained by individual selection (Fig. 1, a–d). *Svetlyachok* cultivar is approved for decorative use in the State Register of Breeding Achievements. *Fonarik* cultivar of the evening primrose was intended for decorative use (Klimakhin et al., 2014; Gryaznov et al., 2016; Totskaya & Gryaznov, 2019; Gryaznov et al., 2021).

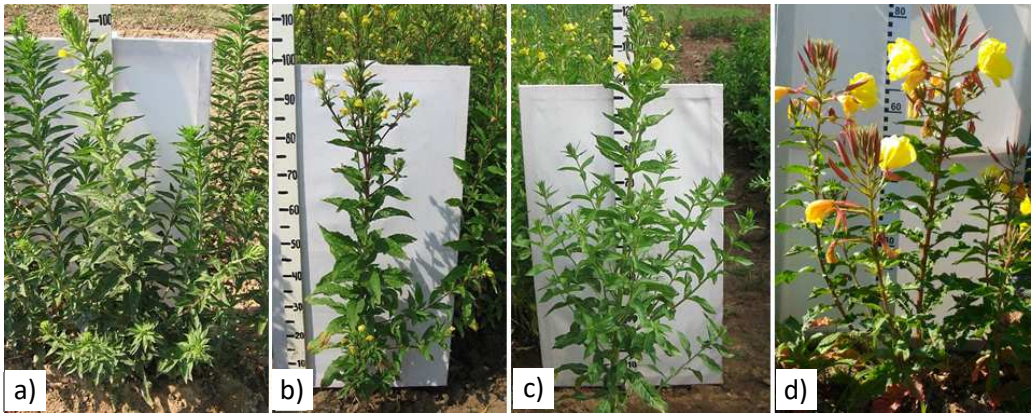

Figure 1. Cultivars of evening primrose. The flowering phase: a) – *Genoteros*; b) – *Tverskoj*; c) – *Svetlyachok*; d) – *Fonarik* [Compiled by the authors].

The research material was collected in the phase of mass flowering, on plants grown from seeds at the collection site of the FGBNU VILAR. The research was carried out on leaf plates of plants of the second year of life. The leaves of the middle part of the shoots were taken from plants in the generative state. Areas of the lower and upper epidermis of the middle part of leaves were studied using temporary specimens (Vorob'yova & Basargin, 2013). The cultivars were examined under light microscope LOMO MIKMED-1. Qualitative and quantitative foliar epidermal anatomical features were examined for both adaxial and abaxial surfaces. Qualitative characters like epidermal cell shape, trichomes type, stomata type and stomata position were examined. Quantitative characters like the length and width of leaf epidermis, stomata, stomatal pore, subsidiary cell and trichomes for both adaxial and abaxial surfaces were studied and measured (Fazal Ullah et al., 2021). The number of cells, stomata, and trichomes in the field of view (0.785 mm^2) was recalculated per 1 mm^2 (Tamahina. & Ahkubekova, 2018). The quantitative parameters and length of trichomes were determined using a

9x Ernst Zeits Wetzlar eyepiece micrometer and an OM-P object micrometer with the main scale length of 1 mm. The samples were prepared according to the methods for light microscopy (Barykina et al., 2004). The description of the evening primrose varieties has been compiled according to the UPOV methodology.

Analysis of microscopic characters of raw materials, morphometric and histochemical studies were carried out according to the methods of the State Pharmacopoeia of the Russian Federation XIV edition and photographed with a 14.0 Mp USB 2.0 C-Mount camera. The sample consisted of 10 measurements; statistical processing was performed in Microsoft Excel. For each studied character, its mean value (M), the error of the mean (m), and the coefficient of variation (Cv) were determined. The anatomical parameters of the epidermis are considered low variable if the coefficient of variation Cv is less than 20%, moderately variable - with Cv > 20%, highly variable - with Cv > 40%. The types of stomatal apparatus were determined according to the special classification (Baranova, 1985), the description of trichomes was done according to the methodology (Aneli, 1975).

RESULTS AND DISCUSSION

It was previously established that the leaves of evening primrose are dorsoventral, amphistomatic. The upper epidermis of the leaf has polyhedral cells, the anticline walls are slightly branched, the trichomes are simple single-celled hairs with a rosette at the base of 7–8 elongated cells and smaller hairs in the shape of a head. Stomata are quite common; the type is anomocytic and anisocytic. The lower epidermis has strongly convoluted anticlinal walls, stomatal apparatus of the anomocytic and anisocytic types, and a large number of simple unicellular hairs were found (Cheryatova, 2014).

The walls of the abaxial epidermis cells of the leaf plates of the evening primrose studied varieties are of rounded-sinuous shape. The cells of the adaxial epidermis are rounded-elongated. The stomatal apparatus is of an anomocytic type. Leaf plates of *Genoteros*, *Tverskoj*, *Fonarik* cultivars are covered with agranular trichomes on the lower and upper epidermis. Trichomes are simple, unicellular, conical in shape, 80 to 190 microns long, located mainly along the veins. Epidermal cells forming a rosette are located around the attachments of cone-shaped pointed trichomes. In some areas of the interveinal space of the *Genoteros* and *Tverskoj* cultivars, a dense localization of trichomes was noted (12–15 pcs mm^{-2}). Papillary unicellular trichomes were also found in the interveinal space of the central part of the evening primrose leaves of *Genoteros, Svetlyachok*, and *Fonarik* cultivars.

Genoteros. The walls of the epidermal cells are sinuous. The size of the stomata on the upper epidermis of the leaf is 30.21 ± 2.76 μm long, 26.34 ± 2.15 μm wide. The number of stomata per 1 mm^2 is 458.67 ± 38.81 pcs. The size of the stomata on the lower epidermis of the leaf has a length of 31.72 ± 3.38 μm and width of 22.18 ± 2.45 μm. The number of stomata per 1 mm^2 comprises 560.57 ± 39.18 pcs.

On the abaxial side of the leaf, papillary unicellular trichomes (2–4 units mm^{-2}) and unicellular cone-shaped trichomes (6–8 units mm^{-2}) 300–500 μm long were found. On the adaxial side of the leaf, cone-shaped trichomes (10–12 pcs mm^{-2}) up to 600 μm in length were revealed. Papillary unicellular trichomes are detected on the lower epidermis (Fig. 2, Tables 1 and 2).

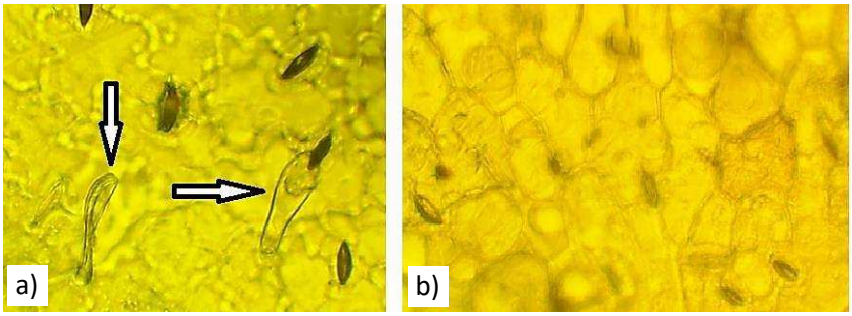

Figure 2. Lower (a) and upper (b) side of the epidermis of the evening primrose's leaf plate *Genoteros* Cultivar. The arrows show papillary unicellular trichomes. Magnification ×280 [Compiled by the authors].

The epidermis of the leaf plate in the *Tverskoj*, *Svetlyachok* and *Fonarik* cultivars are distinguished by large stomata. The stomata of the *Genoteros* cultivar are smaller, their number on the lower epidermis is the lowest: 560.57 ± 39.18 pcs mm^{-2}. The upper epidermis of *Tverskoj* and *Fonarik* cultivars have the lowest stomatal index: 20.45–20.47% (Table 1).

Table 1. Quantifiable and morphometric parameters of the epidermis of the cultivars evening primrose's leaf plate

Cultivars	Side of leaves	Parameters				
		Number of cells, pcs mm^{-2}	Number of stomata, pcs mm^{-2}	S_I, %*	Stomata l ength, microns	Stomata breadth, microns
Tverskoj	upper	1,630.57 ± 144.18	419.88 ± 41.05	20.47	40.22 ± 3.96	31.8 ± 2.54
	Cv%	10.84	19.22		15.09	18.24
	lower	1,704.31 ± 158.96	715.26 ± 63.55	29.56	42.75 ± 3.67	33.85 ± 2.17
	Cv%	10.2	25.94		17.7	11.46
Genoteros	upper	1,579.61 ± 107.22	458.67 ± 38.81	22.5	30.21 ± 2.76	26.34 ± 2.15
	Cv%	21.43	8.56		9.82	10.93
	lower	1,732.48 ± 110.57	560.57 ± 39.18	24.46	31.72 ± 3.38	22.18 ± 2.45
	Cv%	23.22	8.69		6.49	10.12
Svetlyachok	upper	1,477.07 ± 13.86	456.81 ± 41.28	23.62	40.06 ± 4.11	31.79 ± 3.52
	Cv%	26.32	20.71		23.11	19.18
	lower	1,936.13 ± 19.05	613.29 ± 58.73	24	41.08 ± 3.96	34.75 ± 3.66
	Cv%	28.46	26.83		21.13	19.48
Fonarik	upper	1,783.44 ± 16.92	458.59 ± 40.19	20.45	38.16 ± 3.91	32.57 ± 3.08
	Cv%	10.68	19.82		22.24	23.01
	lower	1,885.42 ± 18.09	611.46 ± 54.13	24.48	43.81 ± 4.15	34.63 ± 3.31
	Cv%	13.27	22.07		22.3	23.28

Note: upper – leaf plate upper epidermis; lower – leaf plate lower epidermis; *Stomatal Index, % [Compiled by the authors].

Tverskoj. The walls of the epidermal cells are sinuous. The size of the stomata on the upper epidermis of the leaf is 40.22 ± 3.96 μm long, 31.8 ± 2.54 μm wide. The number of stomata per 1 mm² is 419.88 ± 41.05 pcs. The size of the stomata on the lower

epidermis of the leaf has a length of 42.75 ± 3.67 µm and width of 33.85 ± 2.17 µm. The number of stomata per 1 mm² comprises 715.26 ± 63.55 pcs.

Table 2. Morphometric characteristics of simple unicellular hairs of the epidermis of the varieties evening primrose's leaf plate

Cultivars	Side of leaves	Parameters Number of cone-shaped trichomes, pcs mm^{-2}	Length of cone-shaped trichomes, microns	Number of papillary trichomes, pcs mm^{-2}	Length of papillary trichomes, microns
Tverskoj	upper	8.12 ± 0.77	780.23 ± 60.52	Not available	Not available
	Cv%	28.13	41.17		
	lower	9.25 ± 0.83	774.36 ± 47.61	5.87 ± 0.42	57.24 ± 5.82
	Cv%	23.57	38.83	5.88	4.23
Genoteros	upper	11.76 ± 1.71	600.83 ± 41.68	Not available	
	Cv%	44.02	36.21		
	lower	6.55 ± 1.03	398.67 ± 36.89	4.64 ± 0.45	60.27 ± 6.03
	Cv%	32.55	31.81	6.04	4.89
Svetlyachok	upper	Not available	Not available	3.89 ± 0.27	71.02 ± 6.49
	Cv%			5.15	11.03
	lower	Not available	Not available	3.69 ± 0.26	70.93 ± 6.44
	Cv%			6.35	11.5
Fonarik	upper	6.97 ± 0.52	210.02 ± 18.39	4.50 ± 0.48	64.28 ± 6.04
	Cv%	13.7	19.49	6.22	4.72
	lower	6.39 ± 0.48	283.06 ± 27.96	3.61 ± 0.57	63.85 ± 6.24
	Cv%	14.1	20.94	5.38	5.08

Note: upper – leaf plate upper epidermis; lower – leaf plate lower epidermis [Compiled by the authors].

On the upper and lower sides of the leaf, unicellular cover trichomes were found, the length of which varies from 774 µm to 780 µm (mainly along the veins on the lower side of the leaf) (Fig. 3, Tables 1 and 2).

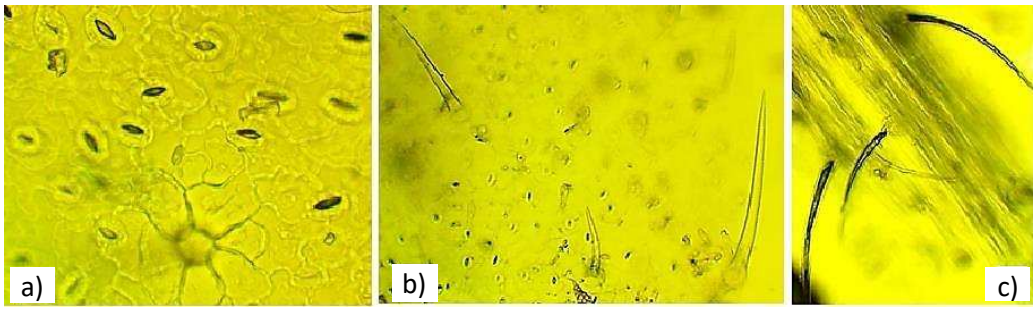

Figure 3. a) – stomata and base of the trichome (magnification ×280). The upper epidermis of the evening primrose's leaf plate the *Tverskoj* cultivar; b) – covering trichomes and stomata. Magnification ×280; c) – The lower epidermis of the leaf of the evening primrose of the *Tverskoj* cultivar, trichomes along the veins (magnification ×70) [Compiled by the authors].

Svetlyachok. The walls of the epidermal cells are sinuous. The size of the stomata on the upper epidermis of the leaf is 40.06 ± 4.11 µm long, 31.79 ± 3.52 µm wide. The number of stomata per 1 mm² is 456.81 ± 41.28 pcs. The size of the stomata on the lower

epidermis of the leaf has a length of 41.08 ± 3.96 μm and width of 34.75 ± 3.66 μm. The number of stomata per 1 mm² comprises 613.29 ± 58.73 pcs.

A few papillary unicellular trichomes, 3–4 pcs mm^{-2}, were found on the adaxial side of the leaf (Fig. 4, Table 1 and 2).

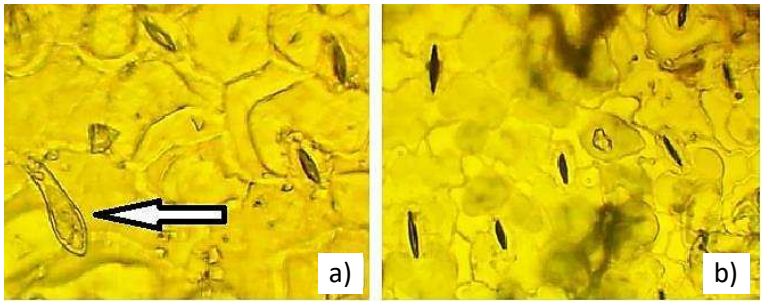

Figure 4. a) – the upper epidermis of the evening primrose's leaf plate, *Svetlyachok* cultivar. The arrow shows a papillary single-celled trichome; b) – the lower epidermis of the leaf of the *Svetlyachok* cultivar. Magnification ×280. [Compiled by the authors].

Fonarik. The walls of the epidermal cells are sinuous. The size of the stomata on the upper epidermis of the leaf is 38.16 ± 3.91 μm long, 32.57 ± 3.08 μm wide. The number of stomata per 1 mm² is 458.59 ± 40.19 pcs. The size of the stomata on the lower epidermis of the leaf has a length of 43.81 ± 4.15 μm and width of 34.63 ± 3.31 μm.

On the upper and lower sides of the leaf, a few (4–6 pcs mm^{-2}) unicellular cover trichomes were found, the length of which is about 200–300 μm. A few papillary unicellular trichomes were revealed on both sides of the leaf (Fig. 5, Tables 1 and 2).

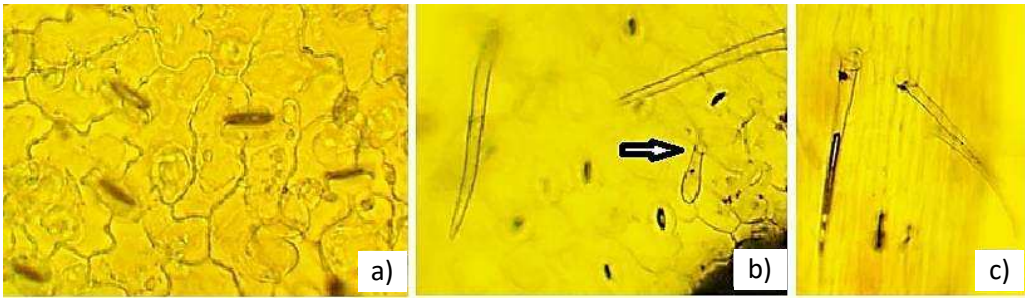

Figure 5. The upper epidermis of the evening primrose's leaf plate, the *Fonarik* cultivar: a) – stomata; b) – cone-shaped and papillary single-celled trichomes (arrow). The lower epidermis of the leaf of the evening primrose *Fonarik*: c) – trichomes along the veins. Magnification ×280 [Compiled by the authors].

The coefficient of variation in the number of cells per 1 mm^2 on the upper and lower epidermis is low in *Tverskoj* and *Fonarik* cultivars, while in *Genoteros* and *Svetlyachok* cultivars this indicator is less variable. The coefficient of variation in the number of stomata per 1 mm^2 on the upper and lower epidermis in *Genoteros* is not very variable. The average length and width of stomata vary on both sides of leaves in *Svetlyachok* and *Fonarik*. The variability of the values of the number of cone-shaped trichomes of the

upper epidermis in the *Genoteros* cultivar is quite significant. In the *Tverskoj* cultivar, the indicator of the length of the cone-shaped trichomes of the upper epidermis also has a high coefficient of variation. *Fonarik* is peculiar for low values of the number and length of the cone-shaped trichomes of the upper and lower epidermis. For the remaining characters of the epidermis (the number and length of papillary unicellular trichomes), all studied varieties showed insignificant variability (Tables 1, 2). *Genoteros* and *Tverskoj* cultivars demonstrate medium and *Fonarik* - weak pubescence.

Morpho-anatomical characteristics of the leaves allow assessing the adaptive capabilities of plants: a limited number of stomata and their small size lead to the constant opening of stomata and excessive transpiration, which indicates low adaptability of plants to light and humidity conditions (Vinogradova et al., 2020). An increase in the number of trichomes may be due to the manifestation of the mechanism of the leaves' protection from overheating, lack of moisture, and other stress factors (Ahkubekova & Tamahina, 2020).

Micromorphological differences between cultivars can be explained by the different origins of the original populations on the basis of which the cultivars were created. Field conditions everywhere differ from the places of natural growth of plants: large open spaces, rapidly drying soil-and at the same time-the absence of competition and shading. Leaf pubescence is one of the main signs of the plant's protective reaction to arid conditions.

The walls of the abaxial epidermis cells of the leaf plates of the evening primrose studied varieties are of rounded-sinuous shape. The cells of the adaxial epidermis are rounded-elongated. The stomatal apparatus is of an anomocytic type. Leaf plates of *Genoteros*, *Tverskoj*, *Fonarik* cultivars are covered with agranular trichomes on the lower and upper epidermis. Epidermal cells forming a rosette are located around the attachments of cone-shaped pointed trichomes. This does not contradict early research (Cheryatova, 2014). Trichomes are simple, unicellular, conical in shape, 80 to 190 microns long, located mainly along the veins.

On the evening primrose leaves of the studied cultivars, the covering trichomes are located mainly along the veins. In *Tverskoj* and *Fonarik* cultivars, their number on the upper and lower epidermis is practically equal. In *Genoteros* cultivar, the covering unicellular trichomes are predominantly located on the upper epidermis of the leaf. Covering trichomes were not found in the *Svetlyachok* cultivar. The papillary unicellular trichomes on the leaves of the studied cultivars of the evening primrose are concentrated in the interveinal space, on the upper and lower epidermis, mainly in the middle part of the leaf.

Papillary trichomes have not been previously detected or described. Papillary unicellular trichomes were also found in the interveinal space of the central part of the evening primrose leaves of *Genoteros, Svetlyachok*, and *Fonarik* cultivars.

The leaves of *Svetlyachok* without of trichomes presumably indicate that insect pests may have been absent or rarely encountered in the places of its original growth.

And so we see that a cultivar that is not from European region continues to have a stress despite a long introduction.

The greater number of stomata in the *Tverskoj* cultivar may indicate a significant potential for photosynthetic activity.

CONCLUSIONS

For the first time, the anatomical differences between new cultivars of the evening primrose of the VILAR biocollection have been studied. The analysis showed that the cultivars of the evening primrose differ not only in the habitus of the bush, indicators of productivity, and the direction of economic use but also in a different number of stomata, and quantity of epidermal cells. The degree of variability of quantitative and morphometric indicators of pubescence in the *Svetlyachok* and *Fonarik* cultivars is lower than that in the *Tverskoj* and *Genotero*s. Number of stomata in plants of the *Genoteros* cultivar is low and their size is smaller in comparison with other studied cultivars, while the number of trichomes is much higher. All these features may indicate the weak potentional ecological flexibility of this cultivar. This should be taken into account when cultivating it. The results obtained may be important taxonomic for the correct identification of problematic *Onagraceae* taxa and in further breeding work.

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REFERENCES

Ahkubekova, A.A. & Tamahina, A.Ya. 2020. Adaptive features of the leaf epidermis of representatives of the *Boraginaceae* family. *Izvestiya Gorskogo gosudarstvennogo agrarnogo universiteta* **57**(2), 188–195 (in Russian).

Aneli, N.A. 1975. *Atlas of the leaf epidermis*. Metsniereba, Tbilisi, Georgia, 112 pp. (in Russian)

Asma, Ayaz, Saddam, Saqib, Haodong, Huang, Wajid, Zaman, Shiyou, Lü, Huayan, Zhao. 2021. Genome-wide comparative analysis of long-chain acyl-CoA synthetases (LACSs) gene family: A focus on identification, evolution and expression profiling related to lipid synthesis. *Plant Physiology and Biochemistry* **161**, 1–11. https://doi.org/10.1016/j.plaphy.2021.01.042

Baranova, M.A. 1985. Classification of morphological types of stomata. *Botanical Journal* **70**(12), 1585–1595 (in Russian).

Barykina, R.P., Veselova, T.D., Devyatov, A.G., Jalilova, Kh. Kh., Ilina, G.M. & Chubatova, N.V. 2004. *Reference book on Botanical micro-technology. Fundamentals and methods.* MSU Publishing House, Moscow, Russia, 312 pp. (in Russian).

Brem, A. 2007. *Life of plants. The newest botanical encyclopedia*. Eksmo, Moscow, Russia, 976 pp. (in Russian).

Cheryatova, Yu. S. 2014. Microscopic examination of the leaves of *Oenothera biennis* L. In: Proc. *International Scientific and Practical, Abstract Book in 11 parts*, Tambov, Russia, pp. 148–150 (in Russian).

Fazal Ullah, Asma Ayaz, Saddam Saqib, Gaurav Parmar, Saraj Bahadur, Wajid Zaman. 2021. Taxonomic implication of leaf epidermal anatomy of selected taxa of *Scrophulariaceae* from Pakistan. *Microscopy Research & Technique* **84**(3), 521–530. https://doi.org/10.1002/jemt.23608

Fiza Liaquat, Muhammad Farooq Hussain Munis, Samiah Arif, Urooj Haroon, Jianxin Shi, Saddam Saqib, Wajid Zaman, Shengquan Che and Qunlu Liu. 2021. PacBio Single-Molecule Long-Read Sequencing Reveals Genes Tolerating Manganese Stress in *Schima superba* Saplings. *Front. Genet.* https://doi.org/10.3389/fgene.2021.635043

Gryaznov, M.Yu., Klimakhin, G.I., Maslyakov, V.Yu., Pimenova, M.E., Semikin, V.V., Totskaya, S.A. & Fonin, V.S. 2015. *Oenothera biennis* L. Cultivar *Tverskoj*. Russian Federation; breeding achievement patent number 7931.

Gryaznov, M.Yu., Makarova, N.V., Savchenko, O.M. & Tockaya, S.A. 2021. *Oenothera biennis* L. Cultivar *Fonarik*. Russian Federation; breeding achievement patent number 11334.

Gryaznov, M.Yu., Makarova, N.V., Romashkina, S.I. & Totskaya, S.A. 2016. *Oenothera biennis* L. Cultivar *Svetlyachok.* Russian Federation; breeding achievement patent number 9255.

Klimakhin, G.I., Semikin, V.V. Totskaya, S.A., Fonin, V.S., Sidel'nikov, N.I. & Khazieva, F.M. 2014. *Oenothera biennis* L. Cultivar *Genoteros*. Russian Federation; breeding achievement patent number 7291.

Klimakhin, G.I., Semikin, V.V., Fonin, V.S., Pimenov, K.S., Golubev, V.P., Kalinina, N.G. & Yershova, G.A. 2006. In: Proc. *Conference Dynamics of the formation of fatty oil in the seeds of the perennial evening primrose in the conditions of culture, Abstract Book*. All-Russian Research Institute of Medicinal Plants, Moscow, Russia, pp. 79–82. (in Russian).

Kuznetsova, T.A. 2015. Adaptations of the leaf surface of *Ribes nigrum* L. depending on the conditions of water supply. *Belgorod State University Scientific Bulletin. Natural sciences* **9**(206), 29–36 (in Russian).

Lonbani, M. & Arzani, A. 2011. Morpho-physiological traits associated with terminal droughtstress tolerance in triticale and wheat. *Agronomy Research* **9**(1–2), 315–329.

Mishurov, V.P., Portnyagina, N.V. & Savinovskaya, N.S. 2005. Ontogenesis of the evening primrose during introduction in the Middle taiga subzone of the Komi Republic. *Bulletin of the Institute of Biology of the Komi Scientific Center of the Ural Branch of the Russian Academy of Sciences* **6**(92), 6–9 (in Russian).

Popov, A.I., Shpan'ko, D.N., Cherno, V.A., Cherkasova, E.A. & Shajdulina, T.B. 2009. Morphological and anatomical study of ornamental plants with medicinal properties. *Tekhnika i tekhnologiya pishchevyh proizvodstv* **4** (15), 74–78 (in Russian).

Saba Gul, Mushtaq Ahmad, Muhammad Zafar, Saraj Bahadur, Shazia Sultana, Shomaila Ashfaq, Fazal Ullah, Omer Kilic, Fayyaz ul Hassan, Zafar Siddiq. 2019. Foliar epidermal anatomy of *Lamiaceae* with special emphasis on their trichomes diversity using scanning electron microscopy. *Microscopy Research & Technique* **82**(3), 206–223. https://doi.org/10.1002/jemt.23157

State Pharmacopoeia of the Russian Federation XIV edition. 2018. FS 1.5.3.0003.15, FS 1.5.1.0002.15 Available from: http://femb.ru/feml. [date of request 01.07.2020]

Sunera Amna, Saddam Saqib, Siraj Uddin, Wajid Zaman, Fazal Ullah, Asma Ayaz, Mehrina Asghar, Shafiq ur Rehman, Muhammad Farooq Hussain Munis, Hassan Javed Chaudhary. 2020. Characterization and phytostimulatory activity of bacteria isolated from tomato (*Lycopersicon esculentum* Mill.) rhizosphere. *Microbial Pathogenesis* **140**, 103966. https://doi.org/10.1016/j.micpath.2020.103966

Tamahina, A.Ya. & Ahkubekova, A.A. 2018. Micromorphological features of the epidermis and histochemical methods for identifying secondary metabolites in the leaves of herbaceous plants of the *Boraginaceae* family. *South of Russia: ecology, development* **13**(3), 31–41. doi:10.18470/1992-1098-2018-3-31-41 (in Russian).

The International Union for the Protection of New Varieties of Plants (UPOV) Available from: https: //www.upov.int/portal/index.html.en [date of request 21.02.2021]

Totskaya, S.A. & Gryaznov, M.Yu. 2019. Some aspects of breeding work with *Oenothera biennis* L. *Voprosy biologicheskoj, medicinskoj i farmacevticheskoj himii* **21**(12), 54–58. doi:10.29296/25877313-2018-12-11 (in Russian).

Totskaya, S.A. & Gryaznov, M.Yu. 2020. Study of the biology of flowering in the process of breeding work with the evening primrose (*Oenothera biennis* L.) in VILAR. In: Proc. *From the plant to the medicinal product*. Abstract Book. All-Russian Research Institute of Medicinal Plants, Moscow, Russia, pp. 110–117 (in Russian).

Vandyshev, V.V., Gritsyuk, Ya.A. & Klimakhin, G.I. 2002. In: Proc. 6th Interd. congress *Actual problems of creating new medicines of natural origin.* S.-Pb, Russia, pp. 202–205 (in Russian).

Vinogradova, Yu. K., Grigor'eva, O.V. & Vergun, E.N. 2020. The structure of the stomatal apparatus of species of the genus *Symphyotrichum* Nees as an additional indicator of their invasiveness. *Russian Journal of biological invasions* **4**, 34–44 (in Russian).

Vorob'yova, A.N. & Basargin, D.D. 2013. Structural features of the leaf epidermis of *Saussurea pulchella* (Fisch.) Fisch. and *S. neopulchella* Lipsch. *Vestnik Tomskogo gosudarstvennogo universiteta. Biologiya* **3**(23), 38–45 (in Russian).

Zalepugin, D.Yu., Tilkunova, N.A., Bykov, V.A., Klimakhin, G.I., Korolev, V.L., Mishin, V.S., Chernova, I.V. 2006. In: Proc. *17th Conference Chemistry, technology, medicine*. All-Russian Research Institute of Medicinal Plants, Moscow, Russia, pp. 79–82 (in Russian).