SHST
Horticulture and Forestry Society of Transylvania

Goncharovska I and Szot I (2023)
Notulae Scientia Biologicae
Volume 15, Issue 1, Article number 11449
DOI:10.15835/nsb15111449
Research Article

NSB
Notulae Scientia Biologicae

Anatomical and morphological structure of the leaf of the genus *Malus* spp.

Inna GONCHAROVSKA[1]*, Iwona SZOT[2]

[1]*National Academy of Sciences of Ukraine, M.M. Gryshko National Botanical Garden, Tymiryazevska str. 1, 01014 Kyiv, Ukraine; Inna_Lera@ukr.net (*corresponding author)*
[2]*University of Life Sciences, Faculty of Horticulture and Landscape Architecture, Lublin, Poland; szoti@autograf.pl*

Abstract

The article describes changes in the structure, leaves, (including mesophyll structure, structure of the midrib, distribution of stomata, and the number of vascular bundles) in *Malus* during the growing season (from June to August) under the influence of prolonged drought. The leaves anatomy and morphology were investigated by methods of light and scanning electron microscopy. The study was carried out on four representatives of the genus *Malus*: *M. niedzwetzkyana* Borkh., *M. baccata* 'Pendula', *M. domestica* 'Eliza Rathke', *M. domestica* 'Renet Simirenko'. The influence of drought on the size of the mesophyll of the leaf plate and the number of stomata along the stretch from June to August was studied. The cell circumference of the sclerenchyma ranged from µm in 27.33 (*M. niedzwetzkyana*) to 81.92 µm (in *M. domestica* 'Elise Rathke'). The number of stomata varied from 214.0 units in (*M. domestica* 'Renet Simirenko') to 304.0 units in (*M. baccata* 'Pendula'), length from 11.6 µm (*M. baccata* 'Pendula') to 28.63 µm (*M. domestica* 'Elise Rathke'), width from 9.4 µm (*M. domestica* 'Elise Rathke') up to 21.8 µm (*M. domestica* 'Elise Rathke') µm. The length of the trichomes varied from 409.92 µm (*M. domestica* 'Renet Simirenko') to 745.09 µm (*M. niedzwetzkyana*). Also, the anocytic stomatal type, the number, different stomata and trichomes, the presence of wax on the cuticle, and the found druses of calcium oxalate in *M. baccata* 'Pendula', can be important for systematic phylogeny. Thanks to these studies, it is possible to suggest physiological adaptability of apple trees to prolonged drought, as well as to identify more resistant phenotypes for the breeding process to develop resistant cultivars.

Keywords: epidermis structure; *Malus*; trichomes; stoma

Introduction

The warming of the last 10 years is really affecting the world around us, including the plants. Long-term monitoring of biological objects provides no less valuable and accurate information about multiyear climate changes than direct meteorological observations. In some cases, living objects are excellent indicators of environmental change and are more sensitive to climate variations than many physical parameters (Beer, et. al., 2010). Long-term studies of the timing of plant development, changes in lifestyle, and dispersal to new areas have now become a scientific priority (Castanheire *et al*., 2016; Goncharovska *et al*., 2021; Goncharovska *et al*.,

Received: 19 Jan 2022. Received in revised form: 09 Feb 2023. Accepted: 07 Mar 2023. Published online: 16 Mar 2023.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

2022). In order to make the right decisions to save biological communities on Earth from the rapid destruction we are witnessing these days, it is important to understand how much of this is due to natural forces and how much is due to human activity (Vlam *et al*., 2018).

In 2018, atmospheric concentrations of carbon dioxide (CO_2), methane, and nitrogen oxide reached their highest levels ever observed for climate (Humphrey *et al*., 2018) so we decided to investigate anatomical changes in the leaf lamina as a result of a prolonged drought.

The anatomical features of the structure of individual plant organs may indicate their suitability for introduction into other environmental conditions (Azevedo *et al*., 2013). When plants are transferred to new conditions, the adaptive potential is realized through anatomical-morphological and physiological-biochemical rearrangements, primarily affecting the leaf, which is one of the most multifunctional plant organs (Mojena *et al*., 1977). However, the nature of the influence of the environment on the organism is determined not only by the nature of the acting factor, but also by the genetic specifics of the organism (the reaction rate). The nature of the organism's reaction to the environment is the most important result of evolution and, at the same time, the most important factor determining its further course (Phipps *et al*., 1990).

Water deficit is considered the primary environmental stress in agriculture, and improving the growth and production of plants under this stress is one of the primary goals of breeding and crop management programs (Potter *et al*., 2007). The apple tree is a plant that is negatively affected by water stress. Plants that develop under a water deficit may develop physiological and anatomical strategies to survive or even produce fruits in these environments (Campben *et al*., 2007; Goncharovska *et al*., 2017). In spite of well described morphology of leaves there are scarce anatomical examinations, which necessitate thorough analysis of the leaf anatomical and surface peculiarities (Medri *et al*., 2011).

The anatomical features of the leaf structure – the thickness and development of mesophyll tissues, the size of the cells of the columnar and spongy parenchyma indicates the level of adaptation to environmental conditions (Barthlott *et al*., 1998). It has been proven that the leaf structure can give a complete picture of the plasticity of the genotype in relation to environmental factors of the environment (Pyykko, 1966).

According to the literature, an increase in the total leaf thickness occurs due to the growth of the mesophyll associated with an increase in the age of plants and with a temperature regime (Xie *et al*., 2020). Respiratory transpiration depends on the number of stomata and especially on the width of the prodigal fissures, which in turn is determined by the light and saturation of the leaves with water (Harbage *et al*., 2019). The stomata are regulators of transpiration. The number of stomata is a trait that varies in a very wide range, since this indicator largely depends on the ecological conditions and the phase of leaf development (Metcalfe *et al*., 1965)

To protect plants, use various preformed chemical or physical barriers. For example, in tobacco plants, toxic chemical nicotine is used as a defense against herbivores. Physical means of protection, one can consider thorns and trichomes that plants use as protection against herbivores insects (Zielinski *et al*., 2010). According to the literature data, calcium oxalate crystals are quite widespread in nature and can be found in more than 215 plant families (Franceschi, 2001). Location, size and shape of calcium oxalate crystals in tissues to serve as protection from both biotic and abiotic factors. The aim was to assess the anatomical and morphological structure of the leaf depending on the response to global warming. In this regard, the structure of the leaf epidermis was studied in four phenotypes of representatives of the genus *Malus* Mill. using light and scanning electron microscopy, the size and number of stomata, as well as the length of the trichomes, were analyzed.

Materials and Methods

Collection of plant material

The study used four genotypes from the genus *Malus* spp. (*Malus niedzwetzkyana*, *M. baccata* 'Pendula', *M. domestica* 'Elise Rathke' and *M. domestica* 'Renet Simirenko') grafted on rootstock M9 the collection of the Department of Acclimatization of Fruit Plants of the National botanical garden (NBG) M.M. Gryshko NAS of Ukraine.

NBG is located on the South-Eastern outskirts of Kyiv on the Pechersk slopes of the low Kyiv hills in the Zverinets tract. The main type of soil on the territory of the NBG is dark gray podzol, which lies on loess and forest-bearing rocks and brown clays (the amount of humus is 0.5-2.0%).

The study was conducted in 2018 during the summer months (June-August). Most of the months this year had a significant excess of average monthly temperatures relative to the climatic norm, on August 15, the temperature in the shade reached + 32.8 °C.

Anatomical studies were carried out on specimens kept in 70% alcohol. The paraffin method was used for the transverse sections of stem, leaf and petiole. The specimens were embedded in paraffin and then sectioned with a Leica RM2125RT rotary microtome. Photographs were taken using a Leica DM1000 binocular light microscope with a Leica DFC280 camera and measurements were determined using the AxioVision 4.8 software.

Statistical analysis

Basic statistical analyses – the minimal and maximal values of the traits and arithmetic means were performed using PAST 2.17 (Norway, 2001).

Results

Examination of the leaf surface of apple trees showed that the stomata are located only on the abaxial side (hypostomatic type). Anatomical and morphological features of the stomata showed that there are significant differences in the number and size of stomata, the number of epidermal cells per 1 mm^2 of leaf surface – from 214.0 to 304.0 pcs.

The stomata are anomocytic type, round of different sizes, there are both small and large – from 14.9 to 23.11 µm in length and from 13.54 to 16.0 µm in width (Figure 1(D), 2(F), 3(C), 4(D)). Stoma sizes varied in *Malus niedzwetzkyana*, stomata length ranged from 14.4 to 22.2 µm, width – from 14.7 to 20.2 µm (Figure 3 D); in hybrid *M. baccata* 'Pendula' stomata were 11.6 to 22.7 µm long, width from 9.96 to 19.1 µm (Figure 4 E), in cultivar *M. domestica* 'Elise Rathke' stomata length varied from 12.4 to 28.63 µm, width from 9.4 to 21.8 µm (Figure 1 D), in hybrid *M. domestica* 'Renet Simirenko' length from 16.8 to 25.4 µm, width from 13.1 to 19.2 µm (Figure 2 F).

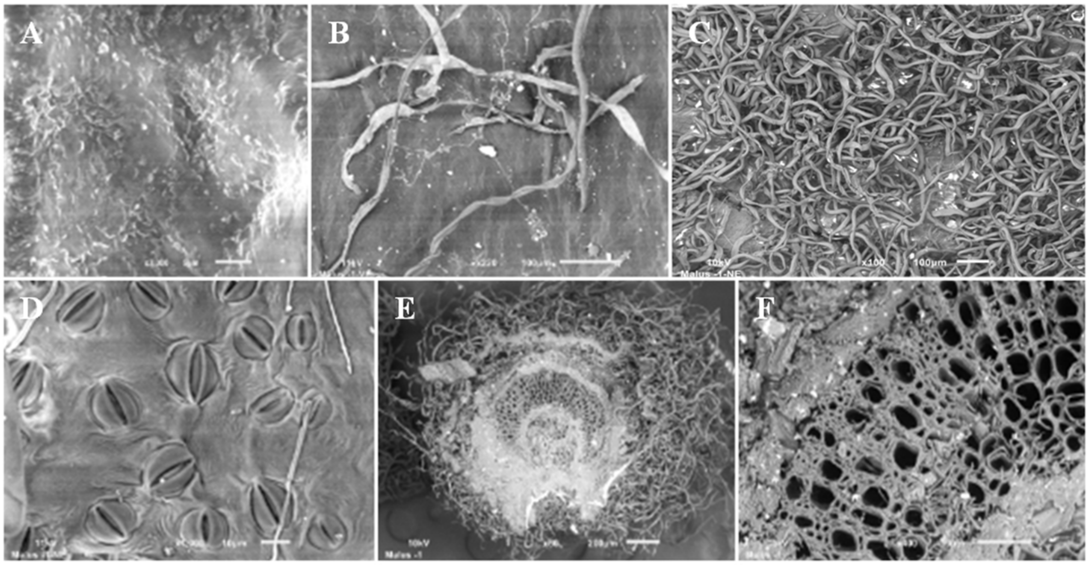

Figure 1. *Malus domestica* 'Elise Rathke': A – upper epidermis, epicuticular waxes; B-C – lower epidermis, trichomes; D – lower epidermis, stoma; E – transverse sections of the petiole; F – parenchyma cell transverse sections of the petiole

The leaves of *M. domestica* ‘Elise Rathke’ turned out to be the most pubescent. *M. domestica* ‘Renet Simirenko’ rather strong pubescence has a central vein (Figure 2 A). Long trichomes in the species *Malus niedzwetzkyana* are – 745.09 µm (Figure 5C), the average trichomes in the apple variety *M. domestica* ‘Elise Rathke’ are – 634.39 µm (Figure 1 (B, C)), the smallest in the hybrid is *M. domestica* ‘Renet Simirenko’ – 409.92 µm (Figure 2 A).

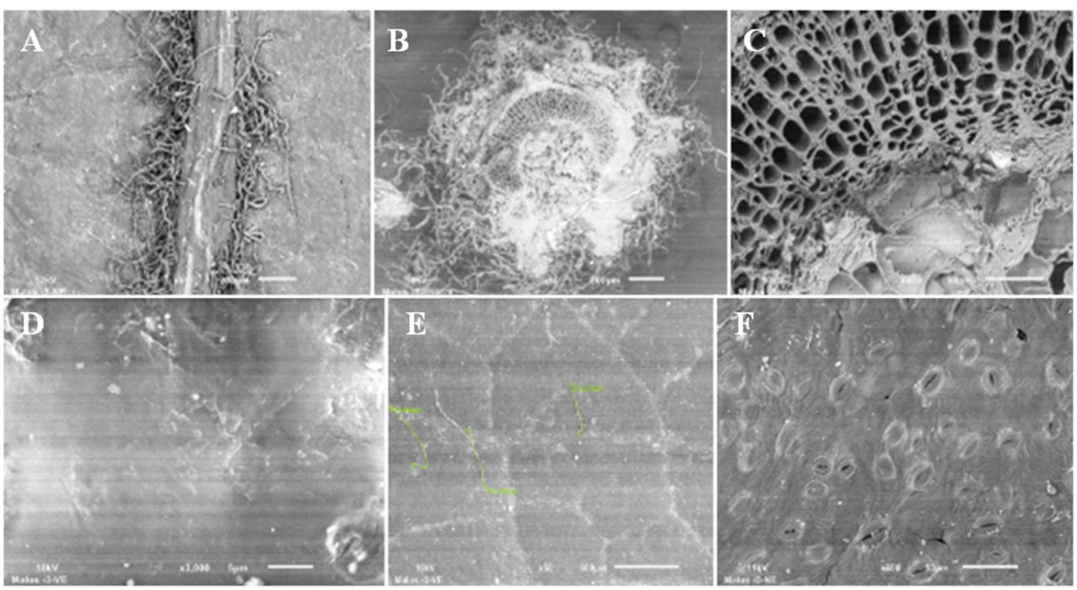

Figure 2. *Malus domestica* 'Renet Simirenko': A – central petiole, trichomes; B – transverse sections of the petiole; C – parenchyma cell transverse sections of the petiole; D – upper epidermis, epicuticular waxes; E – lower epidermis, trichomes; F – lower epidermis, stoma

Transverse sections taken from the petiole of *Malus* showed the following elements. The epidermal cells are oval and rectangular. Adaxial and abaxial epidermis cells are nearly equal in size. One to three layers of collenchyma cells are located under the epidermis (Figure 1 (F); 2 (B,C);.3 (A, B); 4 (A, B)).

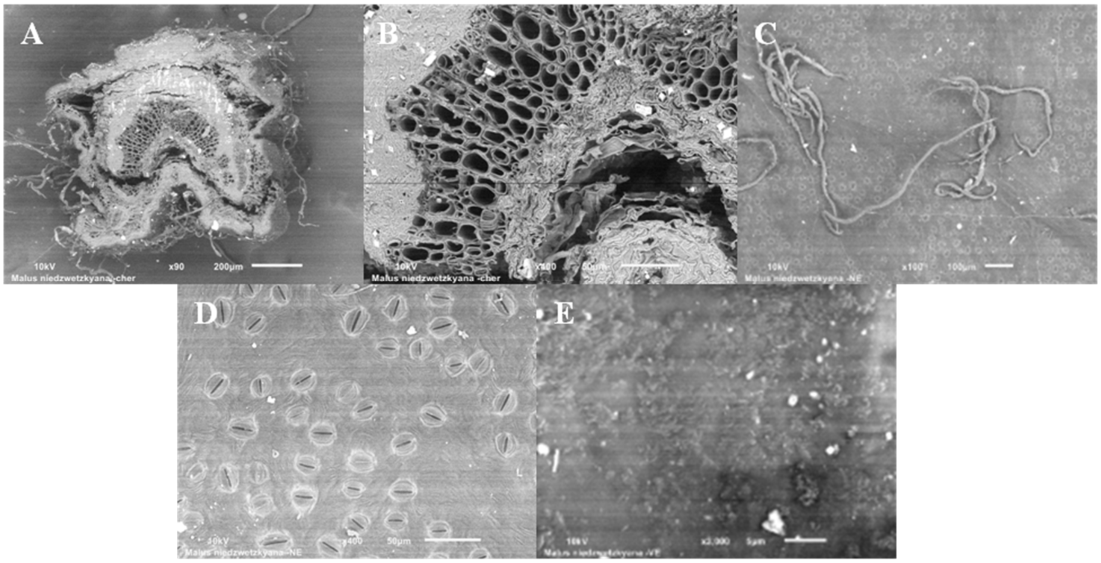

Figure 3. *Malus niedzwetzkyana* Borkh.: A – transverse sections of the petiole; B – parenchyma cell transverse sections of the petiole; C - lower epidermis, trichomes; D – lower epidermis, stoma; E – upper epidermis, epicuticular waxes

The vascular bundle surrounded by parenchymatic cells appears as a shallow arc. A large single vascular bundle is located in the middle, as well as, there are 3 or 4 small subsidiary vascular bundles in each wing. A few sclerenchyma fibers are only observed on the phloem. The cell circumference ranged from 27.33 μm. to 81.92 μm (Figure 1 (F), 2 (C), 3 (B), 4 (B)).

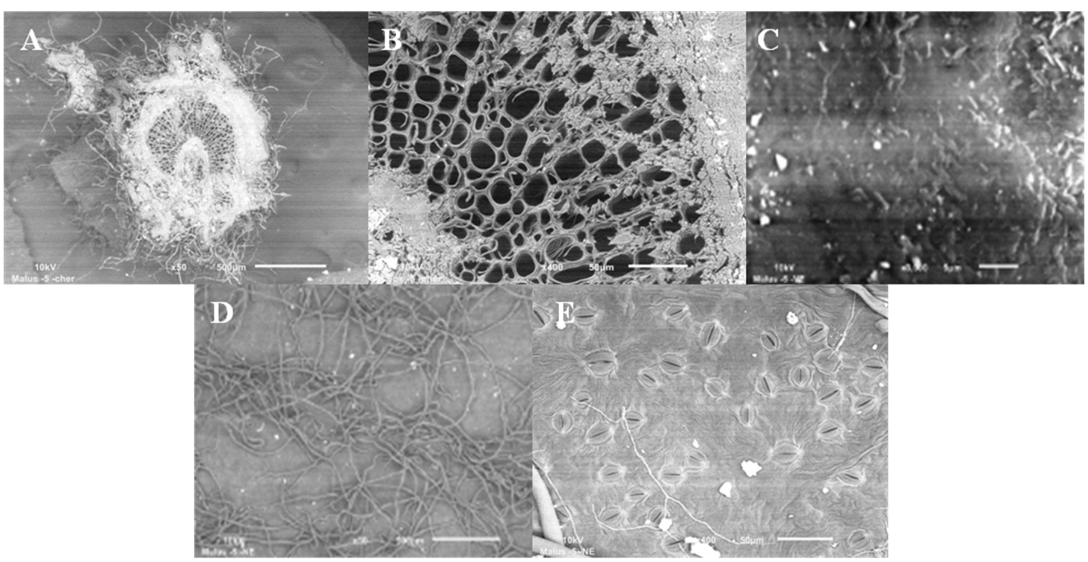

Figure 4. *Malus baccata* 'Pendula': A – transverse sections of the petiole; B – parenchyma cell transverse sections of the petiole; C – upper epidermis, epicuticular waxes; D – lower epidermis, two types of trichomes – ribbon-like twisted and cylindrical-bended; E – lower epidermis, stoma

Varietal and species differences were noted in the size and number of stomata per 1 mm^2 of leaf surface, in the length of guard cells and their shape. It was revealed that the size of the stomata and the degree of their openness depend on the temperature and humidity of the air. During a drought, the degree of stomatal openness decreases sharply.

It was found that from June to August there is an increase in the size of cells of the spongy and columnar parenchyma. The sizes of the leaf mesophyll tissues are probably related to changes in weather conditions, especially temperature rise.

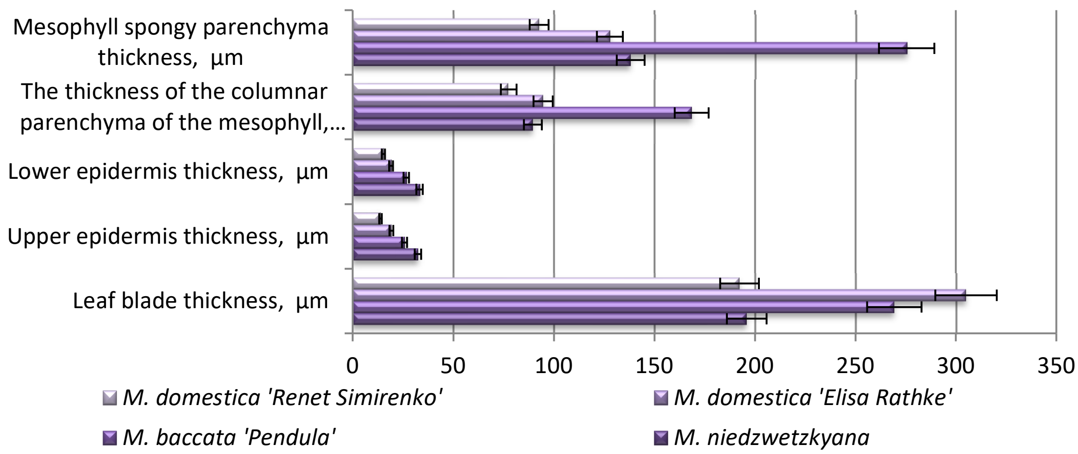

Figure 5. Mesostructure of *Malus* leaves, June

The results obtained agree with the literature data: in drier periods, an increase in leaf mesophyll thickness occurs due to an increase in the volume of air cavities (filled with air rich in moisture), which causes an increase in the ventilation surface in the middle of the leaf. The most dry-looking objects of study were selected based on the results of an increase in the thickness of the mesophyll. In *M. domestica* 'Elise Rathke', the thickness of the leaf blade increased by 12 µm from June to August (Figures 5, 6), in *M. baccata* 'Pendula' hybrids – by 18 µm.

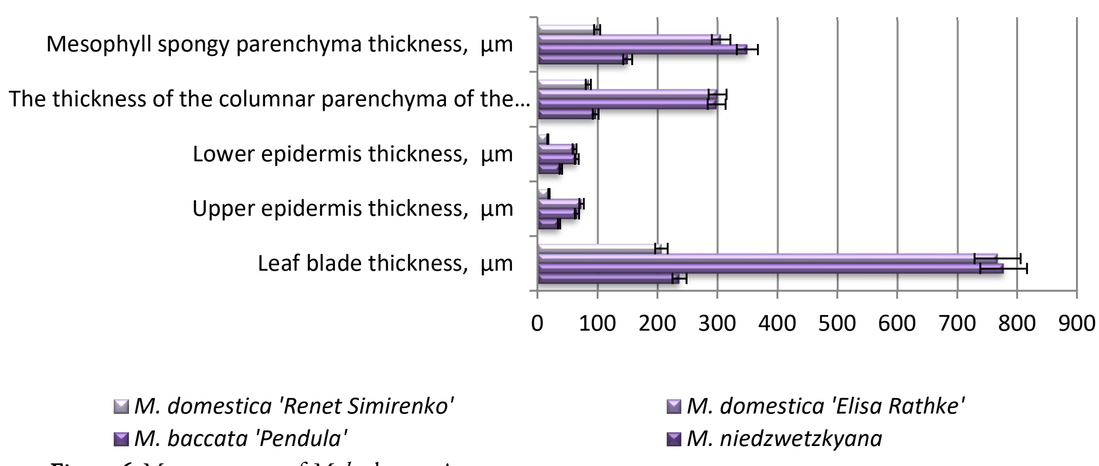

Figure 6. Mesostructure of *Malus* leaves, August

It is obvious that the hybrid *M. baccata* 'Pendula' has a high-water retention capacity of the leaf blade, is sufficiently drought-resistant and can withstand a long time without moisture.

Discussion

It is well known that plants are quite plastic; possess a number of protective reactions, including chemical, physiological and morphological changes in response to environmental stresses. A review of the literature shows that the number of apple stomata ranges from 156 to 405 pcs, their size is 26.1 ± 0.5 – 18.1 ± 0.4 μm, the trichomes are located mainly on the veins, border of the leaf, and sometimes in the areoles. There are two types of simple single trichomes – tapered (some of them are more or less twisted) and cylindrical, curved at the base of the trichome (Forte, 2002).

In our study objects, the number of stomata per 1 mm^2 varied from 214 ± 3.8 to 304 ± 2.2.

According to the opinion of many scientists, the anatomical and morphological characteristics of plants can simultaneously fulfill several functional roles. Grisi *et al*. (2008) evaluated the leaf anatomy of the coffee plants 'catua' and 'siriema' under water stress and found no difference in the thickness of the epidermis of the leaves of the evaluated cultivars. Thickening of the epidermis and palisade parenchyma can increase resistance to water scarcity and promote plant development under these conditions by improving water relations and protecting leaf tissue (Mojena, 1977; Bacelar *et al*., 2004).

Let us give examples of similar studies on various plants, namely, in of the genus *Rosa* L., the number of stomata ranges from 27.1 to 160.0 pcs. / μm^2, length from – 25.2 to 49.7 μm, width – from 17.9 to 35.4 μm (Zieliński, 2010), in *Swida sanguinea* Opiz species the amount per 1 mm^2 – 262.5 pcs., length 15 to 42 μm, width 12.11 μm; *Cornus mas* has 112.5 stomata. per 1 mm^2, the average length of the stomata is 21.30 μm, the width is 13.06 μm (Klimenko, 2016).

An interesting analysis was made to study the size of the stomata in *Chaenomeles* (29.2 × 17) → *Cydonia* (24.5 × 16.5) → *Pseudocydonia* (19.4 × 16.1) μm, in which the stomata in *Chaenomeles japonica* differed in elliptical shape, in *Cydonia oblonga* – obovate and in general. oval in *Pseudocydonia sinensis* (Vinogradova, 2018).

The literature on mesophilic sizes in different plant species was also analyzed, namely, in *Tilia cordata* Mill, the thickness of the leaf blade is 160.23 μm, the parenchyma of the pillar is 69.92 μm, the parenchyma of the lips is 62.35 μm, in representatives of *Persica davidiana* the thickness of the leaf blade is from 151.59 to 173.88 μm (Golubkova, 2015). According to our data, the mesophyll thickness in *Malus* representatives increased with the growth of the leaf blade itself, as well as with moisture retention during drought.

From the above stated research data, it can be seen that in true xerophytes the number of stomata is often small, while in mesophytes, under conditions of impaired water supply, the epidermal cells become smaller and the number of stomata increases. A large number of stomata of mesophytes in arid growth conditions is necessary to enhance transpiration, which weakens the effect of overheating. Therefore, an increase in their number is a positive sign in the structure of introduced species.

Leaf trichomes are associated with herbivore resistance as well as increased water conservation in the plant.

Analyzing the results of our research, in representatives of *Malus*, ribbon trichomes were found on the leaf blades of *M. domestica* 'Elise Rathke' turned out to be the most pubescent. *M. domestica* 'Renet Simirenko' rather strong pubescence with a central vein (Figure 2 A). With the exception of the adaxial leaf surface, all examined tissues were covered with white, long, unicellular trichomes.

The long length of the trichomes provides physical protection for the stomata, as the densely spaced non-glandular trichomes on the leaf surface protect the stomata from extreme heat during dry and hot seasons. It can be concluded that the varieties with the greatest pubescence may be more drought tolerant.

During a review of the literature on trichomes, it was found that the following results were found in representatives of the genus *Barleria albostellata* (Acanthaceae) that nonlandular trichomes "bulge" over the glandular trichomes. The multi-angular-dendritic branched trichomes had a stem length of 554.10 ± 92.27 mm and a width of 28.55 ± 2.48 mm. Due to the longer length of glandular trichomes and their proximity to glandular trichomes, it is likely that these structures may provide physical protection for glandular trichomes (Gangaram, 2020).

Also, during anatomical studies, druses of calcium oxalate were found in the leaf blades of the objects of study, especially in *M. baccata* 'Pendula', which play an important role in maintaining osmotic pressure and acid-base balance in the cell (Figure 4 C). It is possible that crystals contribute to an increase in the osmotic pressure of the cell sap, which, in turn, increases the absorption capacity of cells. On the other hand, the epidermis, which has crystals in its cells, becomes shiny (the function of the cuticle is to protect and waterproof the plant (Robinson *et al*., 2001). As a result, thanks to the wax layer, the sun's rays are reflected more, protecting the plants from overheating and therefore At one time, Castanheira *et al*. (2016) described how, with a lack of water, the genetic variability of apple leaves was higher in terms of anatomical characteristics; Thus, these results can be used to recommend our varieties, which showed leaf plasticity under conditions lack of water.

Conclusions

According to the above, we studied the effect of drought on the size of cells of the spongy and columnar (Palisade) parenchyma and their ratio. The density of the stomata and their sizes were also investigated.
Plants are very plastic and in order to cope with global climate change, they have developed various adaptation mechanisms for themselves at the level of physiology and anatomy. Plant respiratory metabolism is highly flexible due to the presence of various alternative pathways. In the objects studied by us in the Right-Bank Forest-Steppe of Ukraine, changes in the structure of the leaf blade take place: compaction of the adaxial and abaxial cuticles, densification of mesophyll cells, changes in epidermal cells, an increase in the number of stomata, the appearance of calcium oxalate druses in the cells of the epidermis and mesophyll. These changes may indicate an increase in the drought tolerance of plants and their use for further breeding purposes.

Authors' Contributions

Conceptualization: IG; Data curation: IG; Formal analysis: IG; Investigation: IG; Methodology: I; Software: IG; Supervision: IG; Writing – original draft: IG.

Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The publication was prepared with the active participation of researchers in the international network AgroBioNet, and supported by the Visegrad Fund (Slovak Republic).

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Azevedo AM, Andrade Junior VC, Oliveira CM, Fernandes JSC, Pedrosa CE, Dornas MFS, Castro BMC (2013). Selection of lettuce genotypes for protected cultivation: Genetic divergence and importance of characters. Horticultura Brasileira 31:260-265.

Bacelar E, Correia C, Moutinho-Pereira J, Goncxalves B, Lopes J, Torres-Pereira J (2004). Sclerophylly and leaf anatomical traits of five field-grown olive cultivars growing under drought conditions. Tree Physiology 24:233-239. *https://doi.org/10.1093/treephys/24.2.233*

Balkwill MJ, Balkwill K (2002). A preliminary analysis of distribution patterns in a large, pantropical genus, *Barleria* L. (Acanthaceae). Journal of Biogeography 25:95-110.

Barthlott W, Neinhuis C, Cutler D., Ditsch F, Meusel I, Theisen I, Wilhelmi H (1998). Classification and terminology of plant epicuticular waxes. Botanical Journal of the Linnean Society 126:237-260. *https://doi.org/10.1111/j.1095-8339.1998.tb02529.x*

Beer C (2010). Terrestrial gross carbon dioxide uptake: Global distribution and covariation with climate. Science 329:834-838. *https://doi.org/10.1126/science.1184984*

Campbell CS, Evans RC, Morgan DR, Dickinson TA, Arsenault MP (2007). Phylogeny of subtribe Pyrinae (formerly the Maloideae, Rosaceae): limited resolution of complex evolutionary history. Plant Systematics and Evolution 266:119-145. *https://doi.org/10.1007/s00606-007-0545-y*

Castanheira DT, Rezende TT, Baliza DP, Guedes JM, Carvalho SP, Guimaraes RJ, Viana MTR (2016). Potencial de utilizac xao de características anatomicas e fisiol ogicas na selec xao de progenies de cafeeiro. Coffee Science 11:374-385.

Forte AV, Ignatov Ponomarenko AN, Dorokhov VV, Savelyev NI (2002). Phylogeny of the *Malus* (apple tree) species, inferred from the morphological traits and molecular DNA analysis. Russian Journal of Genetics 38(10):1150-1160. *https://doi.org/10.1023/A:1020648720175*

Franceschi V (2001). Calcium oxalate in plants. Trends in Plant Science 6:331.

Gangaram S, Naidoo Y, Dewir YH (2020). Foliar micromorphology, ultrastructure, and histochemical analysis of *Barleria albostellata* C.B. *Clarke*. South African Journal of Botany 135*:*212-224. *https://doi.org/10.1016/j.sajb.2020.09.001*

Golubkova IM (2015). Features of the anatomical structure of the leaf species and varieties of *Persica* Mill. In the right-wing Forest-Steppe of Ukraine 'Young Scientist' 2(17):30-32.

Goncharovska IV, Kuznetsov VV, Galushko VM, Antonyuk GO (2017). Drought resistance of apple hybrids with the participation of the cultivar *Vydubytska plakucha* in the conditions of the Forest-Steppe of Ukraine. Introduction of Plants 3:88-94.

Goncharovska I, Levon V (2021). Content of anthocyanins in the bark of fruit and berry plants due to adaption to low temperatures. Khimiya Rastitel'nogo Syr'ya 1:233-239. *https://doi.org/10.14258/jcprm.2021017747*

Grisi FA, Alves JDA, Castro EC, Oliveira C, Biagiottis G, Melo LA (2008). Leaf anatomical evaluations in 'Catuaí' and 'Siriema' coffee seedlings submitted to water stress. Ciência e Agrotecnologia 32:1730-1736. *https://doi.org/10.1590/S1413-70542008000600008*

Harbage JF, Stimart DP, Evert RF (2019). Anatomy of adventitious root formation in microcuttings of *Malus domestica* Borkh. `Gala'. Journal of the American Society for Horticultural Science 118(5):680-688 *https://doi.org/10.21273/jashs.118.5.680*

Humphrey V (2018). Sensitivity of atmospheric CO_2 growth rate to observed changes in terrestrial water storage. Nature 560:628-631. *https://doi.org/10.1038/s41586-018-0424-4*

Jackson JE, Beakbane AB (1970). Structure of leaves growing at different light intensities within mature apple trees. Annual Report East Malling Research Station 1969:87-89.

Klimenko SV (2016). Anatomy of leaves of members of the family *Cornaceae* Bercht.et J. Presl in the forest-steppe conditions of Ukraine Introduction of plants. Kiev 3:23-37.

Medri C, Medri ME, Ruas EA., Souza LA, Medri PS, Sayhun S, Bianchini E, Pimenta JA (2011). Morpho-anatomy of vegetative organs in seedlings of *Aegiphila sellowiana* Cham. (Lamiaceae) subject to flooding. Acta Botanica Brasilica 25:445-454.

Metcalfe CR, Chalk L (1965). Anatomy of Dicotyledons. Clarendon Press, Oxford, pp 539-550.

Mojena R (1977). Hierarchical grouping methods and stopping rules: An evaluation. The Computer Journal 20:359-363. *https://doi.org/10.1093/comjnl/20.4.359*

Phipps JB, Robertson KR, Smith PG, Rohrer JR (1990). A checklist of the subfamily Maloideae (Rosaceae). Canadian Journal of Botany 68:2209-2269. *https://doi.org/10.1139/b90-288*

Potter D, Eriksson T, Evans RC, Oh S, Smedmark JEE, Morgan DR, … Campbell CS (2007). Phylogeny and classification of Rosaceae. Plant Systematics and Evolution 266:5-43. *https://doi.org/10.1007/s00606-007-0539-9*

Pyykkö M (1966). The leaf anatomy of East Patagonian xeromorphic plants. In: Annales Botanici Fennici, pp 453-622.

Robinson JP, Harris SA, Juniper BE (2001). Taxonomy of the genus *Malus* MIill. (Rosaceae) with emphasis on the cultivated apple, *Malus domestica* Borkh. Plant Systematics and Evolution 226:35-58. *https://doi.org/10.1007/s006060170072*

Sokolova A (2013). Microscopical diagnostics of *Tilia* L. genus species of amur region according to leaf structure Альманах современной науки и образования 11(78):164-167.

Stace C (1965). Cuticular studies as an aid to plant taxonomy. The Bulletin of the British Museum (National History) 4:37-40.

Vinogradova Y, Riabchenko A, Gorbunov Yu, Grygorieva O, Brindza J (2018). Characteristic of stomata for *Cydonia oblonga* Mill., *Pseudocydonia sinensis* (Thouin) C.K. Schneid. and *Chaenomeles japonica* (Thunb.) Lindl. ex Spach species Annals of the Romanian Society for Cell Biology 22(2):18-25. *https://doi.org/10.ANN/RSCB-2018-00017:RSCB*

Vlam M, Baker PJ, Bunyavejchewin S, Zuidema PA (2014). Temperature and rainfall strongly drive temporal growth variation in Asian tropical forest trees. Oecologia 174:1449-1461. *https://doi.org/10.1007/s00442-013-2846-x*

Xie R, Zhao J, Lu L, Brown P, Guo J, Tian S (2020). Penetration of foliar-applied Zn and its impact on apple plant nutrition status: *in vivo* evaluation by synchrotron-based X-ray fluorescence microscopy. Horticulture Research 7:147. *https://doi.org/10.1038/s41438-020-00369-y*

Zieliński J, Guzicka M, Tomaszewski D, Maciejewska-Rutkowska I. (2010). Pericarp anatomy of wild roses (*Rosa* L., Rosaceae). Flora – Morphology, Distribution, Functional Ecology of Plants 205(6):363-369 *https://doi.org/10.1016/j.flora.2009.12.002*