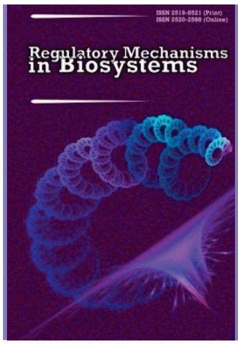

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Anatomical structure of leaves in *Magnolia kobus*, *M. obovata*, *M. denudata* in the first stages of ontogenesis

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In the ontogenesis of woody plants in general, and magnolia species in particular, the virginal age is quite long, and therefore is critically important from the point of view of selecting optimal conditions for growing plants. The study was conducted on biennial plants of deciduous magnolias: *Magnolia kobus* Sarg., *M. obovata* Thunb., *M. denudata* Desr. The studied plants were at the beginning of the virginile stage of development. The material for the investigation is selected from plants, which were planted by us in the research area of A. V. Fomin Botanical Garden of the Taras Shevchenko National University of Kyiv. In this study, we used scanning electron and light microscopes to examine the ultrastructure of the surface of leaves of the studied species. The histological characteristics of the leaves of the studied species are similar. They are hypostomatic. Their stomata are of the paracytic type, they are evenly distributed on the abaxial surface of the leaves. The epidermal cells of the studied species of the genus *Magnolia* have tortuous (cells located on the periphery of the leaf) or straight (cells located on the veins) outlines and rectangular (cells located on the veins) or flattened (cells placed on the periphery of the leaf) projection. The cuticle is relatively thin, located on both the abaxial and adaxial surfaces of the leaves. Epicuticular wax is observed of three types in the studied species: films (*M. denudata*), crust (*M. kobus*, *M. obovata*) and wax granules (*M. denudata*, *M. obovata*). The amount of wax and pubescence is greater on the abaxial surface in *M. obovata*; very weak pubescence and waxy layer is observed only on the abaxial surface of the leaf in *M. kobus*, weak pubescence is present on both sides in *M. denudata*. In all species of the genus *Magnolia* pubescence is simple, formed by hairs that "accompany" the veins. There are two main types of lamina relief in the studied species: reticular (*M. obovata*, *M. denudata* (subtype reticular-collicular)) and pitted (*M. kobus*). The species clearly differ in this feature, so we believe that the type of relief of the lamina can be used as an additional diagnostic feature to distinguish species of the genus *Magnolia*. *Magnolia denudata* is characterized by a small number of stomata, while *M. obovata* and *M. kobus* are characterized by their average number. The Stomata Index varies from 2.8 in *M. denudata* to 1.02 in *M. kobus*. The lamina is the thickest in *M. obovata*, the thinnest is in *M. kobus*, but the total thickness of the epidermal tissue as a percentage of the thickness of the leaf is, in descending order: *M. denudata* (29%), *M. kobus* (24%), *M. obovata* (18%). The mesophyll varies from homogeneous-spongy (in *M. kobus*) to layered (in *M. obovata*, *M. denudata*) type. The number of layers of cells that form the mesophyll in all studied species is from 4 to 6. Thus, *M. kobus* is characterized by the least specialized type of mesophyll. The vascular system in the studied plants is represented by small central and lateral vascular bundles. The ground tissue is present only in the central and large side bundles. In the early stages of ontogenesis the studied plants are typical mesophytes with hypostomatic leaves adapted to exist in sufficiently moist conditions in soil and air. In practical terms, the results of investigation can be used to select optimal conditions for growing plants at the initial stages of ontogenesis.

Keywords: type of stomata; epicuticular wax; lamina relief; mesophyll; spongy tissue; Stomata Index; vascular system; introduction.

Introduction

Adaptation as a result of natural selection is determined by specific mechanisms of reactions and is expressed in a certain coordination of structures and functions between plant organ systems (Butnyk, 1977; Butnyk, 1991). To characterize the adaptive properties of organisms, it is necessary to identify the specifics of their adaptations at the stages of ontogenesis. Study of morphology-anatomical signs of plants of different age conditions is important for determination of effectiveness of introduction of varieties in new ecological conditions and favors the spread of assortments of resistant plants for the use in greenery planting (Boyko, 2017). In addition, the results of such work can be used to assess the range of tolerance and the degree of xeromorphism of species and predict their behavior in order to optimize their wider introduction into landscaping of urban landscapes as promising climate-forming plants (Kazantsev et al., 2018; Svietlova et al., 2020). In addition, information about ontomorphogenesis is especially important for protected species of plants (Gnatiuk et al., 2021). Representatives of the genus *Magnolia* L. (Magnoliaceae) appeared on our planet more than 180 million years ago. Adaptation mechanisms that have allowed Magnoliaceae to adapt to different climatic changes over the course of evolution have attracted the attention of scientists and suggest that certain species of this family may have a high thermoregulatory ability to shape the microclimate of urban landscape (Svietlova et al., 2020). Today, fragmented natural habitats of these plants are preserved in East and Southeast Asia, North and Central America. In total, the genus *Magnolia* includes about 339 species known in the world (Plants of the World Online). Furthermore, many new species of *Magnolia* have been described (Vázquez-García et al., 2022; Morales-Molina et al., 2024). Most of them are rare and endangered and are included in the international red lists. These plants are cultivated in many countries around the world for their decorative flowers, which open in spring or early summer.

As these species have a high ornamental value, the literature deals mainly with their aesthetic qualities (Cvjetićanin et al., 2016). However, some works were devoted to parameters of the functional state of the photosynthetic apparatus (content of photosynthetic pigments and the fluorescence induction parameters of chlorophyll) of species of wood plants. Among them, leafy species, *M. soulangeana* and *M. kobus* are characterized by a high "index of viability" (Svietlova et al., 2018). A study was aimed at evaluating the in situ the physiological responses of some *Magnolia* species to severe drought that frequently occurs in urban environments in Southeastern Europe (Vastag et al., 2020). A comprehensive phylogenetic study of Magnoliaceae on the basis of sequences of the complete chloroplast genomes (Wang et al., 2020) confirmed 15 major clades within the broadly defined *Magnolia*. Seed coat development of *M. stellata* has been studied (Feng et al., 2024).

In A. V. Fomin Botanical Garden of the Taras Shevchenko National University in Kyiv the formation of the plant collection of the genus *Magnolia* started more than 100 years ago. Today, the ancestral complex of *Magnolia* has more than 70 species, forms and varieties (Korshuk & Palagecha, 2007; Demchenko et al., 2019).

The study was conducted on plants collection of deciduous magnolias of the Botanical Garden: *M. kobus* Sarg., *M. denudata* Desr. and *M. obovata* Thunb. The first two species belong to sect. Yulania (Spach) Dandy, the third species - sect. *Rytidospermum* Spach (Wang et al., 2020). *Magnolia obovata* and *M. denudata* are included in the IUCN Red List of Threatened Species (2014).

Magnolia obovata is distributed in Japan on the island of Hokkaido and on the Kuril Islands at an altitude of 1800 m above sea level. The trees reach 30 m in height in natural conditions, in the Botanical Garden 40-year-old specimens have a height of 10 m. They bloom and bear fruit when cultivated (Minchenko & Korshuk, 1987; Korshuk & Palagecha, 2007).

Magnolia kobus is distributed in the northern and central parts of Japan and in Korea. It grows near mountain rivers. In natural conditions, the tree reaches 20 m in height, in the Botanical Garden 45-year-old specimens have a height of about 10 m. It actively blooms and bears fruit every year when cultivated.

Magnolia denudata is distributed in the southern and eastern regions of China at altitudes up to 1200 m above sea level. In natural conditions and when introduced to the Botanical Garden it reaches 9 m in height, blooms annually and bears fruit.

Under natural conditions all studied species grow in moist deciduous forests, often on the slopes of mountains and hills, along rivers. Previously conducted comparative characteristics of climatic conditions of the point of introduction (Kiev) and natural habitats of the studied species allow us to draw conclusions about their relative similarity (Palahecha et al., 2009). Some details that significantly affect the growth and development of the genus representatives are the lack of moisture in the soil and in the air and high temperatures that have been typical for Kyiv in the last decade. Therefore, to grow magnolias in simulated conditions, conditions similar to natural ones are created by keeping magnolias under the canopy of trees and with providision of sufficient moisture.

The anatomical and morphological structure of shoots at high and low temperatures were studied based on the plants collection of the Botanical Garden, differences in the localization and dynamics of lipid compounds and starch were revealed, etc., but most studies were devoted to frost and cold resistance of magnolias (Palahecha et al., 2009; Palahecha, 2011). Some works were devoted to drought resistance of *Magnolia*. However, studies of the histochemical features of the leaves remain insufficient.

One of our works was devoted to the study of variability of anatomical features of leaf blade in species *M. obovata*, *M. cobus* and *M. denudata* at the first ontomorphogenesis stages (Futorna et al., 2020).

In this regard, the aim of the work was to establish a complete histological characteristics of species of the genus *Magnolia* at the early stages of ontomorphogenesis and to identify their adaptive properties.

Materials and methods

The study was conducted on biennial plants of deciduous magnolias: *M. kobus* Sarg., *M. obovata* Thunb., *M. denudata* Desr. The material for the investigation is selected from plants which were planted by us in the research area of A. V. Fomin Botanical Garden of the Taras Shevchenko National University, Kyiv. The studied plants were at the beginning of the virginile stage of development.

Taking into account changes in the structure of the mesophyll and the projection of epidermal cells in different parts of the plant, samples from the middle parts of the leaf were taken for analysis. In order to study the ultrastructure of the surface of leaf blades by scanning, an electron microscope was used. The leaves were dehydrated in silica gel for 2–3 days. Dried samples of leaves for scanning electron microscope research (SEM) (JSM-6060 LA; JEOL company, Tokyo, Japan, 2004) were processed according to standard method: dehydrated fragments of leaves were fixed on brass tables and sprayed with a thin layer of a mixture of gold and platinum in the vacuum chamber. These studies were conducted in the Center for Collective Use of Electron Microscopes of the M. G. Kholodny Institute of Botany of the National Academy of Sciences.

For studies of the epidermal tissue of leaves in the paradermal plane by method of light microscope, the objects were fixed in a mixture of 70% ethanol – acetic acid – formalin followed by tissue maceration. Maceration of plant tissues was carried out in a macerating solution (hydrogen peroxide – glacial acetic acid – water), pieces of leaf lamina were kept at 20 ºC for 5 to 8 days to dissecting the upper (adaxial) and lower (abaxial) epidermis. Two epidermal layers were stripped and stained with 1% hematoxylin solution. Slides were viewed by using the light microscope Carlz Zeiss Primo Star (Carl Zeiss company, Oberkochen, Germany, 2013). The quantitative parameters (length and width of stomata, length of glandular trichomes (glands) along the major and minor axes, length of distal cell of non-glandular trichomes (hairs)) were calculated using AxioVision 4.8 software (Carl Zeiss MicroImaging GmbH, Jena, Germany, 2012. The sample size for calculating the density of trichomes was 20 for the adaxial and abaxial epidermis of each species.

We determined the type of epicuticular wax based on Barthlott et al. (1998), the types of relief are described in Chakrabarty & Mukherjee (1986). To describe outlines of cells and their projection onto the plane we used Zakharevich (1954) terminology. The types of cuticle are determined based on Jeffree (1996) work.

The characteristics (the length and width of the stomata) were measured in 50 repetitions. Data analysis was carried out in the Statistica 10 (Data Analysis Software System, StatSoft, USA, 2011), the results were presented as the mean ± confidence interval with an accepted level of significance ($P < 0.05$).

Results

***Magnolia denudata* Desr.**

Morphological characteristics. The leaves are simple, entire, petiolate. Petioles are 2.5–3.0 cm long, straight. The lamina is reversed broadly lanceolate, up to 20.2 cm long and 10.1 cm wide at its widest part, It is green, shiny on the upper side and matte on the lower side. The apex is sharply pointed, the base is narrowed. Mature leaves are sparsely pubescent along the main veins on the underside. Veining of the lamina is reticulate.

Histological characteristics. Leaves are hypostomatic (Fig. 1a, 1b). The boundaries of the cells are clear. The projections and outlines of epidermal cells vary: cells with flattened projections and tortuous outlines are observed above the mesophyll, cells with elongated projections and tortuous outlines are observed in the area of vascular bundles. The relief of the adaxial surface of the lamina is reticulate (Fig. 1d): anticlinal walls of epidermal cells are located above the level of the main cells of the epidermis. The outer periclinal walls are flat. The cuticle is well developed, of folded type (Fig. 1h).

The abaxial surface of studied species differs from its adaxial surface. The cells of the abaxial epidermis are characterized as very large, their number is 351.55 ± 1.46 per 1 mm^2. The stomata of the paracytic type are present only on the abaxial surface of the lamina (Fig. 1a), and are well visible, not oriented by their longer axis along the midvein of the leaf. The number of stomata is 124.26 ± 0.88 per 1 mm^2. Stomatal index of the abaxial surface is 2.8. They are located above the level of the main cells of the epidermis.

The relief of the abaxial surface of the lamina is reticular-collicular (Fig. 1c): the main cells of the epidermis form a reticular relief, the stomata complexes are located above the level of the main epidermal cells, forming rounded protuberances. The adaxial and abaxial epidermis are characterized by sparse pubescence, which is formed by long multicellular hairs that accompany the central vein (Fig. 1e, 1f). There is a layer of wax, denser on the abaxial epidermis. The wax is represented in a form of wax granules (Fig. 1j).

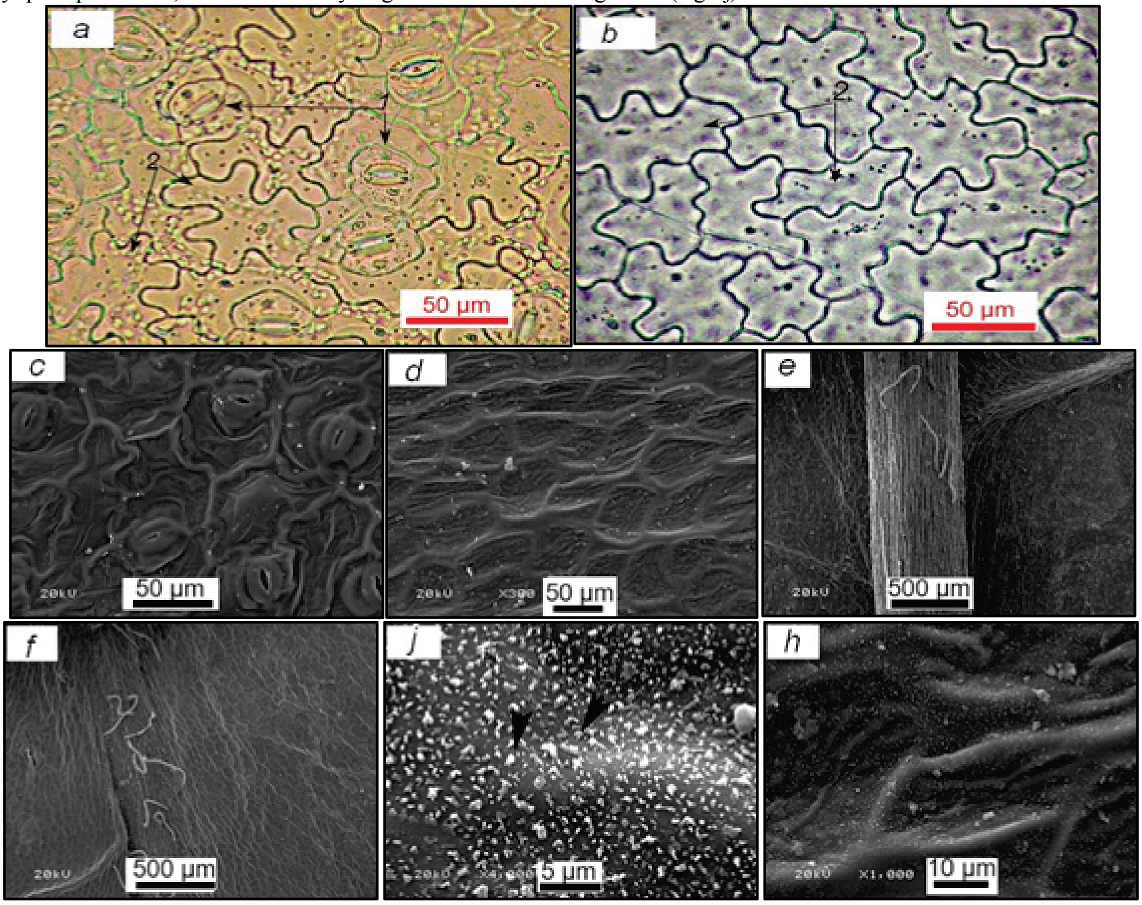

Fig. 1. The structure of the epidermis of the lamina of *M. denudata* Desr.: *a*, *b* – LM micrographs: *a* – stomata of the paracytic type (abaxial epidermis); *b* – adaxial epidermis; *1* – the paracytic stomata, *2* – cells of the epidermis, arrow indicates epicuticular wax; *c*–*h* – SEM micrographs: *c* – abaxial epidermis: reticular-collicular relief; *d* – adaxial epidermis: reticulate relief; *e* – long multicellular hairs (abaxial epidermis); *f* – long multicellular hairs (adaxial epidermis); *j* – wax granules (abaxial epidermis); *h* – wax films and cuticle of folded type (adaxial epidermis)

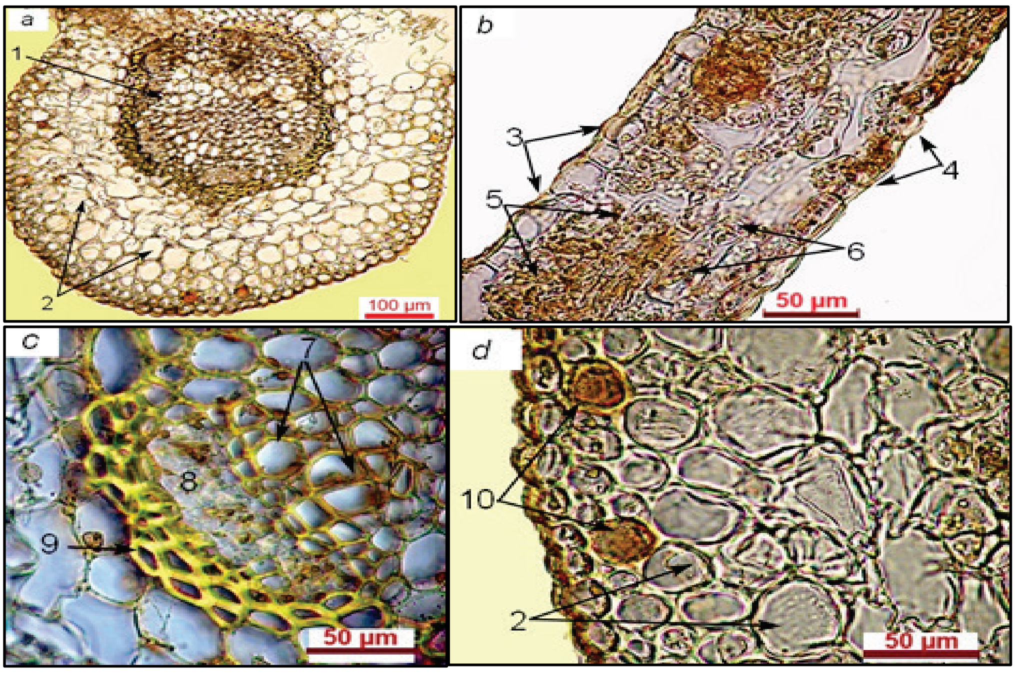

Fig. 2. Anatomical structure of the lamina of *M. denudata* Desr. (LM): *a* – cross section through the center vein of lamina; *b* – cross section of leaf showing the dorsoventral mesophyll; *c* – cross section of leaf showing the vascular bundle; *d* – cross section through the non-chlorophyllous parenchyma of the central vascular bundle; *1* – vascular bundle, *2* – non-chlorophyllous parenchyma, *3* – adaxial epidermis, *4* – abaxial epidermis, *5* – palisade parenchyma, *6* – spongy parenchyma, *7* – xylem, *8* – phloem, *9* – sclerenchyma, *10* – oil drops

The shape of the cross section is linear. The shape of epidermal cells on the same side of the leaf varies in cross section: from elongated-rounded (above and below the mesophyll) to rounded (above and below the midvein). Epidermal tissue is single-layered, large-celled, its total thickness is 35.75 ± 0.93 µm, which is 29.1% of the thickness of the leaf. Both the adaxial and abaxial epidermis have approximately equal thicknesses which are approximately 15.0% and 14.1% of the lamina thickness respectively.

The mesophyll is dorsoventral. It refers to the moderately layered type according to the number of cell layers (4–6). Palisade parenchyma is single-layered, its cells are very wide (coefficient of elongation is 1.6) and are densely arranged. Spongy tissue is represented by two or three layers of rather large round cells (Fig. 2).

The vascular system is represented by vascular bundles of the midvein and a fairly large number of lateral vascular bundles, which vary in size. The midvein is multi-bundle, has five vascular bundles, which are of the same size and are separated from each other by three layers of thin-walled cells of chlorophyll-free parenchyma. The latter is very well-developed, its cells form the keel, and border on both the upper and lower epidermis.

The subepidermal layers of the main parenchyma have a collenchymal thickening of the walls. The xylem vessels in the vascular bundles of the midvein are large, their number is up to 10–12 in each. The phloem is well developed, its elements are quite large and clearly distinguished in cross section. The ground tissue (collenchyma) is poorly developed. It is located in the midvein.

***Magnolia kobus* DC.**

Morphological characteristics. The leaves are simple, entire, petiolate. Petioles are 2.1 cm long, curved. The lamina is oblanceolate about 10.1 cm long and up to 5.2 cm wide at its widest part. The apex is pointed, the base is wedge-shaped. The upper side of the leaf is slightly shiny, the lower is matte. Veining of the lamina is reticulate.

Histological characteristics. Leaves are hypostomatic. Usually the adaxial epidermis is not pubescent (Fig. 3a), but simple trichomes are present in some specimens (Fig. 3g). Cells of epidermal tissue, as in the previous species, isodiametric, polygonal, large (436.61 per 1 mm^2), are characterized by tortuous shapes and flattened projections. Anticlinal walls of epidermal tissue cells are evenly thickened. They are located above the level of the outer periclinal walls. The relief of the adaxial surface of the lamina, in contrast to the representatives of the species *M. denudata*, is pitted (Fig. 3c). The outer periclinal walls are concave and located below the level of the anticlinal walls. The projections and outlines of epidermal cells vary: cells with flattened projections and tortuous outlines are observed above the mesophyll, cells with elongated projections and tortuous outlines are observed in the area of vascular bundles. The boundaries between adjacent cells are well visible. There is a dense layer of wax, which is represented by crusts (Fig. 3e). The cuticle is well developed, mostly smooth.

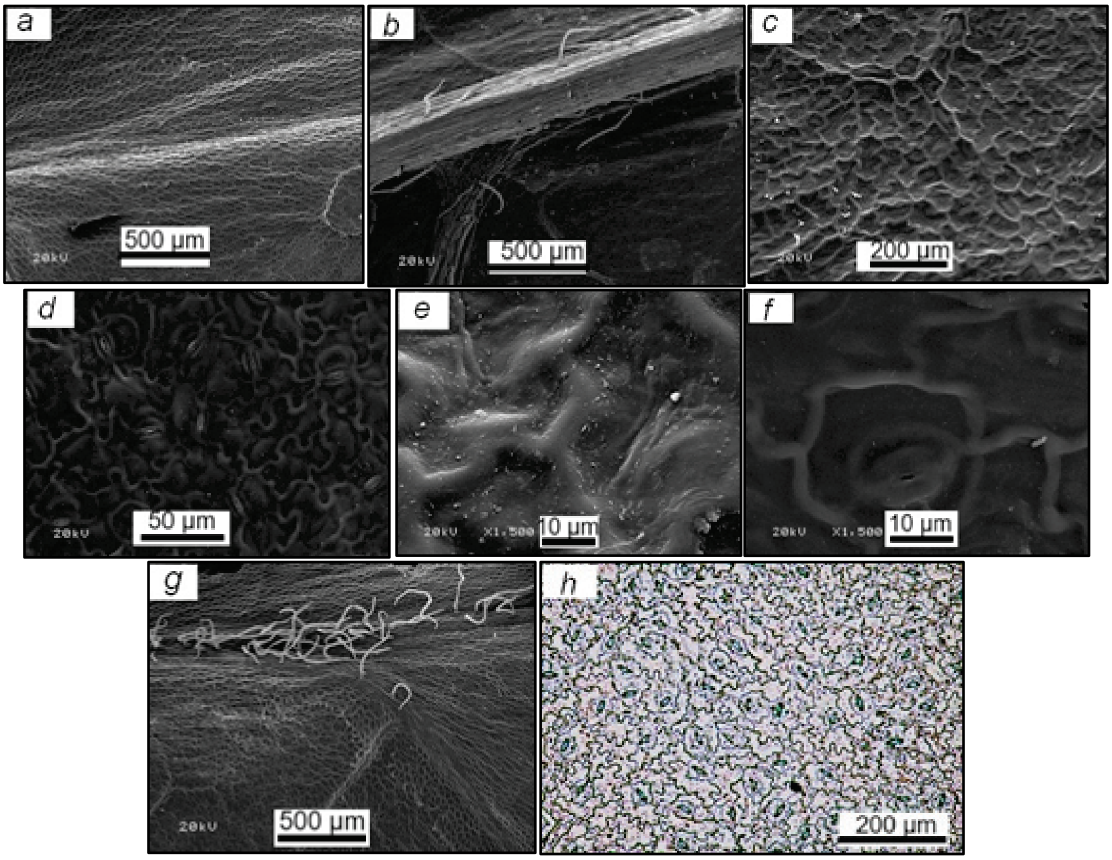

Fig. 3. The structure of the epidermis of the lamina of *M. kobus* DC.: *a–g* – SEM micrographs: *a* – adaxial epidermis; *b* – simple trichomes (abaxial epidermis); *c* – adaxial epidermis: relief pitted; *d* – abaxial epidermis: relief pitted; *e* – wax crusts (adaxial epidermis); *f* – cuticle smooth (abaxial epidermis); *g* – simple hairs (adaxial epidermis); *h* – stomata of the paracytic type (abaxial epidermis) (LM micrographs)

The abaxial surface of studied species differs from its adaxial surface. There are simple trichomes that are located along the midvein of the leaf (Fig. 3b). The stomata of the paracytic type are present only on the abaxial surface of the lamina (Fig. 3f, 3h). They are well visible. They are not oriented by their longer axis along the midvein of the leaf. Their number is 229.13 ± 3.68 stomata per 1 mm^2, the respiratory index is 1.02. They are located on the same level with the main cells of the epidermis. The cells of the abaxial epidermis are large, their number is 531.68 ± 22.43 cells per 1 mm^2. The relief of the abaxial surface of the lamina is pitted (Fig. 3d). The cuticle is well developed, mostly smooth (Fig. 3f). The shape of the cross section is linear. The shape of epidermal cells on the same side of the leaf varies considerably in cross section: from elongated-rounded (above and below the mesophyll) to rounded (above and below the midvein, Fig. 4).

The epidermal tissue is single-layered, its total thickness is 30.25 ± 0.43 µm, which is 24.0% of the leaf thickness. Both the adaxial and abaxial epidermis are approximately of the same thickness, which is approximately 12.1% (each) of the thickness of the lamina. The thickness of the cell walls of the upper and lower epidermis differs slightly: the adaxial epidermis cell walls are of moderate thickness, and the abaxial epidermis cell walls are thin. The mesophyll is of homogeneous – spongy type. It is characterized by large intercellular spaces, its thickness is equal to 100.19 ± 0.01 µm. It refers to the moderately layered type by the number of cell layers (4–6).

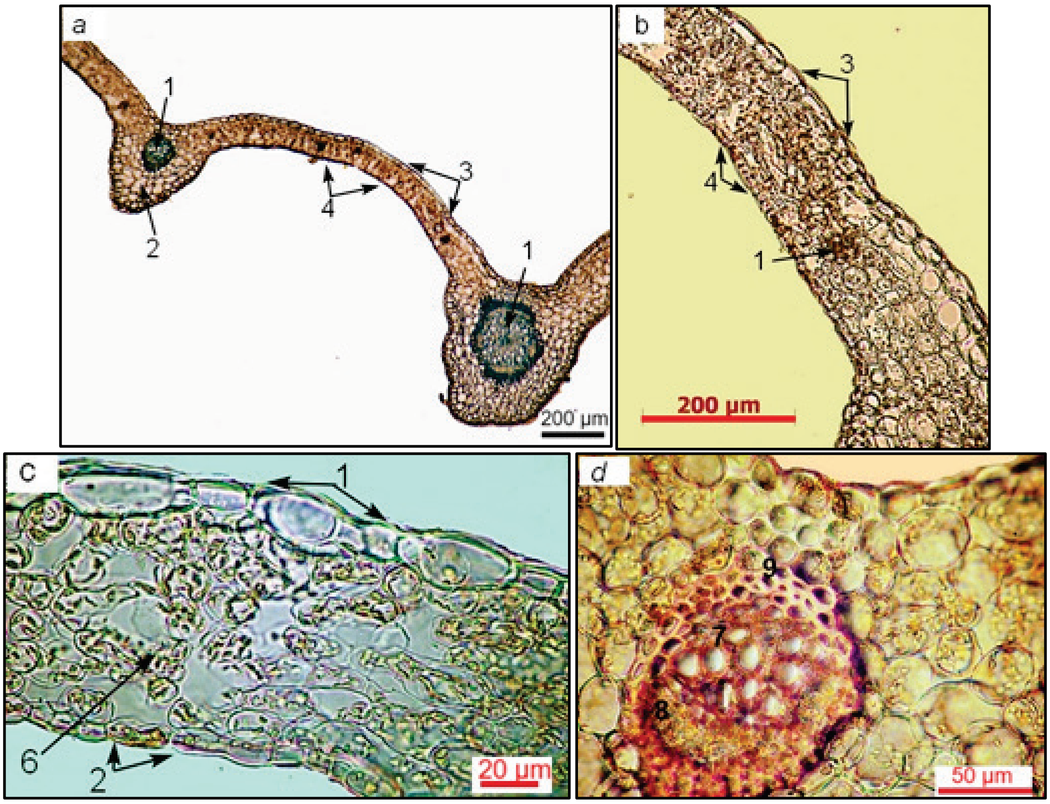

Fig. 4. Anatomical structure of the lamina of *M. kobus* DC. (LM): *a* – cross section through the center vein of lamina; *b*, *c* – cross section of leaf showing the homogeneous mesophyll; *d* – cross section of leaf showing the vascular bundle; *1* – vascular bundle, *2* – non-chlorophyllous parenchyma, *3* – adaxial epidermis, *4* – abaxial epidermis, *6* – spongy parenchyma, *7* – xylem, *8* – phloem, *9* – sclerenchyma

The vascular system is represented by the vascular bundles of midvein and a fairly large number of lateral vascular bundles (up to 12), which vary in size. The midvein, as in the previous species, is multibundle, has five vascular bundles, which are of the same size and separated from each other by three layers of thin-walled cells of chlorophyll-free parenchyma. The latter is very well-developed, its cells form the keel, and border on both the upper and lower epidermis. The subepidermal layers of the main parenchyma have a collenchymal thickening of the walls. The xylem vessels in the vascular bundles of the midvein are large, there are up to 12 pieces in each. The phloem is well developed, its elements are quite large and clearly distinguished in cross section. The ground tissue (collenchyma) is poorly developed. It is located in the midvein.

Magnolia obovata **Ait. ex Link**

Morphological characteristics. The leaves are simple, large, petiolate. Petioles are up to 4.2 cm long and 0.5 mm wide, curved. The lamina is light green, reversed broadly lanceolate, about 40.2 cm long and up to 20.1 cm wide at its widest part. The underside of the leaf is gray-green, matte. Veining of the lamina is reticulate.

Histological characteristics. Leaves are hypostomatic (Fig. 5j, 5h). Cells of adaxial epidermal tissue, as in previous species, are characterized by tortuous outlines and flattened projections. They are evenly thickened over the mesophyll, and are thickenied in the midvein. The anticlinal walls of the epidermal cells of the adaxial surface are located above the level of the main cells of the epidermis, the outer periclinal walls of the epidermal cells are flat. The projections and outlines of epidermal cells vary: cells with flattened projections and tortuous outlines are observed above the mesophyll, cells with elongated projections and tortuous outlines are observed in the area of vascular bundles. The relief of the adaxial surface of the lamina is reticulate (Fig. 5c). The cuticle is well-developed and smooth. There is a well-developed wax layer on the surface. Wax on the periclinal walls of cells is represented by crusts, wax on the anticline walls of cells is represented by wax granules (Fig. 5e).

The abaxial surface of studied species differs from its adaxial surface. The cells of the abaxial epidermis are very large (392.89 ± 11.43 per 1 mm^2). The stomata of the paracytic type are present only on the abaxial surface of the lamina (Fig. 5d, 5h). They are well visible. They are not oriented by their longer axis along the midvein of the leaf. They are located on the same level with the main cells of the epidermis. Their number is 184.46 ± 11.01. The epidermis is characterized by the average number of stomata. Its stomatal index is 2.12. The cuticle is well developed and smooth (Fig. 5d). The wax is represented in a form of granules (Fig. 5f).

The shape of the cross section is linear. The shape of epidermal cells on the same side of the leaf varies considerably in cross section: from elongated-rounded (above and below the mesophyll) to rounded (above and below the midvein). The epidermal tissue is single-layered, its total thickness is 44.22 ± 0.43 μm, which is 18.0% of the leaf thickness.

The degree of development of the adaxial and abaxial epidermis is approximately the same, they do not differ in thickness and are approximately 9.1% (each) of the thickness of the lamina. Cell walls in both epidermis are of moderate thickness.

The mesophyll is dorsoventral. It refers to the moderately layered type according to the number of cell layers (4–6). Spongy tissue is represented by three or four layers of fairly large rounded cells (Fig. 6).

The vascular system is represented by vascular bundles of the midvein and a fairly large number of lateral vascular bundles, which vary in size. The midvein is multi-bundle, has five vascular bundles, which are of the same size and separated from each other by three layers of thin-walled cells of chlorophyll-free parenchyma. The latter is very well developed, its cells form the keel, and border on both the upper and lower epidermis. The subepidermal layers of the main parenchyma have a collenchymal thickening of the walls. The xylem vessels in the vascular bundles of the midvein are large. Their number is up to 18 pieces in each. The phloem is well developed, its elements are quite large and clearly distinguished in cross section. The ground tissue (collenchyma) is poorly developed. It is located in the midvein.

Discussion

Today it is already known that ontogenesis is divided into stages of development, each of which takes place in certain conditions that are special only for it (Ochoa-Lopez et al., 2020). It has been confirmed by many authors that each phase of ontogenesis is adapted to specific environmental conditions, and stages of ontogenesis differ not only in their organization, but also in ecology (Gagliano et al., 2007). Each stage of ontogenesis has its key structural and functional features, which are responsible for the adaptation of organisms at a certain stage of development (Boege & Marquis, 2005; Barton & Koricheva, 2010). Today, it is already clear that in order to characterize the adaptive features of organisms, it is necessary to identify the specifics of their adaptation at the stages of ontogenesis (Gagliano et al., 2007). Perennial woody plants show the ontogenetic variation that extends over many years. They gradually develop from seedling through the reproductive and ageing stages until they finally begin to die. Along with the successive stages of development, the appearance and vigour of individuals changes (Boege & Marquis, 2006; Lundgren & Des Marais, 2020).

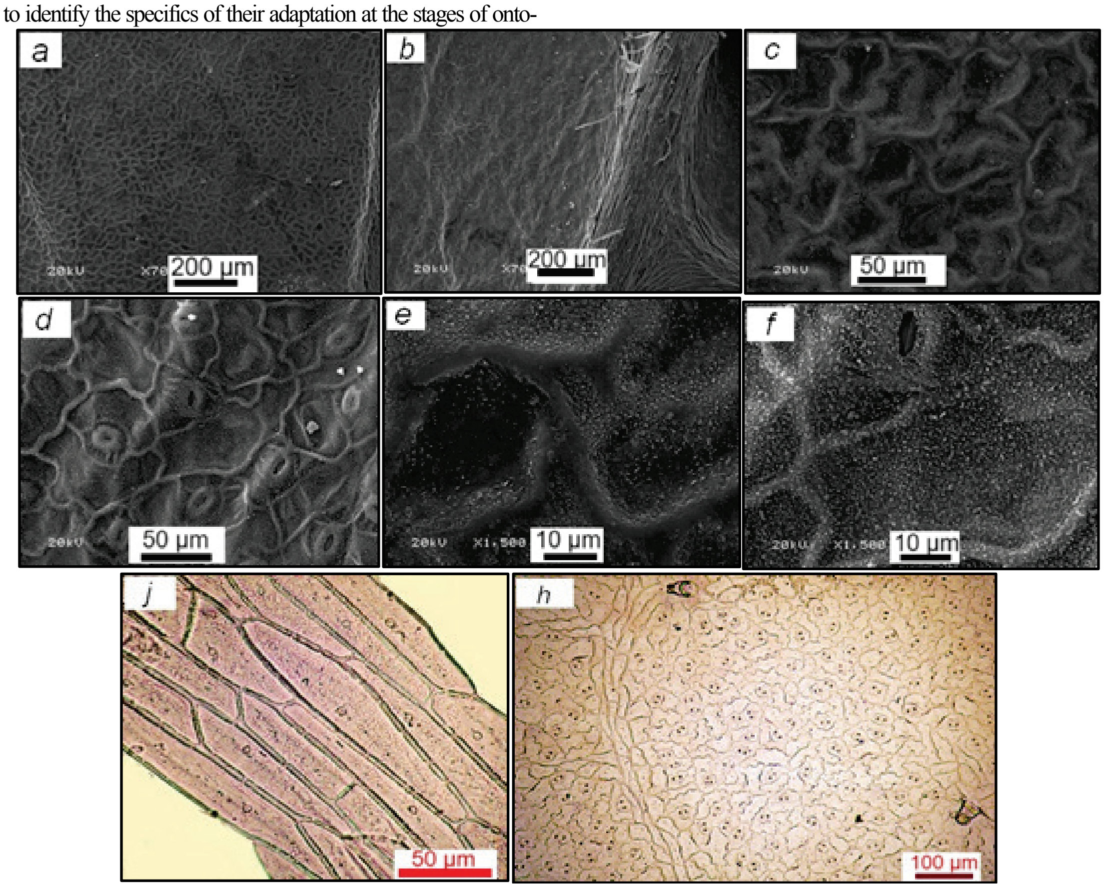

Fig. 5. The structure of the epidermis of the lamina of *M. obovata* Ait. ex Link.: *a–f* – SEM micrographs: *a* – adaxial epidermis; *b* – simple trichomes (abaxial epidermis); *c* – adaxial epidermis: reticulate relief; *d* – abaxial epidermis: cuticle smooth; *e* – adaxial epidermis: wax crusts and granules; *f* – abaxial epidermis: wax granules; *j*, *h* – LM micrographs: *j* – adaxial epidermis; *h* – stomata of the paracytic type (abaxial epidermis)

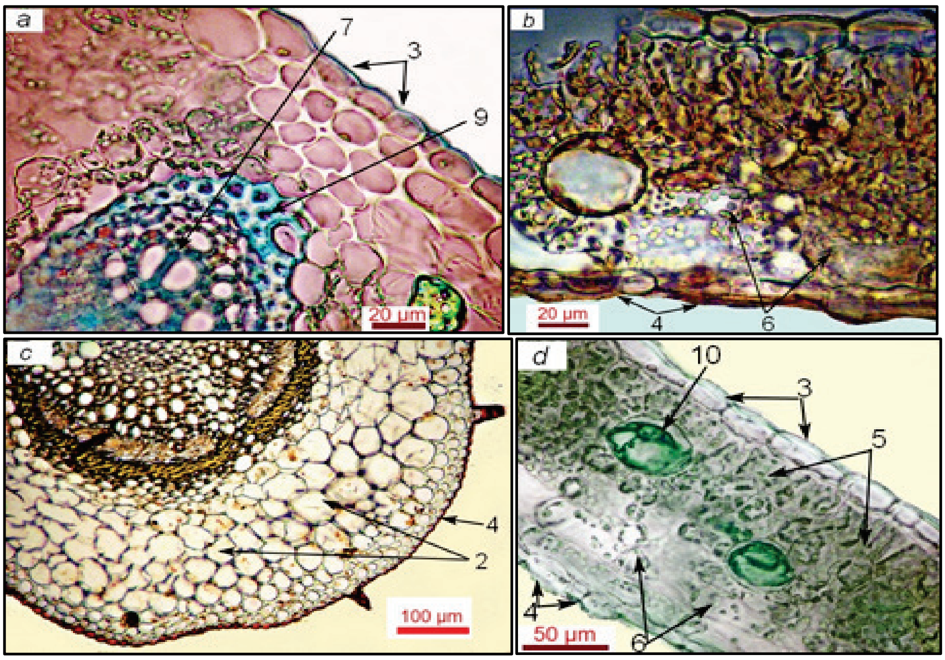

Fig. 6. Anatomical structure of the lamina of *M. obovata* Ait. ex Link (LM): *a* – cross section through the center vein of lamina; *b*, *d* – cross section of leaf showing the dorsoventral mesophyll; *c* – cross section of leaf showing the vascular bundle; *1* – vascular bundle, *2* – non-chlorophyllous parenchyma, *3* – adaxial epidermis, *4* – abaxial epidermis, *5* – palisade parenchyma, *6* – spongy parenchyma, *7* – xylem, *8* – phloem, *9* – sclerenchyma, *10* – oil drops

Different researchers use different signs for analysis at certain stages of ontogenesis. The leaves are the most flexible organs because of their individual structures that are associated with certain features. It was established that the differences between juvenile and other young and adult plants are clearly reflected in the anatomical structure of the leaves (Boyko, 2017). Corresponding comparative anatomical studies were performed for many types of plants, especially woody ones (Ferchaud et al., 2021; Shekhawat, 2021; Raeva-Bogoslovskaya, 2023).

At the same time, in the ontogenesis of woody plants in general, and magnolia species in particular, the virginal age is quite long, and therefore critically important from the point of view of selecting optimal conditions for growing plants. So, the results of correlation analysis of leaf blade anatomical features of the species of *Magnolia* genus plants confirmed that the studied species suffer from a lock of moisture and high temperatures on the first ontomorphogenesis stages (Futorna et al., 2020). Of the three studied species only in *M. obovata* are the vast majority of the studied anatomical features characterized by high correlation coefficients on the first stages of ontomorphogenesis. The plants *M. cobus* and *M. denudata* have stronger consistency between the features of epidermal tissue.

As a result of the study of the ultrastructure of leaves it was found that the studied species of the genus *Magnolia* introduced in Ukraine at the first stages of ontogenesis are characterized by the following features: the leaves of the studied species differ in linear dimensions: *M. kobus* leaves are the smallest: up to 10.1 x 5.2 cm, *M. obovata* leaves are the largest: up to 40.2 x 2.1 cm. Leaves of *M.obovata* differ in color: they are light green, while in the other two species the leaves are dark green. It is noted that the whole lamina with a pointed apex and a narrow base, as in magnolias, is characteristic of most temperate deciduous woody plants (Vasiliev,1988).

The type of stomatal apparatus and stomata size, the shape and height of the epidermal cell, and the structure of the mesophyll can be not only taxonomic characteristics, but also characterize the plant's adaptability to various environmental conditions. The histological characteristics of the leaves of the studied species are similar. They are hypostomatic. Their stomata are of the paracytic type, they are evenly distributed on the abaxial surface of the leaves. Hypostomatic leaves are not common among dicotyledons, but for such plants as *Cornus*, *Valeriana*, *Malus* similar features are noted, which is associated with the processes of adaptation to certain natural conditions (Klymenko & Klymenko, 2016). Cells of abaxial and adaxial epidermis are similar in projections and outlines. When observed in the paradermal plane, the epidermal cells of the studied species of the genus *Magnolia* have tortuous (cells located on the periphery of the leaf) or straight (cells located on the veins) outlines and rectangular (cells located on the veins) or flattened (cells placed on the periphery of the leaf) projection. The cuticle is relatively thin, located on both the abaxial and adaxial surfaces of the leaves. In our opinion, it has functioned as the barrier effect against excessive water loss to maintain turgescence and prevent desiccation. This is important on the first ontomorphogenesis stages.

Waxes are an essential structural element of the surface and of fundamental functional and ecological importance for the interaction between plants and their environment (Barthlott et al., 1998). General patterns of the leaf epicuticular waxes' modification due to increased solar radiation and air temperature can indicate the adaptive metabolic responses of woody plants to changing climatic conditions (Lykholat et al., 2020). Epicuticular wax is observed of three types in the studied species: films (*M. denudata*), crust (*M. kobus*, *M. obovata*) and wax granules (*M. denudata*, *M. obovata*). The amount of wax and pubescence is greater on the abaxial surface in *M. obovata*; very weak pubescence and waxy layer is observed only on the abaxial surface of the leaf in *M. kobus*, weak pubescence is present on both sides in *M. denudata*. A waxy layer predominates on the abaxial surface of leaves, as in all studied species. In all species of the genus *Magnolia* pubescence is simple, formed by hairs that "accompany" the veins.

There are two main types of lamina relief in the studied species: reticular in *M. obovata*, *M. denudata* (subtype reticular-collicular) and pitted in *M. kobus*. The species clearly differ in this feature, so we believe that the type of relief of the lamina can be used as an additional diagnostic feature to distinguish species of the genus *Magnolia*. It is noteworthy that the studied species differ in the number of stomata. *Magnolia denudata* is characterized by a small number of stomata, while *M. obovata* and *M. kobus* are characterized by their average number. The Stomata Index varies from 2.8 in *M. denudata* to 1.02 in *M. kobus*.

The mesostructural organization of the leaf is an important indicator that determines the efficiency of the photosynthetic apparatus of the plant and significantly affects its productivity. It is found that the epidermis is single-row in the cross section. Its cells are horizontally extended and vary in size (small over the midvein and larger over the mesophyll). The width of epidermal cells correlates with the size of the lamina. The lamina is the thickest in *M. obovata*, the thinnest is in *M. kobus*, but the total thickness of the epidermal tissue as a percentage of the thickness of the leaf is, in descending order: *M. denudata* (29.1%), *M. kobus* (24.0%), *M. obovata* (18.0%). It is known from the literature that a larger average stomatal area, a thicker epidermis, a greater height of the palisade chlorenchyma cells, and a higher coefficient of palisade ratio indicate greater resistance to changes in atmospheric precipitation at all stages of ontogenesis (Bogoslovskaya et al., 2023).

The mesophyll varies from homogeneous-spongy (in *M. kobus*) to layered (in *M. obovata*, *M. denudata*) type. The number of layers of cells that form the mesophyll in all studied species is from 4 to 6. Thus, *M. kobus* is characterized by the least specialized type of mesophyll.

The degree of development of strands of mechanical tissue around the vascular bundle differs in different ecological groups of plants. Thus, veins with a covering of mechanical tissue in the form of powerful strands from the vascular bundle to the epidermis on both sides of the leaf or with strands of mechanical tissue usually occur in the leaves of xerophytic plants and many heliophyte plants, while in mesophytes, hygrophytes and shade plants, the mechanical tissue around the vascular bundles is almost undeveloped (Futorrna et al., 2017). The vascular system in the studied plants is represented by small central and lateral vascular bundles. The ground tissue is present only in the central and large side bundles.

Conclusion

The study of anatomical and morphological features of the leaves of three species of *Magnolia* in the early stages of ontogenesis showed their similarity. It was established that the abaxial and adaxial sides of the leaves perform different functions. The adaxial side of the leaf has a cuticle and a small amount of wax which protects the leaf from sunburn and water loss, the abaxial side has stomata which provide gas exchange and transpiration. It can be predicted that the presence of a few trichomes on both sides of the leaves are remnants of more numerous trichomes on the leaves in the past, which were lost due to adaptation to conditions with less solar insolation, which is typical for the studied species. Thus, in the early stages of ontogenesis, the studied plants are typical mesophytes with hypostomatic leaves adapted to exist in sufficiently moist conditions in soil and air.

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