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## Structural features of the leaf in some cultivated species of the genus *Citrus*

This article discusses the significance and relevance of studying the vegetative organ of the leaf in addressing various fundamental questions. *Citrus* crops cultivated worldwide are representatives of the genus *Citrus* L. This genus, along with its wild and semi-wild relatives (*C. trifoliata* L. and *C. ichangensis* Sw.), belongs to the subfamily Aurantioideae Eng. of the family Rutaceae Juss. Almost all are evergreen plants and are considered important fruit crops in tropical and subtropical regions of the world. The classification and systematics of citrus are quite problematic. To this day, there is no universally accepted botanical classification, likely due to the extreme polymorphism of the genus *Citrus*. The three botanical genera of the most economic and practical significance are *Citrus*, *Fortunella*, and *Poncirus*. The fruits, flowers, and leaves contain a wide range of biologically active substances (organic acids, bioflavonoids, essential oils, and 12 vitamins). Citrus essential oils are widely used to produce natural perfumes and as flavoring ingredients in food, pharmaceutical, and cosmetic products. Leaves from plants such as pomelo, orange variety Uzbekistan, and mandarin variety Medovka were studied. A single leaf type was established — hypostomatic. The mesophyll type of the leaf contains various inclusions, with the presence of water-storing cells and a large number of intercellular spaces. It was found that pomelo has the largest leaf blade height, while mandarin Medovka has the smallest one. The presence of trichomes and glandular hairs was noted in the pomelo representative. The smallest number of stomata was observed in mandarin Medovka, while the largest one was found in orange Uzbekistan.

**Keywords:** leaf structure, pomelo, orange, mandarin, stomata, epidermis, trichomes, mesophyll.

### Introduction

Currently cultivated citrus crops worldwide are representatives of the genus *Citrus* L. This genus, along with its wild and semi-wild relatives (*C. trifoliata* L. and *C. ichangensis* Sw.), belongs to the subfamily Aurantioideae Eng. of the family Rutaceae Juss. [1-2]. Citrus species, with the exception of *C. trifoliata*, are evergreen plants and are considered to be among the most important fruit crops in tropical and subtropical regions of the world [3-5].

The systematics and classification of citrus crops are quite problematic and controversial [6]. There is still no universally accepted botanical classification, which is related to the extraordinary polymorphism of the genus *Citrus*. According to V.P. Alekseev (1955) [7], the family Rutaceae includes 7 subfamilies, 150 genera, and approximately 1600 species. The three botanical genera of the greatest economic and practical significance are *Citrus*, *Fortunella*, and *Poncirus*. Currently, two main classifications of the subfamily Aurantioideae have been developed, created by W.T. Swingle (1967) [8] and T.A. Tanaka (1966) [9], each of which has its advantages and disadvantages. Among citrus cultivation specialists, the classification by W.T. Swingle has gained the most recognition. It is widely accepted in many countries, especially in England and the USA, and includes two subgenera represented by 16 species of the genus *Citrus*, eight of which produce edible fruits and have commercial importance: *C. reticulata* (mandarins), *C. medica* (citron), *C. sinensis* (L.) Osb. (sweet oranges), *C. paradisi* Macf. (grapefruits), *C. maxima* (pummelo), *C. limon* (L.) Burm. (lemon), *C. aurantifolia* (Christm.) Swing. (lime), and *C. aurantium* L. (bitter orange) [8, 10, 11]. T.A. Tanaka's classification encompasses more than 147 species of the genus *Citrus* [12]. In distinguishing species, he considered the morphological characteristics of the plants, their ecology, and fruit structure. However, some citrus taxonomists disagree with the existence of such a large number of distinct species, as the differences between some are very minor [11]. The systematics of the genus *Citrus* has also been studied by R.W. Hodgson (1961) [13], P.M. Zhukovsky (1971) [14], and others.

The numerous beneficial properties and qualities of citrus fruits that positively impact human health have contributed to their cultivation and spread worldwide. In terms of distribution among fruit crops, they rank third globally, with an annual production exceeding 137 million tons and an area under cultivation of over 14 million hectares. Currently, citrus cultivation is of industrial significance in more than 142 countries.

The highest gross yields are reported from countries such as China (over 39.3 million tons), Brazil (19.7 million tons), India (11.4 million tons), Mexico, the USA, Spain, Turkey, and Egypt (FAO, 2023).

Citrus plants have the ability to purify indoor air from harmful microorganisms through phytoncides [15]. Currently, there is a growing social demand for environmental improvement technologies that promote active longevity, which were first developed in Russia by A.T. Bolotov in the 18th and 19th centuries [16]. Citrus species are particularly popular in ornamental horticulture. They easily adapt to new growing conditions, have decorative foliage, and are visually striking during flowering [17–20]. Citrus crops are characterized by longevity and high yield, which contribute to rapid cost recovery on an industrial scale. Some citrus species are remarkable for their longevity. For instance, in Rome, there is a bitter orange tree in the grounds of a monastery that is approximately 600 years old [21]. However, the most famous and oldest orange tree grows near the Palace of Versailles in the suburbs of Paris, having been planted around 1421. Due to such longevity and favorable soil-climatic conditions, individual trees can yield exceptionally high harvests, often surpassing the yields of other fruit crops by several times. For example, on the island of Malta and in the vicinity of Naples, trees can produce up to 30,000 fruits [22].

The leaves are simple, thick, leathery, and vary in shape from oval to lanceolate, with varying degrees of serration. The upper side is covered with a cuticle, while the abaxial (lower) side has numerous stomata and cavities containing essential oils. The color of the leaves depends on their age: light green in young leaves and dark green in mature ones, with a lifespan of 2–3 years. Young leaves perform the primary functions necessary for sustaining life, while older leaves accumulate nutrients for the formation of new vegetative and generative organs. Depending on the species, the petioles may have various wing-like structures, ranging from weakly winged (in mandarins) to strongly winged (in pummelos and grapefruits). In citrons, these wings are completely absent, while in plants of the subgenus *Papeda*, they can be comparable in size to the leaf blade. Thorns of various sizes may be present in the leaf axils or on the branches, or they may be completely absent [23, 24].

Uzbekistan, located in Central Asia, boasts a rich agricultural heritage deeply rooted in its warm climate, which fosters the cultivation of a diverse range of species and varieties. This favorable environment allows for the production of numerous fruit crops with exceptional quality and yield. Traditionally, Uzbekistan has relied on conventional methods of cultivation and processing these crops, with a particular focus on local markets. However, against the backdrop of a rapidly changing landscape, the country is poised for significant growth and global recognition, especially in the export of products such as cherries, apricots, and grapes. The shift towards modern farming methods marks a turning point for Uzbekistan's agricultural sector. This transition not only positions the country as a reliable exporter of fruits but also provides lucrative opportunities for nurserymen, including various varieties and rootstocks, as well as for companies engaged in greenhouse technologies worldwide. Furthermore, in regions where agricultural mechanization is underdeveloped, Uzbekistan has significant potential for the implementation of advanced technologies in both cultivation and post-harvest processes. In this context, various international players, including companies from Turkey, the Netherlands, China, and the USA, have begun to capitalize on Uzbekistan's growing agricultural landscape. Thus, Uzbekistan is becoming a beacon of opportunities on the global stage, fostering collaboration and innovation in the field of fruit science.

Citrus fruits primarily grow in countries with a subtropical climate. These bright, juicy fruits are valued for their tangy “sourness”, refreshing aroma, and numerous health benefits.

Many citrus fruits positively influence the functioning of the digestive tract. Citrus juice disinfects mucous membranes and gradually forms a reliable defense against infectious diseases of the upper respiratory tract. In light of the above, the trend of growing these plants in greenhouse conditions has increased recently.

Today, fundamental research on citrus fruits is relevant not only in Uzbekistan but also worldwide for addressing phylogenetic issues and systematic problems. Additionally, leaves are the organs most susceptible to various damages, which are exacerbated by the active spread of various insect pests.

Thus, the aim of our research is to conduct a structural study of the vegetative organ, specifically the leaf, in plants such as orange (Uzbekistan variety), mandarin (Medovka variety), and pomelo.

### *Experimental*

Experimental studies were conducted from 2023 to 2024. The leaves of pomelo, orange (Uzbekistan variety), and mandarin (Medovka variety) were collected from 5–8 sympodial branches for the research. For anatomical analysis, the fresh leaves were fixed in 96 % ethanol. Measurements were taken, and preparations of cross-sections and paradermal sections of the fresh leaves were made. For the analysis of the paradermal

and cross-sections of the leaf, samples with an area of 1 cm<sup>2</sup> were taken from the central zone (on both sides of the main vein of leaves of medium formation).

Young leaves in the phase of most vigorous growth were fixed. The material was washed and preserved in a 70 % alcohol solution. Leaf cuttings of 1x1 cm were placed in a mixture of 23 cm<sup>3</sup> of 96 % alcohol and 2 cm<sup>3</sup> of chloroform for 1-2 days. After rinsing in distilled water, they were placed in an 18 % KOH solution for clarification for 4–6 days. They were then rinsed again and placed on a glass slide. Staining of the epidermal cell walls was performed with methylene blue.

Morphometric processing of the collected material was carried out, photographs were taken, and tables were compiled. A trinocular microscope XSP-500SM (with a 7-inch screen and the ability to connect to a PC) was used for the research. Statistical processing of quantitative data was performed using standard criteria with the help of a personal computer (MS Excel).

### Results and Discussion

Analyzing the obtained data, it was found that the mesophyll of the pomelo leaf is dorsiventral, with two layers of palisade parenchyma. The spongy parenchyma contains from 14 to 18 layers of cells, and in the middle of the mesophyll, there is a presence of water-storing parenchyma. The cells of the water-storing tissue are somewhat larger than those of the spongy parenchyma. Additionally, the spongy parenchyma contains a large number of intercellular spaces. The mesophyll is also rich in lysigenous cavities filled with various essential oils (Fig. 1). The adaxial epidermis is covered with a waxy coating, and no trichomes or glandular hairs were observed on the leaf. The height of the pomelo leaf blade differs from those studied, measuring the largest size at  $224.4 \pm 12.6 \mu\text{m}$  (Tab. 1, Fig. 2, 3). The cells of the palisade mesophyll are elongated-oval in shape. The palisade coefficient is very low at 21 % (according to the scale of B.R. Vasiliev, 1988) (Fig. 4). The spongy mesophyll consists of round-shaped cells. The vascular bundles are of a concentric nature, surrounded by periodic layers.

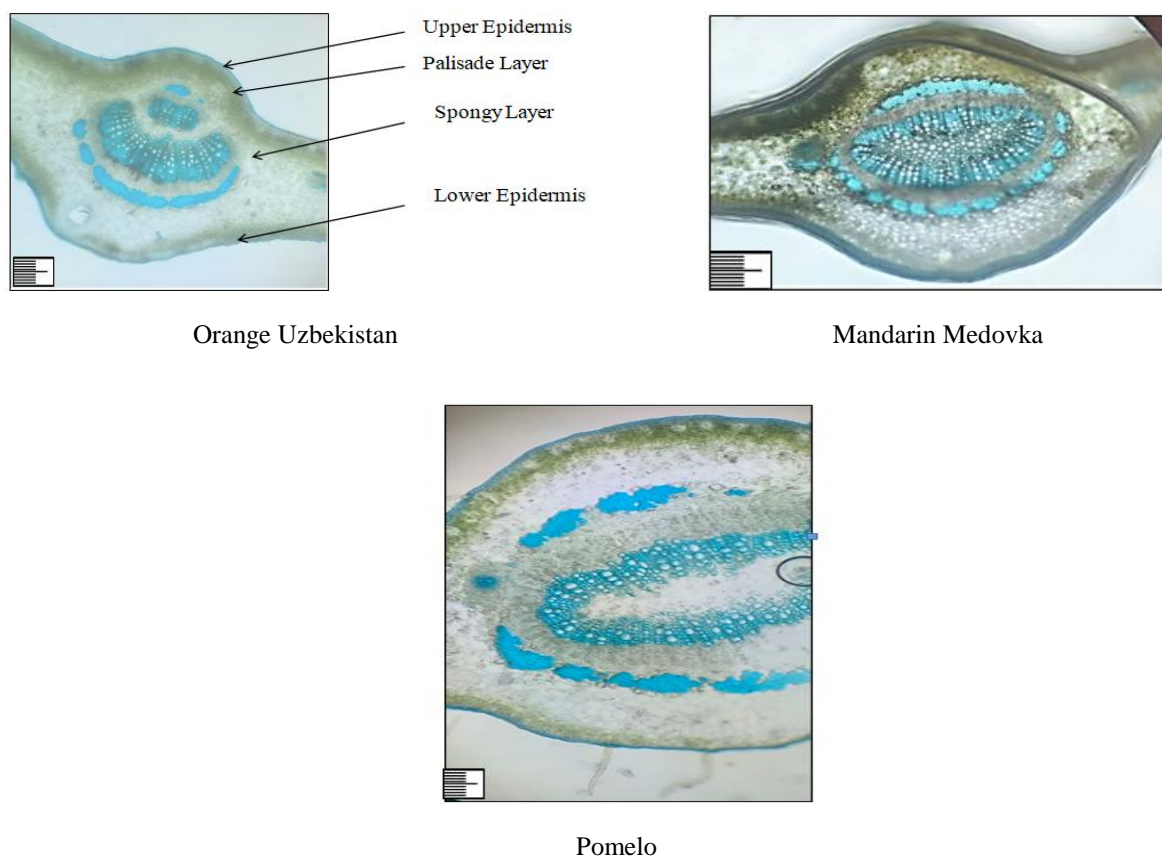
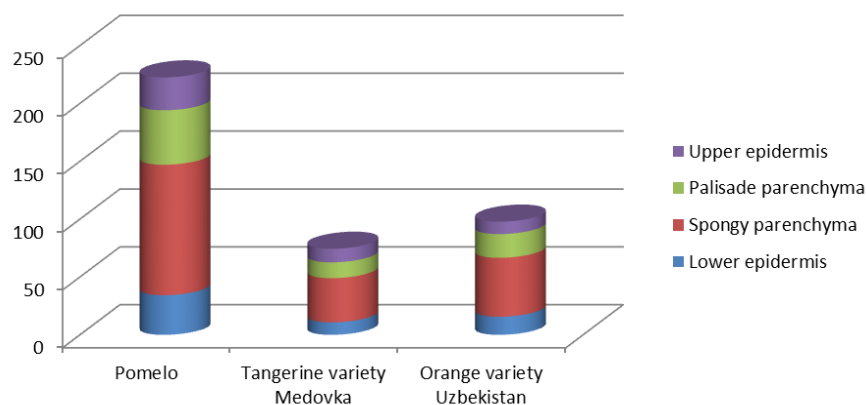
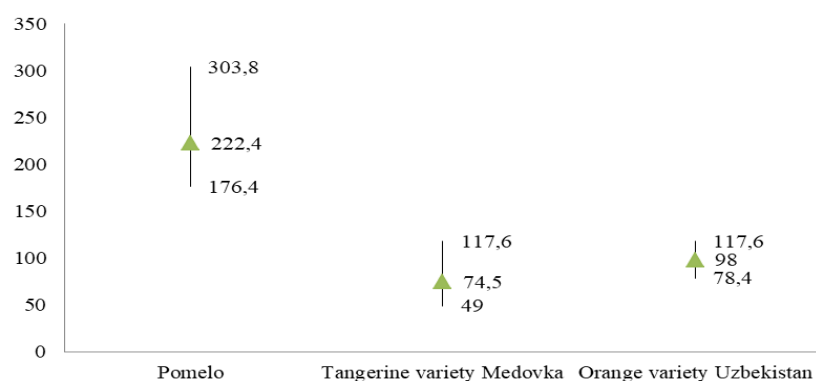
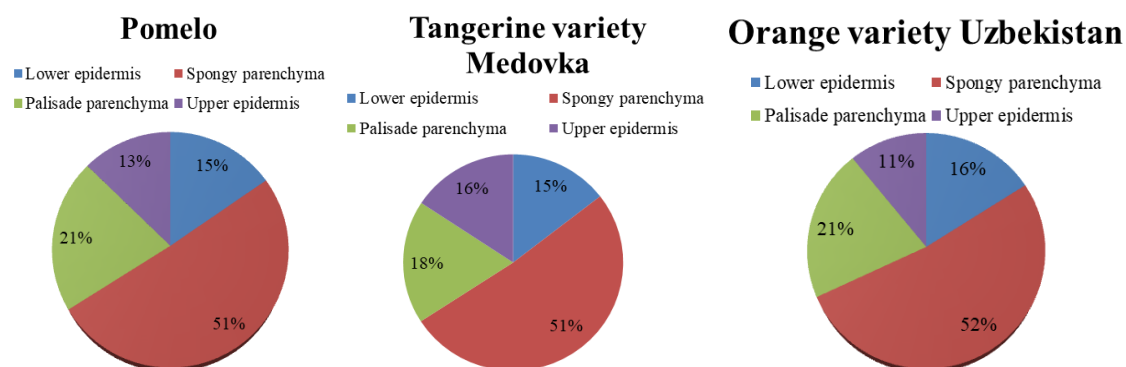


Figure 1. Structure of the leaf blade of some representatives of *Citrus*

Table 1

Structural indicators of the cross-section of the leaf of some representatives of *Citrus* (in  $\mu\text{m}$ )

Types	Upper Epidermis	Palisade Parenchyma	Spongy Parenchyma	Lower Epidermis	Total Leaf Height
Pomelo	28,4 $\pm$ 3,7	47,1 $\pm$ 5,1	112,7 $\pm$ 12,1	34,3 $\pm$ 4,8	222,4 $\pm$ 12,2
Mandarin Medovka	11,7 $\pm$ 1,7	13,7 $\pm$ 2,1	38,2 $\pm$ 4,1	10,8 $\pm$ 0,9	74,5 $\pm$ 6,1
Orange Uzbekistan	10,7 $\pm$ 0,9	20,5 $\pm$ 2,2	50,9 $\pm$ 4,1	15,7 $\pm$ 2,0	98,0 $\pm$ 2,7

Figure 2. Height of individual layers of the leaves of the studied representatives of the genus *Citrus*Figure 3. Indicators of total leaf height and its error in the studied representatives of the genus *Citrus*Figure 4. Indicators of the percentage ratio of leaf tissues in the studied representatives of the genus *Citrus*

When comparing the number of epidermal cells on the adaxial side of the leaf, it was established that the projection of the epidermal cells is flattened, the wall outlines are straight, and the cells are very small, with those on the abaxial epidermis being somewhat larger than those on the adaxial side (Fig. 5, 6). The stomata are slightly sunken, oval in shape, numerous, of the anomocytic type, and randomly oriented. Unicellular trichomes are preferentially located on the leaf veins. In this representative, the adaxial epidermis is the most finely cellular, containing  $7551.3 \pm 14.9$  cells per  $1 \text{ mm}^2$  (Tab. 2). Additionally, the stomatal apparatus is absent on the adaxial side of the leaf. On the abaxial side of the pomelo leaf, the number of epidermal cells is somewhat less compared to the adaxial side, containing  $5443.4 \pm 63.5$  cells per  $1 \text{ mm}^2$ , indicating larger cell sizes. This side also has an anomocytic stomatal apparatus, making the leaf hypostomatic. The size of the stomata varies slightly from one another, with a count of  $802.5 \pm 18.6$  stomata per  $1 \text{ mm}^2$ . Furthermore, on the abaxial side of the pomelo leaf, there are multicellular trichomes ( $89.9 \pm 9.7$  per  $1 \text{ mm}^2$ ) and glandular hairs ( $13.3 \pm 4.6$  per  $1 \text{ mm}^2$ ).

Table 2

**Quantitative indicators of paradermal analysis of the leaf in the studied representatives of the genus *Citrus*, per  $1 \text{ mm}^2$**

Species	Upper Epidermis			Lower Epidermis			
	Number of Epidermal Cells	Number of Stomata	Number of Trichomes	Number of Epidermal Cells	Number of Stomata	Number of Trichomes	Number of Glandular Hairs
Pomelo	$7551,3 \pm 14,9$	-	-	$5443,4 \pm 63,5$	$802,5 \pm 18,6$	$89,9 \pm 9,7$	$13,3 \pm 4,6$
Mandarin Medovka	$3598,6 \pm 267,6$	-	-	$1811,5 \pm 87,7$	$741,5 \pm 62,0$	-	-
Orange Uzbekistan	$2011,3 \pm 63,9$	-	-	$1741,6 \pm 52,8$	$1111,1 \pm 64,4$	-	-

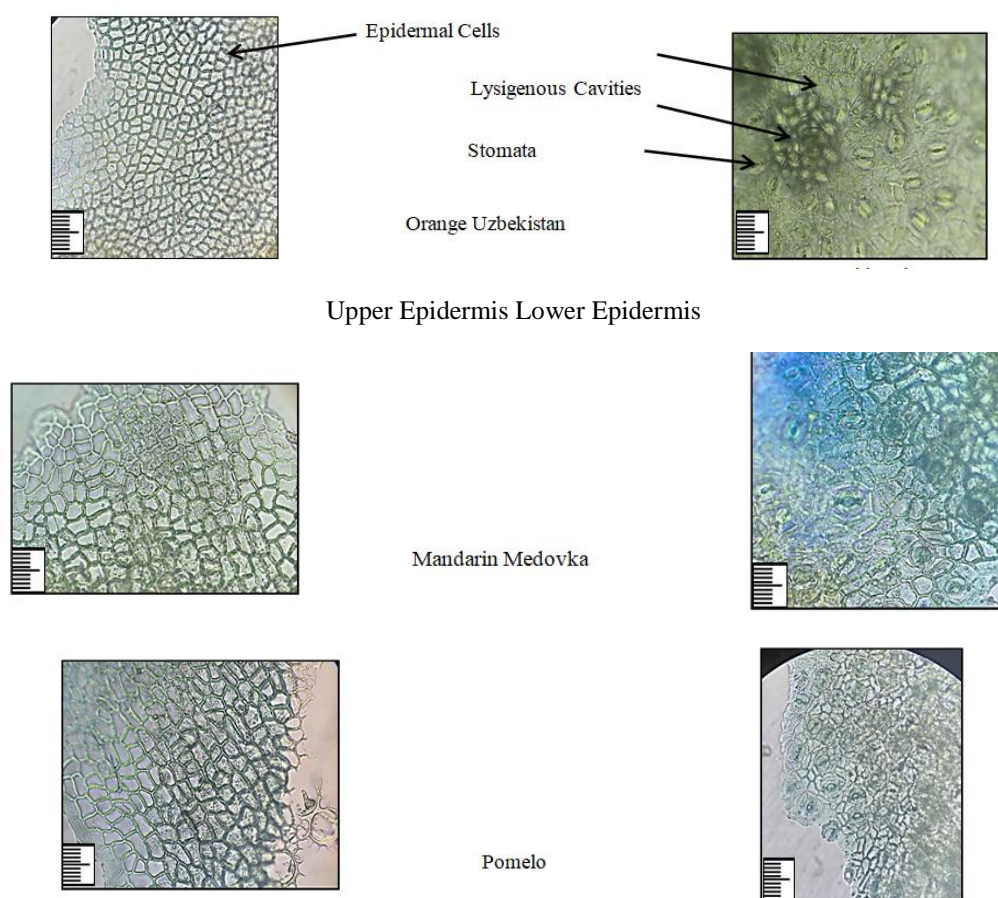


Figure 5. Structure of the paradermal analysis of the leaf in the studied representatives of the genus *Citrus*



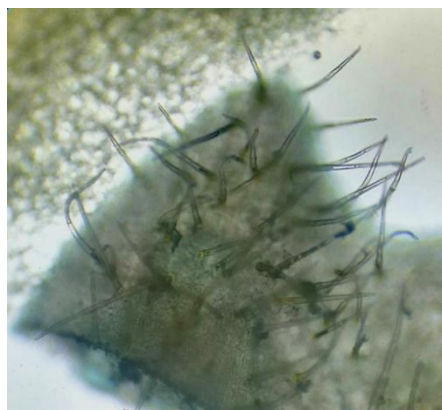


Figure 6. Epidermal Trichomes and Glandular Hairs on the Leaf of Pomelo

The analysis of the structural indicators of the leaf of the Mandarin Medovka showed that the mesophyll of the leaf is dorsiventral (Fig. 1). The total height of the leaf blade is the smallest compared to the studied representatives ( $74.5 \pm 6.1 \mu\text{m}$ ), but the epidermis on the adaxial side of the leaf is somewhat larger compared to the studied representative — the orange variety Uzbekistan (Tab. 1, Fig. 2). All other height indicators of various leaf tissues are significantly lower, practically 2–4 times less than those of other studied representatives, except for the height of the central vein. Its measurements are average among the studied representatives, reaching  $454.7 \pm 15.4 \mu\text{m}$ . Additionally, the mesophyll contains lysigenous cavities. Among the spongy parenchyma, there is a large number of intercellular spaces and water-storing cells.

Upon examining the cells of both epidermal layers, it was found that the cells of the adaxial epidermis are almost twice as small as those of the abaxial epidermis ( $3598.6 \pm 267.7$  per  $1 \text{ mm}^2$  and  $1811.5 \pm 87.7$  per  $1 \text{ mm}^2$ , respectively). The wall contours are somewhat wavy, and the outline of the epidermal cells is polygonal, with 3–5 sides. The stomata are slightly sunken, oval in shape, numerous, of the anomocytic type, and are oriented randomly. The number of stomata is recorded only on the abaxial side of the leaf, indicating that the leaf is hypostomatic. The number of stomata on the abaxial side of the leaf is the lowest compared to the studied representatives ( $741.5 \pm 62.0$  per  $1 \text{ mm}^2$ ).

The obtained data on the structure of the leaf of the orange variety Uzbekistan show that the wall contours of both epidermis layers are straight and polygonal (4–5 sides), and the cells are larger than those of the studied representatives, with the abaxial epidermis being somewhat larger than the adaxial ( $1741.6 \pm 52.8$  per  $1 \text{ mm}^2$  and  $2011.3 \pm 63.9$  per  $1 \text{ mm}^2$ , respectively). The mesophyll of the leaf has a dorsiventral structure, with the palisade layer consisting of 2 rows and the spongy layer being multi-layered (8–10 layers). Lysigenous cavities and water-storing cells are also present in the mesophyll, and calcium oxalates are found in the parenchyma cells. The height of the adaxial epidermal cell in the orange variety Uzbekistan is the smallest among the studied representatives ( $10.7 \pm 0.9 \mu\text{m}$ ). The other indicators are average between the two studied representatives, with the height of the leaf blade reaching  $98.0 \pm 2.7 \mu\text{m}$ . However, the height of the central vein is the maximum among the studied species ( $597.8 \pm 17.1 \mu\text{m}$ ).

When counting the stomata on both sides of the leaf, it was found that stomata are present only on the lower side of the leaf; therefore, the orange variety Uzbekistan, like all studied representatives, has a hypostomatic type of leaf. The number of stomata on the abaxial side of the leaf reaches  $1111.1 \pm 64.4$  per  $1 \text{ mm}^2$ , which is the highest indicator among the studied representatives.

The mesophyll is penetrated by veins, which consist of bundles of conducting tissues made up of primary xylem and phloem. Citrus plants exhibit pinnate venation. In pinnate venation, the main vein and secondary veins are well developed. In the Mandarin Medovka and Pomelo, the vascular bundle has a concentric structure. The central vein of the leaf contains xylem and phloem and is connected to the conducting system of the stem.

It has been identified as belonging to the hygrophytic group of plants, as indicated by the very thin dorsiventral structure of the leaf, the unilayered epidermis, the positioning of the stomata at the same level as the epidermis, and their absence on the upper side of the leaf.

### Conclusions

In Mandarin Medovka and Pomelo, the vascular bundle has a concentric structure, indicating a greater primitiveness compared to the orange variety Uzbekistan. In all studied representatives, there are no stomata and trichomes on the upper epidermis, which is evidence of the hypostomatic nature of the leaf. The leaf blade of Pomelo has the greatest thickness, which contributes to biological protection against insect pests.

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## ***Citrus* тұқымдасының кейбір түрлерін жасанды жағдайда өсіруде жапырақтың құрылымдық ерекшеліктері**

Мақалада әртүрлі іргелі мәселелерді шешу үшін жапырақтың вегетативті мүшесін зерттеудің маңыздылығы мен өзектілігі қарастырылған. Бүкіл әлемде жасанды жағдайда өсірілетін цитрус дақылдары *Citrus* L. туысына жатады. Бұл тұқымның жабайы және жартылай жабайы туыстары (*C. trifoliata* L. және *C. ichangensis* Sw.) Rutaceae Juss тұқымдасының Aurantioideae Eng тұқымдас тармағына жатады. Олардың барлығы дерлік мәңгі жасыл және әлемнің тропикалық және субтропикалық аймақтарында маңызды жеміс дақылдары болып саналады. Цитрус жемістерінің жіктелуі мен систематикасы өте өзекті. Бүгінгі күнге дейін жалпы қабылданған ботаникалық классификация жоқ, бұл *Citrus* туысының арнайы полиморфизміне байланысты болуы мүмкін. *Citrus*, *Fortunella* және *Poncirus* — ең үлкен экономикалық және практикалық маңызы бар үш ботаникалық тұқымдастар. Жемістер, гүлдер мен жапырақтарда биологиялық белсенді заттардың кең спектрі бар (органикалық қышқылдар, биофлавоноидтар, эфир майлары және 12 витамин). Цитрустық эфир майлары табиғи парфюмерия өндіру үшін және тамақ, фармацевтика және косметикалық өнімдерде хош иістендіргіш ретінде кеңінен қолданылады. Помело, Өзбекстан апельсині, «Медовка» мандарині сұрыптары өсімдіктерінің жапырақтары зерттелді. Жапырақтардың бірыңғай түрі анықталды, яғни гипостоматикалық. Жапырақ мезофилінде әртүрлі қосындылар бар; оның құрамында су сақтайтын жасушалар және көптеген жасушааралық кеңістіктер бар. Помелода жапырақ алақанының (тактасы) ең үлкен биіктігі бар екендігі, ал «Медовка» мандаринінде ең кішкентай екені анықталды. Помелолар өкілінде трихомалар мен безді түктердің болуы кездеседі. «Медовка» мандаринінде аз, ал Өзбекстан апельсинінде көп мөлшерде екендігі байқалды.

*Кілт сөздер:* жапырақ құрылымы, помело, апельсин, мандарин, саңылау, эпидермис, трихома, мезофилл.

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## **Структурные особенности листа у некоторых культивируемых видов рода *Citrus***

В данной статье рассматривается значение и актуальность изучения вегетативного органа листа для решения различных фундаментальных вопросов. Цитрусовые культуры, культивируемые во всем мире, являются представителями рода *Citrus* L. Этот род, наряду с его дикими и полудикими родственниками (*C. trifoliata* L. и *C. ichangensis* Sw.), относится к подсемейству Aurantioideae Eng. семейства Rutaceae Juss. Почти все они являются вечнозелеными растениями и считаются важными плодовыми культурами в тропических и субтропических регионах мира. Классификация и систематика цитрусовых весьма проблематичны. По сей день не существует общепринятой ботанической классификации, что, вероятно, связано с чрезвычайным полиморфизмом рода *Citrus*. Три ботанических рода, имеющие наибольшее экономическое и практическое значение, — это *Citrus*, *Fortunella* и *Poncirus*. Плоды, цветки и листья содержат широкий спектр биологически активных веществ (органические кислоты, биофлавоноиды, эфирные масла и 12 витаминов). Эфирные масла цитрусовых широко используются для производства натуральных духов и в качестве ароматизаторов в пищевой, фармацевтической и косметической продукции. Были изучены листья таких растений, как помело, апельсин сорта Узбекистан и мандарин сорта Медовка. Установлен единый тип листьев — гипостоматический. Мезофилл листа содержит различные включения, в нем присутствуют водозапасающие клетки и большое количество межклеточных пространств. Установлено, что у помело наибольшая высота листовой пластинки, а у мандарина Медовка — наименьшая. У представителя помело отмечено наличие трихом и железистых волосков. Наименьшее количество было отмечено у мандарина Медовка, а наибольшее — у апельсина Узбекистан.

*Ключевые слова:* структура листа, помело, апельсин, мандарин, устьице, эпидермис, трихома, мезофилл.



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