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Comparative anatomical and morphological characteristics of two subspecies of *Melissa officinalis* L. (Lamiaceae)

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Abstract. The aim of the research is to comprehensively compare the anatomical and morphological structures of plants of *Melissa officinalis* subsp. *officinalis* ('Krymchanka' and 'Lada' varieties) and of *M. officinalis* subsp. *altissima* (Sm.) Arcang. These plants have pronounced differences in morphological features and production indexes. They are grown in the collection of aromatic plants of the Research Institute of Agriculture of the Crimea (Krymskaya Roza village, Belogorsky district of Crimea). These studies will allow us to supplement the botanical characteristics of both subspecies of *M. officinalis*, and also to evaluate their plasticity and possibilities of adaptation to growing conditions. For examination we collected plants in the flowering phase. Anatomical study was carried out using a fixed (alcohol: glycerin : water in a ratio of 1: 1: 1) and native preparation. A complex of mesomorphic and xeromorphic characters is established, that confirms the high plasticity and determines the adaptive capabilities of the species when introduced into various soil and climatic conditions. A variety of epidermal structures was revealed, which are represented by single and multicellular non-glandular trichomes and various types of glandular structures. Among them, trichomes with a multicellular pedicle and a unicellular head, which we found only in *M. officinalis* subsp. *officinalis*.

Key words: *Melissa officinalis*, anatomical structures, vegetative organs, morphology, pubescence.

INTRODUCTION

Melissa officinalis L. (Lamiaceae) is the valuable culture, widely utilized as spicy and medicinal plant both in healthcare and for food purposes. Its essential oil is used in the production of high-end perfumes (Denisova, 1989; Voitkevich, 1999; Ehsani et al., 2017; Calejaa et al., 2018; Abdel-Naime et al., 2019). *M. officinalis* subsp. *officinalis*, a type subspecies that has been well considered in terms of the content of a number of bioactive and aromatic compounds, is mentioned as cultivated (Chizzola et al., 2018). In the rank of the subspecies, M. *officinalis* subsp. *altissima* (Sm.) Arcang. is being considered

(World Checklist…, http://wcsp.science.kew.org/namedetail.do?name_id=124060). The synonym is *M. bicornis* Klokov (Mosyakin & Fedoronchuk, 1999), described on the Crimean preparation. However, such synonymizing with *M. officinalis* is not possible to assume as unequivocally accepted (Flora Yevropeiskoy chasti SSSR, 1978; World Flora.., http://www.worldfloraonline.org). *M. officinalis* subsp. *altissima* has a typical Mediterranean-Western Asian habitat, covering Spain, Italy, the Balkan countries, Turkey and the Caucasus region (The information resource…, http://www.emplantbase.org/home.html). Standard *M. officinalis* subsp. *officinalis* has the smaller habitat, which includes front Asia and Caucasian region, but more extensive artificial area, appeared, apparently, as a result of the introduction and subsequent naturalizing of plants, covering almost all of Europe and part of North Africa (The information resource…, http://www.emplantbase.org/home.html).

There were significant differences between the two subspecies in the study of the main indicators of productivity and biochemical parameters, including the component composition of essential oil (Nevkrytaya et al., 2020).

There is data concerning the inner structure of vegetative organs of *M. officinalis* without specifying the intraspecific membership (Birulova & Petrishina, 2014; Nikitina et al., 2018). The anatomical and morphological feature of M. officinalis subsp. *altissima* in the literature it is not revealed. In a number of sources there is some evidence of bioactive substances of the plants of this subspecies (Božović et al., 2018).

The aim of the research is to comprehensively compare the anatomical and morphological structures of plants of *M. officinalis* subsp. *officinalis* and *M. officinalis* subsp. *altissima* having pronounced differences in morphological features. The data obtained will complement the botanic feature of *M. officinalis*; to estimate its flexibility and adaptivity to the conditions of cultivation.

MATERIALS AND METHODS

The research was performed in 2018–2019 on plant preparation from the collection nursery of the department of essential oil and medicinal cultures of the Research Institute of Agriculture of Crimea. Experimental site is located in the foothill zone of the Crimea, in its eastern part (Krymskaya Roza village, Belogorsky district). Climate of this region is moderately continental. This territory belongs to one of the five agroclimatic regions - the upper foothill, warm, not humid enough; to the northern subarea with moderately mild winters (Savchuk, 2006).

Research material - plants of two subspecies *M. officinalis* subsp. *officinalis* (Krymchanka and Lada varieties) and *M. officinalis* subsp. *altissima* (Tavrida variety). Krymchanka and Tavrida are clonal varieties. Lada is a population variety. When laying the field plot, seedlings of all varieties derived by the method of propagation by herbaceous cuttings were used. For maximum coverage of the genetic diversity of Lada, the green cuttings were harvested from 100 plants. Samples were placed in three repetitions on the two-row plots. Plot length - 5 running meters, planting width - 0.6 m. Each row contains 17 plants. The experiment was conducted in the spring of 2017. The required processings and measurements were performed in accordance with the methodological guidelines (Essential oil…, 1977).

Plants of both subspecies of *M. officinalis* of the same age were used for the anatomical and morphological description. Anatomical studies were made in specimen

prepared on fixed (alcohol: glycerin: water in a ratio of 1: 1: 1) and native material (Barykina et al., 2004). Anatomical and morphological description was carried out using generally accepted methods (Lotova, 2001; Serebryakova et al., 2006; Timonin, 2007; Evert, 2016). Epidermal leaf structures of subspecies *M. officinalis* were described on temporary preparations according to the methods of Aneli (1975) and Zakharevich (1954). The anatomical structures of vegetative organs were investigated in the permanent and temporary micro-preparations, obtained with the use of a microtome Rotmik 2- P and made by hand a dangerous razor. The study of the preparations was performed on Olympus CX31RTSF microscope with photographic recording of objects and with Olympus digital camera (Industrial Digital Camera TOUPCAMTM U3CMOS10000KPA). Magnification on the microscope: 4×10, 10×10, 20×10. The quantitative properties of individual anatomical and morphological elements (stomata, main cells of the epidermis, etc.) were specified in 30 replicates. Data obtained was statistically processed using Microsoft Office Excel 2010 software package (Lakin, 1980).

RESULTS AND DISCUSSION

The anatomical structure of the stem and root of *M. officinalis* was studied earlier (Birulova & Petrishina, 2014). A comparative analysis of the anatomical structure of the axial organs of the two subspecies *M. officinalis* showed that *M. officinalis* subsp. *officinalis* and *M. officinalis* subsp. *altissima* have similar anatomical structure of root and stem.

The most plastic organ of plants is the leaf. The peculiarities of its anatomical and morphological structure response the reaction of plants to environmental conditions (Serebryakova et al., 2006). This enables to supplement the ecological characteristics of the species and to indicate the degree of its adaptability to specific growing conditions. The anatomical structure of the leaf of both subspecies is consistent to the general plan of *M. officinalis* structure (Birulova & Petrishina, 2014). The differences between them were revealed in the dimensional parameters (linear and quantitative parameters of leaf cells). The leaves of the studied varieties are covered with one layer of epidermal cells. On the abaxial and adaxial sides of the leaf, the basic cells of epidermis in the paradermal plane have a flattened or elongated shape with more wavy anticlinal walls, obtuse or acute angles in adjacent boundaries (Fig. 1).

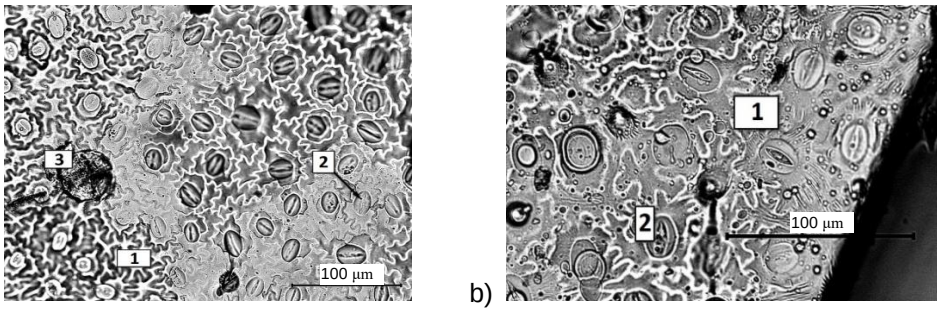

Figure 1. Lower epidermis of a leaf of *M. officinalis*: a – subsp. *altissima*; b – subsp. *officinalis*. (1 – main epidermal cells; 2 – stomata; 3 – glandular trichome).

The main epidermal cells on the abaxial side of the leaf are characterized by more wavy outlines. The size of the epidermal cells of the adaxial side along the long axis is in *M. officinalis* subsp. *altissima*, on average, 64.74 ± 1.61 μm, while subsp. *officinalis* - 38.65 ± 0.99 microns. The number of cells per 1 mm², on average, is 749.45 ± 9.09 and 1372.1 ± 19.26, respectively. The average size of epidermal cells on the abaxial side of the leaf along the long axis is in subsp. *altissima* 50.36 ± 2.09 μm, while subsp. *officinalis* – 46,53 ± 1.51 μm. The number of cells per 1 mm² in subspecies, on average, 1,125 ± 5.83 and 1,138.22 ± 9.71 pcs respectively. Therefore, the large sizes of cells of integumentary structures in subsp. *altissima* induce their smaller number per unit area.

A leaf of *M. officinalis* is hypostomatic (stomata are located only on the lower side of the leaf). The type of stomatal complex is diacytic. The number of stomata on the underside of the leaf is on average 436.67 ± 16.83 pcs mm^{-2} in subsp. *altissima* and 354.44 ± 18.79 pcs mm^{-2} in subsp. *officinalis*.

In the transversal section of the leaf, the epidermal cells of the adaxial and abaxial sides have a rounded or elongated shape along the leaf surface with uniformly thickened walls (Fig. 2).

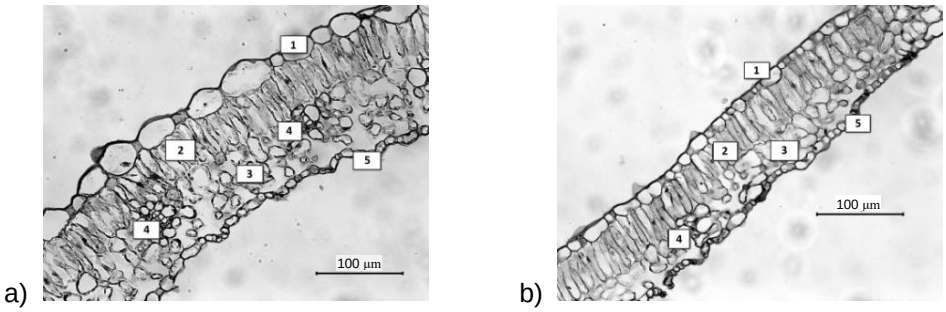

Figure 2. Anatomical structure of the leaf of *M. officinalis* (mesophyll): a – subsp. *altissima*; b – subsp. *officinalis*. (1 – upper epidermis; 2 – columnar mesophyll; 3 – spongy mesophyll; 4 – vascular bundle; 5 – lower epidermis).

The cells of the upper epidermis are of unequal size; the external walls have a thin cuticle layer. The size of the tangent walls of the cells of the upper epidermis of *M. officinalis* subsp. *altissima* varies from 19.12 to 63.59 microns, and anticlinal - from 18.73 to 30.49 microns. Linear parameters of epidermal cells of *M. officinalis* subsp. *officinalis* are smaller. Tangent walls are 6.65–35.34 microns; anticlinal ones - 13.80–21.97 microns.

Lower epidermis of *M. officinalis* is small-celled. Tangent and anticlinal walls of cells in subsp. *altissima* are range from 12.99 to 29.72 μm and from 3.20 to 10.30 μm, correspondingly; as for subsp. *Officinalis* - it is substantially less: from 4.49 to 15.55 μm and from 5.05 to 14.71 μm, respectively. In this case, the dimensional parameters of the cells of the upper and lower epidermis of subsp. *officinalis* rarely exceed 10 microns.

The thickness of the leaf of *M. officinalis* subsp. *altissima* - is 140.98 ± 1.81 microns, which significantly exceeds the thickness of the subsp. *officinalis* - 101.06 ± 1.70 μm. The degree of development of palisade chlorenchyme is described by the palisade coefficient, which in subsp. *altissima* is 42% and in *officinalis* is 48%. It is an average indicator and confirmation of the belonging of this species to mesophytes.

The complex of mesomorphic qualities is native for both subspecies. The leaf is very thin, bifacial. It has a small number of mechanical elements. The leaf's system of intercellular spaces is developed. The stomata is on its lower side; the sheath of small bundles is poorly expressed and is represented by cells that do not differ from the main assimilation cells. Parenchymisation of tissues of axial organs is also may be seen. Additionally, there are a number of xeromorphic signs: a well-developed cuticle, thickened outer walls of the epidermis, the presence of pubescence, and the concentration of essential oil.

One of the areas in the anatomical methods of study is a petiolar anatomy (anatomical structure of petiole) (Kurkin et al., 2014; Gavrilenko & Novozhilova, 2015; Motorykina, 2015; Gavrilenko & Novozhilova, 2017). Literature contains only brief data concerning petiole structure of *M. officinalis* (Nikitina, 2018). Study of the anatomical structure of the cross section of the middle part of petiole of both the *M. officinalis* subspecies showed that it was covered with the small epidermal cells of rounded form with the thickened outer walls, which have the thin cuticle (Fig. 3). In the subepidermal zone of the central part of the petiole with its abaxial and adaxial sides you can find from 1 to 3 layers; in the corners - 4–5 layers of angular collenchyma. Nearer to the peripheral part of the petiole the assimilative tissue is located. It consists of 3–5 the layers of chlorenchima cells.

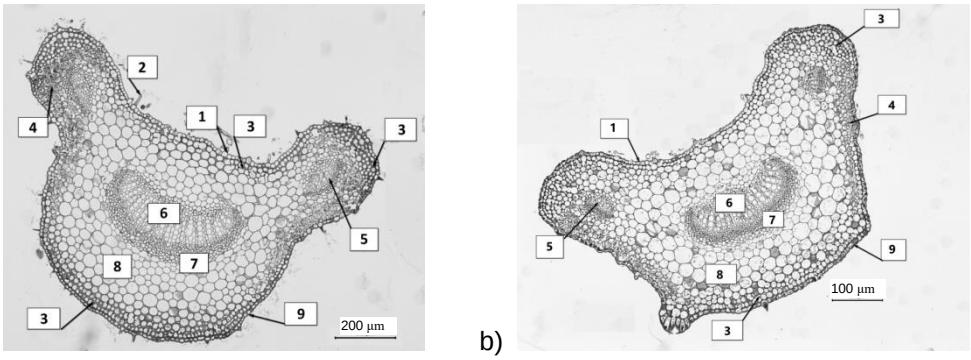

Figure 3. Anatomical structure of the petiole of *M. officinalis*: a – subsp. *altissima*; b – subsp. *officinalis*. (1 – upper epidermis; 2 – covering trichomes; 3 – angular collenchyma; 4 – chlorenchyma; 5 – vascular bundle; 6 – xylem; 7 – phloem; 8 – parenchyma cells; 9 – lower epidermis).

In the cross section the petiole has a round-sinuate shape. There are three vascular bundles. One is large, median (basic) and two are additional (lateral). The bundles are closed and collateral. They are represented by phloem and xylem. Median bundles are of the ring sector shape. They are inverted to the abaxial surface of the petiole. The pulps of the mechanical tissue were revealed from the side of the floem. The main parenchem in the petioles of the studied species is well developed and separates the lateral bundles from the central. In the cells of parenchyma, a small quantity of starch grains is contained.

The similarities of the anatomical structure of the petioles of the subspecies *M. officinalis* affirm data concerning that the petiolar anatomy is promising only for the

development of diagnostic signs at the level of form and larger taxons (Kurkin et al., 2014; Gavrilenko & Novozhilova, 2015; Motorykina, 2015; Gavrilenko & Novozhilova, 2017).

The studied plants of both subspecies have a pubescence, presented by Among the small one- and two-cell non-glandular trichomes there are multicellular trichomes consisting of three to eight cells (Fig. 4).

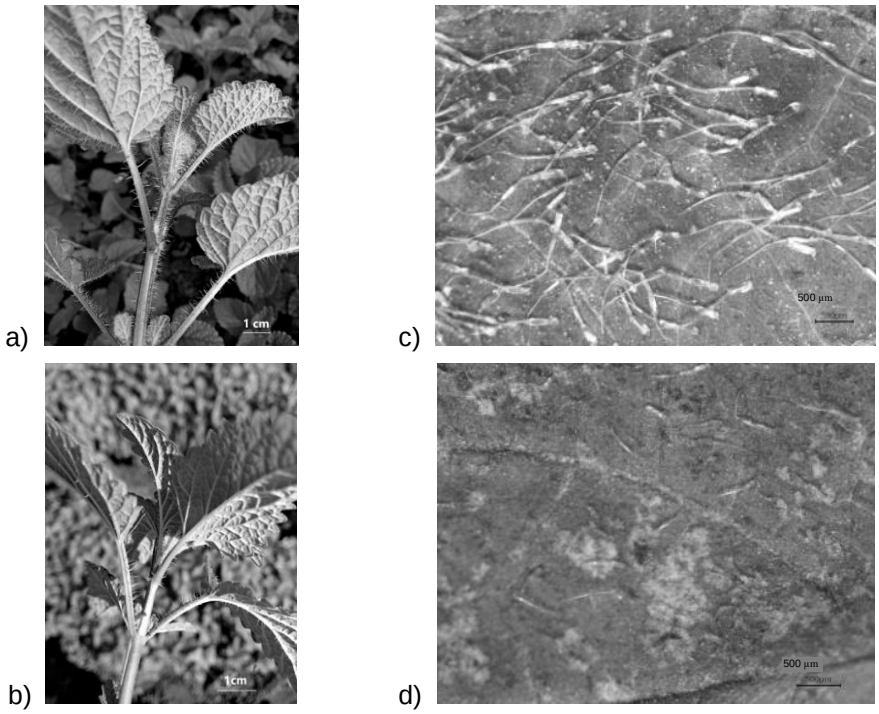

Figure 4. Multicellular trichomes of *M. officinalis*: subsp. *altissima*: a – fragment of the stem; c – adaxial side of the leaf; subsp. *officinalis*: b – fragment of the stem d – adaxial side of the leaf.

The number of multicellular non-glandular trichomes at the number of subsp. *altissima* for 1 mm is 4.66 ± 0.18 pcs; in subsp. they are single. The length of multicellular trichomes in subsp. *altissima* is on the average 1018.39 ± 49.11 µm with the diameter of the base of basal cell 95.53 ± 3.65 µm; in subsp. *officinalis* - 793.85 ± 45.04 µm and 33.45 ± 1.78 µm, respectively. Therefore, the multicellular trichomes, which are found in *M. officinalis* subsp. *officinalis* is considerably thinner in comparison with *M. officinalis* subsp. *altissima*.

According to the classification of terpenoid-containing structures of Denisova (1989), Werker (1993), on the organs of the studied *M. officinalis* subspecies were revealed: capitate glandular trichomes and peltate glandular trichomes. Capitate glandular trichomes with a short unicellular pedicel and the one or two-celled head (Fig. 5, a – d); glandular trichome with the long two-celled pedicel and the unicellular head (Fig. 5, e, f). Peltate glandular trichomes consists of a basal cell, a short pedicel and a multicellular head (four to eight cells) (Fig. 5, g, h).

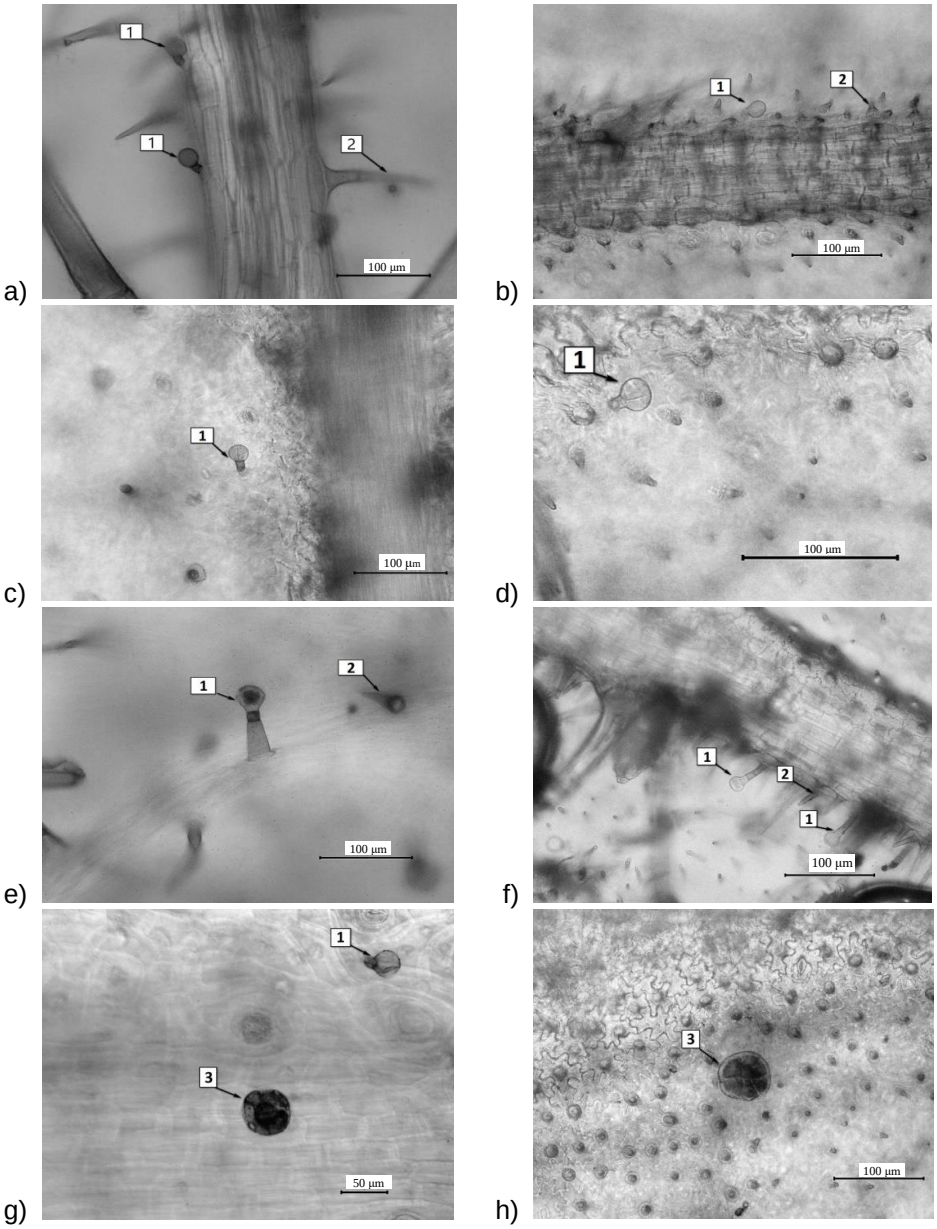

Figure 5. Glandular structures of the organs of *M. officinalis*: a, c, e, g – subsp. *altissima*; b, d, f, h – subsp. *officinalis*; a, b – glandular capitate trichome with a short 1–2 – cellular pedicel and unicellular head; c, d – glandular trichome with a short unicellular pedicel and unicellular head; e, f – glandular trichome with a long 2 – cellular pedicel and unicellular head; g, h – gland with multicellular head (1 – capitate glandular trichome; 2 – non-glandular trichome; 3 – peltate trichomes).

For *M. officinalis* subsp. *officinalis*, the presence of capitate glandular trichome with a multicellular pedicel (3–5 cell) and a unicellular head (Fig. 6) has also been revealed.

Additionally, to micromorphological differences, the samples studied were characterized by differences in the structure of the superius labium of calyx. Therefore, in subsp. *officinalis* the superius labium of calyx of flower has three well expressed triangular teeth, and in subsp. *altissima* they are reduced or missed. The superius labium of calyx is ended with two cusps. This corresponds to the description in the literature of *M. bicornis* 12, which we examine in our paper as the synonym to *M. officinalis* subsp. *altissima* (Fig. 7).

Figure 6. Glandular capitate trichomes with a multicellular (3–5 cell) pedicel and a unicellular head on the stem of *M. officinalis* subsp. *officinalis.*

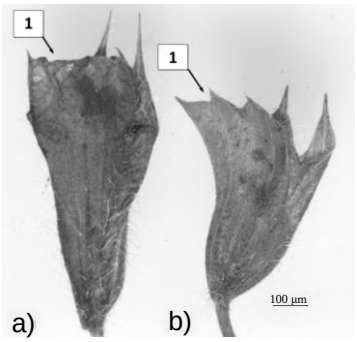

Figure 7. Structure of the upper flower calyx (1) of *M. officinalis:* a – subsp. *altissima;* b – subsp. *Officinalis*.

The habitus of subsp. *altissima* plants is characterized by a higher capacity compared to subsp. *officinalis*, due to a more developed shoot system. Height of subsp. *officinalis* plants (Krymchanka and Lada varieties) lagged appreciably to this index of the plants of the subspecies *altissima* (Tavrida variety). It is about 49.9 ± 2.0 cm; 55.4 ± 4.0 cm and 82.0 ± 5.6 respectively. The diameter of plants differs less significantly. It is about 65.7 ± 3.6 cm; 67.1 ± 4.6 cm and 73.4 ± 6.8 respectively.

Literary sources indicate that the chromosomal complement of subsp. *altissima* has 64 chromosomes; in subsp. *officinalis* - 32 chromosomes (Flora Yevropeiskoy…, 1978; Kittler et al., 2015). It is known that polyploidy causes an increase in plant size (Breslavec, 1963; Fomin, 2009; Gosenova & Kolesova A, 2004; Kittler et al., 2015). Apparently, this particular circumstance explains the above-indicated differences in the quantity indicators of the compared subspecies, including: habitus of plants, thickness of the leaf and compared microstructures.

CONCLUSIONS

Comparative anatomical and morphological study of *Melissa officinalis* subsp. *officinalis* and *M. officinalis* subsp. *altissima*. showed the similarity in anatomical and morphological structures of the vegetative organs of plants.

A complex of mesomorphic features has been established, confirming the belonging of subspecies to the ecological group of mesophytes, characterized by high flexibility. In addition to mesomorphic features, there is a presence of xeromorphic ones, providing their adaptive capabilities in introduction.

Epidermal structures, represented by uni- and multicellular non-glandular trichomes, as well as different types of glandular structures (capitate glandular trichomes and peltate glandular trichomes) have been found. Meanwhile, the glandular trichomes with a multicellular pedicel and unicellular head is noted only in *M. officinalis* subsp. *officinalis*.

An increase in the number of indicators: thickness of a leaf, the palisade ratio, high density of large multicellular covering trichome and habitus of the plants is noted in *M. officinalis* subsp. *altissima*. The explanation of this is in the literature. It is all because of different ploidy of the subtypes indicated: the chromosomal collection of subsp. *altissima* includes 64 chromosomes and subsp. *officinalis* - 32 chromosomes.

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