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Comparative leaf epidermis study in species of genus *Malus* Mill. (Rosaceae)

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ABSTRACT: The leaf epidermis structure of six *Malus* species was studied by light and scanning electron microscopy. Special emphasis was put on *Malus trilobata* (Labill.) C. K. Schneid. Anomocytic stomatal type, different types of simple single trichomes, cuticle ornamentation and waxes were described. An attempt was made to estimate all features of taxonomic value which are relevant for further clarification of the relationships on inter- and intrageneric level within subfamily Maloideae.

Key words: epidermis structure, cuticle, *Malus*

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INTRODUCTION

Genus *Malus* Mill., traditionally referred to subfamily Maloideae (Rosaceae), comprises 25 to 47 species (Robinson *et al.* 2001), widely distributed in the northern hemisphere. The apple fruit crops are of important economic value. Recent studies, based on molecular and morphological characters, lead to a new classification of the Rosaceae family, which includes subfamily Maloideae in tribe Pyreae, subtribe Pyrinae (Campbell *et al.* 2007; Potter *et al*. 2007). The complex taxonomical relevance within the genus and the subfamily Maloideae is due to the hybridization and introgression (Robinson *et al.* 2001). In spite of well described morphology of leaves there are scarce anatomical examinations, which necessitate thorough analysis of the leaf anatomical and surface peculiarities.

The leaf epidermis structure of six *Malus* Mill. species namely *M. dasyphylla* Borkh., *M. domestica* Borkh., *M. praecox* (Pall.) Borkh., *M. pumila* Mill., *M. sylvestris* Mill. and *M. trilobata* (Labill.) Schneider is studied using light and scanning electron microscopy. The aim is to estimate all features of taxonomic value and to provide information on the relationships on inter- and intrageneric level.

MATERIAL AND METHODS

Five of the investigated species from genus *Malus* are referred to section *Malus*, series *Malus* – *M. dasyphylla*, *M. domestica*, *M. praecox*, *M. pumila*, *M. sylvestris* and one is in section *Eriolobus* (DC.) Schneider – *M. trilobata* (Phipps *et al.* 1990).

Preparations were made from herbarium specimens preserved in the collections of the Institute of Botany of the Bulgarian Academy of Sciences (SOM) and the Agriculture University Plovdiv (SOA).

List of investigated samples includes samples: *M. dasyphylla*: SOM – 152044; SOA – 33896, 33969, 35686, 29044, 29053, 56001; *M. domestica*: SOM – 36683, 36689, 36888, 36682; *M. praecox*: SOM – 36628, 36629, 152043; SOA – 33912, 37328, 37014, 29078, 29074; *M. pumila*: SOA – 29029, 29022, 37338; *M. sylvestris*: SOA – 29043, 36490, 36398, 43339, 37306, 37299, 37317; *M. trilobata*: SOM – 145178, 153493.

The cuticle membranes for light microscopy observation, after maceration procedure with Jeffrey solution as modified by Stace (1965) were stained with Sudan IV. Preparations for SEM were made from herbarium material without any treatment.

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RESULTS

Section *Malus*, series *Malus*. The investigated species from this series have little epidermis differences, so we give a general description. The cuticle of the upper epidermis is smooth or thick striated (Fig. 1), covered with fine wax granules. Epicuticilar wax platelets are observed only in *M. domestica* (Fig. 2). The epidermal cells above veins are elongated with straight anticlinal cell-walls. The epidermal cells in the areolae are polygonal with straight to curved anticlinal walls (fig. 3). The trichomes are simple single non-glandular. The trichomes arise from cutinized base surrounded by a rosette of 5-7 radial cells. In mature leaves the trichomes shed and only the pores and the rosettes of radial cells are visible (Fig. 3). The trichomes are located mainly on the veins, the leaf rim and sometimes also in the areolae.

The cuticle of the lower epidermis is smooth, covered with wax granules. Fine parallel striations are observed especially radial to the stomata (Fig. 4). The epidermal cells above veins are elongated, with straight anticlinal walls. The epidermal cells in the areolae are irregular with curved to undulate anticlinal cell-walls (Fig. 5). The stomata are evenly distributed in the areolae with navicular form. The guard cells have prominent outer stomatal rims (Fig. 4). The stomatal type is anomocytic (Fig. 5). The size of the stomata allows division in two groups (31,7±0,8 – 20,4±0,6 and 23,4±0,6 – 16,6±0,5 μm)[1]. They are observed together especially in *M. dasyphylla*, *M. praecox* and *M. sylvestris*. Two types of simple single trichomes are observed - ribbon-like (some of them more or less twisted) and cylindrical-bended near the trichome base (Fig. 6). The trichomes are located on the veins and also in the areolae.

Section *Eriolobus*. The cuticle of the upper epidermis of *M. trilobata* is rough striated and covered with dispersed wax granules (fig. 7). The areolae are conspicuous outlined by 2-4 rows of elongated cells with straight anticlinal walls. The epidermal cells are irregular in form with curved anticlinal cell-walls (Fig. 7). The trichomes are simple single non-glandular with a rosette trichome base of 5-6 cells (Fig. 7). Most of the trichomes shed while the leaves mature. The trichome bases are predominantly on the leaf veins.

The cuticle of the lower epidermis is rough striated. The striations are thicker especially on the epidermal cells in the areolae round the stomata. The cuticle is covered with epicuticular wax granules and platelets (Figs. 8, 9). The epidermal cells above veins are polygonal, elongated with straight anticlinal walls in contrast to the ones in the areolae, which are with irregular form and deeply undulate anticlinal cell-walls (Fig. 8). The stomata are with navicular form. Rough parallel striations and prominent outer stomatal rim are observed on the guard cells (Fig. 9). The stomatal type is anomocytic. The size of the stomata is 26,1±0,5 – 18,1±0,4 μm. The types of the trichomes are the same as seen in the series *Malus* species. The trichomes arise from cutinized base surrounded by a rosette of 5-7 radial cells and they are more abundant on the veins and the leaf rim (Fig. 10).

DISCUSSION

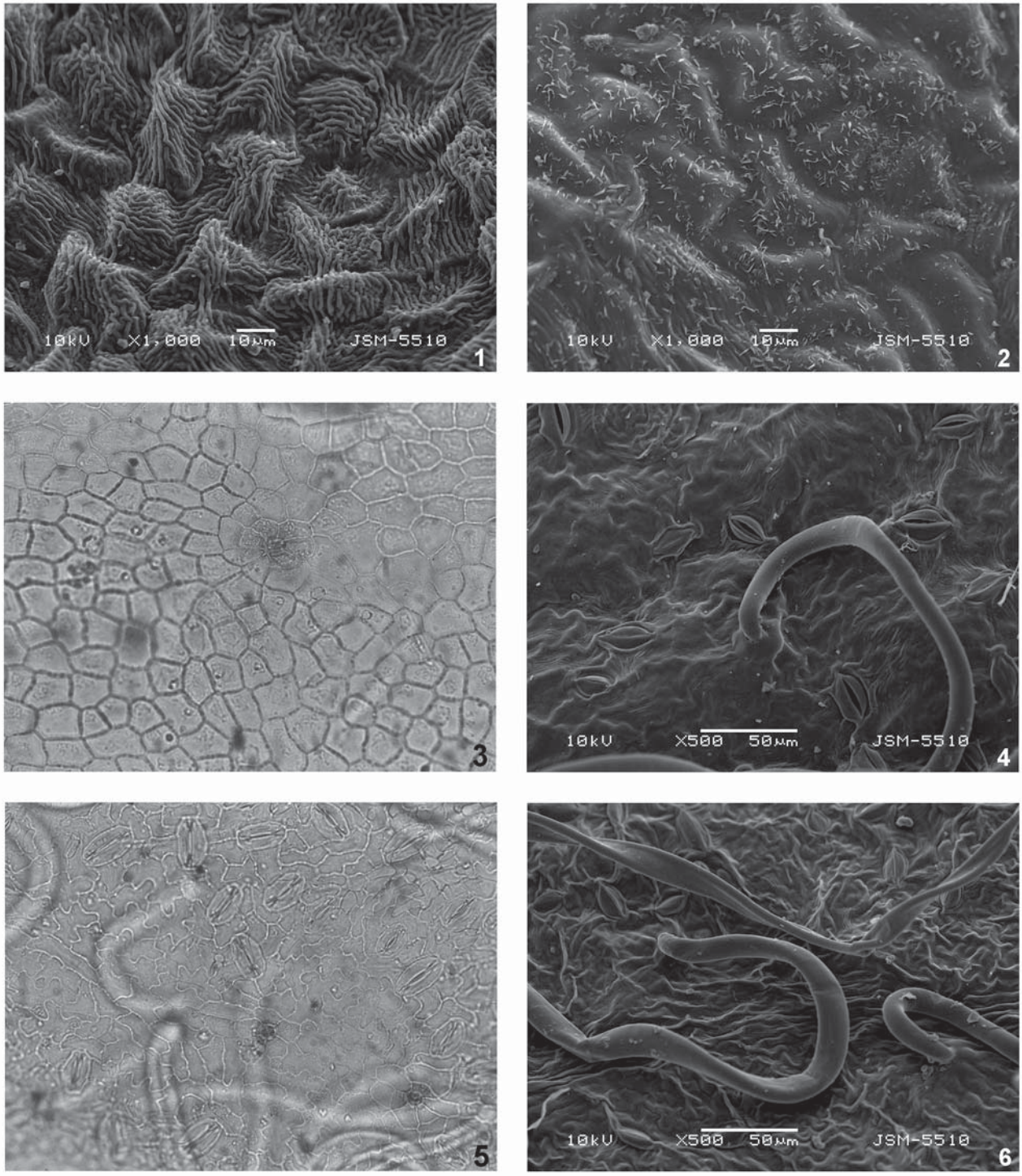
The investigated species of the genus *Malus* possess common epidermal features, but also have shown certain differences in structure. The anomocytic stomatal type and simple single non-glandular trichomes are characteristic of all the representatives. The stomata are with distinctive navicular form and have thickened outer stomatal rim. However, the carried out measurements show significant difference in the stomatal size between the representatives of the two sections. The two described types of trichomes, ribbon-like and cylindrical, are observed together on both upper and lower epidermis. In mature leaves on the upper leaf surface they usually shed and only the pores and rosette trichome base are visible. Metcalfe & Chalk (1965) described simple trichomes with variation in structure and anomocytic (ranunculaceous) stomatal type for Rosaceae, although they pointed out that few anatomical characters are common for the whole family.

Under SEM observation we found out certain differences in cuticle ornamentation and wax deposition. The cuticle of the upper epidermis is smooth covered with amorphous wax granules, but in *M. dasyphylla* is thick striated. Epicuticilar wax platelets are observed only in *M. domestica.* According to Barthlott *et al.* (1998), the size, shape and orientation toward the surface of the crystalloid epicuticular waxes are characteristic for the certain taxon. On the lower epidermis the cuticular striations are distinct radial to the stomata, but in *M. trilobata* they are thicker on the epidermal cells in the areolae and on the guard cells. In the latter platelets of epicuticular wax are observed.

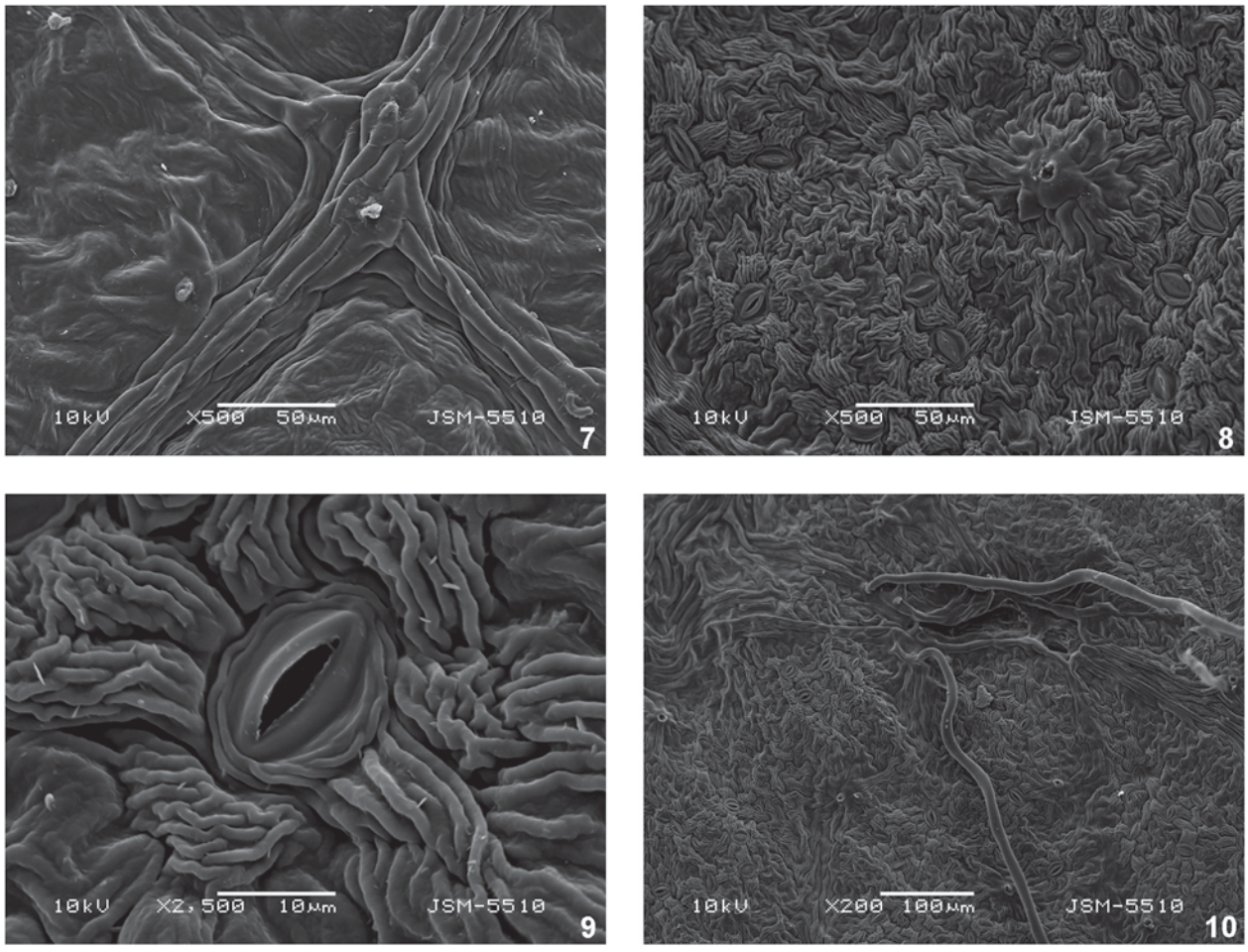
The epidermal cells are usually irregular in form with curved to deeply undulate (in *M. trilobata*) anticlinal cell-walls.

Our study of the leaf epidermis structure in species of series *Malus* is in agreement with the statement that the morphological characters used to delimit species in this series are continuous and overlapping (Robinson *et al.*

1. Lenght $\bar{x}\pm s$ – width $\bar{x}\pm s$, $\bar{x}$- mean (μm), s – standard deviation of the mean



Figs. 1-6. 1 *M. dasyphylla* – upper epidermis, striations (SEM); **2** *M. domestica* – upper epidermis, epicuticular waxes (SEM); **3** *M. pumila* – upper epidermis, epidermal cells, rosette trichome base (LM x 400); **4** *M. praecox* – lower epidermis, striations, stomata (SEM); **5** *M. pumila* – lower epidermis, epidermal cells, stomata, trichomes (LM x 400); **6** *M. sylvestris* – lower epidermis, two types of trichomes – ribbon-like twisted and cylindrical-bended (SEM)



Figs. 7-10. *M. trilobata* **7** - upper epidermis, striations, epidermal cells, rosette trichome bases (SEM); **8** - lower epidermis, striations, epidermal cells with deeply undulated anticlinal walls, rosette trichome base (SEM); **9** - lower epidermis, stoma, striations, platelets of epicuticular wax (SEM); **10** – lower epidermis, trichomes (SEM).

2001). On the other hand, *M. trilobata* posses combination of epidermal features that allow clear delimitation. Cladistic analysis based on molecular features (Robinson *et al.* 2001; Forte *et al.* 2002) place *M. trilobata* in particular clade, but they did not support its generic status as *Eriolobus* (DC.) Roem. (Robinson *et al.* 2001).

CONCLUSION

The epidermis structure studies of *Malus* species provide important information that can elucidate the complex taxonomy of subfamily Maloideae in which morphology and molecular analysis do not yield a fully satisfactory solution.

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REZIME

Komparativna studija lisnog epidermisa kod vrsta roda *Malus* Mill. (Rosaceae)

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Uovom radu izučavana je epidermalna struktura lista na šest vrsta roda *Malus* putem svetlosnog i skening elektornskog mikroskopa. Posebna paznja posvecena je vrsti *Malus trilobata* (Labill.) C. K. Schneid. Anomocitični stomaterni tip, različiti tipovi jednostavnih pojedninačnih trihoma, ornamentacija kutikule i voskova je opisana. Procenjene su sve karakteristike koje imaju taksonomski značaj, i koje su važne za dalje razjašnjenje odnosa na inter i intrageneričkom nivou unutar familije Maloideae.

Ključne reči: epidermalne structure, kutikula, *Malus*