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Anatomical study of the leaves and petioles of scab resistant and susceptible apple cultivars

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Summary: Anatomic studies have been performed on the leaf blade, petiole and annual shoot on six apple cultivars by means of scanning electron as well as light microscope. Four of the cultivars examined are resistant to scab (Florina, Freedom, MR-10, MR-11), whereas two of them are susceptible (Jonathan and Idared). Preliminary results suggest that differences in the width of cross sections of leaf blades, in hairyness, in the shape and size of epidermal cells, moreover, in the cross sections of petioles and shoots are considerable. Some of the anatomical properties seem to be correlated with scab resistance or susceptility of the respective cultivars. Therefore, further studies extending to other cultivars may corroborate our claims to find causal relations between anatomical traits of the leaves and disease, especially scab resistance of apple cultivars.

Key words: *Malus x domestica*, cultivar, leaf, petiole, tissue, apple

Introduction

Observations in the field (*Tóth* et al., 2005) as well as detailed pomological descriptions (*Bereczki*, 1886, *Tomcsányi*, 1979) prove the inter-varietal variability of cultivated apples regarding leaf shape and size, hairyness of the adaxial and abaxial surface, being glossy or leathery, often perceived with unarmed eye or palpation. The relation between the anatomy and the photosynthetic activity of leaves has been explored around the first half of the 20th century (*Picket*, 1933, 1937), moreover, considerable varietal differences have been described in the thickness of leaf blades, size and density of stomata. Later, the anatomical structure of the leaf blade affected by the scab fungus (*Venturia inaequalis* Cke.Wint.) has been studied and followed up by tracing the development of symptoms of the disease (*Kline* et al., 1964) presented also recently (*Jones & Aldwinckle*, 1990, *Holb*, 2002). Our own observations (*Tóth* et al., 2004a) proved substantial differences between the leaf structure of the apple cultivars 'Jonathan', 'Starking' and 'Golden Delicious'.

In our practice of acclimatizing scab resistant foreign apple cultivars and breeding work (*Tóth* et al., 2004b), perceived differences of the leaves on resistant as well as on susceptible cultivars called our attention to the anatomical studies of the leaves in order to predict etiological expectations after scab infection.

Material and method

As cultivars susceptible to scab 'Jonathan' and 'Idared', whereas as scab resistant cultivars the French 'Florina' and the American 'Freedom' cultivars, moreover, breeding stocks of our Department of Fruit Science MR-10 and MR-11 cultivar candidates have been chosen for the examinations. The scab-resistant genotypes owed their resistance to the Vf gene originating from a *Malus floribunda* 821 clone, but that of the cultivar 'Freedom' inherited it from its ancestor, the cultivar 'Antonovka' as documented in the pedigree.

The shoots for the studies were collected in the experimental farm of the Corvinus University of Budapest, where the trees are grown on M4 rootstocks. Leaf samples were taken on June 13, 2005.

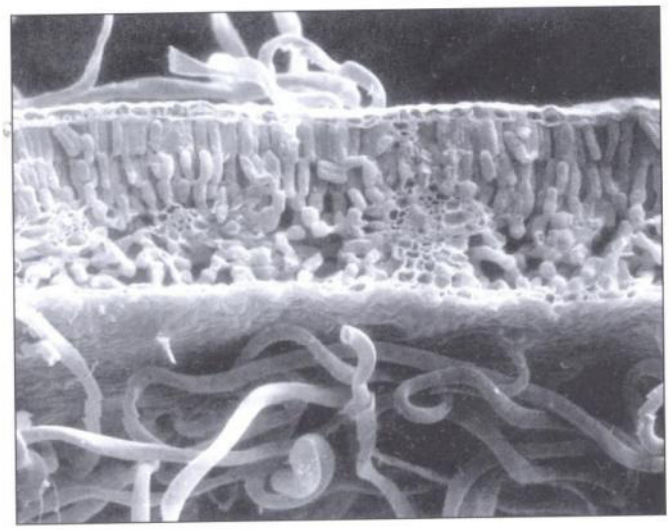
The surface as well as sections made by rasor blades of the respective organs were examined by scanning electron microscope (SEM) and alternatively by light microscope (LM). Leaves were examined and measured for the following traits: diameter of the blade, palisad and spongy parenchyma, hairyness, petiole, epiderm and their ratio as well as histological structures.

Results

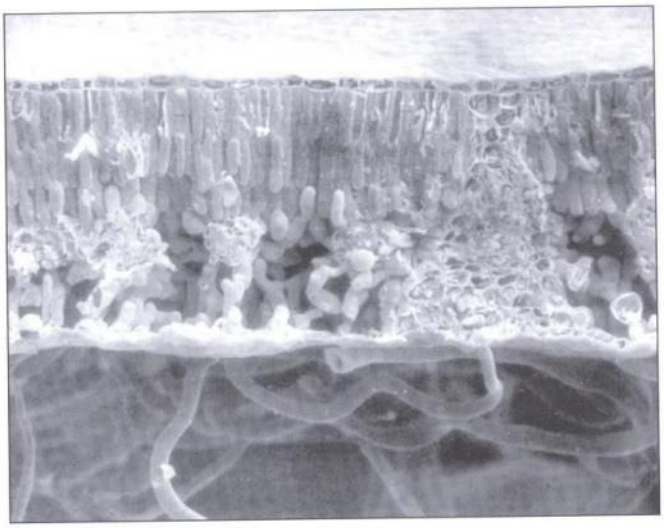
The thickness of the leaf blade in a cross section

Substantial differences appeared in the cross section of the cultivars (*Figure 1*). A leaf of the cultivar 'Jonathan' represented the thin category with 931 µm. Similar measures were found for the cultivars 'Idared' and 'Florina'. The most thick cross sections have been found among others at the clone MR-10 with 1270 µm, being similar to MR-11 and 'Freedom'.

The measures of the palisad parenchyma and the spongy parenchyma were equally variable, but the variability

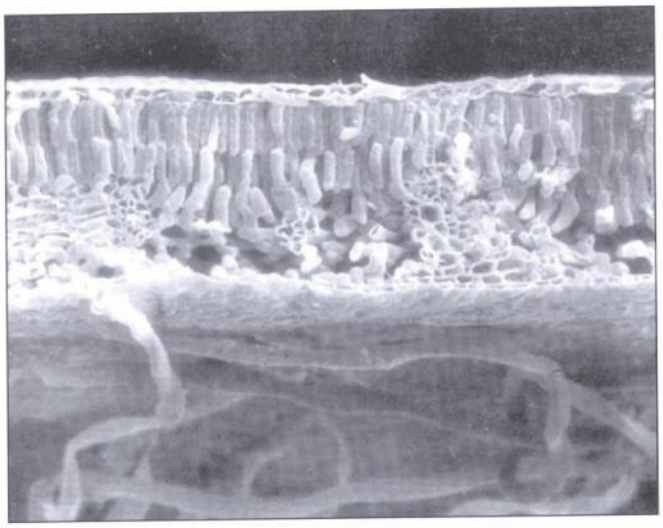


Jonathan

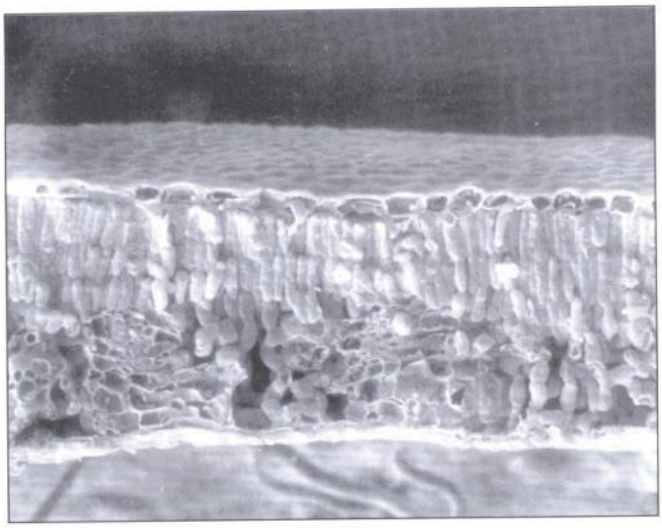


MR-10

Figure 1 Thickness of the leaf blade

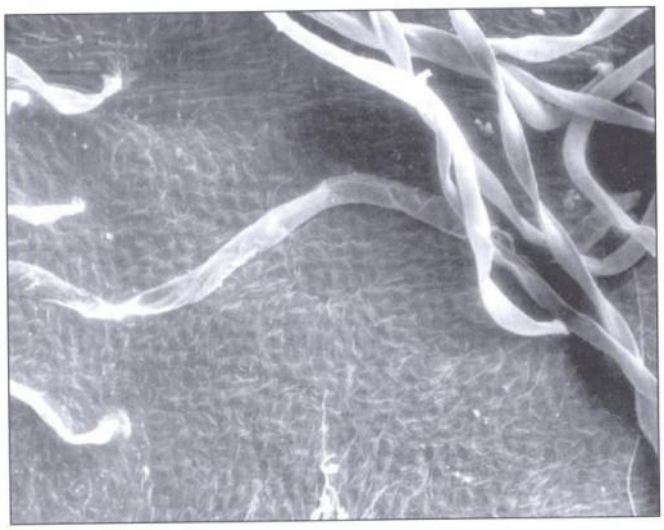


Florina

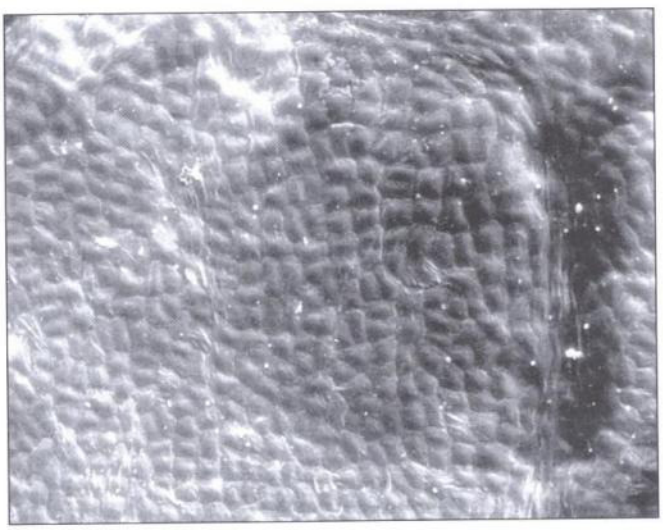


MR-11

Figure 2 The palisad parenchyma



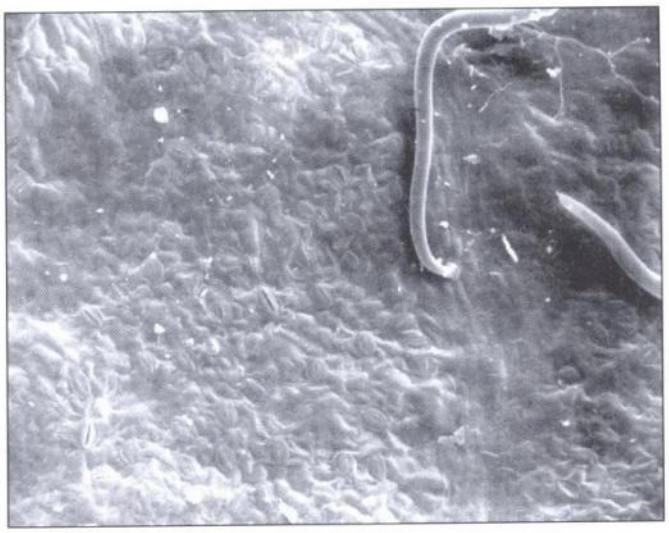
Jonathan



Freedom

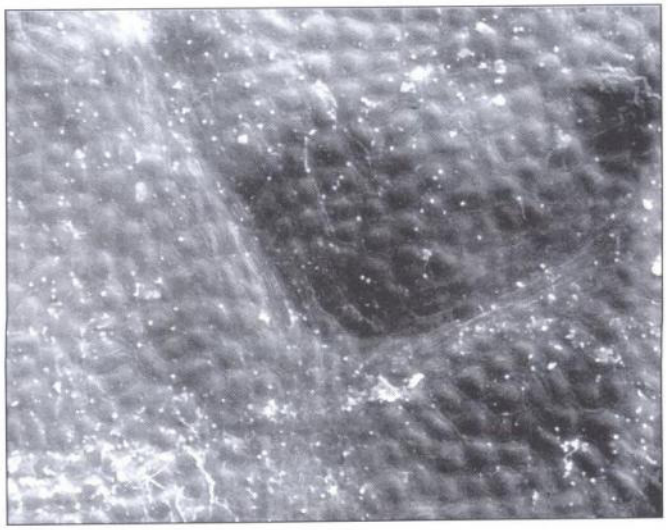
Figure 3 Hairyness of the adaxial surface of leaves

Jonathan

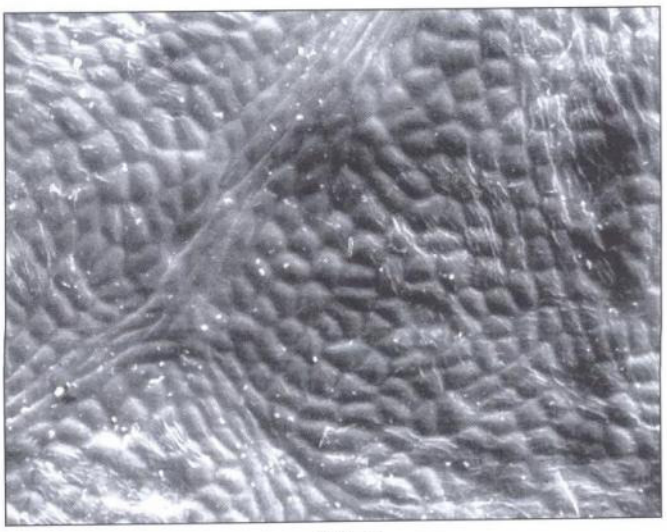


MR-11

Figure 4 Hairyness of the abaxial surface of leaves

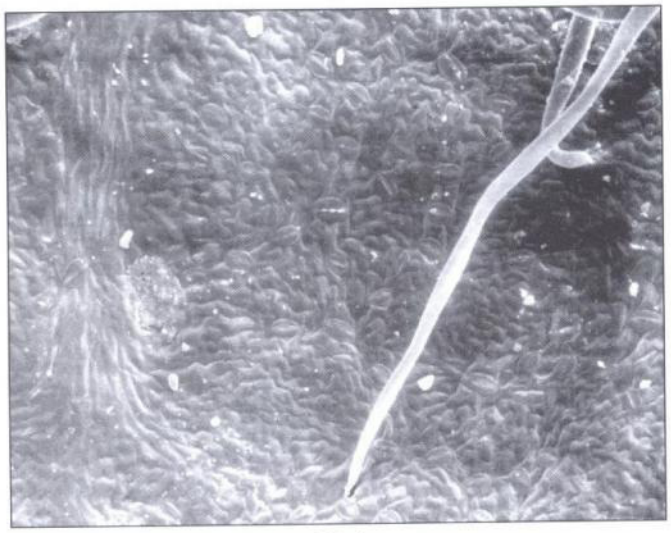


Freedom

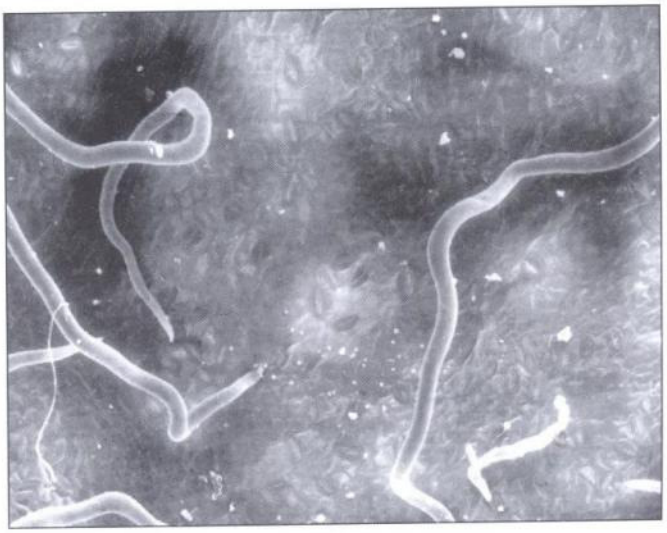


MR-11

Figure 5 Size and shape of the adaxial epiderm

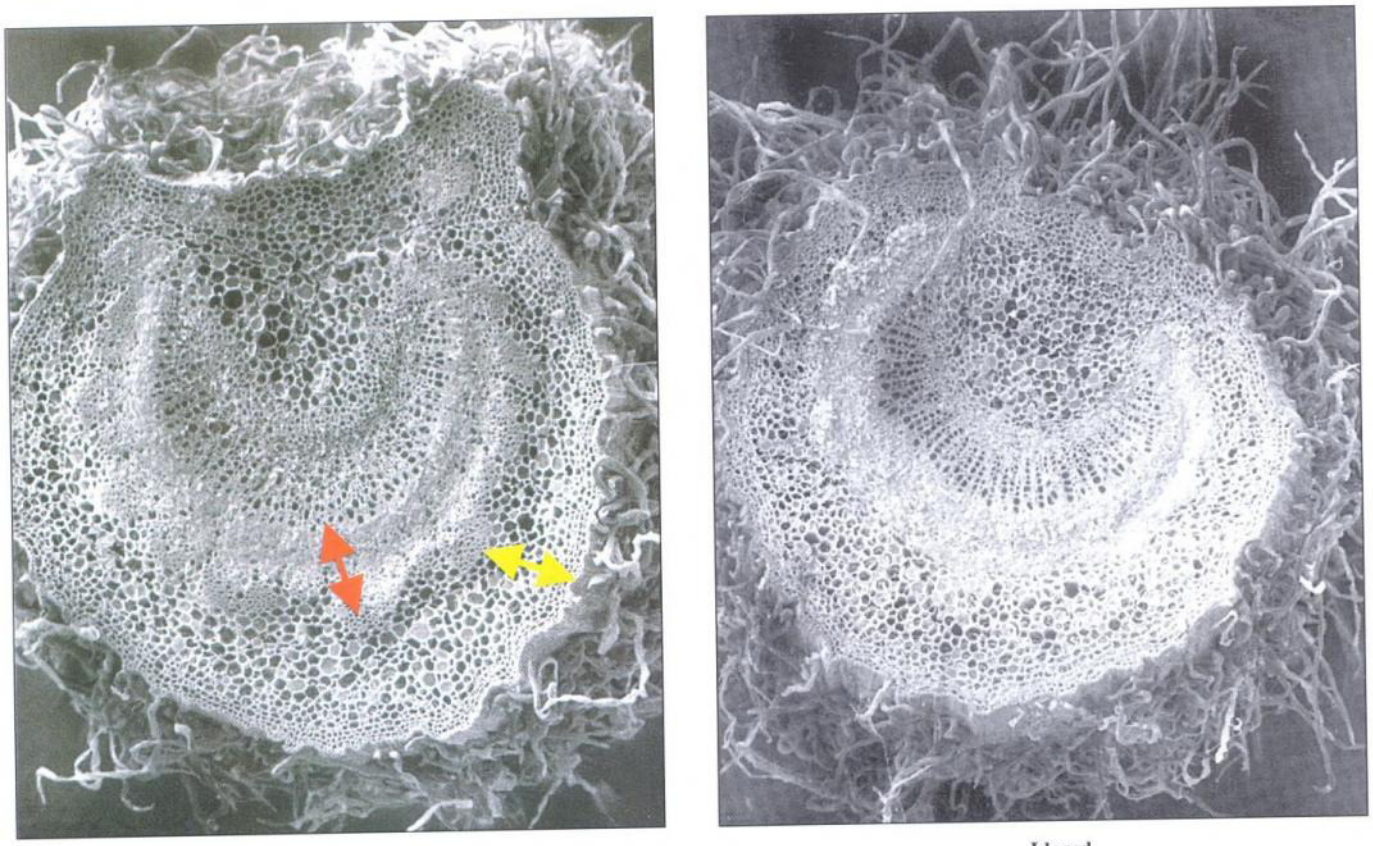


Florina



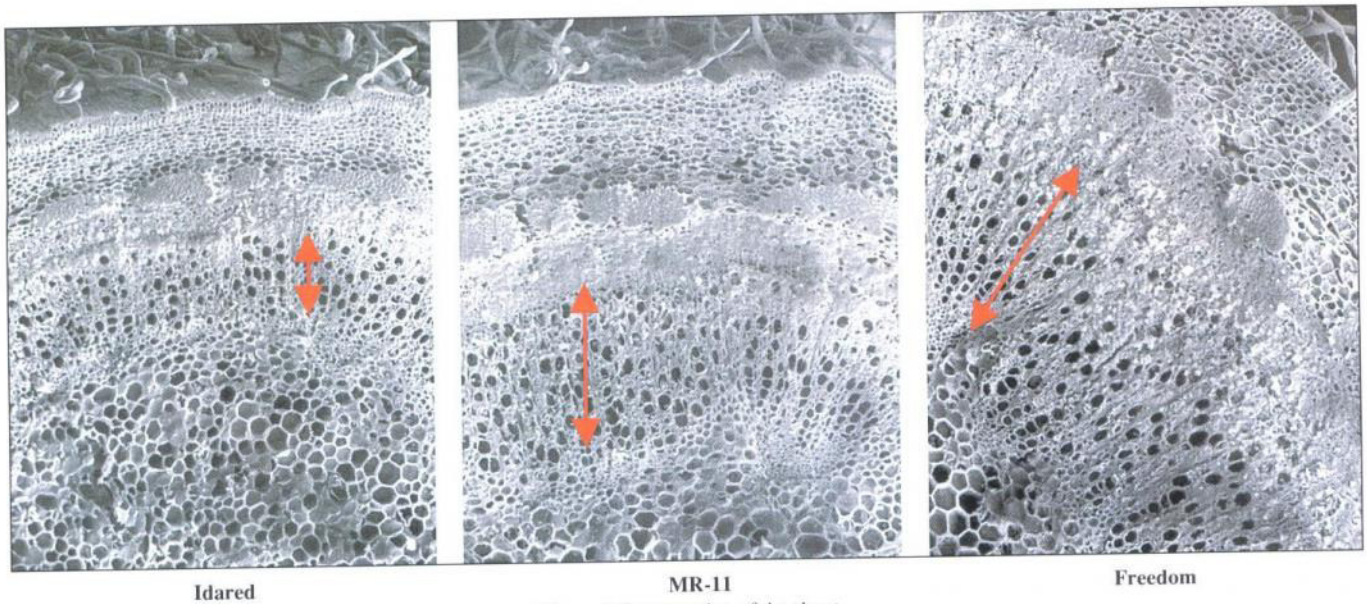
Freedom

Figure 6 Size and shape of the adaxial epiderm



Florina Idared

Figure 7 Cross section of the petiole
Legend: Yellow arrow: collenchyma; Red arrow: semicircular bundle of conductive tissues



Idared MR-11 Freedom

Figure 8 Cross section of the shoot
Legend: Red arrow = xylem

correlated with the thickness of the leaf blade. (*Table 1*). In the leaves of 'Jonathan' the upper (adaxial) epiderm with the palisad measured 496 µm, whereas the spongy parenchyma with the abaxial epiderm 434 µm. Approximately the same data were raised in 'Florina' and 'Idared', or somewhat larger.

The same parameters obtained in leaves of MR-10 cultivar candidate are 744 µm and 527 µm taking the upper and lower part of the leaf, respectively. Nearly the same data are obtained in the leaves of 'Freedom' and MR-11.

Figure 2 presents observations on the structure of palisad parenchyma layers. In 'Florina' and 'Idared', there are two layers, whereas in 'Jonathan', 'Freedom', MR-10 and MR-11 the number of palisad layers is three. Individual cells of the palisad parenchyma are long (248–280 µm) in 'Idared'

Table 1 Cross section of the leaves

Cultivar	Thickness of the leaf blade µm	Palisad parenchyma + epiderm µm	Spongy parenchyma + epiderm µm
Jonathan	930	496	424
Idared	1092	537	455
Florina	1035	558	455
Freedom	1209	620	651
MR-11	1209	620	589
MR-10	1270	744	527

and 'Freedom' leaves. As a rule, cells of the upper (abaxial) layer are longer than those of the layers underneath, the shortest are the palisad cells of the third layer.

The cells of the three-layer palisad parenchyma are somewhat shorter, i.e. between 162 and 207 µm ('Jonathan' cultivar and MR-10, MR-11 cultivar cadidates).

The intercellulary space is narrow in 'Florina', 'Idared' cultivars and MR-10 and MR-11 cultivar candidate, whereas rather spacious in 'Jonathan' and 'Freedom' cultivars.

Hairyness of the leaves

The leaves of 'Jonathan' are strongly hairy on both the adaxial and abaxial surface. All other genotypes examined are considered to be bald on the adaxial surface of the leaf. Moderately hairy are 'Idared' and 'Freedom' on the abaxial surface, and the rest of the genotypes develop there hairs but scarcely, along the veins only (*Figure 3–4*).

Shape and size of the epidermal cells

There is also an important diversity among the genotypes examined. On the adaxial surface, the cells are polygonal isodiametric in 'Florina', 'Idared', 'Freedom', whereas in the rest of cultivars they are slightly elongated: 'Jonathan', MR-10, MR-11.

On the adaxial surface of 'Jonathan', the epidermal cells are small, in 'Idared' they are of intermediate size, whereas larger by about 90% in 'Florina', MR-10 and MR-11 (*Figure 4*).

The cells of the upper (adaxial) epiderm are polygonal in 'Freedom', elongated in MR-10 cultivar candidate (*Figure 5*). Cells of the abaxial epiderm are particularly variable in size as well as in shape. For the examination of details we need more magnification (*Figure 6*).

The cross section of the petiole and the shoot

The cross section of the petiole shows, generally, the shape of a hand bag. The abaxial surface is semicircular and the two edges of the adaxial side are elongated. They are especially characteristic in 'Florina' and MR-10. Much smaller and less pronounced continuations are observed in the petioles of 'Jonathan', 'Idared', 'Freedom' and MR-11 (*Figure 7*).

The size and length of the petiole is hardly variable. Larger and relatively broad are the cross sections of 'Florina' and MR-10. Below the hairy epiderm, the collenchyma is more extended in 'Florina' and MR-10 than in the rest of genotypes.

The conductive tissues are in the cross section of the petiole in a semicircular arc. Its two edges are turned adaxially. The xylem is in the inner zone, the phloem is in the outer one. The cross section area of the xylem varies according to the cultivar. It is most extended in 'Florina' and in 'Idared'.

In the anatomic structure of annual shoots, differences are observed in the extension of the collenchyma beneath the epiderm and the width of the xylem cylinder. The collenchyma of 'Idared' shoots is conspicuously narrow, similarly to that of the cultivar candidate MR-10. The continuous ring of the xylem is extremely narrow in 'Jonathan' and 'Idared', intermediate in 'Florina', 'Freedom' and MR-11 shoots. The xylem is the widest in 'Freedom' (*Figure 8*).

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