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Anatomical and morphological parameters of leaves and leaf petioles of *Actinidia deliciosa*

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Abstract

Differences in anatomy and morphology of the kiwifruit leaves and leaf petioles might play a considerable role in the sex-determination. Three months after bud break (June), the kiwifruit leaves of both male and female plants, grown on the vegetative and generative shoots showed different leaf area (128.6 ± 13.45 cm^2 in male and 104.5 ± 4.02 cm^2 in female plants) and shape. The most frequently leaf shape was determined as "*folium cordatum*" and "*folium rotundato-cordatum*". Higher values of total leaf thickness of the female leaves (190 ± 3.84 μm) in comparison to male leaves (174 ± 3.52 μm) were estimated, resulting in the thicker adaxial leaf epidermis and especially in thicker palisade parenchyma in female leaves (136 ± 2.76 μm in comparison to 104 ± 1.61 μm in male leaves). Typically bifacial leaves were observed in both male and female leaves. Anomocytic stomata in hypostomatic leaves were found. The reticulate venation appears to be the main type of leaf venation. Stalked stellate multicellular trichomes on the abaxial leaf side were frequently observed in the leaves of both sexes. No important differences between male and female plants were found in the structures of vascular system in leaves and leaf petioles. Thus leaf thickness and surface morphology of adaxial leaf epidermis can be considered as important structural parameters in the sex determination.

Additional key words: kiwifruit, mesophyll, stomata, trichomes, vascular system.

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This paper is dedicated to the memory of Mr. M. Strižko (1910 - 1994) who was the first man engaged in cultivation of several cultivars of *Actinidia deliciosa* in the south-west Slovakia since the time more than 30 years ago and without whose work our research would be not conceivable.

Introduction

A world-wide commercially important fruit, the kiwifruit, has been the matter of a wide range of consequent studies. The studies were focused on the physiological features (Buwalda and Smith 1990, Oláh 1992, Buwalda 1993, Buwalda and Meekings 1993, Oláh and Masarovičová 1994, Savé *et al.* 1994, Webby *et al.* 1994, Oláh and Masarovičová 1995, 1996), anatomical and morphological parameters, description of the distribution and accumulation of calcium in leaves, *etc.*, with regard to taxonomic classification, description of genus and cultivars (Li 1952, Ferguson 1984, Clark *et al.* 1987). However, there are still lacks of appropriate set of parameters in sex-determination, unless the blossoms appear after the necessary 5 - 7 years of growth. Nevertheless, several attempts to find some biochemical markers (*e.g.* isoperoxidases and isopolyphenol-oxidases) of sexual differentiation have been already performed using tissue culture method of *Actinidia chinensis* (Hirsch and Fortune 1984) and *Actinidia deliciosa* (Auxtová *et al.* 1994).

The presented paper was performed as a further contribution to the physiological analysis (Oláh 1992, Oláh and Masarovičová 1994, 1995, 1996) implying an overview of the anatomical (leaf thickness, thickness of the adaxial epidermis and palisade parenchyma, the stomata density and the length of guard cells, and structure of the vascular system) and morphological features of the kiwifruit leaves. The aim was to show binding of changes in anatomy, morphology and physiology in responses of plants to exogenous (environmental) and endogenous factors during the growing season.

Materials and methods

Light microscopy: Mature male and female leaves and leaf petioles of 6-year-old plants were sampled for light microscopy, fixed in the FAA (mixture of 2 % formaldehyde, 5 % acetic acid and 70 % ethanol) or mixture of ethanol-acetic acid (3:1) for 12 h, dehydrated in a graded buthanol series with 30 min intervals and embedded in paraffin wax. After dewaxing and hydration through ethanol series the sections (15 ± 1 μm thick) were stained with periodic acid - Shiff reaction or basic fuchsin and picroindigocarmine (O'Brien and McCully 1981). The stomatal complex was investigated also by maceration method described by Przywara *et al.* (1988) and replica method of Inamdar *et al.* (1969). The stomata density was determined using 45 × 20 magnification with objective micrometer. The analysis of leaf thickness, leaf adaxial epidermis and palisade parenchyma thickness were gained from 60 measurements of each leaf segment within 5 leaves. The entire set of samples was examined and photographed using the *Reichert Zetopan* microscope, *Wild M7A* binocular loupe, and a *Wild MPS 55* photoautomat (*Wild*, Herbruggs, Switzerland).

Scanning electron microscopy (SEM): Samples for SEM have been taken from mature male and female leaves and petioles of adult (6-year-old) plants. They were fixed in the FAA (mixture of 2 % formaldehyde, 5 % acetic acid and 70 % ethanol) for 12 h and rehydrated with series of ethanol and water. Subsequently they were air dried for

48 h at 20 °C, mounted on metal blocks by silver paint (Balzers) and covered with gold using a *Jeol Sputter Coater* (25 kV, 200 pA, *Jeol*, Tokyo, Japan). Finally, samples were examined and photographed in a *Jeol JXA 840B* scanning electron microscope.

Results

In dependence on the ontogenetic stage and the insertion of leaves on shoots, the leaves possessed a different shapening. Ontogenetically younger leaves, grown on the higher located shoots, were determined as *"folium cordatum"*. Another tendency was observed in the older leaves on middle and lower located shoots, where the leaves possessed a main leaf shape, determined as *"folium rotundato-cordatum"*. Both leaf shapes were observed as the most frequent ones within male and female sex (Fig. 1*A,B*). The male leaves possessed a higher total leaf area (128.6 ± 13.45 cm^2) compared to the female leaves (104.5 ± 4.02 cm^2). The total leaf thickness was lower in male leaves (174 ± 3.52 μm) than in the female leaves (190 ± 3.84 μm). The thickness of the epidermis on the adaxial leaf side was 18.5 ± 0.31 μm in the male leaves and 19.25 ± 0.42 μm in the female leaves. Even without measuring of cuticular thickness it was evident that cuticule was thicker in female leaves comparing to male ones, due to the extra wax layer on the female leaves which enhanced the gleaming effect on the leaf surface (Fig. 1*A,B*). Thicker cuticular layer in female leaves was also observed using SEM method (data not shown). The different wax content as well as shape and surface morphology of adaxial and abaxial epidermal cells, respectively, were different in female and male leaves (Fig. 1*A,B,C,D*; 2*C,D*).

Within the mesophyll, 1 or 2 layers of palisade parenchyma and continuous layer of spongy parenchyma (Fig. 2*A,B,E*) could be distinguished in dependence on the ontogenetic stages. Such mesophyll structure appears to be a typical bifacial leaf type, observed in both male and female leaves (Fig. 2*E*). The estimated values of palisade parenchyma thickness were 104 ± 1.61 μm in male leaves and 136 ± 2.76 μm in female leaves, which involves 75 % of the whole leaf mesophyll. The observed leaf features indicates the xeromorphic character of both male and female leaves. In the tangential leaf sections, considerable amount of chloroplasts, arranged more marginally, within the cell of the palisade parenchyma was observed, while chloroplasts in the cells of spongy parenchyma occupied more central location (data not shown).

Both male and female leaves were hypostomatic. The stomata belong to the anomocytic type and their guard cells seem to be surrounded by seven subsidiary cells (Fig. 2F). The stomata density was 500 ± 2.97 per mm^2 in the male leaves and 520 ± 4.03 per mm^2 in the female leaves. The stomata length, derived from the length of the guard cells was 24.75 ± 0.37 μm in male leaves and 25.13 ± 0.42 μm in the female leaves.

The abaxial leaf side as well as the epidermis of the leaf petioles are covered by numerous multicellular trichomes. Such non-glandular stellate trichomes have

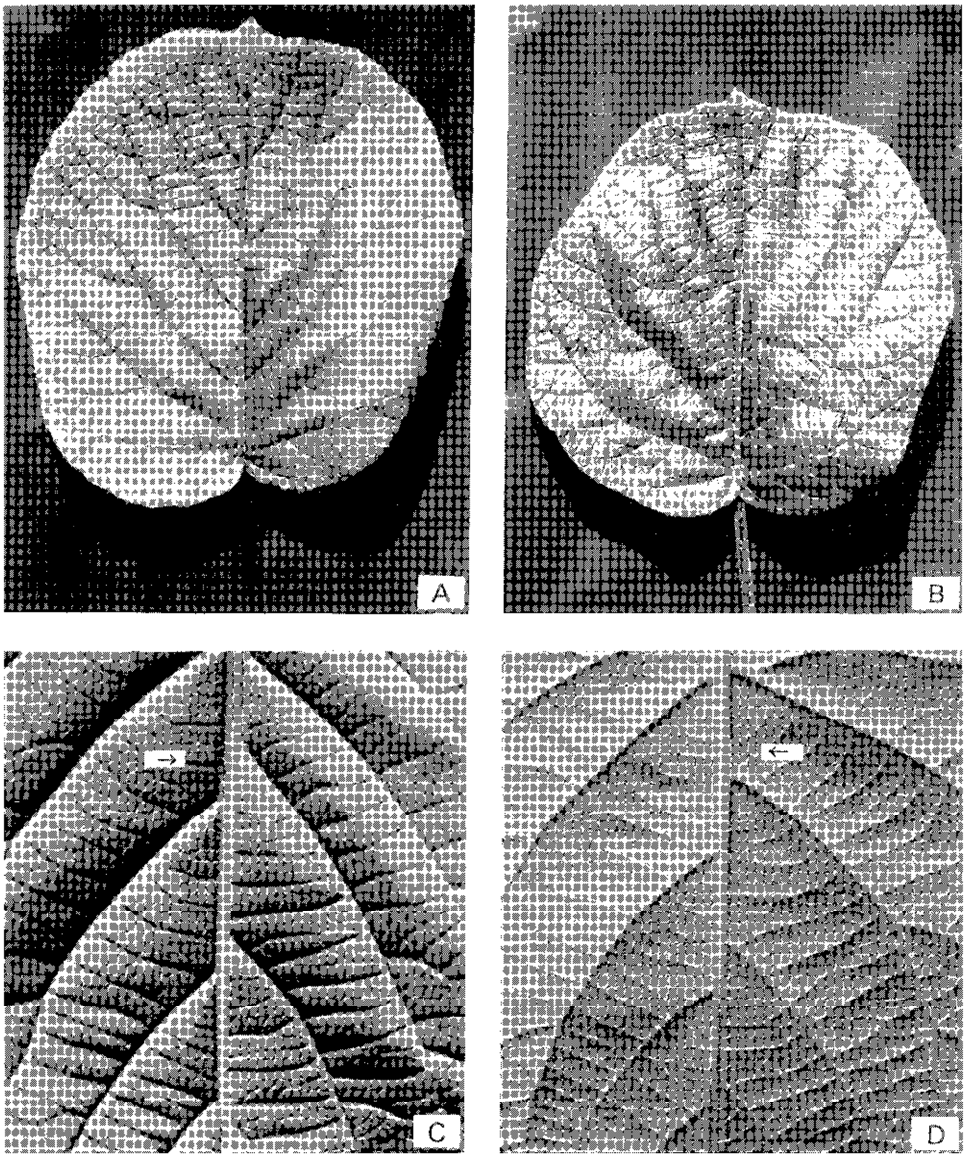

Fig. 1. Leaf morphology of *Actinidia deliciosa*: *A* - shape of male leaf (*folium rotundato-cordatum*), *B* - shape of female leaf (*folium cordatum*), *C* - abaxial surface of male leaf (*arrows* - red coloured epidermis along the main vein), *D* - abaxial surface of female leaf (*arrows* - green coloured epidermis along the main vein).

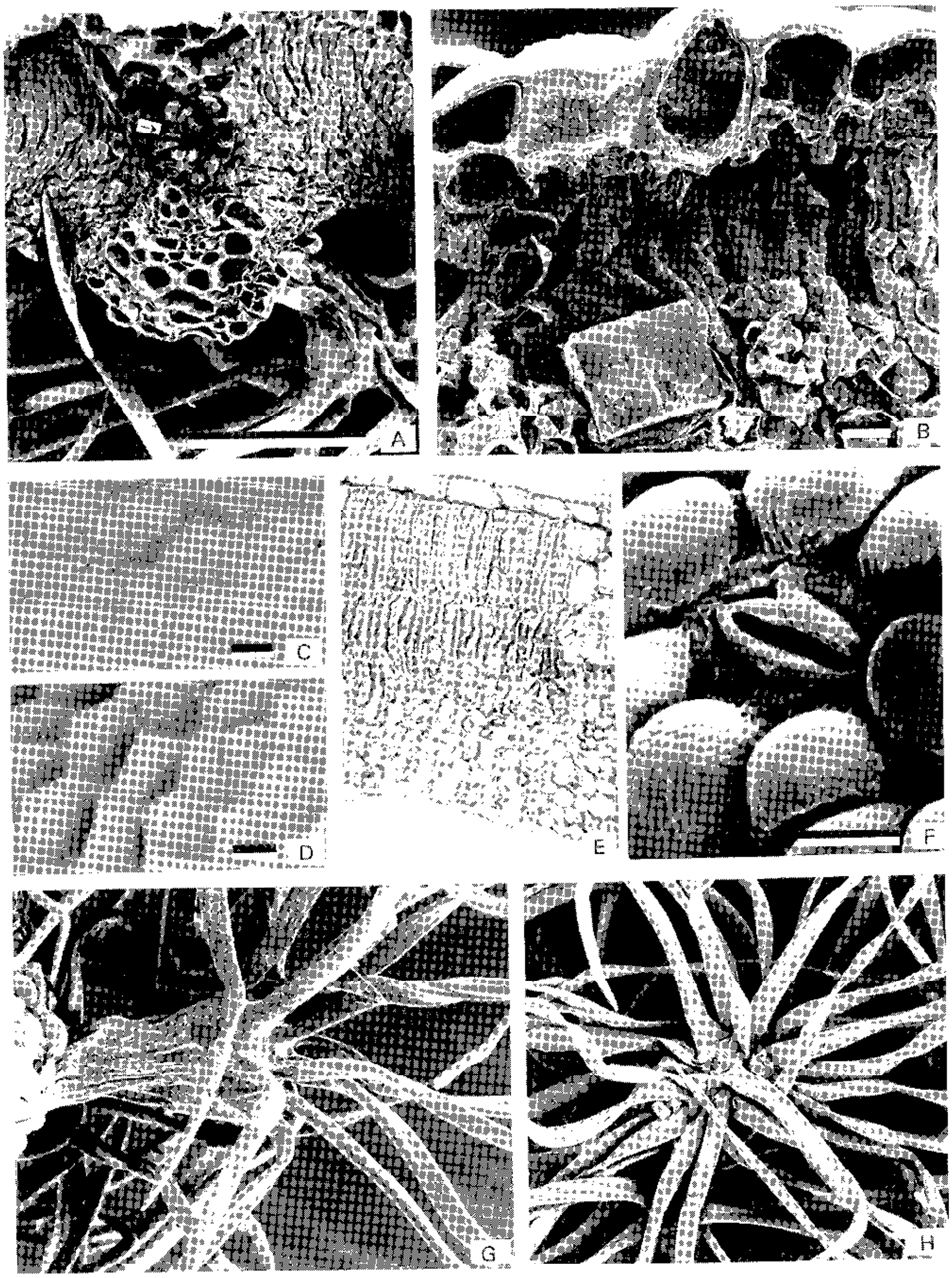

Fig. 2. For legend see page 277.

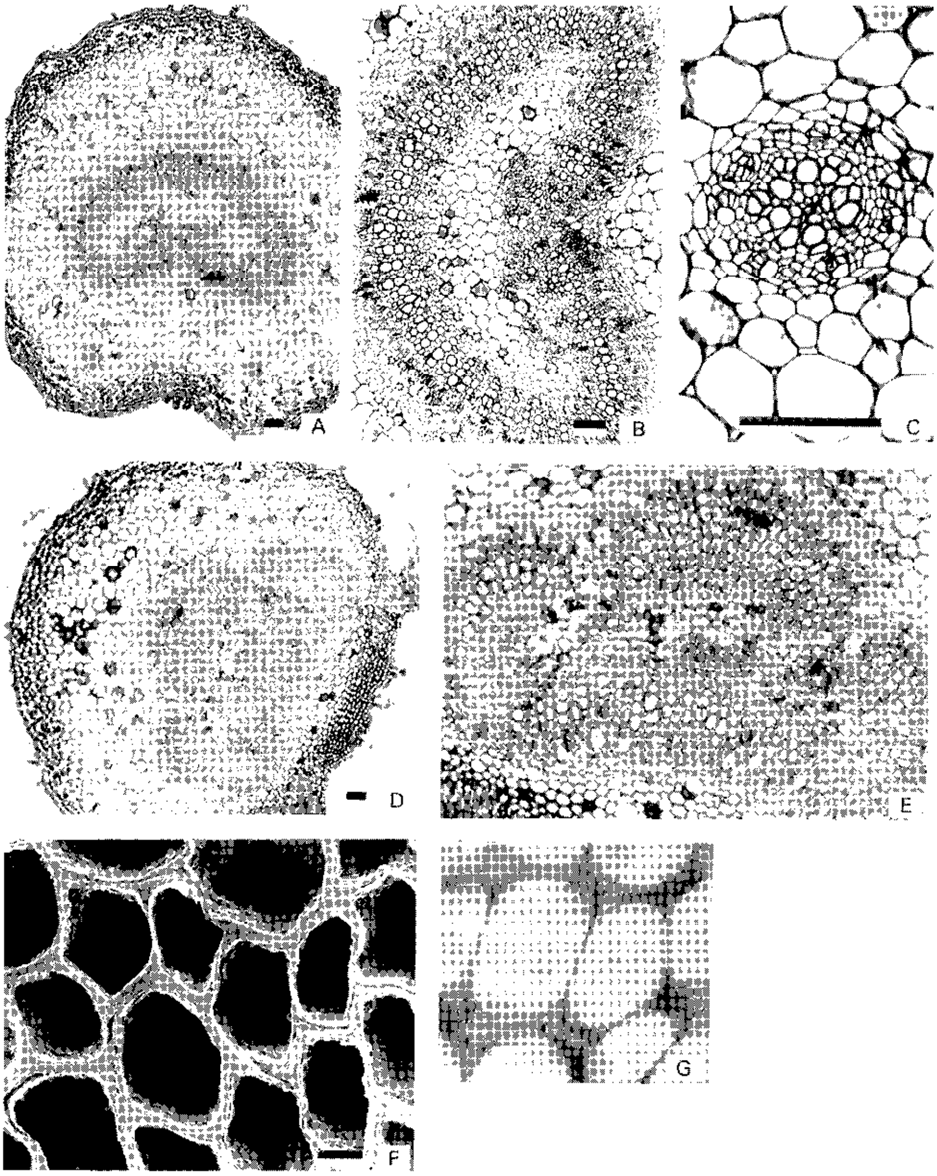

Fig. 3. For legend see page 277.

Fig. 2. Leaf anatomy of *Actinidia deliciosa*: *A* - scanning electron micrograph (SEM) of a cross section of the mature leaf blade with view of lateral vein, upper epidermis and lower epidermis, palisade and spongy parenchyma, bundle sheath extension, and stellate stalked trichomes (*arrows* point to calcium oxalate crystals, *bar* = 100 µm); *B* - SEM of a cross section of the young leaf blade with view of upper epidermis, palisade and spongy parenchyma, bundle sheath extension, cuticular layer (*arrows* point to calcium oxalate crystals, *bar* = 10 µm); *C* - upper epidermis in male leaf prepared by replica method (*bar* = 10 µm); *D* - upper epidermis in female leaf prepared by replica method (*bar* = 10 µm); *E* - paraffin cross section showing bifacial leaf type and special view of upper and lower epidermis, palisade and spongy parenchyma, bundle sheath extension, idioblast with calcium oxalate crystals (*arrows*) (*bar* = 100 µm); *F* - stomatal complex (guard cells, stomatal pore and seven subsidiary cells) in female leaf (*bar* = 10 µm); *G* - multicellular stalked stellate trichome located on the abaxial leaf side of female leaf in a lateral view (*bar* = 100 µm); H - multicellular stalked stellate trichome on the abaxial leaf side of female leaf in a superficial view (*bar* = 100 µm).

Fig. 3. Anatomy and histology of leaf petiole and leaf mid vein: *A* - cross-section through leaf petiole with distinguished collenchyma, cortical parenchyma, mid vein, and lateral minor vein (*bar* = 100 µm); *B* - detail of the Fig. *A* with petiole vein (*bar* = 100 µm); *C* - detail of the Fig. *A* with petiole lateral minor vein showing hadrocentric vascular bundle type (*bar* = 100 µm); D - cross-section through the leaf mid vein with distinguished collenchyma, cortical parenchyma, and mid vein (*bar* = 100 µm); *E* - detail of the Fig. *D* with leaf mid vein (*bar* = 100 µm); *F* - SEM of collenchyma located under petiole epidermis (*bar* = 10 µm); G - paraffin cross-section through the leaf mid vein collenchyma (*bar* = 10 µm).

multicelular stalks (Fig. 2*A*,*G*,*H*). We suppose that they take control, especially those located on the abaxial leaf epidermis, on the microclimatic environment changes throughout the stomata.

The leaves of kiwifruit expressed a reticulate venation as the main type (Fig. 1*A*,*B*). The vascular system was frequently accompanied by idioblasts. Such idioblasts containing presumably calcium oxalate crystals were located mainly in the bundle sheath extensions within the leaf (Fig. 2*A*,*B*,*C*). We have focused on vascular system structure in the leaves and leaf petioles. The cross-sections of the leaf petioles of both male and female plants involve three vascular bundles arranged in one main central ring and two smaller lateral rings (Fig. 3*A*,*B*,*C*). Similar structural arrangement was observed in the main leaf vein, however, two small hadrocentric vascular bundles were not present in the leaf (Fig. 3*D*,*E*). In the cross-sections of the leaf petioles as well as leaf main veins, the ring-arranged collenchyma tissue is present below the epidermis (Fig. 3*A*,*D*,*F*,*G*).

Discussion

The application of several anatomical and/or morphological features in determination of taxonomical classes of plants, their sexes or ploidity were reviewed in many earlier publications (Li 1952, Tichá 1982, Ferguson 1984, Ridge *et al.* 1984, Clark *et al.* 1987, Przywara *et al.* 1988, Buwalda and Smith 1990, Hall *et al.* 1993). Nevertheless, there are still many lacks in understanding how these parameters may play a role in presumption of sex and/or ploidity of plants considering their relation to ontogenetic stage or endogenous and exogenous factors. Comparative studies of

Table 1. Observed anatomical characteristics of kiwifruit leaves and their comparison with that observed by other authors on the same plant species or on other plant species.

Characteristics	Mean ± S.E.
Average leaf area [cm²]	128.60 ± 13.45 (male) 104.54 ± 4.02 (female) 131 (generative, Buwalda and Smith 1990) 197 (vegetative, Buwalda and Smith 1990)
Leaf thickness [μm]	174 ± 3.52 (male) 190 ± 3.84 (female) 288 - 400.5 (*Eucalyptus calophylla, E. resinifera, E. saligna, E. marginata,* Ridge *et al.* 1984)
Upper epidermis [μm]	18.51 ± 0.31 (male) 19.25 ± 0.423 (female) 15.4 - 23.2 *(Eucalyptus calophylla, E. resinifera, E. saligna, E. marginata,* (Ridge *et al.* 1984)
Palisade parenchyma [μm]	104 ± 1.61 (male) 136 ± 2.76 (female)
Stomata density [mm⁻²]	500 ± 2.97 (male) 520 ± 11.21 (female) 510 ± 3.56 (*Quercus palustris*, Tichá 1982) 315 (*Populus alba*, Hall *et al.* 1993) 471.5 ± 12.1 (*Eucalyptus calophylla*, Ridge *et al.* 1984) 383.4 ± 1.7 (*Eucalyptus saligna*, Ridge *et al.* 1984)
Stomata length [μm]	24.75 ± 0.37 (male) 25.13 ± 0.42 (female) 26.40 ± 2.4 (seedlings of *Actinidia deliciosa*, Przywara *et al.* 1988) 28.20 (*Vitis vinifera*, Němec *et al.* 1958)
Other features	bifacial leaf type anomocytic stomata hypostomatic leaf idioblasts in the leaf mesophyll crystals of calcium oxalate along the boundary system calcium oxalate accounting up to 79 % of the total Ca content after leaf emerge, and up to 42 % at leaf fall (Clark *et al.* 1987) seasonal changes in Ca and Mg content of leaf blades (Clark *et al.* 1987) 3 boundary rings in the leaf petioles trichomes on the surface of the abaxial leaf epidermis *folium cordatum* and *folium rotundato- cordatum* as the most frequent leaf shape reticulate venation

kiwifruit plants and their cultivars, growing in different environmental conditions (*e.g.* New Zealand compared with Central Europe), showed some differences in relation to parameters such as stomata density and their length. Przywara *et al.* (1988) estimated somewhat higher values of stomata length for seedlings of *Actinidia* *deliciosa,* cv. Hayward in comparison to our study with 7 - 8 years old plants (Table 1), sustained occasionally due to different ontogenetic stages of the plants. Although the analysed leaves in both male and female plants appeared to be an evident heliophytic leaf type, resulting from rather similar ambient (light) conditions, the analysis showed the biggest differences in total leaf thickness, palisade parenchyma thickness, and thickness of cuticular layer, but less differences in the stomata density, and guard cell length. No considerable differences have been observed within structural arrangement and morphology of stomatal complex, and multicellular trichomes between leaves of both sexes. In regard to vascular system arrangement within leaf petioles of kiwifruit, the compareable pattern was also observed within the taxon *Vitis vinifera* (Němec *et al.* 1958). Accordingly, leaf and cuticular thickness together with surface morphology of adaxial epidermis can serve as structural markers helpful in sex determination. The general structural tendency also improve our earlier supposition that the female leaves have a higher physiological (photosynthetic) activity (Oláh and Masarovičová 1995). This resulted in their different acclimation, primarily driven according to Arp (1991) through the source-sink relationship, and through the endogenous control of photosynthesis, respectively, however, always in relation to the ambient enviromental conditions. Since strongly limitation of bibliographical references with anatomical and morphological reviews of kiwifruit, our results are compared with those of only few authors dealing with kiwifruit or other plant taxons, respectively (Table 1). In conclusion, the presented data presumably do not fulfill completely our purpose to find a wide set of anatomical and morphological parameters regarding sex determination except of few above mentioned parametcters (leaf thickness, surface morphology of adaxial leaf epidermis), nevertheless they might also provide a general overview of some anatomical and morphological features of kiwifruit leaves.

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