The in vitro and *in vivo* anatomical structure of leaves *of Prunus x Davidopersica 'Piroska' and Sorbus rotundifolia L. 'Bükk szépe'*

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Summary: Immature in vitro leaves showed similar structure of the mesophyll tissue to the immature field-grown (in vivo) leaves of Prunus x davidopersica 'Piroska'. Mature leaf anatomical characteristics of in vitro plantlets differ from the field-grown plants. The mesophyll tissue of in vitro plantlets were thinner than the in vivo plants and consisted of only one layer palisade parenchyma, the shape of the cells and the structure of spongy parenchyma basically differed from the field-grown plants. In the case of Sorbus rotundifolia similar anatomical differences were found both in vitro and in vivo as in the case of Prunus x davidopersica 'Piroska'.

Introduction

Anatomical studies of some species have reported that the *in vitro* grown plantlets differ from that of field- or greenhouse grown plants. The anatomical changes can be observed in all organs but first of all in the leaves. On the surface of *in vitro* leaves, the waxy layer and the cuticle are thinner *(Grout* and *Aston* 1988, *Sutter* and *Langhans* 1982).

The structural modifications have also been described during *in vitro* propagation in the case of some woody plants. In the mesophyll of microcultured plantlets the palisade parenchyma is less developed and the air-space of the spongy parenchyma was less than in field-grown plants. *(Brainerd* and *Fuchigami,* 1981, *Schmidt* and *Waldenmaier,* 1992, *Kiss* et al. 1994). The anatomy and functioning of stomata also change *(Capelledes* et al. 1990, *Sallanon* et al. 1993).

The multiplication of both of the examined Hungarian cultivars were studied and published earlier *(Jámbor-Benczúr* et al., 1995, *Jámbor-Benczúr* et al., 1997, *Kissimon* et al., 1998). The objectives of our study were to determine and compare the leaf anatomical features of immature and mature *in vitro* and field-grown *(in vivo)* leaves of *Prunus x davidopersica 'Piroska'* and *Sorbus rotundifolia L. 'Bükk szépe'.*

Material and methods

For the histological studies tissue cultures of *Prunus x davidopersica 'Piroska'* were cultured on S medium containing BM macro-elements *(Jámbor-Benczúr* and *Marta-Riffer,* 1990), Heller micro-elements *(Heller,* 1953) and MS vitamins *(Murashige* and *Skoog,* 1962). The S medium was supplemented with 5 g/l glucose.

For micropropagation of *Sorbus rotundifolia L. 'Bükk szépe'* Murashige and Skoog (1962) macro-elements (in half concentrations), micro-elements and vitamins were used. The culture medium was supplemented with 0.75 mg/l BA, 0.1 mg/l IBA, 0,1 mg/l GA_3. In the range of 5, 10, 15, 20, 25 and 30 mg/l sucrose or glucose were added for studying the effect of sugar.

In both cases the media were solidified with agar-agar 7 g/l. The pH was adjusted to 5.6. The cultures were illuminated by white light of 40 $\mu M/m^2/s$ using 16/8h light/dark cycles for 6 weeks. For the experiments 100 ml Erlenmeyer flasks were used as culture vessels and each flask contained 25 ml of medium. The flasks were covered with three layer of 0.017 mm plastic foil. The temperature was 22–25 °C and 16–18 °C during the light and dark periods respectively.

The mature leaves were collected from the middle nodes and the juvenile leaves from the second upper nodes of the

shoots. Leaf samples for light microscopy were fixed in 3% glutaraldehyde for 2 hours followed by 1 hour treatment with 1% osmium tetroxide, then samples were stained with toluidin blue. The cross sectional area of leaves was studied by light microscopy.

Results

1. The anatomical structure of immature and mature Prunus x davidopersica 'Piroska' leaves

The immature field-grown leaves were compact *(Fig. 1A)*. The upper and lower epidermis were uniseriate, the epidermis cells were not elongated and the cell walls were thin. There were few immature stomata present on the abaxial side of the leaves. The mesophyll was homogenous, the palisade and spongy mesophyll layers were not distinguishable. The juvenile *in vitro* leaves *(Fig. 1B)* showed similar structure but the mesophyll was a little bit thinner than the immature field-grown leaves.

Transverse section of mature field-grown leaves showed a well-developed structure *(Fig. 1C)*. The upper and lower epidermis consisting of a single layer of cells with thick cell-walls and a waxy layer. Stomata were found in the lower epidermis level with the epidermal cells and were closed. The mesophyll tissue consisted of two well-defined layers of palisade cells and 3–4 cell layers of spongy parenchyma with large intercellular spaces. The structure of developed *in vitro* leaves *(Fig. 1D)* were different in the comparison to the leaf structure of field-grown leaves. The cell-walls of the

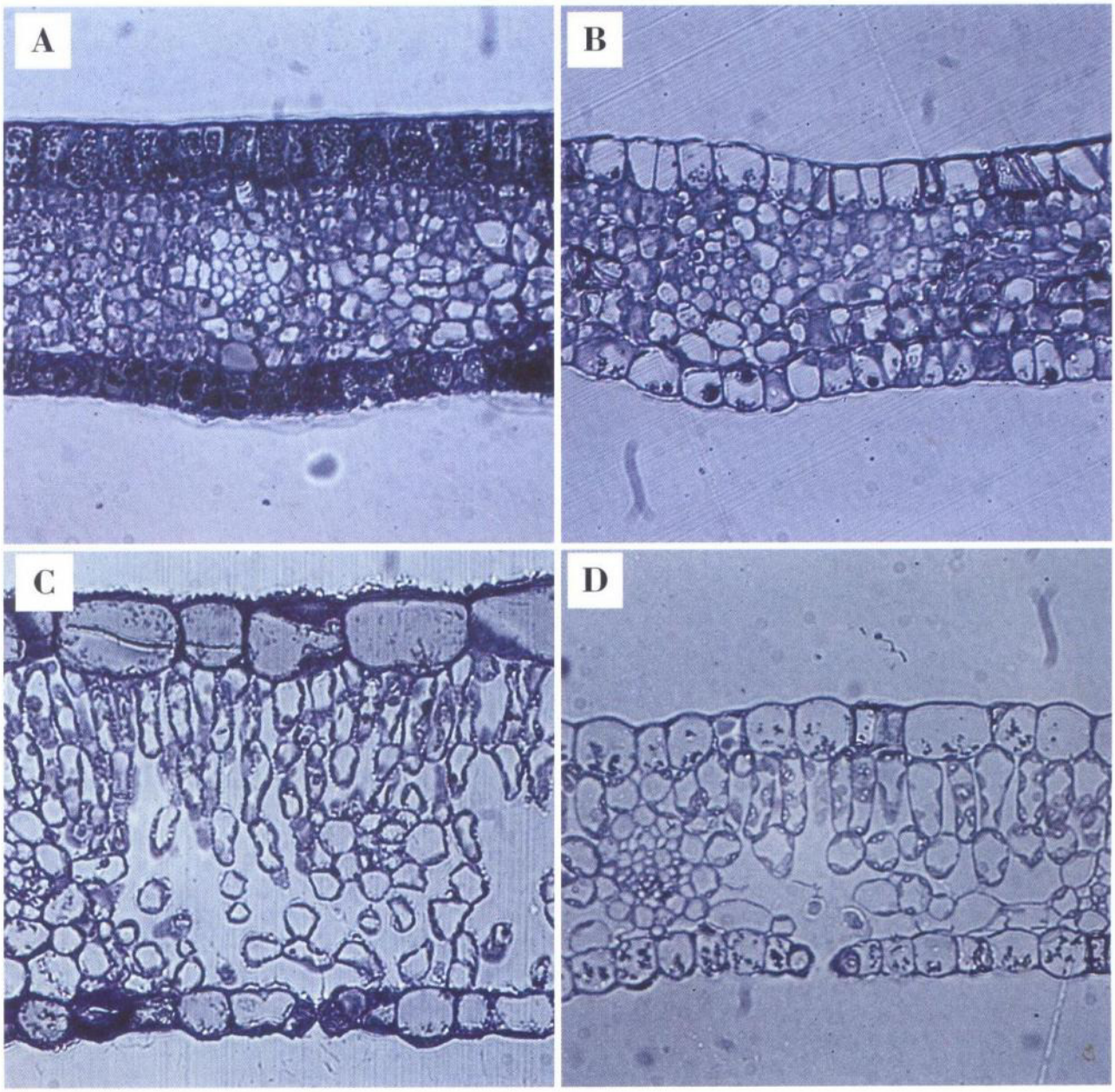

Figure 1 Cross sections of the leaves of *Prunus x davidopersica 'Piroska'*. A immature field grown leaf, B immature *in vitro* leaf, C mature *in vivo* leaf, D mature *in vitro* leaf, X 40.

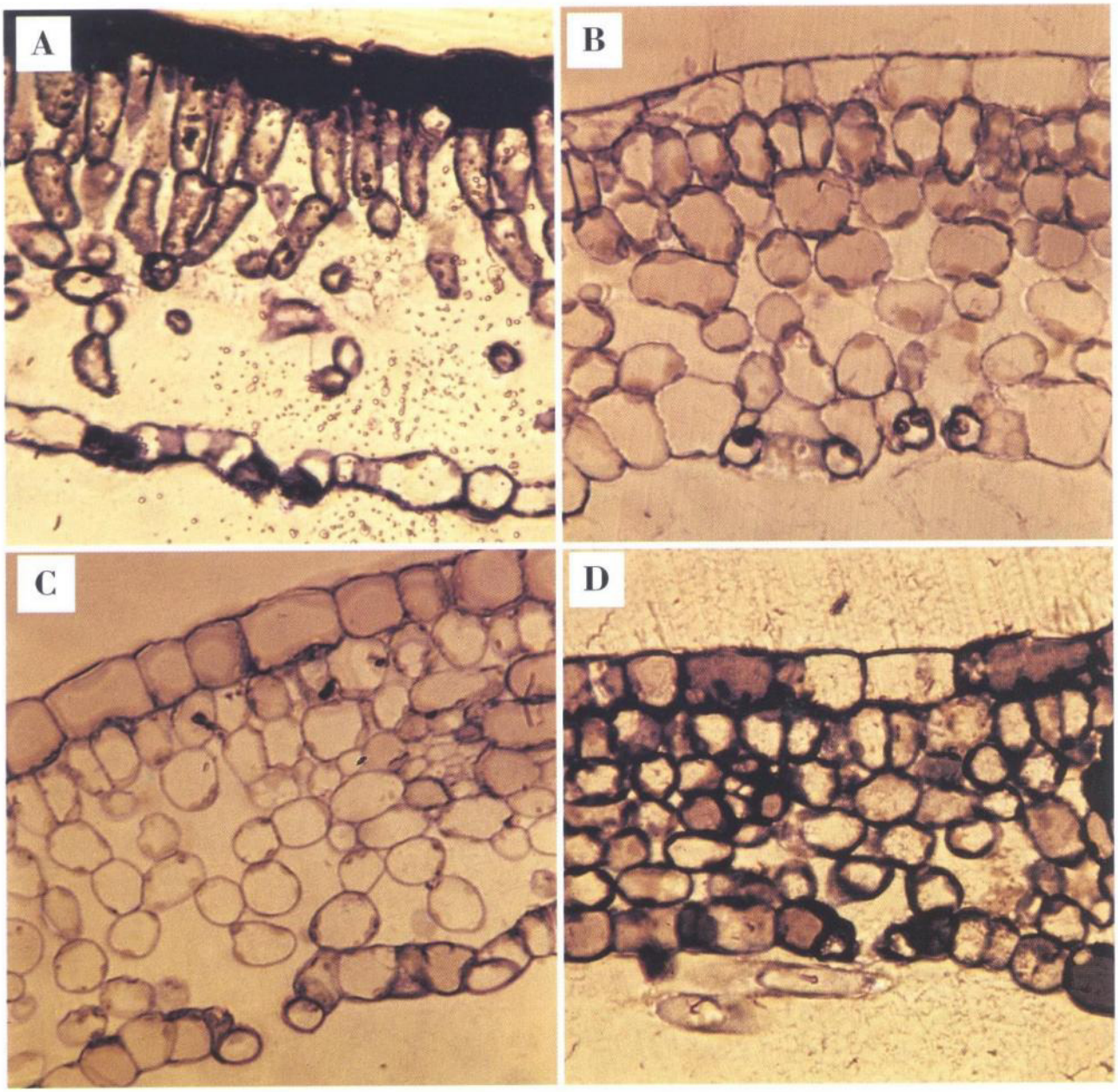

Figure 2 Cross sections of the leaves of Sorbus rotundifolia L. 'Bükk szépe' grown in vivo: A and in vitro: B, C, D cultured with the use of 5, 15 and 30 g/l sucrose, X 40.

epidermal cells were thinner and the stomata were found open. The palisade parenchyma was less developed, consisted of only one layer of palisade cells, the spongy parenchyma had less intercellular spaces. The leaves were thinner than field-grown leaves.

2. The anatomical structure of Sorbus rotundifolia L. 'Bükk szépe' leaves

The cross-section of the field-grown leaves *(Fig. 2A)* exhibited an upper and a lower epidermis consisting of a layer uniform cells. There were stomata on the lower epidermis and they were found closed. The mesophyll contained two rows of palisade layers. The palisade cells were characteristically elongated. The spongy parenchyma contained very large intercellular spaces.

Leaves grown *in vitro* basically differed from field-grown leaves *(Fig. 2B,C,D)*. The leaf cross-section of the *in vitro* plantlets compared to those of field-grown plants showed a different structure. The walls of epidermal cells were thinner the stomata were open. The mesophyll tissue was not clearly differentiated consisting of only one layer of palisade parenchyma with less elongated cells. The spongy parenchyma had less air spaces and parallel with the raising of the sucrose concentration become more compact.

Discussion

Comparing the cross sections of immature leaves the structure was almost the same in the case of *in vitro* developed leaves as in the field-grown leaves. The differences of the internal structure between *in vitro* and *in*

vivo mature leaves were almost the same in both examined species. The field-grown leaves had two layers of palisade cells with a significant amount of mesophyll air space. The *in vitro* leaves had only one layer of palisade cells and its shape differed from the leaves grown *in vivo*. The spongy parenchyma had less air space and more cells compared to field-grown leaves.

Concerning the epidermis and palisade parenchyma of the *in vitro* leaves similar structure was found as described previously *(Brainerd* and *Fuchigami,* 1981, *Schmidt* and Waldenmajer, 1992, Kiss et al. 1994), but in our experiment the intercellular spaces were less in the spongy parenchyma. *Sorbus rotundifolia L. 'Bükk szépe'* cultured on medium containing different concentrations of carbohydrates (5–30 g/l) showed differences in the leaf structure. The anatomical differences were the greatest using 30 g/l sucrose.

The differences in the leaf structure of *in vitro* and *in vivo* developing leaves can be explain by the different environmental conditions. The open and a little bit raised position of the stomata are induced by vapour-saturated atmosphere of the culture vessel, according to *Capellades* et al. (1990) and *Brainerd* and *Fuchigami* (1981).

Reduction in the rows of palisade parenchyma of plum, red raspberry, birch and Rhododendron cultured *in vitro* were reported by *Donelly* and *Vidaver* (1984), *Smith* et al. (1986) and *Schmidt* and *Waldenmaier* (1993). Similarly the palisade layer was shorter and cells lacked the the elongated appearance. According to as described with plum by *Brainerd* et al. (1981) we found decreased mesophyll air space in both rosaceous species. We suppose that the main reason of the dramatic change in the mesophyll structure of *in vitro* plants can be explain mainly by the significant reduction of the light intensity *in vitro*. *Esau* (1962) showed significant differences in the structure of the mesopyll tissue in the case of *Acer platanoides* leaves grown in full light, shaded and strongly shaded parts of the crown. In the case of leaves grown in shaded areas of the crown of the tree, she found only one layer of palisade parenchyma with much shorter cells and a compact spongy parenchyma. The structure of the mesophyll was very similar to that of *S. rotundifolia* and *P. x davidopersica* leaves grown *in vitro*.

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