In vitro rooting and anatomical study of leaves and roots of *in vitro* and *ex vitro* plants of *Prunus x davidopersica 'Piroska'*

INTERNATIONAL JOURNAL OF HORTICULTURAL SCIENCE

AGROINFORM Publishing House, Hungary

Jámbor-Benczúr E.[1], Kissimon J.[2], Fábián M.[1], Mészáros A.[2], Sinkó Z.[1], Gazdag Gy.[1] and Nagy T.[1]

Szent István University, Faculty of Horticultural Science
[1]Department of Floriculture and Dendrology
[2]Department of Molecular Plant Biology,
H-1118 Budapest, Villányi út 35–43
E-mail: ebenczur@omega.kee.hu

Key words: Prunus, rooting, *in vitro*, sucrose, glucose, IBA, NAA, leaf and root anatomy

Summary: The process of *in vitro* rooting and the anatomical characters of *in vitro* and *ex vitro* leaves and roots of *Prunus x davidopersica 'Piroska'* were studied. Best rooting percentage (50%) and highest root number (5.0) was achieved in spring on a medium containing 0.1 mg/l NAA + 30 g/l glucose. At the end of rooting the parenchyma of the *in vitro* leaves was more loose and spongy, than during the proliferation period. In the first newly developed leaf of an acclimatised plant, the parenchyma was much more developed, contained less row of cells and less air space too, compared to the leaves developed in the field. The *in vitro* developed root had a broad cortex and narrow vascular cylinder with less developed xylem elements, but at the end of the acclimatisation the vascular system became dominant in the root.

Abbreviations: IBA: indole-3-butyric-acid, NAA: α-naphthalene-acetic-acid AC: active carbon, MG: malachite green

Introduction

Prunus x davidopersica 'Piroska' (accepted as *X Prunus 'Rubin'*, *Schmidt*, 1998) is a Hungarian cultivar with bright reddish leaves during the vegetation period and large pale pink flowers in spring. The establishment of its *in vitro* culture and micropropagation was successfully carried out on S medium, containing 2iP. To enhance the rate of multiplication and to prevent vitrification BA-riboside was successfully used *(Jámbor-Benczúr* et al., 1995).

Several publications deal with the rooting of peach and almond-peach hybrids. The authors had different results depending on the cultivar *(Rugini* et al., 1988, *Damiano*, 1992, *Koronova*, 1995). Our preliminary results with *P. x davidopersica* showed that this tree is a "difficult to root plant" and it was impossible to root it *ex vitro*.

Anatomical changes of the leaves of woody plants during micropropagation and acclimatization have been described by some authors *(Schmidt & Waldenmeyer*, 1992, *Kiss* et al., 1994, *Jámbor-Benczúr* et al. 1997, *Kiss* et al., 1999), but only a few references can be found concerning anatomical structure of *in vitro* and *ex vitro* roots *(McClelleand & Smith*, 1988, *Roger & Smith*, 1992).

The objectives of the present study were to find the optimal period and medium for rooting and to describe the anatomical changes of leaves and roots during *in vitro* and *ex vitro* phases.

Material and methods

In the first rooting experiment twelve different media were used *(Table 1.)* Two kinds of auxin (IBA and NAA 0.1–0.2 mg/l), two kinds of sugar (sucrose and glucose 30 g/l), malachite green (3 mg/l) and active carbon (1 g/l) were added to the S basic medium *(Jámbor-Benczúr & Márta-Riffer*, 1990). The experiment was started on August 4., 1998 and evaluated twice, first after 4 weeks and second after the following 3 weeks. The second experiment was carried out in spring of 1999. This time only those 6 kinds of

Table 1 Successful rooting of micropropagated *Prunus x davidopersica 'Piroska'* on different culture media and different starting seasons

	Percentage of rooting				
	1998		1999		
Type of the media with the additives	25 August	14 Sept.	24 March	07 April	28 April
G0 30 g/l glucose	16.6	25.0	0	0	0
S0 30 g/l sucrose	20.8	20.8	8.3	16.6	16.6
G1 0.1 mg/l IBA+30 g/l glucose	8.3	12.5	–	–	–
G2 0.1 mg/l IBA+30 g/l sucrose	0	0	–	–	–
G3 0.2 mg/l IBA+30 g/l glucose	20.8	25.0	8.3	8.3	12.5
G4 0.2 mg/l IBA+30 g/l sucrose	12.5	25.0	8.3	20.8	20.8
G5 0.2 mg/l IBA+30 g/l gl.+1 g/l AC.	0	12.5	–	–	–
G6 0.2 mg/l IBA+30 g/l su.+1 g/l AC.	0	25.0	–	–	–
G7 0.1 mg/l NAA+30 g/l glucose	0	12.5	50.0	50.0	50.0
G8 0.1 mg/l NAA+30 g/l sucrose	4.1	12.5	0	0	0
G9 0.1 mg/l IBA+30 g/l gl.+3 mg/l MG.	16.6	25.0	–	–	–
G10 0.1 mg/l IBA+30 g/l su.+3 mg/l MG.	8.3	–	–	–	–

media were used, which proved to be the best during the autumn. The media were solidified with Difco-bacto agar (7 g/l) and the pH was adjusted to 5.6. The 100 ml Erlenmeyer flasks contained 50 ml of medium and were covered with three layers of 0.017 mm plastic foil.

Cultures were illuminated with white light of 40 $\mu M/m^2/s$ using 16/8 hour light/dark cycles for 7 week. The temperature was 23–25 °C and 18–20 °C during the light and dark periods respectively.

Every treatment contained 30 plantlets. Data of the number of primary and lateral roots, the length of roots and shoots were evaluated and summarized. The statistical analysis of the data was made by *Fig.* P. programme.

For the anatomical studies, leaf samples were collected from plants grown on the rooting medium that contained 0.1 mg/l NAA + 30 g/l glucose at the end of the rooting and acclimatisation period in 1998. Root samples were taken from the plants grown on the medium that contained 0.2 mg/l IBA + 30 g/l glucose at the end of the rooting and acclimatisation period in 1998. The samples were analysed by Tesla BS 300 scanning electron microscope.

Results

Concerning the percentage of rooting, the best results (25%) were achieved in autumn on the G3 and G4 media, which contained 0.2 mg/l IBA + 30 g/l glucose or sucrose. In contrast, in spring the G7 medium which contained 0.1 mg/l NAA + 30g/l glucose proved to be the best with 50% rooting *(Table 1)*.

The rooting of plants was most succesful in spring. The highest root number (5.0) could be achieved with the use of 0.1 mg/l NAA differing significantly from all of the other media used. The medium containing IBA gave significantly lower root number but it still resulted in better rooting in spring, than in autumn. Roots developed on media containing NAA were shorter than on media with IBA. From the view-point of acclimatisation the shorter roots are more advantageous and from this point the effect of NAA proved to be better. Concerning the number of lateral roots it was interesting, that in autumn we did not find lateral roots on the medium containing NAA but they appeared in spring. On the medium containing auxin the shoots were longer, much more developed, and looked more vigorous than on the hormon free control *(Fig. 1, 2, 3)*.

Anatomical characteristics of the *in vitro* leaves were different in certain propagation phases. Besides the one row of palisade parenchyma, the spongy parenchyma showed a

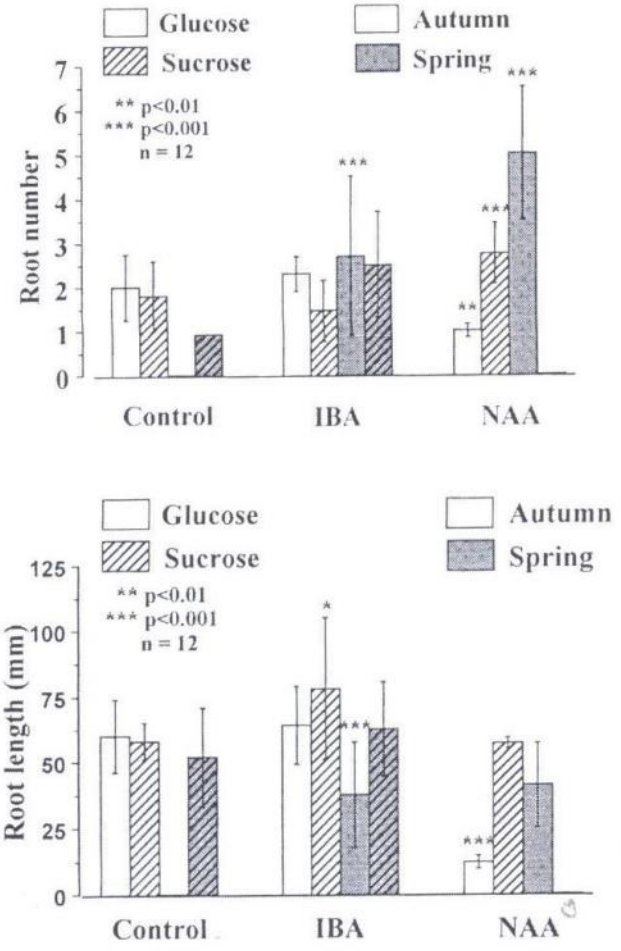

Figure 1 Development of the number and length of roots in micropropagated *Prunus x davidopersica 'Piroska'* on different culture media and at different starting seasons.

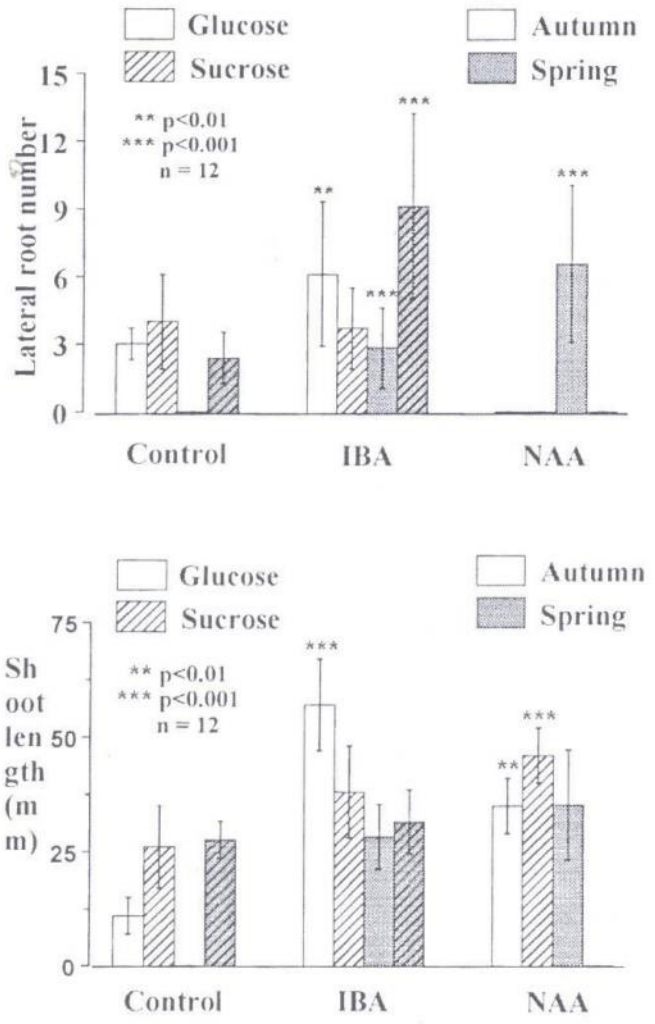

Figure 2 Development of lateral roots and shosts of micropropagated *Prunus x davidopersica 'Piroska'* on different culture media and at different starting seasons.

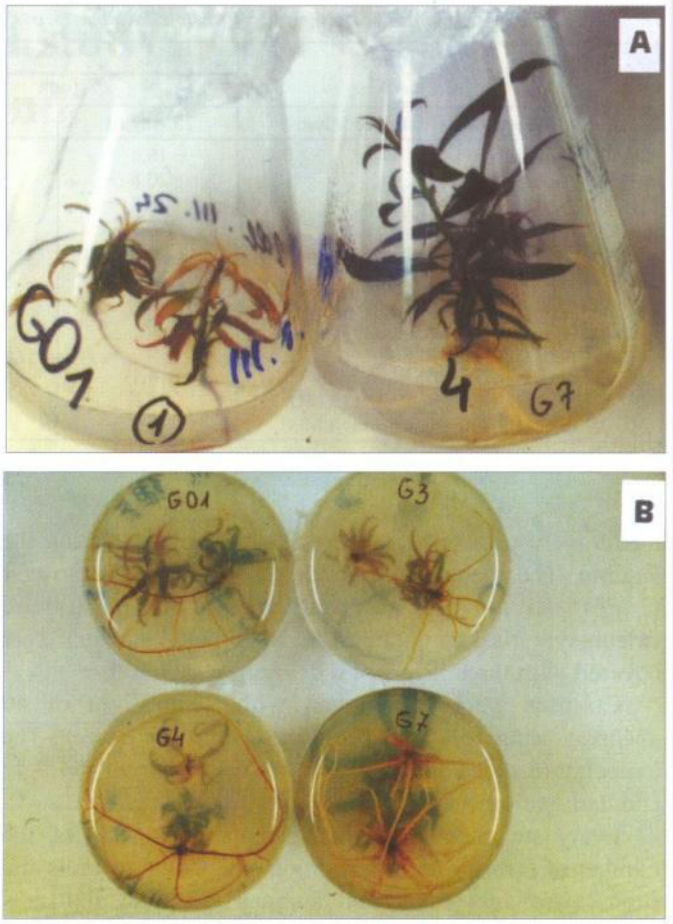

Figure 3 In vitro rooting of *Persica x davidopersica 'Piroska'*. On the medium containing auxin the shoots (A) and the roots (B) were more developed and looked more vigorous

looser character with four row of cells and air spaces, the stomata were open at the end of rooting.

At the end of the acclimatisation period the newly developed leaves were similar to those of the *ex vitro* leaves described by *Kiss* at al, 1999. The only difference was that the spongy parenchyma was less developed. It had less rows of cells than in the field grown leaf *(Fig. 4)*.

The *in vitro* grown roots had a very broad primary cortex and a narrow vascular cylinder with a few slightly developed xylem elements. One cell row of endodermis and one row of pericycle are found between the cortex and the vascular

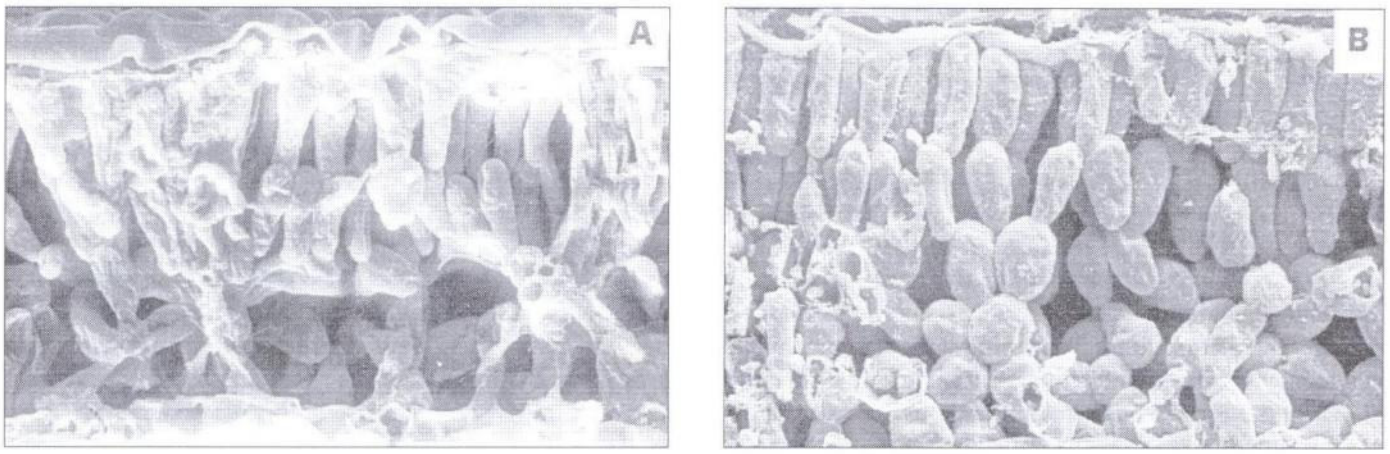

Figure 4 Comparison of the in vitro and ex vitro leaf strucure of *Persica x davidopersica 'Piroska'* A. Cross section of a leaf grown on G7 medium at the and of the rooting period. B. Cross section of a leaf of an *ex vitro* plant at the end of the acclimatisation period, (X 250)

elements. The cross section of the plantlets rooted on both types of rooting media was similar with a small difference. Cortex cells of the plants grown on auxin free media were roundish and less in diameter as compared with the plants growing on media supplemented with auxin.

The roots of the acclimatised plants showed a significant difference compared to the one mentioned above. The roots had a narrow primary cortex and the cells began to die. The pericycle turned to be pericambium and begin to produce a periderm. The cambium became activated and produced the secondary phloem and xylem elements. The diameter of the vascular cylinder was three times larger than the primary cortex. Parallel with this, xylem elements became much larger and better developed as well *(Fig. 5)*.

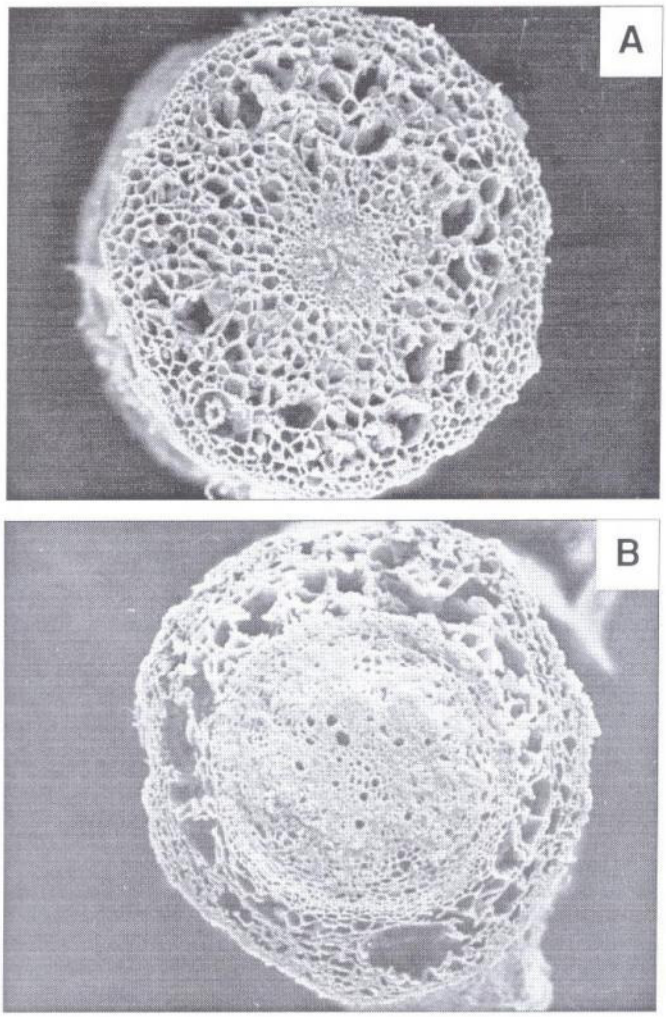

Figure 5 Comparison of the root structure of the *in vitro* and *ex vitro* plants of *Persica x davidopersica 'Piroska'*. A. Cross section of a root grown on G7 medium at the end of the rooting period. B. Cross section of a root of an *ex vitro* plant at the end of the acclimatisation period, (X 50)

Conclusions

It can be concluded that the best period for rooting was the spring, the best auxin in spring was NAA and in autumn was IBA in low (0.1 or 0.2 mg/l) concentrations. Glucose proved to be the best in both periods. The reason of this phenomenon can be that the chlorophyll content of the *in vitro* plantlets was very low *(Kissimon* et al., 1999). In case of using sucrose the plantlets did not have sufficient energy for rooting, but supplying them with glucose -which can be utilised by the plantlets without conversion - much better results could be achieved.

Concerning the anatomical characteristics of the leaves, our results were similar to those described by *Kiss* et al., 1999. In the case of *in vitro* leaves the spongy parenchyma is compact, with no or very little air spaces. In the first newly developed leaf of an acclimatised plant, the spongy parenchyma was more developed, but there were less air spaces and contained less rows of cells, compared to the leaves developed in the field.

The *in vitro* developed roots had a narrow vascular cylinder with less developed xylem elements, because the relative humidity in the flasks is high and there is no need to take up much of water. During the acclimatisation the secondary growth began and the plants had to take up much larger quantities of water. That is why the vascular elements became dominant during the acclimatisation.

Acknowledgement

We would like to express our thanks to *Barbara Nagy, Anikó Csillag* & *László Bogár* for their assistance in the Central Laboratory of our University.

This work was supported by OTKA (Hungarian Sci. Res. Fund) project No. T 026420.

References

Damiano, C. (1992): Some factors affecting the induction and the expression of rooting in different fruit species in vitro. Acta Horticult. 300. 211–224.

Jámbor-Benczúr E. & Márta-Riffer A. (1990): In vitro propagation of *Philodendron tuxtlanum* Bunting with benzylaminopurine. Acta Agronomica Hungarica 39. 341–348.

Jámbor-Benczúr E., Tilly Mándy A. & Szafián Zs. (1995): Effect of Growth Regulators on *in Vitro* Propagation of *Prunus x davidopersica 'Piroska'* Med. Fac. Landbouw. Univ. 60/4a. 1665–1668.

Jámbor-Benczúr E., Ónadi K., Kissimon J. & Horváth G. (1997): The effect of carbon source, on *in vitro* multiplication, photosynthesis and anatomical structure of *Sorbus rotundifolia* L. Acta Horticult. 447. 157–159.

Kiss I., Jámbor-Benczúr E., Fehér T. & Csillag F. (1994): Anatomical Characteristics of Leaf surface and Stomata of Hydrangea Plant grown in vitro, in vivo and transplanted. Horticultural Sci. 26:2. 98–100.

Kiss I., Fábián M., Gazdag Gy., Jámbor-Benczúr E. & Ónadi K. (1999): The *in vitro* and *in vivo* anatomical structure of leaves of *Prunus x davidopersica* 'Piroska' and *Sorbus rotundifolia* L.

'Bükk Szépe'. Intern. J. of Horticultural Sci. 5, (1-2) 92-95.

Kissimon J., Tantos Á., Mészáros A., Jámbor-Benczúr E. & Horváth G. (1999): Stress alterations in growth parameters, pigment content and photosynthetic functions of *in vitro* plants. Z. Naturforsh., 54c. 834–839.

Koronova, K. (1995): Study of in vitro rooting in some peach varieties. Rastenievdni Nauki. 32:7-8. 109–111.

McClelland, M.T. & Smith, M.A.L. (1988): Response of woody plant microcuttings to in vitro and ex vitro rooting methods. Comb. Proc. Int. Plant. Prop. Soc. 38. 593–599.

Rogers, R. B. & Smith, M.A.L. (1992): Consequences of in vitro and ex vitro root initiation for miniature rose production. Journal of Horticult. Sci. 67:4. 535–540.

Rugini, E., Bazzofia, A. & Jacobini (1988): A simple in vitro method to avoid the initial dark period and to increase rooting in fruit trees. Acta Horticult. 227. 438–440.

Schmidt G. (1991): A hagyományos szaporításról, szemzéssel (The conventional propagation by grafting) in: Nagy, B., Schmidt, G. Dendrology. University of Horticulture and Food Industry Budapest 149.

Schmidt G. & Waldenmaier, S. (1993): Histological studies on in vitro and ex vitro leaves during the adaptation phase of Rhododendron 'Pink Pearl'. Acta Agr. Hung. 42. 11–21.

Schmidt G. (1998): Selection and breeding of woody ornamentals in Hungary. Hungarian Agricultural Research. 3. 9–12.