CORVINUS UNIVERSITY OF BUDAPEST

THE EFFECT OF DIFFERENT ACCESSORIES OF MEDIUM ON PHYSIOLOGY, ANATOMY AND MORPHOLOGY DURING MICROPROPAGATION OF *SORBUS* TAXA

Doctoral Thesis

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1. INTRODUCTION AND THE AIMS OF THE STUDY

Several kinds of ornamental plants were kept in large-scale production in favour of producing more and more pathogen-free, true to variety offspring. Stress-effects (which can appear during the whole process of micropropagation) can decisively influence the success of *in vitro* propagation of the plants. We have to guarantee sterility and optimal abiotic conditions for the purpose of not only the largest degree of multiplication but better rooting and higher percentage of surviving rate during acclimatisation.

There are numerous taxa in the genus of *Sorbus* but only few were propagated *in vitro* (principally the economically important species like *S. domestica, S. aucuparia*). Nevertheless some endemic hybrid (for example the decorative *S. redliana* 'Burokvölgy' and *S. borbasii* 'Herkulesfürdő' with white flowers, bright and colourful crops and autumn foliages) were micropropagated.

Earlier studies generally examined the effects of different kinds of sucrose (glucose, fructose, saccharose), and some growth regulators (BA, BAR, 2iP) in case of micropropagation of these two sorb. In my trials, new accessories (for example M-TOP, Titavit, HUMUS^{R} FW) and combinations (BA + KIN, BA + M-TOP) were applied during *in vitro* propagation and rooting in order to obtain more and at the same time well-developed, stronger shoots or higher rate of rooting (with shorter and more roots which are optimal for acclimatizing).

In the course of research the following aims were proposed:

- To find optimum concentration and type of accessories (BA, BAR, KIN, M-TOP, Titavit; and combinations of BA + KIN, BA + M-TOP) during *in vitro* propagation.

- To ascertain the optimum concentration of accessories (IBA, AC, Titavit, HUMUS^{R} FW) and the more successful way of *in vitro* rooting.

- To find morphological, anatomical and physiological differences between *in vitro* (multiplicated and rooted) and *ex vitro* plants (and between groups from medium containing variant accessories).

- To find medium-accessories and methods of *in vitro* propagation and rooting which effect tissue features mostly similar to *ex vitro*s.

- To detect unambiguous coherences between the examined morphological, anatomical, physiological characteristics (for example degree of shoots and roots differentiation, total chlorophyll content of leaves, peroxidase activity).

2. MATERIAL AND METHOD

Both *in vitro* groups of sorbs were started from mother-trees in the Botanical Garden of Corvinus University (Buda) - *S. redliana* 'Burokvölgy' by Jámborné et al. (1998) and *S. borbasii* 'Herkulesfürdő' by Kukor (2003). *In vitro* cultures (with few plants) were originated and used in the laboratory of the Department of Floriculture and Dendrology where previous and further studies of *in vitro* propagation and rooting were done.

2.1. *In vitro* propagation

During *in vitro* propagation Murashige and Skoog (1962) basal medium (with macro- and microelements) was used. For alimentation of carbohydrate, 20 g/l saccharose was applied and 11 g/l agar-agar solidified every medium (which contained 100 mg/l inosit, too). The pH was adjusted to 5,6-5,7 using KOH, certainly in every cases of medium applied for *in vitro* propagation and rooting. Every accessory (**table 1**.) was added after autoclaving (120 ^{o}C, 10^{5}Pa pressure unto 30-40 minutes).

2.2. *In vitro* rooting

S-media with BM (JÁMBOR-MÁRTA, 1990) macro- and HELLER (1952) microelements were used for *in vitro* rooting (accessories were shown in **table 2**.). Every rooting medium was supplemented with 30 g/l saccharose, 11 g/l agar-agar and 100 mg/l inosit. In case of induction, at first, shoots were kept for 2 days on medium with extra concentration of IBA, after this, inducated shoots were transplanted on medium containing AC (active charcoal, *S. redliana* 'Burokvölgy') or Titavit, HUMUS^{R} FW (*S. borbasii* 'Herkulesfürdő').

Table 1: accessories of MS media during *in vitro* propagation of sorbs

	Accessories (mg/l)												
Sign of medium	**BA**	**BAR**	**KIN**	**M-TOP**	**IBA**	**Titavit**	**Sign of medium**	**BA**	**BAR**	**KIN**	**M-TOP**	**IBA**	**Titavit**
A0 (control)	-	-	-	-	-	-	**A0 (control)**						
A1	0,25	-	-	-	0,05	-	**BAK1**	0,25	-	0,5	0	0,05	-
A2	0,5	-	-	-	0,05	-	**BAK2**	0,5	-	0,5	0	0,05	-
A3	0,75	-	-	-	0,05	-	**BAK3**	0,75	-	0,5	0	0,05	-
A4	1,0	-	-	-	0,05	-	**BAK4**	1,0	-	0,5	0	0,05	-
R1	-	0,25	-	-	0,05	-	**BAT1**	0,25	-	-	0,5	0,05	-
R2	-	0,5	-	-	0,05	-	**BAT2**	0,5	-	-	0,5	0,05	-
R3	-	0,75	-	-	0,05	-	**BAT3**	0,75	-	-	0,5	0,05	-
R4	-	1,0	-	-	0,05	-	**BAT4**	1,0	-	-	0,5	0,05	-
AK1	-	-	0,5	-	0,05	-	**T1**	-	-	-	-	-	0,5
AK2	-	-	0,75	-	0,05	-	**T2**	-	-	-	-	-	2,0
AK3	-	-	1,0	-	0,05	-	**T3**	-	-	-	-	-	5,0
AK4	-	-	2,0	-	0,05	-	**T4**	-	-	-	-	-	10,0
AT1	-	-	-	0,5	0,05	-							
AT2	-	-	-	0,75	0,05	-							
AT3	-	-	-	1,0	0,05	-							
AT4	-	-	-	2,0	0,05	-							

Table 2: accessories of S media during *in vitro* rooting of sorbs

		Accessories			
Sign of medium	**Name of sorb (abbreviation)**	**IBA (mg/l)**	**AC (g/l)**	**Titavit (mg/l)**	**HUMUS^{R} FW (ml/l)**
S	**S. RÉ**	-		-	-
SIBA10	**S. RÉ**	1	-	-	-
SIBA15	**S. RÉ**	1,5	-	-	-
SIBA20	**S. RÉ**	2,0	-	-	-
SACIBA15	**S. RÉ**	1,5	1,0	-	-
Medium for induction	**S. RÉ, S. BORB**	15,0	-	-	-
AC0,5	**S. RÉ**	-	0,5	-	-
AC0,75	**S. RÉ**	-	0,75	-	-
AC1	**S. RÉ**	-	1,0	-	-
K	**S. BORB**	-	0,75	-	-
T1	**S. BORB**	-	0,75	1,0	-
T2	**S. BORB**	-	0,75	2,0	-
T3	**S. BORB**	-	0,75	4,0	-
H1	**S. BORB**	-	0,75	-	1,0
H2	**S. BORB**	-	0,75	-	2,0

2.3. Physical conditions of micropropagation

Cultures were kept in air-conditioned room in the laboratory of the Department, 16/8 hours photoperiod (with 10 W/m^2 efficiency of illumination), at 20-25 ^oC temperature. Plants were grown in Erlenmeyer flask (which were covered with clear and air permeable plastic film), and the shelves of flasks were illuminated with type 'F30' fluorescent lamps from 40 cm distance. 2 shoots were placed in every flask.

2.4. Measured morphology features

50-60 (*in vitro* propagation) or 79-95 days (*in vitro* rooting - as the case of species and method of process) after transplanting the following features were examined or determined:

- the number of shoots (*in vitro* propagation and rooting)
- the length of shoots (mm; *in vitro* propagation and rooting)
- average length of leaves (mm; *in vitro* propagation and rooting)
- the number and length (mm) of roots (*in vitro* rooting)
- percentage of rooting (%, *in vitro* rooting)

2.5. Determining chlorophyll content

Examination processes were done in the Central Laboratory of Department of Food Chemistry and Nutrition (BCE). For examination 2 samples per treatment were used. Before investigating, leaf-samples were arranged (homogenization, filtrating, leaching with acetone, separating, repeated rinsing in water and filtrating), and after this, extractions had to be attenuated until the solution's absorbance reached optimum degree for spectrophotometric tests (at 660 nm). Measuring was done at wavelength 660 and 642,5 nm. Observed absorbance values (A_{660} és $A_{642,5}$) were suffected in formula of determining total chlorophyll content (mg/g fresh weight = 7,12 A_{660} + 16,8 $A_{642,5}$**)** (HELRICH, 1990).

2.6. Examination of peroxidase activity

Enzyme activities were measured in the laboratory of Department of Applied Chemistry, Faculty of Food Science. Before examinations homogenized tissue-extracts were prepared from in vitro leaves (3 samples/treatment were used). After centrifuging (4 ^oC, 20 minutes, 13500 rpm) carefully separated, clear extracts (without solid particles) were used for spectrophotometric investigations (type of spectrophotometer: Varian UV, adjusted wavelength: 440 nm). Either cuvette (blind) contained potassium phosphate buffer (0,1 M; pH=6), 0,2 ml H_2O_2 (0,015 M), 0,5 ml guaiacol (0,02 M) and 1,7 ml

distilled water (same solution was applied in case of another cuvette). Reaction was started with 0,2 ml plant-extract (which was added for the second cuvette); during this reaction peroxidase enzyme reduce hydrogen peroxide and rusty-colored tetraguaiacol evolved from guaiacol substrate. Reaction can be traceable by absorbance change at wavelength 440-470 nm. Enzyme activity (μkat/g fresh weight) was calculated with formula $[(\Delta A_{1min}/60) * \text{attenuation} * 4]/\varepsilon$ (CHANCE and MAEHLY, 1955; STEFANOVITSNÉ and HEGEDŰS, 2004).

2.7. Method of anatomical examinations

Some days after morphological measuring leaf-samples were arranged for anatomical investigation. Leaves from *in* (*vitro* propagated and rooted) and *ex vitro* plants were collected for anatomical comparison. Arranging of samples (clearing, slicing, fixating, dehydration, embedding, polymerisation, carving, preparing and painting of microscope slides), investigations (with TESLA BS-500 scanning electron microscope and LEITZ LABORLUX S light microscope), taking and choosing photos were done in the Central Laboratory of Department of Food Chemistry and Nutrition (BCE).

2.8. Statistical analysis

Datas were evaluated by one and two-way analysis of variance, Tukey-Kramer and Games Howell tests. Software of Ropstat (VARGHA 2002, 2007 and 2008) was used for statistical calculations.

3. RESULTS

3.1. Conformation of morphological features during *in vitro* propagation and rooting of *Sorbus* taxa

During *in vitro* propagation of *S. redliana* 'Burokvölgy' 0,75 mg/l BA resulted (considering every medium applied in this phase) the largest number (8,93) of shoots. In case of *S. borbasii* 'Herkulesfürdő the largest multiplication was observed when BA and M-TOP were combined (especially 0,5 mg/l BA + 0,5 mg/l M-TOP: 10,23 shoots). The least propagation was experienced in case of using KIN, Titavit. These accessories affected less than 2 shoots (1-1,67) in every case. Significant difference was not observed between these datas and control.

When compared with control, both sorbs have developed longer shoots on every medium. The highest concentrations of accessories resulted in decreased length of shoots almost in every cases. Distinct elongation was observed in case of applying BA, M-TOP (*S. redliana* 'Burokvölgy'), and – during *in vitro* propagation of both sorbs – BAR (which

resulted shoots longer than 30 mm, as good as every time). Mostly, positive coherence was experienced between the number and length of shoots.

The length of leaves was in inverse proportion to the number of shoots. KIN, Titavit (and in some measure M-TOP) resulted few shoots, but these accessories were optimal for developing longer leaves (longer than 10 mm). Both sorbs formed the largest leaves on medium with KIN (1 mg/l: 17,8 mm – S. redliana; 0,5 mg/l: 12,8 mm – S. borbasii). For developing the longest (usually longer than 12 mm) leaves, Titavit was better for *S. borbasii* 'Herkulesfürdő' (2 mg/l: 16,67 mm) and M-TOP (0,5 mg/l: 16,2 mm) was advantageous for *S. redliana* 'Burokvölgy'. The effects of variant type (but similar concentration) of accessories on morphological differences are nicely shown on **figure 1.** and **2**.

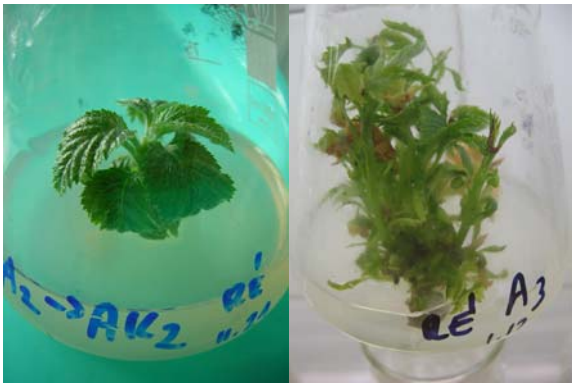

Figure 1: *Sorbus redliana* 'Burokvölgy' on medium with 0,75 mg/l KIN (left) or 0,75 mg/l BA (right)

Figure 2: *Sorbus borbasii* 'Herkulesfürdő' on medium with 0,5 mg/l KIN (left) or 0,5 mg/l BA (right)

During *in vitro* rooting, the highest percentage (79,31%) of *S. borbasii* 'Herkulesfürdő' rooting was achieved on 30 g/l saccharose supplemented control medium having neither Titavit nor HUMUS^{R} FW (**figure 3**). Increasing concentration of Titavit decreased the number (5,21-2,75) and percentage (53,84-40%) of rooting, while applying 1 and 2 ml/l HUMUS^{R} FW had significantly stimulated rooting (64,7 and 69,%) –

furthermore the longest (76,18 and 84,78 mm) roots were developed in this case. Ideal size of roots (45 and 45,5 mm) were observed on medium with 4 mg/l Titavit and control. Higher concentration of Titavit (as compared with control and especially HUMUS-applying) was increased the length of leaves (from 17,4 to 19,48 mm) – that was another advantage of this accessory. Few shoots (1,24-1,52) were developed on every rooting medium and that is understandable, because of ignoring citokinins.

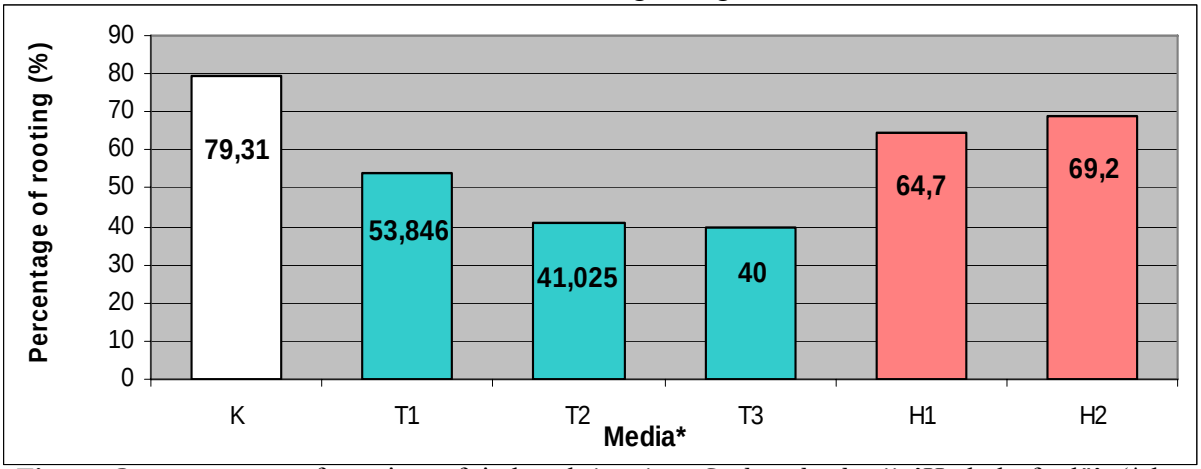

Figure 3: percentage of rooting of induced *in vitro Sorbus borbasii* 'Herkulesfürdő' (*the accessories of medium were shown in **table 2**)

Induction resulted better rooting in case of *Sorbus redliana* 'Burokvölgy' as well, but only if 0,75 (46,42%) and 1 g/l (31,7%) AC was added to the hormone-free medium. In that case the most number of roots (3,84 and 3,3) were developed on an average. Good results (33,33% and 2,77 root) were achieved on medium supplemented 1 g/l AC + 1,5 mg/l IBA (*in vitro* rooting without induction), but rooting was decreased on medium with only IBA (figure 4). Usually, every medium with IBA and/or AC resulted more than 80 mm roots – in proportion to the optimum length.

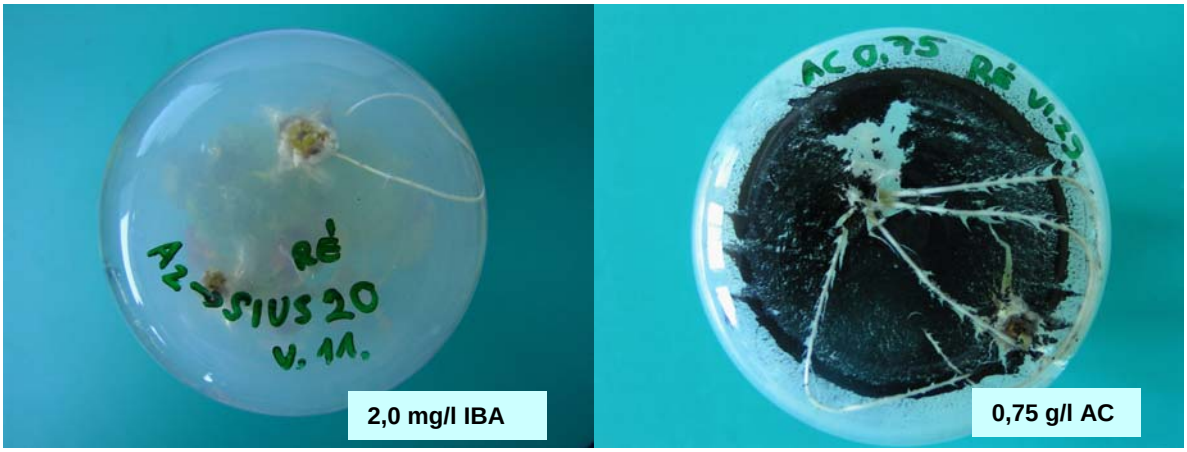

Figure 4: *In vitro* rooted (without induction – left) and induced (right) *Sorbus redliana* 'Burokvölgy'

In case of induced groups of *S. borbasii* (with almost equal rooting time) Titavit (and control) resulted significantly shorter roots. As another sorb, only few (1-1,167) shoots were observed on cytokinin-free rooting medium. At the same time induced plants have grown significantly higher and developed leaves more than 17 mm when 0,5 and 0,75 g/l AC was added to the medium. Medium containing 1 g/l AC + 1,5 mg/l IBA (without induction) resulted almost this length (16,6 mm), while in another treatments (applying only IBA) higher concentrations decreased the length of leaves.

3.2. Changes of chlorophyll content during *in vitro* propagation and rooting of *Sorbus* taxa

During *in vitro* propagation of sorbs, increasing concentration of cytokinins generally decreased chlorphyll content of leaves. The highest concentration of chlorophyll (1,872 mg/g) was detected in case of *in vitro* propagated *S. redliana* 'Burokvölgy' on medium with 10 g/l Titavit and the other concentrations resulted significantly higher values compared to control. This accessory (with 2 mg/l concentration) generated the highest average (1,684 mg/g) at *S. borbasii* 'Herkulesfürdő', too.

KIN (especially at higher concentrations) proved to be good for both sorb (*S. redliana*: 1 and 2 mg/l – 1,08 and 1,176 mg/g; *S. borbasii*: 0,75 and 2 mg/l – 1,397 and 1,339 mg/g). In case of multiplication of *S. redliana* M-TOP (0,25 mg/l) it resulted higher chlorophyll content (1,119 mg/g). There were further differences; whereas BAR resulted values closer to (or more than) control in case of *S. redliana*, similar results were achieved when BA was added to the medium for *in vitro* propagating of *S. borbasii*. In case of the latter sorb, BA + KIN, BA + M-TOP combinations increased chlorophyll content insignificantly in general – compared to medium with BA.

During non-induced rooting of *S. redliana* 'Burokvölgy', only 1 g/l AC + 1,5 mg/l IBA resulted higher chlorophyll content (0,961 mg/g) than that of the control. Furthermore, increasing the auxin concentration decreased values of chlorophyll when only IBA was added to the medium. On the other hand, higher averages were observed in case of induced shoots grown on hormone-free medium containing the higher concentration of AC (**figure 5**).

Both concentration of HUMUS^{R} FW resulted (as compared to applying Titavit) higher values (1,098 and 1,166 mg/g) in case of *in vitro* rooting of induced *S. borbasii* 'Herkulesfürdő' – significant difference was detected only if 2 ml/l HUMUS^{R} FW was

used. As for Titavit, only 2 mg/l resulted higher chlorophyll content (0,893 mg/g) than that of the control.

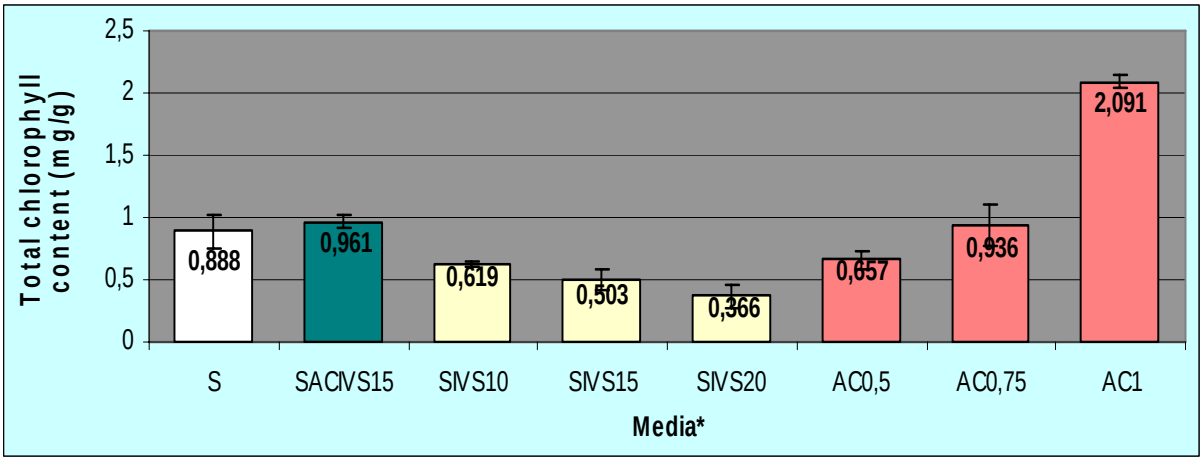

Figure 5: The effect of IBA or AC contained media on total chlorophyll content during *in vitro* rooting of *Sorbus redliana* 'Burokvölgy' (*the accessories of medium are shown in **table 2**)

3.3. Changes of peroxidase activity during *in vitro* propagation and rooting of *Sorbus* taxa

During *in vitro* propagation of sorbs peroxidase activity was the highest (more than 10 nkat/g) in case of using BA, BAR (moreover these accessories increased multiplication). BA + KIN, BA + M-TOP decreased activity (but only in case of *S. redliana* 'Burokvölgy'). In case of *in vitro* propagation of *S. borbasii* 'Herkulesfürdő' these cytokinin combinations (particularly BA + KIN) lead to higher enzyme activity and more shoot (similar to BA, BAR). As compared to control, Titavit resulted lower activity (except concentration 2 mg/l: 11,04 nkat/g), then again higher peroxidase activities (more than 20 nkat/g) were detected when this accessory was used (especially in dose 0,5-5 mg/l) in case of *S. redliana* 'Burokvölgy' - furthermore the highest chlorophyll contents of leaves were observed on these media.

In proportion to experiments of *in vitro* propagations, higher enzyme activity was noticed in case of *in vitro* rooting of both *Sorbus*. Media which were better for rate of rooting (*S. redliana*: without induction, 1 g/l AC + 1,5 mg/l IBA; after induction, 0,75 and 1 g/l AC; induced *S. borbasii*: HUMUS^{R} FW and control) resulted higher activities (more than 30 nkat/g, **figure 6**.). Pronounced positive coherence was not experienced between enzyme activity and number (and especially) the length of roots, principally in case of *S. borbasii* 'Herkulesfürdő'. During *in vitro* rooting of this sorb Titavit decreased peroxidase activity (as compared with control), but significantly less shoots developed roots on medium with this accessory.

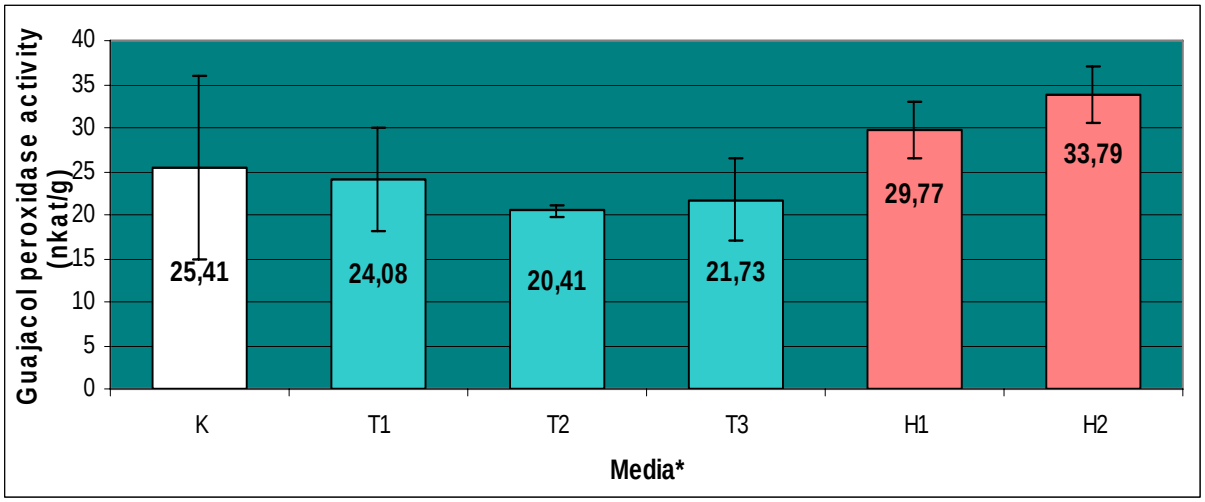

Figure 6: The effect of AC and Titavit or HUMUS^{R} FW contained media on guaiacol peroxidase activity during *in vitro* rooting of *Sorbus borbasii* 'Herkulesfürdő' (*the accessories of medium were shown in **table 2**)

3.4. Anatomical changes during *in vitro* propagation and rooting of *Sorbus* taxa

On medium control both sorbs developed leaves with compact mesophyll and with less differentiated tissue texture, invisible or quite narrow intercellular (palisade and spongy parenchyma was divided easily in case of S. *borbasii* 'Herkulesfürdő'). Chiefly KIN, M-TOP and Titavit resulted more trichome (small, hair-like appendage) especially on the lower surface of leaves, and the baldest leaves were found on medium containing BAR. There were less trichome on (*in* and *ex vitro*) leaves of *S. borbasii* in every cases.

In case of BAR and BA, more differentiated leaf tissue structure, but less number of chloroplast was observed than in the case of applying KIN, M-TOP and particularly Titavit. There was decide difference between palisade (what was consisted of narrower cells) and spongy parenchyma (which was composed of more roundish cells and segmented wider intercellulars). Additionally, M-TOP resulted more compact mesophyll (in both cases of the sorb), and well-developed, larger stomatas of *S. borbasii* were more or less closed.

In case of applying Titavit the tissue structure of *in vitro* leaves most of all neared to the characteristic of leaf anatomy of *ex vitro* (with high content of chloroplast, well-divided tissue parts, and more or less closed, long-shaped, less bulged stomatas). The only distinction between the sorbs were the concentrations: 0,5 mg/l (*S. redliana* 'Burokvölgy') and 10 mg/l (*S. borbasii* 'Herkulesfürdő') Titavit resulted anatomical features similar to *ex vitro* leaves.

During trials of *in vitro* rooting non-closed stomatas were found on every medium (in case of either sorb). Especially non-induced groups of *S. redliana* 'Burokvölgy' (which

were grown on medium with AC + IBA or only IBA) and developed conspicuously wide-opened stomatas which already deformed in case of the highest concentration of IBA. In case of this sorb increasing of IBA's concentration resulted wider intercellulars, less chloroplast and trichome; but after induction (using hormone-free media with AC) tissue structure was most of all similar to *ex vitro* (blurred contours of epidermis cells by reason of thicker cuticle, numerous chloroplast, less bulged stomatas and – in line with increasing AC-level – fewer trichome).

Distinctly sized stomatas were wide-opened especially in case of applying 2 mg/l Titavit (during *in vitro* rooting of *S. borbasii* 'Herkulesfürdő'). Form of epidermis cells and stomatas were rounded under treatment 4 mg/l Titavit; degree of hairy was the highest on medium containing 1 mg/l Titavit and trichomes were developed on the upper surface of leaves, too (by the way, *S. redliana* 'Burokvölgy' had more hairy leaves in every cases). Summarized, significant differences of anatomical features were detected between *in* and *ex vitro* leaves (and between the accessories of medium), in case of this two sorbs (**figure 7** and **8**).

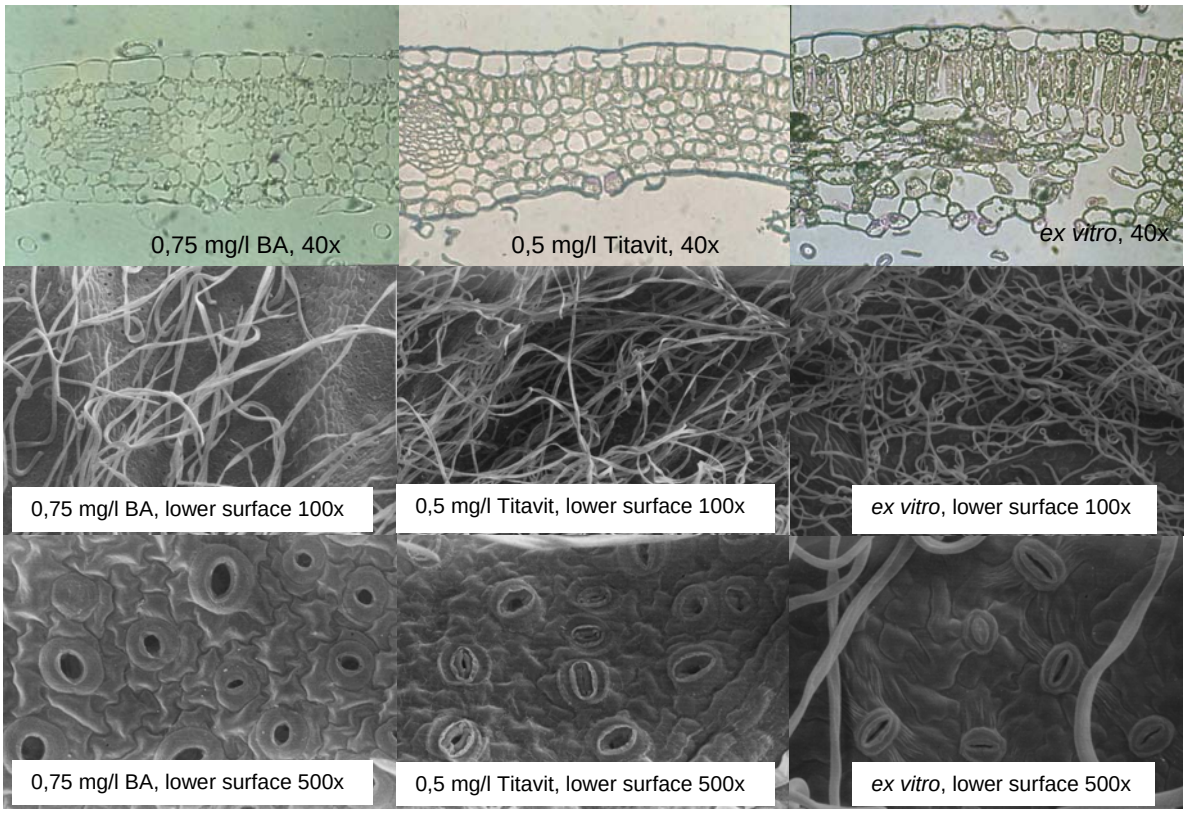

Figure 7: anatomical changes of *in vitro* (propagated in 0,75 g/l BA or KIN supplemented medium) and *ex vitro* leaves of *Sorbus redliana* 'Burokvölgy'

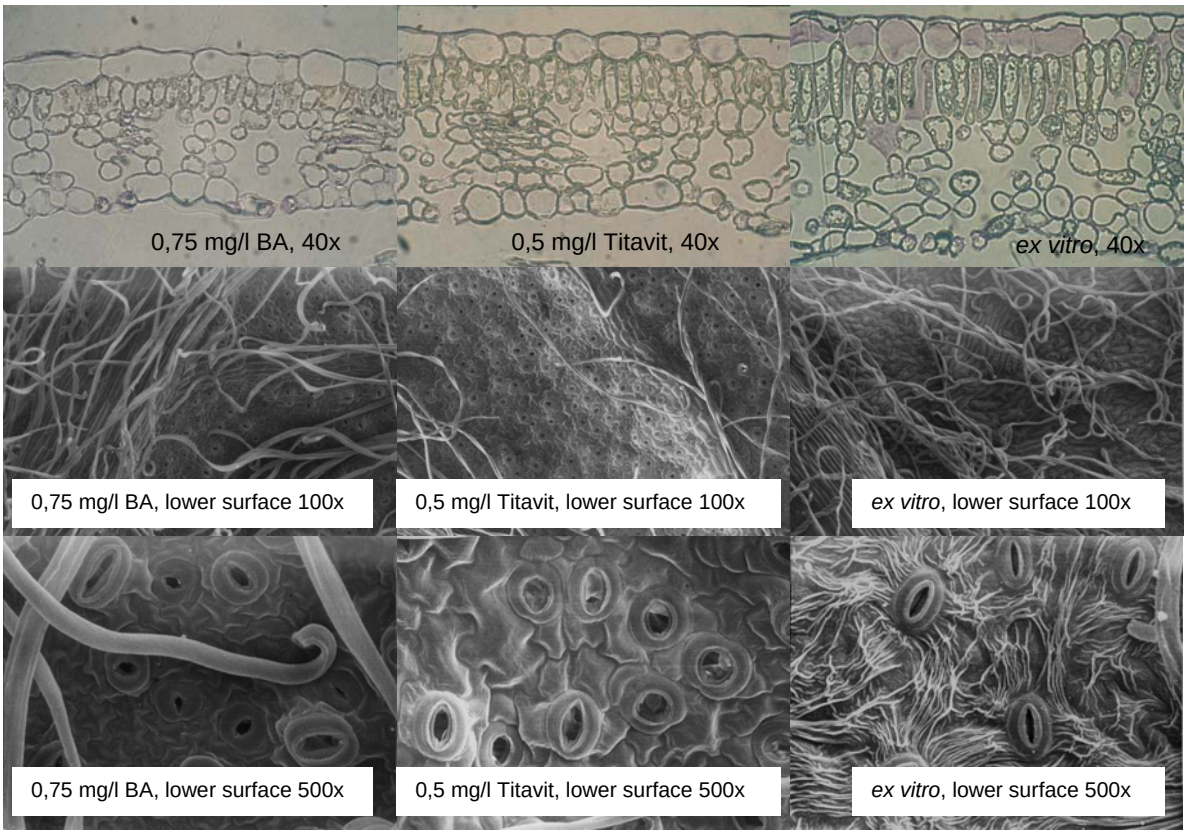

Figure 8: anatomical changes of *in vitro* (propagated in 0,75 g/l BA or KIN supplemented medium) and *ex vitro* leaves of *Sorbus borbasii* 'Herkulesfürdő'

3.5. New scientific results

1. During *in vitro* propagation of sorbs medium (with BA) increased multiplication resulted shorter leaves, but longer leaves were found on media with accessories (KIN, Titavit) resulted few shoots. Consequently, the length of leaves was generally in inverse proportion to the number of shoots.

2. Induction way of *in vitro* rooting resulted significantly more roots in case of *Sorbus redliana* 'Burokvölgy'.

3. Distinct anatomical differences were shown between *in vitro* (propagated and rooted) and *ex vitro Sorbus* plants' leaves. Various type and concentration of medium accessories resulted similar differences.

4. In case of *in vitro* propagation of either sorbs on medium with Titavit, tissue structure of *in vitro* leaves most of all neared the characteristic of leaf anatomy as of *ex vitro.* Similar experiments were observed during in vitro rooting of induced *S. redliana* 'Burokvölgy'.

5. During multiplication there was negative coherence between chlorophyll content of leaves and number of shoots. On the other hand, during *in vitro* rooting chlorophyll content was directly proportional to the rate of rooting.

6. Higher enzyme activity was obtained on the medium which mostly stimulated rooting, namely there was positive coherence between percentage of rooting and degree of enzyme activity.

4. CONCLUSIONS, RECOMMENDATIONS

Media containing BA was the best for increasing number of shoots (in either sorbs) but in that cases lower quality (shorter leaves with fewer chloroplast and lower concentration of chlorophyll) could be improved by another type cytokinin (KIN, M-TOP) or especially Titavit. Titavit was not enough for optimal multiplication, so a combination with cytokinins is recommendable – in order to obtain higher number of shoot.

In case of both sorbs, induction was optimal for *in vitro* rooting (especially for the number of roots) and good results of percentage of rooting and leaves' chlorophyll content were achieved on medium supplemented with AC – directly proportional to the concentration.

Non-induced *S. redliana* 'Burokvölgy' shoots were rooted well on medium with 1 g/l AC + 1,5 mg/l IBA. Irrespectively of this, applying of AC is recommended during in vitro rooting after induction (optimum quantity: 0,75 g/l). Furthermore, increasing concentration of AC (from 0,5 to 0,75 and 1 g/l) enhanced chlorophyll content of leaves.

Az akklimatizálás szempontjából döntő fontosságú a növények kondíciója, illetve megfelelő habitusa. A *S. borbasii* 'Herkulesfürdő' gyökeresítése során a HUMUS^{R} FW kiegészítés ugyan magasabb klorofill tartalmat és nagyobb gyökeresedési arányt eredményezett, de a gyökerek hosszát a Titavit csökkentette az akklimatizáláshoz optimálisan, és a levelek hosszát is növelte. A rövidebb, sűrűbb gyökérzet ugyanis az akklimatizáláskor elvégzett műveletek során fellépő (és elkerülhetetlen) kisebb-nagyobb sérülések dacára nagyobb garanciát jelenthet a növények sikeresebb túléléséhez. Mindent összevetve, a két kiegészítő anyag együttes alkalmazását javaslom, a kísérletek során kapott különféle (sarj- és gyökérfejlődési) jellemzők értékeinek figyelembe vételével 1,0 mg/l (Titavit), illetve 1,0 ml/l (HUMUS^{R} FW) koncentrációkban.

State of health and good habit is very important when plants get into acclimatization. During in vitro rooting of *S. borbasii* 'Herkulesfürdő' HUMUS^{R} FW resulted higher chlorophyll content and percentage of rooting, but the length of roots was

advantageously shorter (and the leaves was longer) in case of applying Titavit – these morphological features were better for acclimatization. Shorter and denser rootage – in spite of injuries of acclimatizing – will guarantee higher rate of survival during this critical phase. Taken it all round, in virtue of values of multiplication and rooting, combination of these two accessory is recommended (1,0 mg/l Tivatit + 1,0 mg/l HUMUS^{R} FW).

5. PUBLICATIONS IN RELATION TO THE PhD THESIS

Publications with IF:

Ördögh, M., Jámbor-Benczúr, E., Tilly-Mándy, A., Lelik, L. 2009. Effects of different cytokinins on proliferation of *Sorbus borbasii* 'Herkulesfürdő'. Propagation of Ornamental Plants 9 (1): 43-46. (IF 0,333)

Publications without IF:

Ördögh, M., Jámbor-Benczúr, E., Tilly-Mándy, A., Lelik, L. 2006. The effects of growth regulators in proliferation of *Sorbus redliana* 'Burokvölgy'. International Journal of Horticultural Science 12 (1): 77-83.

Ördögh M., Jámborné Benczúr E., Tillyné Mándy A., Lelik L. 2006. Különféle citokininek hatásainak vizsgálata *Sorbus redliana* 'Burokvölgy' *in vitro* tenyészetében. Kertgazdaság 38. (1): 56-60.

Conference papers (Hungarian, abstracts)

Ördögh M., Jámborné Benczúr E. 2005. Meta-topolin (M-TOP), kinetin (KIN) és benziladenin (BA) hatása mikroszaporított *Sorbus redliana* 'Burokvölgy' hajtássokszorozódására. Erdei Ferenc Tudományos Konferencia (Kecskemét, 2005. aug. 23-24), II. kötet 659-663.

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Ördögh M., Jámborné Benczúr E., Tillyné Mándy A., Lelik L. 2006. Különféle citokininek hatása egy hazai berkenyefajta, a *Sorbus redliana* 'Burokvölgy' *in vitro* szaporítására. XII. Növénynemesítési Tudományos Napok (Budapest, MTA, 2006. márc. 7-8), Összefoglalók, 45.

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Ördögh M., Jámborné Benczúr E., Tillyné Mándy A., Lelik L. 2007. Különféle növekedésszabályozók hatása a *Sorbus borbásii* 'Herkulesfürdő' *in vitro* tenyészeteiben. XIII. Növénynemesítési Tudományos Napok (Budapest, MTA, 2006. márc. 12), Összefoglalók, 111.

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