International Journal of Horticultural Science 2006, 12 (1): 77–83
Agroinform Publishing House, Budapest, Printed in Hungary
ISSN 1585-0404

The effects of growth regulators in proliferation of *Sorbus redliana* 'Burokvölgy'

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Summary: The Hungarian cultivar Sorbus redliana 'Burokvölgy' was proliferated on Murashige and Skoog (MS, 1962) medium with half-strength macroelements and 100 mg/l meso-inositol, 20 g/l sucrose, 11 g/l agar-agar. Different combinations of kinetin (KIN), metatopolin (mT), benzyladenine (BA), benzyladenine-ribosid (BAR) and indolebutiric acid (IBA) were tested, and pH was adjusted to 5.6 every case using KOH. The cultures were incubated at 20–24 °C in 8/16 hours dark/light photoperiod for 50–52 days. The main aim of our research was to find the optimal growth regulator and its optimum concentration. Furthermore, to determine the chlorophyll contents of the in vitro propagated plants' leaves. During the proliferation, the highest number of shoots were observed in the case of using BA + IBA, and on the medium containing 0.75 mg/l BA + 0.05 mg/l IBA 8.93 shoots were found. The addition of KIN + IBA decreased the number of shoots and increased the sizes of leaves – the widest (11.2 mm) and longest (17.8 mm) leaves were obtained on the medium containing 1.00 mg/l KIN + 0.05 mg/l IBA. The longest shoots (36.46 mm) were found in the case of applying 0.75 mg/l BAR + 0.05 mg/l IBA. The BA + KIN + IBA combination resulted the shortest shoots. Sometimes not only shoot regeneration but spontaneous rooting was observed during the multiplication. The highest chlorophyll content (1.569 mg/g total chlorophyll, 1.132 mg/g chlorophyll-a, 0.437 mg/g chlorophyll-b) was obtained in the presence of 1.0 mg/l KIN + 0.05 mg/l IBA.

Key words: *Sorbus,* proliferation, growth regulator, KIN, mT, BA, BAR, IBA, chlorophyll content

Introduction

There are various microspecies of *Sorbus* in Hungary. One of them, the Hungarian cultivar *Sorbus redliana* 'Burokvölgy' is a drought tolerant, decorative small tree with bright, dark green leaves (which alter yellow in autumn), white flowers and reddish crops (*Figure 1–2*). This plant belongs to the *Section of Torminaria* (*Sorbus torminalis* and their hybrids) and it is suitable for garden or street planting (*Schmidt & Tóth*, 2005; *Jámbor-Benczúr & Sinkó*, 2005). *Sorbus*es are traditionally propagated by seed, but this method often eventuated wide variability of the progeny. Moreover it is difficult to find the suitable rootstock (*Piagnani & Bassi*, 2000; *Schmidt & Tóth*, 2005).

Fig. 1. *Sorbus redliana* 'Burokvölgy'

Fig. 2. Mature crops of *Sorbus redliana* 'Burokvölgy'

In vitro propagation of *Tilia cordata*, *Robinia pseudoacacia* and *Sorbus aucuparia* was achieved by organ cultures grown on MS medium with cytokinin and auxin. Shoot proliferation was observed by the addition of BA and/or thidiazuron. Shoot formation was stimulated on media containing 0.2–1.0 mg/l BA. Thidiazuron was active at low concentrations (0.005-0.05 mg/l). Rooting of shoots was induced on media with low concentrations of mineral nutrients and IBA, NAA (*Chalupa*, 1988).

Apical and lateral buds of 6 cultivated forms of interspecific or intergeneric origin of *Sorbus* were cultured on MS medium at 18-25 °C in a 16 h photoperiod (2500–5000 lx). The best-growing primary cultures were transferred to medium containing BA and KIN in which the shoots produced 3–5 to 20–25 offshoots. Optimal rooting was facilitated by treatment with IAA and IBA at 1 mg/l (*Suvorova* et al., 1990).

Shoot multiplication of *Sorbus domestica* was searched by placing shoot apices or nodal segments on a modified *Schenck & Hildebrandt* medium (1972) with BA. Regenerated shoots were excised and induced to root on media with auxins. In the best treatments (5.2–26 µM IBA or NAA) 75–85% of shoots from juvenile material rooted. Rooting capacity of shoots from mature explants was lower and was not improved by dipping the base of shoots in concentrated solutions of IBA or NAA (*Arrilaga* et al., 1991).

Arrilaga et al. (1992) described a rapid method to break seed dormancy of *Fraxinus ornus* and *Sorbus domestica* by using embryo culture techniques. In this trial, germinating seeds or embryos were transferred to glass tubes containing *Murashige & Skoog* (MS, 1962) medium with 3% sucrose and 0.7% agar (pH 5.8). Germination of seeds of these 2 species was improved mostly by isolation and in vitro culture of embryos. Effects of growth regulators (NAA, IAA, BA) and NO_3:NH_4 ratio were investigated to determine the morphogenetic capacity of hypocotyl explants of *Sorbus domestica* (*Arrilaga* et al., 1992). Further experiments were carried out in this year. NAA induced root differentiation whereas IAA added to MS media with BA promoted adventitious shoot regeneration. Best results were obtained with 2,5 µM each of BA and IAA (19% caulogenic explants). This response was increased (up to 44%) by varying nitrate to ammonium ratio from 2:1 to 4:1.

During micropropagation of *Sorbus rotundifolia*, the best results were obtained on half-strength MS medium supplemented with 0.75 mg/l BA, 0.1 mg/l IBA, 0.1 mg/l GA_3 and 20 g/l sucrose (*Molnár* et al., 1994).

In another work, *Arrilaga* et al. (1995) examined callus and somatic embryogenesis from cultured anthers of *Sorbus domestica*. For the experiments including a cold pre-treatment, detached flower buds were kept in darkness at 4–6 °C for 1 week. After the sterilisation, anthers from several buds were dissected, filaments removed and placed onto the MS medium with 3% sucrose, 0.7% Difco Bacto-agar and growth regulators (2,4-D, IBA, NAA, IAA, BA) in different concentrations. Callus proliferation required the presence of growth regulators in the medium, with the best results obtained with combinations of auxins and BA. Sometimes direct embryogenesis was recorded.

Mándy et al. (1997) examined *in vitro* proliferation of *Sorbus degenii* 'Csákvár'. The number of shoots was the greatest when 0.5 mg/l thidiazuron was added to half-strength MS medium, but the shoots were not elongated and some of them vitrified. With the use of BAR (BA-ribosid) almost no vitrification occurred and the shoot proliferation rate exceeded 4 shoots per inoculum. Vitrification was also decreased by 2 mg/l $AgNO_3$ or cooling the medium to 15 °C.

Shoots of *Sorbus rotundifolia* 'Bükk Szépe' were cultured on 1/2 MS medium containing 0.75 mg/l BA, 0.1 mg/l IBA and GA_3, together with 5–30 g/l sucrose or glucose. The best result was obtained on the medium with 15 g/l glucose. With sucrose, the highest chlorophyll contents were 1.8–2.1 mg/g fresh weight (the chlorophyll content was estimated in 80% acetone extract). The increase of sugar concentration resulted decreases of chlorophyll contents of cultures (*Jámbor-Benczúr* et al., 1997).

The *in vitro* propagation of *Sorbus redliana* 'Burokvölgy' was started from buds in early spring. For studying the effect of growth regulators BA + IBA, BAR and 2 iP were added in concentrations of 0.25, 0.5, 0.75, 1.0 mg/l. As carbon source 20 g/l sucrose was used. Beside, to find the best carbon source glucose, sucrose and fructose (5–30 g/l) was added to the MS medium with 0.25 mg/l BA + 0.1 mg/l IBA. For proliferation the best result (3.2 shoots) was found on MS medium with 0.25 mg/l BA + 0.05 mg/l IBA. The same result (3.3 shoots) was achieved on S medium (which consists of BM – *Jámbor & Márta*, 1990 – macroelements and *Heller*, 1953 microelements) with the use of 0.5 mg/l BAR. Comparing the influence of carbon sources, much better results were obtained by glucose and sucrose (15–20 g/l) than fructose. The best proliferation (32.6 shoots) was found with using 15 g/l glucose and 30.5 shoots observed on medium with 20 g/l sucrose (*Jámbor-Benczúr* et al., 1998).

The effect of different concentrations of glucose, sucrose, triaconthanol were studied under the unfavorable stress conditions of micropropagated *Sorbus rotundifolia* 'Bükk Szépe' and *Prunus x davidiopersica* 'Piroska'. As shown by the growth parameters (the number and length of shoots), the optimal carbohydrate concentration was 1.5–2.5%, whereas at higher concentrations there was a definite inhibition. A similar response was found in changes in the anthocyanin content in *P. x d.* 'Piroska', but this effect was less pronounced with the photosynthetic pigments in both species. In all cases, the addition of 2–4 µg/l triacontanol further enhanced the stimulating effect of the optimal carbohydrate concentration (*Kissimon* et al., 1999).

Explants from lower branches and from epicormic shoots of mature trees (*S. aucuparia*) exhibited high multiplication coefficients of michroshoots cultured on modified MS agar nutrient medium supplemented with cytokinin (BA, TDZ) and auxin (IBA). Microshoots produced from juvenile parts of mature strees exhibited good rooting response and the produced plants were well adapted to grow in forest soils (*Chalupa*, 2002).

In Hungary, metatopolin (mT) was used during shoot regeneration from *in vitro* apple leaves (*Dobránszki* et al., 2002; 2005).

The main aim of our research was to find the optimal growth regulator and its optimum concentration. Furthermore, to determine the chlorophyll contents of the in vitro propagated plants' leaves.

Material and method

The experiments were carried out in the laboratory of the Department of Floriculture and Dendrology, Corvinus University Budapest.

During the proliferation of *Sorbus redliana* 'Burokvölgy', Murashige & Skoog (MS, 1962) medium at half-strength macroelements was used. For examining the effect of growth regulator 0.50-2.00 mg/l KIN + 0.05 mg/l IBA; 0.50–1.00 mg/l mT + 0.05 mg/l IBA; 0.25–1.00 mg/l BA + 0.05 mg/l IBA; 0.25–1.00 mg/l BAR + 0.05 mg/l IBA, and a combinations of 0.25–1.00 mg/l BA + 0.50 mg/l KIN + 0.05 mg/l IBA were added. Beside, 100 mg/l meso-inositol, 20 g/l sucrose and 11 g/l agar-agar were used in every case. The pH was adjusted to 5.6 in every case using KOH. The media used are summarized in *Table 1*.

The shoots were placed in Erlenmeyer flasks (2–3 shoot/test-tube) and the cultures were incubated at 20–24 °C in 8/16 hours dark/light photoperiod for 50–52 days (*Figure 3–8*). At the end of the proliferation period, the number of shoots was counted, the length (mm) of shoots, the length and width (mm) of leaves was measured. Data were evaluated by two-sample analyis (t- and F-test).

For studying the chlorophyll contents (total chlorophyll, chlorophyll-a and chlorophyll-b; mg/g), extraction of chlorophyll from fresh leaves was used. The chlorophyll contents was determined by spectrophotometric absorbancy reading at *A660 nm* and *A642.5 nm* (*Helrich*, 1990).

Table 1. Additives of the *Murashige & Skoog* (MS, 1962) medium for proliferation of *Sorbus redliana* 'Burokvölgy'

medium	Accessories of the Murashige and Skoog (MS, 1962) medium				
	KIN (mg/l)	mT (mg/l)	BA (mg/l)	BAR (mg/l)	IBA (mg/l)
AK1	0.50	–	–	–	0.05
AK2	0.75	–	–	–	0.05
AK3	1.00	–	–	–	0.05
AK4	2.00	–	–	–	0.05
AT1	–	0.50	–	–	0.05
AT2	–	0.75	–	–	0.05
AT3	–	1.00	–	–	0.05
A1	–	–	0.25	–	0.05
A2	–	–	0.50	–	0.05
A3	–	–	0.75	–	0.05
A4	–	–	1.00	–	0.05
R1	–	–	–	0.25	0.05
R2	–	–	–	0.50	0.05
R3	–	–	–	0.75	0.05
R4	–	–	–	1.00	0.05
BAK1	0.50	–	0.25	–	0.05
BAK2	0.50	–	0.50	–	0.05
BAK3	0.50	–	0.75	–	0.05
BAK4	0.50	–	1.00	–	0.05

Fig. 3. *Sorbus redliana* 'Burokvölgy' on MS medium with 0.75 mg/l mT + 0.05 mg/l IBA

Fig. 4. *Sorbus redliana* 'Burokvölgy' on MS medium with 0.75 mg/l KIN + 0.05 mg/l IBA

Results and discussion

The effects of KIN + IBA on the proliferation of Sorbus redliana 'Burokvölgy'

During the proliferation, MS media with KIN + IBA effected the fewest shoots (1.13–1.33 – *Fig 9*), but on the other hand, fairly large (8.73–11.2 mm width and 14.27–17.8 mm length) leaves were obtained in this case. The longest (17.8 mm) and widest (11.2 mm) leaves were found in the case of using 1.00 mg/l KIN + 0.05 mg/l IBA (*Figure 10–11*). Furthermore, the highest chlorophyll content (1.569 mg/g total chlorophyll, 1.132 mg/g chlorophyll-a, 0.437 mg/g chlorophyll-b) was achieved in this case (chlorophyll contents are summarized in *Table 2*).

Table 2. Chlorophyll contents of leaves of *in vitro* propagated *Sorbus redliana* 'Burokvölgy'

medium	total chlorophyll (mg/g)	chlorophyll-a (mg/g)	chlorophyll-b (mg/g)
AK1	0.598	0.433	0.166
AK2	0.883	0.635	0.248
AK3	1.569	1.132	0.437
AK4	1.386	1.013	0.374
AT1	0.271	0.188	0.082
AT2	0.396	0.265	0.132
AT3	0.146	0.095	0.052
A1	0.628	0.448	0.180
A2	0.311	0.223	0.088
A3	0.333	0.239	0.095
A4	0.403	0.285	0.118
R1	0.569	0.463	0.106
R2	0.681	0.503	0.178
R3	0.880	0.635	0.246
R4	0.517	0.399	0.118
BAK1	1.038	0.768	0.272
BAK2	0.501	0.353	0.148
BAK3	0.655	0.470	0.185
BAK4	0.762	0.572	0.190

The effects of mT + IBA on the proliferation of Sorbus redliana 'Burokvölgy'

Significantly more shoots (2.47–3.2) were observed on the MS media containing mT + IBA (*Figure 9*); using 0.75 mg/l mT + 0.05 mg/l IBA gave the best proliferation with 3.2 shoots. Additionally, every concentration of mT + IBA resulted much longer shoots (33.07–35.47mm), and the longest ones (35.47 mm) were found on MS medium with 1.00 mg/l mT + 0.05 mg/l IBA (*Fig 12*). Furthermore, the least chlorophyll values were obtained in the case of using 0.50 mg/l mT + 0.05 mg/l IBA (0.271 mg/g total chlorophyll, 0.188 mg/g chlorophyll-a, 0.082 mg/g chlorophyll-b) and 1.00 mg/l mT + 0.05 mg/l IBA (0.146 mg/g total chlorophyll, 0.095 mg/g chlorophyll-a, 0.052 mg/g chlorophyll-b). Besides the low chlorophyll contents, the sizes of leaves were considerably great (9.07–11.07 mm width and 12.53–16.2 mm length). The lowest concentration (0.50 mg/l mT + 0.05 mg/l IBA) eventuated the widest (11.07 mm) and longest (16.2 mm) leaves (*Fig 10–11*).

Fig. 5. Sorbus redliana 'Burokvölgy on MS medium with 0.75 mg/l BAR + 0.50 mg/l IBA

Fig. 6. Sorbus redliana 'Burokvölgy' on MS medium with 0.75 mg/l BA + 0.05 mg/l IBA + 0.5 mg/l KIN

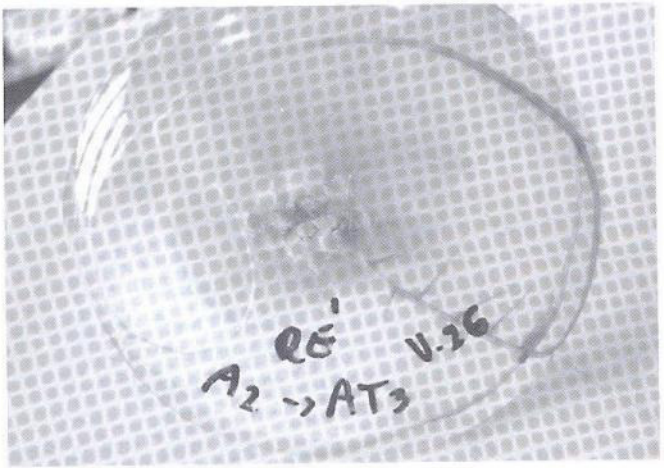

Fig. 7. Sorbus redliana 'Burokvölgy' on MS medium with 1.00 mg/l mT + 0.05 mg/l IBA (spontaneous rooting)

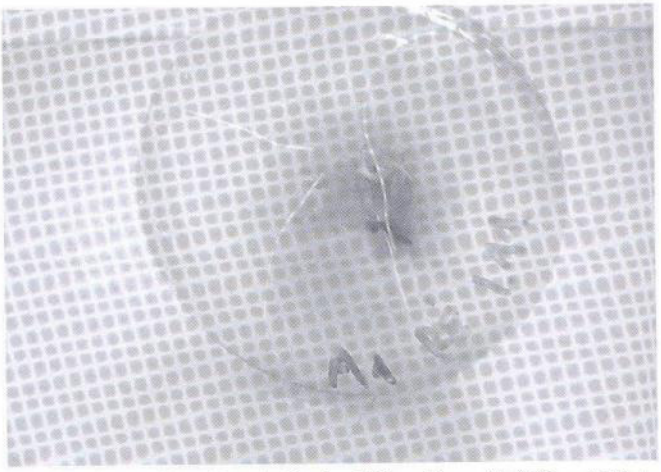

Fig. 8. Sorbus redliana 'Burokvölgy' on MS medium with 0.25 mg/l BA + 0.05 mg/l IBA (spontaneous rooting)

The effects of BA + IBA on the proliferation of Sorbus redliana 'Burokvölgy'

The outstanding proliferation was observed on MS medium containing BA + IBA (4.8-8.93 shoots); 0.75 mg/l BA + 0.05 mg/l IBA resulted the highest number of shoots (8.93 – *Figure 9*), whereas in another trial (*Jámbor-Benczúr et al.*,1998) 0.25 mg/l BA + 0.05 mg/l IBA effected the greatest number of shoots (3.2). Furthermore, BA + IBA eventuated significantly smaller (3.6–5.8 mm width and 6.93–10.73 mm length) leaves. The widest (5.8 mm) and longest (10.73 mm) leaves were found in the case of using 0.50 mg/l BA + 0.05 mg/l IBA (*Fig 10–11*). The lowest concentration (0.25 mg/l BA + 0.05 mg/l IBA) effected the highest chlorophyll concentration (0.628 mg/g total chlorophyll, 0.448 mg/g chlorophyll-a, 0.180 mg/g chlorophyll-b).

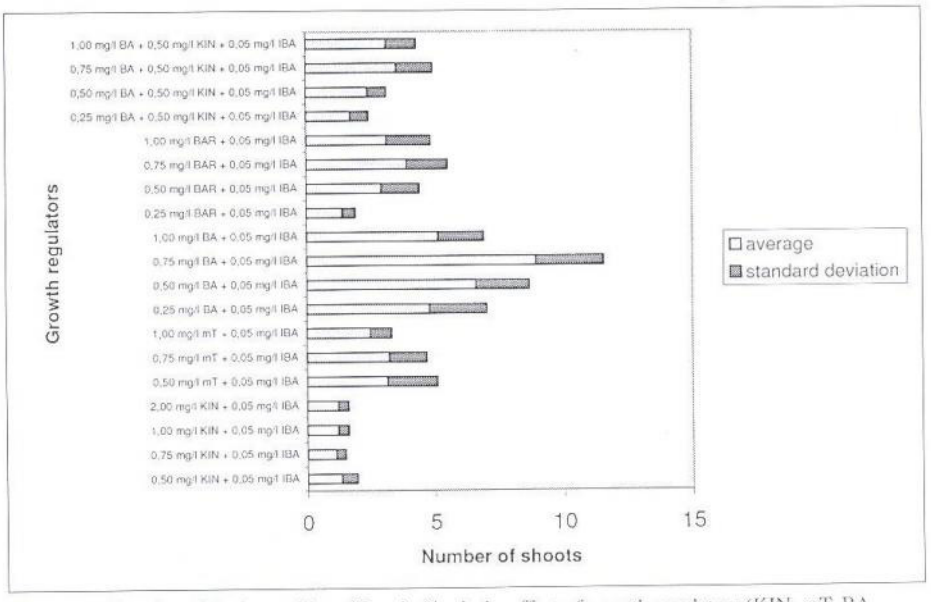

Fig. 9. Proliferation of *Sorbus redliana* 'Burokvölgy': the effect of growth regulators (KIN, mT, BA, BAR, IBA) on the number of shoots

The effects of BAR + IBA on the proliferation of Sorbus redliana 'Burokvölgy'

BAR + IBA resulted significantly less shoots (1.4–3.93) than BA + IBA; the best proliferation (3.93 shoots) was observed in the case of using 0.75 mg/l BAR + 0.05 mg/l IBA (*Fig 9*). In another research, similar result (3.3 shoots) was achieved on the S medium combined with 0.50 mg/l BAR (*Jámbor-Benczúr et al.*,1998). The sizes of leaves were rather small (5.26-6.26 mm width and 7.86-9.13 mm length); the widest (6.26 mm) and longest (9.13 mm) leaves were found in the case of using 0.25 mg/l BAR + 0.05 mg/l IBA (*Fig 10-11*). Using 0.75 mg/l BAR + 0.05 mg/l IBA eventuated the longest shoots (36.46 mm – *Fig 12*) and the highest chlorophyll contents were observed in this case (0.880 mg/g total chlorophyll, 0.635 mg/g chlorophyll-a, 0.246 mg/g chlorophyll-b).

The effects of BA + KIN + IBA on the proliferation of Sorbus redliana 'Burokvölgy'

The shortest shoots (19.86–24.13 mm) were obtained on MS medium with BA + KIN + IBA (*Figure 12*). Additionally, this combination resulted only 1.73–3.53 shoots (0.75 mg/l BA + 0.50 mg/l KIN + 0.05 mg/l IBA effected the best proliferation with 3.53 shoots) and significantly smaller leaves (5.06-6.8 mm width and 7.6–9.53 mm length) than using KIN + IBA (*Fig 9–11*). The widest (6.8 mm) and longest (9.53 mm) leaves were found in the case of using 0.50 mg/l BA + 0.50 mg/l KIN + 0.05 mg/l IBA. The highest chlorophyll contents were obtained in case of using 0.25 mg/l BA + 0.50 mg/l KIN + 0.05 mg/l IBA (1.038 mg/l total chlorophyll, 0.768 mg/l chlorophyll-a, 0.272 mg/l chlorophyll-b).

Summarizing, significant difference was observed among the plants growing on different media containing diverse concentration of growth regulators. The highest number of shoots (8.93) were found on medium

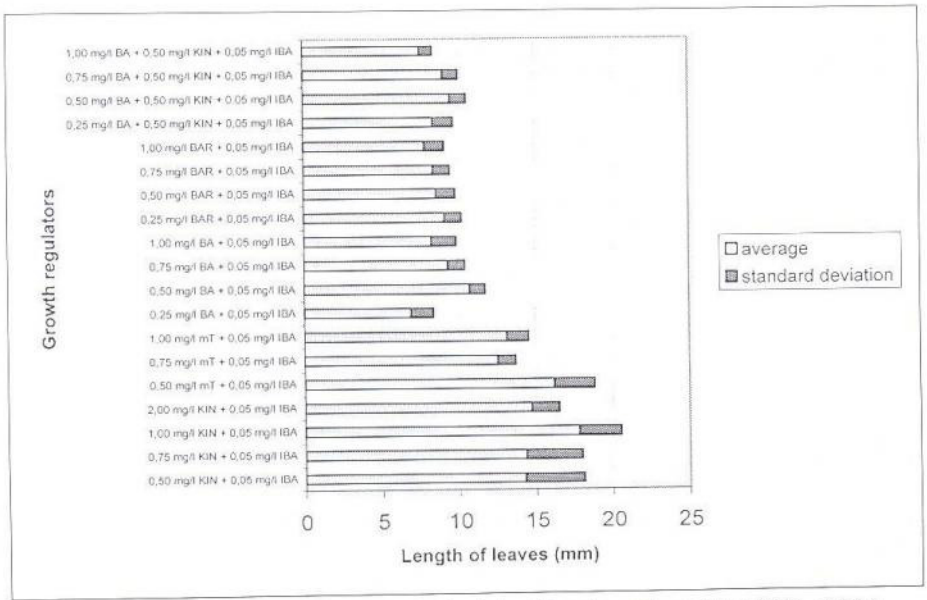

Fig. 10. Proliferation of *Sorbus redliana* 'Burokvölgy': the effect of growth regulators (KIN, mT, BA, BAR, IBA) on the length of leaves (mm)

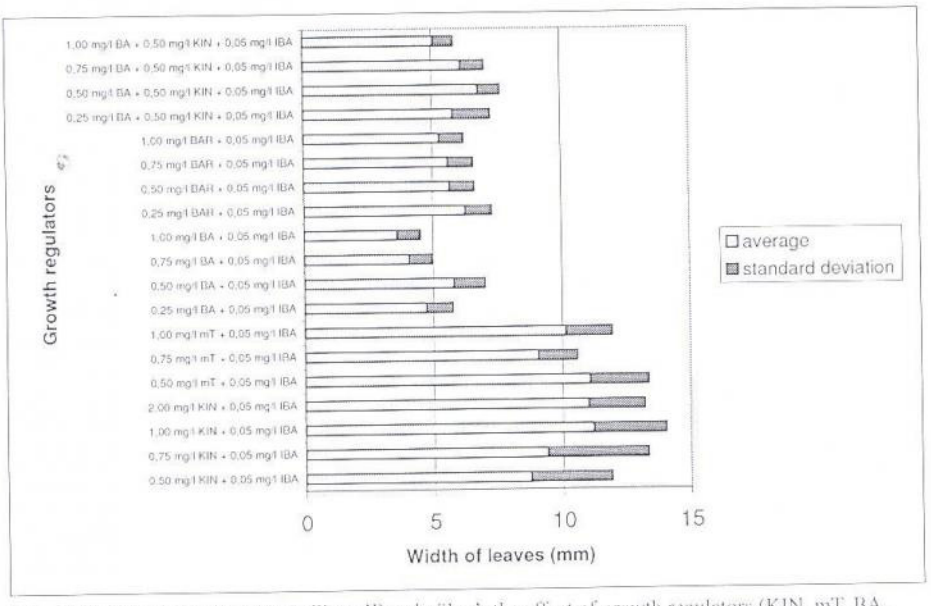

Fig. 11. Proliferation of *Sorbus redliana* 'Burokvölgy': the effect of growth regulators (KIN, mT, BA, BAR, IBA) on the width of leaves (mm)

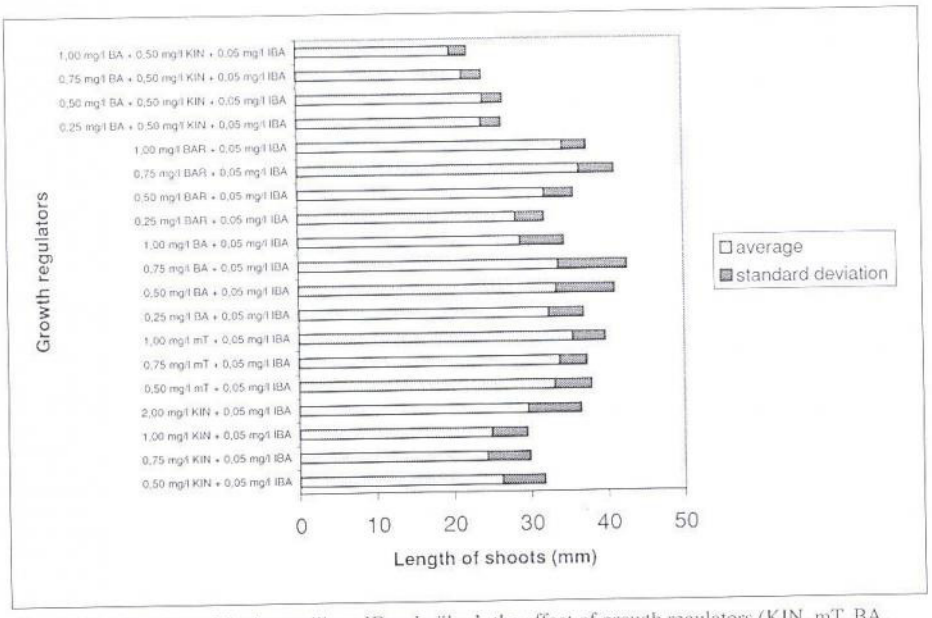

Fig. 12. Proliferation of *Sorbus redliana* 'Burokvölgy': the effect of growth regulators (KIN, mT, BA, BAR, IBA) on the length of shoots (mm)

with 0.75 mg/l BA + 0.05 mg/l IBA; and the largest leaves (17.8 mm length and 11.2 mm width) were obtained in the case of using 1.00 mg/l KIN + 0.05 mg/l IBA. Using 0.75 mg/l BAR + 0.05 mg/l IBA eventuated the longest shoots (36.46 mm). The chlorophyll content was the highest with the use of KIN and the lowest in the case of mT.

Acknowledgements

This work was supported by OTKA (Hungarian Scientific Research Fund), project No. TO49642.

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