Effects of tuberization conditions on the microtuber yield and on the proportion of microtuber tissues

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Summary: The production facilities of large-sized microtubers in three potato varieties (cv. *Desiree, Boró, Gülbaba*) and the effects of the applied tuberization conditions on the proportion of microtuber tissues, especially on the perimedullary region were investigated in present work. *In vitro* tuberization was induced on explants with 2 or 5 nodes layered on MS medium supplemented with 8% sucrose. Induced cultures were exposed to short days (8 h) for 2 weeks, then to total darkness for further 11 weeks. For volume calculations of different tissue regions, the formula for ellipsoids ($V=4/3\pi 1/8lw^2$) was used. The number of large-size tubers (> 8 mm, up to 16 mm) reached 53%, 59% and 44% in cvs. *Desiree, Gülbaba* and *Boró*, respectively, which indicate that the size of microtubers could be increased by appropriate sucrose support and explant type. Microtubers produced on hormone-free medium have well-developed perimedullary region, and its volume rate seemed to be important in the final size of tubers. The increase in the rate of volume of the perimedulla was connected to the increase of tuber size until tubers reached 12 mm diameter. In microtubers larger than 12 mm in diameter, the volume rate of the pith was increased.

Key words: potato, *in vitro*, tuber volume, tuber size, perimedulla

Introduction

The use of microtubers, as final products of potato micropropagation, has several advantages both in the storage of germplasm and in seed-potato production *(Hussey & Stacy,* 1981, *Tovar* et al., 1985, *Seabrook* et al., 1993, *Ranalli* et al., 1994) because microtubers can be stored longer, handled and transported easier than plantlets *(Struik and Lommen,* 1991).

The economical use of microtubers is mainly depends on their size because larger microtubers have greater early vigour, emergence and performance and they are able to produce larger crop than small ones *(Wiersema* et al., 1987, *Ranalli* et al., 1994, *Tábori* et al., 1999). The size of microtubers can be increased by applying an adequate photoperiod regime *(Seabrook* et al., 1993, *Dobránszki,* 1996), culture density *(Forti* et al., 1991, *Tábori* et al., 2000) type of explants *(Nowak & Colborne,* 1989, *Leclerc* et al., 1994) or proper nitrogen and sucrose concentrations in the medium *(Stallknecht & Farnsworth,* 1982, *Garner & Blake,* 1989, *Slimmon* et al., 1989, *Perl* et al., 1991, *Charles* et al., 1992) etc. As a results of above-mentioned manipulations, some of the microtubers developed were larger sized but their final size seldom exceeded 10 mm *(Tovar* et al., 1985, *Slimmon* et al., 1989, *Struik & Lommen,* 1991, *Charles* et al., 1992, *Seabrook* et al., 1993).

Considering the tuberization pattern, it is well known, that after stolon swelling the tuber growth continues, especially in the perimedullary region of tubers. However, this tissue region did not develop (or slightly) in *in vitro* grown tubers and this might be the reason, which limited the final size of *in vitro* tubers around 10 mm. *(Struik* et al., 1999). Nevertheless, earlier we have produced *in vitro* tubers on hormone-free medium, from which 69–79% was larger than 6 mm, 53–55% was larger than 8 mm and 11–29% of the microtubers were produced with a diameter larger than 10 mm up to 16 mm *(Magyar-Tábori & Dobránszki,* 2002) in cv. *Desiree.*

The aims of present work include the investigation of production facilities of large sized microtubers in other potato varieties (cv. *Boró,* cv. *Gülbaba*) and the study of the effects of the applied tuberization conditions on the proportion of microtuber tissues, especially on the perimedullary region.

Material and method

Shoot cultures of *Solanum tuberosum* L. cv. *Desiree, Boró* and *Gülbaba* grown in Kilner jar (400 ml, 75 x 85 mm) on 40 ml of the medium with Murashige-Skoog (MS) salts and vitamins (1962) supplemented with 3% sucrose and 0.8% agar-agar at long day conditions (16 h) and at 22 °C temperature, were used as initial explants.

Tuberization was induced on fully developed potato plantlets by pouring % sucrose solution onto the cultures as described earlier *(Dobránszki* et al., 1999) in the control treatment. Explants with 2 or 5 nodes were layered on a medium containing MS salts and vitamins supplemented with 8% sucrose and 0.8% agar-agar in the other two

treatments: 15 or 6 explants per jar was cultured containing 2 or 5 nodes, respectively, thus the total number of nodes per jar were the same (30 nodes) for each treatment. Induced cultures were exposed to short days (8 h) for 2 weeks, then to total darkness for further 11 weeks *(Dobránszki* et al., 1999).

At the end of the experiments microtubers were harvested and graded by their smallest diameter, and the number of tubers per jar, their size distribution, their fresh weight and the multiplication rate defined as number of microtubers per explants were recorded. Fifteen jars were observed in each treatment. After grading of harvested tubers, 10 microtubers from each size fraction in every treatment were cut in half longitudinally and they were used for calculation of volume of fresh tubers and their tissues. For volume calculations, we used the method described by Liu & Xie (2001), which was based on the formula for ellipsoids: $V=4/3\pi 1/8 l w^2 = 0.52 l w^2$. *Figure 1* illustrates the parameters measured and used for calculation of volumes of different tuber tissues and the way of calculations. Experiments were repeated three times. Data were analysed by ANOVA followed by Tukey's test using SPSS 7.5 for *Windows* programme *(Figure1)*.

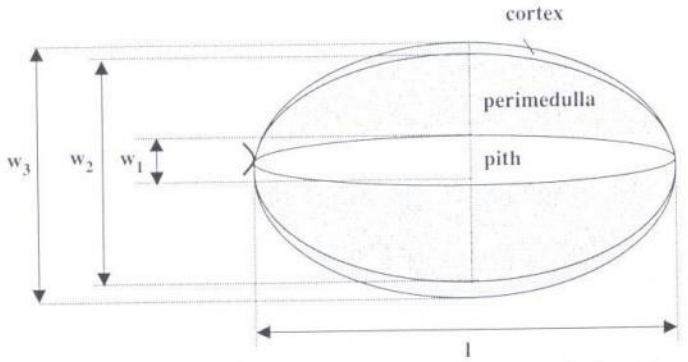

Figure 1 Parameters measured in the longitudinal section of microtubers according to Liu & Xie (2001). *l*: length of tuber, w_1: width of pith tissue, w_2: total width of the perimedulla and pith tissues, w_3: total width of the cortex, perimedulla and pith tissues. Calculations of the volumes: cortex: $V_{co}= 0.52l(w_3^2-w_2^2)$, perimedulla: $V_{pe}= 0.52l(w_2^2-w_1^2)$, pith: $V_{pi}= 0.52lw_1^2$

Results and Discussion

Number, size and fresh weight of microtubers

The multiplication rate (MR) was significantly influenced by treatments and varieties *(Table 1)*. If explants with 2 nodes were layered on tuberization medium, the MR decreased with 10–22% in *Desiree* and *Gülbaba* but it increased with 26% in *Boró* compared to the control treatment. The highest MR (1.98, 1.88 and 1.89 depending on cultivar) was obtained for explants with 5 nodes in each cultivar.

Average fresh weight of microtubers (AFW) increased significantly compared to the control treatment in each cultivar. If explants with 2 nodes were cultured on tuberization medium, increase of AFW was 2.5–4-fold but in the case of explants with 5 nodes it was 3–7-fold depending on cultivars. The total number of microtubers per jar (TNT) was always the highest in the control treatment and it decreased with the increase of the size of explants. The number of tubers larger than 8 mm per jar (NLT) was only 2% in the control treatment of each cultivar. However, in the other two treatments NLT increased up to 38–59% depending on cultivars and on the size of the explants.

Table 1 Effect of different tuberization treatments on the multiplication rate, average fresh weight of microtubers, on the total number of microtubers per jar and on the number of large-size microtubers per jar*

Cultivar	Treatment	Multiplication rate	Average fresh weight of tubers (mg)	Tuber number per jar	
				Total	> 8 mm
Desiree	Control	1.35 b	64 a	36.5 c	0.5
	Explant with 2 nodes	1.06 a	199 b	15.9 b	8.5
	Explant with 5 nodes	1.62 c	368 c	9.7 a	5.2
Gül-	Control	1.46 b	68 a	43.8 c	0.9
baba	Explant with 2 nodes	1.32 a	294 b	19.7 b	10.8
	Explant with 5 nodes	1.88 c	493 c	11.3 a	6.7
Boró	Control	1.06 a	82 a	31.7 c	0.6
	Explant with 2 nodes	1.34 b	208 b	20.1 b	7.6
	Explant with 5 nodes	1.89 c	278 c	12 a	5.3

*: Means within columns followed by the same letter were not significantly different at $p < 0.01$

Volumes of fresh microtubers and their tissue regions

Sizes of tissue regions measured (l, w_1, w_2 and w_3) and their volumes calculated (V_{co}, V_{pe} and V_{pi}) are presented in *Tables 2–4* in every size fractions for each cultivar. Data indicate significant effects of treatments and tuber size on the volumes of tubers and on the volumes of the different tissue regions. The correlations between the volume rate of different tissue regions (cortex, perimedulla and pith) or whole volume of tubers and tuber size are presented in *Table 5*.

Between the tuber size and the tuber volume, a tight correlation could be detected at $p < 0.01$ in every treatment and in every cultivar examined by applying power function *(Table 5)*.

In correlation analyses between the volume rate of different tuber tissues and tuber size, only tubers between 4–12 mm were considered (see *Table 5)* because under 4 mm tuber diameter the volume rate of the cortex whereas above 12 mm tuber diameter, the volume rate of the pith were too high, which would have distorted the correlation between the volume rate of the tuber tissues and the tuber size. The volume of tubers is related significantly to tuber size by power function in every treatment in each cultivar. The value of allometric exponent b was higher with 19–45% if explants with 2 nodes were tuberized and with 14–80% if explants with 5 nodes were tuberized than in the control treatment.

Table 2 Size and volume of different tissues in microtuber of cv. *Desiree*.

(*l*: length of tuber, w_1: width of pith tissue, w_2: total width of the perimedulla and pith tissues, w_3: total width of the cortex, perimedulla and pith tissues; calculations of the volumes: cortex: $V_{co}= 0.52l(w_3^2$*: Different small letters in the columns mean the significant differences (P<0.05) between the size fractions in the same treatment, different block capitals in the columns indicate differences between treatments in the same size fraction.w_2^2), perimedulla: $V_{pe}= 0.52l(w_2^2-w_1^2)$, pith: $V_{pi}= 0.52lw_1^2$, according to Liu & Xie, 2001)*

Size fractions	Tuberization treatments	Desiree						
		l (mm)	w_1 (mm)	w_2 (mm)	w_3 (mm)	V_{co} (mm^3)	V_{pe} (mm^3)	V_{pi} (mm^3)
I.fraction	Control treatment	4.0 a, A	1.4 a, A	2.9 a, A	3.1 a, A	2.5	13.42	4.08
< 4 mm	Explant with 2 nodes	3.2 a, A	1.7 a, A	2.7 a, A	2.8 a, A	0.92	7.32	4.81
	Explant with 5 nodes	4.5 a, A	1.5 a, A	2.9 a, A	3.0 a, A	1.38	14.41	5.27
II.fraction	Control treatment	7.1 b, B	2.6 b, A	4.3 b, A	5.4 b, A	39.39	43.31	24.96
4–6 mm	Explant with 2 nodes	6.0 b, A	2.2 ab, A	3.8 b, A	4.7 b, A	23.87	29.29	15.10
	Explant with 5 nodes	6.0 a, A	2.5 ab, A	3.9 a, A	4.7 b, A	21.47	27.96	19.5
III.fraction	Control treatment	8.3 c, A	2.6 b, A	5.9 c, A	7.1 c, A	67.33	121.07	29.18
6–8 mm	Explant with 2 nodes	9.1 c, B	2.7 ab, A	5.6 c, A	6.7 c, A	64.02	113.89	34.50
	Explant with 5 nodes	9.2 c, A	3.4 b, B	6.1 b, A	7.2 c, A	69.99	122.71	55.30
IV.fraction	Control treatment	8.5 c, A	2.9 b, A	6.0 c, A	7.5 c, A	89.51	121.95	37.17
8–10 mm	Explant with 2 nodes	10.6 c, B	3.1 bc, A	6.5 d, A	7.6 c, A	85.49	179.91	52.97
	Explant with 5 nodes	11.0 bc, B	4.6 c, B	7.5 c, B	8.5 cd, A	91.52	200.71	121.04
V.fraction	Control treatment	–	–	–	–	–	–	–
10–12 mm	Explant with 2 nodes	11.0 c, A	4.0 c, A	7.7 e, A	8.7 d, A	93.81	247.62	91.52
	Explant with 5 nodes	11.9 c, A	4.8 c, B	8.3 c, A	9.4 d, B	120.48	283.72	142.57
VI.fraction	Control treatment	–	–	–	–	–	–	–
> 12 mm	Explant with 2 nodes	–	–	–	–	–	–	–
	Explant with 5 nodes	12.7 c	7.0 d	10.0 d	11,0 e	138.68	336.80	323.60

*: Different small letters in the columns mean the significant differences (P<0.01) between the size fractions in the same treatment, different block capitals in the columns indicate differences between treatments in the same size fraction.

Table 3 Size and volume of different tissues in microtuber of cv. *Boró*.

(*l*: length of tuber, w_1: width of pith tissue, w_2: total width of the perimedulla and pith tissues, w_3: total width of the cortex, perimedulla and pith tissues; calculations of the volumes: cortex: $V_{co}= 0.52l(w_3^2-w_2^2)$, perimedulla: $V_{pe}= 0.52l(w_2^2-w_1^2)$, pith: $V_{pi}= 0.52lw_1^2$, according to Liu & Xie, 2001)*

Size fractions	Tuberization treatments	Boró						
		l (mm)	w_1 (mm)	w_2 (mm)	w_3 (mm)	V_{co} (mm^3)	V_{pe} (mm^3)	V_{pi} (mm^3)
I.fraction	Control treatment	3.8 a, A	1.2 a, A	2.4 a, A	3.6 a, A	14.87	8.63	3.02
< 4 mm	Explant with 2 nodes	4.4 a, A	1.3 a, A	2.3 a, A	3.0 a, A	8.17	8.39	4.16
	Explant with 5 nodes	3.0 a, A	1.3 a, A	2.3 a, A	3.0 a, A	6.05	5.46	2.54
II.fraction	Control treatment	5.4 b, A	2.0 b, A	3.6 b, A	5.0 b, B	34.37	24.35	11.48
4–6 mm	Explant with 2 nodes	6.0 b, A	1.8 a, A	3.4 b, A	4.8 b, B	36.62	26.49	10.21
	Explant with 5 nodes	5.7 ab, A	2.0 a, A	3.4 b, A	4.4 b, A	24.15	24.41	11.49
III.fraction	Control treatment	6.9 c, A	2.9 c, A	4.7 c, A	6.2 c, A	61.93	47.94	30.73
6–8 mm	Explant with 2 nodes	8.5 c, AB	3.7 b, B	5.7 c, C	7.5 c, B	102.17	86.37	62.35
	Explant with 5 nodes	8.0 bc, B	3.3 b, AB	5.2 c, B	6.0 c, A	38.66	64.92	46.18
IV.fraction	Control treatment	–	–	–	–	–	–	–
8–10 mm	Explant with 2 nodes	9.7 cd, A	4.7 c, A	6.7 d, A	8.3 d, A	125.36	109.94	111.54
	Explant with 5 nodes	10.4 c, A	4.6 c, A	6.8 d, A	8.2 d, A	118.02	131.57	115.08
V.fraction	Control treatment	–	-	–	–	–	–	–
10–12 mm	Explant with 2 nodes	10.3 d, A	5.5 d, A	7.8 e, A	9.7 e, A	176.07	166.82	163.07
	Explant with 5 nodes	13.5 d, B	5.4 cd, A	8.5 e, B	10.1 e, A	212.41	303.64	203.89
VI.fraction	Control treatment	–	–	–	–	–	–	–
> 12 mm	Explant with 2 nodes	–	–	–	–	–	–	–
	Explant with 5 nodes	14.3 d	6.0 d	9.3 e	11.5 f	333.06	379.21	268.97

*: Different small letters in the columns mean the significant differences (P<0.01) between the size fractions in the same treatment, different block capitals in the columns indicate differences between treatments in the same size fraction.

Table 4 Size and volume of different tissues in microtuber of cv. *Gülbaba*.
(*l*: length of tuber, w_1: width of pith tissue, w_2: total width of the perimedulla and pith tissues, w_3: total width of the cortex, perimedulla and pith tissues; calculations of the volumes: cortex: $V_{co}= 0.52l(w_3^2-w_2^2)$, perimedulla: $V_{pe}= 0.52l(w_2^2-w_1^2)$, pith: $V_{pi}= 0.52lw_1^2$, according to Liu & Xie, 2001)*

Size fractions	Tuberization treatments	Gülbaba						
		l (mm)	w_1 (mm)	w_2 (mm)	w_3 (mm)	V_{co} (mm^3)	V_{pe} (mm^3)	V_{pi} (mm^3)
I.fraction	Control treatment	4.4 a, B	1.0 a, A	1.8 a, A	3.4 a, A	19.81	5.04	2.29
< 4 mm	Explant with 2 nodes	4.3 a, B	1.0 a, A	1.8 a, A	3.2 a, A	15.73	5.07	2.24
	Explant with 5 nodes	3.0 a, A	1.0 a, A	1.8 a, A	3.0 a, A	8.71	3.77	1.56
II.fraction	Control treatment	6.2 b, A	2.4 b, A	3.8 b, B	4.7 b, A	24.73	26.86	19.66
4–6 mm	Explant with 2 nodes	5.6 a, A	1.9 a, A	3.3 b, A	4.7 b, A	31.76	21.31	12.25
	Explant with 5 nodes	5.5 b, A	1.9 ab, A	3.0 b, A	4.4 b, A	28.94	15.82	10.53
III.fraction	Control treatment	8.1 c, A	2.7 b, A	4.4 c, A	5.4 c, A	43.12	52.3	31.04
6–8 mm	Explant with 2 nodes	7.5 b, A	3.8 b, B	5.8 c, B	7.1 c, C	63.86	74.15	59.33
	Explant with 5 nodes	7.8 c, A	2.4 b, A	4.6 c, A	6.4 c, B	77.62	62.61	24.28
IV.fraction	Control treatment	–	–	–	–	–	–	–
8–10 mm	Explant with 2 nodes	10.1 c, A	4.5 bc, B	7.1 d, B	8.2 d, A	86.98	162.67	107.85
	Explant with 5 nodes	10.3 d, A	3.4 c, A	6.1 d, A	8.3 d, A	168.95	136.34	63.13
V.fraction	Control treatment	–	–	–	–	–	–	–
10–12 mm	Explant with 2 nodes	13.5 d, B	5.0 c, A	8.0 e, A	10.0 d, A	252.72	273.78	175.5
	Explant with 5 nodes	12.3 e, A	5.1 d, A	8.3 e, A	10.2 e, A	221.26	275.44	169.1
VI.fraction	Control treatment	–	–	–	–	–	–	–
> 12 mm	Explant with 2 nodes	–	–	–	–	–	–	–
	Explant with 5 nodes	12.7 e	5.4 d	9.0 e	12.3 f	462.87	334.14	203.32

*: Different small letters in the columns mean the significant differences ($P<0.01$) between the size fractions in the same treatment, different block capitals in the columns indicate differences between treatments in the same size fraction.

In the case of cv. *Desiree* the volume rate of cortex region (V_{co}/V) varied between 37–44% in the control treatment but it decreased with the increase of the tuber size when explants with 2 nodes (from 35% to 25%) or explants with 5 nodes (from 37% to 20%) were used and if the size of tubers was larger than 4 mm (from the II. fraction). However, no significant correlation could be detected. The volume rate of perimedulla (V_{pe}/V) varied between 34–55% depending on tuberization treatments but no significant correlation could be obtained between this and the tuber size. If the size of tubers was larger than 4 mm, the volume rate of the pith (V_{pi}/V) depended on the tuberization treatment appreciably. In the control treatment V_{pi}/V varied between 14–22% but if explants with 2 nodes were tuberized, it decreased from 23% to 16% till tubers reached 10 mm but in the case of tubers larger than 10 mm it was 22% again. Tuberization of explants with 5 nodes resulted in tubers, in which V_{pi}/V increased with the increase of tuber size from 18% up to 41% and this correlation proved to be significant at $p < 0.01$ *(Tab. 2)*.

V_{co}/V showed a decreasing tendency if the size of tubers increased in all of the treatments in the case of cv. *Boró* but the correlation was not significant. V_{pe}/V varied between 33-43% and no important relationship with the tuber size could be detected. However, V_{pi}/V increased with the increase of tuber size from 16% to 22% in the control treatments; from 15% to 33% if explants with 2 nodes were tuberized and from 20% up to 32% if explants with 5 nodes were tuberized and these correlations proved to be statistically significant at $p < 0.01$ *(Tab. 3)*.

V_{co}/V decreased but V_{pe}/V and V_{pi}/V increased significantly with the increase of tuber size in all of the treatments in the case of cv. *Gülbaba*, except V_{pi}/V when explants with 5 nodes were tuberized *(Tab. 4)*.

Statistical analysis proved a significant correlation between V_{co}/V and V_{pe}/V in every cultivar and treatment; moreover, correlation between V_{pe}/V and V_{pi}/V was significant in cv. *Desiree* in every treatment and in cv. *Gülbaba* in the control treatment *(Tab. 5)*.

In these experiments, the production of microtubers of cvs. *Boró* and *Gülbaba* occurred on hormone-free medium by different treatments, in which different way of sucrose support and different types of explants were applied, as earlier in the case of cv. *Desiree (Magyar-Tábori & Dobránszki*, 2002). According to the results, it can be concluded, that the size of microtubers could be increased also in the other two cultivars by appropriate ways of sucrose support and explant type. The number of large-size tubers (> 8 mm, up to 16 mm) reached 59% and 44% in cvs. *Gülbaba* and *Boró*, respectively. If explants with 2 nodes were cultured on tuberization medium, both AFW (294 or 208 mg) and NLT (55% or 38%) were high enough besides satisfying TNT (19.7 or 20.1) as described earlier in the case of cv. *Desiree* and as presented in *Table 1*.

Table 5 Effects of tuberization treatments on the correlations between the parameters of microtubers.
(Abbreviations applied in the table: V_{co}/V: volume rate of the cortex, V_{pe}/V: volume rate of the perimedulla, V_{pi}/V: volume rate of the pith, F: tuber size, V: volume of the microtuber, *n.s.*: non significant, **: significant at $p < 0.05$, ***: significant at $p < 0.01$)

Correlations	Tuberization treatment	Desiree	Boró	Gülbaba
V – F	control treatment	$V=22.40\ F^{1.92}$ $r^2=0.904^{***}$	$V=24.72\ F^{1.54}$ $r^2=0.873^{***}$	$V=25.73\ F^{1.41}$ $r^2=0.823^{***}$
	explant with 2 nodes	$V=13.86\ F^{2.29}$ $r^2=0.932^{***}$	$V=19.73\ F^{2.07}$ $r^2=0.946^{***}$	$V=19.74\ F^{2.05}$ $r^2=0.945^{***}$
	explant with 5 nodes	$V=17.86\ F^{2.19}$ $r^2=0.889^{***}$	$V=9.84\ F^{2.58}$ $r^2=0.948^{***}$	$V=10.49\ F^{2.54}$ $r^2=0.976^{***}$
V_{co}/V – F	control treatment	*n.s.* *n.s.*	$V_{co}/V=65.18\ F^{-0.69}$ ***	
V_{pe}/V – F		*n.s.* *n.s.*	$V_{pe}/V=18.30\ F^{0.79}$ ***	
V_{pi}/V – F		*n.s.*	$V_{pi}/V=10.71\ F^{0.62}$ ***	$V_{pi}/V=9.79\ F^{0.96}$ ***
V_{co}/V – V_{pe}/V	4mm<F<12mm	$V_{pe}/V=-0.88\ V_{co}/V+78.18^{***}$	$V_{pe}/V=-0.63\ V_{co}/V+65.21^{***}$	$V_{pe}/V=-0.61\ V_{co}/V+61.27^{***}$
V_{pi}/V – V_{pe}/V		$V_{pe}/V=-0.80\ V_{pi}/V+56.16^{***}$	*n.s.*	$V_{pe}/V=0.51\ V_{pi}/V+6.76^{***}$
V_{co}/V – F	explant with 2 nodes	*n.s.* *n.s.*	$V_{co}/V=69.38\ F^{-0.70}$ ***	
V_{pe}/V – F		*n.s.* *n.s.*	$V_{pe}/V=21.45\ F^{0.47}$ ***	
V_{pi}/V – F		*n.s.*	$V_{pi}/V=13.78\ F^{0.50}$ ***	$V_{pi}/V=9.73\ F^{0.80}$ ***
V_{co}/V – V_{pe}/V	4mm<F<12mm	$V_{pe}/V=-0.91\ V_{co}/V+77.92^{***}$	$V_{pe}/V=-0.36\ V_{co}/V+49.05^{***}$	$V_{pe}/V=-0.56\ V_{co}/V+58.70^{***}$
V_{pi}/V – V_{pe}/V		$V_{pe}/V=-0.90\ V_{pi}/V+66.42^{***}$	*n.s.* *n.s.*	
V_{co}/V – F	explant with 5 nodes	*n.s.* *n.s.*	$V_{co}/V=60.80\ F^{-0.27}$ ***	
V_{pe}/V – F		*n.s.* *n.s.*	$V_{pe}/V=25.25\ F^{0.24}$ ***	
V_{pi}/V – F		$V_{pi}/V=17.29\ F^{0.32}$ **	$V_{pi}/V=17.30\ F^{0.34}$ ***	*n.s.*
V_{co}/V – V_{pe}/V	4mm<F<12mm	$V_{pe}/V=-0.70\ V_{co}/V+64.33^{***}$	$V_{pe}/V=-0.48\ V_{co}/V+55.57^{***}$	$V_{pe}/V=-0.69\ V_{co}/V+67.05^{***}$
V_{pi}/V – V_{pe}/V		$V_{pe}/V=-0.55\ V_{pi}/V+57.96^{**}$	*n.s.* *n.s.*	

The main aim of the present work was to study the presence and size of perimedullary region of microtubers with different sizes and produced under different conditions. Our results suggest, that microtubers produced on hormone-free medium have well-developed perimedullary region, and its volume rate seemed to be important in the final size of tubers. Correlation analysis proved that with the decrease of the volume rate of the cortex region (V_{co}/V), the volume rate of the perimedullary region (V_{pe}/V) increased. Increase of V_{pe}/V was connected to the increase of tuber size until tubers reached 12 mm diameter. Between V_{pe}/V and tuber size, a direct relationship was detected in cv. *Gülbaba*, but it was indirect in the other two cultivars (Table 5). It could be supposed, that, as in the case of field-grown tubers, the increase of V_{pe}/V could be one of the important factors influencing the capacity of microtubers to act as sink for assimilates. It could be particularly true if the increase of V_{pe}/V is connected with the increase of the cell number, which was not examined in these experiments, but was proved by others under *in vitro* conditions (Liu & Xie, 2001). However, in microtubers larger than 12 mm the V_{pe}/V did not increase any more but the V_{pi}/V increased and the maximal tuber size was 16 mm (w_3). It seems, that this is the maximum tuber size, which could be reached on hormone-free medium, if we would like to obtain also economically sufficient MR and TNT in a jar.

References

Charles, G., Rossignol, L., & Rossignol, M. (1992): Environmental effects on potato plants *in vitro*. J. Plant Physiol. 139(6): 708–713.

Dobránszki J. (1996): Effects of dark treatment on tuber initiation and development of induced potato plantlets cultured *in vitro*. Acta Agronomica Hungarica. 44(4): 377–386.

Dobránszki J., Magyar-Tábori K. & Ferenczy A. (1999): Light and genotype effects on *in vitro* tuberization of potato plantlets. Potato Research. 42 (3–4): 483–488.

Magyar-Tábori K. & Dobránszki J. (2002): How to produce large sized microtubers of potato cv. Desiree. Int. J. Hortic. Sci. 8(3–4) 33–36.

Forti, E., Mandolini,, G. & Ranalli, P. (1991): *In vitro* tuber induction: Influence of the variety and of the media. Acta Horticulturae. 300: 127–132.

Garner, N. & J. Blake, (1989): The induction and development of potato microtubers *in vitro* on media free of growth regulating substances. Ann. Botany. 63: 663–674.

Hussey, G. & N. J. Stacy (1981): *In vitro* propagation of potato (*Solanum tuberosum* L.). Ann. of Botany. 48: 787–796.

Leclerc, Y., Donnelly, D. J. & Seabrook, J. E. A. (1994): Microtuberization of layered shoots and nodal cuttings of potato: The influence of growth regulators and incubation periods. Plant Cell, Tissue and Organ Culture. 37: 113–120.

Liu, J. & Xie, C. (2001): Correlation of cell division and cell expansion to potato microtuber growth *in vitro*. Plant Cell, Tissue and Organ Culture. 67: 159–164.

Murashige, T. & F. Skoog, (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473–497.

Nowak, J. & Colborne, D. (1989): *In vitro* tuberization and tuber proteins as indicators of heat stress tolerance in potato. American Potato Journal. 66: 35–44.

Ranalli, P., Bizzari, M., Borghi, L. & Mari, M. (1994): Genotypic influence on *in vitro* induction, dormancy length, advancing age and agronomical performance of potato microtubers (*Solanum tuberosum* L.). Ann Appl Biol 125: 161–172.

Perl, A., Aviv, D., Willmitzer, L. & Galun, E. (1991): *In vitro* tuberization in transgenic potatoes harboring β-glucuronidase linked to a patatin promoter: effects of sucrose levels and photoperiods. Plant Science. 73: 87–95.

Seabrook, J. E. A., S. Coleman & D. Levy (1993): Effect of photoperiod on *in vitro* tuberization of potato (*Solanum tuberosum* L.). Plant Cell, Tissue and Organ Culture. 34(1): 43–51.

Slimmon, T., Souza Machado, V., & Coffin, R. (1989): The effect of light on *in vitro* microtuberization of potato cultivars. Am. Potato Journal. 66: 843–848.

Stallknecht, G. F. & Farnsworth, S. (1982): General characteristics of coumarin-induced tuberization of axillary shoots of *Solanum tuberosum* L. cultured *in vitro*. American Potato Journal. 59: 17–31.

Struik, P.C. & Lommen, W. J. M. (1991): Production, Storage and use of Micro- and Minitubers. Proceedings 11th Triennial Conference of EAPR, Edinburgh. 122–133.

Struik, P. C., Vreugdenhil, D., VanEck, H. J., Bachem, C. W. & Visser, R. G. F. (1999): Physiological and genetic control of tuber formation. Potato Research. 42: 313–331.

Tábori K. M., Dobránszki J. & Ferenczy A. (1999): Some sprouting characteristics of microtubers. Potato Research. 42 (3–4): 611–617.

Tábori K. M., Dobránszki J. & Ferenczy A. (2000): Effects of culture density on growth and *in vitro* tuberization capacity of potato plantlets. Acta Agronomica Hung. 48(2): 185–189.

Tovar, P., Estrada, R., Schilde-Rentschler, L., & Dodds, J. H. (1985): Induction and use of *in vitro* potato tubers. CIP Circular 13(4): 1–5.

Wiersema, S. G., Cabello, R., Tovar, P. & Dodds, J. H. (1987): Rapid seed multiplication by planting into beds microtubers and *in vitro* plants. Potato Research. 30: 117–120.