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EFFECT OF CYTOKININS ON *in vitro* MULTIPLICATION OF RHUBARB (*Rheum rhaponticum* L.) 'KARPOW LIPSKIEGO' SHOOTS AND *ex vitro* ACCLIMATIZATION AND GROWTH

Danuta Kozak, Andrzej Sałata
University of Life Sciences in Lublin

Abstract. The influence of four cytokinins: BA (4.4, 11.1, 22.2 μmol·dm^{-3}), kinetin (4.7, 11.6, 23.3 μmol·dm^{-3}), 2iP (4.9, 12.3, 24.6 μmol·dm^{-3}), TDZ (4.5, 11.4, 22.7 μmol·dm^{-3}) on shoot multiplication of *Rheum rhaponticum* 'Karpow Lipskiego' on Murashige and Skoog (MS) medium was studied. The shoots used in the experiment were obtained from aseptically grown shoot clusters. Rooted shoots (except treatment with TDZ) after acclimatization were transplanted to pots into a peat substrate where they were grown for 4 weeks. Plants were then cultivated in the field for 8 months. During *in vitro* studies the cytokinins promoted the development of leaves as well as the formation of new shoots. The highest average number of axillary shoots was found on the medium with BA 11.1–22.2 μmol·dm^{-3}. The use of kinetin (4.7–11.6 μmol·dm^{-3}) or 2iP 12.3 μmol·dm^{-3} stimulated strong growth of shoots expressed in their length and size of the leaf blade. All TDZ concentrations caused a significant inhibition of shoot elongation. BA and TDZ had a harmful effect on the rooting of multishoots. On the control medium (hormone-free) and on the media containing kinetin or 2iP, 100% of shoots produced roots. After a period of 8 months, following overwintering and start of vegetation growth, the plant survival rate was 80–100%. It was found that 2iP 12.3 μmol·dm^{-3} had the highest after effect on plant growth. The plants derived from this medium had uniform and compact growth and they developed more leaves in the rosette with significantly larger laminae as well as longer and thicker petioles, than plants obtained from others used media.

Key words: branching, rooting, consequent effect, acclimatization, growth in the field

INTRODUCTION

Rheum rhaponticum L. (Polygonaceae) is a popular vegetable crop used in domestic cooking and in the food industry. Rhubarb is usually propagated vegetatively, but asex-

Corresponding author – Adres do korespondencji: Danuta Kozak, Department of Ornamental Plants and Landscape Architecture, University of Life Sciences in Lublin, Leszczyńskiego 58, 20-068 Lublin, Poland, e-mail: danuta.kozak@up.lublin.pl

ual reproduction can be attributed to virus diseases perpetuated in the clone by these propagation method so *in vitro* cultures are used for elimination of viruses and rapid multiplication [Walkey 1968, Roggemans and Claes 1979, Walkey and Matthews 1979, Pierik et al. 1989, Lassus and Voipio 1994]. Cytokinins are required in tissue culture for cell division and shoot multiplication. *In vitro* propagation of *Rheum* has been done using mostly BA [Lal and Ahuja 1989, 1993, Camara-Machado et al. 1990, Lassus and Voipio 1994, Xu et al. 2004]. Transfer and acclimatization to the *ex vitro* environment is frequently most difficult step in a successful micropropagation system [Pospisilowa et al. 1999, Kadlecek et al. 2001, Hazarika 2003]. Many authors [Maene and Debergh 1983, Podwyszyńska and Hempel 1989, D'Arth et al. 2002, Kozak 2006] have observed the residual effect of growth regulators used for multiplication of shoots *in vitro* cultures on their rooting, adaptation to greenhouse condition and further growth.

The current study was performed in order to compare four cytokinins in three concentrations in multiplication stage and to estimate the survival rate and *in vivo* growth of plants coming from three cytokinins (BA, kinetin, 2iP).

MATERIALS AND METHODS

Rhubarb shoots isolated from multishoots grown *in vitro* were the object of the experiment. The culture were initiated from buds isolated from three-year-old crowns. They were disinfected in sodium hypochlorite containing 2% of active chlorine for 45 min. and rinsed 3 times in sterilized water. The explants were cultivated on the basic Murashige and Skoog (MS) [1962] medium containing: mineral salts and thiamine – 0.4 $mg \cdot dm^{-3}$, pyridoxine – 0.5 $mg \cdot dm^{-3}$, nicotinic acid – 0.5 $mg \cdot dm^{-3}$, glycine – 2 $mg \cdot dm^{-3}$, myo-inositol – 100 $mg \cdot dm^{-3}$, sucrose – 30 $g \cdot dm^{-3}$, Agar-Agar (Sigma) – 6.5 $g \cdot dm^{-3}$, and supplemented with benzyladenine (BA) 8.8 $\mu mol \cdot dm^{-3}$ and IAA 2.9 $\mu mol \cdot dm^{-3}$. After several months of multiplication, shoots of 10 mm in length were dissected from the shoot clusters and used in the experiment. Four cytokinins: BA (4.4, 11.1, 22.2 $\mu mol \cdot dm^{-3}$), kinetin (KIN – 4.7, 11.6, 23.3 $\mu mol \cdot dm^{-3}$), isopentenyladenine (2iP – 4.9, 12.3, 24.6 $\mu mol \cdot dm^{-3}$), thidiazuron (TDZ – 4.5, 11.4, 22.7 $\mu mol \cdot dm^{-3}$) were used to examine the production of new shoots. A control medium without cytokinin was included. The pH of the media was adjusted to 5.7 before autoclaving. After 4 weeks of culture the leaves were trimmed to 1 cm, the roots were removed and shoots base were subcultured on the same medium for 4 weeks for further proliferation. There were eight replications per treatment, each consisting of 5 explants/Erlenmeyer flask. The cultures were maintained at 22°C and light intensity of 35 $\mu mol\ m^{-2}\ s^{-1}$ and 16 h photoperiod.

The following characters were evaluated after 8 weeks (2 passages) using half of cultures: height of shoot clusters; fresh weight of shoot clusters and basal tissue; number of leaves, length of leaf petiole and size of leaf blade on main and axillary shoot; number of axillary shoots and number, length, fresh weight of roots and % of shoot clusters rooted.

Plants from treatments with kinetin and 2iP and from control medium were transplanted into plastic vessels (16 × 10 × 9.5 cm) containing a mixture of 1 peat : 1 perlite (by volume). For shoots derived from treatments with BA rooting powder was used. All

plants were hardening on the bench under polyethylene sheeting. They were grown for 4 weeks. Then young plants were planted in the 1st decade of August 2007 in 8 cm diameter pots. A substrate manufactured by Klasmann-Deilmann was used for planting. For 1 month, the plants grew in a greenhouse on tables, with average temperature of 22°C and natural light. Before transplanting the plants into field, the survival rate was estimated relative to the number of plants planted in pots as well as the number of leaves and plant height were determined. In the 1st decade of September, the plants were planted in a nursery in field at a spacing of 40×40 cm. In the 1st decade of May 2008, the condition of overwintered plants was evaluated as well as the leaf rosette diameter was measured, the number of leaves per plant was counted; measurements were also made of lamina length and width as well as of petiole length and width, measured at the base of all leaves on a particular plant.

The results of the experiment were analyzed statistically using a standard statistical procedure with one factorial design and the Tukey test was used to estimate the differences between the means at a 5% level of significance.

RESULTS AND DISCUSSION

Cytokinins affected the growth and development of shoots of *Rheum rhaponticum* 'Karpow Lipskiego'. The strongest elongation growth of shoots was obtained on growth media containing kinetin at a concentration of 4.7 and 11.6 µmol·dm^{-3} or BA at 4.4 µmol dm^{-3} (respectively: 114.4 mm, 119.5 mm, 101.5 mm). It was observed that TDZ, in all the studied concentrations, as well as BA, kinetin and 2iP at the highest concentration, strongly inhibited the shoot elongation growth (tab. 1).

A significant influence of the type of cytokinin on the number of leaves in a shoot cluster was found. BA at 4.4–22.2 µmol·dm^{-3} most favourably affected the leaf production (11.4–16.8 leaves) (tab. 1). The cultures growing on the media containing 2iP or kinetin produced the smallest number of leaves.

When assessing the fresh weight of shoot clusters, a significant influence of cytokinins was observed. On the medium with 4.4 µmol·dm^{-3} BA, the shoot clusters achieved the highest value of fresh weight. The further increase of the concentration of this cytokinin inhibited the growth in fresh weight. On the media with kinetin 23.3 µmol·dm^{-3} or 2iP at an amount of 24.6 µmol·dm^{-3}, as well as in the presence of TDZ (4.5–22.7 µmol·dm^{-3}), shoot clusters characterised by the lowest fresh weight were obtained (tab. 1).

Cytokinins strongly affected the growth of the basal tissue at the base of the shoots. BA had the strongest effect on its formation. The amount of the basal tissue on the control medium was minimal.

A significant influence of cytokinins on the production and growth of leaves on the main shoot was observed (tab. 2). On the medium supplemented with BA (4.4–22.2 µmol· dm^{-3}), the main shoot was characterised by the largest number of leaves (4.1–6.3). The average length of the petiole was the highest on the media with 11.6 and 4.7 µmol·dm^{-3} kinetin, as well as in the presence of 2iP at 12.3 µmol·dm^{-3} (respectively: 77.3 mm, 64.0 mm, 59.1 mm). Leaves with the largest sizes of the lamina were also

Table 1. The influence of cytokinins on the growth and development of *Rheum rhaponticum* 'Karpow Lipskiego' shoot clusters after 2 passages in culture *in vitro*

Tabela 1. Wpływ cytokinin na wzrost i rozwój pędów *Rheum rhaponticum* 'Karpow Lipskiego' po 2 pasażach w kulturze *in vitro*

Growth regulators Regulatory wzrostu	Concentration Stężenie µmol·dm^{-3}	Height of shoot cluster Wysokość zespołów pędów mm	Number of leaves/ shoot cluster Liczba liści w zespole pędów	Fresh weight of shoot cluster Świeża masa zespołu pędów mg	Fresh weight of basal tissue Świeża masa tkanki bazalnej mg
Control Kontrola	0.0	88.9 a-d*	5.1 cd	832.1 de	91.5 e
BA	4.4	101.5 abc	11.4 abc	2051.5 a	735.8 a
	11.1	71.2 b-e	14.8 ab	1676.4 ab	681.6 ab
	22.2	57.3 cde	16.8 a	1485.5 bc	794.2 a
Kinetin Kinetyna	4.7	114.4 ab	3.8 cd	1645.3 ab	502.3 bcd
	11.6	119.5 a	3.9 cd	1671.0 ab	619.0 ab
	23.3	70.1 b-e	5.1 cd	769.9 de	353.1 cd
2iP	4.9	82.7 a-e	3.6 d	1039.5 cd	313.9 de
	12.3	97.3 abc	4.8 cd	1472.6 bc	483.3 bcd
	24.6	73.1 a-e	3.6 d	409.5 e	487.8 bcd
TDZ	4.5	37.0 e	8.0 bcd	554.6 e	317.2 de
	11.4	46.3 de	8.2 bcd	745.5 de	564.2 abc
	22.7	45.0 de	7.0 cd	528.5 e	618.3 ab

* Means in vertical columns followed by the same letter are not significantly different at α = 0.05
Średnie w kolumnach oznaczone tą samą literą nie różnią się między sobą istotnie przy α = 0,05

Table 2. The influence of cytokinins on the growth and development of *Rheum rhaponticum* 'Karpow Lipskiego' main shoot after 2 passages in culture *in vitro*

Tabela 2. Wpływ cytokinin na wzrost i rozwój pędu głównego *Rheum rhaponticum* 'Karpow Lipskiego' po 2 pasażach w kulturze *in vitro*

Growth regulators Regulatory wzrostu	Concentration Stężenie µmol·dm^{-3}	Number of leaves on main shoot Liczba liści na pędzie głównym	Length of leaf petiole Długość ogonka liściowego mm	Length of leaf blade Długość blaszki liściowej mm	Width of leaf blade Szerokość blaszki liściowej mm
Control Kontrola	0.0	3.7 ab*	45.9 bcd	20.5 bcd	14.0 bcd
BA	4.4	4.6 ab	53.0 a-d	19.2 cd	13.2 bcd
	11.1	4.1 ab	36.4 bcd	15.5 cd	10.1 cd
	22.2	6.3 a	31.3 cd	18.5 cd	12.5 bcd
Kinetin Kinetyna	4.7	3.3 b	64.0 ab	34.9 a	26.5 a
	11.6	3.1 b	77.3 a	35.5 a	28.5 a
	23.3	2.7 b	39.6 bcd	23.7 a-d	19.2 abc
2iP	4.9	2.8 b	46.3 a-d	26.8 abc	20.6 ab
	12.3	3.1 b	59.1 abc	32.1 ab	26.5 a
	24.6	2.7 b	46.9 a-d	25.6 a-d	19.4 abc
TDZ	4.5	3.2 b	23.9 d	14.7 d	9.8 d
	11.4	3.5 ab	28.6 cd	16.1 cd	10.6 cd
	22.7	3.1 b	28.3 cd	17.8 cd	11.1 cd

*See explanation table 1 – Patrz objaśnienia do tabeli 1

Fig. 1. Plants of *Rheum rhaponticum* L. 'Karpow Lipskiego' obtained after 2 passages on the MS medium containing different concentrations of BA
Ryc. 1. Rośliny *Rheum rhaponticum* L. 'Karpow Lipskiego' uzyskane po 2 pasażach na pożywce MS uzupełnionej BA w różnych stężeniach

Fig. 2. Plants of *Rheum rhaponticum* L. 'Karpow Lipskiego' obtained after 2 passages on the MS medium containing different concentrations of kinetin (KIN)
Ryc. 2. Rośliny *Rheum rhaponticum* L. 'Karpow Lipskiego' uzyskane po 2 pasażach na pożywce MS uzupełnionej kinetyną w różnych stężeniach

observed on these media. In the presence of TDZ, the average length of the petiole, as well as the size of the lamina, had the lowest value.

The study results showed a significant influence of cytokinins on the number of axillary shoots regenerated from 1 explant (tab. 3). It was found that BA had the most favourable effect. On the media containing 11.1 and 22.2 μmol·dm^{-3} BA, the largest number of shoots was obtained (respectively: 4.8, 4.4). BA at 4.4 μmol·dm^{-3} and TDZ (4.5–22.7 μmol·dm^{-3}) also affected the induction of axillary shoots. Kinetin and 2iP sporadically stimulated the growing of axillary shoots. Shoots regenerating in the presence of BA at 4.4 μmol·dm^{-3}, kinetin at 11.6 μmol·dm^{-3} or 2iP at 12.3 μmol·dm^{-3}, were characterised by the strongest growth of the petiole and the lamina.

Root regeneration was observed during the study. TDZ and BA inhibited root development, whereas in the presence of 2iP and kinetin, as well as on the control medium without growth regulators, 100% of shoots produced roots. The largest number of roots characterised by the highest fresh weight and length was obtained in the presence of kinetin at 4.7 and 11.6 μmol·dm^{-3} (tab. 4).

The results of the experiment showed that BA plays a key role in the process of induction of shoots of *Rheum rhaponticum* 'Karpow Lipskiego'. In *in vitro* cultures, the multiplication of rhubarb shoots was carried out on MS medium with 4.4 μmol·dm^{-3} BA and 4.9 μmol·dm^{-3} IBA [Roggemans and Claes 1979, Lassus and Voipio 1994], 8.8 μmol·dm^{-3} BA and 0.5 μmol·dm^{-3} IBA [Camara-Machado et al. 1990], as well as 8.8 μmol·dm^{-3} BA and 4.9 μmol·dm^{-3} IBA [Lal and Ahuja 1993]. BA was also used for the induction and multiplication of shoots of other plants from the family Polygonaceae: *Coccoloba uvifera* [Wojtania and Gabryszewska 2000], *Rumex acetosella* and *Rumex acetosa* [Culafic et al. 1987], *Polygonum aubertii* [Dąbski and Kozak 1998).

The shoot multiplication ratio depended on the genotype, and it was 3.2 for *R. rhaponticum* clone AF [Lassus and Voipio 1994], 7 for the variety 'Holsteiner Blut' [Camara-Machado et al. 1990] and 8.8 for *R. emodi* [Lal and Ahuja 1993]. Lassus and Voipio [1994] observed a different reaction of the studied genotypes to the medium used. On the medium supplemented with 4.4 μmol·dm^{-3} BA and 4.9 μmol·dm^{-3} IBA, clone AF shoots propagated correctly, whereas the variety 'Victoria' produced large amounts of callus and the problem of vitrification occurred. In the conducted experiment on *R. rhaponticum* 'Karpow Lipskiego', 4.8–4.4 shoots were obtained in the presence of BA at 11.1 μmol·dm^{-3} and 22.2 μmol·dm^{-3}. On these media, a strong growth of the shoot base, on which shoot buds formed, was also observed.

On the media with kinetin or 2iP, a very low shoot multiplication ratio (0.3–1.3) was obtained, but these cytokinins strongly stimulated the growth of the petiole and the leaf blades. In addition, they had a favourable effect on root production and growth. Some researchers recommend the introduction of an additional passage in which plantlets would get longer and increase the surface area of their leaf blades [Maene and Debergh 1983, Podwyszyńska and Hempel 1989]. Based on the studies conducted, it may be suggested that the multiplication of *Rheum rhaponticum* shoots should be carried out on media enriched with 11.1–22.2 μmol·dm^{-3} BA, but media with 4.7–11.6 μmol·dm^{-3} kinetin or with 2iP 12.5 μmol·dm^{-3} should be used in the passage preceding shoot rooting.

Table 3. The influence of cytokinins on the induction and growth of *Rheum rhaponticum* 'Karpow Lipskiego' axillary shoots after 2 passages in culture *in vitro*

Tabela 3. Wpływ cytokinin na indukcję i wzrost pędów kątowych *Rheum rhaponticum* 'Karpow Lipskiego' po 2 pasażach w kulturze *in vitro*

Growth regulators Regulatory wzrostu	Concentration Stężenie µmol·dm^{-3}	Number of axillary shoots/explant Liczba pędów kątowych z 1 eksplantatu	Number of leaves on axillary shoots Liczba liści na pędach kątowych	Length of leaf petiole Długość ogonka liściowego mm	Length of leaf blade Długość blaszki liściowej mm	Width of leaf blade Szerokość blaszki liściowej mm
Control Kontrola	0.0	0.2 e*	1.0 c	13.1 bc	7.2 c	4.8 d
BA	4.4	2.8 b	2.7 a	34.6 a	14.3 a	9.7 abc
	11.1	4.8 a	2.5 ab	19.9 bc	10.6 abc	6.5 bcd
	22.2	4.4 a	2.5 ab	14.6 bc	9.8 abc	6.3 bcd
Kinetin Kimetyna	4.7	0.3 e	1.0 c	8.0 c	-	-
	11.6	0.4 e	1.8 abc	27.7 ab	13.3 ab	10.3 ab
	23.3	1.3 b-e	1.7 abc	13.2 bc	10.3 abc	8.4 a-d
2iP	4.9	0.6 e	1.0 c	11.5 bc	8.8 bc	7.6 a-d
	12.3	0.9 cde	1.9 abc	28.3 a	13.8 ab	10.8 a
	24.6	0.7 de	1.5 bc	10.0 c	10.0 abc	8.3 a-d
TDZ	4.5	2.2 bc	2.1 ab	12.4 bc	8.1 c	5.6 cd
	11.4	2.4 bc	2.2 ab	12.9 bc	9.3 abc	6.9 a-d
	22.7	2.3 bc	2.1 ab	14.7 bc	10.3 abc	6.8 a-d

*See explanation table 1 – Patrz objaśnienia do tabeli 1; unfolded leaves – liście nierozwinięte

Table 4. The influence of cytokinins on the regeneration and growth of *Rheum rhaponticum* 'Karpow Lipskiego' roots after second passage in culture *in vitro*

Tabela 4. Wpływ cytokinin na regenerację i wzrost korzeni *Rheum rhaponticum* 'Karpow Lipskiego' po 2 pasażach w kulturze *in vitro*

Growth regulators Regulatory wzrostu	Concentration Stężenie µmol·dm^{-3}	Percentage of shoot clusters rooted Procent ukorzenionych zespołów pędów	Number of roots/shoot cluster Liczba korzeni z 1 zespołu pędów	Length of roots Długość korzeni mm	Fresh weight of roots/shoot cluster Świeża masa korzeni z 1 zespołu pędów mg
Control Kontrola	0.0	100	5.2 ab*	44.4 ab	163.8 cd
BA	4.4	50	2.3 cd	24.1 cd	82.7 cd
	11.1	40	3.0 bc	18.3 d	31.9 d
	22.2	20	2.0 cd	26.0 cd	35.0 d
Kinetin Kinetyna	4.7	100	7.7 a	49.2 ab	875.3 a
	11.6	100	7.4 a	54.5 a	619.6 ab
	23.3	100	6.1 a	38.5 abc	370.5 bcd
2iP	4.9	100	6.1 a	42.2 ab	461.0 bc
	12.3	100	5.8 a	41.8 ab	295.2 bcd
	24.6	100	6.1 a	36.2 bc	126.3 cd
TDZ	4.5	0.0	0.0 d	-	-
	11.4	0.0	0.0 d	-	-
	22.7	0.0	0.0 d	-	-

*See explanation table 1 – Patrz objaśnienia do tabeli 1

Table 5. Survival and growth of micropropagated plants of *Rheum rhaponticum* 'Karpow Lipskiego' 1 month after transplanting to the greenhouse

Tabela 5. Przeżywalność i wzrost roślin *Rheum rhaponticum* 'Karpow Lipskiego' rozmnażanych *in vitro*, 1 miesiąc po przeniesieniu do warunków szklarniowych

Growth regulators in multiplication stage Regulatory wzrostu stosowane w etapie namnażania	Concentration Stężenie µmol·dm^{-3}	Survival of plants Przeżywalność roślin %	Number of leaves/plant Liczba liści na roślinie	Height of plant Wysokość roślin cm
Control Kontrola	0.0	88	4.6 bc*	12.1 e
BA	4.4	100	4.5 bc	17.5 c
	11.1	100	5.0 a	19.1 bc
	22.2	77	4.8 b	17.6 c
Kinetin Kinetyna	4.7	100	4.3 b	15.8 d
	11.6	100	5.5 a	18.6 c
	23.3	100	4.6 bc	17.0 cd
2iP	4.9	100	4.5 bc	22.1 ab
	12.3	100	5.3 ab	24.1 a
	24.6	100	4.1 c	25.0 a

*See explanation table 1 – Patrz objaśnienia do tabeli 1

Table 6. Survival and growth micropropagated plants of *Rheum rhaponticum* 'Karpow Lipskiego', 8 months after transplanting to the field. Plants were transferred to the field in September 2007 and recorded in May 2008

Tabela 6. Przeżywalność i wzrost roślin *Rheum rhaponticum* 'Karpow Lipskiego' rozmnażanych *in vitro*, 8 miesięcy po posadzeniu w polu. Rośliny posadzono we wrześniu 2007 i oceniano w maju 2008 r.

Growth regulators in multiplication stage Regulatory wzrostu stosowane w etapie namnażania	Concentration Stężenie µmol·dm^{-3}	Survival of plants Przeżywalność roślin %	Diameter of leaf rosette Średnica rozety liściowej cm	Number of leaves/rosette Liczba liści w rozecie	Length of leaf petiole Długość ogonka liściowego cm	Width of leaf petiole Grubość ogonka liściowego cm	Length of leaf blade Długość blaszki liściowej cm	Width of leaf blade Szerokość blaszki liściowej cm
Control Kontrola	0.0	100	36.2 c*	3.3 d	6.9 c	0.5 f	11.9 c	13.5 c
BA	4.4	80	34.8 d	3.5 c	6.7 c	0.9 de	12.6 c	11.1 c
	11.1	90	38.6 c	6.6 b	10.9 b	1.1 cd	15.4 b	10.8 c
	22.2	100	33.9 d	5.6 b	7.9 c	0.8 e	14.1 bc	11.0 c
Kinetin Kinetyna	4.7	100	40.5 c	4.0 c	8.7 c	0.5 f	16.8 b	11.2 c
	11.6	100	45.5 bc	5.0 c	12.4 b	1.2 bc	16.8 b	13.4 c
	23.3	100	34.5 cd	5.5 c	7.1 c	1.4 ab	13.2 c	10.4 c
2iP	4.9	100	48.8 b	6.0 b	9.2 bc	1.2 bc	17.5 b	18.3 b
	12.3	100	62.5 a	12.5 a	22.7 a	1.6 a	28.5 a	31.3 a
	24.6	100	52.2 b	7.7 b	10.5 b	1.5 a	14.5 b	22.2 b

*See explanation table 1 – Patrz objaśnienia do tabeli 1

Fig. 3. Plants of *Rheum rhaponticum* L. 'Karpow Lipskiego' obtained after 2 passages on the MS medium containing different concentrations of 2iP
Ryc. 3. Rośliny *Rheum rhaponticum* L. 'Karpow Lipskiego' uzyskane po 2 pasażach na pożywce MS uzupełnionej 2iP w różnych stężeniach

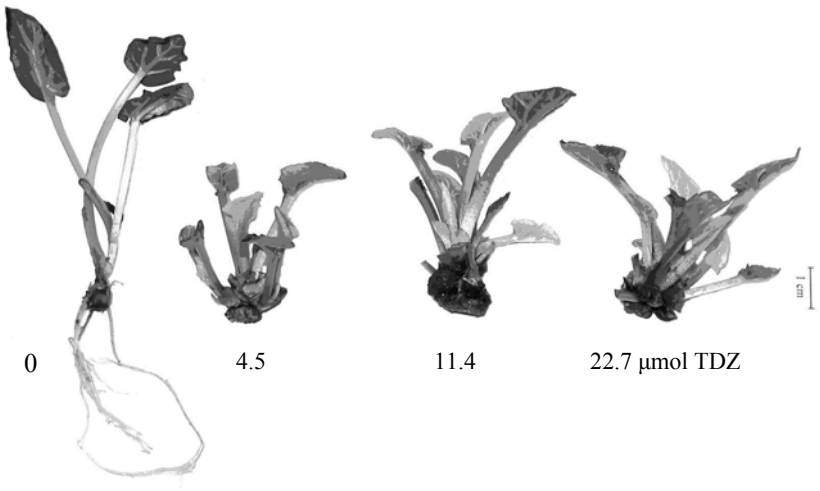

Fig. 4. Plants of *Rheum rhaponticum* L. 'Karpow Lipskiego' obtained after 2 passages on the MS medium containing different concentrations of TDZ
Ryc. 4. Rośliny *Rheum rhaponticum* L. 'Karpow Lipskiego' uzyskane po 2 pasażach na pożywce MS uzupełnionej TDZ w różnych stężeniach

One of more difficult stages of micropropagation is the transfer of plants from *in vitro* cultures to a growing substrate in a greenhouse or in field. The data presented in Table 5 show that the degree of plant survival after a 1-month period of adaptation under greenhouse climate conditions was dependent on growth regulators applied. In the case of plantlets multiplied on medium supplemented with BA at a concentration of 22.2 μmol·dm^{-3}, the plant survival rate was 77%, whereas for the plants from the control treatment, in which no growth regulators were applied, this rate was 88%. 100% of plants survived in the other treatment combinations.

After a period of 8 months, following overwintering and the start of vegetation growth, the plant survival rate, in the treatments in which the plantlets had been raised on medium supplemented with BA at a concentration of 4.4 and 11.1 μmol·dm^{-3}, was 80 and 90%, respectively. The plantlets multiplied on the growing medium with the addition of kinetin or 2iP at all studied concentrations were rooted in 100% and no losses were recorded after an 8-month period of the plants' growth in field.

In literature, there are few reports on the acclimatization of rhubarb plants obtained from *in vitro* cultures. Lal and Ahuja [1989] report that *R. emodi* plants originating from liquid medium survived in 91.7%, whereas those raised on agar solidified medium in 89%. A study of Zhao et al. [2003] showed that the acclimatization and further growth of plantlets in the growing substrate depend on their size. Plants obtained from small plantlets survived the winter period in 26.8%, whereas plants raised from larger sized plantlets in 88.2%.

The growth regulators applied in the experiment at a medium concentration significantly increased the number of leaves produced by the plants after a 1-month period of acclimatization in the greenhouse climate. 2iP, applied at different concentrations, had the largest effect on the size of the plants. The application of BA and kinetin resulted in a smaller increase in plant height.

According to Zhao et al. [2004], rhubarb plants are not stabilised phenotypically after *in vitro* treatments. Zhao et al. [2006] explained that applied growth regulators and their different concentrations may cause morphological deformations and changes of rhubarb plants as well as they may result in low frost resistance of plants.

The number of leaves per plant, leaf size and petiole length as well as plant health decide about the potential yield-forming value of rhubarb. In the conducted experiment, the applied growth regulators affected at different degrees some morphological traits of the plants after 8 months of growth and overwintering in the nursery. It was demonstrated that 2iP applied at a concentration of 12.3 μmol·dm^{-3} had the highest residual effect on plant growth. The plants from plantlets cultured on the medium containing 2iP had a well-balanced and compact habit and they developed more leaves in the rosette with significantly larger lamina as well as longer and thicker petioles. The plants obtained from plantlets cultured on the medium with kinetin and BA applied, as well as in the control treatment, grew less intensively. A part of the plants from these treatments was characterised by distorted habit and tiny, often deformed leaves. In effect, the plants in these experimental plots developed, on the average, fewer leaves in the rosette, with a smaller petiole length and thickness.

In an earlier study of Zhao et al. [2005a] on *in vitro* propagated rhubarb, the resultant somaclonal changes and plant deformations in the later period of growth and devel-

opment were termed "bushiness" by the authors. Changes in rhubarb plant morphology may also take place under the influence of environmental factors in a nursery or in field [Lassus and Voipio 1994], as well as they may be caused by an inappropriate selection of a given variety for *in vitro* propagation [Zhao et al. 2005b; 2007].

CONCLUSIONS

1. Cytokinins promoted the development of leaves as well as the formation of new shoots.

2. The highest average number of axillary shoots was found on the medium with BA 11.1–22.2 μmol·dm^{-3}.

3. The use of kinetin (4.7–11.6 μmol·dm^{-3}) or 2iP 12.3 μmol·dm^{-3} stimulated strong growth of shoots expressed in their length and size of the leaf blade.

4. All TDZ concentrations caused a significant inhibition of shoot elongation.

5. BA and TDZ had a harmful effect on the rooting of multishoots.

6. On the control medium (hormone-free) and on the media containing kinetin or 2iP, 100% of shoots produced roots.

7. After a period of 8 months, following overwintering and start of vegetation growth, the plant survival rate was 80–100%.

8. It was found that 2iP 12.3 μmol·dm^{-3} had the highest after effect on plant growth. The plants derived from this medium had uniform and compact growth and they developed more leaves than plants obtained from others used media.

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WPŁYW CYTOKININ NA NAMNAŻANIE PĘDÓW RABARBARU (*Rheum rhaponticum* L.) 'KARPOW LIPSKIEGO' *in vitro* ORAZ AKLIMATYZACJĘ I WZROST *ex vitro*

Streszczenie. Badano wpływ 4 cytokinin: BA (4,4; 11,1; 22,2 µmol·dm^{-3}), kinetyny (4,7; 11,6; 23,3 µmol·dm^{-3}), 2iP (4,9; 12,3; 24,6 µmol·dm^{-3}), TDZ (4,5; 11,4; 22,7 µmol·dm^{-3}), zastosowanych w pożywce Murashige i Skoog (MS), na namnażanie pędów rabarbaru (*Rheum rhaponticum* 'Karpow Lipskiego'). Pędy użyte do doświadczenia pochodziły

z aseptycznych kultur zespołów pędów. Ukorzenione pędy (z wyjątkiem kombinacji z TDZ) po aklimatyzacji sadzono do doniczek do substratu torfowego, gdzie rosły 4 tygodnie. Następnie przesadzano je w pole i uprawiano przez 8 miesięcy. Stwierdzono korzystny wpływ cytokinin na liczbę liści i formowanie nowych pędów w kulturze *in vitro*. Największą liczbę pędów kątowych uzyskano na pożywce uzupełnionej BA 11,1–22,2 µmol·dm^{-3}. Zastosowanie kinetyny (4,7–11,6 µmol·dm^{-3}) lub 2iP 12,3 µmol·dm^{-3} wpływało silnie stymulująco na wzrost elongacyjny pędów oraz wielkość blaszki liściowej. TDZ we wszystkich badanych stężeniach powodował istotne zahamowanie wzrostu pędów. BA i TDZ wywierały niekorzystny wpływ na ukorzenianie pędów. Na pożywce kontrolnej (bez regulatorów wzrostu) oraz na pożywkach zawierających kinetynę lub 2iP, 100% pędów formowało korzenie. Po okresie 8 miesięcy, obejmującym zimę i początek wegetacji, przeżywalność roślin wynosiła 80–100%. Stwierdzono, że 2iP 12,3 µmol·dm^{-3} wykazywała największy wpływ następczy na wzrost roślin. Rośliny otrzymane z mikrosadzonek pochodzących z pożywek z 2iP miały wyrównany i zwarty pokrój, wykształciły więcej liści w rozecie, o istotnie większych blaszkach liściowych oraz dłuższych i grubszych ogonkach liściowych niż rośliny pochodzące z innych zastosowanych pożywek.

Słowa kluczowe: rozkrzewianie, ukorzenianie, wpływ następczy, aklimatyzacja, wzrost w polu

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