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THE EFFECT OF CYTOKININ TYPES AND THEIR CONCENTRATION ON *in vitro* MULTIPLICATION OF *Clematis viticella* (L.) AND *Clematis integrifolia* 'Petit Faucon'

Marzena Parzymies, Marek Dąbski
University of Life Sciences in Lublin

Abstract. Determination of the optimal types and concentrations of plant growth regulators as medium constituents is one of the most important factors of successful micropropagation. With the aim of optimization of *in vitro* multiplication of *Clematis viticella* and *Clematis* 'Petit Faucon' the effect of following cytokinins on growth and branching was studied: benzyladenine (BA), isopentenyl adenine (2iP), kinetin (KIN) and thidiazuron (TDZ). The obtained results show that KIN at concentration of 10 mg·dm^{-3}or 5 mg 2iP·dm^{-3} are the best for proliferation of *Clematis viticella* shoot tips while nodal parts produce more axillary shoots on the media with 2iP at concentration of 0.62 mg·dm^{-3}. Shoot tip explants of *Clematis* 'Petit Faucon' produce the most axillary shoots in the presence of 20 mg 2iP·dm^{-3} or 10 mg KIN·dm^{-3} and nodal ones branch best on the media with addition of 1.25 mg 2iP·dm^{-3}.

Key words: shoot tips, nodal explants, cytokinins, micropropagation, branching

INTRODUCTION

Clematises (*Clematis* sp.) are vines, perennials and shrubs [Isnard et al. 2003], which can be a beautiful decoration in any garden. Nowadays clematises are propagated mainly through stem cuttings [Grey-Wilson 2000]. However, this method is ineffective. Thanks to the *in vitro* propagation it is possible to fasten the production of plants, improve the health and quality of plantlets and produce healthy mother plants for nurseries [Kreen et al. 2002]. The latest research on *Clematis* describes micropropagation through somatic embryogenesis and callus cultures [Zhang et al. 2011, Raja Naika and Krishna 2008]. This method however does not guarantee that the plant material will be identical to mother plants, thus propagation through parts of plants with merystematic tissue seems better. Micropropagation needs specific medium and culture conditions [Giri et al. 2004]. The use of cytokinins allow to increase the number of young plants. Cyto-

Corresponding author – Adres do korespondencji: Marzena Parzymies, Institute of Ornamental Plants and Landscape Architecture, University of Life Sciences in Lublin, Leszczyńskiego 58, 20-068 Lublin, Poland, e-mail: marzena1122@tlen.pl

kinins most often used in tissue cultures are BA, KIN and 2iP as well as thidiazuron which has cytokinin-like activity. The effectiveness of BA to stimulate growth of axillary shoots *in vitro* is well described [Nobre et al. 2000, Sahoo and Chand 1998]. However, high concentration of BA may cause shoots vitrification [Huang et al. 1998]. Studying propagation of plants in tissue cultures it can be observed that growth and multiplication of explants of the same species is positively influenced by different cytokinins. Kim et al. [1998] observing effect of BA and TDZ on shoots induction of three clones of *Fraxinus pennsylvanica* obtained good results using 2.2 mg TDZ·dm^{-3} or 9 mg BA·dm^{-3}. Both cytokinins had also positive effect on propagation of *Acacia sinuata* shoots [Vengadesan et al. 2002]. It happens that cytokinins added to media have a disadvantageous influence on explants growth in tissue cultures. Palacios et al. [2002] described that kinetin added to the media used for initiation of *Lonicera tatarica* shoots lowered effectiveness of induction from 55 to 30%.

The aim of the undertaken studies was to estimate the influence of cytokinins: KIN, 2iP, BA and TDZ at concentrations of 0,62–20 mg·dm^{-3} on growth and propagation of *Clematis viticella* and *Clematis integrifolia* 'Petit Faucon' shoots *in vitro*. The presented results are part of experiments leading to development of micropropagation protocol for *Clematis*.

MATERIAL AND METHODS

The experiments were conducted in the years 2003–2007 in the laboratory of the Institute of Ornamental Plants and Architecture of Landscape in the University of Life Sciences in Lublin. The plant material was excised from aseptically grown tissue cultures of *Clematis viticella* and *Clematis integrifolia* 'Petit Faucon'. The explants used in the experiments were shoot tips 15–20 mm long with one pair of developed leaves or one-node cuttings. Explants were placed into 300 ml Erlenmayer flasks filled with modified Murashige and Skoog (MS) medium [1962]. Shoot tips of *Clematis viticella* (L.) and *Clematis* 'Petit Faucon' were placed in the MS medium supplemented with the following cytokinins: kinetin (KIN), isopentenyl adenine (2iP), benzyladenine (BA) and thidiazuron (TDZ) at concentrations: 0.62; 1.25; 2.5; 5; 10 and 20 mg·dm^{-3}. Nodal parts of *Clematis viticella* shoots were placed in the MS medium supplemented with cytokinins: KIN 0.62, 1.25, 2.5, 5 and 10 mg·dm^{-3}, 2iP 0.62, 1.25, 2.5, 5 and 10 mg·dm^{-3} and BA at concentrations 0.62, 1.25, 2.5, 5 and 10 mg·dm^{-3}. Nodal parts of shoots of *Clematis* 'Petit Faucon' were placed in the MS media supplemented with cytokinins: KIN 1.25, 2.5 and 5 mg·dm^{-3}, 2iP 0.62, 1.25 and 2.5 mg·dm^{-3} and BA 0.62 and 1.25 mg·dm^{-3}. As a control medium the MS without growth regulators was used. Medium pH was adjusted to 5.8 prior to the addition of 6,5 g·dm^{-3} of agar and subsequently autoclaved in the temperature 121ºC for 20 min. The cultures were incubated in a culture room at a temperature of 22ºC during the day and 20ºC at night and 16-h photoperiod with irradiance of 35 μmol·m^{-2}·s^{-1}. Each combination included 21 shoots. One flask with 7 shoots was treated as a replication. Each experiment was repeated twice and lasted four weeks.

The following multiplication parameters were monitored: length (mm), weight (mg) and number of leaves and nodes of main shoots, percentage of shoots with axillary

shoots, number of axillary shoots, length (mm), weight (mg) and number of leaves of axillary shoots, percentage of main shoots with callus and weight of callus (mg). Some specific issues, such as colour, leaf and callus size, leaf roll, incidence of chlorosis, necrosis or deformations were also monitored. The results obtained in the experiments were evaluated statistically with the use of analysis of variance and Tukey t-test at 5% level of significance. For use in the analysis of variance all percentage values were transformed on square degrees according to Bliss method [1938] and they were retransformed after calculations had been done.

RESULTS

Cytokinins used in the experiment affected growth and development of *Clematis viticella* shoot tips (tab. 1).

Table 1. Growth and development of *Clematis viticella* shoot tips in the presence of cytokinins
Tabela 1. Wzrost i rozwój eksplantatów wierzchołkowych *Clematis viticella* w obecności cytokinin

Cytokinin Cytokinina	Concentration Stężenie ($mg \cdot dm^{-3}$)	Main shoot length Wysokość pędu głównego (mm)	Number of leaves on the main shoot Liczba liści pędu głównego	Number of nodes Liczba węzłów	Main shoot weight Masa pędu głównego (mg)	Percentage of the shoots with callus Procent pędów z kalusem	Callus weight Masa kalusa (mg)
Control Kontrola	0	8.0_{c-f}*	4.5_{bc}	2.2_{bc}	13.8_{b}	0_{f}	-
KIN	0.62	8.4_{b-f}	4.5_{bc}	2.3_{bc}	13.8_{b}	0_{f}	-
	1.25	12.3_{ab}	5.8_{a-c}	2.9_{a-c}	17.6_{ab}	24_{d-f}	9.7_{ab}
	2.5	14.4_{a}	6.1_{a-c}	3.0_{a-c}	20.2_{ab}	19_{ef}	3.8_{b}
	5	7.2_{d-f}	4.9_{a-c}	2.4_{a-c}	17.1_{ab}	19_{ef}	3.8_{b}
	10	7.1_{d-f}	5.1_{a-c}	2.5_{a-c}	15.3_{ab}	38_{b-f}	4.4_{b}
	20	6.4_{ef}	4.3_{c}	2.1_{c}	15.3_{ab}	52_{a-e}	6.3_{b}
2iP	0.62	10.6_{a-d}	5.5_{a-c}	2.8_{a-c}	21.4_{ab}	36_{b-f}	5.2_{b}
	1.25	10.7_{a-d}	6.0_{a-c}	3.0_{a-c}	22.8_{a}	67_{ab}	11.0_{ab}
	2.5	9.6_{b-f}	6.1_{a-c}	3.0_{a-c}	17.3_{ab}	82_{a}	23.8_{a}
	5	9.9_{b-e}	6.1_{a-c}	3.0_{a-c}	16.7_{ab}	63_{a-c}	11.1_{ab}
	10	8.1_{b-f}	5.6_{a-c}	2.8_{a-c}	13.5_{b}	57_{a-e}	9.2_{ab}
	20	8.3_{b-f}	5.5_{a-c}	2.7_{a-c}	14.3_{ab}	57_{a-e}	5.5_{b}
BA	0.62	11.8_{a-c}	6.8_{a}	3.4_{a}	17.0_{ab}	61_{a-d}	4.0_{b}
	1.25	8.0_{c-f}	6.0_{a-c}	3.0_{a-c}	16.9_{ab}	62_{a-d}	3.5_{b}
	2.5	8.2_{b-f}	6.1_{ab}	3.1_{a-c}	18.3_{ab}	70_{ab}	9.7_{ab}
	5	9.1_{b-f}	6.4_{ab}	3.2_{ab}	15.6_{ab}	43_{b-e}	3.2_{b}
	10	7.0_{d-f}	5.7_{a-c}	2.8_{a-c}	14.5_{ab}	38_{b-f}	3.1_{b}
	20	7.2_{d-f}	5.8_{a-c}	2.9_{a-c}	14.6_{ab}	36_{b-f}	3.7_{b}
TDZ	0.62	7.4_{d-f}	5.2_{a-c}	2.6_{a-c}	15.4_{ab}	44_{a-e}	14.3_{ab}
	1.25	8.4_{b-f}	5.8_{a-c}	2.9_{a-c}	17.3_{ab}	36_{b-f}	12.5_{ab}
	2.5	8.7_{b-f}	6.1_{a-c}	3.0_{a-c}	18.5_{ab}	56_{a-e}	3.2_{b}
	5	5.7_{f}	4.8_{a-c}	2.4_{a-c}	13.6_{b}	26_{c-f}	8.8_{ab}
	10	5.8_{ef}	4.9_{a-c}	2.4_{a-c}	15.6_{ab}	33_{b-f}	17.5_{ab}
	20	5.8_{ef}	4.8_{a-c}	2.4_{a-c}	15.7_{ab}	20_{ef}	22.5_{a}

* means followed by the same letter do not differ significantly at $\alpha = 0.05$ – średnie oznaczone tą samą literą nie różnią się istotnie przy $\alpha = 0,05$

The elongation was the best when kinetin at concentration of 2.5 mg·dm^{-3} (14.4 mm) was used. Similar results were observed on media supplemented with 1.25 mg KIN·dm^{-3} (12.3 mm), 2iP at concentrations of 0.62 mg·dm^{-3} and 1.25 mg·dm^{-3} (respectively 10.6 and 10.7 mm) and 0.62 mg BA·dm^{-3} (11.8 mm). It was observed that BA at concentrations of 0.62, 2.5 and 5 mg positively influences development of nodes in comparison to the control medium and combinations with 0.62 and 20 mg KIN·dm^{-3}.

Multiplication of *Clematis viticella* depended on type and concentration of cytokinin added to the media (tab. 2).

Table 2. Growth and development of axillary shoots of *Clematis viticella* shoot tips in the presence of cytokinins

Tabela 2. Wzrost i rozwój pędów pachwinowych z eksplantatów wierzchołkowych *Clematis viticella* w obecności cytokinin

Cytokinin Cytokinina	Concentration Stężenie (mg·dm^{-3})	Percentage of branched shoots Procent pędów rozkrzewionych	Number of axillary shoots per explant Liczba pędów kątowych / eksplantat	Length of axillary shoots Długość pędów kątowych (mm)	Number of leaves on axillary shoots Liczba liści na pędach kątowych	Weight of axillary shoots Masa pędów kątowych (mg)
Control Kontrola	0	5_{cd}*	1.0_{c}	$2.0_{d\text{-}f}$	2.0_{d}	$3.6_{c\text{-}f}$
KIN	0.62	$18_{b\text{-}d}$	1.0_{c}	$2.5_{b\text{-}f}$	$2.7_{b\text{-}d}$	$2.2_{d\text{-}g}$
	1.25	$18_{b\text{-}d}$	1.0_{c}	$2.3_{c\text{-}f}$	2.7_{cd}	$2.4_{d\text{-}g}$
	2.5	$24_{b\text{-}d}$	1.1_{bc}	$2.6_{b\text{-}f}$	2.4_{cd}	$4.3_{b\text{-}d}$
	5	$28_{b\text{-}d}$	$1.5_{a\text{-}c}$	$2.7_{b\text{-}e}$	$2.8_{b\text{-}d}$	6.9_{ab}
	10	$37_{a\text{-}c}$	$1.4_{a\text{-}c}$	$2.8_{b\text{-}e}$	$2.7_{b\text{-}d}$	$4.1_{b\text{-}e}$
	20	$19_{b\text{-}d}$	$1.6_{a\text{-}c}$	1.6_{ef}	2.0_{d}	$1.5_{d\text{-}g}$
2iP	0.62	7_{cd}	1.0_{c}	7.5_{a}	4.0_{a}	8.0_{a}
	1.25	$28_{b\text{-}d}$	$1.2_{a\text{-}c}$	$3.4_{b\text{-}d}$	$2.9_{b\text{-}d}$	6.4_{ab}
	2.5	$26_{b\text{-}d}$	$1.2_{a\text{-}c}$	$2.3_{c\text{-}f}$	2.0_{d}	6.8_{ab}
	5	43_{ab}	1.7_{ab}	1.5_{ef}	2.1_{cd}	$1.1_{e\text{-}g}$
	10	5_{cd}	1.0_{c}	1.0_{f}	2.0_{d}	0.3_{g}
	20	3_{d}	1.0_{c}	1.0_{f}	2.0_{d}	0.3_{g}
BA	0.62	50_{ab}	1.8_{a}	4.0_{b}	$3.1_{a\text{-}c}$	$4.0_{b\text{-}f}$
	1.25	67_{a}	$1.6_{a\text{-}c}$	$2.8_{b\text{-}e}$	$2.8_{b\text{-}d}$	$2.9_{d\text{-}g}$
	2.5	51_{ab}	$1.6_{a\text{-}c}$	$2.5_{b\text{-}f}$	$2.8_{b\text{-}d}$	$2.1_{d\text{-}g}$
	5	$26_{b\text{-}d}$	$1.5_{a\text{-}c}$	1.4_{ef}	2.5_{cd}	$1.8_{d\text{-}g}$
	10	$27_{b\text{-}d}$	$1.5_{a\text{-}c}$	1.7_{ef}	2.5_{cd}	$2.1_{d\text{-}g}$
	20	$21_{b\text{-}d}$	$1.6_{a\text{-}c}$	$1.4_{b\text{-}f}$	2.2_{cd}	0.9_{fg}
TDZ	0.62	$18_{b\text{-}d}$	$1.4_{a\text{-}c}$	3.7_{bc}	3.7_{ab}	$3.4_{c\text{-}f}$
	1.25	$18_{b\text{-}d}$	$1.2_{a\text{-}c}$	3.6_{bc}	2.5_{cd}	$3.1_{d\text{-}g}$
	2.5	$21_{b\text{-}d}$	$1.2_{a\text{-}c}$	$2.1_{c\text{-}f}$	2.3_{cd}	$1.5_{d\text{-}g}$
	5	$20_{b\text{-}d}$	$1.3_{a\text{-}c}$	$2.1_{c\text{-}f}$	2.4_{cd}	$1.6_{d\text{-}g}$
	10	$31_{b\text{-}d}$	$1.5_{a\text{-}c}$	$2.3_{b\text{-}f}$	2.3_{cd}	$2.5_{d\text{-}g}$
	20	$29_{b\text{-}d}$	1.7_{ab}	$1.9_{d\text{-}f}$	2.3_{cd}	$2.6_{d\text{-}g}$

* means followed by the same letter do not differ significantly at $\alpha = 0.05$ – średnie oznaczone tą samą literą nie różnią się istotnie przy $\alpha = 0{,}05$

The most plants with axillary shoots were observed on the media supplemented with BA at concentration of 1.25 mg·dm^{-3} (67%). Similar results were obtained in the presence of BA at concentrations of 0.62 and 2.5 mg·dm^{-3} (50 and 51% respectively) and KIN at concentration of 10 mg·dm^{-3} (37%). The least shoots with axillary shoots formed on the media supplemented with 20 and 10 mg 2iP·dm^{-3} (3% and 5% respectively) and in the control media (5%).The most axillary shoots per explants were formed in the presence of 0.62 mg BA·dm^{-3} (1.8). Good results were also observed in combinations with KIN at concentrations of 5–20 mg·dm^{-3}, 2iP at concentrations 1.25 and 2.5 mg·dm^{-3}, BA at concentrations of 1.25–20 mg·dm^{-3} and TDZ at all concentrations used. The longest axillary shoots formed in the presence of 0.62 mg 2iP·dm^{-3} (7.5 mm).

Table 3. Growth and development of *Clematis* 'Petit Faucon' shoot tips in the presence of cytokinins

Tabela 3. Wzrost i rozwój eksplantatów wierzchołkowych *Clematis* 'Petit Faucon' w obecności cytokinin

Cytokinin Cytokinina	Concentration Stężenie (mg·dm^{-3})	Main shoot length Wysokość pędu głównego (mm)	Number of leaves on the main shoot Liczba liści pędu głównego	Number of nodes Liczba węzłów	Main shoot weight Masa pędu głównego (mg)	Percentage of the shoots with callus Procent pędów z kalusem	Callus weight Masa kalusa (mg)
Control Kontrola	0	7.9_{a-d}*	4.8_{a-c}	2.4_{a-c}	31.6_{a}	31_{d}	4.6_{c}
KIN	0.62	8.3_{ab}	4.2_{a-c}	2.1_{a-c}	23.8_{a}	35_{cd}	4.4_{c}
	1.25	10.5_{a}	5.7_{a}	2.8_{a}	25.7_{a}	55_{a-d}	7.8_{b-c}
	2.5	7.6_{a-d}	4.9_{a-c}	2.4_{a-c}	41.0_{a}	86_{ab}	12.7_{a-c}
	5	7.2_{a-d}	4.9_{a-c}	2.4_{a-c}	44.1_{a}	83_{a-c}	14.9_{a-c}
	10	7.1_{a-d}	4.8_{a-c}	2.4_{a-c}	32.5_{a}	82_{a-c}	28.1_{ab}
	20	5.1_{b-d}	4.3_{a-c}	2.2_{a-c}	23.0_{a}	83_{a-c}	17.9_{a-c}
2iP	0.62	6.6_{a-d}	4.1_{a-c}	2.1_{a-c}	30.1_{a}	78_{a-d}	15.1_{a-c}
	1.25	6.3_{a-d}	4.5_{a-c}	2.3_{a-c}	29.1_{a}	85_{ab}	15.6_{a-c}
	2.5	5.8_{b-d}	4.5_{a-c}	2.2_{a-c}	31.4_{a}	85_{ab}	24.0_{a-c}
	5	4.9_{b-d}	4.7_{a-c}	2.3_{a-c}	42.6_{a}	88_{a}	31.7_{a}
	10	4.7_{b-d}	4.2_{a-c}	2.1_{a-c}	39.9_{a}	93_{a}	31.7_{a}
	20	4.7_{b-d}	4.2_{a-c}	2.1_{a-c}	21.7_{a}	84_{a-c}	17.1_{a-c}
BA	0.62	8.2_{a-c}	5.4_{ab}	2.7_{ab}	31.2_{a}	66_{a-d}	22.6_{a-c}
	1.25	7.8_{a-d}	5.4_{ab}	2.7_{ab}	30.4_{a}	67_{a-d}	23.9_{a-c}
	2.5	6.4_{a-d}	5.4_{ab}	2.7_{ab}	29.7_{a}	75_{a-d}	23.9_{a-c}
	5	5.9_{b-d}	5.0_{a-c}	2.5_{a-c}	29.5_{a}	76_{a-d}	20.4_{a-c}
	10	5.6_{b-d}	5.1_{a-c}	2.5_{a-c}	28.4_{a}	80_{a-d}	20.3_{a-c}
	20	5.4_{b-d}	4.9_{a-c}	2.5_{a-c}	27.7_{a}	60_{a-d}	16.1_{a-c}
TDZ	0.62	5.7_{b-d}	4.7_{a-c}	2.3_{a-c}	26.5_{a}	57_{a-d}	21.3_{a-c}
	1.25	5.5_{b-d}	4.7_{a-c}	2.3_{a-c}	28.8_{a}	57_{a-d}	20.5_{a-c}
	2.5	4.9_{b-d}	4.3_{a-c}	2.1_{a-c}	21.5_{a}	63_{a-d}	14.7_{a-c}
	5	3.9_{c-d}	3.5_{c}	1.7_{c}	21.8_{a}	64_{a-d}	14.5_{a-c}
	10	3.7_{d}	3.6_{bc}	1.8_{bc}	23.3_{a}	69_{a-d}	11.4_{a-c}
	20	3.6_{d}	3.5_{c}	1.7_{c}	22.1_{a}	52_{a-d}	10.2_{b-c}

*means followed by the same letter do not differ significantly at α = 0.05 – średnie oznaczone tą samą literą nie różnią się istotnie przy α = 0,05

On the basis of visual observations it was found that good quality shoots formed in the media supplemented with 2iP and KIN at concentrations of 0.62–5 mg·dm^{-3}. Increasing concentration of these cytokinins lowered the quality of axillary shoots. In the presence of BA at higher concentrations many shoots were deformed. Presence of TDZ stimulated growth of deformed axillary shoots and leaves rolled.

Cytokinins used in the research influenced growth and development of *Clematis* 'Petit Faucon' shoot tip explants (tab. 3).

Table 4. Growth and development of axillary shoots of *Clematis* 'Petit Faucon' shoot tips in the presence of cytokinins

Tabela 4. Rozkrzewianie eksplantatów wierzchołkowych *Clematis* 'Petit Faucon' w obecności cytokinin

Cytokinin Cytokinina	Concentration Stężenie (mg·dm^{-3})	Percentage of branched shoots Procent pędów rozkrzewionych	Number of axillary shoots per explant Liczba pędów kątowych / eksplantat	Length of axillary shoots Długość pędów kątowych (mm)	Number of leaves on axillary shoots Liczba liści na pędach kątowych	Weight of axillary shoots Masa pędów kątowych (mg)
Control Kontrola	0	0_{d}*	-	-	-	-
KIN	0.62	0_{d}	-	-	-	-
	1.25	0_{d}	-	-	-	-
	2.5	7_{b-d}	1.0_{e}	2.2_{c-f}	2.0_{c}	4.4_{b-g}
	5	9_{b-d}	1.0_{e}	2.3_{b-e}	2.0_{c}	6.3_{a-d}
	10	26_{a-d}	1.4_{b-e}	2.8_{b-e}	2.0_{c}	5.2_{a-e}
	20	9_{b-d}	1.2_{de}	3.1_{bc}	2.0_{c}	5.4_{a-d}
2iP	0.62	0_{d}	-	-	-	-
	1.25	2_{cd}	1.0_{e}	1.0_{fg}	3.0_{a}	1.1_{gh}
	2.5	2_{cd}	1.0_{e}	6.2_{a}	2.0_{c}	8.4_{a}
	5	9_{b-d}	1.0_{e}	1.7_{ef}	2.0_{c}	6.6_{ab}
	10	8_{b-d}	1_{e}	1.0_{f}	2.0_{c}	0.2_{h}
	20	14_{a-d}	2_{ab}	1.0_{f}	2.0_{c}	0.2_{h}
BA	0.62	29_{a-d}	1.6_{a-e}	3.5_{b}	2.6_{ab}	4.5_{b-g}
	1.25	45_{a}	1.7_{a-e}	3.0_{b-d}	2.3_{bc}	2.8_{c-h}
	2.5	31_{a-b}	1.6_{a-e}	1.9_{c-f}	2.3_{bc}	2.6_{d-h}
	5	28_{a-d}	2.2_{a}	1.7_{ef}	2.0_{c}	1.6_{f-h}
	10	29_{a-d}	1.8_{a-d}	1.8_{d-f}	2.1_{c}	1.8_{d-h}
	20	23_{a-d}	1.3_{c-e}	1.7_{ef}	2.0_{c}	1.2_{gh}
TDZ	0.62	26_{a-d}	1.4_{b-e}	2.6_{b-e}	2.2_{bc}	4.8_{b-f}
	1.25	34_{a-c}	1.4_{b-e}	2.5_{b-e}	2.2_{bc}	3.2_{b-h}
	2.5	37_{a-c}	1.4_{b-e}	2.1_{c-f}	2.2_{bc}	1.5_{f-h}
	5	26_{a-d}	1.5_{b-e}	1.8_{d-f}	2.0_{c}	1.5_{f-h}
	10	20_{a-d}	1.9_{a-c}	1.6_{ef}	2.0_{c}	1.7_{e-h}
	20	15_{a-d}	1.5_{b-e}	1.6_{ef}	2.0_{c}	1.5_{f-h}

* means followed by the same letter do not differ significantly at $\alpha = 0.05$ – średnie oznaczone tą samą literą nie różnią się istotnie przy $\alpha = 0,05$

It was observed that KIN at concentration of 1.25 mg·dm^{-3} had the biggest effect on elongation of shoots (10.5 mm) in comparison to 2iP at concentrations 2.5–20 mg·dm^{-3}, BA at concentrations of 5–20 mg·dm^{-3}, KIN at concentration of 20 mg·dm^{-3} and TDZ at all concentrations used.

The multiplication of *Clematis* 'Petit Faucon' explants also depended on the type and concentration of cytokinin used in the experiment (tab. 4). The most shoots with axillary shoots were formed in the media supplemented with 1.25 mg BA·dm^{-3} (45%). Similar results were obtained on the media with BA at all other concentrations, 2iP at concentration of 20 mg·dm^{-3}, KIN at concentration of 10 mg·dm^{-3} and TDZ at concentrations of 0.62–20 mg·dm^{-3}. The biggest number of axillary shoots was observed in the presence of BA at concentration of 5 mg·dm^{-3}. Many axillary shoots developed also on the media with BA at concentrations of 0.62–2.5 mg·dm^{-3} and 10 mg·dm^{-3} and 2iP at concentration of 20 mg·dm^{-3}. The axillary shoots were the longest at presence of 2.5 mg 2iP·dm^{-3} (6.2 mm).

On the basis of visual observations it was found that in the presence of 2iP and KIN at concentrations of 0.62–2.5 mg·dm^{-3}and on the control media it was possible to get good quality plants. Higher concentrations of these cytokinins lowered the quality of microshoots. In the presence of BA and TDZ many shoots were deformed.

Table 5. Growth and development of axillary shoots of *Clematis viticella* nodes in the presence of cytokinins

Tabela 5. Rozkrzewianie eksplantatów węzłowych *Clematis viticella* w obecności cytokinin

Cytokinin Cytokinina	Concentration Stężenie (mg·dm^{-3})	Percentage of branched shoots Procent pędów rozkrzewionych	Number of axillary shoots per explant Liczba pędów kątowych / eksplantat	Length of axillary shoots Długość pędów kątowych (mm)	Number of leaves on axillary shoots Liczba liści na pędach kątowych	Weight of axillary shoots Masa pędów kątowych (mg)
Control Kontrola	0	81_{bc}*	1.3_{cd}	5.4_{c-e}	3.8_{a-c}	5.6_{bc}
KIN	0.62	62_{c}	0.9_{e}	5.6_{c-e}	3.9_{a-c}	7.0_{bc}
	1.25	76_{bc}	1.2_{de}	6.4_{b-d}	3.9_{a-c}	7.4_{bc}
	2.5	83_{a-c}	1.2_{de}	6.6_{bc}	4.5_{ab}	7.3_{bc}
	5	100_{a}	1.3_{cd}	9.6_{ab}	4.9_{a}	10.5_{ab}
	10	71_{c}	1.4_{b-d}	2.8_{e}	2.9_{c}	4.5_{c}
2iP	0.62	100_{a}	1.8_{a}	11.2_{a}	4.6_{ab}	12.9_{a}
	1.25	94_{ab}	1.5_{a-d}	4.3_{c-e}	3.7_{a-c}	7.4_{bc}
	2.5	84_{a-c}	1.4_{b-d}	4.5_{c-e}	3.5_{a-c}	7.9_{bc}
	5	85_{a-c}	1.4_{b-d}	3.2_{de}	3.4_{bc}	6.0_{bc}
	10	71_{bc}	1.4_{b-d}	3.4_{c-e}	3.4_{bc}	5.7_{bc}
BA	0.62	66_{c}	1.7_{a-c}	3.4_{c-e}	2.8_{c}	4.0_{c}
	1.25	82_{bc}	1.8_{a}	5.9_{c-e}	4.3_{a-c}	6.1_{bc}
	2.5	83_{a-c}	1.9_{a}	4.6_{c-e}	3.7_{a-c}	7.6_{bc}
	5	90_{a-c}	1.8_{ab}	4.6_{c-e}	3.8_{a-c}	8.0_{a-c}
	10	90_{a-c}	1.8_{ab}	3.0_{e}	3.0_{c}	7.2_{bc}

* means followed by the same letter do not differ significantly at $\alpha = 0.05$ – średnie oznaczone tą samą literą nie różnią się istotnie przy $\alpha = 0,05$

Multiplication of *Clematis viticella* from nodal explants depended on the type and concentration of cytokinins added to media (tab. 5). It was observed that axillary shoots were formed in each combination. In the presence of KIN at concentration of 5 mg·dm^{-3} and 2iP at concentration of 0.62 mg·dm^{-3} 100% of explants formed axillary shoots. The most axillary shoots per explant formed on media supplemented with BA at all concentrations used and 0.62 1.25 mg 2iP·dm^{-3}. The longest axillary shoots were observed in the presence of 2iP at concentration of 0.62 mg·dm^{-3} (11.2 mm). On the basis of visual observations it could be stated that good quality axillary shoots were formed on the media supplemented with 2iP at concentrations of 0.62–2.5 mg·dm^{-3} and on the control media. In the presence of BA shoots were smaller, deformed and yellowish.

On the basis of the statistical analysis it was proven that nodal explants of *Clematis* 'Petit Faucon' formed axillary shoots in all combinations (tab. 6).

Table 6. Growth and development of axillary shoots of *Clematis* 'Petit Faucon' nodes in the presence of cytokinins

Tabela 6. Rozkrzewianie ekplantatów węzłowych *Clematis* 'Petit Faucon' w obecności cytokinin

Cytokinin Cytokinina	Concentration Stężenie (mg·dm^{-3})	Percentage of branched shoots Procent pędów rozkrzewionych	Number of axillary shoots per explant Liczba pędów kątowych / eksplantat	Length of axillary shoots Długość pędów kątowych (mm)	Number of leaves on axillary shoots Liczba liści na pędach kątowych	Weight of axillary shoots Masa pędów kątowych (mg)
Control Kontrola	0	100_a*	1.2_{bc}	3.6_{ab}	3.1_a	7.2_{ab}
KIN	1.25	83_a	0.8_c	5.7_{ab}	3.9_a	15.6_a
	2.5	100_a	1.2_{bc}	3.4_{ab}	3.1_a	7.6_{ab}
	5	100_a	1.2_{bc}	4.0_{ab}	3.7_a	12.8_{ab}
2iP	0.62	89_a	2.1_a	3.8_{ab}	3.5_a	11.0_{ab}
	1.25	100_a	1.8_{ab}	5.1_{ab}	3.8_a	12.6_{ab}
	2.5	100_a	1.7_{a-c}	2.6_b	3.2_a	4.6_b
BA	0.62	92_a	2.0_{ab}	5.7_a	4.2_a	11.3_{ab}
	1.25	100_a	2.0_{ab}	3.9_{ab}	3.6_a	9.5_{ab}

* means followed by the same letter do not differ significantly at α = 0.05 – średnie oznaczone tą samą literą nie różnią się istotnie przy α = 0,05

Number of axillary shoots per node depended on the cytokinin added to the media. The most axillary shoots were observed on the media supplemented with 0.62 mg 2iP·dm^{-3} (2.1) in comparison to KIN at concentrations of 1.25 mg·dm^{-3} (0.8) and 2.5–5 mg·dm^{-3} (1.2) and control media (1.2). On the basis of visual observations there were not morphological differences between combinations.

DISCUSSION

There is very little information on propagation of *Clematis* sp. in tissue culture. Most of available papers describe micropropagation of *Clematis* through callus cultures. This method does not guarantee production of plants identical to mother plants. Technology based on the explants with meristematic tissue would allow to get clonally identical and healthy plant material.

Growth regulators have very big effect on development of explants in tissue cultures. Undertaken experiments also proved significant influence of cytokinins on branching of *Clematis* depending on genotype.

Shoot tips of *Clematis* 'Petit Faucon' branched most on the media supplemented with BA at concentration of 1.25 mg·dm^{-3}, but taking into consideration quality of plants it was decided that KIN at concentration of 20 mg·dm^{-3} or 2iP at concentration of 2.5 mg·dm^{-3} are better. In presence of these cytokinins many axillary shoots were formed. Nodal explants of *Clematis* 'Petit Faucon' branched in all combinations. Taking into consideration number and quality of axillary shoots the best results were achieved when 1.25 mg BA·dm^{-3}or 1.25 mg 2iP·dm^{-3} were used. Growth and development of *Clematis viticella* shoot tips and nodes also depended on type and concentration of cytokinin added to the media. The best branching was observed in the presence of BA at concentrations of 0.62–2.5 mg·dm^{-3}, but axillary shoots were deformed and were not suitable for farther propagation. Many good quality axillary shoots were obtained on the media supplemented with 5 mg 2iP·dm^{-3} and 20 mg KIN·dm^{-3}. 100% nodal explants formed axillary shoots in presence of 2iP at concentration of 0.62 mg·dm^{-3} and KIN at concentration of 5 mg·dm^{-3}.

In the previous research it was observed that branching of *Clematis integrifolia* was best on the media supplemented with KIN 10 mg·dm^{-3} and 2iP 20 mg·dm^{-3} [Dąbski and Parzymies 2006]. The most often used cytokinin in tissue cultures is BA. It's effectiveness is well proven. It was used for *in vitro* propagation of *Arbutus unedo* [Mereti et al. 2002], *Vitis thunbergii* [Lu 2005], *Forsythia koreana* 'Suwon Gold' [Kyung-Ku Shim and Yoo-Mi Ha 1997], *Ranunculus lyalii* [Bicknell et al. 1996]. However, it may cause vitrification of shoots and inhibit elongation [Hosoki et al. 2003], which was also observed in this research. 2iP and KIN seems to be the best cytokinins to obtain many good quality shoots. The superiority of kinetin over other cytokinins was proven in many experiments. It was used for micropropagation of many ornamental plants [Sansberro et al. 2003; Ibrahim and Debergh 2000], while 2iP was used for propagation of *Syringa* × *chinensis* [Nesterowicz et al. 2006].

CONCLUSIONS

1. Obtained results suggest that *Clematis* genotypes have different media requirements as regarding to the type and concentration of cytokinins.

2. To obtain the biggest number of good quality axillary shoots from *Clematis* 'Petit Faucon' shoot tips 20 mg 2iP·dm^{-3}or 10 mg KIN·dm^{-3} is recommended. For nodal explants 2iP in concentration of 1.25 mg·dm^{-3} seems better.

3. Shoot tips of *Clematis viticella* form the biggest number of good quality axillary shoots in the presence of 10 mg KIN·dm^{-3} or 5 mg 2iP·dm^{-3} and nodes in the presence of 0.62 mg 2iP·dm^{-3}.

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WPŁYW RODZAJU I STĘŻENIA CYTOKININ NA ROZKRZEWIANIE *in vitro Clematis viticella* (L.) I *Clematis integrifolia* 'Petit Faucon'

Streszczenie. Określenie rodzaju i stężenia regulatorów wzrostu w pożywce jest jednym z najważniejszych czynników udanego mikrorozmnażania. W celu optymalizacji rozmnażania *in vitro Clematis viticella* i *Clematis integrifolia* 'Petit Faucon' zbadano wpływ następujących cytokinin: kinetyny (KIN), izopentenyloadeniny (2iP), benzyloadeniny (BA) oraz tidiazuronu (TDZ) na wzrost i rozkrzewianie pędów *Clematis viticella* i *Clematis* 'Petit Faucon' *in vitro*. Stwierdzono, że do rozkrzewiania ekplantatów wierzchołkowych *Clematis viticella* najbardziej odpowiednią cytokininą jest KIN w stężeniu 10 mg·dm^{-3} lub 5 mg 2iP·dm^{-3}, natomiast dla eksplantatów węzłowych lepsza okazała się 2iP w stężeniu 0,62 mg·dm^{-3}. Ekplantaty wierzchołkowe *Clematis* 'Petit Faucon' najlepiej rozkrzewiają się w obecności 2iP w stężeniu 20 mg·dm^{-3} lub KIN w stężeniu 10 mg·dm^{-3}, a węzłowe na pożywce zawierającej 2iP w stężeniu 1,25 mg·dm^{-3}.

Słowa kluczowe: eksplantaty wierzchołkowe, eksplantaty węzłowe, cytokininy, mikrorozmnażanie, rozkrzewianie

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