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The influence of cytokinins on *in vitro* multiplication of *Aeschynanthus hybridus* 'Carina' shoots

Wpływ cytokinin na namnażanie *in vitro* pędów
Aeschynanthus hybridus 'Carina'

Summary. Shoot tips of *Aeschynanthus hybridus* 'Carina' taken from aseptically grown shoot clusters were cultured 5 weeks *in vitro* in MS medium supplemented with cytokinins: BA (4.4, 8.9, 22.2 µM), kinetin (4.7, 9.3, 23.3 µM), 2iP (4.9, 9.9, 24.6 µM). Explants cultured on the medium without cytokinins were used as a control. A significant influence of cytokinin type and their concentration on the multiplication of shoots were observed. BA at the concentration of 22.2 µM gave the highest multiplication rate. Axillary shoots regenerated sporadically on media containing 24.6 µM 2iP. No regeneration of new shoots was obtained in the presence of kinetin. The best rooting was on the control medium and on the media with 2iP.

Key words: *Aeschynanthus hybridus*, cytokinins, micropropagation

INTRODUCTION

Aeschynanthus is a perennial plant with branched erect or hanging shoots. The foliage is composed of ovate, leathery and shiny leaves, opposite or whorled. But the greatest value of this plant is its flowers with a tubular corolla, orange, red or yellow, grouped in several-flowered clusters at the end of the shoot. Stamens and the pistil stick out above the calyx, and for many it is reminiscent of a lipstick emerging from a tube. *Aeschynanthus* is propagated from herbacerous or semi-hardwood tip cuttings. However, in mass production, particularly of new varieties, these plants can be propagated in *in vitro* cultures. Micropropagation of plants from Gesneriaceae family has been reported by many researches, e.g. *Aeschynanthus* [Dąbski and Kozak 1996, 1998, Cui *et al*. 2009], *Chirita* [Tang *et al.* 2007a, b, Li *et al*. 2009, Nakano *et al.* 2009], *Episcia* [Chee-Kok 1980], *Kohleria* [Kozak *et al.* 2006], *Lysionatus* [Lu *et al.* 2006, Godo *et al.* 2010], *Nema-*

tanthus [Li *et al*. 2001], *Saintpaulia* [Lo 1997, Khan *et al.* 2007, Daud and Taha 2008], *Sinningia* [Palazetti de Almeida and Kirszenzaft Shepherd 1999, Xu *et al.* 2009], *Streptocarpus* [Afkhami-Sarvestami *et al*. 2006]. Benzyladenine is most frequently used for shoot induction and multiplication of these species. Earlier studies of Dąbski and Kozak [1996, 1998] on micropropagation of plants of the genus *Aeschynanthus* showed that there was a great influence of the genotype on the value of the multiplication ratio and different requirements of species and varieties with respect to the cytokinin type and concentration.

The aim of the study was to compare the influence of cytokinins (BA, kinetin, 2iP) on *in vitro* multiplication and growth of *Aeschynanthus hybridus* 'Carina' shoots.

MATERIAL AND METHODS

Shoots *Aeschynanthus hybridus* 'Carina' taken from aseptically grown shoot clusters were used in this experiment. Shoot tips 5 mm long with 2 pairs fully developed leaves were isolated as a initial explants. They 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 cytokinins: BA (benzyladenine) (4.4, 8.9, 22.2 μM), kinetin (4.7, 9.3, 23.3 μM), 2iP (isopentenyladenine) (4.9, 9.9, 24.6 μM). Explants cultured on the medium without growth substances were used as a control.

Five shoot tips were incubated per 250 ml Erlenmeyer flask. Twenty five shoot tips were used for each combination. Each flask with 5 explants was a replicate.

The cultures were maintained at 22°C ±2°C with a photon flux of 35 $\mu M \cdot m^{-2} \cdot s^{-1}$ and a 16-h photoperiod. The following characters were evaluated after 5 weeks: length of main shoot, number of leaves and nodes on main shoot, length and width of 3rd from shoot tip upper pair of leaves on main shoot; number of new (axillary, base-adjoin adventitious shoots) shoots from 1 explant, their length and number of roots.

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

The analysis of the results of the conducted study demonstrated significant differences in the main shoot length, depending on the type and concentration of cytokinins. After 5 weeks of *in vitro* growth, the shoots reached the length of 8.5 up to 14.6 mm (Tab. 1). The significantly longest shoots were obtained when the explants were cultivated on the culture medium containing 2iP 24.6 μM (14.6 mm). Shoots growing in the presence of the highest BA concentration (22.2 μM), on the control medium and on the media containing Kin 9.3, 23.3 μM or 2iP 4.9 μM were characterised by the weakest elongation growth (8.5–9.8 mm). In analysing the number of leaves and the number of nodes on the main shoot, it was found that the medium supplemented with 2iP 24.6 μM had the most beneficial effect (10.4 and 5.2, respectively). The size of the 3rd pair of

leaves was the largest in the case of the shoots growing on the control medium (11.2 mm length and 8.4 mm width), however, in the presence of 2iP 9.9 µM, the size of leaves was only slightly smaller. The medium containing 22.2 µM BA suppressed the growth of leaves the most (5.6 mm length and 4.3 mm width).

Table 1. The influence of cytokinins on the growth of *Aeschynanthus hybridus* 'Carina' main shoot after 5 weeks of culture *in vitro*
Tabela 1. Wpływ cytokinin na wzrost pędu głównego *Aeschynanthus hybridus* 'Carina' po 5 tygodniach kultury *in vitro*

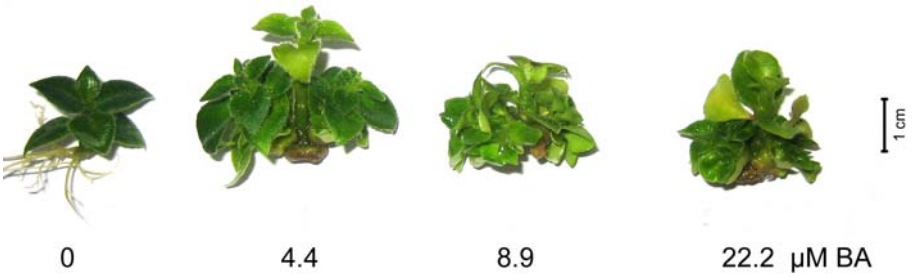
Cytokinin	Concentration µM	Length of main shoot mm	Number of leaves on main shoot	Number of nodes on main shoot	Length of 3rd pair of leaves mm	Width of 3rd pair of leaves mm
Control	0	8.9 b*	6.0 d	3.0 d	11.2 a	8.4 a
BA	4.4	12.6 ab	9.6 ab	4.9 ab	9.6 ab	7.4 ab
	8.9	11.1 ab	10.0 a	5.0 ab	8.3 bc	6.2 b
	22.2	8.5 b	8.9 abc	4.4 abc	5.6 c	4.3 c
Kinetin	4.7	10.2 ab	7.0 cd	4.0 a-d	9.8 ab	6.8 ab
	9.3	9.3 b	6.8 cd	3.4 cd	9.2 ab	6.2 b
	23.3	9.8 b	6.9 cd	3.3 cd	9.4 ab	6.7 ab
2iP	4.9	9.8 b	7.1 cd	3.8 bcd	10.3 ab	7.4 ab
	9.9	10.4 ab	7.6 bcd	3.8 bcd	10.8 ab	7.6 ab
	24.6	14.6 a	10.4 a	5.2 a	9.2 ab	7.2 ab
Mean		10.5	8.0	4.1	9.3	6.8

*Values in vertical columns followed by the same letter do not differ significantly at P = 0.05/Wartości w kolumnach oznaczone tą samą literą nie różnią się istotnie przy P = 0,05

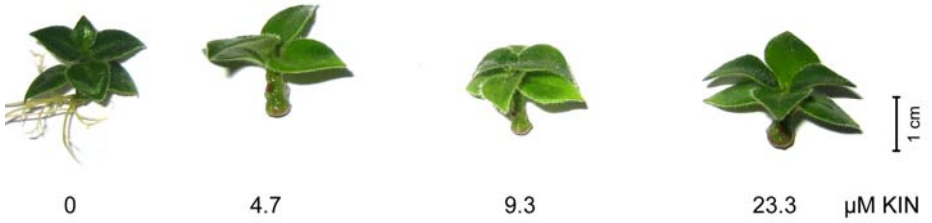
Table 2. The influence of cytokinins on the regenerative capabilities of *Aeschynanthus hybridus* 'Carina'shoots after 5 weeks of culture
Tabela 2. Wpływ cytokinin na zdolności regeneracyjne *Aeschynanthus hybridus* 'Carina' po 5 tygodniach kultury *in vitro*

Cytokinin	Concen-tration µM	Axillary shoots		Base-adjoin shoots		Roots	
		number per explant	length mm	number per explant	length mm	shoots forming roots, %	number per explant
Control	0	0 c*	-	0 b	-	100	3.0 a
BA	4.4	3.8 b	7.7 a	1.1 a	7.0 a	0	0 c
	8.9	4.6 b	4.9 b	1.1 a	6.0 a	0	0 c
	22.2	6.5 a	5.0 b	1.2 a	3.3 b	0	0 c
Kinetin	4.7	0 c	-	0 b	-	44	1.2 b
	9.3	0 c	-	0 b	-	48	1.7 b
	23.3	0 c	-	0 b	-	40	2.8 a
2iP	4.9	0 c	-	0 b	-	100	2.0 a
	9.9	0 c	-	0 b	-	100	2.4 a
	24.6	0.1 c	5.0 b	0.1 b	4.4 b	100	2.5 a
Mean		1.5	5.7	0.4	5.2	53.2	1.6

*See explanation Table 1/Patrz objaśnienie do tabeli 1



Phot. 1. Shoot regeneration from shoot tips of *Aeschynanthus hybridus* 'Carina' on MS medium supplemented with different concentration of BA after 5 weeks in tissue culture *in vitro*
Fot. 1. Regeneracja pędów z wierzchołkowych fragmentów pędów *Aeschynanthus hybridus* 'Carina' na pożywce MS z dodatkiem BA w różnym stężeniu po 5 tygodniach kultury *in vitro*



Phot. 2. Growth of shoot tips of *Aeschynanthus hybridus* 'Carina' on MS medium supplemented with different concentration of kinetin after 5 weeks in tissue culture *in vitro*
Fot. 2. Wzrost wierzchołkowych fragmentów pędów *Aeschynanthus hybridus* 'Carina' na pożywce MS z dodatkiem kinetyny w różnym stężeniu po 5 tygodniach kultury *in vitro*

Phot. 3. Growth of shoot tips of *Aeschynanthus hybridus* 'Carina' on MS medium supplemented with different concentration of 2iP after 5 weeks in tissue culture *in vitro*
Fot. 3. Wzrost wierzchołkowych fragmentów pędów *Aeschynanthus hybridus* 'Carina' na pożywce MS z dodatkiem 2iP w różnym stężeniu po 5 tygodniach kultury *in vitro*

During the maintenance of shoot cultures, the regeneration of axillary shoots and base-adjoin adventitious shoots was observed, and it was often preceded by the regeneration of the callus tissue at the place where the shoot was cut. A significant influence of cytokinins was found on the number of shoots produced by 1 explant (Tab. 2). Among 3 cytokinins investigated, BA had the most beneficial influence on regeneration abilities of shoots cultured on the growth medium (Phot. 1, 2, 3). It was observed that the number of shoots significantly increased together with the increase in BA concentration, but their growth was inhibited and the shoots were tiny. Axillary shoots (3.8–6.5) and single base-adjoin shoots (1.1–1.2) mainly regenerated. No regeneration of new shoots was obtained in the presence of kinetin, and axillary shoots regenerated sporadically (0.1) on media containing 2iP 24.6 µM. Opposite trends were observed in the case of root regeneration (Tab. 2). The best rooting was on the control medium and on the media with 2iP (Phot. 3).

Lo [1997], Afkhani-Sarvestani *et al*. [2006], Tang *et al.* 2007, Li *et al.* [2009], Nakano *et al.* [2009] obtained *in vitro* organogenesis from leaf explants of plants from Gesneriaceae family on media supplemented with 0.4–4.4 µM BA and 5.7–11.4 µM IAA or 0.5–2 µM NAA. Their results showed production of adventitious shoot buds directly from the surface of explants. Shoot development of *Sinningia allagophylla* was only observed on media containing BA alone (0.04–0.4 µM) [Palazetti de Almeida and Kirszenzaft Shepherd 1999]. Also for *Kohleria amabilis* medium with BA 4.4 µM was the best for shoot multiplication [Kozak *et al*. 2006]. Dąbski and Kozak [1996] reported that *Aeschynanthus hybridus* 'Purple Star', *A*. *hybridus* 'Rubens' and *A*. *speciosus* shoots multiplied better in the presence of BA 4.4 µM, whereas *A*. 'Mira' with the addition of 2iP 24.6 µM. In the present study, the usefulness of BA for multiplication of *Aeschynanthus hybridus* 'Carina' shoots has been demonstrated. For root induction the best was control medium and media with 2iP. Dąbski and Kozak [1996] observed root regeneration of *A*.'Purple Star', *A*.'Rubens' and *A*. *speciosus* on the all studied media (control, BA 4.4 µM, Kin 23.3 µM or 2iP 24.6 µM). Shoots of *Kohleria amabilis* formed the biggest number of roots on the control medium and with presence of 4.9 µM 2iP. Induction of roots was completely inhibited at media with 22.2 µM BA, 23.3 µM Kin or 24.6 µM 2iP [Kozak *et al.* 2006].

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Streszczenie. Wierzchołki pędów *Aeschynanthus hybridus* ‘Carina’ pochodzące ze sterylnych kultur *in vitro* rosły 5 tygodni na pożywce MS uzupełnionej cytokininami BA (4.4, 8.9, 22.2 µM), kinetin (4.7, 9.3, 23.3 µM), 2iP (4.9, 9.9, 24.6 µM). Eksplantaty wyłożone na pożywkę bez cytokininy stanowiły kontrolę. Stwierdzono istotny wpływ rodzaju i stężenia cytokininy na namnażanie pędów. Na pożywce z dodatkiem BA 22.2 µM uzyskano najwyższy współczynnik namnażania. W obecności 2iP 24.6 µM pędy kątowe regenerowały sporadycznie, natomiast nie uzyskano nowych pędów na pożywce z dodatkiem kinetyny. Najlepsze ukorzenianie pędów obserwowano w kontroli oraz na pożywkach z dodatkiem 2iP.

Słowa kluczowe: *Aeschynanthus hybridus,* cytokininy*,* mikrorozmnażanie