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**The effect of benzyladenine on shoot regeneration *in vitro*
of *Zantedeschia aethiopica* ‘Green Goddess’**

Wpływ benzyloadeniny na regenerację pędów *Zantedeschia aethiopica*
‘Green Goddess’ *in vitro*

Summary. Shoots of *Zantedeschia aethiopica* ‘Green Goddess’ obtained from aseptically grown shoot clusters were cultured *in vitro* on a Murashige and Skoog [1962] medium containing BA (benzyladenine) in concentrations: 5, 10, 25 and 50 μM . The control was a medium without of cytokinin. The highest number of axillary shoots was found on media with BA at concentration of 25 and 50 μM . The treatment with BA 25 μM stimulated the most formation of rhizomes. The roots occurred on the all of studied media. The best rooting was on the media with 5 or 10 μM of BA.

Key words: *Zantedeschia aethiopica* ‘Green Goddess’, BA, axillary shoots, *in vitro*

INTRODUCTION

The genus *Zantedeschia* (family Araceae) is native to South Africa and it includes about 8 species which are used as pot plants, but also for cut flowers. New cultivars have recently appeared, among others, ‘Green Goddess’ with deep green leaves and a glorious, large green flower with white throat. Their multiplication by using *in vitro* culture techniques enables the quick introduction of these new cultivars into production. *In vitro* propagation of rhizomatous plants involves the induction of shoots from terminal or nodal buds of rhizomes, e.g. *Aglaonema* [Podwyszyńska 1992], *Alstromeria* [Gabryszewska 1995], *Zantedeschia* [Fan *et al.* 2005, Koech *et al.* 2005] or from portions of shoots of *Spathiphyllum* [Ramirez-Malagon *et al.* 1989] as well as shoot regeneration from leaf explants of *Achimenes* [Vlahos 1988]. BA has been used most frequently to initiate *Zantedeschia* cultures [Chang *et al.* 2003, Fan *et al.* 2005, Koech *et al.* 2005, Peng *et al.* 2006].

The aim of the experiment was to determinate the effect of four BA concentrations on *in vitro* induction of *Zantedeschia aethiopica* ‘Green Goddess’ shoots.

MATERIAL AND METHODS

Shoots of *Zantedeschia aethiopica* 'Green Goddess' taken from aseptically grown shoot clusters were the object of experiment. Aseptic cultures were established from rhizome – bud explants which 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 – $0.4 \text{ mg} \cdot \text{dm}^{-3}$, pyridoxine – $0.5 \text{ mg} \cdot \text{dm}^{-3}$, nicotinic acid – $0.5 \text{ mg} \cdot \text{dm}^{-3}$, glycine – $2 \text{ mg} \cdot \text{dm}^{-3}$, myo-inositol – $100 \text{ mg} \cdot \text{dm}^{-3}$, sucrose – $30 \text{ mg} \cdot \text{dm}^{-3}$ and Agar-Agar (Sigma) – $6.5 \text{ mg} \cdot \text{dm}^{-3}$, and supplemented with benzyloadenine (BA) $2 \text{ mg} \cdot \text{dm}^{-3}$ and IAA $0.5 \text{ mg} \cdot \text{dm}^{-3}$. After several months of multiplication, with 6 weeks intervals, shoots of 15 mm in length were dissected from the shoot clusters and used in the experiment. BA at the concentrations of 5, 10, 25 and 50 μM was 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. There were four replications per treatment, each consisting of 5 explants/Erlenmeyer flask. The experiment was repeated twice. The cultures were maintained at 22°C and light intensity of $35 \mu\text{M m}^{-2} \text{ s}^{-1}$ and 16-h photoperiod.

The following characters were evaluated after 8 weeks of culture: length (with leaves) and fresh weight of main shoot, number of leaves on main shoots, number of axillary shoots and their length (with leaves) and fresh weight, number, length and fresh weight of roots, fresh weight rhizomes (basal tissue). 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 5% level of significance.

RESULTS AND DISCUSSION

In analysing the effect of BA on the main shoot length, a beneficial influence of this cytokinin was found *at all* investigated concentrations. The shoots reached a significantly larger length compared to the control. At the highest BA concentration (50 μM), the inhibition of elongation growth of the shoots was observed (Tab. 1).

Table 1. The effect of BA on the growth and development of main shoot of *Zantedeschia aethiopica* 'Green Goddess' after 8 weeks of culture *in vitro*
Tabela 1. Wpływ BA na wzrost i rozwój pędu głównego *Zantedeschia aethiopica* 'Green Goddess' w 8-tygodniowej kulturze *in vitro*

Cytokinin μM	Length of main shoot mm	Fresh weight of main shoot mg	Number of leaves on main shoot	Fresh weight of rhizome/explant mg
0	30.3 c	229.1 c	1.4 a	259.6 c
5	40.8 a	387.9 b	1.6 a	329.5 bc
10	43.7 a	426.1 b	1.8 a	336.9 bc
25	46.4 a	371.2 b	1.3 a	427.3 a
50	38.5 b	874.4 a	1.3 a	328.5 bc
Mean	39.9	457.7	1.5	336.4

*Values in vertical columns followed by the same letter do not differ significantly at $p = 0.05$

*Wartości w kolumnach pionowych oznaczone tą samą literą nie różnią się istotnie przy $p = 0,05$

There were significant differences in the fresh weight of the main shoot, depending on the cytokinin concentration (Tab. 1). On the control medium, the shoots reached the lowest fresh weight, whereas cytokinin addition resulted in its strong increase. In the presence of BA 50 μM , the fresh weight of the shoots was 3.8 times larger compared to the control.

No influence of the addition of cytokinin and its concentration was found on the number of leaves produced on the main shoot (Tab. 1). In analysing the fresh weight of rhizomes, formed the base of the shoot, the most beneficial effect of BA was found at a concentration of 25 μM , whereas at the other concentrations the fresh weight did not differ significantly from the value in the control.

A significant effect of the presence of cytokinin in the culture medium was found on the regeneration of axillary shoots (Tab. 2). It was observed that the number of axillary shoots increased together with the increase in BA concentration. Similar trends occurred in the case of the length and fresh weight of axillary shoots.

The shoots cultivated on all investigated culture medium produced roots (Tab. 3). A beneficial effect of BA was found on the number, length and fresh weight of the roots at a concentration of 5 and 10 μM . BA at an amount of 50 μM inhibited strongly rhizogenesis.

Table 2. The effect of BA on the regeneration and growth of axillary shoots of *Zantedeschia aethiopica* 'Green Goddess' after 8 weeks of culture *in vitro*
Tabela 2. Wpływ BA na regenerację i wzrost pędów kątowych *Zantedeschia aethiopica* 'Green Goddess' w 8-tygodniowej kulturze *in vitro*

Cytokinin μM	Explants regenerating axillary shoots %	Number of axillary shoots/explants	Length of axillary shoots mm	Fresh weight of axillary shoots/explants mg
0	0	0	-	-
5	48	1.2 b	10.1 b	55.8 c
10	60	1.7 ab	11.0 b	107.7 c
25	80	2.1 a	17.3 a	209.4 b
50	88	2.4 a	20.7 a	339.0 a
Mean	55.2	1.5	14.8	178.0

*See explanation Tab. 1/Patrz objaśnienie do tab. 1.

Table 3. The effect of BA on the regeneration and growth of roots *Zantedeschia aethiopica* 'Green Goddess' after 8 weeks of culture *in vitro*
Tabela 3. Wpływ BA na regenerację i wzrost korzeni *Zantedeschia aethiopica* w 8-tygodniowej kulturze *in vitro*

Cytokinin μM	Rooted shoots mm	Number of roots/explant	Length of roots mm	Fresh weight of roots/explant mg
0	92	5.9 b	16.0 b	73.0 c
5	88	9.7 a	23.2 a	200.9 a
10	80	7.3 ab	26.1 a	252.9 a
25	84	5.4 b	16.7 b	123.5 b
50	60	3.1 b	14.6 b	47.6 c
Mean	80.8	6.3	19.3	139.6

*See explanation table 1/Patrz objaśnienie do tab. 1.

The results of the experiment showed that BA *at all* investigated concentrations had a positive effect on the induction and growth of axillary shoots of *Zantedeschia aethiopica* ‘Green Goddess’. BA had the most beneficial effect at a concentration of 25 and 50 μM . In *Zantedeschia in vitro* cultures at the initiation stage, MS medium supplement with BA $1 \text{ mg} \cdot \text{dm}^{-3}$ [Fan *et al.* 2005], BA $8.87 \mu\text{M}$ ($2.5 \text{ mg} \cdot \text{dm}^{-3}$) [Change *et al.* 2003] or B 5 [Gamborg *et al.*, 1968] medium with the addition of BA $3 \text{ mg} \cdot \text{dm}^{-3}$ [Peng *et al.* 2005] were used. But Ebrahim [2004] obtained the highest multiplication ratio using for *Z. aethiopica* ‘Spreng’ MS medium with the addition of 2iP $12.3 \mu\text{M}$ ($2.5 \text{ mg} \cdot \text{dm}^{-3}$).



Fig. 1. Plants of *Zantedeschia aethiopica* ‘Green Goddess’ obtained on MS medium with different concentration of BA, after 8 weeks of *in vitro* culture

Rys. 1. Rośliny *Zantedeschia aethiopica* ‘Green Goddess’ pochodzące z pożywki MS z różnymi stężeniami BA w 8-tygodniowej kulturze *in vitro*

In the present experiment, on the media supplemented with BA at amounts from 1 up to 50 μM , 1.2 up to 2.4 shoots of *Zantedeschia aethiopica* ‘Green Goddess’ were obtained from 1 explant. Similar regeneration results were obtained by Koech *et al.* [2005] from *Z. albomaculata* ‘Black Magic’. Ebrahim reports that for *Z. aethiopica* ‘Spreng’ the multiplication ratio was 3.0 (cultured on a medium supplemented with BA 22.2 and 44.4 μM or kinetin 11.6 and 23.2 μM) up to 4.0 (in the presence of 12.3–49.2 μM 2iP). Change *et al.* [2003] obtained 3.8 shoots of *Z. albomaculata* on a medium with the addition of 8.87 μM BA and observed that the increasing of concentrations of cytokinin often led to lower proliferation rate and stunted growth.

Z. aethiopica ‘Green Goddess’ shoots rooted on the control medium and with the addition of BA, with the best results in the presence of BA 5 and 10 μM . Koech *et al.* [2005] report the rooting of *Z. albomaculata* ‘Black Magic’ on the control medium, whereas Ebrahim [2004] recommends MS medium containing a half of mineral salts and 5.4 μM NAA for the rooting of *Z. aethiopica* ‘Spreng’.

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Streszczenie. Pędy *Zantedeschia aethiopica* 'Green Goddess' pochodzące z kultur aseptycznych *in vitro* kultywowano na pożywce Murashige and Skoog [1962] zawierającej BA w stężeniach 5, 10, 25 i 50 μM . Kontrolę stanowiła pożywka bez cytokininy. Największą liczbę pędów kątowych uzyskano na pożywkach z dodatkiem BA 25 i 50 μM . Pożywka uzupełniona 25 μM BA stymulowała najsilniej formowanie kłączy. Korzenie tworzyły pędy rosnące na wszystkich badanych pożywkach, najlepiej w obecności BA 5 i 10 μM .

Słowa kluczowe: *Zantedeschia aethiopica* 'Green Goddess', BA, pędy kątowe, *in vitro*