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Micropropagation of calla lily (*Zantedeschia rehmannii*)

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ABSTRACT

The aim of this study was to develop methods for the *in vitro* propagation of *Zantedeschia rehmannii*. *Zantedeschia rehmannii* tuber fragments (1 cm^2) containing eyes were soaked for 30 s in a solution containing 100 mg dm^{-3} L-ascorbic acid (AA) before transfer to culture vessels containing an MS medium supplemented with BAP (0 to 3 mg dm^{-3}). Cultures were maintained in darkness. Soaking explants in an L-ascorbic acid solution improved the establishment of explants. Culture initiation should be conducted on media supplemented with 3 mg dm^{-3} BAP. On a multiplication stage, adventitious shoots were placed on MS media supplemented with cytokinin: BAP (0.5 to 5 mg dm^{-3}), KIN (0.5 to 5 mg dm^{-3}), TDZ (0.1 to 1 mg dm^{-3}) and 2iP (2.5 to 15 mg dm^{-3}) or BAP (0.5 to 7.5 mg dm^{-3}) with IAA (0.5 to 2 mg dm^{-3}). The highest coefficient of multiplication for *Zantedeschia* was obtained on the medium with the addition of 2.5 mg dm^{-3} BAP, which positively affected the shoot length (3.41 cm) and the number of adventitious shoots (4.13). Rooting took place on media supplemented with IBA, IAA and NAA at a concentration of 0.1 to 2 mg dm^{-3}. The most numerous and the longest roots were found in plants placed on a medium with the addition of 0.1 mg dm^{-3} IBA.

Key words: antioxidant, Araceae, L-ascorbic acid, propagation

INTRODUCTION

Zantedeschia rehmannii, a member of Araceae, grows naturally in Middle and South Africa (Perry 1989). It has pink flowers with orange spadices blooming in the summer and lancet-like leaves growing out of a small tuber (Funnell 1993, Kuehny 2000). The species *Z. rehmannii* has been used for obtaining cultivars with decorative colourful inflorescence spathes through crosses with other species of the genus *Zantedeschia* (Corr 1993, Yao et al. 1996). Calla lily may be grown for cut or pot flowers or container cultivation on balconies and terraces.

Up till now, *Zantedeschia* was propagated vegetatively by tuber division. However, the propagules are often contaminated with bacteria (especially with *Erwinia carotovora* subsp. *carotovora* and *E. chrysanthemi*) and viral organisms which are transferred to the plants (Joubert and Truter 1972, Wright 1998, Huang and Chang 2005, Snijder and van Tuyl 2002). Tissue culture propagation is the best option to rapidly obtain a large number of pathogen-free plants. Previous studies have focused on propagation of other species including *Zantedeschia aetiopica* (Cohen 1981, Clemens and Welsh 1993, Fang et al. 1999, Ebrahim 2004), *Zantedeschia elliotiana* or *Z. albomaculata* (Jerzy and Pawlak-Arend 2002, Chang at al. 2003). Therefore, the aim of this study was to develop the methods for *in vitro* propagation of *Zantedeschia rehmannii*.

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MATERIAL AND METHODS

Culture initiation

Tubers of *Zantedeschia rehmannii* with a diameter 2.5-3 cm were sterilized in tempered water (40°C) with a few drops of detergent for 20 min followed by several rinses under running water. Washed tubers were immersed in 70% (v/v) ethanol for 30 seconds followed by immersion in 0.5% $HgCl_2$ for 15 minutes and rinsed three times in deionized, autoclaved water. Initial explants were 1 cm^2 tuber fragments containing at least one tuber eye. Prior to being placed on the media, the explants were soaked for 1 min in a 100 mg dm^{-3} of L-ascorbic acid solution. Next, they were transferred to four combinations of initiation media containing macro- and micro-elements according to MS medium (Murashige and Skoog 1962) with various BAP concentrations (Tab. 1). The cultures were maintained in a growth chamber at 23°C, in darkness. The initiation stage lasted six weeks.

Multiplication stage

Effect of cytokinins. Explants, initiated for growth, were first proliferated on the media with 3 mg dm^{-3} 6-benzylaminopurine **(**BAP). Next, adventitious shoots were placed on 16 kinds of proliferation media: MS medium, supplemented with cytokinin: BAP (0.5, 1, 2.5 and 5 mg dm^{-3}), kinetin – KIN (0.5, 1, 2.5 and 5 mg dm^{-3}), thidiazuron – TDZ (0.1, 0.2, 0.5 and 1 mg dm^{-3}) and isopentenyl adenine – 2iP (from 2.5, 5, 7.5 and 15 mg dm^{-3}).

Effects of cytokinins and auxins. In the next experiment, the total effects of the addition of indole-3-acetic acid (IAA) (0.5, 1 and 2 mg dm^{-3}) with BAP (0.5, 2,5 and 7.5 mg dm^{-3}) to the media were determined. The multiplication stage lasted six weeks.

Rooting stage

In vitro proliferated shoots on the MS medium with 2.5 mg dm^{-3} BAP were transferred to 12 kinds of rooting media. MS media supplemented with various concentrations of indole-3-butyric acid (IBA), 1-naphthaleneacetic acid (NAA) and IAA (0.1, 0.5, 1 and 2 mg dm^{-3}, respectively) were used for rooting. The rooting stage lasted six weeks.

The media were supplemented at all stages with 8 g dm^{-3} agar (Sigma Aldrich), 30 g dm^{-3} sucrose and 100 mg dm^{-3} inositol, and their pH was adjusted to 5.7 with 0.1M NaOH and HCl solutions prior to autoclaving. After the addition of growth regulators, the media were sterilized by autoclaving for 20 minutes at a temperature of 121°C. The plants on the MS medium without growth regulators constituted the control at all stages of the experiment. The cultures were incubated at 23°C under a 16/8 h light/dark regime, and at a photosynthetic photon flux density of 35 µmol m^{-2} s^{-1}, provided by cool-white fluorescent tubes.

Statistical analysis

The experiment at the initiation stage was conducted in a two-factor completely randomised design (1st factor – BAP concentration, 2nd – L-ascorbic acid treatment). The experiment was performed in three replications with 50 explants each. The experiment on the multiplication and rooting stage was conducted in a one-factor completely randomised design. Each treatment in this stage consisted of 75 explants (3 replications of 25 explants each). An analysis of variance (ANOVA) followed by Tukey's test ($p \leq 0.05$) were performed. Homogenous groups between the examined combinations were labelled with successive letters of the alphabet. The percentage data were transformed to arc-sin before analysis.

RESULTS AND DISCUSSION

A great problem for the initiation of cultures of plants belonging to the family Araceae is the occurrence of infections at this stage, connected with the presence of endogenous bacteria in zantedeschia tubers (Kritzinger et al. 1998). The studies of Riuz et al. (1996) show that the

Table 1. Percentages (%) of initiated explants from media with varying BAP content

BAP concentration (mg dm^{-3})	Not soaked in L-ascorbic acid solution	Soaked in L-ascorbic acid solution	Mean
0.0 – control	18.0 c*	26.0 c	22.0 c
1.0	16.0 c	40.0 b	28.0 bc
2.0	20.0 c	50.0 ab	35.0 b
3.0	40.0 b	75.0 a	57.5 a
Mean	23.5 b	47.8 a	

*Means in a column followed by different letters are significantly different according to Tukey's test ($p \leq 0.05$)

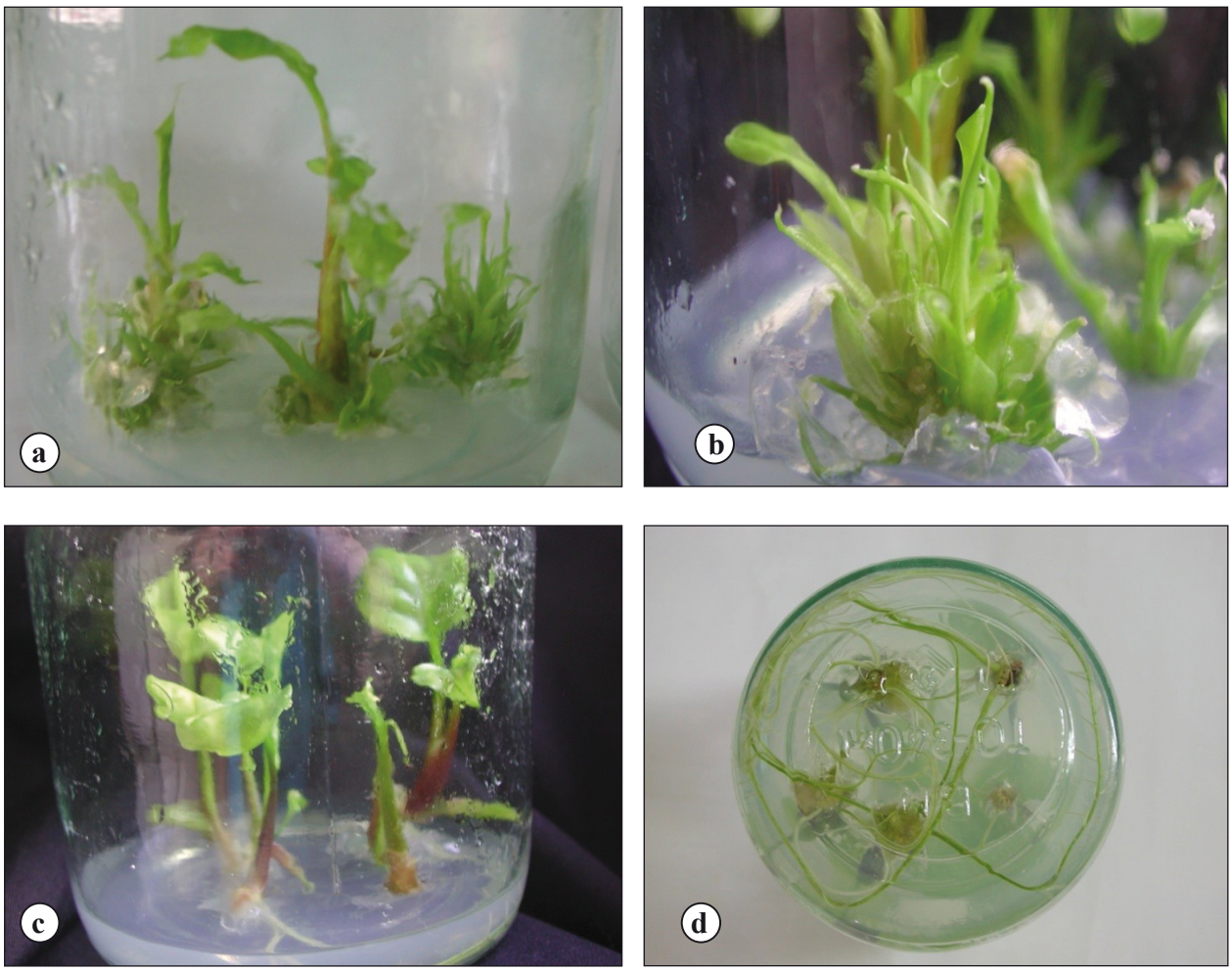

Figure 1. *Zantedeschia rehmannii* propagated on the medium with 1.0 BAP (a) and 2.5 BAP (b) and rooted on the medium with 0.1 mg dm^{-3} IBA (c and d)

sterilisation of explants isolated from *Zantedeschia* tubers in a 10% solution of natrium hypochlorite results in 20% of 'clean' uninfected explants. In my studies, soaking in a solution of 0.5% mercuric chloride ($HgCl_2$) for 15 minutes was used to disinfect tuber fragments. Infections were only found in 27% of the explants, on average (data unpublished).

Antioxidants such as L-ascorbic acid or citric acid reduce the exudation of phenol compounds inhibiting plant growth at the initiation stage. Thus, they increase the frequency of explants initiating growth and accelerate their development. The beneficial effects of L-ascorbic acid on culture initiation have been described by Panda and Hazra (2010), Muthan et al. (2006) and Dhavala and Rathore (2010). In my studies a positive influence of treating explants with L-ascorbic acid solution, prior to transferring them to the media, was observed; in all cases, irrespective of the initiation medium, the percentage of initiated explants was higher. When they were soaked in the L-ascorbic acid solution, 47.8% of explants initiated growth, whereas only 23.5% of those not treated did so (Tab. 1).

Initiating *Zantedeschia aethiopica*, Ruiz et al. (1996) supplemented the media with 1 mg dm^{-3} BAP. In studies on culture initiation of the same species, Wu et al. (1999) considered the medium with the addition of both 1 to 2 mg dm^{-3} BAP and 0.1 mg dm^{-3} NAA the best for callus formation and shoot regeneration from tuber fragments. According to Jerzy and Pawlak-Anhalt (2002), the best growth regulators for initiating *Zantedeschia elliotiana* are BAP and IAA at the concentration of 3 and 2 mg dm^{-3}, respectively, applied simultaneously. In my study, I recorded more effective initiation in plants from the MS media with 3 mg dm^{-3} BAP. In plants treated with L-ascorbic acid, the percentage of initiated explants was 75%, while it was 40% without that treatment.

The growth regulators used in the first experiment in the multiplication stage are cytokinins. Xiao et al. (1998) applied 3 mg dm^{-3} zeatin for proliferating *Zantedeschia*. The effectiveness of BAP for the

Table 2. Effects of different cytokinin and their concentrations on *Zantedeschia* multiplication

Cytokinin concentration (mg dm^{-3})		Shoot length (cm)	Number of shoots	Plant weight (g)
BAP	0.5	3.28 a*	2.47 cd	1.01 b
	1.0	2.61 b	3.71 ab	0.90 b
	2.5	3.41 a	4.13 a	0.82 b
	5.0	2.78 b	3.93 a	1.14 b
KIN	0.5	1.90 d	1.30 f	0.26 d
	1.0	2.61 bc	2.13 de	0.32 d
	2.5	2.15 cd	1.80 ef	0.35 d
	5.0	1.90 d	1.33 f	0.40 cd
TDZ	0.1	2.69 bc	2.23 d	0.54 bcd
	0.2	2.42 bc	1.67 f	0.66 bcd
	0.5	2.44 bc	1.93 e	0.92 b
	1.0	2.23 cd	1.72 ef	0.64 bcd
2iP	2.5	2.84 b	2.77 c	1.69 a
	5.0	2.52 bc	2.90 c	1.46 a
	7.5	2.56 bc	3.40 b	1.12 b
	15.0	2.32 bcd	1.82 ef	1.53 a
Control – MS	0.0	2.03 cd	0.67 efg	0.28 d

*Means in a column followed by different letters are significantly different according to Tukey's test ($p \leq 0.05$)

multiplication of plants of the family *Aracaeae* was confirmed by Benczur and Riffer (1990) and Sanchez et al. (2000). Chang et al. (2003) added 2 mg dm^{-3} BAP for the proliferation of *Z. albomaculata*, whereas Cohen and Yao (1996) proliferated species of *Zantedeschia – Z. elliottiana, Z. rehmannii, Z. albomaculata* and *Z. pentlandii* – on media with the addition of 3 mg dm^{-3} BAP. According to Jerzy and Pawlak-Anhalt (2002), the highest coefficient of shoot proliferation from explants isolated from *Z. elliotiana* tubers was obtained on the medium supplemented with 3 mg dm^{-3} BAP. A higher concentration of this growth regulator in the medium considerably reduces plant regeneration abilities. My studies show that BAP is the most effective cytokinin for the stage of multiplication (Tab. 2, Fig. 1). In comparison with other cytokinins (KIN, TDZ, 2iP), plants from the media supplemented with BAP were taller and produced the largest number of adventitious shoots. The highest coefficient of multiplication for *Zantedeschia* was obtained on the medium with the addition of 2.5 mg dm^{-3} BAP, which positively affected the shoot length (3.41 cm) and the number of adventitious shoots (4.13). TDZ application resulted in a poorer induction of adventitious shoots

Table 3. Effects of BAP applied with IAA on *Zantedeschia* multiplication

Plant growth regulators (mg dm^{-3})		Shoot length (cm)	Number of shoots	Plant weight (g)
IAA	BAP			
0.5	0.5	2.22 ef*	2.33 abc	0.46 ab
1.0	0.5	2.53 de	1.73 bcd	0.31 ab
2.0	0.5	2.99 bc	2.00 abcd	0.39 ab
0.5	2.5	2.65 cd	2.42 abc	0.43 ab
1.0	2.5	1.88 fg	1.85 bcd	0.21 b
2.0	2.5	3.77 a	3.33 a	0.56 a
0.5	7.5	2.11 f	3.07 ab	0.55 a
1.0	7.5	3.20 b	2.27 abc	0.48 ab
2.0	7.5	1.55 g	1.55 cd	0.20 b
Control – MS		2.03 fg	0.67 d	0.28 ab

*Means in a column followed by different letters are significantly different according to Tukey's test ($p \leq 0.05$)

Table 4. Influence of auxins on rooting of *Zantedeschia*

Auxin concentration (mg dm^{-3})		Rooting (%)	Shoot length (cm)	Root length (cm)	Number of roots	Plant weight (g)
IBA	0.1	100	2.98 a*	4.56 a	4.23 a	0.89 ab
	0.5	95	3.08 a	4.53 a	1.69 d	0.31 a
	1.0	90	2.85 ab	3.96 b	2.37 cd	0.35 b
	2.0	89	2.66 bc	3.01 c	0.85 ef	0.68 ab
IAA	0.1	79	2.03 e	1.05 e	1.89 de	0.46 b
	0.5	69	1.49 f	3.98 b	2.80 bc	0.36 b
	1.0	59	2.45 c	3.93 b	1.62 de	0.37 b
	2.0	60	2.39 c	3.24 c	1.67 de	0.56 ab
NAA	0.1	79	1.56 e	1.73 d	2.03 cd	0.59 ab
	0.5	100	2.37 cd	1.43 d	4.03 a	0.88 ab
	1.0	98	2.09 de	1.09 e	4.27 a	0.22 b
	2.0	87	2.04 e	0.78 e	3.44 ab	0.49 b
Control – MS		3	2.41 c	0.41 f	0.11 f	0.42 b

*Means in a column followed by different letters are significantly different according to Tukey's test ($p \leq 0.05$)

– from 1.67 to 2.23 depending on its concentration. The shoots from the media with thidiazuron had a larger fresh weight but were light green, deformed and also hyaline.

The simultaneous addition of BAP and IBA did not increase the number of adventitious shoots (Tab. 3). The studies showed that the plants proliferated on the media supplemented with cytokinin BAP and auxin IBA had the highest proliferation coefficient on the medium with 2.5 mg dm^{-3} BAP and 2 mg dm^{-3} IBA. The plants from that medium were the tallest, had the largest weight and produced the most adventitious shoots.

Rooting is a critical stage of micropropagation. Plants devoid of a well-developed root system have no ability to adapt themselves to glasshouse conditions. Low concentrations of auxin are usually added to induce rhizogenesis. Among the three auxins used in our experiments at the stage of proliferation, the largest number of shoots, height, fresh weight and the longest roots and leaves were obtained in plants from the media supplemented with 0.1 mg dm^{-3} IBA. Atta et al. (1998) also used 0.25 mg dm^{-3} IBA for rooting explants of *Anthurium sp.* Apart from inhibiting root formation and their growth, higher concentrations of IBA or NAA in the medium also reduced plant height and the number of adventitious shoots of zantedeschia under study (Tab. 4, Fig. 1). In those studies, the shoots rooted on the media with NAA at the concentration 1 and 2 mg dm^{-3} produced the shortest and the least numerous roots. In the opinion of many authors (Cohen, 1981, Xiao et al. 1998, Jerzy and Pawlak-Anhalt 2002), the addition of BAP in high concentrations may have a negative influence on the rooting of micro-plantlets. In my study, shoots proliferated on MS medium with 2.5 mg dm^{-3} BAP were rooted successfully.

This study describes the protocol for the micropropagation of *Z. rehmannii.* Results indicate that it is possible to start large-scale production of this very beautiful ornamental plant. Results and approaches from this study may prove useful for the propagation of *Z. rehmannii*.

CONCLUSIONS

1. Soaking explants in a solution of 100 mg dm^{-3} L-ascorbic acid, prior to their transfer onto the initiation medium, has a positive effect on the in vitro initiation process.
2. Culture initiation should be conducted in darkness on media supplemented with 3 mg dm^{-3} BAP.
3. Zantedeschia should be proliferated on media with 2.5 mg dm^{-3} BAP, whereas rooting should take place on Murashige and Skoog with the addition of 0.1 mg dm^{-3} IBA.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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