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Shoot Propagation of *Zantedeschia* spp. in a Temporary Immersion System – Effect of Culture Parameters on Plant Proliferation and Quality

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ABSTRACT

Callas (*Zantedeschia* spp.), belonging to the *Araceae* family, are ornamental plants with high commercial demand as pot plants and cut flowers. Traditional propagation techniques are not able to satisfy the rapid introduction of new hybrids onto international markets. Thus, *in vitro* culture techniques are useful tools for the propagation of new varieties with a wide range of colours. Aiming to establish a simplified and more efficient protocol for the *in vitro* propagation of callas, the effect of medium volume (10, 20 and 40 ml/explant) and explant wounding (dissected or undissected shoots) on shoot proliferation and quality, were evaluated. The ideal protocol for the efficient in *vitro* propagation of *Zantedeschia* var. 'Treasure' used 20 ml/explant when dissected shoots were grown in a temporary immersion system.

Keywords: calla, dissection, medium volume, ornamentals, temporary immersion system

INTRODUCTION

Araceae plants can be perennial or terrestrial with tubers or rhizomes. This family is the most diverse in the tropics of the New World, but members are also distributed throughout the old world. In both tropical and sub-tropical areas, this family is comprised of over 107 genera and about 3000 species. The family also includes known ornamentals such as *Anthurium* and *Philodendron* (de la Luz and Ortega 1985).

The genus *Zantedeschia* is spread primarily through seeds (in breeding programs), separation (following the formation of the tuber, stems are separated once they have rooted) and by the division of tubers (practiced to increase the number and size of flowering). However, the divisions of tuber crops can be infected by soft rot *Erwinia*, which causes serious losses (Chen *et al.* 2000). This genus is very susceptible to bacteria that can cause losses of up to 100% of the plantation (Etcheverría 2002). The use of *in vitro* propagation allows for the immediate production of homogeneous plants free of viruses (Funnell *et al.* 1998).

Callas (*Zantedeschia albomaculata*) are propagated by micropropagation using conventional plant growth regulators such as 6-benzyladenine (BA) and thidiazuron (Chang *et al.* 2003). Tissue culture techniques have been developed as an alternative method for the production of *Zantedeschia aethiopica* tubers (Clemens and Welsh 1993; Fang *et al*. 1999).

In vitro propagation on solid medium is labor intensive and therefore results in high production costs while a temporary immersion system (TIS) was successfully used for the propagation of several plants species and is considered to be less labor intensive and therefore, less expensive (Etienne and Berthouly 2002).

In the micropropagation of *Zantedeschia* in a TIS, Ruffoni *et al.* (2002) and Ruffoni and Savona (2005) achieved very low rates of multiplication. Sánchez *et al*. (2009) used a TIS and paclobutrazol (PBZ) to evaluate the micropropagation of three *Zantedeschia* varieties on semi-solid, liquid and TIS. The purpose of this study was to establish a new protocol to assess medium volume and explant management for the *in vitro* propagation of *Zantedeschia* sp. in TIS.

MATERIALS AND METHODS

Effect of TIS medium volume

Zantedeschia var. 'Treasure' shoots were immersed in a TIS for 4 min every 4 h in MS basal culture medium (Murashige and Skoog 1962) supplemented with 100 $mg.L^{-1}$ *myo*-inositol, 1 $mg.L^{-1}$ thiamine, 30 $g.L^{-1}$ sucrose, 4 $mg.L^{-1}$ BA and 0.3 $mg.L^{-1}$ PBZ (Sánchez *et al.* 2009). For all TIS experiments, medium was sterilized at 120°C at 1 Kg/cm^2 for 45 min. Cultures were maintained under cool white fluorescent lamps (80 µmol m^{-2} s^{-1} PAR) in a 16-h photoperiod and at 25 ± 2°C.

Each TIS had a 250-mL capacity to which the following quantities of culture medium were added: 10, 20 (Sánchez *et al.* 2009) and 40 mL. Five shoots were added to each TIS.

After inoculation, shoots that were proliferated for 28 days were transfer to culture MS medium supplemented with 0.5 $mg.L^{-1}$ gibberellic acid (GA_3) for another 28 days. The multiplication rate (i.e. number of shoots formed per shoot) and the quality of shoots were visually determined (number of leaves and shoot height) in all shoots obtained from any TIS.

Fig. 1 Effects of different volumes of culture medium on shoots proliferation of *Zantedeschia* var. 'Treasure'. (**A**) 10 mL/explant; (**B**) 20 mL/explant; (**C**) 40 mL/explant.

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Research Note

Effect of explant sectioning

Using the optimized medium above, two explants were tested: 1) shoots sectioned transversally (Sánchez *et al*. 2009) and 2) individual shoots sectioned transversally then again longitudinally in half. Five shoots per TIS were cultured. Shoots were proliferated and multiplication and quality were assessed as for the establishment culture.

We used a monofactorial completely randomized design with three replicates per treatment. Variations among treatments means were analyzed using according to KW non-parametric tests and the Dunnet C test at the 5% level using SPSS, version 1.8 for Windows.

RESULTS AND DISCUSSION

Effect of TIS medium volume

Medium volume had a significant effect on the rate of proliferation and the quality of 'Treasure' shoots in TIS, maximum (23.6) with 40 mL/explant (**Table 1**).

There were no significant differences in the number of shoots with leaves or shoot height between any of the three treatments (**Table 2**).

Kozai *et al.* (1995) argued that nutrient content in the culture medium may vary due to changes in concentration or changes in the volume of medium at a fixed concentration of nutrients (Williams 1995) and thus comparing data obtained from plants produced *in vitro* in TIS and plants produced *in vitro* on semi-solid medium is unrealistic.

Most plant tissue culture studies test the effect of initial concentration of nutrients but the initial volume per explant is usually completely ignored. There are few studies that have dealt with this issue in the ornamental literature to some extent, one on *Lilium longiflorum* (Nhut *et al*. 2002) and the second on *Cymbidium* hybrids (Teixeira da Silva *et al*. 2006), both studies finding significant effects of medium volume.

Lorenzo *et al*. (1998) tested this variable in a TIS, finding that 50 mL was the optimal volume for the shoot proliferation of *Saccharum* spp. with an increase from 8.3 to 23.9 shoots when volume was increased from 5 to 50 mL/explant. However, this variable did not affect shoot height. Escalona *et al.* (1999) found that the optimum volume for the cultivation of pineapple in a TIS was 200 mL/explant and larger volumes were less effective; results were genotype-dependent. Basail *et al.* (2003) claimed 20 mL/explant to be sufficient to mass propagate *Manihot esculenta* in TIS. This form of cultivation in a liquid culture medium such as TIS may result in the dilution of plant growth factors excreted by the explants that might stimulate the formation of shoot buds (Lorenzo *et al.* 1998; de Feria *et al.* 2002). Banana (*Musa* sp.) shoots required 30 mL/explant in TIS for maximum rate of multiplication (Roels *et al*. 2005). Surminski-Saare *et al.* (2008) showed that *Gentiana* spp. could be propagated in a TIS created from a ventilated culture flask, although the exact medium per explant was not defined.

Effect of explant sectioning

Longitudinal sectioning of individual shoots significantly increased the rate of shoot production, 16.33 shoots/explant vs explants without sectioning where the value was 10. This is the type of explants that has been used in previous experiments to begin cultivation in the TIS.

In the treatment there were no statistical differences in the number of leaves and the height of the shoots. Both sections and no sections the shoot height was higher than two (2.53 and 2.63, respectively), similar to the one presented in the previous experiments. It should be noted that this species is of poor leaf development, less than two. Shoots showed similar response without regard to sections in the height of the shoots. The number of leaves and height of the shoots are two variables that are closely related.

Table 1 Effect of medium volume on the multiplication rate of *Zantedeschia* sp. var. Treasure shoots after 28 days in TIS.

Medium volume/explant (mL)	No. shoots/explant
10	6.4 c
20	15.8 b
40	23.6 a

Means with different letters of the alphabet are statistically different (KW non-parametric tests and Dunnet C, $P \geq 5\%$).

Table 2 Effect of medium volume on the number of leaves and shoot height of *Zantedeschia* sp. var. Treasure shoots after 28 days in TIS.

Medium volume/explant (mL)	No. leaves/shoot	Shoot height (cm)
10	1.28 c	1.43 a
20	1.71 b	1.5 a
40	2.21 a	1.3 a

Means with different letters of the alphabet within each column are statistically different (KW non-parametric tests and Dunnet C, $P \geq 5\%$).

The selection of the type of explant for growing *Zantedeschia* in TIS has precedent in the results already obtained for the cultivation of banana (Roels *et al.* 2005) and some bromeliads (Daquinta *et al.* 2001, 2007) in this form of cultivation. In growing shoots is important to stimulate the growth of side shoots, but the genotypes vary in their degree of apical dominance. This phenomenon is explained in some genotypes with strong apical dominance have a high content of endogenous auxin or a natural increased sensitivity to auxin receptor systems (George 1993).

Although cytokinins are frequently used to promote the formation of side shoots, other techniques can be used to enhance this process. Among the procedures that are used to reduce the apical dominance in the *in vitro* culture are applying cuts explants, removal of the apical shoot manually and the application of some compounds in the culture medium (George 1993).

Although several decades of banana micropropagation has been achieved by decapitation of the pseudostem of shoots, Madhulatha *et al*. (2004) used shoot apices of decapitated cv. 'Nendran'. In addition to decapitate the shoots of banana and plantain micropropagation to a commercial level, these shoots were sectioned longitudinally into two, four and even eight sections depending on the size of the explants. Parthanturarug *et al*. (2003) to study the type of explants, achieved the best results in terminal buds of *Curcuma longa* decapitated and divided lengthwise into four equal parts.

Conducting a longitudinal cut in shoots that led to *Zantedeschia* sectioned in half, causing a significant increase in the formation of buds. In shoots were decapitated and sectioned longitudinally obtained the highest number of shoots cast. These variables differ statistically with regard to just where the shoots were decapitated, in this type of explant was regenerated again the beheaded. Undoubtedly, the main effect of the cutting was damaging the tissue explant and perhaps an increase in ethylene release from injured cells (George 1993). TIS is a suitable culture system for the *in vitro* multiplication of *Zantedeschia* spp. shoots, while the optimization of culture condition lead to an increase of shoot quantity.

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