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Frequency of Immersion and Paclobutrazol Application Affect the Propagation of *Zantedeschia* sp. Var. 'Treasure' Shoots in a Temporary Immersion System

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ABSTRACT

Calla lily (*Zantedeschia* sp. Var. 'Treasure'; Araceae family) is an ornamental plant with high commercial demand as pot plants and for cut flowers. Traditional propagation techniques are not able to satisfy the fast introduction of new hybrids to the market. Thus, *in vitro* culture techniques are useful tools for the propagation of new varieties with a wide range of colours. Aiming to establish a more efficient protocol for the *in vitro* propagation of callas in a Temporary Immersion System (TIS), the effect of immersion frequency and paclobutrazol (PBZ) on shoot proliferation and quality were evaluated. This protocol showed greatest biological efficiency when shoots were immersed every 4 hours (or 6 immersions per day) and when 0.3 mg.L^{-1} PBZ was included in the culture medium.

Keywords: callas, growth retardants, immersion frequency, ornamentals

INTRODUCTION

Araceae plants are perennial, terrestrial, and are often tuberous or rhizomatous; this family of plants is the most diverse in the New World tropics, although they can also be found in the old world in areas that are both tropical and warm, comprised of over 107 genera and 3000 species. *Anthurium* and *Philodendron* are two well-known members that belong to this family (de la Luz and Ortega 1985).

The propagation of the genus *Zantedeschia*, a new important model plant for developmental studies (Tavares *et al*. 2008), is achieved through seeds (in breeding programs), through separation (allowing the tuber to form and then separating the stems once they have sprouted roots) and through the division of the tuber (done in order to increase the number and size of its flowering). However, the division of the tuber can subject the plants to the soft rot infection *Erwinia*, which causes serious losses (Chen *et al.* 2000). The genus is very susceptible to this bacterium, and it can cause up to 100% loss of the crop (Etcheverría 2002). The use of *in vitro* multiplication allows the immediate production of homogeneous plants free of the virosis (Funnell *et al.* 1998).

Zantedeschia albomaculata (spotted arum lily) has been reproduced through conventional micropropagation with the use of growth regulators, such as 6-benzyladenine and thidiazuron (Chang *et al.* 2003). Tissue cultivation techniques have been developed as a viable planting alternative for the production of *Zantedeschia aethiopica* (giant white arum lily or common arum lily) tubers (Clemens and Welsh 1993; Fang *et al.* 1999).

The *in vitro* propagation of plants on a solid medium is labor intensive and therefore causes high production costs, while Temporary Immersion Systems (TIS) were successfully used in the propagation of several plant species (but not calla lily) and were considered less labor intensive and therefore less expensive (Etienne and Berthouly 2002).

Few cases of *Zantedeschia* micropropagation in TIS exist. One of these was carried out by Ruffoni *et al.* (2002), who achieved very low rates of reproduction, both with the use of the conventional system as well as the TIS (Ruffoni and Savona 2005). The purpose of this study was to evaluate different frequencies of immersion and concentration of paclobutrazol (PBZ) in the *in vitro* propagation of *Zantedeschia* sp. in a TIS.

MATERIALS AND METHODS

Culture conditions and effect of immersion frequency

In this experiment, the effect of different frequencies of immersion on the proliferation of shoots in TIS was evaluated, using a MS basal culture medium (Murashige and Skoog 1962) modified with 100 mg.L^{-1} *myo*-inositol, 1 mg.L^{-1} thiamine, 30 g.L^{-1} sucrose and 4 mg.L^{-1} benzyl amino purine (BAP). Medium was sterilized at 120°C, at a pressure of 1 Kg/cm^2 for 45 min. Each TIS had a 250 mL capacity with 100 mL of culture medium and five explants (shoots of var. 'Treasure' derived from four sub-cultures on semi-solid medium). The 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.

For trials, three frequencies of immersion were used: intervals of 3, 4, and 6 h, with an immersion time of 4 min for all trials based on previous experience on other ornamentals such as *Caladium* and *Anthurium*. A completely randomized monofactorial design was used, with three repetitions per trial. Immersion was selected over agar-based medium since the former performed better than the latter in initial trials.

After 28 days of proliferation, the multiplication rate (i.e. number of shoots formed per shoot) and the quality of the shoots were determined. All the shoots obtained through TIS were analyzed (number of leaves and shoot height) in order to determine the quality indicators.

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Research Note

Effect of paclobutrazol

PBZ was added to the medium under the exact same conditions as the immersion frequency trials at 0.0, 0.3, and 0.6 mg.L^{-1}. The experimental design and parameters analyzed were the same as those used/assessed in the immersion frequency trials.

RESULTS AND DISCUSSION

Effect of immersion frequency

Among the most important factors in cultivation using temporary immersion is the frequency of immersion. In evaluating the effect of this variable during the proliferation of 'Treasure' shoots in TIS, it was discovered that the immersion frequency of every 4 h significantly increased the multiplication rate after 28 days, reaching a value of 12.53, compared to 11.30 and 9.30 after 3 and 6 h immersion frequency, respectively (**Table 1**).

There were no significant differences in the two shoot quality indicators assessed, i.e. the number of leaves per shoot and the height (**Table 2**).

In temporary immersion culture, the frequency of immersion is one of the factors that has the greatest influences on an explant's morphogenetic response since it is the medium by which they receive nutrients and plant growth regulators, achieve aeration and gaseous exchange, and how the level of shoot hyperhydricity is regulated (Rademacher 2000). The effectiveness of temporary immersion comes from the fact that this technique combines plant tissue ventilation with intermittent contact between the tissue surface and the liquid medium (Etienne and Berthouly 2002), which favors the multiplication rate. Under our experimental conditions there was an increase in multiplication rate as was obtained by Daquinta *et al.* in bromeliads and other Araceae plants in 2001 and 2007, respectively when TIS was used.

The ideal immersion conditions were every four hours, or six immersions per day.

Effect of paclobutrazol

When PBZ was applied at 0.3 mg.L^{-1}, an average of 9.7 shoots per shoot formed, significantly more than the 6.3 and 1.1 shoots which formed with 0.0 and 0.6 mg.L^{-1}, respectively (**Table 3**). PBZ thus stimulated the shoot formation rate. However, in terms of the number of leaves and height of the shoots, the treatment without PBZ resulted in the best results (**Table 4**).

Growth retardants such as PBZ block gibberellin biosynthesis through the competitive inhibitors of the enzyme P450 mono-oxygenaze, which catalyzes the oxidation reactions which causes the formations of kaurenoic acid from *ent*-kaurene (Rademacher 2000). Therefore, the differences observed in the height of the explant may be explained by the presence, or lack thereof, of PBZ.

A strong synergistic effect of BAP and PBZ on the multiplication and length of the shoots of *Spathiphyllum floribundum* was observed by Werbrouck and Debergh (1996). Similarly, the combination of BAP with PBZ positively affected the proliferation and growth of *Zantedeschia* shoots (**Fig. 1**).

Tefera and Wannkrairoj (2006) found that the addition of PBZ at 3 mg.L^{-1} to the culture medium produced the greatest number of shoots and dry weight in *Aframomum corrorima* seedlings. The combined use of cytokinin (BAP) and PBZ in the culture medium had a positive effect on the number and length of the shoots, and plant dry weight. Ziv (2008), by adding 0.7 mg.L^{-1} of PBZ together with cytokinin (BAP) could increase the number of meristematic clusters of *Ornithogalum dubium*.

Table 1 Effect of the frequency of immersion on the multiplication rate of *Zantedeschia* sp. 'Treasure' shoots in TIS, 28 days from the beginning of the culture.

Frequency of immersion	Multiplication rate
Every 3 hours	11.30 b
Every 4 hours	12.53 a
Every 6 hours	9.30 c
S.E.	0.472

Averages with similar letters do not differ statistically according to KW non-parametric tests and Dunnet C, for p≥0.05.

Table 2 Effect of the frequency of immersion on the quality of *Zantedeschia* sp. 'Treasure' shoots in TIS.

Frequency of immersion	№ of leaves/shoot	Height (cm)
Every 3 hours	1.75 a	1.98 a
Every 4 hours	1.59 a	1.69 a
Every 6 hours	1.90 a	2.29 a
S.E.	0.119	0.149

*Averages with similar letters do not differ (KW, C Dunnett, p≥0.05).

Table 3 Effect of paclobutrazol (PBZ) on *Zantedeschia* sp. 'Treasure' multiplication rate in TIS, 28 days from the beginning of the culture.

PBZ concentrations (mg.L^{-1})	Multiplication rate
0,0	3.4 c
0.3	9.7 a
0.6	8.6 b
S.E.	0.972

Averages with similar letters do not differ statistically according to KW non-parametric tests and Dunnet C, for p≥0.05

Table 4 Effect of different concentrations of paclobutrazol (PBZ) on the quality of *Zantedeschia* sp. 'Treasure' shoots in TIS.

PBZ treatments (mg.L^{-1})	№ of leaves per shoot	Height (cm)
0	1.93 a	1.67 a
0,3	1.52 ab	0.88 b
0,6	1.38 b	0.60 b
S.E.	0.103	0.166

Averages with similar letters do not differ statistically according to KW non-parametric tests and Dunnet C, for p≥0.05

Fig. 1 ***Zantedeschia*** **sp. 'Treasure' shoots in TIS.**

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