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Shoot Proliferation of *Caladium* × *hortulanum* in a Temporary Immersion System

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ABSTRACT

Caladiums are highly prized ornamental plants whose high cost derives from their beautiful leaves. Within this group of plants *Caladium* × *hortulanum* commands a prominent market position. In this study, the petioles of young leaves were used to establish *in vitro* cultures. We were able to multiply this species on semi-solid Murashige and Skoog (MS) medium supplemented with 2.0 mg/L 6-benzylaminopurine (BAP). Moreover, a unique procedure for the mass propagation of caladium plants using a temporary immersion technique is described. This procedure involved an initial sprouting phase in an automated temporary immersion system followed by an elongation phase using conventional culture methods. To establish this protocol, *in vitro* shoots developed from petioles cultured on a semi-solid medium were used as starting materials. When using temporary immersion the multiplication rate was more than 12 times higher than under a conventional propagation system after 45 days. The highest multiplication rate was found when explants were cultured in sprouting medium (MS + 2.0 mg/l 6-BAP) in the temporary immersion system for four weeks. The highest number of competent (i.e. ready for acclimatization) and uniform plants was achieved when bud clusters were subcultured for four weeks on MS medium without plant growth regulators. Plantlets could be effectively acclimatized (92%) on a 1:1 zeolite : sugarcane filter (i.e. derived from the sugar milling process) substrate. Although these results are preliminary, the methodology is already being employed at a commercial level.

Keywords: Araceae, bioreactor, ornamentals plants
Abbreviations: 6-BAP, 6-benzylaminopurine

INTRODUCTION

Caladiums (Araceae) are tropical plants indigenous to Brazil and areas of South and Central America and are grown for their beautiful foliage. The size of the heart-shaped leaves may vary from 15 to 60 cm in length while wild plants grow 40-90 cm tall, although several dward cultivars exist. The colours vary from green and white, green and red, white with red blotches or green veins, and some even have lavender spots. Tubers are prepared in February or March. They are first shaken free of any old dirt and then placed in a flat of peat moss and covered. As soon as roots have formed, they are taken out of the box and potted in 10 cm pots. A few of the feeble or insignificantly coloured leaves may be removed if the plant becomes overcrowded. Conventional clonal propagation is by root division after plants have started to grow in the spring. The root pieces should be set in sifted leaf mold or peat moss and kept warm and damp. When roots have emerged and grown, they should be transplanted into 7.5 cm pots. In order to rapidly multiply the plant other methods are necessary.

Caladium plants can be propagated asexually, by rhizome division. However, asexual reproduction is inca-pable of producing the large numbers of plants needed in commercial nurseries. Micropropagation would easily be applied to the ornamental *Caladium* industry, as is already the case in the production of other desired ornamental hybrids (Zhu *et al*. 1992; Gliozeris *et al*. 2001).

The aim of this study was to evaluate the simplicity and effectiveness of temporary immersion systems in the clonal propagation of *Caladium* × *hortulanum*.

MATERIALS AND METHODS

Culture establishment

Caladium (*Caladium* × *hortulanum* cv. 'John Peed') plants were obtained from established cultures grown on a sprouting medium, which consisted of full Murashige and Skoog (MS) salts (Murashige and Skoog 1962) supplemented with 2.0 mg/L 6-benzylaminopurine (6-BAP) and 3% sucrose, as recommended by Daquinta (unpublished data). The cultures were maintained under cool white fluorescent lamps (80 μmol m^{-2} s^{-1} PAR; daylight F 40 T 12/D 40 W, Sylvania, Cuba), in a 16-h photoperiod, and at 25°C. Medium was sterilized at 120°C, at a pressure of 1 kg/cm^2 for 45 min.

Temporary immersion system

The temporary immersion system consisted of two containers: one for growing plants (glass culture container, 300 mL) and a separate reservoir for liquid medium (glass stock container, 300 mL). The two containers were connected by silicone and glass tubes. In each case, the airflow was sterilized by passage through a 0.2 μm hydrophobic filter. Air pressure from an air compressor pushed the medium from the stock container to the culture container, allowing the plants to be completely immersed. The airflow was reversed to withdraw the medium from the culture container. Electronic timers controlled the frequency and length of the immersion period. Three-way solenoid valves provided an on/off control (**Fig. 1**). The culture vessel for conventional micropropagation, the control, was also 300 mL. The culture container held 150 mL of recirculated medium at any one time. Five explants (shoots with two small leaves) were cultured per vessel. For the temporary immersion

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Short Communication

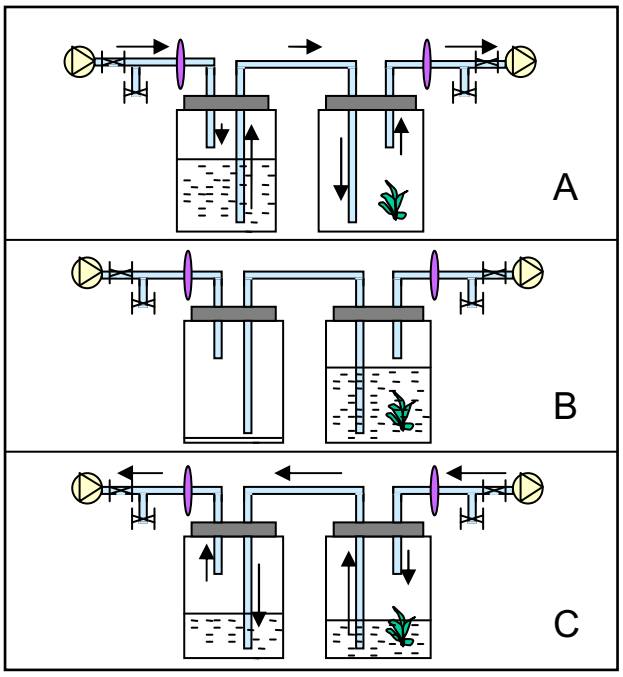

Fig. 1 Description of an automated system for temporary immersion. (**A**) An electric valve is opened and the compressed air pushes the culture medium to the plant vessel (**B**). (**C**) After immersion, a second electric valve is opened and the culture medium returns to its vessel. 1) Air compressor, 2) Electric valve, 3) Hydrophobic filter (0.2 µm).

system, shoots were immersed for 2 min every three hours. Cultures were incubated at 25°C under cool white fluorescent lamps (80 µmol m^{-2} s^{-1} PAR). The multiplication rate (final number of shoots minus initial number of shoots) was evaluated after 45 d of culture.

In vitro rooting and acclimatization of cultured plants

Shoots were removed from the temporary immersion system after 45 days and subcultured onto MS medium without plant growth regulators for 30 days to elongate. Rooted plantlets were transplanted into a substrate consisting of zeolite mixed in a 1:1 ratio with sugarcane filter (i.e. sugarcane filter-cake derived from the sugar clarification process). The trays were placed in a greenhouse under humidity tents to prevent desiccation during the first 10 days. Plantlets were placed in multi-tray containers with 144 holes (l × w × h = 52.5 cm × 29.5 cm × 4 cm) under 90% relative humidity. Survival was evaluated after 30 days.

Statistics

Variance was analyzed by a completely random design. Duncan's test was applied to compare the means at $P < 0.05$.

RESULTS AND DISCUSSION

An automated system, functioning on the principle of temporary immersion, was first designed for the large-scale propagation of pineapple (Escalona *et al*. 1998; Firoozabady and Gutterson 2003). This micropropagation system enabled a constant supply of nutrients and aeration to plants without the use of sophisticated technology. In this study, a similar temporary immersion system was designed and implemented for *Caladium*.

The temporary immersion culture combines the advantages of semi-solid and liquid medium. Solid culture allows for aeration, but does not provide full contact with nutrient media. Liquid culture medium on the other hand permits an efficient nutrient uptake, but often with resulting hyperhydricity. However, hyperhydricity was not reported in *Caladium* agitated liquid cultures (Mujib *et al*. 1999).

In this study, the first ever reported for caladium, we

Fig. 2 Conventional micropropagation of *Caladium* 'John Peed' (left). Temporary immersion system for *Caladium* (right).

describe the simple, but efficient mass production of *Caladium* × *hortulanum* shoots using a temporary immersion system. The use of temporary immersion resulted in a 43.5 multiplication rate (**Fig. 2**, right), more than 12 times higher than the use of conventional micropropagation (**Fig. 2**, left), suggesting a strong relation between culture system and proliferation rate.

In the temporary immersion system, the use of paclobutrazol for bromeliads and banana micropropagation promoted the formation of compact bud clusters with limited leaf development, avoiding unnecessary leaf growth during the shoot formation stage (Daquinta *et al*. 2001a, 2001b). However, in caladium the use of paclobutrazol or other growth retardants was not necessary to promote the multiplication rate in the temporary immersion system.

Shoots are not adequate for direct *ex vitro* rooting and acclimatization because of their small size (only 2 cm long). Such shoots need further elongation, which, due to the high propagation rate, does not increase production costs. The reason for the efficiency of the temporary immersion culture system is probably its ability to efficiently aerate plant tissue and provide contact between all parts of the explants and the liquid medium. These two features are usually not combined in a classic liquid culture procedure (Alvard *et al*. 1993).

From our results several factors might be considered to affect the efficiency of the temporary immersion system: higher proliferation rates are associated with a pH around the equilibrium point, which might facilitate the availability of some ions. One of the advantages of temporary immersion culture on *in vitro* nutrition can be that temporary immersion limits the movement of ions associated with pH change out of the plants.

A four week interval resulted in the best multiplication rate, but the plant container was not large enough to allow for shoot elongation. The use of MS medium without plant growth regulators (and only 3% sucrose) allowed for the elongation of bud clusters derived from the temporary immersion system. Culture over 30 days provided the best shoot elongation and plant uniformity. This culture period corresponds to that now used in conventional commercial laboratories.

New methods for pineapple micropropagation have been developed to reduce the cost for its commercial application (Firoozabady and Gutterson 2003). A retardant, paclobutrazol, was used at 0.5 mg/L to increase the axillary shoot multiplication rate of pineapple during the shooting stage in a temporary immersion system; shoots were elongated in the same container after separation from shoot clusters and placed directly on substrate (zeolite and sugarcane filter-cake mixture, 1:1) for simultaneous *ex vitro* rooting and acclimatization over a period of four months (Escalona *et al*. 1998). However, in our case, for caladiums, shoot elongation could be achieved simply on MS medium without plant growth regulators, but with 3% sucrose. Similar results were obtained in sugarcane by Lorenzo *et al*. (1998).

Liquid medium generally increases the rates of growth and multiplication, and this has been clearly demonstrated for several ornamentals, but often with the risk of hyperhydricity and resulting poor acclimatization (Teixeira da Silva 2006). Temporary immersion systems, in contrast, provide an excellent method of using liquid medium while controlling the gaseous environment, often preventing problems such as hyperhydricity. Increasing the growth and multiplication rate by using these systems produces more plants per unit area in the growth room, thus reducing the cost per plant per unit space of growth room, making it a financially sound option. If multiple transfers without separation of the propagules are needed, then labor costs can also be reduced. However, there are also some problems with the use of temporary immersion bioreactors (Etienne and Berthouly 2002).

This system is now being operated in several commercial laboratories, and production is just starting in one of these laboratories. In general, we think that once we overcome contamination problems, temporary immersion systems will play a major role in finally industrializing commercial micropropagation.

Caladium plantlets were completely hardened after three months (**Fig. 3**), without leaf color variation, as observed by Zhu *et al*. (1993) in some *Caladium* varieties propagated *in vitro* by conventional methods. Variation in leaf colour was observed in plants regenerated on medium containing 2,4-D (Ahmed *et al*. 2004). However, Gliozeris *et al*. (2001) did not observe any variation with similar explants.

Micropropagation through temporary immersion systems has already proved its worth in the small-scale production of several plant species, including caladium (this study), but should find a ready application in the ornamental caladium industry as well as in the production of other desired ornamental hybrids. To date, and unlike conventional solid, agar-based micropropagation systems, only temporary immersion systems have been found to permit a rapid multiplication and production of caladiums in sufficient number to merit the use of this system cost-effectively. Vegetative multiplication of individual caladium plants remains a promising possibility for the clonal production of homogeneous plant material and for efficient, competitive and cost-effective mass propagation.

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