Plant Tissue Cult. **15**(1): 1-6, 2005 (June)

PTC

Effect of Physical Factors on Micropropagation of *Anthurium andreanum*

Jaime A. Teixeira da Silva*, S. Nagae and Michio Tanaka

Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa, 761-0795, Japan

Key words : Acclimatization, Aeration, Rooting

Abstract

Anthurium shoot tips were successfully micropropagated on either agar- or rockwool-based substrates in either conventional glass bottles with (Milliseal® added) or without aeration, or within a Neoflon® PFA film Culture Pack®, with modified half strength of MS as the medium of choice. Rockwool proved to be a far better substrate than agar, while aeration, provided either by the presence of a Milliseal® on the cap or by the use of Neoflon® PFA film, enhanced the growth, rooting and subsequent acclimatization (100%) of this popular ornamental plant. A scaled-up model was then applied for the mass production (96 plants per vessel) of *Anthurium* with similar positive growth results.

Introduction

Micropropagation industries and plant tissue culture scientists have always three important factors to consider when assessing the efficiency of a micropropagation system: capacity to induce organogenesis with subsequent successful regeneration, efficient acclimatization and cost. The culture vessel used for micropropagation may be thought of as a miniature greenhouse or growth chamber with *in vitro* culturing of the miniature vegetative cutting or explant. However, the differences in the *in vitro* physical environment of conventional tissue culture systems from that found in greenhouses often results in undesirable physiological aberrations, and even alteration at the genetic level. The conventional vessel environment has been characterized as having high humidity, constant temperature, low photosynthetic photon flux density, large diurnal fluctuations in CO_2 concentration, the presence of high concentrations of exogenously applied substances, and the accumulation of ethylene (Aitken-Christie et al. 1995), conditions which often affect the uptake of water, nutrients and CO_2, transpiration, dark respiration and development of photosynthetic machinery, resulting in poor plantlet growth. Dufour and Guérin (2005) showed that high nitrogen and potassium levels in hydroponic systems resulted in superior growth of *A. andreanum*, while the vase-life of inflorescences was

*Author for corresponding. E-mail: jaimetex@lycos.com

enhanced by the application of 100 mg/l BA (Paull and Chantrachit 2001). A few studies exist on the tissue culture of *Anthurium*, whose callus has been induced from shoot cuttings and young parts of greenhouse plants, and from excised embryos (Pierik et al 1974; Pierik and Steegmans 1976). Teng (1997) used liquid medium or membrane rafts in the regeneration of *Anthurium* adventitious shoots, whose rate and frequency was affected by inoculation size and volume (Teng 1997). Leaf-induced somatic embryogenesis in *A. scherzerianum* could be obtained on Gelrite-solidified, 2,4-D supplemented medium, while a conversion to plantlets occurred with the application of kinetin (Hamidah et al. 1997). The genetic transformation of *A. andreanum* 'Alii' root-induced shoots was possible, although the protocol was unsuccessful for four other tested hybrids (Chen et al. 1997).

The film culture vessel used in this study, the Culture Pack (CP), is made of fluorocarbon polymer film (Neoflon® PFA, a tetrafluoroethylene perfluoroakyl vinyl ether copolymer; 25 μm thick; O_2 permeability: 15,000 $cm^3/m^2/d/Pa$; CO_2 permeability: 34,200 $cm^3/m^2/d/Pa$; water vapour permeability: 4.2 $g/m^2/d/Pa$), supported by a stainless frame (Tanaka et al. 1988). This film possesses superior thermal stability, high light transmittance, low water vapor transmittance, chemical inertness and most notably, high gas permeability, allowing gases to diffuse across the vessel wall to compensate for differences in gas concentrations internal and external to the vessel (Tanaka 1991).

This study deals with simple systems that overcome many of these limitations to growth, and allow for the rapid and efficient mass production of ornamental *Anthurium*.

Materials and Methods

Commercially obtained *Anthurium andreanum* 'Elizabeth' shoot tips containing three leaves were used as initial explant material. Sixteen shoot tips were placed in either: (a) glass bottles (BO) with rockwool multiblock (RW; Grodania, Denmark); (b) BO-RW with a Milliseal® (Millipore, Japan), BOM-RW; (c) Neoflon® PFA film Culture Pack® (CP) with RW, CP-RW, or (d) CP with agar (8 g/l, Wako, Japan), CP-A. Media (modified half strength of MS, $MgSO_4.7H_2O$ and NH_4NO_3, 100 mg/l each, Fe-EDTA 30 mg/l, thyamine 1 mg/l, 30 g/l sucrose), liquid in all cases except for CP-A, was added at 100 ml per vessel. All media were adjusted to pH 5.3 with 1N NaOH or HCL prior to autoclaving at 121 KPa for 17 min, prior to adding to the vessels. Vessels were placed at 25°C, under a 16 h photoperiod with a light intensity of 45 μmol m^{-2} s^{-1} provided by plant growth fluorescent lamps. For acclimatization, individual *in vitro* plantlets were transferred to course Metromix® (USA) and placed in a greenhouse for 60 days with regular watering, and no additional fertilizer application.

Root samples were fixed in 30% FAA (90:5:5, 70% ethanol : formalin : acetic acid) for two days, 50% FAA for two days and 70% FAA for two days. Fixed specimens were dehydrated through an ethanol/acetone series, critical point dried, coated with platinum and examined by a scanning electron microscope (Hitachi S-2150, Japan).

Experiments were organized according to a randomized complete block design (RCBD) with three blocks of 16 replicates per treatment. Data was subjected to analysis of variance (ANOVA) with mean separation ($P < 0.05$) by Duncan's New Multiple Range test (DMRT) using SAS® vers. 6.12 (SAS Institute, Cary, NC, USA).

Results and Discussion

Anthurium shoot tips could form roots and develop into plants under all the conditions studied. However, BOM-RW produced a higher number of leaves per plant (5.9) than other treatments (Table 1; Fig. 1A, B). In terms of plant height, and shoot fresh and dry weights, CP-RW had higher values than the other culture vessels; BOM-RW also had higher values in these parameters than non-ventilated culture bottles (BO; Table 1), indicating that aeration, proportionally

Table 1. Effects of culture medium and substrate on the growth of *Anthurium* plants *in vitro*.

Culture system		Leaves				Leaf :	Roots			Plant height
Culture vessel	Medium substrate	No.	FW*1	DW*1	FW:DW	Root*2	FW*1	DW*1	FW:DW	(cm)
BO	Agar	4.7 c	153.8 c	14.0	10.99	1.03 a	40.7 c	3.8	10.71	40.9 c
BO	Rockwool	5.3 b	175.4 c	15.3	11.46	0.91 b	45.4 c	3.6	12.61	40.5 c
BOM	Agar	4.7 c	161.3 c	14.1	11.44	0.98 ab	105.6 ab	9.0	11.73	39.4 c
BOM	Rockwool	5.9 a	252.3 b	21.7	11.63	1.07 a	82.8 b	7.6	10.89	55.2 b
CP	Agar	5.2 b	249.7 b	21.2	11.78	1.04 a	135.9 a	12.0	11.33	44.1 c
CP	Rockwool	5.3 b	295.1 a	24.8	11.90	0.99 ab	96.9 b	8.1	11.96	63.8 a

Data presented as means, with different letters within a column indicating significant differences at $P < 0.05$ according to Duncan's New Multiple Range test. Abbreviations: BO = bottle, BOM = BO with a Milliseal®, CP = Culture Pack®, FW = fresh weight, DW = dry weight. *1 = mg; *2 = (Leaf FW:DW) : (Root FW:DW), or measurement of carbon partitioning.

much higher in CP than in BOM, is an important factor resulting in superior growth of the plant. This aeration can be achieved to some extent with Milliseal®, and to a greater extent when the entire vessel is surrounded by a semi-permeable film, such as in the CP. Such a selectively permeable film, Neoflon® PFA, allows for efficient gaseous exchange, with limited or no loss in water vapour. Lucchesini and Mensuali-Sodi (2004) demonstrated that increased vessel ventilation improved the *in vitro* growth of *Phillyrea*. Forced ventilation also improved the *in vitro* growth of cauliflower (Zobayed et al. 1999.

Agar was found to be more efficient in producing roots than RW, but roots had a fewer root hairs than in RW-derived plants (Fig. 1D, E), independent of the culture vessel (BO vs. RW). Significantly fewer roots were formed in non-aerated BOs, indicating that aeration is important for effective root formation. As with shoots, higher root and fresh and dry weight values were obtained in aerated (hgher in CP than BOM) vessels (Table 1). An analysis of the shoot : root fresh : dry weight ratios basically indicate carbon partitioning with a 1.0 value indicating equal shoot and root carbon partitioning, and >1.0, the allocation of carbon gains to the shoot, and <1.0, the allocation of carbon gains to the root. BOM-RW resulted in greatest shoot carbon partitioning, while BO-RW resulted

Table 2. Effects of culture medium and substrate on the growth of *Anthurium* plants *ex vitro* after two months.

Culture system		Leaves		Roots	Plant height (mm)
Culture vessel	Medium substrat	No.	FW*[1]	FW*[1]	
BO	Agar	6.1 b	367.9 c	272.2 c	50.3 c
BOM	Agar	6.4 a	618.4 b	477.9 b	56.2 b
CP	Rockwool	6.8 a	757.3 a	591.6 a	65.0 a

Data presented as means with different letters within a column indicating significant differences at $P < 0.05$ according to Duncan's New Multiple Range test. BO = bottle, BOM = BO with a Milliseal®, CP = Culture Pack®, FW = fresh weight.

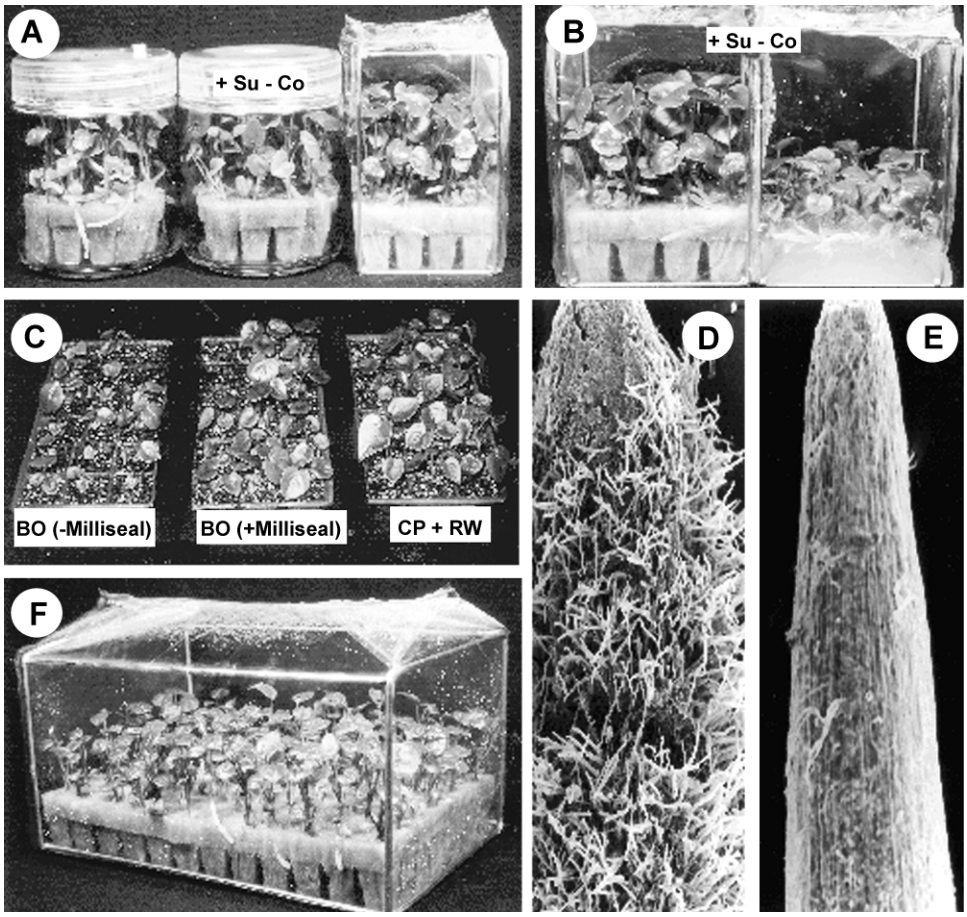
Table 3. Effects of a large-sized CP-RW on the growth of *Anthurium* plants *in vitro*.

Culture system		Leaves					Roots		
Culture vessel	Medium substrate	No.	FW*[1]	DW*[1]	FW : DW	Leaf:Root*[2]	FW*[1]	DW*[1]	FW:DW
BOM	Agar	4.6 b	120.8 b	10.6	11.40	0.91 b	80.5 a	6.4	12.58
CP	Rockwool	5.0 a	141.5 a	11.4	12.41	0.96 ab	51.6 b	4.0	12.90
CP-large	Rockwool	4.9 b	147.1 a	10.9	13.50	1.07 a	64.4 ab	5.1	12.63

Data presented as means with different letters within a column indicating significant differences at $P < 0.05$ according to Duncan's New Multiple Range test. BOM = bottle with a Milliseal®, CP = Culture Pack®, FW = fresh weight, DW = dry weight. *[1] = mg; *[2] = (Leaf FW:DW):(Root FW:DW), or measurement of carbon partitioning.

in greatest allocation of carbon resources to the roots. *Arabidopsis* grown in airtight containers was stunted (Soga et al. 1999) showing the importance of gas-permeability for effective plant growth. Larger vessels stimulated more vegetative growth in lettuce, while high plant density decreased it (Tisserat and Silman 2000).

In order to test whether there was an influence by the size of the vessel on plant growth parameters, a much larger CP-RW system was developed, in which 96 plants were placed (Fig. 1F). Plants performed equally well under the small CP, as in the larger CP (Table 3), with similar carbon partitioning as observed in Table 2.



Figs. A-F. *In vitro* growth of *Anthurium* in different growth vessels and on different substrates and subsequent acclimatization. (A) culture in BO-RW (left), BOM-RW (middle) and CP-RW (right); (B) culture in CP-RW (left) and CP-A (right); (C) Subsequent acclimatization after two months from BO (left), BOM (middle) and CP-RW (right); (D) SEM of root from CP-R plants and (E) from CP-RW plants; (F) 96 *Anthurium* plants rooted in a large CP-RW.

Subsequent growth data of plantlets acclimatized to the greenhouse (Table 2; Fig. 1C) indicate that CP-RW-derived plants showed higher growth values than either BOM-A or BO-A, demonstrating that the greater the aeration the more efficient the survival, and the higher the growth and vigour of the plant.

This study demonstrates, in a simple way, the importance of including aeration (achieved either by including a Milliseal® or by using a gas-permeable film, such as in the Culture Pack®) in the *in vitro* growth vessel for successful development of *Anthurium* and *ex vitro* successful acclimatization.

References

Aitken-Christie J, Kozai T and **Smith MAL** (1995) Automation and environmental control in plant tissue culture. Kluwer Academic Publishers, Dordrecht., pp. 1 - 493.

Chen F-C, Kuehnle A and **Sugii N** (1997) *Anthurium* roots for micropropagation and *Agrobacterium tumefaciens*-mediated gene transfer. Plant Cell Tiss. Organ Cult. **49** : 71 -74.

Dufour L and **Guérin V** (2005) Nutrient solution effects on the development and yield of *Anthurium andreanum* Lind. in tropical soilless conditions. Sci. Hort. **105 :** 269 - 282.

Hamidah M, Karim AGA and **Debergh P** (1997) Somatic embryogenesis and plant regeneration in *Anthurium scherzerianum*. Plant Cell Tiss. Organ Cult. **48 :** 189 - 193.

Lucchesini M and **Mensuali-Sodi A** (2004) Influence of medium composition and vessel ventilation on *in vitro* propagation of *Phillyrea latifolia* L. Sci. Hort. **100 :** 117 - 125.

Paull RE and **Chantrachit T** (2001) Benzyladenine and the vase life of tropical ornamentals. Postharv. Biol. Technol. **21 :** 303 - 310.

Soga K, Wakabayashi K, Hoson T and **Kamisaka S** (1999) Inhibition of reproductive growth of *Arabidopsis* in airtight vessels. Adv. Space Res. **23 :** 2037 - 2040.

Tanaka M, Goi M and **Higashiura T** (1988) A novel disposable film culture vessel in micropropagation. Acta Hortic. **226 :** 663 - 670.

Tanaka M (1991) Disposable film culture vessels. *In*: Biotechnology in Agriculture and Forestry. High-Tech and Micropropagation, I vol. **17**, Bajaj YPS (Ed.), Spinger-Verlag, Berlin, pp. 212 - 228.

Teng W-L (1997) Regeneration of *Anthurium* adventitious shoots using liquid or raft culture. Plant Cell Tiss. Organ Cult. **49 :** 153 - 156.

Tisserat B and **Silman R** (2000) Interactions of culture vessels, media volume, culture density, and carbon dioxide levels on lettuce and spearmint shoot growth *in vitro*. Plant Cell Rep. **19 :** 464 - 471.

Zobayed SMA, Armstrong J and **Armstrong W** (1999) Evaluation of a closed system, diffusive and humidity-induced convective throughflow ventilation on the growth and physiology of cauliflower *in vitro*. Plant Cell Tiss. Organ Cult. **59 :** 113 - 123.