Photoautotrophic micropropagation of *Spathiphyllum*

J.A. TEIXEIRA DA SILVA*, D.D.T. GIANG, and M. TANAKA

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

Abstract

In order to maximize yield, *Spathiphyllum*, an ornamental plant, was cultured *in vitro* in novel culture vessels termed *Vitron*. The best growth was obtained by culturing plantlets on sugar-free liquid medium under CO_2 enrichment (3 000 μmol mol^{-1} 24 h^{-1} d^{-1}) at a low photon flux density (PPFD of 45 μmol m^{-2} s^{-1}), suggesting that the novel *Vitron* culture system is suitable for the photoautotrophic micropropagation of *Spathiphyllum*.

Additional key words: fresh and dry mass; leaf number; plant height; root number and length.

Introduction

Photoautotrophic micropropagation requires cultivation of the micropropagated material under sufficient CO_2 supply, achieved by culturing explants in (*1*) gas-permeable film as vessel enclosures, (*2*) large vessel with a forced ventilation or CO_2 supply system, (*3*) use of chemicals to produce CO_2 inside the vessel, and (*4*) film culture vessels in a CO_2 enrichment room (Kozai 1991b, Tanaka 1991a). Tanaka *et al*. (1988a,b) first developed a film culture vessel, the *Culture Pack* (CP), which is made of fluorocarbon polymer films (*Neoflon*® PFA films) and supported by a stainless frame. Tanaka *et al.* (1996) later developed the *Miracle Pack* (MP), the practical model of the CP. The MP-PFA system made of PFA film and supported by a clear polycarbonate frame with RW as supporting material, placed in CO_2 enrichment, had enhanced *in vitro* growth of many plant species such as *Anthurium*, *Syngonium*, *Spathiphyllum*, *Agapanthus*, and hascup (Tanaka *et al*. 1996), *Cymbidium* (Tanaka *et al*. 1999), and *Eucalyptus* (Nhut *et al*. 2002b). However, this MP-PFA vessel is very expensive due to the high price of the PFA film and the polycarbonate frame, making it ill-suited for widespread usage in commercial plant tissue culture laboratories.

In the present study, we applied and assessed the disposable gas-permeable *Vitron* culture vessel, which is of similar size and shape as the MP-PFA, but which can be produced at lower cost. The *Vitron* is made from a novel *OTP*® film, a multi-layer film consisting of TPX (4-methyl-1-pentane polymer) and CPP (a polypropylene), which has similar physical characteristics as PFA film (Table 1). The frame of the *Vitron* apparatus is made of polypropylene, which also greatly reduces the cost. In other experiments conducted within our lab, the *Vitron* vessel has already shown to be beneficial for the photoautophophic micropropagation of *Epidendrum* orchid and sweet potato.

The aim of this study was to examine the applicability of the *Vitron* vessel to the growth and development of *Spathiphyllum* cv. Merry shoots in the rooting stage by comparing the *in vitro* and *ex vitro* growth and net photosynthetic rate (P_N) achieved in three culture vessels: CP, MP-PFA, and *Vitron*. Effects of CO_2 concentration and duration of enrichment as well as the effect of photosynthetic photon flux density (PPFD) on the *in vitro* growth of *Spathiphyllum* cultured in the *Vitron* vessels were also examined.

Materials and methods

Plants: Explants used in this study were terminal apices containing three leaves obtained from a mass of shoots derived from the *in vitro* culture of *Spathiphyllum* cv. Merry shoot tips. Twelve shoots were cultured in each culture vessel for two months, and two culture vessels were used for each treatment. To acclimate the shoots, groups of 24 *in vitro* plantlets cultured in one culture vessel were transferred to soil (*Jiffy-Garden* series, USA) and placed in a greenhouse for two months.

Received 7 October 2004, *accepted* 20 June 2005.
Fax: +81 (0)87 898 8909, e-mail: jaimetex@yahoo.com

Table 1. Characteristics of films used for the *Miracle Pack* and the *Vitron*.

Kind of film	Film thickness [μm]	Oxygen permeability [$cm^3 m^{-2} 24 h^{-1} kPa^{-1}$]	CO_2 permeability [$cm^3 m^{-2} 24 h^{-1} kPa^{-1}$]	Water vapour permeability [$g m^{-2} d^{-1}$]
OTP®	30–35	107.6	297.1	38.0
Neoflon® PFA	25	148.1	337.6	4.2

Culture vessels: Two types of film were used to make the vessels: PFA and *OTP*®. The PFA (*Neoflon*® PFA) film is made of a fluorocarbon polymer (*Daikin Industries*). The *OTP*® (*Otsuka Technology Production*, Japan) is a multi-layer film, which consists of three layers. The outer layer is made of TPX (4 methyl-1-pentane polymer), the inner layer is made of CPP (a polypropylene), and the middle is an adhesive layer, which is made of polyolephin resins.

The *Vitron* (Fig. 1) consists of a 3-dimensional injection-molded polypropylene frame covered by a heat-sealed *OTP* film on all sides except the top. The top seal film (*OTP*) is affixed to the top of the vessel after removing the paper backing to expose the adhesive. The sealed polycarbonate vessel is made of polycarbonate and has the similar shape and size as the *Vitron* and MP-PFA vessel (Fig. 2).

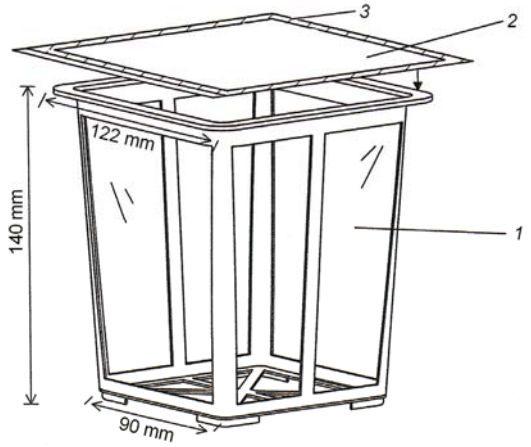

Fig. 1. Diagram of the *Vitron*: *1*, main frame; *2*, top seal film; *3*, adhesive area.

Culture medium: Shoots were cultured in Murashige and Skoog (1962; MS) sugar-free liquid medium, except one treatment of polycarbonate vessel in the first experiment in which 2 % sugar-containing MS medium was used. The pH of the medium was adjusted to 5.7 before autoclaving at 121 kPa for 17 min. Rockwool (RW; 25 joined-blocks, 5×5, *Grodan A/S*, Denmark) substrate was sterilized in a dry sterilizer at 150 °C for 1 h prior to being placed in autoclaved polycarbonate, MP-PFA, or *Vitron* culture vessels. 160 cm^3 of sterile liquid medium was then poured over the RW.

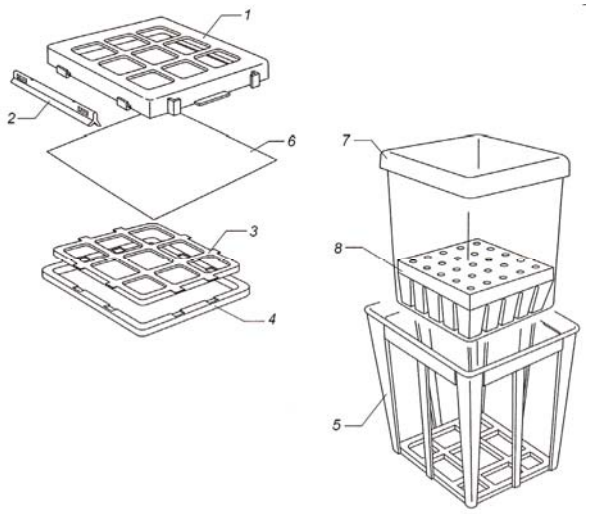

Fig. 2. Diagram of *Miracle Pack*® film culture system (Tanaka *et al*. 1996). *1*, Lid (polycarbonate); *2*, clamp (polycarbonate); *3*, frame for lining film to the lid (polycarbonate); *4*, silicon foam band; *5*, main frame (108×108×130 mm; polycarbonate); *6*, *Neoflon*® PFA film (25 μm) or *OTP*® film (30 μm) sheet; *7*, *Neoflon*® PFA film bag (25 μm) or *OTP*® film bag (30 μm); *8*, rockwool multiblock.

Culture conditions: The first experiment using the polycarbonate and the *Vitron* culture vessels was conducted under the following conditions: temperature (25±1 °C), photoperiod (16 h per d), PPFD (45 μmol $m^{-2} s^{-1}$; *Homo-Lux*, *National Electric Co.*, Tokyo, Japan), and CO_2-enrichment (3 000 μmol mol^{-1} 24 h^{-1} d^{-1}).

The second and third experiments using only the *Vitron* vessels were conducted under the same experimental conditions as the first experiment, but CO_2-enrichment was 3 000 μmol mol^{-1} for either 16 (matching the natural light period) or 24 h d^{-1} in the second experiment, and various CO_2 concentrations for 24 h d^{-1} in the third experiment. In these two experiments *Vitrons* were placed in small chambers and CO_2 concentration was controlled as described by Tanaka *et al*. (1992b).

The fourth experiment using only the *Vitron* vessels was conducted under the same conditions as the first experiment, but PPFD was 30, 45, 60, 75, or 90 μmol $m^{-2} s^{-1}$ (*Homo-Lux*, *National Electric Co*., Tokyo, Japan).

Plantlet growth was assessed using the following parameters: plant height, number of leaves, number of roots, root length, and shoot and root fresh and dry masses.

Chlorophyll content of the third leaf counting downwards from the top of the plantlets was measured by a chlorophyll meter (*SPAD-502, Minolta Co.*, Japan) and reported as the SPAD value.

P_N of the leaves was measured using a *LI-COR* portable gas exchange system (*LI-6400, LICOR*, Lincoln, USA) at 25 °C and the vapour pressure deficit at the leaf surface maintained between 2.3 and 3.1 kPa. The CO_2 concentration in the sample chamber was set at 400 μmol m^{-2}. CO_2 uptake between 0 and 300 μmol m^{-2} s^{-1} was measured using a built-in red emitting diode (LED) lamp.

Data analysis: Duncan's multiple range test was used to assess the means using *IRRISTAT* version *3.0*.

Results and discussion

Growth and photosynthesis of *Spathiphyllum* plantlets cultured in the three culture systems: The *in vitro* growth of *Spathiphyllum* plantlets cultured in the *Vitron* (Table 2, Fig. 3) was not significantly different from MP-PFA in terms of plant height, number of leaves, number of roots, root length, and SPAD value of leaves. The shoot total fresh and dry masses of the plantlets cultured in *Vitron* and MP-PFA were similar. The root fresh mass of plantlets cultured in the *Vitron* were slightly lower than those in the MP-PFA, however, there was no significant difference in root dry mass of plantlets in the two systems. The numbers of leaves and roots of the plantlets cultured in the PC were equivalent to those of plantlets cultured in MP-PFA and *Vitron*. However, all other parameters of plantlet cultured in the PC were lower than those of plantlets grown in the two film culture systems.

Table 2. *In vitro* and *ex vitro* (2 months after transfer to greenhouse) growth (plant height [cm], masses [mg]) of *Spathiphyllum* plantlets cultured in different culture systems. *Different letters* within a column indicate for each group significant differences at p=0.05 by Duncan's multiple range test. Chlorophyll content (SPAD) was determined in the third leaf counted from top.

Culture systems		Plant height	Number of Leaves	roots	Root length	SPAD	Fresh mass of shoot	root	Dry mass of shoot	root
PC	*in vitro*	5.1b	6.2a	4.1a	4.1b	39.5b	323.4b	100.5b	33.4b	8.6b
MP-PFA		6.1a	6.5a	3.9a	7.2a	42.4a	438.3a	130.9a	48.1a	10.2a
Vitron		6.2a	6.2a	3.6a	6.4a	42.2a	459.5a	102.1b	45.6a	9.2a
PC	*ex vitro*	6.2a	7.2b	7.1b	16.3a	45.2a	2011.1b	1034.5b	193.4b	72.2b
MP-PFA		10.5a	8.8a	7.8a	15.1a	46.5a	2498.7a	1283.8a	247.2a	96.7a
Vitron		10.2a	8.5a	8.2a	16.4a	47.8a	2649.4a	1138.1a	267.3a	94.2a

P_N of *Spathiphyllum* plantlets cultured in *Vitron* was similar to that of plantlets cultured in MP-PFA regardless of the difference in PPFD of 0–300 μmol m^{-2} s^{-1} while these values were higher than those of plantlets cultured in PC.

A 100 % survival of plantlets was obtained in all treatments. All plantlets were normal and vigorous (Table 2, Fig. 3). There were no significant differences in all growth parameters among the *Vitron* and MP-PFA. The plantlets cultured in *Vitron* and MP-PFA were significantly greater than those in the PC. However, regardless of the culture system, root length and SPAD values of leaves were not significantly different.

These results indicate that the gas-permeable *Vitron* as well as MP-PFA, placed in a CO_2 enriched environment at low PPFD and supplied with sugar-free medium on RW as a substrate, are efficient in promoting *in vitro* photoautrophic growth of *Spathiphyllum* plantlets compared to plantlets cultured photomixotrophically in the sealed PC. This could be due to: (*1*) The ventilation of *Vitron*, since in conventional culturing conditions, explants and culture media are protected against contamination and desiccation in a way that may often unintentionally lead to restricted gas exchange between the vessel atmosphere and the outside air (Buddendorf-Joosten and Woltering 1994). *Vitron*, which is almost entirely made of a film material with high gas permeability, allows CO_2 to diffuse into the vessel when the vessel is placed in high CO_2 concentration and out of the vessel when the internal CO_2 concentration exceeds the external gas concentration. (*2*) The closure of *Vitron*: PPFD is often very different between the inside and outside of culture vessels. The distribution of PPFD in the culture vessel largely depends upon characteristics of the vessel, closure type (Aitken-Christie *et al.* 1995, Solárová *et al.* 1996), and the spatial arrangement of the vessels (Aitken-Christie *et al.* 1995). *Vitron* has large smooth closures made of *OTP* film, allowing high radiation transmittance. Therefore, plantlets in the *Vitron* receive more photons than in other types of vessels since the radiation source is usually installed above the vessel so that plantlets receive downward irradiation (Kozai *et al.* 1997). (*3*) Relative humidity in *Vitron*: The exchanges of water in both gas and liquid phases between the plant, air, and culture medium in the vessel, as well as the characteristics of the air outside the vessel play an important role in plant

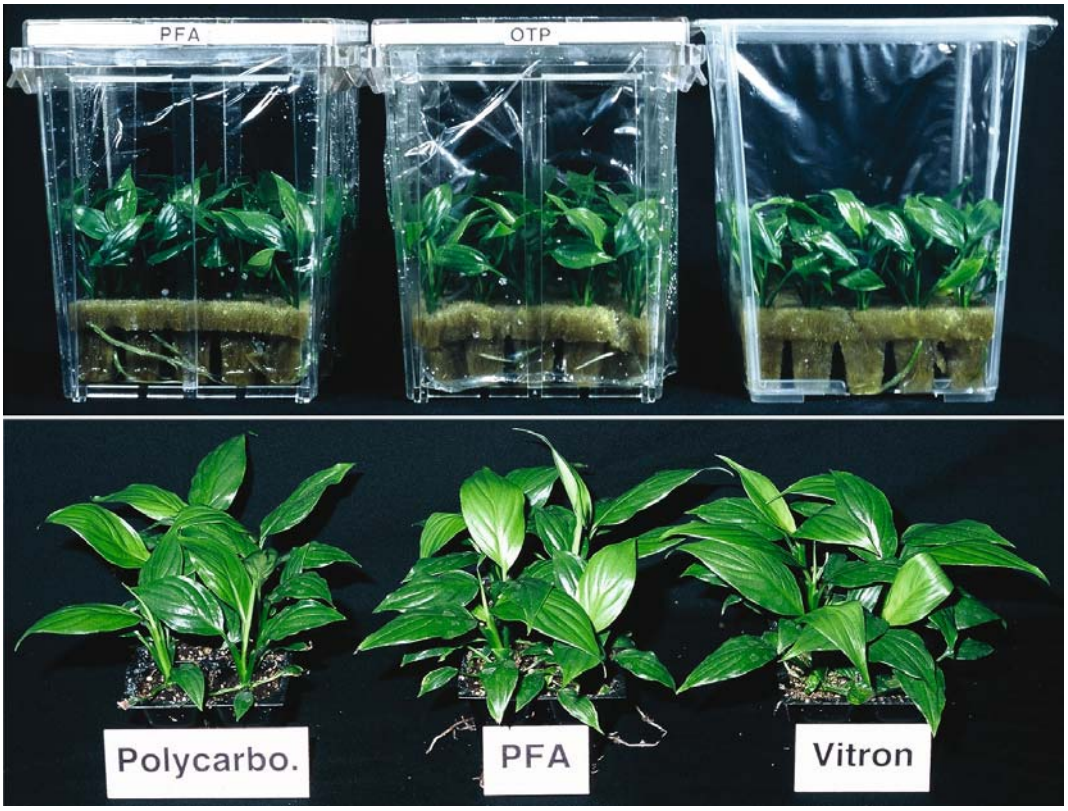

Fig. 3. *In vitro* (*top*, after 4 weeks of culturing) and *ex vitro* (*bottom*, 4 weeks after transferring to soil) growth of *Spathiphyllum* cv. Merry plantlets cultured in different film culture systems (*left*: MP-PFA, *middle*: MP-OTP, *right*: *Vitron*).

Fig. 4. *In vitro* (*top*, after 5 weeks of culturing) and *ex vitro* (*bottom*, after 2 weeks of acclimation) growth of *Spathiphyllum* cv. Merry plantlets cultured in *Vitron* under various CO_2-enrichments (from *left* to *right*: control, 1 000, 2 000, and 3 000 μmol mol^{-1}).

growth and development (Kozai *et al.* 1997). Relative humidity in conventional culture vessels is normally high, which can lead to abnormal leaf development (Ziv *et al.* 1983). Vessel relative humidity is highly dependent on the number of air changes per hour in the culture vessel and the relative humidity of the culture room (Kozai *et al.*

1993, Aitken-Christie *et al*. 1995). The *Vitron*, which is well ventilated, is likely to have lower relative humidity than other vessels.

The results confirm that the enhanced growth of *in vitro Spathiphyllum* plantlets cultured in MP-PFA and *Vitron* which, compared to plantlets cultured in PC, is in accordance with the enhancement of photosynthetic capacity observed in the treatments. Plantlets growing heterotrophically *in vitro* have low P_N. This is due to low PPFD, low CO_2 concentrations (Infante *et al*. 1989), and inhibition of photosynthesis by high sugar concentrations in the medium (Sheen 1990, Lees *et al*. 1991, Reuther 1991). In this research, the plantlets which were cultured photoautrophically in gas permeable film culture vessels, under CO_2-enrichment, and without exogenous sugars in the medium, regardless of the low PPFD had a profound impact on the photosynthetic capacity of *Spathiphyllum* plantlets. These results agree with the observation on *Cymbidium* (Tanaka *et al*. 1996, Kozai *et al*. 1997) and coffee (Nguyen *et al*. 1999).

The plantlets were grown photoautotrophically in the film culture systems, therefore they would have fewer changes in the physical, physiological, and nutritional environments after transferring from *in vitro* to *ex vitro* conditions. Thus, higher growth and survival rates might be expected after being transferred to the outside. The positive *ex vitro* growth of plantlets in all treatments obtained in this experiment is in agreement with the results of Kozai (1991a) which indicated that *ex vitro* growth can be achieved more easily with photoautotrophic micropropagation. In addition, the film vessels have a large opening enabling to avoid damages to plantlets when they were taken out for transferring to soil.

Effect of duration of CO_2 enrichment on the *in vitro* growth of *Spathiphyllum* plantlets in *Vitron*: *In vitro* plantlets cultured in 24-h CO_2 enrichment were greater than those cultured in the 16-h CO_2 enrichment in the following parameters: plant height, number of leaves and roots, root length, and shoot and root fresh and dry masses of plantlets (Table 3). On the other hand, SPAD value of leaves did not differ between the two conditions.

Generally, CO_2 concentrations inside culture vessels increase due to respiration during the dark period of the light cycle (Solárová *et al*. 1989, Jackson *et al*. 1991) and decrease during the light period depending on photosynthetic activity of the plants (Buddendorf-Josten and Woltering 1994). CO_2 concentration inside the vessel often decreases to 100–200 μmol mol^{-1} or lower at the onset of the photoperiod; this concentration is approximately 150–250 μmol mol^{-1} lower than the normal atmospheric CO_2 concentration. During the dark period, CO_2 concentration increases with time to up to 3 000–9 000 μmol mol^{-1} (Kozai *et al.* 1987a,b, Fujiwara *et al*. 1988, Solárová 1989). Theoretically, employing high CO_2 enrichment (3 000 μmol mol^{-1}) for 16 h should increase the CO_2 concentration inside the *Vitron* due to the diffusion of gas from the external atmosphere to inside the vessel through the *OTP* film walls; in the dark period, it is probably not necessary to supply CO_2 since concentrations are elevated due to respiration, with less losses due to diffusion. However, in practice, *Spathiphyllum* cultured in 24-h CO_2 enrichment showed greater growth than those in the 16-h CO_2 enrichment. The reason for this difference remains unclear. However, this result indicates that a longer CO_2 enrichment (24-h) is necessary for obtaining better *in vitro* growth of *Spathiphyllum*.

Effect of CO_2 concentration on the *in vitro* growth of *Spathiphyllum* plantlets cultured in the *Vitron*: The *in vitro* growth of plantlets cultured in the *Vitron*, which was placed in culture chambers with 24-h CO_2 enrichment at concentrations of 350–400 (control), 1 000, 2 000, and 3 000 μmol mol^{-1}, is shown in Table 4 and Fig. 4. Growth of plantlets cultured in the control condition (non-CO_2 enrichment) was inhibited. All growth parameters were highest and lowest among the four treatments for plantlets cultured in the 3 000 μmol mol^{-1} CO_2 enrichment and the control condition, respectively.

Subsequent growth of plantlets (Fig. 4) derived from those cultured in various CO_2-enrichments three weeks after transplanting to soil is shown in Table 4. There was no significant difference in SPAD value of leaves regardless various CO_2-enrichments. The number of leaves and the root length was lowest in the control conditions. The values of the other three treatments were significantly equal. All other growth parameters were highest among the four treatments for plantlets cultured at 3 000 μmol mol^{-1} CO_2 enrichment. In conclusion, the subsequent growth of plantlets, which were cultured at different CO_2 enrichments, was most enhanced at 3 000 μmol mol^{-1} CO_2-enrichment.

The *in vitro* growth and subsequent transfer to soil of plantlets cultured in the *Vitron* under 3 000 μmol mol^{-1}

Table 3. Effect of duration of applied CO_2 enrichment on *in vitro* growth (plant height [cm], masses [mg]) of *Spathiphyllum* plantlets cultured in the *Vitron* for 2 months. *Different letters* within a column indicate for each group significant differences at p=0.05 by Duncan's multiple range test. Chlorophyll content (SPAD) was determined in the third leaf counted from top.

CO_2 duration [h]	Plant height	Number of leaves	Number of roots	Root length	SPAD	Fresh mass of shoot	Fresh mass of root	Dry mass of shoot	Dry mass of root
24	7.5a	7.6a	6.9a	12.3a	43.8a	685.3a	256.7a	69.4a	26.3a
16	6.7b	6.9b	4.6b	8.9b	41.7a	578.7b	211.4b	55.9b	19.9b

Table 4. *In vitro* and *ex vitro* growth (plant height [cm], masses [mg]) of *Spathiphyllum* plantlets cultured in the Vitron at various CO_2 concentrations. *Different letters* within a column indicate for each group significant differences at p=0.05 by Duncan's multiple range test. Chlorophyll content (SPAD) was determined in the third leaf counted from top.

CO_2 [μmol mol^{-1}]		Plant height	Number of leaves	roots	Root length	SPAD	Fresh mass of shoot	root	Dry mass of shoot	root
Control	*in vitro*	4.9c	6.5c	2.9d	3.8d	33.2c	318.9d	102.9d	34.7d	3.1d
1 000		5.2c	6.3c	4.7c	8.7c	39.6b	383.7c	115.2c	48.1c	12.9c
2 000		6.7b	6.9c	5.1b	10.2b	42.1a	491.6b	170.4b	62.9b	22.9b
3 000		7.3a	7.8a	5.9a	12.3a	41.8a	697.1a	264.7a	79.4a	28.3a
Control	*ex vitro*	9.5d	8.2b	7.7c	18.5b	46.7a	2342.5c	972.5c	332.8d	138.1c
1 000		10.5c	9.2a	7.8c	21.8a	47.2a	2214.9c	1513.2b	397.9c	170.6b
2 000		11.7b	9.3a	8.8b	22.7a	47.7a	3891.7b	1636.5b	455.9b	158.8b
3 000		12.3a	9.2a	9.7a	21.3a	46.9a	4310.7a	1783.2a	571.1a	235.9a

Table 5. *In vitro* and *ex vitro* growth (plant height [cm], masses [mg]) of *Spathiphyllum* plantlets cultured in the *Vitron* under various PPFD. *Different letters* within a column indicate for each group significant differences at p=0.05 by Duncan's multiple range test. Chlorophyll content (SPAD) was determined in the third leaf counted from top.

PPFD [μmol m^{-2} s^{-1}]		Plant height	Number of leaves	roots	Root length	SPAD	Fresh mass of shoot	root	Dry mass of shoot	root
30	*in vitro*	5.8c	6.1a	3.5b	5.6b	40.1a	374.8c	178.9c	35.7c	16.7c
45		6.7a	6.4a	4.7a	6.5a	41.2a	697.1a	284.7a	64.8a	27.4a
60		6.6a	6.3a	4.4a	6.7a	39.7a	658.2a	245.1a	59.6a	25.9a
75		6.1b	5.2b	3.2b	4.3c	32.4b	457.5b	200.1b	42.3b	20.7b
90		5.7c	4.1c	3.3b	4.5c	30.1c	324.2d	155.2d	31.7d	15.2d
30	*ex vitro*	10.5b	7.9b	8.1b	16.5b	47.1b	2719.6b	836.7b	282.5b	79.4b
45		12.3a	8.5a	7.6b	16.1b	50.9a	3236.8a	1030.1a	337.3a	108.8a
60		12.9a	8.7a	8.7a	18.5a	49.3a	3367.8a	1148.9a	363.2a	115.7a

Fig. 5. *In vitro* (*top*, after 5 weeks of culturing) and *ex vitro* (*bottom*, 2 months after acclimation) growth of *Spathiphyllum* cv. Merry plantlets cultured in *Vitron*, at various PPFD (from *left* to *right*: *top* 30, 45, 60, 75, and 90 μmol m^{-2} s^{-1}; *bottom*: 30, 45, and 60 μmol m^{-2} s^{-1}).

CO_2-enrichment and low PPFD was the best. The atmosphere inside the vessel is controlled mainly through exchanges with the external atmosphere and by the *in vitro* growth of plantlets. Positive growth results were obtained under 3 000 μmol(CO_2) mol^{-1}, probably as a result of a greater difference in CO_2 concentration between the internal and external atmosphere than in the other conditions. Thus, as discussed above, the gas permeability of the vessel probably prevents the depletion of CO_2 inside the vessel during the light period to concentrations lower than the level saturating plantlet photosynthesis, possibly resulting in the observed superior growth. As there is a very large diurnal fluctuation of CO_2 concentration inside the vessel, it may require a very high external CO_2 concentration (3 000 μmol mol^{-1}) to compensate for the very low internal CO_2 concentration during the photoperiod. This finding is in agreement with those of other authors who found that using gas permeable film culture vessels in the presence of high CO_2 enrichment and low PPFD enhanced *in vitro* growth of several plant species: *Spathiphyllum* (Tanaka *et al*. 1992b), *Anthurium, Syngonium, Agapanthus,* and hascup (Tanaka *et al*. 1996), *Azadirachta excelsa* L. (Kool *et al*. 1999), banana (Nhut *et al*. 2002a), and strawberry (Nhut *et al*. 2003). The most suitable CO_2 enrichment in this study (3 000 μmol mol^{-1}) was relatively high as compared to other reports: in carnation, 1 000–1 500 μmol mol^{-1} CO_2 (Kozai and Iwanami 1988) and *Cymbidium,* 950–1 000 μmol mol^{-1} CO_2 (Kozai 1991b) were applied. However, these studies also used very high PPFD (150 and 230 μmol m^{-2} s^{-1}, respectively). Recently, the use of a forced ventilation method (Nguyen *et al*. 2001) was shown to enhance the growth of coffee plantlets under 1 000–1 200 μmol mol^{-1} CO_2 enrichment and 100–150 μmol m^{-2} s^{-1} PPFD. Since high PPFD potentially limits plant growth due to the elevation in temperature inside the vessel and increases production costs as a cooling system becomes necessary in the culture chamber, the cost of electricity for high irradiance is increased.

Effect of PPFD on the *in vitro* and *ex vitro* growth of *Spathiphyllum* plantlets cultured in the *Vitron*: The *in vitro* growth of *Spathiphyllum* plantlets cultured in the *Vitron* after two months under irradiances of 30–90 μmol m^{-2} s^{-1} (here named 30, 45, 60, 75, and 90 condition) is shown in Table 5. Plantlets from the 45 and 60 conditions were highest while plantlets from the 30 and 90 conditions were shortest among all treatments (Fig. 5). The number of leaves of plantlets from the 30, 45, and 60 conditions were highest while the value of those from the 90 condition was lowest. Roots were longest in the 45 and 60 conditions and shortest in the 75 and 90 conditions among all treatments. The SPAD values of leaves of plantlets from the 30, 45, and 60 conditions were the highest, while that of plantlets from the 90 condition was the lowest among all treatments. Yellow leaves were observed in the 75 and 90 conditions. The shoot and root fresh and dry masses of plantlets cultured in the 45 and 60 conditions were the highest, and values were found lowest in plantlets cultured in the 90 condition. These values were higher for plantlets cultured in the 30 than the 90 condition. In brief, the growth of plantlets cultured in the 45 or 60 conditions were the best. The growth of plantlets from the 75 and 90 conditions was inhibited, having yellow leaves. Therefore, in the next experiment of acclimatization, these treatments were rejected.

The subsequent growth of *Spathiphyllum* plantlets derived from the *Vitron* under the 30, 45, and 60 conditions, two months after transplanting to soil, is shown in Table 5 and Fig. 5. The plantlets cultured in the 45 and 60 conditions were significantly greater when compared to those derived from the 30 condition. The root number and length of the plantlets cultured in the 30 and 45 conditions were equivalent and lower than those of plantlets cultured in the 60 condition. However, all other parameters of plantlets cultured in the 45 and 60 conditions were equal and higher than those plantlets cultured in the 30 condition. In conclusion, the subsequent growth in soil of plantlets from the 45 or 60 conditions was most enhanced. The *in vitro* growth and subsequent growth after transplanting to soil of plantlets cultured in the *Vitron* vessels was best under the 45 or 60 conditions. These conditions are not as high as in other reports, where the irradiances often ranged between 100–150 μmol m^{-2} s^{-1} (Kozai *et al*. 1987a,b, Nguyen *et al*. 2001). These low irradiance requirements may be due to the suitable combination of environmental factors for *in vitro* growth (3 000 μmol mol^{-1} CO_2-enrichment). In addition, the *Vitron* has a large opening (122×122 mm), which is made of high transmittance OTP film, therefore plantlets receive more downward radiation, consequently they do not demand a high irradiance. However, Desjardins (1995) suggested that P_N of many tissue cultured species might saturate at a relatively low PPFD after a successive growth in heterotrophic or photo values mixotrophic conditions.

We showed that the growth of *Spathiphyllum* plantlets cultured photomixotrophically in the *Vitron* as well as MP-PFA under CO_2-enrichment can be enhanced at a low PPFD as compared to conventional polycarbonate vessels. The best condition for *Spathiphyllum* plantlets cultured photomixotrophically in the *Vitron* is 3 000 μmol mol^{-1} CO_2-enrichment for 24 h d^{-1} and 45 μmol m^{-2} s^{-1} PPFD.

By employing the disposable *Vitron* for *Spathiphyllum* micropropagation, the *in vitro* plantlets had a high photosynthetic capacity, were vigorous, and became high quality/healthy *ex vitro* plantlets as compared to those from conventional vessels. The most important characteristic of the *Vitron* is disposability, which reduces labour costs for washing while keeping the environment clean. In addition, *Spathiphyllum* plantlets were able to grow photoautotrophically in the *Vitron* under a low

PPFD. This is an important consideration, since high PPFD results in increased electricity costs for lighting as well as increased temperature in the culture room, which in turn increases the additional cost of cooling systems. Moreover, the new *Vitron* is very light (only 25 g per *Vitron* compared to 144 g per MP-PFA or 178 g per PC), These attractive features of the *Vitron* help to reduce both production and labour costs, and overcome many difficulties encountered with using other existing conventional vessels, thereby promoting effectiveness of micropropagation. In conclusion, the *Vitron* vessel placed under 3 000 μmol mol^{-1} CO_2-enrichment for 24 h d^{-1} at 45 μmol m^{-2} s^{-1} PPFD is recommended for the photoautotrophic micropropagation of *Spathiphyllum* cv. Merry.

References

Aitken-Christie, J., Kozai, T., Smith, M.A.L. (ed.): Automation and Environmental Control in Plant Tissue Culture. – Kluwer Academic Publ., Dordrecht – Boston – London 1995.

Buddendorf-Joosten, J.M.C., Woltering, E.J.: Components of the gaseous environment and their effects on plant growth and development *in vitro*. – In: Lumsden, P.J., Nicholas, J.R., Davies, W.J. (ed.): Growth and Development of Plants in Culture. Pp. 95-125. Kluwer Academic Publ., Dordrecht – Boston – London 1994.

Desjardins, Y.: Photosynthesis *in vitro* – on the factors regulating CO_2 assimilation in micropropagation systems. – Acta Hort. **393**: 45-61, 1995.

Fujiwara, K., Kozai, T., Watanabe, I.: Development of a photoautotrophic tissue culture system for shoots and/or plantlets at rooting and acclimatization stages. – Acta Hort. **230**: 153-158, 1988.

Infante, R., Magnanini, E., Righetti, B.: The role of light and CO_2 in optimising the conditions for shoot proliferation of *Actinidia deliciosa in vitro*. – Physiol. Plant. **77**: 191-195, 1989.

Jackson, M.B., Abbott, A.J., Belcher, A.R., Hall, K.C., Butler, R., Cameron, J.: Ventilation in tissue cultures and effects of poor aeration on ethylene and carbon dioxide accumulation, oxygen depletion and explant development. – Ann. Bot. **67**: 229-237, 1991.

Kool, L.T., Keng, C.L., Hoe, C.T.K.: *In vitro* rooting of Sentang shoots (*Azadirachta excelsa* L.) and acclimatization of plantlets. – *In Vitro* cell. develop. Biol. Plant. **35**: 396-400, 1999.

Kozai, T.: Micropropagation under photoautotrophic conditions. – In: Debergh, P., Zimmerman, R. (ed.): Micropropagation. Technology and Application. Pp. 447-469. Kluwer Academic Publ., Dordrecht – Boston – London 1991a.

Kozai, T.: Photoautotrophic micropropagation. – *In Vitro* cell. develop. Biol. Plant. **27**: 47-51, 1991b.

Kozai, T., Iwanami, Y., Fujiwara, K.: [Environment control for mass propagation of tissue cultured plantlets. (1) Effects of CO_2 enrichment on the plantlet growth during the multiplication stage.] – Plant Tissue Cult. Lett. **4**: 22-26, 1987a. [In Jap.]

Kozai, T., Kubota, C., Jeong, B.R.: Environmental control for large-scale production of plants through *in vitro* techniques. – Plant Cell Tissue Organ Cult. **51**: 49-56, 1997.

Kozai, T., Oki, H., Fujiwara, K.: Effect of CO_2 enrichment and sucrose concentration under high photosynthetic photon fluxes and on growth of tissue cultured *Cymbidium* plantlets during preparation. – Plant Microprop. Hort. Ind. **11**: 135-141, 1987b.

Kozai, T., Tanaka, K., Jeong, B.R., Fujiwara, K.: Effect of relative humidity in the culture vessels on the growth and shoot elongation of potato (*Solanum tuberosum* L.) plantlets *in vitro*. – J. jap. Soc. hort. Sci. **62**: 413-417, 1993.

Lees, R.P., Evans, E.H., Nicholas, J.R.: Photosynthesis in *Clematis* 'The President' during growth *in vitro* and subsequent *in vivo* acclimatization. – J. exp. Bot. **42**: 605-610, 1991.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. – Physiol. Plant. **15**: 473-497, 1962.

Nagae, S., Takamura, T., Tanabe, T., Murakami, A., Murakami, K., Tanaka, M.: *In vitro* shoot development of *Eucalyptus citriodora* on rockwool in the film culture vessel under CO_2 enrichment. – J. Forest Res. **1**: 227-230, 1996.

Nguyen, Q.T., Kozai, T., Heo, J., Thai, D.X.: Photoautotrophic growth response of *in vitro* cultured coffee plantlets to ventilation methods and photosynthetic photon fluxes under carbon dioxide enriched condition. – Plant Cell Tissue Organ Cult. **66**: 217-225, 2001.

Nguyen, Q.T., Kozai, T., Niu, G., Nguyen, V.U.: Photosynthetic characteristics of coffee (*Coffea arbusta*) plantlets *in vitro* in response to different CO_2 concentration and light intensities. – Plant Cell Tissue Organ Cult. **55**: 133-139, 1999.

Nhut, D.T., Hong, L.T.A., Watanabe, H., Goi, M., Tanaka, M.: *In vitro* growth of banana plantlets cultured under red and blue light-emitting diode (LED) irradiation source. – Acta Hort. **575**: 117-123, 2002a.

Nhut, D.T., Takamura, T., Watanabe, H., Murakami, A., Murakami, K., Tanaka, M.: Sugar-free micropropagation of *Eucalyptus citriodora* using light-emitting diodes (LEDs) and film-rockwool culture system. – Environ. Contr. Biol. **40**: 147-155, 2002b.

Nhut, D.T., Takamura, T., Watanabe, H., Okamoto, K., Tanaka, M.: Responses of strawberry plantlets cultured *in vitro* under superbright red and blue light-emiiting diodes (LEDs). – Plant Cell Tissue Organ Cult. **73**: 43-52, 2003.

Reuther, G.: Stimulation of the photoautotrophy of *in vitro* plants. – Acta Hort. **300**: 59-75, 1991.

Sheen, J.: Metabolic repression of transcription in higher plants. – Plant Cell **2**: 1027-1038, 1990.

Solárová, J.: Photosynthesis of plant regenerants. Diurnal variation in CO_2 concentration in cultivation vessels resulting from plantlets photosynthetic activity. – Photosynthetica **23**: 100-107, 1989.

Solárová, J., Součková, D., Ullmann, J., Pospíšilová, J.: *In vitro* culture: environmental conditions and plantlets growth as affected by vessel and stopper types. – HortScience **23**: 51-58, 1996.

Tanaka, M.: Disposable film culture vessels. – In: Bajaj, Y.P.S. (ed.): Biotechnology in Agriculture and Forestry. Pp. 212-228. Springer-Verlag, Berlin 1991a.

Tanaka, M., Goi, M., Higashiura, T.: A novel disposable film culture vessel in micropropagation. – Acta Hort. **226**: 663-670, 1988a.

Tanaka, M., Hirano, T., Goi, M., Higashiura, T., Sasahara, H., Murasaki, K.: Practical application of a novel disposable film

culture vessel in micropropagation. – Acta Hort. **300**: 77-84, 1991b.

Tanaka, M., Ikeda, M., Fukai, S., Goi, M.: Effect of different films used for film culture vessels on plantlet development of *Phalaenopsis* and *Cymbidium*. – Acta Hort. **319**: 225-230, 1992a.

Tanaka, M., Jinno, K., Goi, M., Higashiura, T.: The use of disposable fluorocarbon polymer film culture vessel in micropropagation. – Acta Hort. **230**: 73-80, 1988b.

Tanaka, M., Nagae, S., Fukai, S., Goi, M.: Growth of tissue-cultured *Spathiphyllum* on rockwool in a novel film culture vessel under high CO_2. – Acta Hort. **314**: 139-146, 1992b.

Tanaka, M.; Nagae, S., Takamura, T., Kusanagi, N., Ujike, M., Goi, M.: Efficiency and application of film culture system in the *in vitro* production of plantlets in some horticultural crops. – J. Soc. high Technol. Agr. **8**: 280-285, 1996.

Tanaka, M., Yap, D.C.H., Ng, C.K.Y., Hew, C.S.: The physiology of *Cymbidium* plantlets cultured *in vitro* under conditions of high carbon dioxide and low photosynthetic photon density. – J. Hort. Sci. Biotech. **74**: 632-638, 1999.

Ziv, M., Meir, G., Halevy, A.H.: Factors influencing the production of hardened glaucous carnation plantlets *in vitro*. – Plant Cell Tissue Organ Cult. **2**: 55-63, 1983.