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Micropropagation of Sweetpotato (*Ipomoea batatas*) in a novel CO_2-Enriched Vessel

Jaime A. Teixeira da Silva*, Dam Thi Thanh Giang, Michio Tanaka

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

Abstract

To overcome various disadvantages of conventional culture vessels for micropropagation, a novel disposable vessel, the "Vitron", made of a multi-layered OTP® film and supported by a polypropylene frame, was developed. The film possesses superior properties such as: high light transmittance, low water vapor transmittance and thermal stability and in particular, high gas-permeability. Single nodal explants, which were excised from the multiple shoots derived from shoot-tip culture, were cultured in Vitron and polycarbonate vessels on 3% sugar-containing agar on MS medium and placed at 3000 ppm CO_2-enrichment at a low photosynthetic photon flux density (PPFD) (45 μmol m^{-2} s^{-1}). The *in vitro* and *ex vitro* growth, and the net photosynthetic rate of *in vitro* and *ex vitro* plantlets were significantly enhanced in the Vitron compared to those cultured in a polycarbonate vessel. Explants that were cultured on the same MS medium under low PPFD at various CO_2 concentrations were also cultured at 3000 ppm CO_2- enrichment at various PPFD: 30, 45, 60, 75 and 90 μmol m^{-2} s^{-1}. The best in vitro and *ex vitro* growth obtained for 3000 ppm CO_2-enrichment at 75 μmol m^{-2} s^{-1} PPFD. The novel Vitron vessel, when placed under the two conditions, may replace conventional culture vessels for the successful micropropagation of sweetpotato.

Key words: carbon dioxide, film vessel, photomixotrophic, sweet potato, tissue culture

* Corresponding author, E-mail: jaimetex@lycos.com
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Introduction

Glass or plastic conventional culture vessels, which are being used throughout the world nowadays for micropropagation, are air-tight containers. The conventional protective conditions, under which the explants are grown to prevent contamination and retard desiccation of the plant and the nutrient medium may often unintentionally cause a restriction of gas exchange between the vessel atmosphere and the outside air (Buddendorf-Joosten and Woltering 1994). It has been found that the CO_2 concentration in an air-tight vessel containing chlorophyllus plantlets is often as low as the CO_2 compensation points during the most of photoperiod, and much lower than normal atmospheric CO_2 concentration (Kozai 1991). These features cause the physical environment *in vitro* in a conventional tissue culture system is quite different from that in greenhouse and often results in undesirable physiological and pathological problem (Debergh and Maene 1984).

Many researchers achieved enhanced gas exchange between the culture vessel atmosphere and growth chamber environment by using a ventilated culture vessel (Kozai et al. 1987; Tanaka et al. 1988, 1992, 1999; Desjardins 1995). These findings have stimulated the development of culture containers and container enclosures that facilitate control of the vessel atmosphere.

Tanaka (1991) reviewed the use of film culture vessel for micropropagation by increasing CO_2 concentrations inside the vessel through using gas-permeable Neoflon® PFA (tetrafluoroethylene perfluoroakyl vinyl ether copolymer) film. In the present research, a novel film culture vessel, developed by using another kind of film, was examined. The vessel, termed the "Vitron", is made of a multi-layered OTP® film (Figure 1A) and is supported by a polypropylene

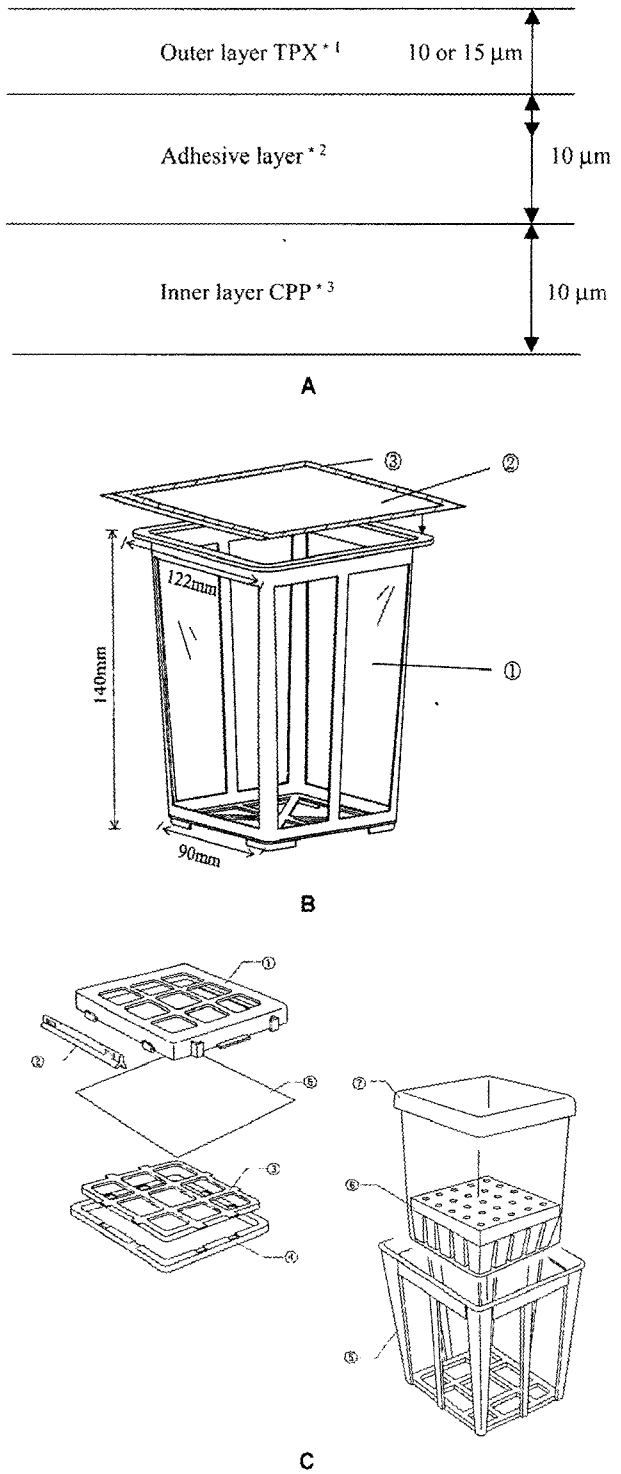

Figure 1. A; Structure of OTP film. 1, 4 methyl-1-pentane polymer; 2, polyolephin resins; 3, polypropylene. B; Diagram of Vitron vessel. 1, main frame; 2, top seal film; 3, adhesive area. C; Diagram of Miracle Pack® film culture system. 1, lid (polycarbonate); 2, clamp (polycarbonate); 3, frame for lining film to the lid (polycarbonate); 4, silicon foam band; 5, main frame (108 x 108 x 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 (Grodan, Denmark) multiblock.

frame (Figure 1B). The film possesses superior properties such as: high light transmittance, high gas-permeability, low water vapor transmittance and thermal stability. Superior gas permeability is the unique characteristic of this vessel; that is, gases can diffuse accross the vessel wall to compensate for differences in gas concentrations internal and external to the vessel.

The purpose of this study is to investigate the applicability of the Vitron vessel for the micropropagation of sweetpotato (cv. Naruto Kintoki) by comparing the *in vitro* and *ex vitro* growth of plantlets cultured photomixotrophically (CO_2-enriched and sugar-containing medium) in the gas permeable Vitron and cultured heterotrophically (non CO_2-enriched and sugar-containing medium) in a sealed polycarbonate vessel. The effects of various CO_2 concentrations and light intensities on the *in vitro* and *ex vitro* growth of plantlets by using this novel Vitron vessel were also examined.

Materials and Methods

Plant material

The explants used in this study were single nodes of sweetpotato (*Ipomoea batatas* cv. Naruto Kintoki), which were excised from the multiple shoots derived from a shoot-tip culture. Twelve explants were cultured in each culture vessel (Figure 1B, 1C). Three vessels were used for each treatment. For acclimatization, 24 sweetpotato *in vitro* plantlets were transferred to soil (Jiffy®-mix, USA) and placed in a greenhouse for three weeks.

Media and culture conditions

A 3% sugar-containing agar MS (Murashige and Skoog 1962) medium was used. The pH of the medium was adjusted to 5.7 before autoclaving at 121 kPa for 17 min. The culture conditions were: temperature, 25±1℃; photoperiod, 16 h/day; light intensity, 45 μmol m^{-2} s^{-1} (Homo-Lux. National Electric Co., Ltd., Japan); and CO_2 enrichment, 3000 ppm/24 h/day. For the experiment of various CO_2 concentrations, Vitron vessels were placed in different chambers at controlled CO_2 concentrations (Tanaka et al. 1992).

Culture vessels

OTP® film (Otsuka Technology Production, Ltd) is used to make the Vitron vessel. OTP® is a multi-layer film (Figure 1A) consisting of three layers. The outer layer of TPX (4-methyl-1-pentane polymer, 15 μm in thickness) and the inner layer of CPP (a polypropylene, 10 μm in thickness)

Table 1. Characteristics of OTP film.

Thickness of film (μm)	35
Oxygen permeability (cm^3/m^2. 24 hr. atm)	10,900
Carbon dioxide permeability (cm^3/m^2. 24 hr. atm)	30,100
Water vapor permeability (g/m^2/day)	38

are bonded together by the middle layer of polyolephin resins (10 μm in thickness). The characteristics of OTP film are demonstrated in Table 1. The Vitron (Figure 1B) consists of a 3-dimensional injection-molded polypropylene frame, covered by an OTP film sheet heat-sealed on all sides except the top. A top seal film (OTP) was affixed to the top of the vessel after removing the paper backing to expose the adhesive. The film was secured on the flange of the vessel, and the edges of the film were folded to the underside of the flange to achieve a hermetic seal. The sealed polycarbonate vessel is made of polycarbonate and has the same shape and size as the Vitron vessel (Figure 1C).

Morphogenic and photosynthetic analysis

Plantlet growth was quantified by the number of leaves, plant height, fresh and dry weight of shoots, root length and fresh and dry weight of roots. Chlorophyll content in the third leaf (counting downward from the top) of 30 plantlets was measured as the SPAD value by a chlorophyll meter (SPAD-502, Minolta Co., Ltd., Japan). The photosynthetic rate of the leaves of 30 plantlets were measured using a LI-COR portable gas exchange system (LI-6400, LI-COR, USA). Measurements were performed at 25℃ and the vapor pressure deficit at the leaf surface was maintained between 2.3 and 3.1 kPa. The CO_2 concentration in the sample chamber was set at 400 $\mu l\ l^{-1}$. Measurement of CO_2 uptake between the range of 0 $\mu mol\ m^{-2}\ s^{-1}$ and 300 $\mu mol\ m^{-2}\ s^{-1}$ was conducted using a built-in red light-emitting diode (LED) lamp.

Statistical analysis

Data analysis was carried out using the IRRISTAT, version 3.0. Duncan's multiple range tests at $p = 0.05$ were used for statistical comparisons.

Results and Discussion

Growth of sweetpotato plantlets cultured in the ventilated Vitron and sealed polycarbonate vessels

The *in vitro* growth of sweetpotato plantlets cultured in Vitron and polycarbonate vessels after five weeks is shown in Table 2 and Figure 2. The plantlets were significantly enhanced in the Vitron when compared to those cultured in the polycarbonate vessel. The plant height, root length, the root fresh and root dry weights of plantlets cultured in Vitron were significantly much higher than those in the polycarbonate vessel. In particular, the top fresh and dry weight of those from Vitron vessel was almost 2-fold higher than those

Figure 2. *In vitro* growth of sweet potato plantlets cultured in the polycarbonate and Vitron vessels, 5 weeks after culturing (*left*, polycarbonate vessel; *right*, Vitron).

Table 2. *In vitro* growth of sweet potato plantlets cultured in the Vitron and polycarbonate vessel.

Vessels	Plant height (cm)	Root length (cm)	SPAD value[3]	Number of leaves	Fresh weight (mg)		Dry weight (mg)	
					Top	Root	Top	Root
Poly.[1]	3.7b[2]	12.7b	38.9a	9.1a	640.6b	193.6b	52.8b	14.3b
Vitron	4.8a	15.3a	41.2a	9.3a	1122.8a	254.3a	101.1a	20.3a

[1]Polycarbonate vessel

[2]Different letters within a column indicate significant differences at $p = 0.05$ by Duncan's multiple range test.

[3]Chlorophyll content in the third leaf, counted from top downward of the plantlets.

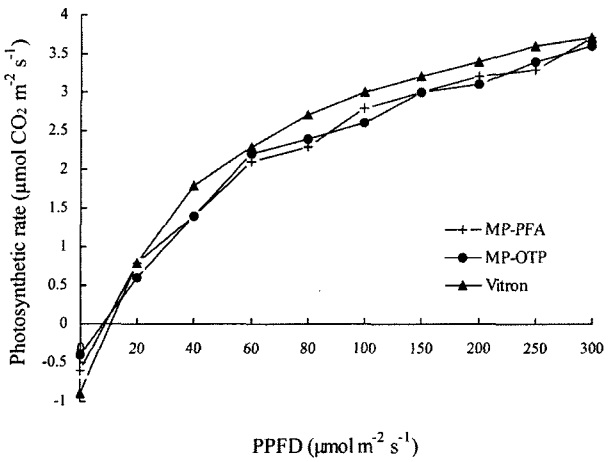

Figure 3. Photosynthetic rate of sweet potato plantlets cultured in the polycarbonate and Vitron vessels.

Figure 4. *Ex vitro* growth of sweet potato plantlets cultured in polycarbonate and Vitron vessels, 3 weeks after transferring to soil (*left*, polycarbonate vessel; *right*, Vitron).

cultured in the polycarbonate vessel. However, the number of leaves and SPAD value was almost equal between the two treatments.

Net photosynthetic rate of *in vitro* plantlets cultured in the two vessels is shown in Figure 3. The photosynthetic performance of plantlets from the Vitron vessel were greater than those in the polycarbonate vessel regardless of the difference in light intensity from 0 to 300 μmol m^{-2} s^{-1}.

Subsequent growth of sweetpotato plantlets derived from the two culture vessels three weeks after transplanting to soil, is shown in Table 3 and Figure 4. The plantlets cultured in the Vitron vessel were significantly greater when compared to those derived from the polycarbonate vessel. The plant height, number of leaves and top, root fresh weight as well as the top, root dry weight of those from the Vitron were much higher than those from the polycarbonate vessel. However, regardless of the kind of culture vessel, root length and SPAD value of leaves were not significantly different.

Sweetpotato plantlet explants grew better in the Vitron vessel under photomixotrophic condition (CO_2-enriched and sugar-containing medium), even at a low PPFD, as compared to those from polycarbonate vessel under heterotrophic condition (non CO_2-enriched and sugar-containing medium). This could be due to the better gas permeability of the Vitron vessel, which is made of superior gas permeable film, that is, gases can diffuse across the vessel wall to compensate for differences in gas concentrations internal and external to the vessel. This is a very important consideration, since it has been found that the CO_2 concentration in a sealed vessel containing chlorophyllus plantlets is often as low as the CO_2 compensation points during most of the photoperiod, and much lower than normal atmospheric CO_2 concentration (Kozai 1991).

It should be noted that both treatments in these experiments used 3% sugar-containing medium, since in the sugar-free medium, the single nodal explants did not induce shoots, even under photoautotrophic condition. This observation is contrary to Kozai et al. (1987), who suggested that sucrose, an essential exogenous carbon source in conventional systems, can be replaced by elevated CO_2 within the culture atmosphere. The observation is, however, in agreement with Tanaka et al. (1999), who indicated that exogenous sucrose for micropropagation of *Cymbidium* is required, even in the presence of elevated CO_2. This may be indicative of the dependence on an exogenous sugar source during the early stages of culture, before the plantlets have achieved a positive carbon balance. Further studies are needed to provide a better understanding of the role of exogenous sugars on the growth of sweetpotato plantlets *in vitro* under CO_2-enrichment.

Table 3. *Ex vitro* growth of sweet potato plantlets cultured in the Vitron and polycarbonate vessel.

Vessels	Plant height (cm)	Root length (cm)	SPAD value[3]	Number of leaves	Fresh weight (mg)		Dry weight (mg)	
					Top	Root	Top	Root
Poly.[1]	11.8b[2]	19.3a	29.5a	10.9b	2179.3b	552.2b	171.9b	36.5b
Vitron	13.4a	21.8a	32.1a	12.1a	2986.4a	852.3a	230.5a	59.4a

[1,2,3]See legends to Table 2.

The net photosynthetic rate of plantlets from the ventilated Vitron vessel was greater than those in the sealed polycarbonate vessel. The result is supported by Fujiwara et al. (1988), who indicated that the CO_2 concentration in a sealed vessel containing chlorophyllous plantlets is often as low as the CO_2 compensation point (less than 100 ppm) during most of the photoperiod, and much lower than the normal atmospheric CO_2 concentration of 345 ppm. Even in loosely capped vessels capped with gas permeable film, the concentration is often lower than 200 ppm (Kozai 1991). Therefore, the photosynthesis of plantlets is restricted by a low CO_2 concentration inside the vessel. By using ventilating the Vitron vessel, which has superior gas-permeable characteristics, the CO_2 concentration in the Vitron during the photoperiod may be higher than in the polycarbonate vessels, as a result of an increased photosynthetic rate.

The *ex vitro* growth of plantlets cultured in the Vitron were also better than from the polycarbonate vessel. All the plantlets from the Vitron were vigorous and normal. These results strongly confirm that the plantlets cultured in a ventilated Vitron vessel, under CO_2-enriched and sugar-containing medium, also have enhanced growth as compared to those from conventional vessels. This finding is different from the results of Kozai (1991), who indicated that the *ex vitro* growth of several plant species can be achieved more easily with photoautotrophic micropropagation. In addition, the Vitron vessels have a large opening, which makes it easy to avoid damaging the plantlets when they were removed for transfer. Thus, higher growth rate and plantlet quality are expected following transfer as compared to conventional culture vessels.

Effect of CO_2 concentrations on the *in vitro* and *ex vitro* growth of plantlets cultured in the Vitron vessel

The *in vitro* growth of plantlets cultured in the Vitron vessel after 5 weeks under various CO_2-enrichment (1000, 2000, or 3000 ppm, and the control i.e., 400 ppm), hereafter named 1000, 2000, 3000 and control condition, is shown in Table 4 and Figure 5. Plantlets cultured in control condition were lowest, while that of plantlets cultured in the 3000 condition were highest. Such a trend was also observed with number of leaves. The plant heights and number of leaves of plantlets cultured in the 1000 and 2000 conditions were equal. The root length of plantlets cultured in control and the 1000 conditions were equal to or lower than the other two treatments. These values of those cultured in the 3000 and 2000 conditions were equal. The SPAD value of leaves was highest in plantlets cultured in the 3000 condition, while these values were equal in the other treatments. The top fresh weights of plantlets cultured in the 3000 and control conditions were highest and lowest, respectively, while those of the two other treatments were equal. The root fresh weights of those cultured in 3000 condition were greatest, while the other treatments were equal. The top, root dry weight of plantlets cultured in 3000 and control conditions were highest and lowest, respectively, while the values of the other treatments were equal. In brief, the *in vitro* growth of plantlets cultured in 3000 condition is greatest, and the *in vitro* growth of plantlets from other treatments were better than those in the control treatment.

Figure 5. *In vitro* growth of sweet potato plantlets cultured in Vitron vessel, in various CO_2-enriched conditions, 5 weeks after culturing (from *left* to *right*, Cont.; 1000; 2000; 3000 ppm).

Subsequent growth of plantlets derived from various CO_2-enrichment conditions after three weeks transplanting to soil

Table 4. *In vitro* growth of sweet potato plantlets cultured in the Vitron under various CO_2-enriched conditions.

CO_2 Conc.[1] (ppm)	Plant height (cm)	Root length (cm)	SPAD value[3]	Number of leaves	Fresh weight (mg)		Dry weight (mg)	
					Top	Root	Top	Root
Control	4.4c[2]	9.8b	35.5b	8.2c	788.3c	206.9b	55.6c	15.1c
1000	4.7b	10.8b	35.7b	8.7b	907.2b	223.3b	68.1b	16.7b
2000	4.6b	12.3a	35.5b	9.0b	923.2b	223.8b	66.8b	17.8b
3000	5.5a	12.1a	38.3a	10.7a	953.5a	241.3a	97.8a	18.8a

[1]Concentration [2,3]See legends to Table 2.

is shown in Table 5 and Figure 6. The plant heights of plantlets increased as CO_2-enriched levels increased. There is no significant difference in SPAD value of leaves regardless of various CO_2-enriched levels. The number of leaves was highest in the 2000 and 3000 conditions, and lowest in the control condition. The values of plantlets in the 1000 conditions were higher than those in the control condition. The root length values of plantlets in CO_2-enriched treatments were equal and higher than those in the control condition. The top fresh and dry weights of plantlets cultured in the 3000 and control conditions were highest and lowest, respectively. The values of the other two treatments were equal. The root fresh and root dry weights of plantlets cultured in the 3000 condition were highest while these values of those in control and the 1000 conditions were lowest. In conclusion, the subsequent growth of plantlets, which cultured at different levels of CO_2 enrichment was most enhanced in the 3000 CO_2-enriched condition.

The *in vitro* growth and subsequent transplantation and acclimatization to soil of plantlets cultured in the Vitron vessel under 3000 ppm CO_2 and low PPFD (45 μmol m^{-2} s^{-1}) was the best. It has been found that the CO_2 concentration inside the vessel during the dark period reaches 3000 to 9000 ppm, but decreases sharply with time to 100 to 200 ppm within a few hours after the onset of the photoperiod and remains almost the same until the dark period begins (Kozai et al. 1987). By using the gas permeable Vitron vessel, where the gas can diffuse across the vessel wall to compensate for big differences in gas concentrations of internal and external of the vessel during the photoperiod, the photosynthetic capacity of plantlets could be enhanced to obtain better 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 (3000 ppm) to compensate for the very low internal CO_2 concentration during the photoperiod.

The most suitable CO_2-enrichment of this study is relatively high as compared to other reports, which, in the case of carnation, suggested that the explants grown photoautotrophically enhanced under 1000-1500 ppm CO_2-enrichment (Kozai et al. 1988) and 950-1000 ppm CO_2-enrichment in *Cymbidium* (Kozai 1991). However these studies used very high PPFD of 150 or 230 μmol m^{-2} s^{-1}, respectively. Recently, by using a forced ventilation method (Nguyen et al. 2001), the growth of coffee plantlets under 1000-1200 ppm CO_2-enrichment and 100-150 μmol m^{-2} s^{-1} PPFD could be enhanced. This is a very important consideration, since the high PPFD is a limiting factor of plant growth due to the increase in the temperature inside the vessel, and moreover increasing production costs for a cooling system in the culture chamber. However, since the present research used sugar-containing medium, and thus an additional cost was needed for sugar.

Figure 6. *Ex vitro* growth of sweet potato plantlets cultured in Vitron vessel, following various CO_2-enriched conditions, 2 weeks after acclimatization (from *left* to *right*, Cont.; 1000; 2000; 3000 ppm).

Effect of light intensity on the *in vitro* and *ex vitro* growth of plantlets cultured in the Vitron vessel

The *in vitro* growth of sweetpotato plantlets cultured in the Vitron vessel after five weeks under various light intensities (30, 45, 60, 75 and 90 μmol m^{-2} s^{-1}), hereafter named 30, 45, 60, 75 and 90 condition, is shown in Table 6 and Figure 7. Plantlets from the 90 condition were highest while plantlets from the 30 and 45 conditions were shortest among all treatments. Plantlets cultured under the 60 and 75 conditions were equal in height. The number of leaves of plantlets from the 90 conditions were highest while there

Table 5. *Ex vitro* growth of sweet potato plantlets cultured in the Vitron under various CO_2-enriched conditions.

CO_2 Conc.[1] (ppm)	Plant height (cm)	Root length (cm)	SPAD value[2]	Number of leaves	Fresh weight (mg)		Dry weight (mg	
					Top	Root	Top	Root
Control	11.8d	17.3b	28.1a	9.3c	2860.7c	482.3c	206.8c	31.5c
1000	13.4c	19.4a	28.2a	10.3b	3515.6b	464.9c	253.7b	32.2c
2000	16.2b	20.7a	28.8a	12.4a	3735.1b	614.2b	274.4b	41.2b
3000	17.1a	19.2a	28.9a	13.3a	4544.9a	896.9a	369.9a	62.9a

[1,2]See legends to Table 4.

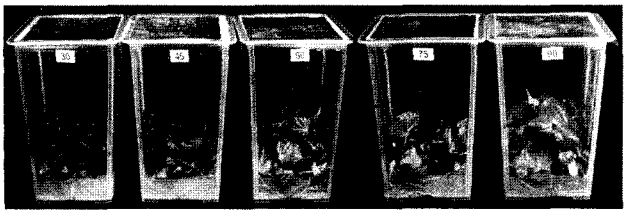

Figure 7. *In vitro* growth of sweet potato plantlets cultured in Vitron vessel, at various PPFD, 5 weeks after culturing (from *left* to *right*, 30; 45; 60; 75; 90 μmol m^{-2} s^{-1}).

Figure 8. *Ex vitro* growth of sweet potato plantlets cultured in Vitron vessel, at various PPFD, 2 weeks after acclimatization (from *left* to *right*, 30; 45; 60; 75; 90 μmol m^{-2} s^{-1}).

was now significant difference between those from other treaments. Roots were longest in the 90 condition, but shortest in plantlets from the 30 condition. Root of plantlets from the 75 condition were longer than those from the 45 and 60 conditions, while plantlets from the other two conditions had a similar root length. The SPAD value of leaves of plantlets from the 90 condition was highest, while that of plantlets from the 30 condition was lowest among all treatments. The SPAD values of the other three treatments were equal. The top fresh weight of plantlets cultured in the 90 condition was highest, and lowest in plantlets cultured at the 30 condition. Top fresh weight values were equal in plantlets cultured under the 60 and 75 condition and higher than plantlets in the 45 condition. The root fresh weights of plantlets in the 90 condition were also highest while lowest values were obtained in the 30 and 45 conditions. Plantlets from the 60 and 75 conditions had similar root fresh weights. The top, root dry weights of plantlets in the 75 and 90 conditions were highest among all treatments and lowest values obtained in plantlets in the 30 condition. Plantlets from the 45 and 60 conditions had a similar top dry weights. Plantlets from the 75 and 90 conditions had a similar top and root dry weights. The growth tendency of plantlets decreased with the decreasing of light intensity from 90 to 30 μmol m^{-2} s^{-1}. However the growth of plantlets from the 75 conditions was similar to those from the 90 condition.

The subsequent growth of sweetpotato plantlets derived from the Vitron vessel under various light intensities three weeks after transplanting to soil is shown in Table 7 and Figure 8. Plantlets derived from the 75 and 90 conditions were highest, decreasing with a decrease in light intensity.

Table 6. *In vitro* growth of sweet potato plantlets cultured in the Vitron under various PPFD.

PPFD[1]	Plant height	Root length	SPAD value[3]	Number of leaves	Fresh weight (mg)		Dry weight (mg)	
(cm)	(cm)				Top	Root	Top	Root
30	4.2c[2]	12.8d	40.7c	9.2b	923.3d	201.5c	66.7d	17.9d
45	4.8c	15.3c	41.2b	9.3b	1122.8c	254.3c	101.1c	20.3c
60	6.9b	16.1c	41.9b	9.7b	1397.8b	351.8b	107.1c	26.1b
75	7.1b	20.5b	42.2b	9.8b	1464.9b	407.2b	125.6a	31.6a
90	7.8a	23.1a	43.3a	10.3a	1922.5a	500.2a	141.8a	37.6a[1]

Photosynthetic photon flux density (μmol m^{-2} s^{-1}) [2,3]See legends to Table 2.

Table 7. *Ex vitro* growth of sweet potato plantlets cultured in the Vitron under various PPFD.

PPFD[1]	Plant height	Root length	SPAD value[3]	Number of leaves	Fresh weight (mg)		Dry weight (mg)	
	(cm)	(cm)			Top	Root	Top	Root
30	11.8d[2]	21.4b	29.9b	11.9b	2135.2d	518.1d	149.5e	38.2d
45	13.4c	21.8b	31.1b	12.1b	2986.1c	852.3c	230.5d	59.4c
60	15.1b	22.6b	31.2b	10.4b	3489.3b	831.8c	257.8c	56.3c
75	17.1a	27.5a	32.8a	9.1b	4667.5a	1226.6a	370.5a	98.7a
90	17.4a	27.8a	33.1a	11.3a	3706.8b	937.8b	294.8b	75.6b

[1,2,3]See legends to Table 6

The number of leaves of plantlets from the 90 condition was highest. These values were similar to other treatments. Roots were highest in the 75 and 90 conditions, but were equal in the other treatments. Such a trend was also true for the SPAD values of leaves. The top, root fresh weights of plantlets from the 75 and 30 conditions were highest and lowest, respectively. The top fresh weights of plantlets from the 60 and 90 conditions were equal and higher than plantlets in the 45 condition. The top, root dry weights of plantlets from the 75 and 30 conditions were highest and lowest, respectively. The root dry weights of plantlets from the 45 and 60 conditions were equal to and lower than plantlets in the 90 conditions. In brief, the subsequent growth to soil of plantlets from the 75 condition was most enhanced.

The *in vitro* growth and subsequent growth after transplanting to soil of plantlets cultured in the Vitron vessel was best under 75 μmol m^{-2} s^{-1} PPFD. This condition is not as high as compared to other reports, where the light conditions were often ranged from 100-150 μmol m^{-2} s^{-1} (Kozai et al. 1987; Nguyen et al. 2001). This low light requirement may be due to the suitable combination of environmental factors for *in vitro* growth (3000 ppm CO_2-enrichment on a sugar-containing medium). In addition, the Vitron vessel has a large opening (122 x 122 mm), which is made of high light transmittance OTP film, therefore plantlets could receive more downward light, consequently not demanding a high light intensity. However, Desjardins (1995) suggested that the net photosynthetic rate of many tissue cultured species might saturate at a relatively low PPFD after a successive growth in heterotrophic or photomixotrophic conditions.

We have shown that the growth of sweetpotato plantlets cultured photomixotrophically in the Vitron vessel u CO_2-enriched condition can be enhanced at a low PPFD (Figure 7) as compared to conventional polycarbonate vessel. The best condition for sweetpotato plantlets cultured photomixotrophically in the Vitron vessel is 3000 ppm CO2-enrichment and 75 μmol m^{-2} s^{-1} PPFD.

By employing the disposable Vitron vessel for sweetpotato micropropagation, the *in vitro* plantlets had a high photosynthetic capacity and were vigorous, and became high quality/healthy *ex vitro* plantlets as compared to a conventional vessel. These attractive features of the Vitron vessel help to reduce both production and labor costs, and overcome many difficulties encountered with using other existing conventional vessels. In conclusion, the Vitron vessel is recommended for sweetpotato micropropagation.

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