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Commercial Application of a Photoautotrophic Micropropagation System Using Large Vessels with Forced Ventilation: Plantlet Growth and Production Cost

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Abstract. **A photoautotrophic or sugar-free medium micropropagation system (PAM) using five large culture vessels (volume = 120 L each) with a forced ventilation unit for supplying CO_2-enriched air was developed and applied to commercial production of calla lily (*Zantedeschia elliottiana*) and china fir (*Cunninghamia lanceolata*) plantlets. The culture period of calla lily plantlets in the PAM was reduced by 50%, compared with that in a conventional, photomixotrophic micropropagation system (PMM) using small vessels each containing a sugar-containing medium. Percent survival ex vitro of calla lily plantlets from the PAM was 95%, while that from the PMM was 60%. The production cost of calla lily in the PAM was reduced by about 40%, compared with that in the PMM, and the initial investment per plantlet for the PAM was ≈10% lower than that for the PMM. The sales price of *ex vitro* acclimatized calla lily plantlet was increased by 25% due to its higher quality, compared with plantlets produced in the PMM.**

A photoautotrophic micropropagation system (called a PAM hereafter) that uses a sugar-free culture medium has many advantages over the conventional, photomixotrophic micropropagation system (called a PMM hereafter) that uses a sugar-containing culture medium (Kozai, 1991). The advantages include the use of large culture vessels with minimum risk of microbial contamination and the enhancement of plantlet growth at a high photosynthetic photon flux (PPF) and a high CO_2 concentration inside the vessel (Fujiwara et al., 1988; Kozai and Iwanami, 1988).

To increase CO_2 concentration in the vessel under pathogen-free conditions, both natural and forced ventilation methods have been employed. Putting gas-permeable filter disks on the vessel lid enhances natural ventilation (Aitken-Christie et al., 1995). Forced ventilation can be conducted by supplying CO_2-enriched air with an air pump into the vessel through a gas-permeable filter disk (Kozai et al., 2000). The forced ventilation rate can be easily controlled during the production process by using an airflow controller, while the natural ventilation rate is difficult to change with passage of days (Aitken-Christie et al., 1995). In addition, it is difficult to obtain a high natural ventilation rate for a large vessel. Thus, for commercial production, forced ventilation is more convenient and practical than natural ventilation in a PAM that uses large vessels. Furthermore, many reports have shown that a PAM with forced ventilation considerably enhances the growth of plantlets compared with a PAM with natural ventilation. Fujiwara et al. (1988) developed a 20-L vessel with forced ventilation for enhancing the photoautotrophic growth of strawberry (*Fragaria ×ananassa* Duch.) plantlets during the rooting and acclimatization stages. Kubota and Kozai (1992) used a 2.6-L vessel containing a multi-cell tray with forced ventilation for photoautotrophic growth of potato (*Solanum tuberosum* L.) plantlets. Heo and Kozai (1999) developed a similar system using a 12.8-L vessel for the photoautotrophic growth of sweetpotato [*Ipomoea batatas* (L.) Lam.] plantlets. Heo et al. (2000) developed another type of a 11-L vessel containing air distribution pipes to improve an airflow pattern in the vessel for obtaining the uniform growth of sweetpotato plantlets. Zobayed et al. (1999) engineered a 3.5-L vessel with units of forced ventilation and sterile nutrient solution supply for uniform and enhanced growth of sweetpotato plantlets. Commercial application of the PAM may be advantageous to additional ornamental species such as calla lily (*Zantedeschia elliottiana*) and china fir (*Cunninghamia lanceolata* (Lamb.) Hook). However, economic analysis of the PAM has rarely been conducted until recently (Kubota and Kozai, 2001). The objectives of this study were 1) to assess plantlet growth of calla lily and china fir in the PAM in comparison with that in the PMM, and 2) to assess the possibility of commercialization of the PAM based on the estimation of production cost of calla lily plantlets.

Calla lily, a herbaceous flowering plant for which there is currently a large demand, is conventionally propagated by tubers, resulting in a limited multiplication rate. The multiplication rate can be improved by a conventional micropropagation system but wide application has been limited by its high production costs, which are mainly due to poor plantlet growth, high percent of biological contamination of the medium, and labor intensive work (Lorenzo et al., 1998). China fir is a rapid-growing woody plant that is used in the timber, furniture, and ornamental industries. In recent years, large quantities of china fir plantlets have been produced by PMM. However, china fir, like many other woody plant species, does not easily develop roots in vitro. Plant growth regulators have been supplied to the medium to promote in vitro rooting of woody plantlets, often without success (Kozai et al., 2000). In addition, sugar-containing medium often causes callus formation at the base of shoots and a low percentage of survival for many plant species during the ex vitro acclimatization (Kozai and Zobayed, 2000).

Table 1. Basic specifications of the PAM (photoautotrophic micropropagation) and the PMM (photomixotrophic micropropagation) systems.

Item (Unit)	PAM	PMM
Vessel volume (L)	120	0.37 (ϕ = 7 cm)
Vessel bottom area (cm^2)	5980	38.5
Number of vessels per module	5	500
Ventilation type	Forced	Natural
Vessel ventilation rate ($mL \cdot s^{-1}$)	0–60 (controllable)	0.05 (fixed)
Supporting material	Vermiculite	Agar (6 $g \cdot L^{-1}$)
Medium sucrose concentration ($g \cdot L^{-1}$)	0	30
Nutrient solution	Murashige and Skoog (1962)	
Light source	Cool white fluorescent lamps	
Floor area of culture room (m^2)	20	
No. of modules per culture room	9	
No. of shelves per module	5	
Area per shelf (cm^2)	6760 (130 cm wide × 52 cm deep)	
Height of module (cm)	220	
Room air temperature (C)	22–23 °C	
Room relative humidity	70% to 80%	

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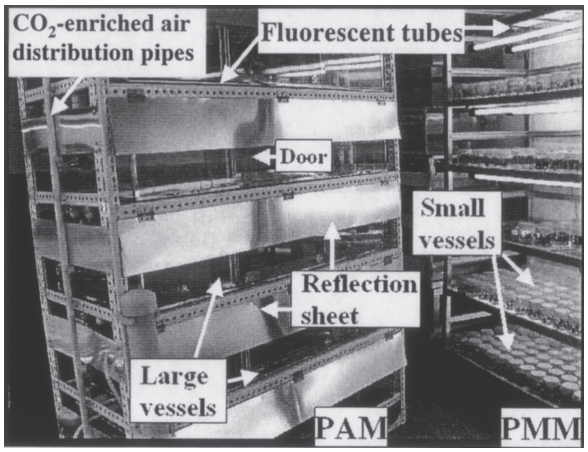

Fig. 1. A culture module of the photoautotrophic micropropagation system using large culture vessels with forced ventilation (PAM system) at the center and a culture module of the photomixotrophic micropropagation system using small vessels with natural ventilation (PMM system) in the right.

Materials and Methods

The commercial production site was owned and operated by a research institute where the first author was working for, as a production manager.

PAM and PMM system configurations. Specifications of the PAM and PMM are summarized in Table 1. The PAM consisted of a culture room and nine culture modules. Each culture module consisted of a five-shelf unit (each shelf having a culture vessel and a lighting unit) and a forced ventilation unit for supplying CO_2-enriched air. The five-shelf unit consisted of a steel frame supporting five shelves (Fig. 1). The culture vessel unit consisted of a plexiglass box with two air inlets (diameter = 5 mm) and six air outlets (diameter = 20 mm) for forced ventilation. The culture vessels, supporting material and MS (Murashige and Skoog, 1962) nutrient solution were sterilized according to Xiao et al. (2000). A forced ventilation unit for supplying CO_2-enriched air to each of the vessels consisted of a CO_2 container with gas tubes, pressure gauges, airflow meters, an air pump and valves, an air disinfection tank and a humidification tank, and a CO_2 concentration controller (Fig. 1). To increase the uniformity of PPF over the shelf and the ratio of light energy received by the plantlets to the light energy emitted from the lamps, the vessel was surrounded by white reflective sheets. A five-shelf unit of the PMM was identical to the one used in the PAM, although the ventilated vessels, white reflective sheets and forced ventilation unit were not used. A detailed description of the PAM will be given elsewhere.

Calla lily plantlet growth. The growth of calla lily plantlets in the PAM was compared with that in the PMM. Experimental conditions are described in Table 2. Twenty plantlets were harvested at random from each of five vessels in the PAM on day 15, and two vessels each containing ten plantlets were selected at random from each of five culture shelves and harvested on days 15 and 30 in the PMM for destructive measurements of shoot length, number of shoots, leaf area, fresh weight and dry weight. Each culture shelf was considered as a replication in both systems. ANOVA (Analysis of Variance) was run and then treatment means were compared using a least significant difference (LSD) test ($P < 0.05$). Percent loss of plantlets in vitro due to microbe contamination of the medium was calculated by counting dead or heavily damaged plantlets among all the plantlets.

China fir plantlet growth. The growth, percent loss in vitro and percent in vitro rooting of china fir plantlets in the PAM under the presence/absence of NAA (1 mg·L^{-1}) in the medium (called PAM-1 and PAM-0, respectively, hereafter) were compared with those in the PMM. Experimental conditions are described in Table 2. Forty plantlets were selected at random from each of two vessels in the PAM-0 and the PAM-1, respectively, and five vessels each containing eight plantlets were selected at random from each of two culture shelves in the PMM for destructive measurements of shoot length, number of shoots, leaf area, shoot and root fresh weight, and shoot and root dry weight of plantlets. Each culture shelf was considered as a replication. The PMM treatment was considered as a control. ANOVA was conducted for the PAM-0 and the PAM-1, in which the presence/absence of NAA was considered as a factor. Treatment means were compared using a LSD test ($P < 0.01$).

Percent survival during the acclimatization ex vitro. In vitro rooted plantlets were transplanted for acclimatization ex vitro in a bamboo-structured plastic greenhouse with a simple shading screen in September 2001 in Kunming, China. Three thousand calla lily plantlets and 2,000 china fir plantlets from the PAM were transplanted with substrate (vermiculite), while 3,000 calla lily plantlets and 700 china fir plantlets from the PMM were transplanted without substrate (agar). Transplanting the plantlets with substrate reduces the amount of labor hour and root damage, and makes it possible to use automatic transplanting instead of manual transplanting. During the ex vitro acclimatization, average air temperature was 17 °C, the highest and lowest daily average air temperatures were 23 and 14 °C, and average relative humidity during daytime was 76%. PPF was not measured due to a technical problem. Twelve days after transplanting ex vitro, all the dead plantlets were counted in each treatment, and the percent survival ex vitro was determined. This experiment was conducted only once. In addition, 4000 in vitro rooted calla lily plantlets produced in the PAM were sold without ex vitro acclimatization directly to a farmer, just as a trial. Then, the farmer transplanted the plantlets in a bamboo-structured plastic greenhouse without any shading screen (i.e., without ex vitro acclimatization). The number of dead plantlets was counted 12 d after transplanting to determine the percent survival ex vitro.

Table 2. Conditions in calla lily and china fir experiments using the PAM (photoautotrophic micropropagation) and the PMM (photomixotrophic micropropagation) systems.[z]

Experimental conditions	PAM				PMM	
Common to calla lily and china fir	Day 0–3	Day 4–5	Day 6–9	Day 10–12	Day 13–15	Day 0–15/30
PPF (μmol·m^{-2} s^{-1})	50	50	70	100	100	50
Photoperiod (h)	12	12	14	16	16	14
CO_2 concentration (μmol·mol^{-1})	1,500	1,500	1,500	1,500	1,500	400
Vessel ventilation (mL·s^{-1})	0	5–8	13–20	25–30	50–60	0.05
RH in vessels (%)	95	95	90-95	80–90	80	95–100
Calla lily			PAM	PMM		
Days of culture		15			30	
No. of explants/vessel		1500			10	
No. of explants/ treatment		7500			5000	
Medium NAA (mg·L^{-1})		0			1	
Type of explants		Shoots each with one unfolded leaf				
Leaf area, fresh and dry weights per explant		657 mm^2, 243 mg and 13 mg				
Nutrient solution supplied on day 0 per plantlet (mL)					6	6
China fir	PAM-0		PAM-1		PMM	
Days of culture	28		28		28	
No. of explants/vessel	1200		1200		8	
No. of explants/treatment	2400		2400		1600	
Medium NAA (mg·L^{-1})	0		1		1	
Type of explants		Single-node leafy cuttings				
Nutrient solution supplied on day 0 per plantlet (mL)		8 (and 4 mL was added on day 18)				8

[z]RH = relative humidity, NAA = α-naphthaleneacetic acid.

Table 3. Growth and development of calla lily (*Zantedeschia elliottiana*) plantlets during in vitro rooting stage in the PAM (photoautotrophic micropropagation) and PMM (photomixotrophic micropropagation) systems.

Treatment code	Shoot length (mm)	No. of leafy shoots	Leaf area (cm^2)	Fresh wt (mg)	Dry wt (mg)
PAM (on day 15)	91.4 a	3.7 a	12.8 a	674 a	45 a
PMM (on day 15)	51.3 c[z]	3.3 a	7.3 b	395 b	23 b
PMM (on day 30)	76.3 b	3.4 a	9.8 b	579 ab	36 a

[z]Mean separation within columns by LSD test at $P < 0.01$ (n = 100).

Table 4. Production performance and sales price of the PAM (photoautotrophic micropropagation) and PMM (photomixotrophic micropropagation) systems.

Species	PAM (A)		PMM (B)	A to B ratio
Calla lily				
Percent loss in vitro	0% on day 15		5% on day 30	0
Percent rooting in vitro	98% on day 15		98% on day 30	1.0
Multiplication or rooting cycle	15 d		30 d	0.5
Percent survival ex vitro on day 12	95%		60%	1.6
Price per in vitro rooted plantlet	7.23¢		6.02¢	1.09
Price per ex vitro acclimatized plantlet	18.1¢		14.5¢	1.25
Yearly production capacity of in vitro plantlets per module	152,000		52,000	2.92
China fir	PAM-0 (A)	PAM-1		
Percent rooting in vitro on day 28	91%	93%	65%	1.4
Percent survival ex vitro on day 12	95%	97%	16%	5.9

Table 5. Growth and development of china fir (*Cunninghamia lanceolata*) plantlets on day 28, in the PAM-0, PAM-1 and PMM systems. PAM and PMM denote photoautotrophic and photomixotrophic micropropagation, respectively. PAM-0: PAM with absence of NAA in the medium, PAM-1: PAM with presence of NAA in the medium.

Treatment code	Shoot length (mm)	No. of shoots	Leaf area (cm^2)	Fresh wt (mg)		Dry wt	
				Shoot	Root	Shoot	Root
PAM-0	77.2 a[z]	68 a	3.88 a	299 a	84 a	58 a	4.7 a
PAM-1	73.5 a	56 a	3.13 a	272 a	78 a	57 a	4.1 a
PMM	46.1 b[z]	33 b	7.30 b	105 b	51 b	19 b	2.8 b

[z]Mean separation within columns and experiment by LSD test at $P < 0.01$ (n = 80).

Production cost of calla lily plantlets: A case study. Production cost was divided into the investment (or indirect) cost and the direct production cost. The direct production cost was further divided into in vitro multiplication, in vitro rooting, and ex vitro acclimatization costs (The greenhouse construction cost for ex vitro acclimatization was included in the acclimatization cost). In vitro multiplication cost was divided into labor, medium, electricity and other costs. The amounts of electric energy consumed for lighting, cooling, air pumping and autoclaving during the experiments were measured separately by watt meters (DD282, Shanghai Sanxing Ammeter Co., Ltd.). Electricity cost per kWh was 8.1 US cents. The costs of labor, electricity, medium, acclimatization and initial investment for in vitro rooting and ex vitro acclimatization in the PMM and the PAM were recorded and/or calculated based upon their prices as of 2001 in Kunming, China. The number of labor hours, production costs by components, and sales prices were recorded. Costs for supervision, administration, sales and transportation of plantlets to farmers were not included in the calculation of production cost. In this experiment, the cost of in vitro multiplication was equal to the cost of in vitro rooting in both the PMM and the PAM, because these two processes differed only by the combination of plant growth regulators in the medium only.

Results and Discussion

Calla lily plantlet growth. Shoot length, leaf area, fresh and dry weight per plantlet on day 15 were 1.8, 1.8, 1.7, and 2.0 times greater in the PAM than in the PMM, respectively (Table 3). The growth on day 15 in the PAM was similar to or greater than the growth on day 30 in the PMM (Table 3). Most of the plantlets on day 15 in the PAM nearly reached the inner surface of the vessel lid (≈15 cm from the medium surface), and their morphology and quality of plantlets seemed suitable for ex vitro acclimatization, according to visual observation. The PAM shortened the period of in vitro multiplication as well as rooting by half (from 30 to 15 d), compared with that in the PMM. The greater plantlet growth in the PAM than in the PMM was mainly due to the increased photosynthesis and transpiration under high PPF, high CO_2 concentration, enhanced air movement, and low relative humidity in the vessel (Aitken-Christie et al., 1995). Under such environmental conditions, the plantlets generally develop physiologically and morphologically normal stomata. Low relative humidity enhances transpiration, and thus nutrient uptake. The percent loss of in vitro plantlets due to contamination was 0% on day 15 in the PAM, and 5% on day 30 in the PMM (Table 4). Therefore, the monthly production capacity of calla lily plantlets in the PAM is about 3 times (=30/15 × 67,500/(0.95 × 45,000) higher than that in the PMM (The factor of 0.95 in the above expression comes from the 5% loss of in vitro plantlets in the PMM). Percent rooting in vitro was 98% in the PAM (day 15) and in the PMM (day 30).

China fir plantlet growth. Stem length, number of shoots, leaf area, fresh and dry weight of plantlets on day 28 were 1.7, 2.1, 5.3, 2.5, and 2.9 times greater in the PMM-0 than in the PMM (Table 6). There were no significant differences in shoot growth, number of shoots or leaf area between the PAM-1 and the PAM-0. The percentages of rooted plantlets in vitro on day 28 in the PAM-0, PAM-1, and PMM, were 91%, 93% and 65%, respectively. In the PAM, the presence of NAA in the medium had little effect on the increase in percent in vitro rooting, although roots formed 2 to 3 d earlier in the PAM-1 than in the PAM-0. The higher percent of in vitro rooting in the PAM-0 and the PAM-1 than in the PMM was probably due to the absence of sugar in the medium, the use of porous supporting material and enhanced photosynthesis. Sugar in the medium can inhibit adventitious root development in the early stage (Jarvis, 1986). Porous supporting materials such as vermiculite, perlite or mixtures of these materials promoted the in vitro rooting of plant species such as sweetpotato (Afreen-Zobayed et al., 1999), coffee (Nguyen et al., 1999) and sugarcane (Xiao et al., 2003). Endogenous phytohormones such as auxin necessary for rooting and carbohydrates must be more produced by plantlets in the PAM than by plantlets in the PMM.

Percent survival during acclimatization ex vitro. Percent survival of calla lily plantlets during the ex vitro acclimatization was 95% in the PAM and 60% in the PMM, and that of china fir was 95% in the PAM-0, 97% in the PAM-1, and 16% in the PMM. The percent survival was ≈6 times higher in the PAM-0 and the PAM-1 than in the PMM. The lower percent survival in the PMM was probably due to the malfunction of stomata in leaves (Aitken-Christie et al., 1995) and callus formation at the base of the nodal cuttings. Callus formation has been considered a cause of poor root initiation and limited uptake of nutrient and water (Nguyen, et al., 1999). The higher percent survival in the PAM was probably because the plantlets were already acclimatized and functionally photoautotrophic in vitro (Kozai and Zobayed, 2000). The percent survival of calla lily plantlets transplanted in the farmer's greenhouse without shading (i.e., no acclimatization procedure) was 80%, compared with 95% in the greenhouse with a shading screen. Thus, by further improving the in vitro acclimatization method in the PAM, the ex vitro acclimatization process could be eliminated under moderate weather conditions (Heo and Kozai, 1999). This simplification is one of the advantages of the PAM.

Production cost of calla lily plantlets: A case study. The investment cost of the module (excluding the culture room) was $15,180 or 126,000 Chinese Yuan for the PAM ($1 = 8.3 CNY as of 2003), and was $5,807 for the PMM. Since the lifetime of the PAM and PMM was considered to be 10 years, yearly depreciation was $1,518 for the PAM and $581 for the PMM. The yearly production capacity per module of the PAM was ≈152,000 plantlets (= 365/18 × 7,500), since one multiplication cycle was 18 d; 15 d for multiplication, 1 day for harvesting and 2 d for cleaning, preparation and transplanting. On the other hand, the yearly production capacity of the PMM was ≈52,000 plantlets (= 365/33 × 5,000 × 0.95), since one multiplication cycle was 33 d; 30 d for multiplication, 1 day for harvesting and 2

Table 6. A comparison of production cost, labor time and electricity consumption per plantlet of calla lily in the PAM (photoautotrophic micropropagation) and the PMM (photomixotrophic micropropagation) systems.

Cost[z]	PAM (A)	PMM (B)	A to B ratio
Cost per ex vitro acclimatized plantlet (¢)			
Investment cost	1.00 (18%)	1.12 (12%)	0.89
in vitro multiplication	0.84 (16%)	1.44 (16%)	0.58
in vitro rooting	0.84 (16%)	1.44 (16%)	0.58
ex vitro acclimatization	2.65 (50%)	5.06 (56%)	0.52
Total	5.33 (100%)	9.06 (100%)	0.59
In vitro multiplication cost per plantlet (¢)			
Labor cost	0.35 (42%)	0.75 (52%)	0.47
Electricity cost	0.22 (26%)	0.36 (25%)	0.61
Medium cost	0.16 (19%)	0.22 (15%)	0.73
Others	0.11 (13%)	0.11 (8%)	1.00
Total	0.84 (100%)	1.44 (100%)	0.58
Ex vitro acclimatization cost per plantlet (¢)			
Investment for greenhouse	0.53 (20%)	0.56 (11%)	0.95
Labor	1.48 (56%)	1.92 (38%)	0.77
Supplies	0.50 (19%)	0.56 (11%)	0.89
Others	0.13 (5%)	2.02 (40%)	0.06
Total	2.65 (100%)	5.06 (100%)	0.52
Labor time for in vitro multiplication per plantlet (s)			
Vessel washing	0.48 (2.4%)	3.84 (9%)	0.13
Harvesting	0.48 (2.4%)	7.68 (18%)	0.06
Medium preparation	0.96 (4.8%)	3.84 (9%)	0.25
Excising/transplanting	17.28(85.6%)	25.92 (60%)	0.67
Others	0.96 (4.8%)	1.92 (4%)	0.50
Total	20.16 (100%)	43.2 (100%)	0.47
Electricity consumption for in vitro multiplication per plantlet (Wh)			
Lighting	18.7 (69%)	24.1 (56%)	0.65
Cooling	6.49 (24%)	9.1 (21%)	0.71
Air pumping	0.56 (2%)	---	---
Autoclaving	1.50 (5%)	9.6 (23%)	0.20
Total	27.2 (100%)	42.8 (100%)	0.16

[z]$1 = 8.3 CNY (Chinese Yuan), as of 2001. Wh (Watt-hour): 3600 Joules.

d for cleaning, preparation and transplanting. Therefore, if the PAM and PMM were operated throughout the year, the yearly depreciation of investment cost per plantlet was $0.010 (= 1,518/152,000) or 1.00 cent in the PAM, and $0.0112 (= 581/52,000) or 1.12¢ in the PMM. In other words, the investment cost per plantlet was a little lower in the PAM than in the PMM, as shown in Table 6. The above figures indicate that the investment cost accounted for 18% of the production cost in the PAM and 12% of the production cost in the PMM.

Production cost per acclimatized plantlet. The production cost per ex vitro acclimatized plantlet from the PAM was 59% of that from the PMM (Table 6). The cost for ex vitro acclimatization in the PAM and the PMM accounted for 50% and 56% of the production cost, respectively. Thus, an increase in percent survival during ex vitro acclimatization significantly decreases the production cost. Of the total costs for ex vitro acclimatization in the PAM, 20% was spent on the construction of the greenhouse, 56% was spent on labor, 19% was spent on supplies such as substrate, electricity, water, fertilizer and pesticide and 5% was spent on other items. On the other hand, in the PMM, 11% was spent on the construction of the greenhouse, 38% was spent on labor, 11% was spent on supplies such as substrate, electricity, water, fertilizer and pesticide and 40% was spent on other items. The lower cost of ex vitro acclimatization in the PAM than in the PMM was mainly due to a higher percentage of survival ex vitro and less labor.

Cost, labor time and electricity consumption for in vitro multiplication or rooting. The cost for in vitro multiplication, which was equal to that for in vitro rooting, in the PAM was 58% of that in the PMM (Table 6). Labor cost in the PAM was less than half of that in the PMM. This result is consistent with the prediction by Kozai et al. (2000). The reduced labor cost in the PAM significantly reduced the cost for in vitro multiplication and rooting. Electric energy consumption per plantlet during the in vitro multiplication was 27.2 Wh (or 97.9 kJ = 27.2 Wh × 3600 s) in the PAM; 69% for lighting, 24% for cooling, 2% for air pumping and 5% for autoclaving. On the other hand, it was 42.8 Wh in the PMM; 56% for lighting, 21% for cooling, 23% for autoclaving using electricity. Electric energy consumption per plantlet in the PAM, therefore, was 64% of that in the PMM. The lower electricity consumption in the PAM was mainly due to the reduction in multiplication and rooting periods by half, a low electric energy consumption for autoclaving the medium and vessels, and a high percent use of light energy by using white reflective sheets. A preliminary experiment showed that PPF was 1.65 times higher on a shelf with the reflective sheets (86 ± 8 $\mu mol \cdot m^{-2} \cdot s^{-1}$) than on a shelf without the reflective sheets (52 ± 12 $\mu mol \cdot m^{-2} \cdot s^{-1}$) when four fluorescent lamps were turned on. Average air temperature outside the culture room was 18 °C during the experiment. The electricity consumption for cooling increases with increasing outside air temperature. Thus, the cooling cost would be increased by 50% to 60% when outside air temperature was ≈35 °C (Aitken-Christie et al., 1995).

Sales price of in vitro and ex vitro acclimatized plantlets. The sales price of calla lily plantlets in vitro was ≈7.23¢ when produced using the PAM, and was 6.02¢ when produced using the PMM; i.e., the sales price was 20% higher in the PAM than in the PMM because of the higher quality produced by the PAM. The sales price of ex vitro acclimatized plantlets was 18.1 cents when produced in the PAM, and was 14.5 cents when produced in the PMM and acclimatized ex vitro; i.e., the sales price was 25% higher in the PAM than in the PMM because of the higher quality produced by the PAM. In this experiment, it was not possible to record the expenses for supervision, administration, sales, transportation of plantlets to farmers, etc., and thus to calculate the profit per plantlet. This is because the personnel doing were doing other jobs concurrently. According to Chu (1992), the supervision cost accounts for 13% of the total production cost in the micropropagation industry. In any case, The PAM could produce higher quality plantlets at a lower cost than the PMM, which shows that the PAM has a commercial advantage over the PMM.

Conclusions

In comparison with plantlets produced by the conventional micropropagation system using small vessels with sugar-containing medium, plantlets produced by the photoautotrophic micropropagation system using large vessels with sugar-free medium resulted in better growth, lower percent loss due to contamination, higher quality, higher percent survival ex vitro, and lower production costs. Therefore, the photoautotrophic micropropagation system has advantages over the conventional micropropagation system for commercial production of calla lily plantlets and china fir plantlets with respect to production costs and sales price. This system should be useful for commercial production of micropropagated plantlets of other plant species.

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