Biosystems Engineering (2005) **91** (4), 501–511
doi:10.1016/j.biosystemseng.2005.05.005
SE—Structures and Environment

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Physical Properties of Culture Vessels for Plant Tissue Culture

Chingwen Huang; Chiachung Chen

Department of Bio-industrial Mechatronics Engineering, National ChungHsing University, 250 Kuokuang Rd., Taichung Taiwan, 40227;
e-mail of corresponding author: ccchen@dragon.nchu.edu.tw

(Received 14 July 2004; accepted in revised form 3 May 2005; published online 28 June 2005)

Plant tissue culture vessels with their caps or closures made the boundaries between the internal microenvironment and the external environment of outside air. The physical properties of the vessels and caps or closures affected the growth microenvironment of plantlets by the interface between inside and outside environments. The most important specifications for vessels are to provide uniform and adequate light quality, to isolate contaminating of microorganisms and to allow gas exchange. Seven types of culture vessels were selected in this study. Some physical properties are determined including air exchange rate, transmittance distribution, and spectral irradiance. The results indicated that air exchange rates of culture vessels ranged between 0·0145 and 0·0811 h^{-1}. The transmittance distribution of vessels with round bases was more uniform than that of vessels with rectangular bases. Spectral irradiance was significantly affected by its material characteristics.

1. Introduction

The orchid industry has become the most important agricultural bio-industry in Taiwan. There are 40 millions units of orchids for export in 2003. All orchids are cultured from plantlets produced by micropropagation. The requirement of the orchid plantlets enhanced the progress of the tissue culture industry. In order to promote the quality of plantlets and to reduce the product cost, the culture technique of plantlets needs to be improved continuously.

Tissue culture plantlets *in vitro* are planted in a culture vessel. Vessels are placed on the successive horizontal shelves arranged in the culture room. The growth conditions of plantlets are affected by the internal microclimate of vessels, such as light quantity and quality, light distribution, and air temperature. It is impractical to adjust the internal microclimate of the culture vessel by installing some equipment on the culture vessels. The optimum way to modify the internal microclimate of vessel is to adjust the outside environment of culture room indirectly. The internal microclimate of cultures vessels is affected by the outside conditions and the physical properties of culture vessels. The relationship between the internal microclimate and others factors has been studied (Chen & Chen, 2002; Chen, 2003, 2004a, 2004b). The most important physical properties of culture vessels are the air exchange rate (Chen & Chen, 2002; Chen, 2003), the transmittance (Chen, 2003, 2004b), and the spectral irradiance (Dooley, 1991).

The caps or closures and walls of tissue culture vessels isolated the inside environment from the containment of outside environment. By altering the interface between inside and outside environments, the effects of the conditions of culture room on the microenvironment can be regulated. The selective criteria for a tissue culture vessel are: (1) light transmittance; (2) isolation from water loss and contamination; (3) allowance for some gas exchange; and (4) provision of adequate growing area Smith & Spomer, 1995)

The air exchange rate for the culture vessel is defined as the hourly volumetric ventilation rate of the vessel divided by the air volume of the vessel (Kozai *et al.*, 1995). However, the effect of air exchange rate on the growing conditions of plantlets has not been consistently inspected. Lentini *et al.* (1988) reported that tight closure of the vessel would limit air exchange with the outside air and then build up the humidity and ethylene, adversely affecting tissue quality and grow rate. Bottcher *et al.* (1988) found that a tight cap on the culture vessel contributed to the adverse quality of plantlets. However, Kumar *et al.* (1987) indicated that sealed Petri dishes had a favourable effect on bud differentiation in excised cotyledons of *Pinus radiata* and limited air exchange could help the early initiation stages for this plant. Walker *et al.* (1989) compared the effect of ventilation rate on the fresh weights and number of shoots of Rhododendrons 'P. J. M', their results showing that unventilated vessels had significantly higher values than that in the ventilated vessels. Cuello *et al.* (1991) compared the effect of five levels of force ventilated CO_2 on the growth conditions of *Buddleia alternifolia*, their results showing that there were no significant differences between the means of all five CO_2 treatments for several growth indices. Majada *et al.* (2001) observed the leaf surfaces of *Dianthus caryophyllus* plantlets cultured in airtight or ventilated vessels. Plantlets grown in vessels with higher air exchange rates showed better performance of stomatal function than plantlets grown in sealed culture vessels. Jackson *et al.* (1991) evaluated the effects of ventilation in tissue cultures on ethylene and CO_2 accumulation for *Ficus lyrata* Warb. and *Gerbera gamesonnii* Bolus. They found that poor ventilation assisted in the accumulation of ethylene and CO_2 concentration but then retarded the growth of two kinds of plantlets.

The irradiance and spectra of light that reach the top of plantlets are affected by the transmittance of the caps and walls of vessels (Smith & Spomer, 1995). The required characteristic of the materials for caps and walls is to maximise the light quantity that penetrated into the internal space of vessels. Fujiwara *et al.* (1989) investigated the effects of four types of closures and three kinds of vessels on light transmittance in the culture vessels. The light distributions in the vessels were illustrated by graphics. Two closures, from rubber plugs and aluminium foil cap, severely reduced the irradiance of the area that was under the closures. Kozai *et al.* (1992) emphasised that the stainless-steel wire frame stands that were used to support the vessels also reduced the irradiance entering the vessel.

The effect of spectra irradiance on the growth of plantlets has been reviewed (Dooley, 1991; Lees, 1994). The spectral irradiance that reached the plantlets was affected by the light source and the light characteristics of caps and walls of culture vessels. Stasinopoulos and Hangarter (1990) compared the spectra transmittance of several culture vessels. The glass vessels could transmit the light at wavelengths over 300 nm. The spectral irradiance of Petri dishes and Magenta GA7 had the same patterns. However, the average transmittance was lower than that of glass. Hangarter and Stasinopoulos (1991) found that as ultraviolent wavelengths and blue wavelengths entered the medium, the formaldehyde production was induced and iron in the medium could be degraded. They suggested that the materials for the caps and walls of vessels should have the ability to exclude the wavelengths between 290 and 450 nm.

In all the above discussions, the most important physical properties of culture vessels are the air exchange rate, the transmittance, and the spectral irradiance. The objective of this study was to determine these physical properties of several tissue culture vessels that are usually applied in the tissue culture industry.

2. Materials and methods

2.1. *Culture vessels*

Seven types of vessels were selected to determine their physical properties.

Conical glass flask

The cap of the conical glass flask (FA-1, I-Shin Co., Taiwan) is a black rubber stopper. The sketch of this vessel has been described elsewhere (Chen, 2003). The height of this vessel is 0·15 m and the diameter of bottom is 0·10 m.

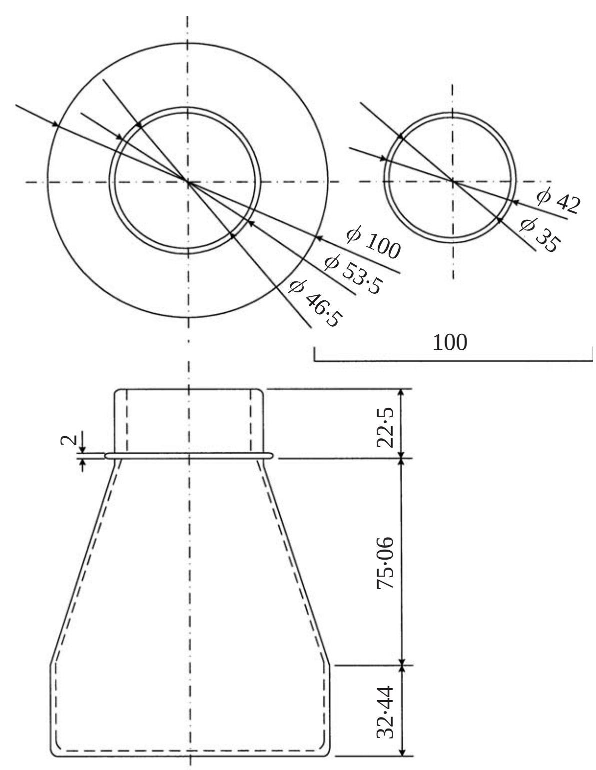

Fig. 1. Schematic diagram and size of conical plastic flask; all dimensions in mm

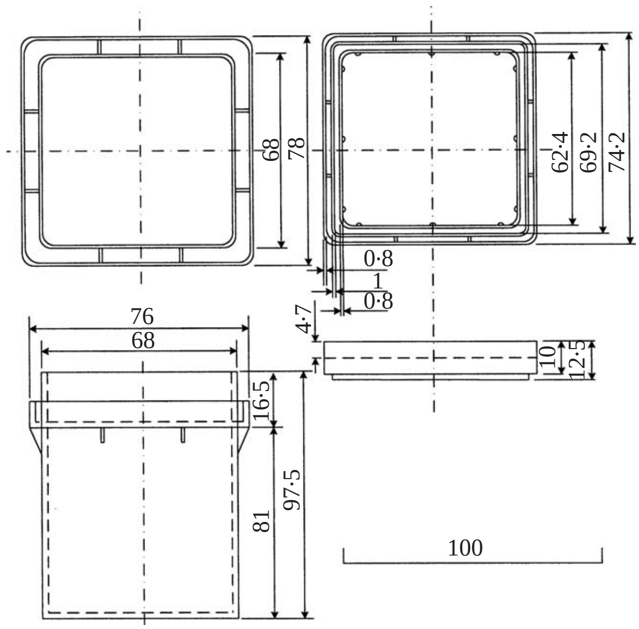

Fig. 2. Schematic diagram and size of GA-7 vessel; all dimensions in mm

Conical plastic flask

The conical plastic vessel (ZP-550, Jiana stationery Co., China) has a plastic screw-cap closure. The cap closure and wall of the vessel are made of polycarbonate (PC). The height and basal diameter are same as the dimensions of the conical glass vessel (*Fig. 1*).

Japanese irregular box

The lids of the Japanese irregular box (Iw-1, Iwan Co., Japan) are made of polypropylene (PP). However, the walls are made of PC. The cap of the vessel is screw cap. The sketch diagram of this vessel is shown elsewhere (Chen, 2003).

GA-7 box

The sketch diagram of the GA-7 box (Magenta Co., USA) is shown in *Fig. 2*. The lid material is PP and the wall material is PC. The heights of lids can be adjusted to control their air exchange rate. In this test, the air exchange rate at the conditions of the tightest and the loosest levels were determined.

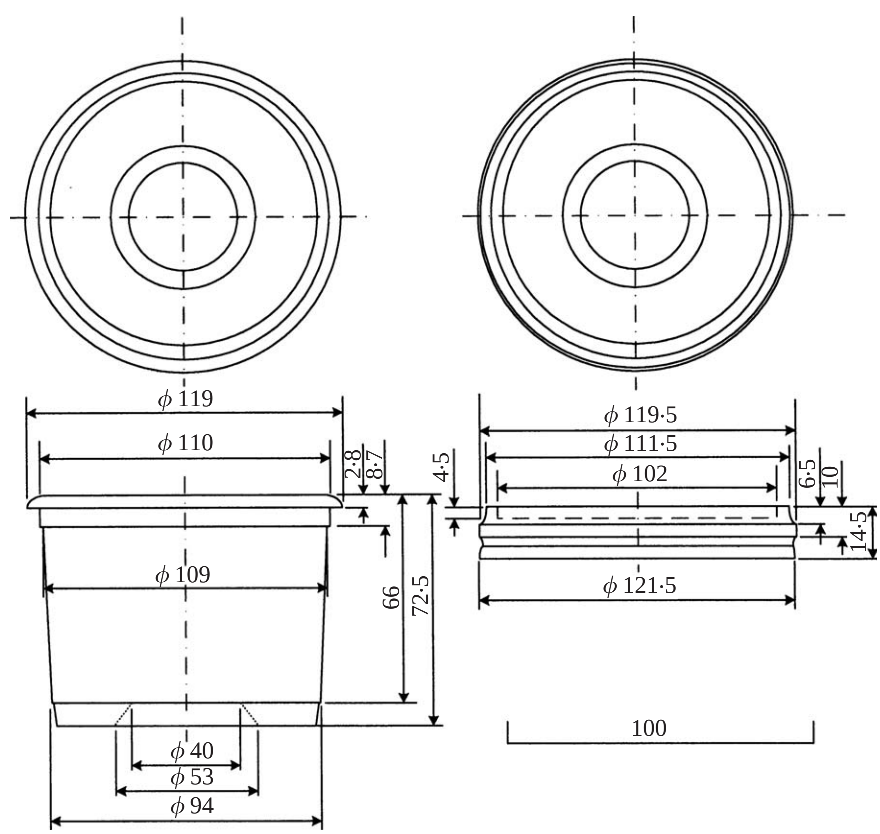

Fig. 3. Schematic diagram and size of round vessel; all dimensions in mm

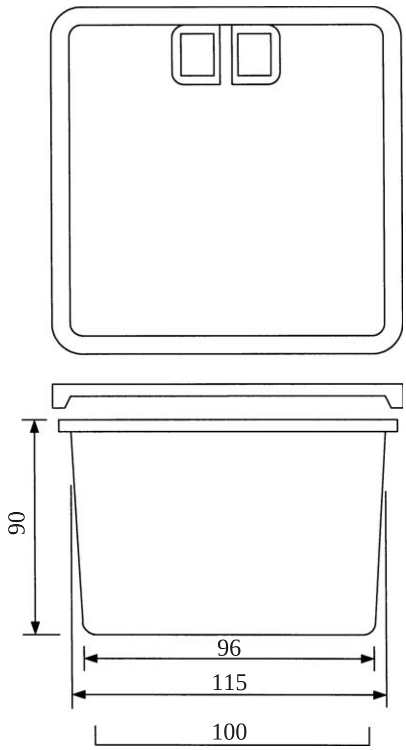

Fig. 4. Schematic diagram and size of square vessel; all dimensions in mm

Round vessel

The sketch diagram of the round vessel (LA-RH, LAB Associate, The Netherlands) is shown in *Fig. 3*. Round lids and walls are made of PP.

Square box

The special design of the square box (Vitro-Vent, Vitro CO., France) is the two hollows at the lid position to permit fingers to grid and remove the cover (*Fig. 4*). Lids and walls are made of PP.

Rectangular box

The special characteristic of the rectangular box (RA-1, LAB Associate, The Netherlands) is the addition of two round holes at the lid (*Fig. 5*). The material of the vessel is PP.

2.2. *Measurement of air exchange rate*

The standard operation procedures for the measurement of air exchange rates of culture vessels have been described in detail in previous study (Chen & Chen, 2002). This method was adopted in this study. The procedure is briefly described as follows.

(1) Calibrate the resistive-type humidity sensors by saturated salt solutions. The Shinyei THP-B7T transmitter

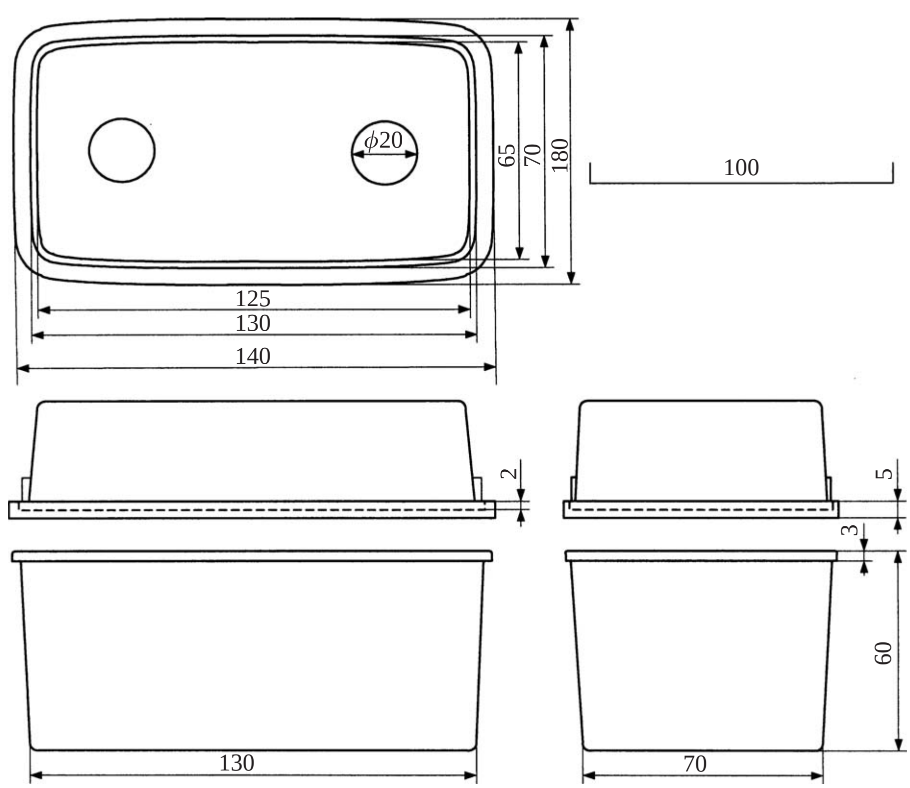

Fig. 5. Schematic diagram and size of rectangle vessel; all dimensions in mm

(Shinyei Kaisha Co., Tokyo, Japan) was adopted. The sensing element was the Macromolecule element. The measuring range was from 20 to 99% relative humidity (RH).

(2) Add the pre-determined water into the culture vessel to keep it in a saturated condition.

(3) Install the sensing element in the culture vessel and place the vessel and humidity sensor in a fixed relative humidity environment. The relative humidity of this environment was maintained by $MgCl_2$ $6H_2O$ salt solutions at the RH value of 32·2%.

(4) The relative humidity of the vessel decreased gradually due to the difference in the vapour pressure between the outside and inside environment. Record the relative humidity of the vessel and then analyse these data to calculate the air exchange rate value.

The air exchange rate measurements for each vessel were repeated six times.

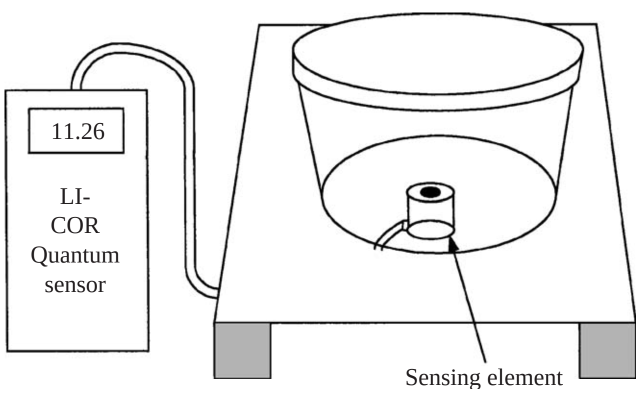

Fig. 6. Measurement of the internal transmittance of round vessel

2.3. *Measurement of transmittance in culture vessels*

A LI-190 SA Quantum sensor (Li-COR Co., USA) was used to measure the light flux density in culture vessel. This sensor was calibrated by Li-COR 1800-02 optical radiation calibrator (Li-COR Co., USA). The accuracy of this meter was $\pm 3\%$ after calibrating. The bottoms of the culture vessels were removed carefully. The LI-190SA quantum sensor was placed at the plantlets level (*Fig. 6*). There are 36 measuring positions in culture vessels to ensure the observation data were enough to evaluate the uniformity of transmittance. The transmittance of vessel was calculated as the ratio between the internal irradiance and external irradiance. The contour lines of transmittance calculated from measured data were drawn using the Sigma plots software version 8·0 (SPSS Inc., USA) to evaluate the uniformity of light irradiance.

2.4. *Measurement of spectral irradiance*

The spectral irradiance of lids and walls of the culture vessels was measured by the LI-1800 Spectroradiometer (Li-COR., Co., USA) with the integrated sphere. The operated wavelength of this device ranged from 380 to 750 nm. This wavelength range covered the UV ray, visible and partial near-infra ray.

2.5. *Experimental arrangement*

The measurement of light flux density and spectral irradiance in culture vessels was executed on a full-scale micropropagation shelf. Two fluorescent luminaries were mounted on the upper side of an identical shelf. The length of fluorescent tubes, Philips TLD 36 w/39, was 1·2 m. The height of tubes was 0·35 m. All vessels were placed in this identical shelf for measuring.

Table 1
The air exchange rates of different vessels

Category	*Vessel type*	*Air exchange rate, h^{-1}*
Low	Conical glass flask	$0{\cdot}015 \pm 0{\cdot}00020$*
	Conical plastic flask	$0{\cdot}022 \pm 0{\cdot}00031$
Intermediate	Japanese irregular box	$0{\cdot}038 \pm 0{\cdot}00070$
	GA-7 box with the tightest state	$0{\cdot}035 \pm 0{\cdot}00415$
	Round vessel	$0{\cdot}037 \pm 0{\cdot}0010$
	Square box	$0{\cdot}034 \pm 0{\cdot}0031$
High	GA-7 box with the loosest state	$0{\cdot}071 \pm 0{\cdot}00315$
	Rectangular box	$0{\cdot}081 \pm 0{\cdot}0005$

*Mean values $\pm$ standard deviations.

3. Results and discussion

3.1. *The gas exchange rates for several vessels*

The gas exchange rates for seven vessels are listed in Table 1. The conical glass flask had the smallest air exchange rate and the rectangle box had the largest values. According to the numerical values of air exchange rate, these vessels can be divided into three categories. The conical glass flask and conical plastic flask had low exchange rates, ranging between 0·015 and 0·022 h^{-1}. The Japanese irregular box, GA-7 box in the tightest state, round vessel, and square box showed intermediate exchange rates, ranging between 0·034 and 0·038 h^{-1}. The GA-7 box in the loosest state and rectangular box had high exchange rates, ranging between 0·071 and 0·081 h^{-1}.

The effect of air exchange rate on the internal air temperature of culture vessels could be neglected (Chen, 2003). However, the air exchange rate is very useful to solve the gases accumulation problems. The driving force for gas exchange was the different gas concentrations between inside microclimate and outside air

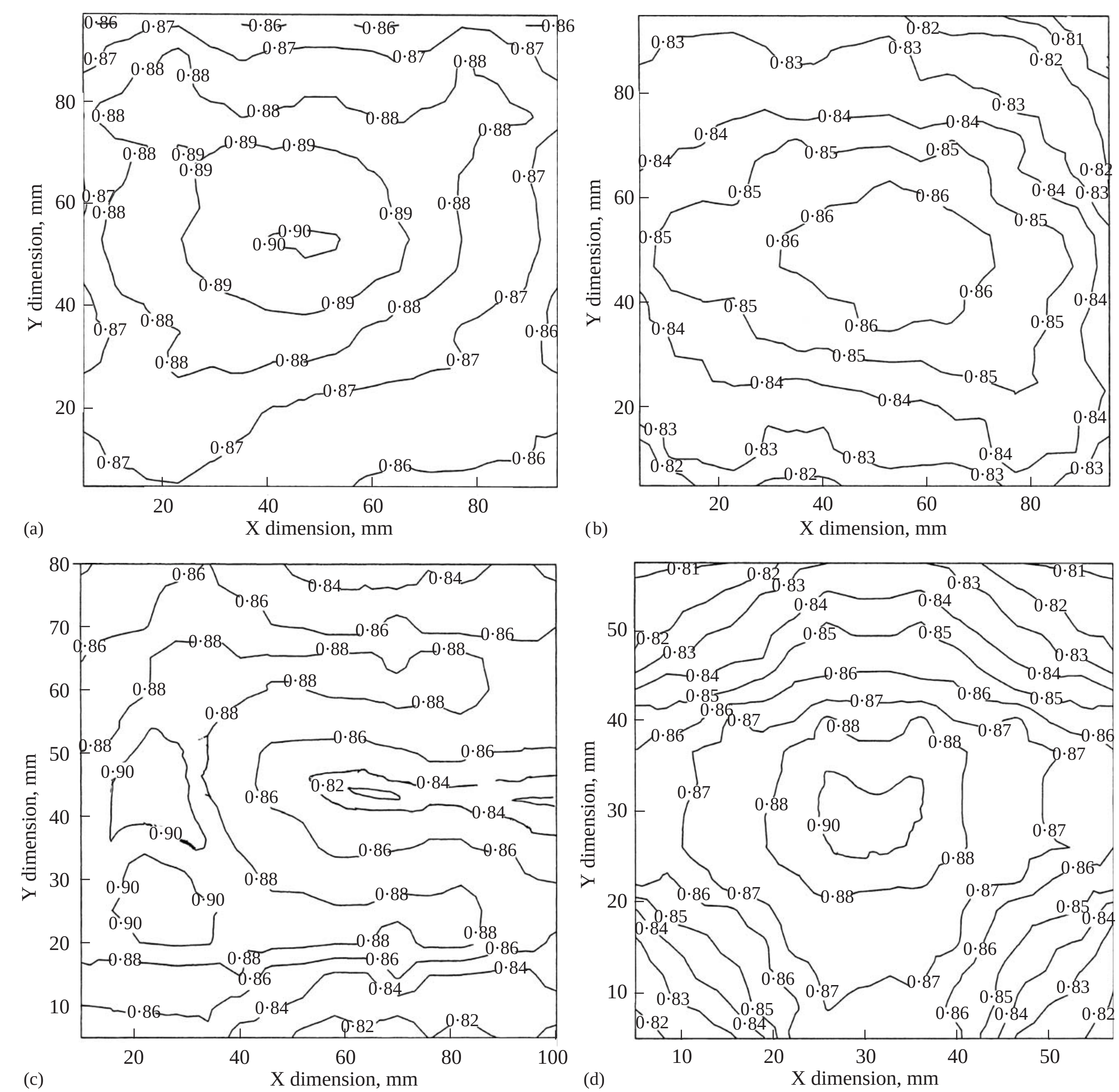

Fig. 7. The contour curves of flux density transmittance of (a) conical glass flask, (b) conical plastic flask, (c) Japanese irregular box, (d) GA-7 vessel, (e) round vessel, (f) square box, and (g) rectangular box

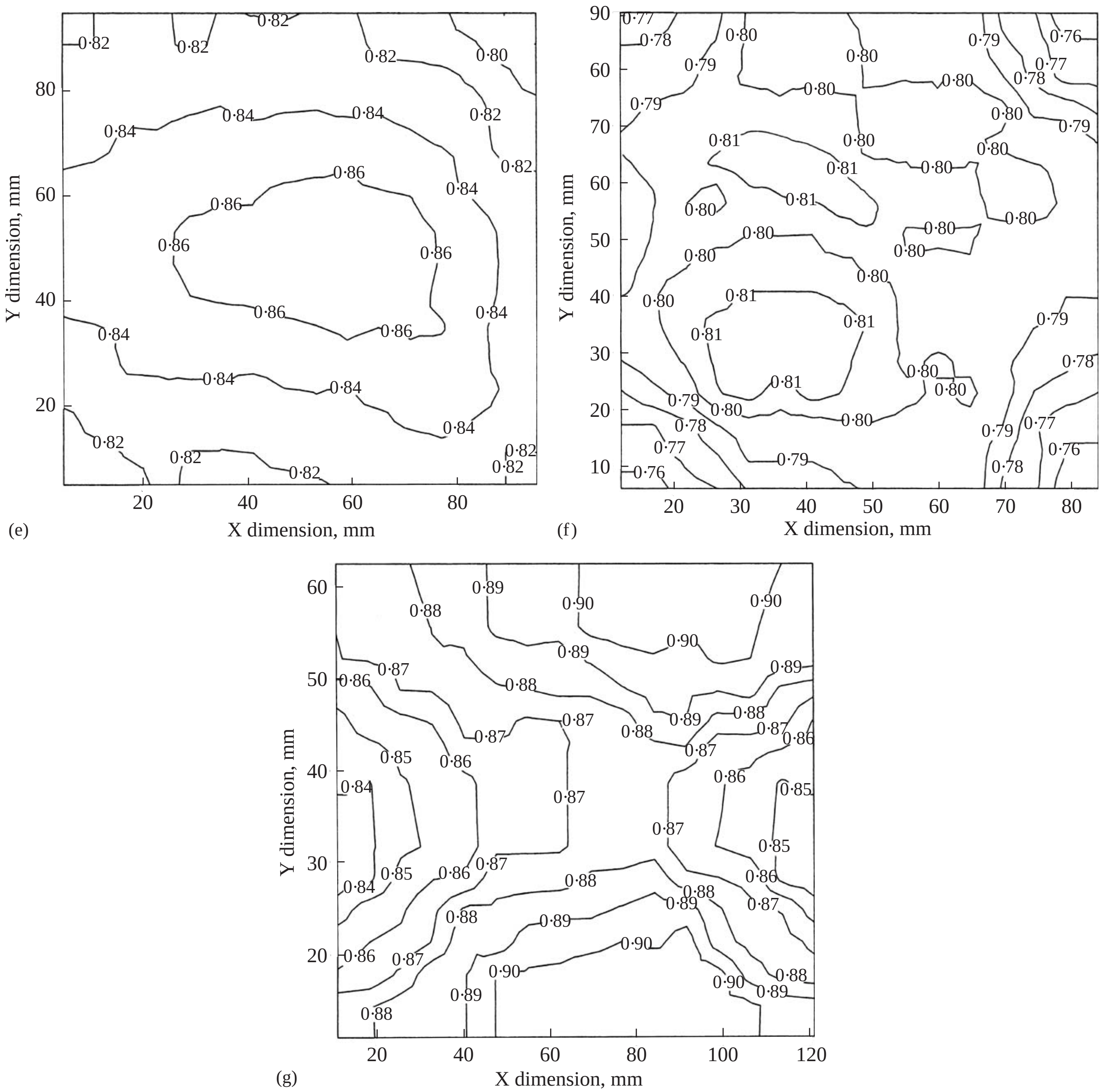

Fig. 7. (Continued)

conditions. The accumulation of CO_2 and ethylene concentrations had been mentioned by many researchers (Bottcher *et al.*,1988; Jackson *et al.*, 1991; Kumar *et al.*, 1987; Lentini *et al.*, 1988; Majada *et al.*, 2001). The ethylene concentration of outside air can be assumed as zero. The CO_2 concentration of air is nearly 350 p.p.m. Compared with the internal concentration of CO_2 and ethylene, these concentrations were pretty low and can be neglected. The accumulations of CO_2 and ethylene are produced by plantlets due to their physiological functions. To avoid these gases accumulating to a plant stress level, the air exchange between outside air and inside air can be interacted to avoid this problem. The air exchange rates that were determined in this study can serve as an index to select the culture vessel according to the optimum gas conditions for plantlets.

3.2. *Light flux density in culture vessels*

The contour curves of light flux density transmittance for various culture vessels are shown in *Fig. 7(a)–(g)*.

Figure 7(*a*) shows the distribution condition of transmittance for conical glass flask. The central position under the black rubber stopper had the highest transmittance. The lowest transmittance was found in the circumference of the bottom.

The numeric values of transmittance ranged between 0·86 and 0·90. It indicated the uniformity performance of light flux density in the conical glass flask. In the study of Fujiwara *et al*. (1989), a significant decrease of transmittance under the central position of their culture vessels was found. In their study, foam rubber plugs and aluminium foil closures were found to be non-translucent for light irradiance. The culture vessels had a right-angled corner. This structure did not permit much diffusive light from entering the internal space of vessels. The shading effect was found under the central position for their closures and vessels. In this study, the spherical shape of the flask walls is similar to the partial construction of a dome. More irradiance could pass the wall of the vessels than is gathered in the central position of base. The transmittance in central positions was higher than that in other positions. The special design of this spherical angle for the flask walls could improve the uniformity of irradiance significantly.

The distribution of the internal transmittance for the plastic flask is presented in *Fig. 7*(*b*). Similar to the conical glass flask, the highest transmittance was found in the central position and the lowest transmittance was found in the peripheral position. The transmittance ranged between 0·82 and 0·86. The internal transmittance indicated the uniform characteristic for both conical flasks with circular base.

Figure 7(*c*) reveals the transmittance of flux density in the Japanese irregular box. The base of this vessel was rectangle. The lids of this vessel were not located at the central position. The transmittance of the lids and other parts of wall was not the same. The highest transmittance value of 0·90 and lowest transmittance of 0·82 were found in the middle position of this vessel. The dome shape of the wall on the left enhanced the transmittance of light irradiance to enter the internal space. However, the lids installed on the right reduced the light irradiance. That is the reason explaining the larger range of transmittance from 0·82 to 0·92 in the vessels.

The transmittance of GA-7 box is shown in *Fig. 7*(*d*). The position of maximum transmittance was located at central position. The transmittance ranged between 0·81 and 0·88. The contour lines indicated systematically decreased for transmittance from the centre to edge. *Figure 7*(*e*) presents the distribution of transmittance for the round vessel. The transmittance ranged from 0·82 to 0·86. *Figure 7*(*f*) indicates the transmittance for the square box. The transmittance ranged from 0·76 to 0·81. The shape for vessel base of the square box is same as that of the GA-7 vessel. However, the base area of square box is larger than that of GA-7 vessels. The transmittance of the square box was more uniform than that of GA-7 vessels.

Figure 7(*g*) presents the transmittance of the rectangular box. The lower transmittance was found in the middle for these types of vessels. However, the sides had lower transmittance. The transmittance ranged from 0·84 to 0·90.

Compared with the above results, round vessels had more uniform transmittance than that of the square or rectangular box. The square base vessel, with smaller base area (GA-7 box), had the larger range of transmittance.

3.3. *Spectra irradiance of culture vessels*

The irradiance that reached the plantlet level was affected by the incident light and vessel materials. The distribution of transmittance at different wavelengths was called the spectral transmittance. It could serve as the indication of the influence of vessel materials on the spectral properties.

Figure 8(*a*) shows the distribution of spectral transmittance of the conical glass flask *versus* the wavelength from 380 to 760 nm. The transmittance was near to 0·925. The range of wavelength did not influence the spectral transmittance. The results indicated that the wall materials of glass had a good penetration ability for irradiance at different wavelengths. The spectral transmittance of conical plastic flask is presented in *Fig. 8*(*b*). For the wavelength ranging between 420 and 760 nm, the transmittance was fairly uniform at 0·90. However, the transmittance drops sharply in the range between 420 and 380 nm. The wavelength of ultraviolet (UV-A) rays ranges from 315 to 400 nm. These results indicated that most of the UV-A irradiance was filtered out by the vessel material.

Figure 8(*c*) indicates the spectral transmittance of the Japanese irregular box. The lids material was PP and the walls material was PC. The spectral transmittance of the lid was higher than 0·90 for wavelengths above 530 nm. In the lower wavelength region, the transmittance of lids decreased gradually. The transmittance of the walls is fairly uniform at 0·90 for wavelengths above 450 nm. In the lower wavelength region, the transmittance drops sharply. The transmittance distribution of the lids is the same as that for the conical plastic flask. The phenomenon can be explained because both vessels used the same materials (PC). The lids did not cover the entire upper side of Japanese irregular box. So the spectral irradiance of interior space would be different.

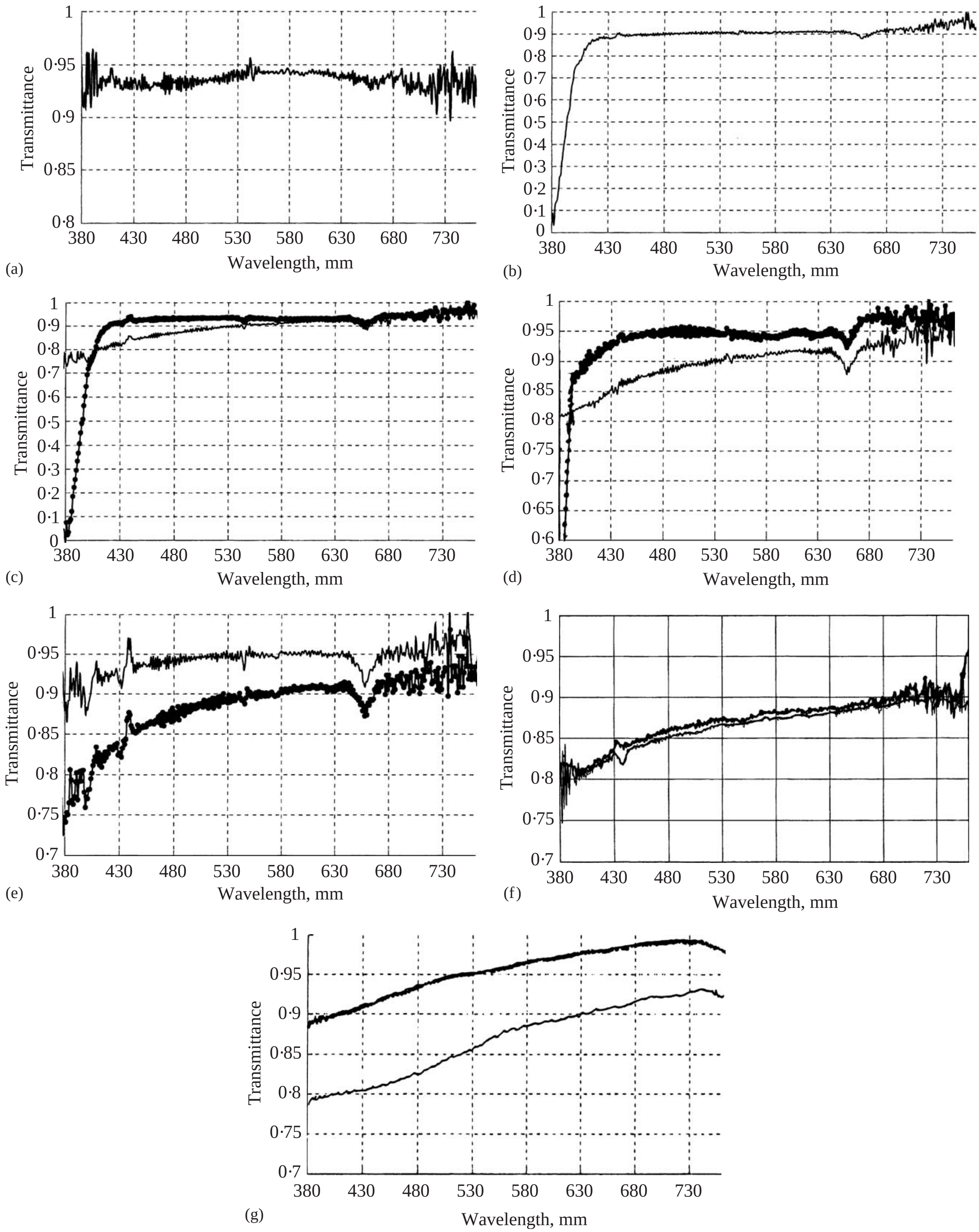

Fig. 8. The distribution of spectral transmittance of (a) conical glass flask, (b) conical plastic flask, (c) Japanese irregular box, (d) GA-7 vessel, (e) round vessel, (f) square box, and (g) rectangular box: ____ ,wall; ______ ,lid

The spectral transmittance of the GA-7 box is presented in *Fig. 8(d)*. The materials for the lids and walls are PP and PC. The distribution of spectral transmittance for the lids is similar to that of Japanese irregular box (*Fig. 8c*). The spectral transmittance of the walls also revealed the typical characteristic of irradiance distribution of PC. In the lower wavelength region, the transmittance reduced rapidly. It indicated the prohibition of the entrance for UV irradiance.

Figure 8(e) shows the spectral transmittance of the round vessels. The materials for the lids and walls were PP. The distribution patterns for the two spectral transmittances were alike. The transmittance reduced for wavelengths of less than 450 nm. The transmittance of the lid is higher than that of the walls. The spectral transmittances of French square box and rectangular box are presented in *Fig. 8(f)* and *(g)*. The materials for lids and walls are PP. The distribution pattern of spectral irradiance is the same as that of the round vessel. The spectral irradiance of the lids and walls is very close for French square box. However, the spectral transmittance of the lid is significantly lower than that of the walls for the rectangular box.

Kitaya *et al.* (1995) have developed a sidewall lighting system to promote the growth of plantlets and to reduce the electrical costs. In a commercial micropropagation company, many sets of horizontal shelves are arranged in the culture room. Culture vessels are placed on the shelves. Several levels of shelves comprise the layout in a culture room. In order to improve the spatial efficiency, these vessels are placed as close as possible. The direct irradiance from the upper fluorescent tubes was almost the only source of light flux density. The spectral transmittance of the lid is more important than that of walls. From the results of this study, PP permits the penetration of irradiance for the wavelength ranging between 380 and 760 nm. However, PC filtered out the irradiance of wavelengths below 400 nm and excludes UV light from the internal space of vessels. The conical glass flask had the most uniform spectral irradiance. However, the major disadvantage of this vessel was the weight of vessels. The handling work of this vessel for plantlet transplant is labour intensive. The wall material of conical plastic flask and Japanese irregular box was PC. The plantlets in this vessel could not receive any irradiance in the UV waveband. The effects of the spectral irradiance on the growth characteristic need to be further studied.

Dooley (1991) had reviewed the effect of light wavelengths on the growth of plantlets *in vitro*. However, many researchers only illustrated the light source but they did not mention the spectral transmittance of culture vessels. The actual spectral irradiance in the plantlet level may not be the same as the light source. This is a partial reason for the different conclusions claimed by different researches (Economou & Read, 1987; Hangarter & Stasinopoulos, 1991; Lees, 1994; Stasinopoulos & Hangarter, 1990). The determined results of spectral irradiance for various kinds of vessels obtained in this study can provide useful information for researchers to study the effect of spectral irradiance on the growth characteristics of plantlets *in vitro*.

4. Conclusions

The physical properties of seven culture vessels were determined in this study. The air exchange rates could serve as an index to release the accumulation of gas in the vessel. The uniformity of internal transmittance for round vessels was better than that of a square or rectangular box. The polypropylene of vessels could pass the wavelength ranged between 380 and 760 nm. However, the polycarbonate of vessels filtered out the transmittance for the wavelength below 400 nm. These physical properties could provide useful information to select the adequate vessels to meet the growth requirements of plantlets.

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