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Humidity in Plant Tissue Culture Vessels

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The relative humidity within the culture vessels is usually very high, resulting in poorly developed epicuticular wax layer and malfunctioning stomata of the plantlets. In this study, the humidity profile in the plant culture vessel was measured by the high polymer humidity sensor. The accuracy of these sensors was improved by calibration equations established using several saturated salt solutions. The tension of the medium was measured by a piezoresistive silicon tension meter. A vapour transfer model was proposed to describe the factors affecting relative humidity in the vessel. The evaporation model of the medium was established to quantify these factors. Several techniques that had been applied for reducing the relative humidity were evaluated by the vapour transfer model.

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1. Introduction

Orchids have become the most important flower industry in Taiwan. Micropropagation is the popular way to produce orchids plantlets. It allows rapid reproduction of disease-free plants of genetically identical origin. To improve the quality and quantity of plantlets, the growth environment must be maintained at the optimum conditions.

Plantlets *in vitro* are planted in a small vessel. The air exchange rate between outside and inside air is very low (Chen & Chen, 2002). As the medium within the vessel had adequate water and nutrients, the internal relative humidity is very high.

As the plantlets are cultured in a closed environment, the culture vessel is analogous to a mini greenhouse. However, the ventilation rate (air exchange rate) is limited. Many vessels are placed on the shelves arranged in the culture room. The important gas environment factors affecting the development of plantlets are light irradiance, air temperature, relative humidity, and carbon dioxide (CO_2) concentration (Aitken-Chrisie *et al.* 1994; Zimmerman, 1995). The best way to modify the internal microclimate of vessels is to adjust the outside environment indirectly. The air temperature and relative humidity of the culture vessel are the important factors which influence plantlet growth. The effect of the external condition on the internal climate of culture vessel has been studied (Chen, 2003). The relative humidity and temperature have an interactive effect. The humidity distribution and the moisture model need to be developed to quantify the effect of several parameters on the internal air relative humidity.

As the water content of medium is very high, the medium was filled into the culture vessel like a closed system. The relative humidity of the inside air is higher than that of a greenhouse (Fujiwara & Kozai, 1995; Gryze *et al.*, 1995). Several researchers have reported that the high relative humidity in culture vessels led to physiological and morphological disorders of plantlets (Ghashghaie *et al.*, 1992; Preece & Sutter, 1990; Ziv, 1990). The epicuticular wax layer and stomata of the plantlets did not have the normal function for evaporation. Owing to the high relative humidity, plantlets do not have the ability to resist water stress after transplanting from *in vitro* to *ex vitro* conditions. Debergh *et al.* (1992) concluded that high relative humidity was the dominant factor for the hyperhydricity of plantlets.

The research results of the effect of relative humidity on the growth conditions of plantlets were not a coincidence for researchers. Tanaka *et al.* (1992) compared the effects of four relative humidities on the growth of potato plantlets. These humidity

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Notation

a_1, a_2	constants
b_0, b_1, b_2	constants
c_0, c_1, c_2,	
c_3, c_4	constants
E_c	condensation rate of moisture vapour, kg h^{-1}
E_f	water evaporation from the medium at equilibrate state, kg h^{-1}
E_v	water evaporation from the medium, kg h^{-1}
E_1	water evaporation from the medium during the first stage, kg h^{-1}
E_2	water evaporation from the medium during the second stage, kg h^{-1}
E_x	air exchange rate of culture vessel, h^{-1}
H_{f-1}	absolute humidity of internal air prior to the equilibrate state, kg [H_2O] kg [dry air]$^{-1}$
H_i	absolute humidity of internal air, kg [H_2O] kg [dry air]$^{-1}$
H_{out}	absolute humidity of external air, kg [H_2O] kg [dry air]$^{-1}$
H_R	air relative humidity in the vessel, decimal
H_{R1}	air relative humidity at the mid position of the plantlets, decimal
H_{R2}	air relative humidity at the top position of the plantlets, decimal
H_{R3}	air relative humidity at the head space, decimal
H_0	absolute humidity of internal air initially, kg [H_2O] kg [dry air]$^{-1}$
H_1	absolute humidity of internal air at the end of the first state, kg [H_2O] kg [dry air]$^{-1}$
I_s	irradiance entering the vessel, W m^{-2}
P_{atm}	air pressure, kPa
P_w	vapour pressure of air, kPa
P_{wd}	vapour pressure deficit, kPa
P_{ws}	saturation water vapour pressure, kPa
R^2	coefficient of determination
s	standard errors of estimated values
t	time, min
t_f	time at equilibrate state, min
T_1	air temperature at the mid position of the plantlets, °C
T_2	air temperature at the top position of the plantlets, °C
T_3	air temperature of the head space, °C
T_i	temperature of internal air, °C
T_k	temperature of internal air, K
T_m	temperature of the medium, °C
X_i	independent variable of regression equation
X_1	measured values of sensor no. 1
X_2	measured values of sensor no. 2
X_3	measured values of sensor no. 3
Y_i	dependent variable of regression equation
Y_1	corrected values of sensor no. 1
Y_2	corrected values of sensor no. 2
Y_3	corrected values of sensor no. 3
V	volume of culture vessel, m^3
W_f	moisture content of the internal air at the equilibrate state, kg h^{-1}
W_{f-1}	moisture content of the internal air before the equilibrate state, kg h^{-1}
W_{out}	moisture migration rates from vessel, kg h^{-1}
W_0	moisture content in the internal air initially, kg h^{-1}
W_1	moisture content of the internal air at the end of the first stage, kg h^{-1}
W_2	moisture content of the internal air at the end of the second stage, kg h^{-1}
ρ	air density, kg m^{-3}

environments were maintained by three saturated salt solutions and water vapour. Their results indicated that the relative humidity of the culture vessel had a significant effect on the plantlet evaporation during the initial 10-day growing period. However, evaporation of older plantlets was not affected by this factor during the subsequent 20-day growing period. Kozai *et al.* (1995) studied the effect of relative humidity (RH) ranging from 80 to 95% on the growth and shoot elongation of potato plantlets in the culture vessel. They found that the shoot length of plantlets decreased with a lower RH environment. No significant differences in dry weight of plantlets were found among the different RH treatments.

Vanderschaeghe and Debergh (1987) proposed a bottom cooling technique to reduce the relative humidity of the culture vessel. Culture vessels were placed on the surface of a double layer metallic plate. Cooling liquid at a lower temperature passed through the interior of the layer to remove the internal energy of culture vessels.

Ghashghaie *et al.* (1992) used the bottom cooling and water vapour permeable lid to reduce the relative humidity. The results indicated that these techniques could increase leaf resistance to dehydration and improve stomata regulative ability. However, roots were shorter with the bottom cooling technique and apices were necrosed with the permeable lid method. In this study, the relative humidity of the internal air was not measured. Sallanon and Maziere (1992) studied the influence of culture room humidity on the *in vitro* development of rose plants. Culture vessels with rose

plantlets were placed in two culture rooms with different relative humidities. A culture room with a lower humidity could enhance the evaporation of plantlets. Larger changes in the growth and morphology of plantlets were observed. Debergh *et al.* (1992) suggested several techniques to reduce air humidity of internal air, *e.g.* using gas-permeable membranes for the vessels, and cooling the shelf on which the vessels were placed. However, no practical experiment was conducted.

Short *et al.* (1987) reduced the relative humidity of the internal air by covering the medium in the vessels with a layer of molten lanolin and by inserting a sachet of silica gel. Lowering the relative humidity could enhance wax production of the leaves but reduced plant growth. Wardle *et al.* (1983) covered the medium with a layer of molten autoclaved lanolin and hung a bag containing silica gel in the headspace of the vessels to reduce the relative humidity. The auxillary buds of chrysanthemum were transplanted in vessels. High mortality was observed in the plantlets by this treatment. Root growth was retarded in the reduced humidity treatment. However, the wax development of the leaves was better than that of the control treatment with high humidity. No actual value for the relative humidity was measured and presented in the above reports.

Honjo and Takakura (1991) applied dew sensors to measure the dew point temperature of the internal air in a micropropagation vessel. Relative humidity was calculated from the dry bulb temperature and dew point temperature. Two kinds of vessels were selected. The air exchange rate for vessels ventilated with a filter cover was $0{\cdot}7\,h^{-1}$ and that for vessels without the filter was $0{\cdot}04\,h^{-1}$. At the same temperature, the relative humidity of the vessels with ventilated filters was lower than that of vessels without filters. However, the difference in relative humidity between the two treatments was less than 0·5%. In this study, only the empty vessel was used to measure the humidity profile.

Spomer (1990) had proposed the use of a tension meter to measure the tension of the medium. In a tension meter, a water-permeable membrane is connected to a manometer. When the tension meter was placed in contact with the medium, water moves from the tension meter into the medium or moves out from the medium into the tension meter until the matric potential drawing water into the medium or into the tension meter is balanced by pressure within the tension meter. The equilibrium pressure within the tension meter equals the matric potential of the medium. This method was confirmed by a drainage column technique (Spomer, 1990). However, only the medium was filled in this column, and no plantlets were transplanted in this container. The pressure was measured by a U-tube manometer, and the accuracy of this meter was limited.

The relative humidity of the internal air in a culture vessel is affected by the internal air temperature, irradiance, and water content of the medium. It is very useful to quantify these factors by a mathematical model. The air temperature model for a plant tissue culture vessel has been developed and validated (Chen, 2003). The objective of this study was to develop a moisture balance model to predict the humidity distribution in a culture vessel planted with plantlets. Several techniques for humidity reduction were evaluated by this model.

2. Materials and methods

2.1. *Culture vessel*

A conical flask (F-1, I-Shin Co., Taiwan) was selected to measure the internal air relative humidity distribution and tension of the medium. The sketch of this vessel has been described elsewhere (Chen, 2003) (*Fig. 1*).

2.2. *Plantlet material*

The usable shoots of *Phalaenopsis* (Dtps. *Ton Jy Pecan x Ruey Lin Stripe* '*TH*') were transplanted on a rooting medium for 21 days. There were 20 plantlets in a flash. Each plantlet had two or three leaves when the experiment began.

2.3. *Measuring equipment*

2.3.1. *Temperature and relative humidity sensors*

The Shinyei THP-B7 T transmitter (Shinyei Kaisha CO., Tokyo, Japan) was adopted to measure the temperature and relative humidity. The sensor probe was composed of both temperature and relative humidity sensing elements. The temperature sensing element was RTD Pt100 ohm. The measuring range was from 0 to 60°C. This sensor was checked using a Microcal 2 Calibrator (Eurotron Instruments, S.P.A. Italy). These sensor probes were calibrated by adjusting a potentiometer located within the probe housing. The accuracy of this sensor can be improved to within $\pm 0{\cdot}15$°C after calibrating.

The relative humidity sensing element was the Macromolecule element. The measuring range for the manufacturer's specification was from 20 to 95% RH. To improve the accuracy of this humidity sensor over the high humidity range, the sensors were calibrated by several saturated salt solutions. The sensor was placed in a closed container with a measured saturated salt solution. As the air within the container reached

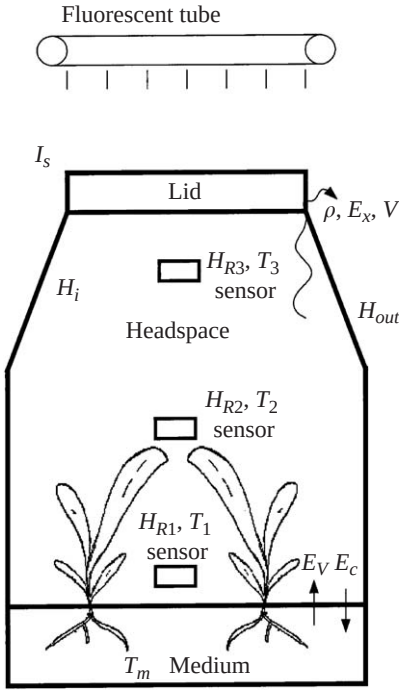

Fig. 1. Schematic diagram of the moisture transfer model for tissue culture vessel; I_s, entrance energy in head space of short wave radiation from light irradiance; H_i, absolute humidity of internal air; H_{out}, absolute humidity of external air; ρ, air density; E_x; air exchange rate of vessel; V, volume of vessel; E_v, evaporation of moisture from medium; E_c, condensation of moisture into medium; H_{R1}, H_{R2}, H_{R3}, relative air relative humidity of the mid position of the plantlets, the top position of the plantlets and the head space; T_m, temperature of the medium; T_1, T_2, and T_3, air temperature at the mid position of the plantlets, at the top position of the plantlets and in the head space

Table 1
The saturated salt solutions and its standard relative humidity value for the calibration of humidity sensors at 25°C

Saturated salt solutions	*Standard relative humidity values*
KI	68·86
NaCl	75·29
KCl	84·34
KNO_3	93·58
K_2SO_4	97·3
Water vapour	100·0

equilibrium, the reading values of sensors were recorded. The humidity produced by saturated salt solutions served as a standard value. Saturated salt solutions used in this study are listed in Table 1.

To establish the calibration equations for the humidity sensors, the standard value was adopted as the dependent variable Y_i. The reading value was used as the independent variable X_i. The standard errors of the estimated value s and the coefficient of determination R^2 were used to evaluate adequate calibration equations for these sensors. All measured values of the relative humidity sensors were transformed into actual values by these calibration equations.

2.3.2. *Irradiance*

Irradiance in $W\,m^{-2}$ from fluorescence tubes was measured by LI-200SA pyranometer (Li-COR Co., USA). This pyranometer was calibrated by a Kipp & Zonen Model CM11 thermopile pyranometer (Kipp and Zonen Ltd; The Netherlands). The accuracy of the meter was $\pm 3\%$ after calibrating.

2.3.3. *Tension meter*

Tension of the medium was measured with a UMS TS miniature pressure tension meter (UMS Co., CA, USA). The sensing element was a ceramic cup and a shaft filled with water. The diameter of this element was 5 mm. The tension of water was transmitted to a piezoresistive pressure transducer. The output signal ranged from 0 to 100 mV. The tension measuring ranged from 0 to 100 kPa. This sensor was calibrated by the pressure chamber technique provided by the manufacturer.

2.3.4. *Data logger*

All signals were connected to a Delta-T2e data logger (Delta devices LTP, UK).

2.4. *Experimental procedures*

Vessels were placed on the horizontal shelves in the culture room. Four fluorescent tubes, Philips TLD 36w/39, were installed under the upper shelves. The length of the tubes was 120 cm. The space between the tubes was 20 cm. The distance between the tubes and the vessels was 30 cm.

Irradiance was measured at the top position of the vessels. The entrance energy could then be calculated by the pre-determined transmittance of the vessel. The surface of the sensing elements for the THP-B7 T transmitter and tension meter was treated with 80% alcohol, placed in the laminar flow cabinet, and then exposed to ultraviolet light for at least 1 h. The vessel lids were removed and these sensing elements were placed at the pre-determined height of the vessel within the laminar flow cabinet. The tension meter was placed at the mid position of medium. Transmitter THP-B7 T no. 1 was placed at the mid position of the plantlets, transmitter no. 2 was placed at the top position of the plantlets, and transmitter no. 3 was placed in the head space of the vessel.

A small hole was drilled in the lid through which the tension meter was passed. The hole was then sealed using silicon. After installing all sensing elements, the vessels were removed from the laminar flow cabinet and placed back on the shelves.

At the different settings of the external air temperature and irradiance, temperatures and relative humidities of the internal air and tension of the mediums were measured and recorded.

3. Results and discussion

3.1. *Calibration equations of humidity transmitters*

Typical calibration data for the three humidity transmitters are shown in *Fig. 2*. The quadratic equation was found to be the adequate calibration equation for this transmitter. The typical calibrations are as follows.

3.1.1. *Sensor no. 1*

$$Y_1 = 4{\cdot}749 + 1{\cdot}049X_1 - 27{\cdot}447 \times 10^{-4}X_1^2 \qquad (1)$$

with a value for R^2 of 0·9991, and for s of 0·42; where: Y_1 is the corrected value of sensor no. 1, and X_1 is the measured value of sensor no. 1.

3.1.2. *Sensor no. 2*

$$Y_2 = 8{\cdot}353 + 0{\cdot}8139X_2 - 1{\cdot}070 \times 10^{-3}X_2^2 \qquad (2)$$

with a value for R^2 of 0·9992, and for s of 0·40; where: Y_2 is the corrected value of sensor no. 2, and X_2 is the measured value of sensor no. 2.

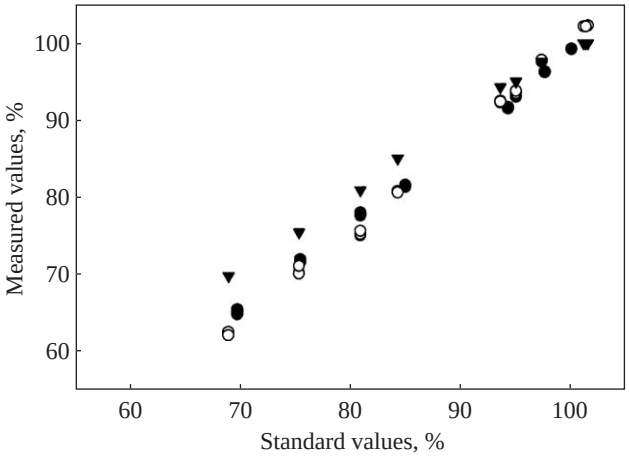

Fig. 2. Relationship between measured values and standard values made by saturated salt solutions for three humidity sensors: ▼, sensor no. 1; ●, sensor no. 2; ○, sensor no. 3

3.1.3. *Sensor no. 3*

$$Y_3 = 5{\cdot}081 + 1{\cdot}174X_3 + 2{\cdot}289 \times 10^{-3}X_3^2 \qquad (3)$$

with a value for R^2 of 0·9987, and for s of 0·45; where: Y_3 is the corrected value of sensor no. 3, and X_3 is the measured value of sensor no. 3.

From the standard errors of the estimated values s for the three calibration equations, the accuracy of the sensors was improved to within 0·45% RH (Chen & Tsao, 1989).

3.2. *Temperature and humidity distribution of culture vessel*

A typical temperature distribution inside the conical culture vessel is shown in *Fig. 3*. The light period is from artificial light for 4 h and irradiance was 13·5 W m^{-2} (67·0 μmol m^{-2} s^{-1}). *Phalaenopsis* plantlets were planted in a medium. During the dark period, all temperatures were similar. As the light period began, air temperature at the mid position of the plantlets T_1, air temperature at the top position of the plantlets T_2, and air temperature at the head space T_3 rapidly increased. The highest temperature was T_3. The maximum difference between the temperature of the medium T_m and T_3 was 1·8°C. The result was similar to that found by Chen (2003).

The typical relative humidity distribution is shown in *Fig. 4*. During the dark period, the three relative humidities were all close to 99·4%. In the light period, the relative humidity at the mid position of the plantlets H_{R1} was maintained close to 99·4%. However, the air

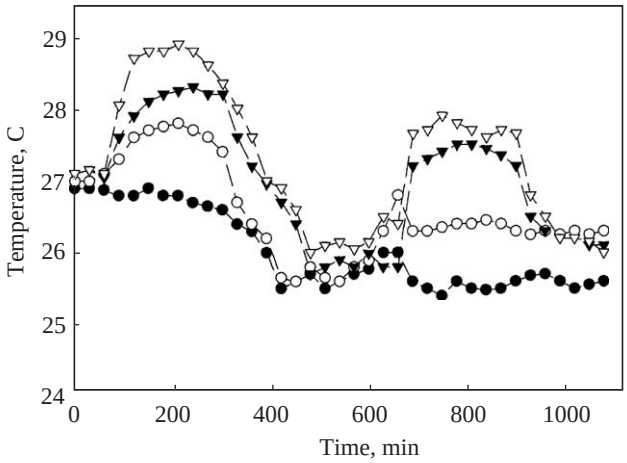

Fig. 3. Temperature distribution curve for a conical tissue culture vessel planted with Phalaenopsis plantlets: ●, temperature of the medium (T_m); ○, temperature at the mid position of plantlets (T_1); ▼, temperature at the top position of plantlets (T_2); ▽, temperature at the head space (T_3)

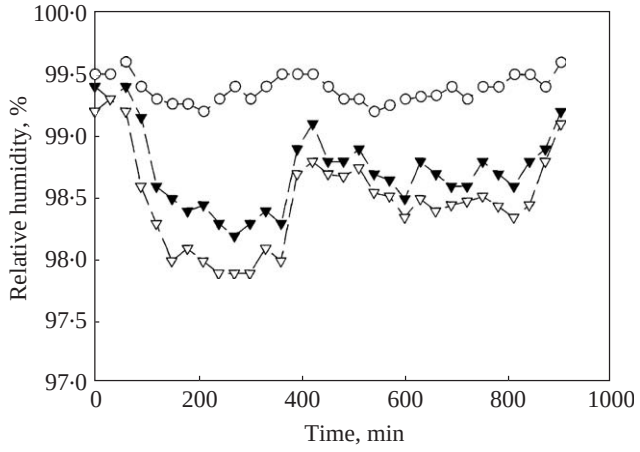

Fig. 4. Relative humidity distribution curve for a conical tissue culture vessel planted with Phalaenopsis plantlets: ○, relative humidity at the mid position of plantlets; ▼, relative humidity at the top position of plantlets; ▽, relative humidity at the head space

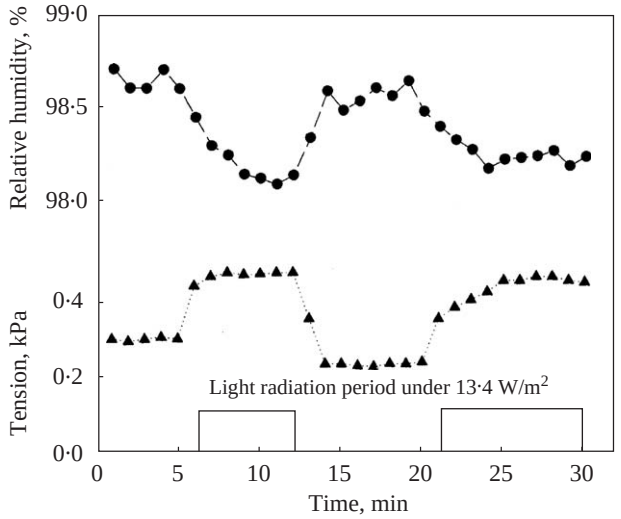

Fig. 5. Relative humidity and tension distribution curve for a conical tissue culture vessel planted with Phalaenopsis plantlets: ●, relative humidity at head space; ▲, tension of medium

relative humidity of the head space of the plantlets H_{R3} and air relative humidity at the top position of the plantlets H_{R2} decreased quickly. The value of H_{R3} was 98·0% in the irradiance of 13·5 W m^{-2} and was 98·3% in the irradiance of 7·2 W m^{-2}. The value of H_{R2} was 98·4% in the irradiance of 13·5 W m^{-2} and was 98·7% in the irradiance of 7·2 W m^{-2}.

This result is similar to that reported by Honjo and Takarura (1991). The humidity profile could be found in the culture vessel. The resistive-type humidity sensing element could serve as an adequate sensor to measure the high relative humidity in the culture vessels.

3.3. *Tension of the medium*

The air relative humidity and tension distribution of the medium is shown in *Fig. 5*. During the light period, the entrance irradiance was increased, then the air relative humidity was decreased and the tension of the medium increased. This indicated that more water flowed out from the medium. The force-driven water leaving the medium was affected by irradiance, temperature, and relative humidity. During the dark period, air relative humidity was increased. Some water began to condense on the wall of the culture vessel. As the water flowed back to the medium, the tension of the medium then decreased to 2·1 kPa.

The effect of irradiance on the tension of the medium is shown in *Fig. 6*. At the irradiance value of 9·8 W m^{-2}, the tension ranged between 3·5 and 3·7 kPa. At the 14·0 W m^{-2} condition, the tension ranged between 3·5 and 4·15 kPa. The more the irradiance, the larger the

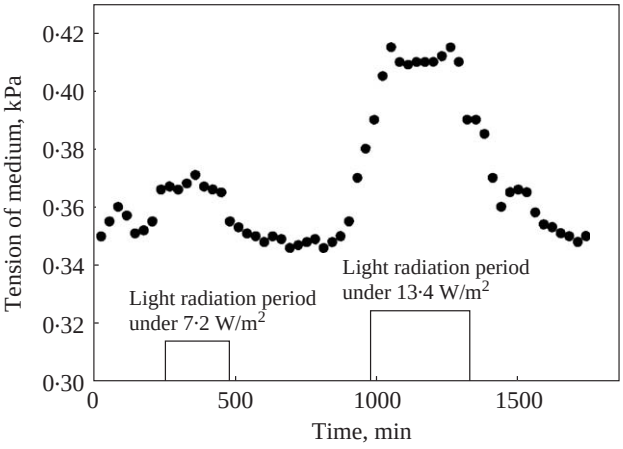

Fig. 6. Tension distribution curve for a conical tissue culture vessel planted with Phalaenopsis plantlets at different light intensities

tension of the medium. This result indicated that the piezoresistive pressure transducer could detect the small change in tension of the medium.

3.4. *Absolute humidity distribution of culture vessel*

The absolute humidity of the internal air can be calculated by the measured value of temperature and relative humidity. The type of distribution for absolute humidity at different irradiance values is shown in *Fig. 7*.

During the dark period, the absolute humidities at the three positions were similar. During the light period,

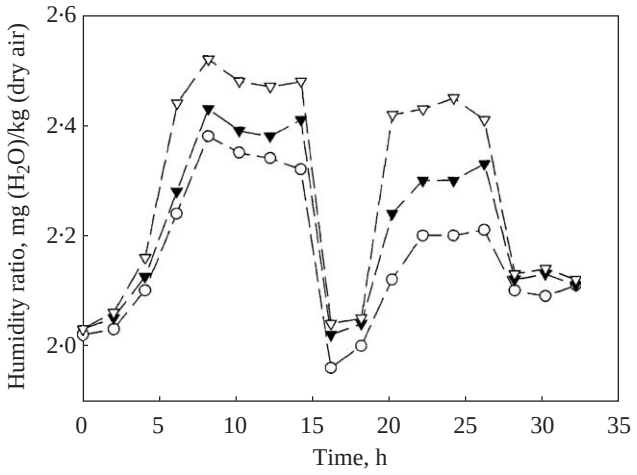

Fig. 7. Absolute humidity distribution curve for a conical tissue culture vessel planted with Phalaenopsis plantlets: ○, absolute humidity at the middle position of plantlets; ▼, absolute humidity at the top position of plantlets; ▽, absolute humidity at head space

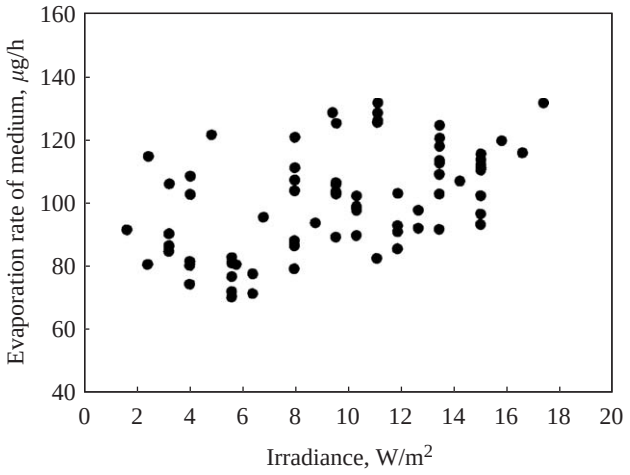

Fig. 8. Relationship between evaporation rate of medium and irradiance

energy was absorbed by the medium and the internal air, and the internal temperature then increased. If no moisture entered the head space of the culture vessel, the absolute humidity of the three positions should be similar. Due to the water vapour released from the medium and entering the head space air, the distribution of absolute humidity indicated the increase in moisture content of the air. The evaporation from the medium could be evaluated by the value of absolute humidity.

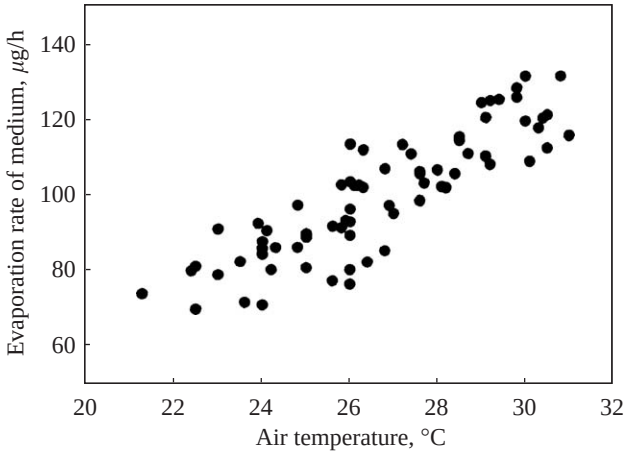

Fig. 9. Relationship between evaporation rate of medium and air temperature

3.5. *Evaporation from the medium*

The function of the stomata for plantlets in the culture vessels was abnormal. The transport resistance of water in the plantlets can be neglected, so the evaporation of plantlets can be neglected (Gryze *et al.*, 1995). The irradiance, temperatures and relative humidities at various conditions were measured and recorded. The evaporation from the medium can be calculated by the moisture transfer model developer in Appendix A. The relationship between evaporation of the medium and influencing factors is presented in *Figs. 8–10*.

The relationship between evaporation of the medium and irradiance is shown in *Fig. 8*. The regression model for evaporation and irradiance was:

$$E_v = 8{\cdot}12 \times 10^{-8} + 1{\cdot}996 \times 10^{-9}\, I_s \qquad (4)$$

with a value for R^2 of 0·37; where: E_v is evaporation in $kg\,h^{-1}$; and I_s is irradiance in $W\,m^{-2}$.

By the *F*-test, E_v has a significant relationship with irradiance. However, irradiance only can explain 37% of

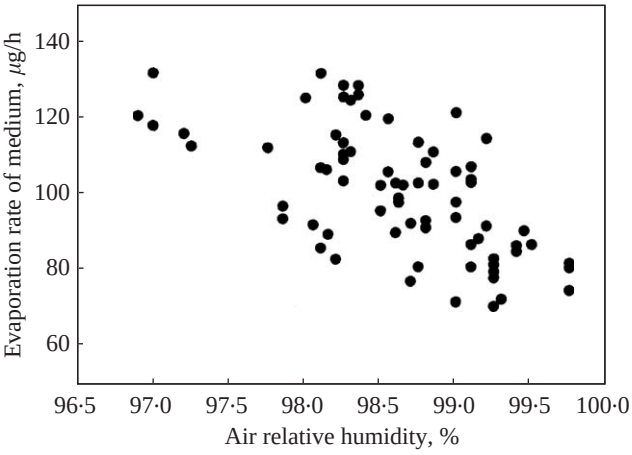

Fig. 10. Relationship between evaporation rate of medium and relative humidity

the variation of evaporation. The irradiance was measured and calculated at the top position of plantlets in the previous study (Chen, 2003). The plantlets planted in the medium have a shading effect on the received irradiance of the medium. This may be the explanation for the lower value of R^2 for this empirical equation.

The relationship between evaporation and internal air temperature is shown in *Fig. 9*. The regression model for evaporation and air temperatures was:

$$E_v = -5{\cdot}70 \times 10^{-8} + 5{\cdot}879 \times 10^{-9}\ T_3 \qquad (5)$$

with a value for R^2 of 0·796; where: T_3 is the internal air temperature of head space in °C.

The value for R^2 indicated that the internal air temperature could explain 79·6% of the variation of evaporation. As the air temperature increased, the evaporation also increased.

The relationship between evaporation and internal air relative humidity is shown in *Fig. 10*. The regression model for evaporation and air relative humidity was:

$$E_v = 1{\cdot}64 \times 10^{-6} - 1{\cdot}562 \times 10^{-8}\ H_{R3} \qquad (6)$$

with a value for R^2 of 0·414; where: H_{R3} is the internal air relative humidity of head space in decimal.

As the relative humidity increased, the vapour pressure deficit decreased and the evaporation decreased. Using Eqn (A5) to evaluate the fitting agreement of evaporation data, the regression equation was:

$$E_v = 9{\cdot}47 \times 10^{-6} + 1{\cdot}203 \times 10^{-7}\ I_s - 7{\cdot}2954 \times 10^{-3} \times (1 - H_{R3}) + 4{\cdot}895 \times 10^{-4}\ T_3(1 - H_{R3}) - 8{\cdot}158 \times 10^{-6}\ {T_3}^2(1 - H_{R3}) \qquad (7)$$

with a value for R^2 of 0·884.

The regression model was significant and revealed the relationship between the evaporation from the medium and irradiance, air temperature, and relative humidity. This model could explain 88·4% of the variation. The results confirmed the validity of the evaporation model developed in this study.

3.6. *Modification relative humidity of culture vessel*

The phenomenon of humidity distribution in culture vessels can be explained from the results of this study. During the dark period, the evaporation of water from the medium still occurs, and the relative humidity of the internal air was maintained nearly saturated. As there was no temperature gradient during this period, the relative humidity was uniform in the vessel. During the light period, the energy entered the culture vessel and increased the temperatures of the internal air and the medium. The saturated vapour pressure increased and enhanced the evaporation from the medium. The moisture leaving the medium then flowed into the internal air of the vessel. Only a little moisture was exhausted to the outside air by the air exchange effect. Most moisture remained in the internal air to increase the air humidity. The entering energy generated the temperature and humidity profile.

Several methods have been applied to reduce the relative humidity in the culture vessel including the use of desiccants (Wardle *et al*., 1983), covering the culture medium with a layer of lanoline (Wardle *et al*., 1983), increasing the ventilation rate (Fujiwara & Kozai, 1995; Ghashghaie *et al*., 1992), and bottom cooling (Ghashghaie *et al*., 1992). These techniques are discussed as follows.

3.6.1. *Use of desiccants*

Desiccants placed in the culture vessel can absorb the moisture from the air and so reduce the humidity directly. However, desiccants will lose their absorbing ability gradually as water gets accumulated in their internal structure. It was impractical to insert replacement desiccants into the culture vessel. If too much desiccant was hung in the vessel, the humidity decreased too quickly and partial irradiance was lost because of the shading effect.

3.6.2. *Covering the medium with a layer of lanoline*

This technique can isolate the transfer of moisture from the medium to the air. The moisture content of the air was reduced and the relative humidity decreased. However, placing a layer of lanoline was labour intensive and restricted plantlet transplanting work. If the lanoline was added before the autoclaving process, the lanoline must be able to resist the damage of the high-pressure and high-temperature treatment.

3.6.3. *Bottom cooling*

Bottom cooling for vessels can reduce the medium temperature and then decrease the internal air temperature. From Eqn (5), temperature has a significant effect on the evaporation. As the evaporation decreased, the air humidity also decreased effectively. When the outside temperature was 20°C, on reducing the bottom temperature to 10°C, the internal relative humidities ranged from 96 to 98% RH. As the bottom temperature was cooled to 4°C, the internal relative humidity ranged from 93 to 97% RH (Honjo & Takarura, 1991). The technique was useful. However, the energy cost of bottom cooling is very high. It is impractical in a culture room to cool so many shelves.

3.6.4. *Vessels with a layer of gas permeable tape*

Fujiwara and Kozai (1995) suggested using a layer of gas permeable tape in the vessel cover to increase the air exchange rate. Wardle *et al*. (1983) used porous closures

with the culture vessels. However, no actual data were available to validate the suitability of their technique. In the study of Honjo and Takarura (1991), the internal relative humidity ranged from 99·0 to 99·7% RH at an air exchange rate of $0{\cdot}04\,h^{-1}$. The relative humidity ranged from 98 to 99% RH at an exchange rate of $6{\cdot}7\,h^{-1}$. The ratio for the two air exchange rates was 167. However, higher air exchange rates vessels did not significantly reduce the relative humidity. As the humidity reached the equilibrant state, Eqn (A6) can be modified as follows:

$$E_v = (H_i - H_{out})\rho E_x V \tag{8}$$

where: E_v is water evaporation from the medium in $kg\,h^{-1}$; H_i is the absolute humidity of internal air in kg $[H_2O]$ kg [dry air]$^{-1}$; H_{out} is the absolute humidity of external air in kg $[H_2O]$ kg [dry air]$^{-1}$; ρ is air density in $kg\,m^{-3}$; E_x is the air exchange rate of the culture vessel in h^{-1}; and V is the volume of the culture vessel in m^3.

Increasing the air exchange rate also increases the evaporation from the medium. More moisture enters the internal air so that the air relative humidity is still maintained at a higher state. In other words, the increased air exchange rate will send out more moisture to the external environment. If the medium loses too much water, its hardness will increase and will retard the root growth and nutrition absorption of the plantlets.

From the above discussion, no practical method can be applied to reduce the relative humidity. The effect of relative humidity on the physiology of plantlets was inconsistent among researchers (Tanaka *et al.*, 1992; Kozai *et al.*, 1993). The domain drawback of a high relative humidity environment was the malfunction of the stomata. As the plantlets were transplanted and placed in a greenhouse, the leaves were readily dehydrated. The solution to this problem was to reduce the light irradiance reaching the leaves and to control the microclimate at a higher relative humidity in this acclimatisation stage.

4. Conclusions

The moisture transfer model developed in this study was used to study the factors affecting evaporation from the medium in the culture vessels. The factors affecting evaporation of medium were internal air temperature, relative humidity, and irradiance. The humidity distribution was found by the measurement of relative humidities using resistive-type humidity sensors. A piezoresistive silicon tension meter could measure the tension of medium accurately. Several techniques for reducing internal humidity were evaluated by this moisture transfer model.

Appendix A: Moisture balance model of plant culture vessel

A.1. *Absolute humidity model of plant culture vessel*

Plant culture vessels are placed on shelves installed in the culture room. The moisture source of internal air in the culture vessel is from the evaporation of the medium and the moisture exchange between internal air and external air. A typical culture vessel for moisture transfer is sketched in *Fig. 1*. The moisture exchange between the internal air of the culture vessel and the external air can be calculated as follows.

$$W_{out} = \rho E_x V(H_i - H_{out}) \tag{A1}$$

where: W_{out} is the moisture migration rates from vessel in $kg\,h^{-1}$; and ρ is air density in $kg\,m^{-3}$; E_x is the air exchange rate of the culture vessel in h^{-1}; V is the volume of the culture vessel in m^3; H_i is the absolute humidity of internal air in kg $[H_2O]$ kg [dry air]$^{-1}$; and H_{out} is the absolute humidity of external air in kg $[H_2O]$ kg [dry air]$^{-1}$.

The light irradiance is the domain heat source for the vessels. The irradiance increased the internal air temperature and enhanced evaporation of from the medium. The water evaporated from the medium during the light period and water condensed from air during the dark period.

The water evaporation from the medium is analogous to the soil evaporation. The water evaporation from the medium was proposed as follows:

$$E_v = a_1 I_s + a_2 P_{wd} \tag{A2}$$

where: E_v is the water evaporation of the medium in $kg\,h^{-1}$; I_s is the irradiance entering the vessel in $W\,m^{-2}$; P_{wd} is the vapour pressure deficit in kPa; and a_1 and a_2 are constants.

$$P_{wd} = P_{ws}(1 - H_R) \tag{A3}$$

where: P_{ws} is the saturated vapour pressure in kPa; and H_R is the relative humidity in decimal.

The air temperature of the culture usually ranges from 20 to 30°C. The relationship between P_{ws} and temperature is assumed to be a polynomial equation for the narrow temperature range.

$$P_{ws} = b_0 + b_1 T_i + b_2 T_i^2 \tag{A4}$$

where: T_i is the internal air temperature in °C; b_0, b_1, and b_2 are constants.

Combining Eqns (A1), (A2) and (A4),

$$E_v = c_0 + c_1 I_s + c_2(1 - H_R) + c_3 T_i(1 - H_R) + c_4 T_i^2(1 - H_R) \tag{A5}$$

where: c_0, c_1, c_2, c_3, and c_4 are constants.

In other words, the evaporation of the medium is a function of irradiance, temperature, and relative humidity.

A.1.1. *Absolute humidity model during the light period*

From the moisture balance principle, the moisture of internal air entering the culture vessel was from the absolute humidity of internal air and the evaporation from the medium. The moisture exchange rate was described as:

$$\rho E_x V \frac{dH_i}{dt} = (H_i - H_{out})\rho E_x V - E_v \quad \text{(A6)}$$

where: t is time in min.

As the external irradiance increased or decreased, several internal microclimatic factors, such as air temperature and relative humidity varied and the evaporation of the medium changed. The variation in humidity can be described as follows.

(1) Initially, when $t = 0$, the moisture content in the internal air was:

$$W_0 = \rho V H_0 \quad \text{(A7)}$$

where: W_0 is the initial moisture content of the internal air at the initial stage in $kg\,h^{-1}$; H_0 is the initial absolute humidity in kg $[H_2O]$ kg [dry air]$^{-1}$.

(2) When $t = 1$ min, the first stage ranged from 0 to 1 min, and the moisture content of air can be computed from Eqn (A6):

$$W_1 = W_0 - \rho V E_x(H_0 - H_{out}) + E_1 \quad \text{(A8)}$$

where: W_1 is the moisture content of air at the end of the first stage in $kg\,h^{-1}$; H_{out} is the absolute humidity of external air in kg $[H_2O]$ kg [dry air]$^{-1}$; and E_1 is the water evaporation of the medium during the first stage in $kg\,h^{-1}$.

(3) When $t = 2$ min, the second stage ranged from 1 to 2 min,

$$W_2 = W_1 - \rho V E_x(H_1 - H_{out}) + E_2 \quad \text{(A9)}$$

where: W_2 is the moisture content of air at the end of the second stage in $kg\,h^{-1}$; H_1 is the absolute humidity of external air at the end of the first stage in kg $[H_2O]$ kg [dry air]$^{-1}$; and E_2 is the water evaporation of the medium during the second stage in $kg\,h^{-1}$.

(4) As the internal environment reached the equilibrate state, $t = t_f$, then:

$$W_f = W_{f-1} - \rho V E_x(H_{f-1} - H_{out}) + E_f \quad \text{(A10)}$$

where: t_f is the time at equilibrate state in min; W_{f-1} is the moisture content of air prior to the equilibrate state in $kg\,h^{-1}$; H_{f-1} is the absolute humidity of external air prior to the equilibrate state in kg $[H_2O]$ kg [dry air]$^{-1}$; E_f is the water evaporation from the medium at the equilibrate state in $kg\,h^{-1}$; and W_f is the moisture content of air at the equilibrate state in $kg\,h^{-1}$.

In this final stage, W_f is equal to W_{f-1}. From the sequence deviation process, E_f can be calculated by summing Eqns (A7)–(A10)

A.1.2. *Absolute humidity model in the dark period*

$$\rho V E_x \frac{dH_i}{dt} = (H_i - H_{out})\rho V E_x + E_c \quad \text{(A11)}$$

where: E_c is the condensation rate of moisture vapour in $kg\,h^{-1}$.

At the equilibrate state,

$$H_i = H_{out} - \frac{E_c}{\rho E_x V} \quad \text{(A12)}$$

A.2. *Relative humidity and absolute humidity*

The humidity of the internal air in the culture vessel can be calculated from the absolute humidity and dry bulb temperature.

A.2.1. *Calculating absolute humidity from relative humidity*

(1) The saturated vapour pressure, P_{ws}, at temperature T_k is given by (Albright, 1990):

$$P_{ws} = \mathrm{Exp}[-5800/T_k + 1{\cdot}3915 - 0{\cdot}04864T_k + 4{\cdot}1765 \times 10^{-5}T_k^2 - 1{\cdot}445 \times 10^{-8}T_k^3 + 6{\cdot}546 \ln(T_k)] \quad \text{(A13)}$$

where: P_{ws} is the saturation water vapour pressure in kPa; and T_k is the temperature in K.

(2) The vapour pressure of air P_w in kPa is given by:

$$P_w = H_R \times P_{ws} \quad \text{(A14)}$$

(3) The absolute humidity is then obtained from:

$$H_i = \frac{0{\cdot}622 \times P_w}{P_{atm} - P_w} \quad \text{(A15)}$$

where: H_i is the absolute humidity in kg $[H_2O]$ kg [dry air]$^{-1}$; and P_{atm} is the air pressure in kPa.

For the standard air pressure, P_{atm} equals to 101·325 kPa at sea level.

A.2.2. *Calculating relative humidity from absolute humidity*

The partial pressure of water vapour is calculated from the absolute humidity:

$$P_w = H_i \times P_{atm}/(H_i + 0{\cdot}622) \quad \text{(A16)}$$

$$H_R = P_w/P_{ws} \quad \text{(A17)}$$

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