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Application of growth models to evaluate the microenvironmental conditions using tissue culture plantlets of *Phalaenopsis* Sogo *Yukidian* 'V3'

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ABSTRACT

Phalaenopsis, an important pot plants worldwide, are propagated with a tissue culture technique. The aim of the present study was to validate growth models for *Phalaenopsis* plantlets with measured data. Effect of temperature and light irradiance on the growth characteristics was investigated by total weight, total leaf area and weight ratio of shoot to root weight for *Phalaenopsis* Sogo *Yukidian* 'V3'. Relationship between culture days and total weight and leaf area of plantlets with was analyzed by nonlinear regression analysis. A four-parameter logistics model for growth rate was selected used as the growth index for further study. The relationships between environmental factors and the total weight, total leaf area of plantlets and the weight ratio of leaf to root were evaluated by multiple regression analysis. These environmental factors all had a significant effect on the growth characteristics. The optimal microclimate conditions for plantlets culture of *Phalaenopsis* Sogo *Yukidian* 'V3' determined from the regression results were light irradiance 40–60 $\mu mol\ m^{-2}\ s^{-1}$, light temperature 29–32 °C and dark temperature 22–25 °C. The results can be useful information for propagating *Phalaenopsis* plantlets.

1. Introduction

In 2014, 80 million units of orchids were produced in Taiwan. Most were *Phalaenopsis*. Commercial *Phalaenopsis* is propagated from tissue culture. The quality and quantity of orchid plantlets needs to be improved continuously.

Orchid tissue culture plantlets are cultivated in a small aseptic culture vessel. The air exchange between inside and outside air is limited because of the need to isolate microorganisms. Water and composition of the medium in the vessel are the main source of nutrition. In the vessels, the aerial environment contains very high relative humidity. From the investigation of the microclimate inside vessel, the CO_2 concentration is decreased in the light period and increased in the dark period (Chen, 2007).

The growth of plantlets inside vessels is affected by the internal microclimate, such as air temperature and humidity, light quality and quantity (Hsu and Chen, 2009; George and Davies, 2008; Fujiwara and Kozai, 1995; Kozai, 2010). In the culture room, all vessels are placed on horizontal shelves. The practical way to control the internal microclimate of the vessel is to modify the outside environment of the culture room. Yao et al. (2007) proposed a model to describe the microclimate inside a glass jar with medium. However, only a simulation result was presented. Models for air temperature, relative humidity and photosynthetic photon flax density (PPFD) was developed and validated with measured data (Chen, 2003, 2004, 2005).

The effect of photosynthesis and other physiological activities on plantlets is of interest. Pospisilova et al. (1997) mentioned irradiance, CO_2 concentration, medium sucrose concentrations and growth regulators as factors. The highest net photosynthetic rate was found with 25 °C and 226 $\mu mol\ m^{-2}\ s^{-1}$ light irradiance. Lim et al. (1992) observed the effect of sugar, light intensity and CO_2 concentrations on the growth and mineral uptake of *Dendrobium* plantlets. The nitrate uptake was increase with increased light intensity, and CO_2 enrichment could not enhance the growth and ion uptake. Yamagishi (1988) examined the effect of the culture temperature on the growth characteristics of *in vitro* bulblets of *Lilium japonicum* Thunb and found that higher bulblets height and sugar uptake at 20 °C than 15 °C or 26 °C. Nguyen et al. (1999) observed the effect of different CO_2 concentrations and light intensities on the photosynthetic characteristics of coffee plantlets *in vitro*. Maximal dry mass, leaf area and photosynthetic rate were

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found at low light intensity (150 μmol m^{-2} s^{-1}) and high CO_2 concentration (1400–1450 ppm). Cui et al. (2000) suggested that increased difference in light period (18 °C) and dark period (26 °C) temperature and high intensity (210 μmol m^{-2} s^{-1}) increased the shoot weight, root weight and total fresh weight of *Rehmannia glutinosa* plantlets.

A crop growth model is useful to describe the crop growth index in the culture period. The effect of growth conditions or climate factors could be observed with the parameters of the growth model. Many crop models have been proposed to describe the growth characteristics of horticultural crops (Thornley and Joshson, 1990). Tei et al. (1996) found that the Gompertz model was the best fit for lettuce and the exponential linear equation for red beet and onion. Karadavut et al. (2010) compared the fitting ability of growth models for leaf growth data of maize and found that the logistic model was the adequate model. Fernamdes et al. (2014) proposed the Gompertz model to describe the growth curves of coffee fruit.

The growth models for plantlets grown in vessels are limited. Niu and Kozai (1997) developed a potato plantlet growth model by assuming that the dry weight of a plantlet at the end of one day consisted of the dry weight of the previous day, the carbon accumulation from the daily net photosynthetic rate and the absorption of sugar and minerals from the medium. However, only two datasets measured at days 8 and 15 were used for validation of the model and important parameter values were not considered. For the growth model for *Oncidium* plantlets (Chen, 2012), the gain in carbon source for *Oncidium* plantlets was assumed as the photosynthesis rate during the light period and the absorption of sugar from the medium. The loss of the carbon source was due to the respiration during the dark period. Three growth equations were proposed and the four-parameter logistic equation was the best equation.

Many studies have investigated about the micropropagation of *Phalaenopsis* plantlets. Tokuhara and Mii (1993) described the multiplication method and the medium compositions. Hahn and Paek (2001) reported that high photosynthetic photon flux density and high CO_2 concentration promoted photosynthesis of *Phalaenopsis* and three other orchids with photoautotrophic culture than heterotrophic culture. Park et al. (2002) described the rapid propagation technique of *Phalaenopsis* plantlets from floral stalk-derived leaves. Sinha et al. (2007) used inflorescence-axis thin sections as explants for micropropagation of *Phalaenopsis*. Balilashaki et al. (2014) reported the micropropagation technique of *Phalaenopsis* by using flower stalk nodes and leaves. Cha-um et al. (2010) tested the effects of temperature and relative humidity during *in vitro* acclimatization. The leaf growth model was proposed and influencing factors of temperature, light intensity and fertilization concentration were evaluated for *Phalaenopsis* plants cultured in 9-cm pots (Chen and Chien, 2012). However, interaction among these factors affecting the growth of *Phalaenopsis* plantlets is limited.

In the previous literatures, the effect of factors on growth characteristics has been studied. The growth characteristics on fixed culture days were measured and then these data sets were usually evaluated by analysis of variance (ANOVA). However, growth characteristics of plantlets such as fresh weight and leaf area are affected by different environmental factors during all culture days. Comparison the growth conditions on fixed days only may be inadequate to evaluate the influencing factors. Factors affecting the growth on all culture day should be studied by regression analysis.

Regression analysis has been proposed by plant biotechnologists to evaluate the related quantitative treatment on the growth characteristics of plantlets (Compton, 1994; Ibanez et al., 2003; Lorenzo and Garcia-Borroto, 2008; Mize and Chun, 1988). Gomes et al. (2010) used the multiple linear regression analysis to study the effect of the different factors on the multiplication rate of *Arutus unedo* L. (strawberry tree). Faria et al. (2004) adopted polynomial regression equations to compare the effect of different concentrations of sucrose on the plant of *in vitro* of *Dendrobium nobile*. Hsu and Chen (2009) studied the effect of light spectrum on the growth characteristics of *in vitro* of *Phalaenopsis*.

The aim of the present study was to validate growth models for *Phalaenopsis* plantlets with measured data. The parameter of the adequate model was then used to evaluate the effect of environmental factors. The experimental design included seven levels of light/dark temperature and three levels of photosynthetic photon flux density (PPFD).

2. Materials and methods

2.1. Plant materials

Samples were multiplicated plantlets of *Phalaenopsis* (Sogo *Yukidian* 'V3'). Plantlets were transplanted into 550 ml conical glass vessels with 120 ml medium in each vessel. Ten plantlets were cultured per flask. Vessels were sealed with a rubber stopper that had a hole with permeable film to provide ventilation.

The root medium was composed of 1/2 MS nutrients (Murashige and Skoog, 1962), supplemented with 200 ml l^{-1} coconut liquid 6.5 g l^{-1} agar, 10 g l^{-1} sucrose, 10 mg l^{-1} 6-benzylaminopurine (BA) and 5.0 mg l^{-1} d-naphthaleneacetic acid (NAA).

Typical figures of shoot proliferation and rooting is shown in Fig. 1.

2.2. Experimental design

The cultures were incubated in a photoperiod of 14 h light and 10 h dark. A total of thirty-six culture vessels were placed on three layers of shelves in a growth chamber, 12 vessels per shelf. Following are the seven experimental designs:

1. Light temperature 32 °C, dark temperature 26 °C, and three irradiance levels (PPFD): 20, 40 and 60 μmol m^{-2} s^{-1}.
2. Light temperature 32 °C, dark temperature 23 °C, three irradiance levels.
3. Light temperature 28 °C, dark temperature 22 °C, three irradiance levels.
4. Light temperature 25 °C, dark temperature 20 °C, three irradiance levels.
5. Light temperature 23 °C, dark temperature 19 °C, three irradiance levels.
6. Light temperature 20 °C, dark temperature 18 °C, three irradiance levels.
7. Light temperature 25 °C, dark temperature 25 °C, three irradiance levels.

2.3. Measurement of the growth parameters

The adequate time for good quality plantlets of *Phalaenopsis* ranged from 63 to 75 days. The growth characteristics were measured at day 15, 30, 40, 50, 60 and 72 days. Two culture vessels were taken out to measure the growth characteristics of plantlets at the time of each interval. The total fresh weight, total leaf area and ratio of leaf weight to the root weight were measured. The ratio of total leaf weight to total root weight (WR) served as a criterion to evaluate the quality of *Phalaenopsis* plantlets.

The fresh weight of roots and leaves was measured by using of an electronic balance (Metter PM 400, HP, Palo Alto, CA). The leaf area was determined with use of an area meter (LI-300 area meter, L2-COR, Inc., Lincoln, NE).