A Simple and Nondestructive Technique for Estimating the Newly Grown Roots of Potted *Phalaenopsis* Plants

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Abstract. A simple and nondestructive technique, based on the line intersect principle, was developed for estimating simultaneously more traits of the newly grown roots of potted *Phalaenopsis* plants. Two kinds of root distribution counting pot (RDCP) were used in the present study, namely, RDCP with a large drawing grid (RDCPL) and RDCP with a small drawing grid (RDCPS). Fifty *Phalaenopsis* plants were randomly sampled during the cultivation period of two to eight months after transplantation for each kind of pots. The grid number of the newly grown roots of each plant was determined by both RDCPS and RDCPL, followed by measurement of other traits such as number, fresh weight, dry weight, and length of the roots. Data were subjected to regression analyses for the selection of predictive equations. The results revealed that the grid number determined by RDCPS correlated better with the traits of the newly grown roots of potted *Phalaenopsis* [coefficients of determination $R^2 = 0.71$ to 0.90 ; low mean squared error (MSE)] than the grid number obtained with RDCPL ($R^2 = 0.66$ to 0.83 ; high MSE). Without tedious and time-consuming measurements, the four linear equations obtained with RDCPS can be a simple and nondestructive estimation for predicting the number, fresh weight, dry weight, and length of the developing roots of *Phalaenopsis* plants grown in pots. Another 48 *Phalaenopsis* plants were randomly sampled during the cultivation period of two to eight months after transplantation for the validation of selected predictive equations. The accuracy of the predictive equations of the traits of newly grown roots obtained with RDCPS was different in the following order: length > dry weight > fresh weight > number.

Roots of terrestrial plants perform many functions such as anchorage, acquisition of water and nutrients, storage, synthesis of growth regulators, propagation, and dispersal (Fitter, 2002). Therefore, assessment of the developing root is important for the proper growth of the plant (Atkinson, 2000).

Plant roots are buried in the ground wherein environmental conditions vary with respect to space and time. In the field, the

experimental design and treatments carried out during root research are often marked by unforeseen changes in the environmental conditions (Polomski and Kuhn, 2002). The known root measurement methods are tedious and time-consuming. The results are often inaccurate (Böhm, 1979). Thus, several pieces of equipment and tools have been developed for root analysis and measurement (McMichael and Person, 1991; Smit et al., 2000; Waisel et al., 2002). The methods of length estimation, based on the line intersect principle, were developed and tested during a program of root growth investigation (Marsh, 1971; Newman, 1966; Tennant, 1975). However, no information is available on nondestructive

techniques based on the line intersect principle that can investigate simultaneously more traits of root growth, including the number, fresh weight, dry weight, and length.

It has been observed that roots of *Phalaenopsis* plants, grown in pots for commercial production, often grow into the gaps between the medium and pot wall. The condition of the newly grown roots is an indicator of not only the growth of *Phalaenopsis* plants, but also its marketable value (van der Knaap et al., 2005). Thus, it is standard practice to measure the newly grown roots of *Phalaenopsis* plants for number of roots, length, and biomass (Kubota and Yoneda, 1993; Wang, 1995, 1998; Wang and Konow, 2002; Yoneda et al., 1997). In addition, the commonly used methods for root measurement of *Phalaenopsis* are tedious and time-consuming. The use of a simple and nondestructive technique for the measurement of developing roots of *Phalaenopsis* remains unexplored. Therefore, the objective of this study was to develop a simple and nondestructive technique based on the line intersect principle for evaluating simultaneously more traits of the newly grown roots of potted *Phalaenopsis* plants.

Materials and Methods

Plant materials. The experiment was conducted in a greenhouse at the Floriculture Research Center (FRC) of Taiwan Agricultural Research Institute, Gukeng, Yunlin, Taiwan. One hundred white-flowered *Phalaenopsis* Sogo Yukidian 'V3' plants were cultivated from 2 Feb. 2010 to 2 Oct. 2010 for the selection of the predictive equations. Another 100 plants of 'V3' were cultivated from 8 July 2011 to 8 Mar. 2012 for the validation of the predictive equation. They were transplanted into 10.5-cm clear polyethylene (PE) pots (0.75 L) containing 560 mL of Chilean sphagnum moss (*Sphagnum magellanicum* Brid.) (Jenn-yiyoung Company, Taichung, Taiwan), one plant per pot. The sphagnum moss was soaked in water overnight before transplanting. Plants were kept in a greenhouse equipped with a pad and fan cooling system with glass panels on the roof and the sides under a natural photoperiod. Two layers of black polypropylene shade fabric were installed inside of the greenhouse. The greenhouse air temperature ranged between 22 and 30 °C and the photosynthetic photon flux was 200 to 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Plants were fertigated with a 20N–8.7P–16.6K fertilizer (Peters Professional 20-20-20; The Scotts Co., Marysville, OH) at 0.5 g·L⁻¹ every 2 to 3 weeks.

Preparation and operation of the root distribution counting pot. The RDCP was designed and prepared by FRC. They were soft, transparent PE pots with the same dimensions as the growth container used in this study (diameter, 10.5 cm; volume, 0.75 L). Two kinds of RDCPs were used, namely, an RDCP with a large drawing grid (grid length $\times$ width = 2.5 cm $\times$ 1.5 cm) and an RDCP with a small drawing grid (grid length $\times$ width = 1.0 cm $\times$ 1.0 cm) (Fig. 1 A). The

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procedure for measuring the newly grown roots of *Phalaenopsis* using RDCP involved 1) replacing the original growth pot or container with RDCP (Fig. 1B–C); 2) marking the grids through which the roots have grown between the growth medium and RDCP (Fig. 1D); and 3) counting the number of marked grids.

Data acquisition for the selected predictive equations. Fifteen plants were randomly sampled at second and fourth months (2 Apr. 2010 and 2 June 2010) and 10 plants were randomly sampled at sixth and eighth months (2 Aug. 2010 and 2 Oct. 2010) after the transplanting. Each *Phalaenopsis* plant sampled was first counted for grid number by both RDCPs (RDCPS and RDCPL), and then the number, fresh weight, dry weight, and length of all new roots were recorded. The data obtained were subjected to a linear regression analysis of grid number vs. each of the root growth parameters. The predictive equations were selected based on the combination of the significance, high R^2 , and low MSE of regression (Cittadini and Peri, 2006; Roupalel et al., 2007; Wu et al., 2010).

Validation of the predictive equation. Twelve *Phalaenopsis* plants were randomly sampled at each second, fourth, sixth, and eighth month (8 Sept. 2011, 8 Nov. 2011, 8 Jan. 2012, and 8 Mar. 2012) after the transplanting for the validation of selected predictive equations obtained with selected RDCP. Each sampled *Phalaenopsis* plant was measured for grid number by selected RDCP, followed by recording the number, fresh weight, dry weight, and length of the newly grown roots. The calculated root growth for these plants was also obtained using the predictive equations. Data of measured and predicted per plant were used to fit a linear regression. The slopes were tested to see if they were significantly different from the slope of the 1:1 correspondence line (Dent and Blackie, 1979). The criteria of validating the accuracy of selected predictive equations were the significance of 1:1 linear regression equation, high R^2 , and no significant difference from the slope of the 1:1 correspondence line. It means the predicted values from predictive equation were very close to the measured values. Regression analyses were conducted using PC Version 9.1 of the Statistical Analyses System for windows (SAS Institute Inc., 2004).

Results and Discussion

Relationships between grid number measured by the various root distributions counting pot and traits of newly grown roots. The regression between the grid number counted on the various RDCPs and the traits of the newly grown roots was highly significantly correlated ($P < 0.01$) (Figs. 2, 3, 4, and 5). Based on the regression analysis, RDCPS ($R^2 = 0.71$ and $MSE = 10.2$) showed a better relationship between grid number and the number of newly grown roots compared with RDCPL ($R^2 = 0.66$ and $MSE = 11.9$) (Fig. 2). A similar finding was also obtained for comparison of grid number with

fresh weight, dry weight, and the length of the newly grown roots, showing that the relationship obtained with RDCPS ($R^2 = 0.85$ to 0.90 ; low MSE) was a better fit than that obtained with RDCPL ($R^2 = 0.79$ to 0.83 ; high MSE) (Figs. 3, 4, and 5). It showed that differences in grid size affected the correlation between measured and predicted values of the traits of newly grown roots. Based on the selective criteria of higher R^2 and lower MSE, the grid number measured by RDCPS showed better correlation with the traits of newly grown roots. The four predictive equations obtained with RDCPS could be used to evaluate the number, fresh weight, dry weight, and length of the developing roots of *Phalaenopsis* plants grown in pots.

Our result is similar to that reported by Marsh (1971), Newman (1966), and Tennant (1975), a method based on the line intersect

principle able to measure the root length of crop. Ichihashi (2006) developed a nondestructive method, based on the Archimedes law, for evaluating the newly grown roots of potted *Phalaenopsis* plants, but it was not able to evaluate more traits of root growth except for fresh weight. Therefore, previous reports provided no viable nondestructive techniques that can simultaneously evaluate more traits of root growth. However, our results demonstrated that a simple and non-destructive technique with RDCPS can simultaneously evaluate more traits of root growth of potted *Phalaenopsis*, including the number, fresh weight, dry weight, and length.

Validation of the predictive equations.

The accuracy of root estimation based on the line intersect principle depends on the root size, orientation of root, and root overlapping (Melhuish, 1968). It is necessary to

Fig. 1. (A) Two kinds of root distribution counting pots (RDCPs) with a large drawing grid (RDCPL) and a small drawing grid (RDCPS); (B–C) replacing the original growth pot by RDCP; (D) marking the grids through which the roots have grown between the growth medium and RDCP.

Fig. 2. Relationships between grid number measured by root distribution counting pots with (A) large and (B) small drawing grid and number of newly grown roots. R^2 = coefficients of determination; MSE = mean squared error. **Significant at the 1% level.

Fig. 3. Relationships between grid number measured by root distribution counting pots with (A) large and (B) small drawing grid and fresh weight of newly grown roots. R^2 = coefficients of determination; MSE = mean squared error. **Significant at the 1% level.

Fig. 4. Relationships between grid number measured by root distribution counting pots with (A) large and (B) small drawing grid and dry weight of newly grown roots. R^2 = coefficients of determination; MSE = mean squared error. **Significant at the 1% level.

Fig. 5. Relationships between grid number measured by root distribution counting pots with (A) large and (B) small drawing grid and length of newly grown roots. R^2 = coefficients of determination; MSE = mean squared error. **Significant at the 1% level.

Fig. 6. Relationships between measured and predicted traits of (A) number, (B) fresh weight, (C) dry weight, and (D) length of newly grown roots. Selected predicted equations obtained with root distribution counting pots with a small drawing grid (RDCPS), “ $Y = 0.081X - 3.996$,” “ $Y = 0.196X - 9.716$,” “ $Y = 0.014X - 0.863$,” and “ $Y = 0.233X - 41.498$,” were used for prediction of number, fresh weight, dry weight, and length of the newly grown roots, respectively. R^2 = coefficients of determination; $P(1:1)$ = probability different from the 1:1 line. **Significant at the 1% level.

validate the predictive accuracy of selected equations obtained with RDCPS for evaluating the roots of *Phalaenopsis* plants. It showed all good fits in a linear regression between measured and predicted values for the four traits of the newly grown roots (all regression coefficients were significant and all $R^2 > 0.92$) (Fig. 6). According to the estimates of regression coefficient shown in Figure 6, the accuracy of the predictive equations of the traits of newly grown roots obtained with RDCPS was different in the following order: length > dry weight > fresh weight > number. Only the regression coefficient between measured and predicted length of the predictive equations of root length was not significantly different from the 1:1 correspondence line ($P = 0.46 > 0.05$) (Fig. 6). This indicated that the predicted root length from the predictive equation obtained with RDCPS was very close to the measured root length during the cultivation of two to eight months after transplantation. The predictions of the number, fresh weight, and dry weight of the newly grown roots were slightly underestimated as a result of the fact that the regression coefficients between measured and predicted values were more than 1. Most of the predicted values deviating from the 1:1 line of the root number, fresh weight, and dry weight were found to be estimated from the plants sampled at the eighth month of post-transplantation (Fig. 6). This was principally caused by the root growth of *Phalaenopsis* plants that often became too extensive with many of them overlapping. Thus, the predictive equations for the number,

fresh weight, and dry weight of the newly grown root obtained with RDCPS were still accurate during the cultivation period of six months after transplantation.

Conclusions

The grid number determined with by RDCPS correlated better with the traits of the newly grown roots of potted *Phalaenopsis* than that obtained with RDCPL. The four linear predictive equations obtained with RDCPS with RDCPS can be used as a simple, time-saving, and nondestructive technique for simultaneously evaluating the number, fresh weight, dry weight, and length of the developing roots of *Phalaenopsis* plants grown in pots. The predictive accuracy of the equations of the traits of newly grown roots obtained with RDCPS was different in the following order: length > dry weight > fresh weight > number. The predictive equation of root length could be satisfactorily used in the estimation of actual root length. The predictions of the number, fresh weight, and dry weight of the newly grown roots were accurate during the cultivation period of six months after transplantation, and were underestimated slightly at the eighth month of post-transplantation.

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