Macronutrient Level for The Highest Seedling Yield of *Phalaenopsis deliciosa*

C.H. Lim, and C.W. Choong

Abstract—A defined medium was found to be suitable for wide range of genotypes of *Phalaenopsis* species. Macronutrient is important for vegetative growth of *Phalaenopsis* seedlings. However, the optimum macronutrient level of the medium is not known. *Phalaenopsis deliciosa* seedlings, an endangered species, were cultured on media with 1/3×, 1/2×, 1×, 2× and 3× macronutrient level with one subculture in between onto the same media before data collection. Seedling growth was measured by measuring fresh weight and dry weight of seedlings. It was found that the maximum seedling yield was obtained at 1× macronutrient level, which is recommended for seedling growth of *P. deliciosa* and possibly other *Phalaenopsis* species.

Keywords—Macronutrient level, *Phalaenopsis deliciosa*, seedling growth, nitrogen concentration, *in vitro* nutrition.

I. Introduction

PREVIOUSLY, a defined medium was found to be suitable for seedling growth and root development of *Phalaenopsis* species [1]. However, the medium has lower nitrogen (N) concentration to support wide range of genotypes of the species. Nitrogen is one of the most critical factors for the growth of orchids and there are numerous biological mechanisms to maximize the efficiency of nitrogen metabolism. Complex system of uptake, absorption and metabolism play role in complete utilization of nitrogen sources [2].

Cibes and colleagues (1949) found that growth increase in *Vanilla* orchid was detected when concentration of nitrogen increased in a gravel culture experiment [3]. In another research, Davidson (1957) found that growth of *Cattleya* in the deficiency of N and phosphorus (P) was more drastically limited compare to deficiency of potassium (K), calcium (Ca) or magnesium (Mg) in gravel culture experiment [3]. While working with *Phalaenopsis* hybrid seedling, it was shown that the best growth normally occurred at high N levels [4].

In more recent works, lower N concentration was found to lead to better growth at 100 ppm for a *Phalaenopsis* hybrid [3] and 7.5 mM for *Phalaenopsis amabilis* [5]. It is noted that *P. amabilis* and *P. deliciosa* (used in this study) were both classified in the same subgenus [6]. Contradictory information on optimum N concentration for *Phalaenopsis* as above made us wonder the optimum N concentration that supports wide range of genotypes of *Phalaenopsis* species.

In order to find N concentration that supports the best seedling growth, N concentration of the defined medium could be altered. However, in order not to disturb N balance with other macronutrients, the macronutrient level was altered instead. Therefore the aim of this study is to determine the optimum macronutrient level for maximum *P. deliciosa* seedling yield.

II. Materials And Methods

Seedpods of 110 days after pollination from *Phalaenopsis deliciosa* Rchb.f. were washed under tap water and then soaked in 5.25% sodium hypochlorite for 5 min to sterilize the outer surface of the seedpods. Then the seedpods were removed and soaked in 70% ethanol for 1 min. The seedpods were then flamed briefly and until traces of ethanol had burnt away. The seedpods were allowed to cool, then excised with a scalpel and seeds within were germinated on modified Choong *et al.* (CCT) defined medium [1], where KNO_3 were altered from 370 to 350 mg/l. Germinated seedlings with shoots and roots were subcultured onto the same medium. These seedlings were subcultured every 90 d until sufficient size was achieved for subsequent steps.

Approximately 0.3 ± 0.05 g of seedlings were inoculated onto modified CCT media with different macronutrient levels of 1/3×, 1/2×, 1×, 2× and 3×. This is done by adjusting NH_4NO_3, KNO_3, $Ca(NO_3)_2{\cdot}4H_2O$, $Mg(NO_3)_2{\cdot}6H_2O$, $CaSO_4{\cdot}2H_2O$ and $MgSO_4{\cdot}7H_2O$ to the levels mentioned above. The experiment was done in 8 replicates. Seedlings were incubated at 25°C and 16 h photoperiod with photon flux density of 30 μmol m^{-2} s^{-1} for 90 d. After 45 d, the seedlings were subcultured onto the same media as the previous ones.

Data collection was done at day 90 and the procedure was previously described by [7]. Roots and shoots of the seedlings were separated and weighed to measure the fresh weight. Then the organs were dried at 70°C for 2 d before measuring the dry weight. Plant fresh weight (FW) was obtained by addition of shoot and root fresh weight. Plant dry weight (DW) were obtained by the addition of shoot and root dry weight. Root to shoot ratio (R/S) was calculated by dividing root dry weight with shoot dry weight. Data were tested for normality based on z-score of *kurtosis* and skewness at α = 0.05. Treatment means of FW, DW and root to shoot ratio (R/S; based on dry weights) were analyzed by single-factor analysis of variance and two-way pairwise comparisons between those treatment means were performed with Fisher's Least Significant Difference (LSD) test at α = 0.05.

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III. Results

As shown in Table 1, there were two distinct groups ($\rho \geq 0.95$) representing the treatment means for FW and DW, which were group a and b, with treatments 1× in group a, 1/2×, 2× and 3× in group ab, and 1/3× in group b. This showed that 1× macronutrient produced the highest seedling yield. There were three distinct group representing the treatment means for R/S, which were group a, b and c, with treatments 1/3× macronutrient in group a, 1/2× macronutrient in group b, 1× macronutrient in group bc, and 2× and 3× macronutrient in group c. This indicated that as macronutrient level increased, shoot growth occurred relatively more than root growth.

TABLE I
Effect of macronutrient levels to seedling yield and root to shoot ratio

Macronutrient level	FW (g)	DW (g)	R/S
1/3×	0.884 ± 0.109 [b]	0.089 ± 0.013 [b]	7.976 ± 2.526 [a]
1/2×	1.024 ± 0.095 [ab]	0.103 ± 0.014 [ab]	6.055 ± 1.255 [b]
1×	1.154 ± 0.139 [a]	0.113 ± 0.013 [a]	4.564 ± 0.259 [bc]
2×	1.126 ± 0.258 [ab]	0.106 ± 0.022 [ab]	3.470 ± 0.596 [c]
3×	1.084 ± 0.226 [ab]	0.104 ± 0.018 [ab]	3.734 ± 0.150 [c]

Effect of macronutrient level on means of *Phalaenopsis deliciosa* Rchb.f. seedling fresh weight (FW), dry weight (DW) and root to shoot ratio (R/S) with respective 95% confidence intervals. Treatments were carried out at 8 replicates for 90 days with subculturing after 45 days. Treatment means were analyzed by single-factor analysis of variance and two-way pairwise comparisons between those treatment means were performed with Fisher's LSD test at $\alpha = 0.05$ that separated treatment means with significant difference ($\rho \geq 0.95$) into distinct groups represented by small cap alphabets.

As macronutrient level increased from 1/3× macronutrient, FW and DW increase until a maximum at 1× macronutrient, after which FW and DW reduce from 1× to 3× macronutrient level (Figure 1). This indicated that 1× macronutrient produced the highest seedling yield. Treatment means for R/S reduced as macronutrient level reduce, indicating that seedlings produced more shoot growth and lesser root growth at higher macronutrient levels as shown in Figure 1, and *vice versa*. However, from 2× to 3× macronutrient, R/S increased slightly. This increase however was not significant ($\rho \geq 0.95$).

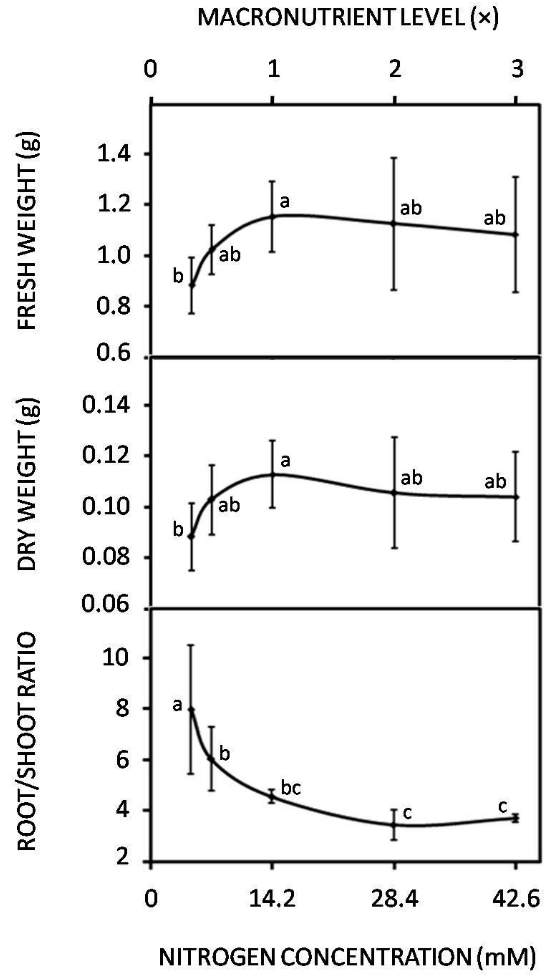

Fig. 1. Effect of macronutrient level (1/3×, 1/2×, 1×, 2× and 3×) on means of *Phalaenopsis deliciosa* Rchb.f. seedling ($n = 40$; $n = 8$ per treatment) fresh weight (FW), dry weight (DW) and root to shoot ratio (R/S) with 95% confidence interval error bars. Treatment means with significant difference ($\rho \geq 0.95$) were separated into distinct groups represented by small cap alphabets.

IV. Discussion

According to Davidson (1957), the most important element in macronutrients is N and P [3]. In the medium used for this study, 1× macronutrient contained 200 mg/l (or 200 ppm) or 14.2 mM of N and 182 mg/l (or 182 ppm) or 5.9 mM of P. High concentration of N is known to affect shoot growth significantly. For *Phalaenopsis* hybrid seedlings, higher N levels resulted in the best growth in a study [4].

The overall highest seedling yield of *P. deliciosa* was observed on 1× macronutrient level (14.2 mM or 200 ppm of N) as shown in Figure 1. The result from this study was different from [5] where lower N concentration (7.5 mM) resulted in the highest *P. amabilis* seedling yield.

In another study, a *Phalaenopsis* hybrid grew well with 100 ppm of N, which provided adequate or almost optimum level of N for the best plant growth. However, 200 ppm of N had shorter, fewer leaves and lower root dry weight compared to 100 ppm of N [3].

The concentration of phosphorus was much lower in [5] and [3] studies, i.e. at 39 mg/l (1.25 mM) and 20 ppm respectively. The medium used in this study at 1× macronutrient level had P concentration of 5.9 mM or 180 ppm. This could explain why maximum seedling yield was obtained at N concentration of 14.2 mM in this study and not 7.5 mM [5] or 100 ppm [3], possibly due to limiting amount of P in the latter studies.

Treatment means for R/S reduced as macronutrient level increase. This indicated that the higher the macronutrient level, the higher was the rate of shoot growth relative to root growth of seedlings. This result was similar to the result by [5], where when N level increase, higher shoot growth was obtained. Both N and P induced shoot growth and could cause the increase of R/S as macronutrient level increased [8]–[10].

V. Conclusion

Optimum macronutrient level for *P. deliciosa* was 1×. Yield reduced with both increase and decrease from the optimum macronutrient level. Increase of macronutrient level increased shoot growth relative to root growth and this observation is similar to the effect of the increase of N concentration. When P is not limiting, 14.2 mM of N was optimum for the highest seedling yield. When P is limiting, optimum N concentration may be lower. Optimum concentration of other macroelements such as P and K will be investigated at 14.2 mM of N in future.

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