Optimum Nitrogen to Sulfur Ratio to Increase *in Vitro* Seedling Yield of *Phalaenopsis bellina*

C.W. Choong, and S.M. Choong

Abstract**—** It is now understood that relative amount of nitrogen (N) to sulfur (S) influence the assimilation of nitrogen in crops, thus affecting crop yield and quality. There is not much attention placed on the N/S ratio of tissue culture medium, which vary considerably among standard media. It is therefore of interest to investigate the optimum N/S ratio in culture medium for *Phalaenopsis bellina*, an endangered species with horticultural value. Media with different N/S ratio was tested and it was found that the optimum N/S ratio for the species is 15. This ratio is recommended for *P. bellina* and possibly other species in the genus *Phalaenopsis*.

Keywords**—** N/S ratio, *Phalaenopsis bellina*, tissue culture, sulfur nutrition.

I. Introduction

EVEN though sulfur (S) is the fourth major plant nutrient after nitrogen (N), phosphorus and potassium, S nutrition has received relatively little attention until recently due to its wide tolerable range in plant nutrition. S is considered the element that would push crop yield further and is known to improve assimilation of other nutrients especially N. N content of plant generally increases with application of both N and S within a narrow range of N/S ratio for optimum crop yield and quality [1].

The amount of S in culture media has not received much attention as well and N/S ratio could range from 26, 12, 5 or even 2.5 in [2]–[5] media respectively. Generally, concentration of sulfate in culture medium should fall within the range of 1-3 mM [6].

With the recognition of S and N interaction in several studies [7], [8], there may be a need to adjust S concentration in relative to N in culture medium for optimum culture yield and quality. Therefore the aim of this study is to determine optimum S concentration of a culture medium for *P. bellina*.

II. Materials and Methods

Seed pod of 150 days after pollination from *Phalaenopsis bellina* (Rchb.f.) E.A. Christ. was cleaned under tap water and then immersed in 5.25% sodium hypochlorite for 5 min followed by 70% ethanol for 1 min. The seedpod was then flamed until traces of ethanol burnt away, excised with a scalpel and seeds within were inoculated onto Choong *et al.* (CCT) medium [9]. Seedlings were subcultured every 90 d.

Approximately 0.2 ± 0.05 g of seedlings were inoculated onto CCT medium with different N/S ratio. Five mediums with N/S ratio of 125, 20, 15, 10, 7.5 and 5 with 16 replicates each were used for the study by adjusting the amount of $CaSO_4$ and $MgSO_4{\cdot}7H_2O$ while maintaining calcium to magnesium ratio at 2. Seedlings were incubated at 25°C and 16 h photoperiod with photon flux density of 30 μmol m^{-2} s^{-1} for 180 d, with one subculturing at 90 d.

After that, roots and shoots of the seedlings were separated and weighed to measure the fresh weight, then dried at 70°C for 2 d before measuring the dry weight. Plant fresh weight (FW) and dry weight (DW) were obtained by the addition of shoot and root fresh weight and dry weight respectively. Data were tested for normality based on z-score of *kurtosis* and skewness at $\alpha = 0.05$. Treatment means of fresh weight, dry weight 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 $\alpha = 0.05$.

III. Results

Sample size was 16 replicates per treatment ($n = 96$). Data of FW, DW and R/S from each of the treatments passed normality test for both *kurtosis* and skewness, and is shown in Table 1. N/S ratio of 15 produced highest FW and DW of seedlings. Mean FW and DW of seedlings slightly increased from N/S ratio of 125 to 15, and then decreased from N/S ratio of 15 to 5 (Figure 1).

Mean FW of seedlings on N/S ratio of 15 were significantly higher ($\rho \leq 0.05$) than those with N/S ratio of 7.5 or lesser. Mean FW of seedlings on N/S ratio of 125, 20 and 10 were significantly higher ($\rho \leq 0.05$) than those with N/S ratio of 5. Mean FW of seedlings on N/S ratio of 7.5 was not significantly higher ($\rho \leq 0.05$) than those with N/S ratio of 5.

Mean DW of seedlings on N/S ratio of 15 and 125 were significantly higher ($\rho \leq 0.05$) than those on N/S ratio of 7.5 or lesser. Mean DW of seedlings on N/S ratio of 20 and 10 were significantly higher ($\rho \leq 0.05$) than those with N/S ratio of 5. Mean DW of seedlings on N/S ratio of 7.5 was not significantly higher ($\rho \leq 0.05$) than those with N/S ratio of 5.

There was no significant difference ($\rho \leq 0.05$) of mean R/S between treatments across N/S ratio as shown in Figure 1.

C.W. Choong is with the Faculty of Science, Technology, Engineering and Mathematics (FOSTEM), INTI International University, Persiaran Perdana BBN, Putra Nilai, 71800 Nilai, Negeri Sembilan, Malaysia. (corresponding author's e-mail: chiehwean.choong@newinti.edu.my).

S.M. Choong is with HELP University, BZ-2 Pusat Bandar Damansara, 50490 KL, Malaysia. (e-mail: choongsm@help.edu.my).

TABLE I
EFFECT OF NITROGEN TO SULFUR (N/S) RATIO

NS ratio	S concentration (mM)	FW (g)	DW (g)	R/S
125	0.12	2.168 ± 0.313 [abc]	0.206 ± 0.029 [ab]	1.896 ± 0.370 [ns]
20	0.72	2.215 ± 0.344 [ab]	0.200 ± 0.029 [ab]	2.131 ± 0.271 [ns]
15	0.96	2.300 ± 0.323 [a]	0.220 ± 0.030 [a]	1.960 ± 0.192 [ns]
10	1.44	2.062 ± 0.309 [abc]	0.192 ± 0.028 [abc]	2.040 ± 0.209 [ns]
7.5	1.92	1.850 ± 0.204 [bc]	0.166 ± 0.017 [bc]	2.058 ± 0.243 [ns]
5	2.88	1.597 ± 0.192 [c]	0.147 ± 0.016 [c]	2.012 ± 0.278 [ns]

ns – not significant

Effect of nitrogen to sulfur (N:S) ratio on mean of *Phalaenopsis bellina* (Rchb.f.) E.A. Christ. seedling fresh weight (FW), dry weight (DW) and root to shoot ratio (R/S) with respective 95% confidence intervals. Treatments were carried out at 16 replicates for 180 days with subculturing after 90 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 $\alpha = 0.05$.

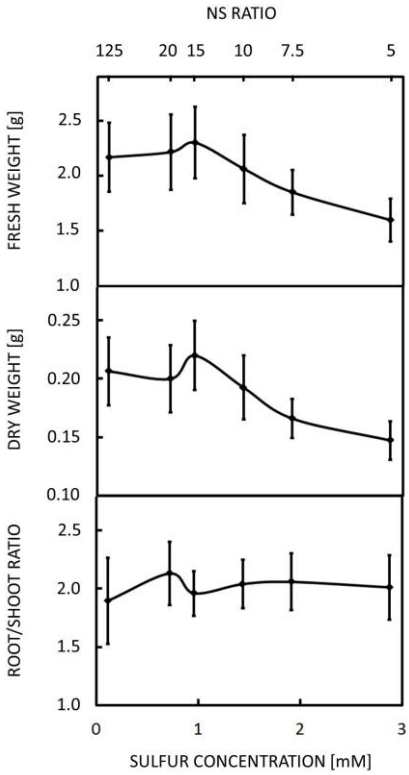

Fig. 1 Effect of nitrogen to sulfur (N:S) ratio on mean of *Phalaenopsis bellina* (Rchb.f.) E.A. Christ. seedling (n = 96; n = 16 per treatment) fresh weight (FW), dry weight (DW) and root to shoot ratio (R/S) with 95% confidence interval error bars.

IV. DISCUSSION

The optimum N/S ratio for seedling growth of *P. bellina* was 15. S was found to improve assimilation of other nutrients, particularly N [7], [8], [10]. However, optimum N/S ratio was found to be different with crop types and growth stages [1]. In *P. bellina*, N/S ratio higher and lower than 15 reduced seedling yield. This could be due to reduced assimilation of nitrogen and could be confirmed with chemical analysis of the seedlings' nutrient content to understand better the nutritional aspect of such seedlings. This finding highlights the importance of supplying the appropriate amount of S in culture media and alerts us on the effect of high S pollutants could have on the growth of the species in the wild.

V. CONCLUSION

Optimum N/S ratio for *P. bellina* was 15. Yield reduced with both increase and decrease from the optimum N/S ratio, more significantly when N/S ratio was increased. This could be due to imbalance in nutrient assimilation. Chemical analysis of the nutrient content of the seedlings would provide clue to the reason behind yield reduction as N/S ratio increase or decrease.

ACKNOWLEDGMENT

This study was financially supported by and conducted at INTI International University, Nilai campus. The authors would like to thank Mr. Sri Viknesh a/l Muniandy for assisting in data collection.

REFERENCES

[1] A. Jamal, Y.-S. Moon, and M. Z. Abdin, "Sulphur -a general overview and interaction with nitrogen," *Aust. J. Crop Sci.*, vol. 4, pp. 523-529, 2010.

[2] T. Murashige, and F. Skoog, "A revised medium for rapid growth and bio assays with tobacco tissue cultures," *Physiol. Plant.*, vol.15, pp. 473–497, 1962.

[3] O. L. Gamborg, R. A. Miller, and K. Ojima, "Nutrient requirements of suspension cultures of soybean root cells," *Exp. Cell Res.*, vol. 50, pp. 148-151, 1968.

[4] L. Knudson, "A new nutrient solution for the germination of orchid seeds," *Amer. Orchid Soc. Bull.*, vol. 15, pp. 214-217, 1946.

[5] E. F. Vacin, and F. W. Went, "Some pH changes in nutrient solutions," *Bot. Gaz.*, vol. 110, pp. 605–613, 1949.

[6] O.L. Gamborg, T. Murashige, T.A. Thorpe, I. K. Vasil, "Plant tissue culture media," *In Vitro*, vol. 12, pp. 473–478, 1976.

[7] S. Ahmad, I. S. Fazli, A. Jamal, M. Iqbal, and M. Z. Abdin, "Interactive effect of sulfur and nitrogen on nitrate reductase and ATP-sulfurylase activities in relation to seed yield from *Psoralea corylifolia* L," *J. Plant Biol.*, vol. 50, pp. 351-357, 2007.

[8] A. Jamal, I. S. Fazli, S. Ahmad, M. Z. Abdin, and S. J. Yun, "Effect of sulphur and nitrogen application on growth characteristics, seed and oil yields of soybean cultivars," *Korean J. Crop Sci.*, vol. 50, pp. 340-345, 2005.

[9] C. W. Choong, S. M. Choong, and W. H. Thong, "A defined culture medium suitable for sensitive Phalaenopsis species seedlings," Advance online publication. Retrieved from http://scialert.net/qredirect.php?doi=biotech.0000.54975.54975&linkid=pdf.

[10] A. Jamal, I. S. Fazli, S. Ahmad, and M. Z. Abdin, "Interactive effect of nitrogen and sulphur on yield and quality of groundnut (*Arachis hypogea* L.)," *Korean J. Crop Sci.*, vol. 51, pp. 519-522, 2006.