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Effect of Two Elicitors on Organogenesis in Protocorm-Like Bodies (PLBs) of *Phalaenopsis* 'Fmk02010' Cultured *In Vitro*

[1]Kazi Sadia Sultana, [2]Kazi Mustafa Hasan, [3]Kazi Mehedi Hasan, [4]Shamima Sultana, [5]Hasan Mehraj, [6]Monjurul Ahasan, [1]Kazuhiko Shimasaki and [5]Sultana Umma Habiba

[1]Faculty of Agriculture,Kochi University, B200 Monobe, Nankoku, Kochi 783-8520, Japan
[2]Dhaka Commerce College, National University, Dhaka, Bangladesh
[3]American International University, Dhaka, Bangladesh
[4]Eden Mohila College, National University, Dhaka, Bangladesh
[5]The United Graduate School of Agricultural Sciences, Ehime University, 3-5-7 Tarami, Matsumaya, Ehime 790-8556, Japan
[6]Department of Molecular Genetics, Graduate School of Medical Sciences, Kanazawa University, Takaramachi Kanazawa, 920-0934, Japan

Abstract: The application of elicitors, which is currently the focus of research, has been considered as one of the most effective methods to improve the synthesis of secondary metabolites in plant tissue culture. This experiment was done to see the effects of growth and development of *Phalaenopsis* 'Fmk02010' using two elicitors: N-Acetyl-D-glucosamine(NAG) and 5-aminolevulinic acid (5-ALA). The maximum average number of Protocorm-like bodies (PLBs) (24.3), highest shoot number (5.1) and fresh weight (0.335 g) was found from 0.1 mg/L NAG treatments. In case of 5-ALA, 0.1 mg/L 5-ALA supplementation in modified MS medium enhanced the best formation of PLBs (20.2), whereas the highest number of shoots (4.66) highest fresh weight (0.312 g) was found at 1mg/L compared with control.Elicitors are substances that induce protective responses in plants. Low concentration of NAG and 5-ALA has been suggested as a new natural and environmentally friendly regulator, which can be widely used in agriculture.

Key words: Protocorm-like bodies (PLBs) ・ N-acetyl-D-glucosamine (NAG) ・ 5-aminolevulinic acid (5-ALA) ・ Organogenesis ・ *Phalaenopsis*

INTRODUCTION

Phalaenopsis comprises one of the most important genera of orchids in horticulture over the past century. Plant growth regulators are widely applied to crops to improve productivity, increase stress resistance and regulate plant growth [1, 2]. Plant tissue cultures are exposed to stresses and stress combinations that they may not have encountered in nature in their long evolution. It is a remarkable reflection on the plasticity of the plant genome that it can decipher and respond to novel in vitro stresses. Today various tissue culture techniques are used to enhance yield of secondary metabolites by trigger stress response like using Elicitors, Precursors and Biotransformation, change in environment conditions, change in medium constituents etc. Elicitors are compounds stimulating any type of plant defense[3]. *N*-acetylgulcosamine (NAG) is a monosaccharide derivative of glucose. They serve as the main source of energy for metabolism and are used in biosynthesis[4]. and there also plays a role in structural stability and interaction with other molecules. In plants, NAG has been found in bromelain, ricin agglutinin and *abrus* agglutinin. Recently, NAG and its derivates were utilized in dietary supplements and for therapeutic development due to its unique characteristic. NAG is non-toxic supporting the essential safety issue. With this reason, these chemicals were used to see its effect on

Corresponding Author: Sultana Umma Habiba, The United Graduate School of Agricultural Sciences, Ehime University, 3-5-7 Tarami, Matsumaya, Ehime 790-8556, Japan. Tel: +819094474448.

organogenesis of *Phalaenopsis*. Another elicitor,5-aminolevulinic acid (ALA), is a key precursor in the biosynthesis of porphyrins such as chlorophyll and heme [5].ALA is synthesized from glutamatein ,a reaction involving a glutamyl-tRNA intermediate and requires ATP and NADPH as cofactors; its formation is the rate-limiting step in chlorophyll biosynthesis[5]. ALA increases the yield of garlic,barley, rice and potato plants by enhancing their photosynthetic capacity and plant biomass [6]. ALA is also known to be effective in improving plant growth and crop yields, as well as carbon and nitrogen assimilation [7] and is involved in regulating plant growth and developmental processes [1, 2, 8, 9]. ALA treatment increases chlorophyll content, photosynthesis and leaf soluble sugar content of Ginkgo biloba [10]. Exogenous ALA can enhance the quality of some medicinal plants [10, 11] and enhance the photosynthetic rate, chlorophyll content andyield of lettuce [12]. ALA has been proposed as a novel natural and environmentally friendly regulator that is widely used in agriculture [13]. This compound appears to act as a hormone like plant growth regulator and is effective at relatively low concentrations [11]. However, whether it can be used in orchid plants such as *Phalaenopsis* has not been reported. *Phalaenopsis* spp. comprises one of the most popular potted plant orchids in Japan. The majority of *Phalaenopsis* spp. are commercially produced using tissue culture methods. Considering its high potential in the ornamental market, are liable propagation method for large-scale production is important for its commercialization.

To date, there has a few report of NAG act as a plant growth stimulator in some plant species including in orchid species [14, 16]. Therefore, the objective of this section was investigated the effect of various concentration of *N*-acetyl-D-glucosamine and 5-ALA on organogenesis of *Phalaenopsis in vitro* culture.

MATERIALS AND METHODS

Plant Materials and Culture Conditionsp: The *Phalaenopsis*'Fmk02010' was used in this work. Protocorm-like bodies (PLBs) derived from meristem cultures of these cultivars were subcultured every two months on modified Murashige & Skoog [17] medium supplement with 412.5 mg /L ammonium nitrate, 950 mg/L potassium nitrate, 20 g/L sucrose and 2.2 g/L Phytagel (Sigma). Modified MS medium was adjusted to pH 5.5- 5.8 with 1 mM 2-(N-morpholino) ethanesulfonic acid sodium salts (MES-Na) before autoclaving at 121 °C for 15 min at 1.5 Kgf cm^{-2}. 250 ml of UM culture bottles (AsOne, JAPAN) with plastic caps were used, each bottle receiving 30 ml of medium. In this study, modified MS medium supplemented with various concentrations of NAG or Marine Sweat (Yaizu Suisan, Japan) and 5-ALA at 0, 0.01, 0.1, 1.0 and 10.0mg/L. Five PLBs explants were put in each culture vessel and three culture vessels were used for each treatment.

Data Collection: The numbers of PLBs, the numbers of shoots, the percentage of PLBs, the percentage of shoot and fresh weight of PLBs were recorded after 6 weeks of culture. The experiment was a completely randomized design with 3 replications and each replicate contained 5 PLBs. Data were statistically analyzed by calculating standard error of the means (means ± SE)

RESULTS

Effect *N*-acetylglucosamine (NAG) on the growth and development of PLBs in *Phalaenopsis*: The effects of different concentration of NAG on growth and development of PLBs and shoot induction *in vitro* of *Phalaenopsis* are shown in Table 1. However, modified MS medium containing 0.1 mg/L NAG was higher effective increased number of PLBs as well as in the percentage of PLBs formation, on which 100%, an average 24.3 PLBs per explants. Meanwhile, supplementation with 0.1 mg/L NAG increased the highest number of shoots (5.1 shoots per explant). This concentration was also found increased the maximum fresh weight of PLBs (0.335 g FW).

Fig. 1, showed that 100% PLBs formation rate were found at low concentration of NAG while maximum percentage of shoots formation (93.3%) was found at concentration 0.1mg/L NAG.The result also showed that NAG at concentration higher than 0.1 mg/L caused decreasing PLBs formation in *Phalaenopsis* 'Fmk02010'.

Effect of 5-Aminolevulinic Acid on organogenesis of PLBs in *Phalaenopsis*: The effects of elicitors (5-ALA) on organogenesis of *Phalaenopsis* were conducted in this study which was added with modified MS medium in PLBs culture. In Phalaenopsis, 0.1 mg/L 5-ALA supplementation in modified MS medium enhanced the best formation of PLBs (20.2); 1 mg/L 5-ALA induced highest formation of shoots (4.66) and produced highest fresh weight (0.322 g). These results suggested that low concentrations of 5-ALA added with culture medium enhance PLBs, shoots formation compare with control treatment.

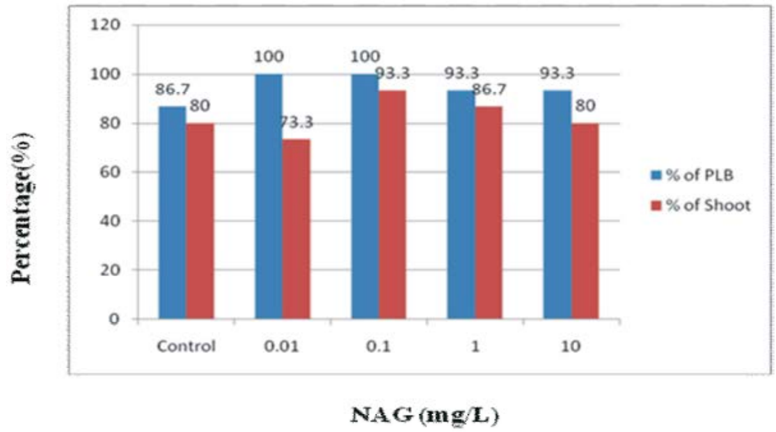

Fig. 1: Effects of NAG on PLBs and Shoot formation rate (%) in *Phalaenopsis* 'Fmk02010'
Percentage of PLBs/ shoot formation (%) = [(Number of cultured explants with new PLBs or shoot) / (Total number of cultured explants)] x 100

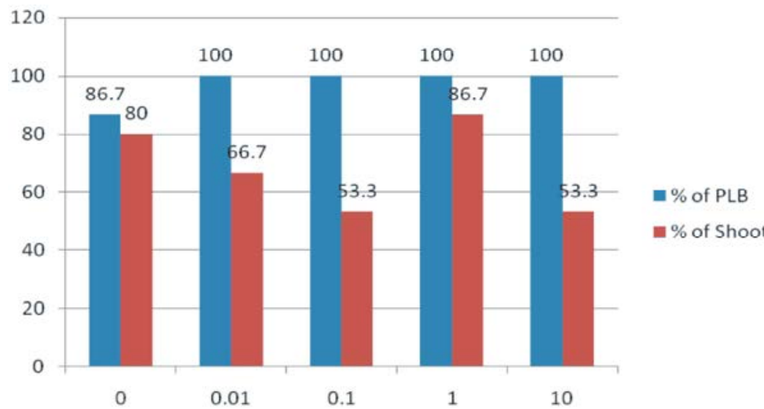

Fig. 2: Effect of 5-ALA on percentage of PLBs and Shoots in *Phalaenopsis* 'Fmk02010'

Table 1: Effect of NAG on organogenesis of PLBs in *Phalaenopsis*

NAG (mg/L)	PLB number	Shoot number	Fresh weight (g)
Control	11.1±0.6	2.5±0.1	0.257±0.01
0.01	13.2±0.6	3.4±0.2	0.262±0.01
0.1	24.3±0.5	5.1±0.2	0.335±0.01
1	12.5±0.6	4.9±0.2	0.236±0.01
10	12.3±0.5	3.8±0.2	0.297±0.00

Data were statistically analyzed by calculating standard error of the means (means ± SE)

Average number = Number of cultured explants with new PLBs or shoot / Total number of cultured explants

Table 2: Effect of 5-Aminolevulinic Acid on organogenesis of PLBs in Phalaenopsis

5-ALA (mg/L)	Average number of PLB	Average number of shoots	Fresh weight (g)
0	9.6±0.6	0.93±0.2	0.286±0.01
0.01	10.7±0.5	2.26±0.2	0.297±0.01
0.1	20.2±0.5	1.06±0.1	0.117±0.01
1	10.7±0.4	4.66±0.1	0.322±0.01
10	9.1±0.7	1.13±0.1	0.236±0.01

Data were statistically analyzed by calculating standard error of the means (means ± SE)

DISCUSSION

Present study investigated the effects of two biopolymer elicitors including NAG and 5-ALA on PLB and shootformation of *Phalaenopsis*. N-acetyl-D-glucosamine (NAG) has been found in bromelain, ricin agglutinin and *abrus* agglutinin in plant [18, 19, 20] and NAG also play a role in plant organogenesis and invertebrate embryogenesis [21]. In the present study, it was found that NAG at each concentration had a little or no enhance the growth and develop PLBs. Nahar *et. al*. [16] reported that modified MS media supplemented with 0.1 mg/L NAG was a suitable concentration used to induce both the highest PLBs formation and shoot formation of *Cymbidium* and the highest root formation was observed at medium containing 1 mg/L NAG. While, Keawjampa N. *et al*. [15] found the effect of NAG on the growth of PLBs and shoot formation of *Epidendrum* Rouge Star 'No.8'. The highest percentage of PLBs formation was found at 1 mg l^{-1} and 10 mg/L NAG and percentage of shoot formation was highest at concentration of 10 mg /L NAG. On the other hand, ALA is a potential plant growth regulator in stress conditions, being an essential biosynthetic precursor of tetrapyrrole compounds such as heme, cytochromes and chlorophyll [22]. In plants ALA is synthesized from glutamate in a reaction involving a glutamyl-tRNA intermediate and requiring ATP and NADPH as cofactors; its formation is the rate limiting step in chlorophyll biosynthesis [23]. (ALA is found in all plants and its concentration is regulated at low concentrations (60μmol) *in vivo* [22]. Same results found in our present study. Unfortunately, commercial ALA is too expensive for many common agricultural applications. Recently it was found that low concentrations of ALA had a promotive effect on growth and yield of several crops and vegetables [1, 9, 22]. During 42 days of culture, there was no malformation observed in regenerated shoots. High concentration of exogenous ALA can be used as non-polluting, non-residual photosensitive herbicides; in low concentration, it can regulate plant growth and development, increase productivity and enhance plant resistance [1].

CONCLUSION

Micropropagation of plants has become a significant technique to reproduce and make the availability of orchids that is otherwise difficult to propagate traditionally by seed or vegetative. This study showed that the choosing an appropriate of concentration of these elicitors. NAG and 5-ALA was effective on traits of organogenesis of *Phalaenopsis* and on the basis of results concluded that low concentrations of NAG and 5-ALA were more effective to promote the organogenesis in PLB cultures of *Phalaenopsis* ‘Fmk02010’.

REFERENCES

1. Watanabe, K., T. Tanaka and Y. Hotta, 2000. Improving salt tolerance of cotton seedlings with 5-aminolevulinic acid. Plant Growth Regul., 32: 99-103.
2. Zhang, Z.J., H.Z. Li, W.J. Zhou, Y. Takeuchi and K. Yoneyama, 2006. Effect of 5-aminolevulinic acid on development and salt tolerance of potato (*Solanam tuberosum* L.) microtubers *in vitro*. Plant Growth Regul., 49: 27-34.
3. Radman, R., T. Sacz, C. Bucke and T. Keshvartz, 2003. Biotehnol. Appl. Biochem., 37: 91-10.
4. Alvarez-Anorve, L.I., M.L. Calcagno and J. Plumbridge, 2005. Why does *Escherichia coli* grow more slowly on glucosamine than on *N*-Acetylglucosamine? Effects of enzyme levels and allosteric activation of GlcN6P deaminase (NagB) on growth rates. J. Bacteriol., 187: 2974-2982.
5. Von Wettstein, D., S. Gough and C.G. Kannangara, 1995. Chlorophyll Biosynthesis. Plant Cell, 7: 1039-1057.
6. Tanaka, Y., A. Tanaka and H. Tsuji, 1992. Stabilization ofapoproteins of light-harvesting chlorophyll-a/b protein complexby feeding 5-aminolevulinic acid under intermittent illumination. Plant Physiol. Biochem., 30: 365-370.
7. Maruyama-Nakashita, A., M.Y. Hira, S. Funada and S. Fuek, 2010. Exogenous application of 5-aminolevulinic acid increases the transcript levels of sulfur transport and assimilatory genes, sulfate uptake and cysteine and glutathione contents in *Arabidopsis thaliana*. Soil Sci. Plant Nutr., 56: 281-288.
8. Kefu, Z., L. Mingliang and L. Jiayao, 1986. Reduction by GA3 of NaCl-induced inhibition of growth and development in *Suaeda ussuriensis*. Aust. J. Plant Physiol., 13: 547-551.
9. Bindu, C.R. and M. Vivekanandan, 1998. Role of 5-aminolevulinic acid in improving biomass production in *Vigna catjung*, *V. mungo* and *V. radiata*. Biol. Plant, 41: 211-215.
10. Xu, F. and S. Cheng, 2011. Effects of 5-aminolevulinic acid on chlorophyll, photosynthesis, soluble sugar and flavonoids of *Ginkgo biloba*. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 39: 41-47.
11. Xu, F., J. Zhu, S. Cheng, W. Zhang and Y. Wang, 2010. Effect of5-aminolevulinic acid on photosynthesis, yield, nutrition andmedicinal values of kudzu (*Pueraria phaseoloides*). Trop.Grassl., 44: 260-265.

12 Xu, D.B., Q.J. Wang, Y.C. Wu, G.H. Yu, Q.R. Shen and Q.W. Huang, 2012. Humic-like substances from different compost extracts could significantly promote cucumber growth. Pedosphere., 22: 815-24.

13. Wang, L.J., W.B. Jiang and B.J. Huang, 2004. Promotion of 5-aminolevulinic acid on photosynthesis of melon (*Cucumismelo*) seedlings under low light and chilling stress conditions.Physiol. Plant, 121: 258-264.
14. Kyrychenko, O.V. and Perkovska G. Yu, 2007. Effect of hapten, N-acetyl-D-glucosamine, on the biological activity of wheat lectin. Gen. APPL. Plant Physiology, 33(3-4): 141-154.

15. Keawjampa, N., K. Shimasaki, C.L. Huang and N.J. Syeda, 2010. Effects of biopolysaccharide on organogenesis in orchid *in vitro*. Proceeding of The 1st Kamphaengsaen International Natural Products Symposium, The relationship between living Organisms and Environment, pp: 37-42.
16. Nahar, J.S., K. Shimasaki, C.L. Huang and N. Kaewjamp, 2011. Effect of plant growth regulators on organogenesis in protocorm-Like Body (PLBs) of *Cymbidium dayanum in vitro*. ARPN Journal of Agricultural and Biological Science, 6: 28-33.
17. Shimasaki, K. and S. Uemoto, 1990. Micropropagation of a terrestrial *Cymbidium* species using rhizomes developed from seeds and pseudobulbs. Plant Cell Tissue Org Cult., 22: 237-244.
18. Liu, C.L., C.C. Tsai, S.C. Lin, L.I. Wang, C.I. Hsu, M.J. Hwang and J.Y. Lin, 2000. Primary Structure and Function Analysis of the *Abrus precatorius* Agglutinin A Chain by Site-directed Mutagenesis. J. Biol Chem., 257: 1897-1901.
19. Lord, J.M., 1985. Precursors of Ricin and *Ricinus communis* Agglutinin. Eur J Biochem., 146: 411-416.
20. Wu, N., K. Fu, Y. Fu, Y.G. Zu, F.R. Chang, Y.H. Chen, X.L. Liu, Y. Kong, W. Liu and C.B. Gu, 2009. Antioxidant activities of extracts and main components of Pigeonpea (*Cajanus cajan* (L.) Millsp.) leaves. Molecules., 14: 1032-1043.
21. Jeen-Kuan, C., S. Chia-Rui and L. Chao-Lin, 2010. *N*-Acetylglucosamine: Production and Applications. Marine Drungs, 8: 2493-2516.
22. Hotta, Y., T. Tanaka, Y. Takeuchi and M. Konnai, 1997b. New physiological effects of 5-aminolevulinic acid in plants: the increase of photosynthesis, chlorophyll content and plant growth. Biosci. Biotech. Biochem., 61: 2025-2028.
23. Beale, S.I and P.A. Castelfranco, 1974. The Biosynthesis of delta-Aminolevulinic Acid in Higher Plants: I. Accumulation of delta-Aminolevulinic Acid in Greening Plant Tissues. Plant Physio., 53(2): 291-306.
24. Balestrasse, K.B., M.L. Tomaro, A. Batlle and G.O. Noriega, 2010. The role of 5-aminolevulinic acid in the response to cold stress in soybean plants.Phytochemistry, 71: 2038-2045.
25. Hotta, Y., T. Tanaka, Y. Takeuchi and M. Konnai, 1997a. Promotive effects of 5-aminolevulinic acid in the yield of several crops. Plant Growth Regul., 22: 109-114.