Notes Plant Tissue Culture Letters, **13**(2), 207-210(1996)

Topsin M® Stimulates the *In vitro* Growth in Some Orchids

Fumiaki KISHI* and Koujiroh TAKAGI**

Some agricultural chemicals such as Topsin M®[1,2] and thidiazuron[3-11], and antibiotics such as carbenicillin, cefotaxime and kanamycin[12-14] have been reported to stimulate the growth of plant tissue cultures. Topsin M® is an agricultural chemical which contain 70% of thiophanate-methyl consisted of 1, 2-bis(3-methoxy carbonyl-2-thioureido)benzene and 30% of mineral powder. The main effect of this compound is the inhibition of mitosis[1]. It has a molecular structure similar to that of some naturally occuring cytokinins. Therefore, it is also thought to have an influence on plant growth and to be of use for plant tissue culture. Xiao and Bao[1] reported that the medium containing less than or equal to 200 mg/*l* Topsin M® stimulated the growth, development and multiplication of autotetraploid rice plantlets and that the multiplication ratio increased from 21. 9 to 42. 7. However, the effect of Topsin M® was unclear in this study because it was used with other plant growth regulators such as KIN and NAA. On the other hand, Linfield and Price[2] reported that the 2000 mg/*l* of Topsin M® was phytotoxic for both the number and the weight of *Narcissus* bulbils.

In the present study, we report the effect of Topsin M® to the node culture of *Dendrobium moniliforme*, the PLB culture of *Darwinara* Pretty Girl and *Brassocattleya* Pastoral 'Innocence' BM/JOGA.

In vitro plantlets of *Dendrobium moniliforme* induced from the shoot apex and maintained by the methods of Kim *et al.*[15] were used as the source of node sections. Proliferation of *in vitro* plantlets from node sections was made for 4 months at 1 month intervals prior to use. One cm long node sections were excised from the *in vitro*-grown plantlets of *Dendrobium moniliforme* and inoculated onto Murashige and Skoog's(MS)[16] medium containing 30 g/*l* sucrose, 2 g/*l* Gelrite®(pH 5. 4)and different concentrations(0, 4, 20, 80 and 400 mg/*l*)of Topsin M®(Kumiai Chemical Industries Co., Ltd, Tokyo, Japan)which contain 70%(W/W)of thiophanate-methyl and 30%(W/W)of mineral powder to give rise to appropriate concentrations. The powder of Topsin M® was sterilized by immersion into 70%(V/V)ethanol solution. It was dryed up in a laminar flow chamber and added to the autoclaved(1 atm/120°C for 15 min.)medium after cooling to approximately 40°C. Plant boxes (L 6×W 6×H 9. 5 cm)containing 50 m*l* aliquot of the medium was used for the culture. Ten node explants were inoculated in each plant box, each explant being stuck vertically into the medium to its midpoint. They were cultured at 25±2°C under a photoperiod of 16 hr with fluorescent illumination at 4000±500 lux. After 2 months of culture, the number of nodes newly developed from a node section was calculated in *Dendrobium moniliforme*. The rate of node propagation slightly increased with the increase of the concentration of Topsin M® and the highest rate of node propagation was obtained at 400 mg/*l* although no statistically significant effect was obtained among the concentrations tested. However, no phytotoxic effect on the growth of *Dendrobium moniliforme* was observed(**Table 1**).

The PLB of *Darwinara* Pretty Girl and *Brassocattleya* Pastoral 'Innocence' BM/JOGA were also

* *Applied Research Center, Kirin Brewery Co., Ltd.*, 3377 Kitsuregawa, Tochigi 329-14, Japan
** *Takagi Orchid Nursery Co.*, 510-3 Sono, Chiba 263, Japan

Table 1. Effect of Topsin M® on the node propagation of *Dendrobium moniliforme*.

Concentration (mg/*l*)	Rate of node propagation*1
0	2.1
4	2.3
20	2.4
80	2.8
400	2.9 NS*2

*1 Number of newly regenerated nodes per explant after 2 months of culture.

*2 Nonsignificant within the column by Duncan's multiple range test ($P=0.05$).

used for the experiments. They were induced from the floral buds and shoot apex, respectively. These meristematic tissues were excised from main shoots and pseudobulbs, respectively, using razor blades. After initial washing with 10% (V/V) benzalkonium chloride solution for 10 min., the explants were surface-sterilized with a solution of 70% (V/V) ethanol for 30 sec. and 0.5% (V/V) sodium hypochlorite solution containing 0.05% (W/V) Tween 20 for 5 min., successively. After surface sterilization, the explants were rinsed 3 times with autoclaved (120°C, 1 atm for 15 min.) distilled water. Using a dissecting microscope, the explants were cut into 0.5 mm cubes using forceps and put on 1/4-strength MS medium containing 10 g/*l* sucrose, in which all the compositions of MS were reduced to 1/4-strength and FeEDTA was replaced by 6.95 mg/*l* $FeSO_4 7H_2O$ (pH 5.8). For the induction of PLB, two explants were inoculated in 80 m*l* of medium in each 300 m*l* flask and cultured on rotary shaker (TB-300 L, Takasaki Kagaku Kikai Co., Ltd., Kawaguchi, Japan). The rotation speed was 90 rpm with a 7 cm stroke. PLB was subcultured on the same medium with that for the induction of PLB. The cultures were made for 4 months at half a month intervals prior to use. Approximately 2 g PLB was inoculated into each 300 m*l* flask containing 100 m*l* aliquot of the same liquid medium as that for the induction and micropropagation of PLB containing Topsin M®. The treatments of Topsin M® were done in the same way as for the node culture of *Dendrobium moniliforme*. PLB of both *Darwinara* Pretty Girl and *Brassocattleya* Pastoral 'Innocence' BM/JOGA were cultured on a rotary shaker at 90 rpm with a 7 cm stroke at 25±2°C under a photoperiod of 16 hr with fluorescent illumination at 500±100 lux. After 2 months of culture, fresh weights of PLB were measured in *Darwinara* Pretty Girl and *Brassocattleya* Pastoral 'Innocence' BM/JOGA. Growth of PLB was evaluated as relative growth rate: fresh weight of PLB after 2 months of culture/fresh weight of PLB (2 g).

Table 2. Effect of Topsin M® on the PLB propagation of *Darwinara* Pretty Girl.

Concentration (mg/*l*)	Relative growth rate*1
0	2.8
4	4.5
20	4.7
80	4.5
400	2.4 NS*2

*1 Fresh weight 2 months after culture/Initial fresh weight (2 g)

*2 Nonsignificant within the column by Duncan's multiple range test ($P=0.05$).

Table 3. Effect of Topsin M® on the PLB propagation of *Brassocattleya* Pastoral 'Innocence' BM/JOGA.

Concentration (mg/*l*)	Relative growth rate*1
0	13. 3^{bc}
4	16. 8^{c}
20	16. 4^{c}
80	10. 4^{ab}
400	6. 5^{a}

*1 Fresh weight 2 months after culture/Initial fresh weight (2 g)
Values within the column followed by different letter are significantly different at P=0. 05 by Duncan's multiple range test.

In *Darwinara* Pretty Girl, Topsin M® at 4 to 80 mg/*l* was promotive for the PLB micropropagation and gave 1. 6 times higher relative growth rate than the control (**Table 2**). At 400 mg/*l*, however, it had no effect on the PLB growth.

In *Brassocattleya* Pastoral 'Innocence' BM/JOGA, 4 to 20 mg/*l* Topsin M® gave a slightly higher relative growth rate than the control (**Table 3**). However, concentrations higher than 80 mg/*l* reduced the relative growth rate, particularly at 400 mg/*l* which significantly (P=0. 05) inhibited the PLB growth.

As shown above, the addition of Topsin M® to the medium was slightly stimulative for the growth and/or propagation of the 3 different types of orchids although the optimum concentration was different from each other (**Table 1, 2, 3**). In *Darwinara* Pretty Girl and *Brassocattleya* Pastoral 'Innocence' BM/JOGA, a relatively lower concentration of Topsin M® (80 mg/*l* ≧) was promotive for the PLBs micropropagation. To our knowledge, this is the first report on the stimulative effect of Topsin M® for the tissue culture of ornamental plants as well as orchids.

As an antimicrobial chemical for encapsulated synthetic seeds of carrot and celery, Sakamoto *et al.*[17] used Topsin M® because it showed low inhibitory effect on their conversion to plantlets. Also, Linfield and Price[2] showed that this compound was effective for a broad spectrum of fungi. Therefore, this compound would also be usable for a wide range of tissue culture as an agent to prevent microbial contaminations. Although the 3 orchids, especially *Dendrobium moniliforme*, showed a tolerance to the high dosage of Topsin M® (400 mg/*l*), the effect of Topsin M® on the induction of somaclonal variation during the prolonged subculture was still unclear. Hence, somaclonal variation of these 3 types of orchids plantlets produced in the presence of Topsin M® in the medium are now being studied.

Acknowledgements

The authors wish to thank Mrs. Y. Hoshino for her excellent assistance with the node culture of *Dendrobium moniliforme*. The authors also wish to thank Dr. M. Mii, Prof. of Plant Cell Technology, Faculty of Horticulture, Chiba Univ. for his revision of this manuscript.

(Accepted April 9, 1996)

References

1) Xiao, S. S., W. K. Bao, 1989. Acta Phytophysiol. Sin., **15**: 329-332.
2) Linfield, C. A., D. Price, 1990. Crop Protec., **9**: 143-147.
3) Niedz, R. P., S. S. Smith, K. B. Dunbar, C. T. Stephens, H. H. Murakishi, 1989. Plant Cell Tissue Org. Cult., **18**: 313-319.

4) Huetteman, C. A., J. E. Preece, 1993. Plant Cell Tissue Org. Cult., **33**: 105-119.
5) Baker, B. S., S. K. Bhatia, 1993. Plant Cell Tissue Org. Cult., **35**: 273-277.
6) Gray, D. J., D. W. McColly, M. E. Compton, 1993. J. Amer. Soc. Hort. Sci., **118**: 425-432.
7) Molle, F., J. M. Dupuis, J. P. Ducos, A. Anselm, I. Crolus-Savidan, V. Petiard, G. Freyssinet, 1993. In "Synseeds" (ed. by Redenbaugh, K.), p. 56-57, CRC Press, Boca Raton.
8) Ernst, R., 1994. Plant Cell Tissue Org. Cult., **39**: 273-275.
9) Gill, R., T. Senaratha, P. K. Saxena, 1994. J. Plant Physiol., **143**: 726-729.
10) Leshem, B., R. Ronen, S. Lurie, 1994. J. Plant Physiol., **143**: 344-348.
11) Ficcadenti, M., G. L. Rotino, 1995. Plant Cell Tissue Org. Cult. **40**: 293-295.
12) Mathias, R. J., L. A. Boyd, 1986. Plant Sci., **46**: 217-223.
13) Zaghmout, O. M. F., W. A. Torello, 1992. J. Plant Physiol., **140**: 101-105.
14) Yepes, L. M., H. S. Aldwinckle, 1994. Plant Cell Tissue Org. Cult., **37**: 257-269.
15) Kim, K. K., J. T. Kunisaki, Y. Sagawa, 1970. Amer. Orchid Soc. Bull., **39**: 1077-1080.
16) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-497.
17) Sakamoto, Y., T. Mashiko, A. Suzuki, H. Kawata, A. Iwasaki, 1993. Acta Hortic., **319**: 71-76.