BIOLOGIA PLANTARUM 52 (4): 723-726, 2008

BRIEF COMMUNICATION

Induction of *in vitro* flowering in the orchid *Dendrobium* Sonia 17

C.S. TEE*,***, M. MAZIAH* and C.S. TAN**

Faculty of Biotechnology and Biomolecular Sciences, Univeristi Putra Malaysia, and*
*Malaysian Agricultural Research and Development Institute**, 43400 Serdang, Malaysia*
*Department of Bioscience and Chemistry, Faculty of Engineering and Science, Universiti Tunku Abdul Rahman, Jalan Genting Kelang, Setapak, 53300 Kuala Lumpur, Malaysia****

Abstract

In this study, *Dendrobium* Sonia 17 plantlets were used to induce *in vitro* flowering. Inflorescences were induced and rooting was inhibited in the half-strength Murashige and Skoog medium containing 20 μM N^6-benzyladenine (BA). The medium with high P and low N contents was effective to induce inflorescences while the medium with low P and high N contents was only effective to promote forming of shoots. In addition, the induced *in vitro* inflorescences were able to multiply and maintain without exhibiting a distinctive vegetative phase. Different morphologies of *in vitro* flowers such as incomplete flower structures, abnormal and unresupinated *in vitro* flowers were observed.

Additional key words: cytokinin, phytohormone, rooting.

In vitro flowering is not a rare phenomenon in plant tissue culture. Plant species such as ginseng (Lin *et al*. 2005), *Kniphofia leucocephala* (Taylor *et al*. 2005), *Orychophragmus violaceus* (Luo *et al*. 2000), *Gentiana* species (Zhang and Leung 2000), *Brassica napus* (Koh and Loh 2000) and *Chamomile recutita* (Kintzios and Michaelakis 1999) were reported to be able to flower *in vitro*. Different plant species require different induction medium to induce *in vitro* flowers. In most of the studies, single cytokinin, such as N^6-benzyladenine (BA), zeatin and kinetin, or combinations of other phytohormone and nutrients were used to induce *in vitro* flowering (Luo *et al*. 2000, Kintzios and Michaelakis 1999). In addition, Groenewald and Van der Westhuizen (2004) reported that various lipid compounds affected *in vitro* flowering of *Pharbitis nil*.

Orchids, one of the largest flowering families, are well known for their unique flower shapes and attractive colours. Generally, orchids have long juvenile phase that requires several years of growing before they flower (Kostenyuk *et al*. 1999, Duan and Yazawa 1994). Many factors such as photoperiod, irradiance, temperature and hormonal control might affect flowering of orchids (Chia *et al*. 1999, Goh 1984).

In order to reduce the juvenile phase, it is important to study *in vitro* flowering of orchids. In addition, the study might facilitate the elucidation of the flowering mechanism. Therefore, the study of *in vitro* flowering of *Dendrobium* was initiated.

In this study, three-leaf stage plantlets of *Dendrobium* Sonia 17 were used. The half-strength Murashige and Skoog (1962, MS) medium with Gamborg's B_5 vitamins (Gamborg *et al*. 1968) containing 20 μM BA (pH 5.8) was used as the induction medium while phytohormone-free half-strength MS medium was used as the control. Before transferring into the medium, the roots of the plantlets used were removed and the cultures were kept at temperature of 25 ± 2 °C and 16-h photoperiod with irradiance of 25 μmol m^{-2} s^{-1}.

Besides, the effects of phosphorus and nitrogen contents in the same medium on induction of *in vitro* flowering were investigated. In the high P/low N medium, KH_2PO_4 was used in concentration corresponding 1.25 × of the full strength MS medium while the nitrogen content [both KNO_3 and $(NH_4)_2NO_3$] was reduced to 0.25 × and *vice-versa* for the medium containing high N/low P. The cultures were kept at the similar conditions as described above.

The plantlets cultured on the medium containing 20 μM BA showed early sign of inflorescence formation after six months. Only the plantlets in the medium

Received 15 November 2006, *accepted* 20 July 2007.
Abbreviations: BA - N^6- benzyladenine; MS - Murashige and Skoog.
*** Address for correspondence; Fax: (+603) 41079803, e-mail: teecs@mail.utar.edu.my

containing BA formed inflorescence. An induction frequency of 14 and 22 % were achieved in two different sets of experiments. Different induction frequency obtained might be due to different numbers of plantlets used in the experiments. In addition, no root was formed in the medium containing BA. Normal rooting was observed in all the plantlets cultured in the BA-free control medium. Thus, BA might play an essential role in inducing the flower inflorescence but inhibiting root formation for *Dendrobium* Sonia 17 at this stage.

In most of the studies, BA was a crucial for induction of *in vitro* flowering of orchids, *e.g.*, *Cymbidium* (Kostenyuk *et al*. 1999), *Doriella, Phalaenopsis* and *Dendrobium* (Duan and Yazawa 1994), and *Dendrobium candidum* (Wang *et al*. 1993). The ability of inducing orchids to flower *in vitro* greatly reduced the time required (from years to months) for reaching the maturity stage necessary for flowering. For examples, *in vitro* flowering of *Oncidium varicosum* was observed after eight to nine months (Kerbauy *et al*. 1984), the *in vitro* flower of *Dendrobium candidum* was induced within three to six months (Wang *et al*. 1993), and *Cymbidium niveo-marginatum in vitro* flowers were observed within three months (Kostenyuk *et al*. 1999).

Phytohormone, polyamines and nutrient composition in the basal medium were studied on their effects to induce *in vitro* flowering of orchids (Kostenyuk *et al*. 1999, Duan and Yazawa 1994, Wang *et al*. 1993). The importance of BA for *in vitro* flowering induction was observed in our study as well as in the *in vitro* flowering study of ginseng (Lin *et al*. 2005) and *Kniphofia leucocephala* (Taylor *et al*. 2005). In addition, different concentrations of P and N in the medium affected the induction of *in vitro* flowering for orchids (Kostenyuk *et al*. 1999, Duan and Yazawa 1994).

In the study of the effects of P/N ratio on *in vitro* flowering induction, 52 % plantlets cultured on the medium containing BA with high P and low N content formed inflorescence while only 20 % plantlets formed inflorescences in the half-strength MS medium containing BA without any modification of the P/N ratio after four months. On the other hand, multiple shoots were observed from plantlets cultured in the half-strength MS medium containing BA with high N and low P contents.

Kostenyuk *et al*. (1999) reported that the root-excised *Cymbidium* plantlets cultured in the medium containing BA with high P and low N content achieved about 2.5 fold higher number of *in vitro* flowers compared to the cultures that were grown in the half-strength MS medium containing BA without modified P/N ratio. Similar results were reported when Duan and Yazawa (1994) studied the *in vitro* flowering in *Doriella*, *Phalaenopsis* and *Dendrobium*. They found that high N in the medium decreased or discouraged the floral buds formation. On the other hand, low N content in the culture medium improved the formation of floral buds.

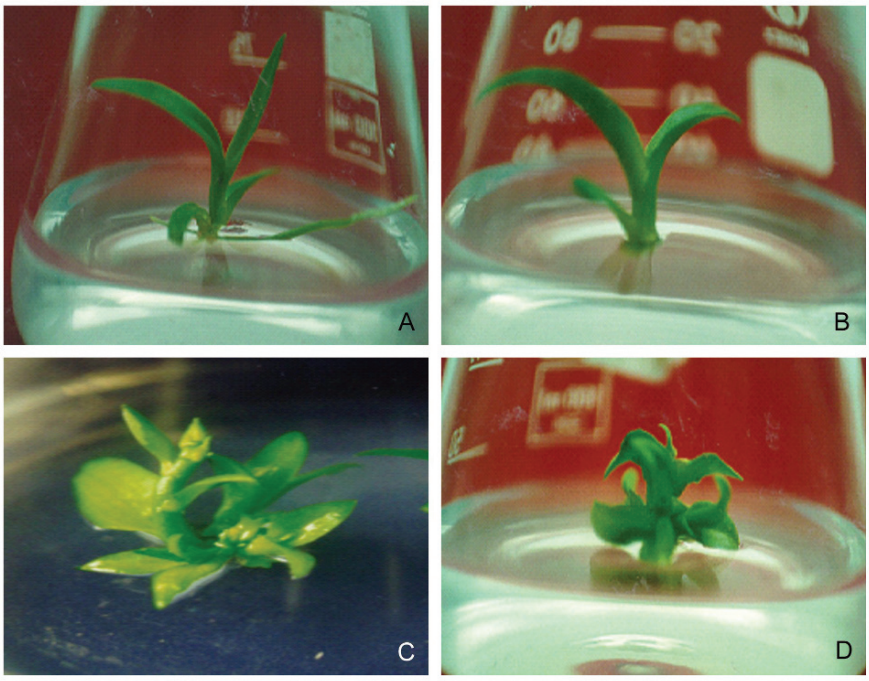

Fig. 1. Effects of the phosphorus and nitrogen content on *Dendrobium* Sonia 17 *in vitro* flowering induction after three months. *A* - The plantlet cultured on BA-free medium was able to root; *B* - No inflorescence was induced for the plantlet cultured in the medium containing BA after three months; *C* - *In vitro* inflorescences were induced in a single plantlet growing in the medium containing BA and high phosphorus and low nitrogen content; *D* - More shoots were formed instead of the inflorescence for the plantlet cultured in the medium containing BA with high nitrogen and low phosphorus content; *B* to *D* - No root was observed in medium containing BA.

Fig. 2. Different morphologies of *Dendrobium* Sonia 17 *in vitro* flowers were observed. *A* to *C* - Abnormal *in vitro* purple flowers with three sepals but all petals were missing; *D*, *E* - Different shapes of purple sepals were observed on the abnormal *in vitro* orchid flowers; *F* - An abnormal *in vitro* flower which has a large lip and three sepals but two main petals were missing; *G* - An inflorescence stalk with three opened flowers; *H* - Unresupinated whitish *in vitro* flower; the reproduction organ, column was clearly observed; *I* - A comparison between the natural flower and *in vitro* flower.

In this study, the induced *in vitro* inflorescence was maintained and multiplied in the induction medium. New inflorescence stalks were formed from the basal part of the mother inflorescence stalks. This phenomenon could be due to exposing of the cultures to the medium containing high concentration of BA continuously and the newly formed shoots were directly induced to form inflorescences without a vegetative phase. Besides, it was observed that rooting of the plantlets was again inhibited in the medium containing 20 μM BA. Only the plantlets cultured in the medium without BA were able to form roots (Fig. 1). This observation further confirmed BA was able to induce the inflorescence and also inhibit the root formation.

A similar observation was reported in ginseng in which the buds flowered without developing vegetative organs (Lin *et al*. 2005). On the other hand, if the *in vitro* inflorescences were transferred to the medium without BA, the inflorescences withered and turned brown. Similar result was observed when the flower stalks of *Doriella* were transferred to the phytohormone-free Vacin and Went medium (Duan and Yazawa 1994).

There were two distinctively different *in vitro* inflorescence morphologies observed, one of them produced larger leaf-like structures (bracts) with smaller flower buds hidden inside the bracts and the other type formed distinct flower buds protected by smaller bracts. *In vitro* flower buds developed from both types of inflorescences were either withered or bloomed into small flowers within one to two months. The size of *in vitro* flowers induced was far smaller than the original flower size. Only 4 % of inflorescence stalks were able to flower. Different types of incomplete floral structures were observed, for examples, the abnormal flowers with

less petals or sepals, flowers without the lip structure, flowers with different colours (white to purple) and sizes (Fig. 2) and resupinated (Fig. 2*F*) and unresupinated (Fig. 2*H*). Similar abnormalities were observed previously (Duan and Yazawa 1994, Chia *et al*. 1999). Various abnormalities of *in vitro* flower buds formed indicated that different conditions might be required for the initiation and development of flowers (Wang *et al*. 1993).

To summarize, a repeatable method for *in vitro* inflorescence induction in *Dendrobium* Sonia 17 was achieved using the medium containing 20 μM BA. The modified half-strength MS medium with high P and low N content further improved the *in vitro* inflorescence induction.

References

Chia, T.F., Arditti, J., Segeren, M.I., Hew, C.S.: Review: *In vitro* flowering of orchids. - Lindleyana **14**: 60-76, 1999.

Duan, J.X., Yazawa, S.: *In vitro* flowering of *Doriella*, *Phalaenopsis* and *Dendrobium*. - In: Ichihashi, N. (ed.): Proceedings of the Nagoya International Orchid Show. Pp. 87-96. Nagoya 1994.

Gamborg, O.L., Miller, R.A., Ojima, K.: Nutrient requirements of suspension cultures of soybean root cells. - Exp. Cell Res. **50**: 151-158, 1968.

Goh, C.J.: Flowering in tropical orchids. - Malayan Orchid Rev. **18**: 84- 91, 1984.

Groenewald, E.G., Van der Westhuizen, A.J.: The effect of various lipids on flowering of *Pharbitis nil* in *in vitro* culture. - Biol. Plant. **48**: 637- 639, 2004.

Kerbauy, G.B.: *In vitro* flowering of *Oncidium varicosum* mericlones (*Orchidaceae*). - Plant Sci. Lett. **35**: 73-75, 1984.

Kintzios, S., Michaelakis, A.: Induction of somatic embryogenesis and *in vitro* flowering from inflorescence of chamomile (*Chamomilla recutita* L.). - Plant Cell Rep. **18**: 684-690, 1999.

Koh, W.L., Loh, C.S.: Direct somatic embryogenesis, plant regeneration and *in vitro* flowering in rapid-cycling *Brassica napus*. - Plant Cell Rep. **19**: 1177-1183, 2000.

Kostenyuk, I., Oh, B.J., So, I.S.: Induction of early flowering in *Cymbidium niveo-marginatum* Mak *in vitro*. - Plant Cell Rep. **19**: 1-5, 1999.

Lin, C.S., Chen, C.T., Hsiao, H.W., Chang, W.C.: Effects of growth regulators on direct flowering of isolated ginseng buds *in vitro*. - Plant Cell Tissue Organ Cult. **83**: 241-244, 2005.

Luo, P., Ye, Q., Lan, Z.: A study on floral biology of seedlings *in vitro* in *Orychophragmus violaceus*: Induction of flowers in seedlings of *O. violaceus* cultured *in vitro*. - Plant Cell Tissue Organ Cult. **63**: 73-75, 2000.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue culture. - Physiol. Plant. **15**: 473-497, 1962.

Taylor, N.J., Light, M.E., Van Staden, J.: *In vitro* flowering of *Kniphofia leucocephala*: influence of cytokinins. - Plant Cell Tissue Organ Cult. **83**: 327-333, 2005.

Wang, G.Y., Xu, Z.H., Chia, T.F., Chua, N.H.: *In vitro* flowering of orchid (*Dendrobium candidum*). - In: You, C.B. (ed.): Biotechnology in Agriculture. Pp. 373-378. Kluwer Academic Publishers, Dordrecht 1993.

Zhang, Z., Leong, D.W.M.: A comparison of *in vitro* with *in vivo* flowering in *Gentiana*. - Plant Cell Tissue Organ Cult. **63**: 223-226, 2000.