Kasetsart J. (Nat. Sci.) 42 : 7 - 12 (2008)

Micropropagation of Bat Flower Plant, *Tacca chantrieri* Andre

Rommanee Charoensub*, Darat Thiantong and Salak Phansiri

ABSTRACT

The protocol for micropropagation of bat flower plant, *Tacca chantrieri* Andre., through axillary and adventitious shoot proliferation was investigated. The sterile seeds for the propagation of sterile seedlings of bat flower were prepared by surface sterilization of loose contaminated seeds in 10% commercial bleach, Haiter® (6%w/w sodium hypochlorite) and by aseptic removal of sterile seeds from fruits pre-sterilized by burning 95% ethanol. The sterile seeds from both sources were germinated and seedlings were cultured on the sterile MS medium containing 30 g/l sucrose, pH 5.6 for 2 months. Two months old sterile seedlings were then cultured on solidified MS medium containing 30 g/l sucrose supplemented with 1, 2 or 3 mg/l of kinetin or BAP for *in vitro* shoot induction and proliferation. By the end of the first month, seedlings produced more new shoots on the medium containing 2 mg/l BAP. However, in further subcultures more normal shoot were obtained from the medium with the addition of 1 mg/l BAP than from other media. The single shoot dissected singly from plantlets clusters formed roots and could survive after transplantation and the maximum numbers of their plantlets growing normally were obtained from solidified MS medium containing 30 g/l sucrose with the addition of 0.1 mg/l NAA.

Key words: medicinal plant, micropropagation, *Tacca chantrieri* Andre

INTRODUCTION

Bat flower plant (*Tacca chantrieri* Andre.) is perennial, distributes in the tropical regions of Asia, southeastern China (Yokosuka *et al*., 2005) and is one of the rare species in Tibet (Dong *et al*., 2007). Its rhizome has been used in Chinese folk medicine (Jiang Su New Medical College, 1997) and recently the isolated compounds of four new spirostanol saponins, along with one known saponin have been evaluated for their cytotoxicity against HL-60 human promyelocytic leukemia cells (Yokosuka *et al*., 2002). In Thailand, bat flower are found in the tropical rain forest areas and have been used in Thai folk medicine as well (Wutthithamawet, 1997). Its values are not only the medicinal properties but also the chemical compounds extracted from rhizome which are able to fight against plant pest (Nuanla and Sruamsiri, 2000). Besides, its flower has outlandish shaped, like bats with wide-spreading wings and long trailing filaments with rich maroon black colour making the plant favorably use as ornamental one. Normally, bat flower propagates from seeds and stem budding but plants and seeds are rarely collected from the natural forest. Moreover, its seed germination rate is quite low.

Scientific Equipment Center, Kasetsart University Research and Development Institute, Bangkok 10900, Thailand.
* corresponding author, e-mail: rdirmc@ku.ac.th

Micropropagation is a positive method to obtain numerous plant materials for mass production serving the future requirement instead of conventional propagation method.

There is a report of using young leaves and leaf stalks of seedling as explants for *in vitro* propagation via callus induction (He *et al*., 2002). However, there are few reports on micropropagation of bat flower. This investigation intended to provide a protocol for micro-propagation of bat flower through axillary and adventitious shoot proliferation.

MATERIALS AND METHODS

Preparation of sterile seedlings

The sterile seeds for the preparation of sterile seedlings were prepared by surface sterilization of loose seeds and by removal of sterile seeds from sterilized freshy fruits (berry like). Mature loose contaminated bat flower seeds were immersed in 70% ethanol for 1 min prior to surface-sterilization in 10% commercial bleach, Haiter® (6%w/w sodium hypochlorite), for 25 – 30 min and rinsed 3 times with sterile water. The whole clean fruits were dipped in 95% ethanol and directly flamed 2 – 3 times prior to dissection and removal of the sterile seeds. The sterilized seeds were germinated on the MS medium (Murashige and Skoog, 1962) containing 30 g/l sucrose and 6.7 g/l agar, pH 5.6. After seeds germination, the seedlings were cultured on the same medium under 50 μmol $m^{-2}s^{-1}$ light intensity, 10 h photoperiod at 25°C for 2 months.

Shoot induction and proliferation

Two months old *in vitro* seedlings, having the leaves and roots removed, were transferred to MS media containing 30 g/l sucrose and 6.7 g/l agar, pH 5.6 and supplemented with 1, 2 or 3 mg/l 6-benzylaminopurine (BAP) or kinetin. The seedlings were cultured under 50 $\mu molm^{-2}s^{-1}$ light intensity, 10 h photoperiod at 25°C. Each medium consisted of seven seedlings. The percentage of seedlings responsive to treatments, new shoots, new leaves and roots per seedling or plantlet were recorded twice, after 1 month of the first and the second subcultures.

Root induction

In vitro plantlet clusters obtained from shoot induction medium containing 1 mg/l BAP were separated into single shoots (with 2 – 3 leaves and without root). Then the rootless shoots were transferred to MS media containing 30 g/l sucrose and 6.7 g/l agar, pH 5.6, supplemented with 0.1, 0.3 or 0.5 mg/l α-naphthaleneacetic acid (NAA) for root induction and without NAA (control). Each medium consisted of seven plantlets. The numbers of root, root length and growth of rooting plantlets were recorded.

The experimental data were analyzed by variance analysis (ANOVA), followed by LSD test at 5% level of probability for mean comparison.

RESULTS AND DISCUSSION

Naturally, bat flower seeds give low rate of germination, about 12% (He *et al*., 2002) and usually take up to 9 months to germinate (Tom Dawn Communications Ltd., 2007). Bat flower seeds sterilized in commercial bleach gave 30% of germination, higher than the germination rate of sterile seeds from fruits, which gave only 5% of germination after one month of seeds culture. It is speculated that the seed coat was softened by the commercial bleach and absorbed more water for germination.

Shoot induction and proliferation

After one month, the sterile seedlings cultured on the MS medium without cytokinin (control) did not produce any new shoot primordia while in the MS media supplemented with cytokinin (kinetin or BAP) showed distinctive increase in growth. BAP induced significantly

Table 1 Responses of bat flower seedling induced by kinetin (K) and BAP supplemented to solidified MS medium after 1 month of the first subculture.

Kind of cytokinin and concentration (mg/l)		Numbers of shoots /plantlet	Responsive seedling (%)	Numbers of new leaves/planlet	Numbers of roots/plantlet
Without cytokinin		0.0^{c}	0.0	1.7^{ab}	0.6
K	1	0.4^{c}	28.6	2.1^{a}	0.0
	2	0.9^{bc}	42.9	1.0^{cd}	0.1
	3	0.7^{bc}	71.4	1.4^{bc}	0.1
BAP	1	1.7^{ab}	71.4	0.4^{de}	0.0
	2	2.3^{a}	71.4	0.1^{e}	0.0
	3	1.9^{ab}	85.7	0.4^{de}	0.0

Mean with different letters are significantly different at P< 5%.

larger numbers (2.3) of new shoots/plantlet than kinetin especially at 2 mg/l BAP and the maximum percentage (85.7%) of responsive seedling was obtained from 3 mg/l BAP (Table 1). The seedlings cultured on the MS medium containing kinetin showed rather normal plantlets with expanded leaves and roots compared to the control. The numbers of new leaves per plantlet were significantly larger on the control and the medium added with 1 mg/l kinetin than the plantlets cultured on the medium containing BAP (Table 1). Plantlet roots were rarely observed in all media containing cytokinin (Table 1).

In the second subculture, the cluster of plantlets with shoot primordia, subcultured onto the MS medium supplemented with 1 mg/l BAP developed new normal shoots while on the media supplemented with 2 and 3 mg/l BAP and all concentrations of kinetin obtained new normal shoots together with new shoot primordia (Figure 1 and 2C). Moreover, the plantlets formed callus (Figure 1 and 2A) and had abnormal shoot

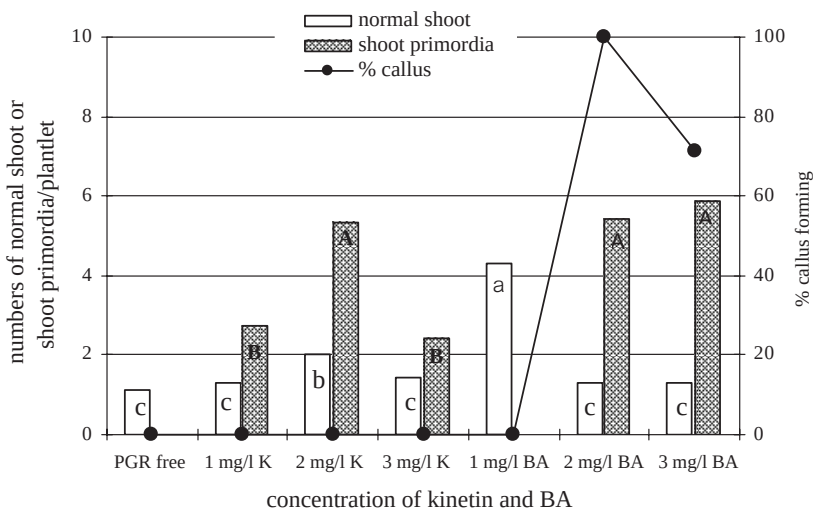

Figure 1 Growth of plantlets after second subculture onto MS medium containing kinetin and BAP at different concentrations and cultured for one month. Numbers of normal shoots or shoot primordia /plantlet and percentage of plantlets forming callus were shown. Column with different letters are significantly different at P< 5%.

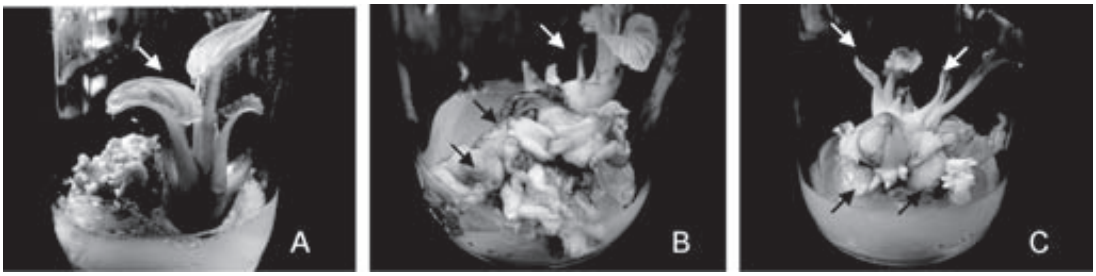

Figure 2 Callus forming (A: black arrow), abnormal plantlets (B: black arrows), shoot primordia (C: black arrows) and normal shoot (A, B and C: white arrows) obtained when bat flower planlets were cultured on MS medium supplemented with 3 mg/l BAP after one month of the second subculture.

with less expanded leaves especially in the media containing 2 and 3 mg/l BAP (Figure 2B). It is speculated that the increasing the concentration of cytokinin decreased shoot elongation and tended to induce callus. According to Leshem *et al.* (1988), although cytokinin is needed to induce shoot proliferation, the supra-optimum concentrations can be toxic. As in fig (*Ficus carica* L.), the increasing level of the kinetin, from 1 up to 4 mg/l, decreased shoot length (Fraguas *et al.*, 2004).

Root induction

Root initiation was observed within 10 – 15 days in all root induction media including the control. Supplementation of NAA resulted in the increase of roots/plantlet compared to the control. The maximum numbers of root obtained from the medium supplemented with 0.3 mg/l NAA. In contrast, maximum length of root was obtained from the control (Figure 3).

After culturing for one month, the rooted plantlets were transplanted into pots. Even though the MS medium supplemented with 0.3 mg/l NAA gave larger numbers of roots, the plantlets in the control and in 0.1 mg/l NAA added media inducing healthier roots had higher percentage of normal growing plantlets (Figure 4). Higher concentrations (0.3 and 0.5 mg/l) of NAA produced thick, fragile,

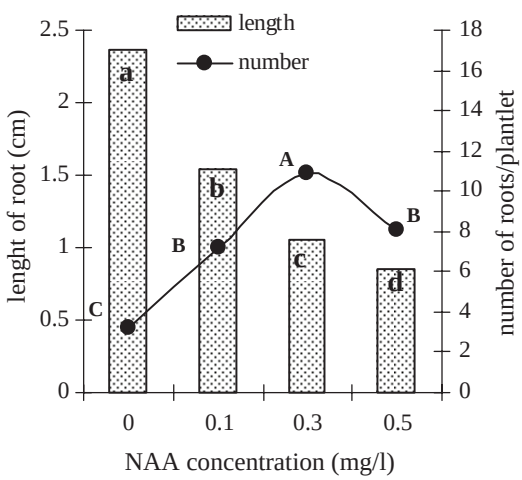

Figure 3 Effect of NAA on root formation after culturing for 1 month. Column or • with different letters are significantly different at P< 5%.

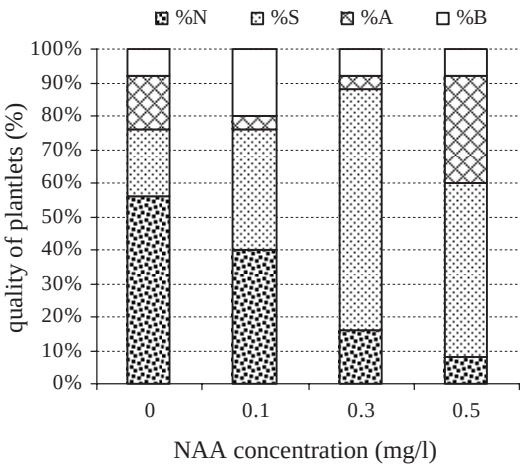

Figure 4 Quality of plantlets after culturing on root induction media for 1 month presented as percentage of normal (%N), small (%S), abnormal (%A) and large (%B) growing plantlets.

short roots and forming callus with low survival rate (data not show) after being transplanted. The use of exogenous auxin to induce root of culturing shoots is a common practice. As in *Decalepis arayalpathra* plantlets, "amarithapala" a rare endermic medicinal plant, the presence of auxin greatly increased the rooting and survival plantlets, however, callus formation with short roots occurred in the high auxin concentration (> 0.2 mg/l NAA) and reduced the survival rate (Sudha *et al*., 2005).

CONCLUSION

Micropropagation of bat flower can be done by culturing sterile seedlings. To obtain normal plantlets of bat flower, two culture steps of shoot induction and proliferation were needed. The first step was shoot primordia induction by culturing sterile seedling on the MS medium containing 30 g/l sucrose supplemented with 2 mg/l BAP. The second step, the plantlets were subcultured onto 1 mg/l BAP added medium to improve shoot elongation. Then the roots of isolated plantlets were induced by using control medium or the MS medium containing 0.1 mg/l NAA. Finally, rooted plantlets were successfully field established.

ACKNOWLEDGEMENT

This study was supported by Kasetsart University Research and Development Institute.

LITERATURE CITED

Dong, K., X. Zhong and L. Shuzhen. 2007. Quantitative assessment on endangerment degree of rare animal and plant species in Tibet, China. **Wuhan Univ. J. Nat. Sci.** 12: 684-688.

Fraguas, C. B., M. Pasqual, L. F. Dutra and J. O. Cazetta. 2004. Micropropagation of fig (*Ficus carica* L.) 'Roxo de Valinhos' plants. **In Vitro Cell. Dev. Biol.-Plant** 40: 471-474.

He, H., Q. Lan and Y. Zhang. 2002. In vitro propagation of *Tacca chantrieri*. **J. Guangxi Agric. Biol. Sci.** 2: 108-110.

Jiang Su New Medical College. 1997. **The Dictionary of Chinese Herbal Medicines**, Vol. 1. Shanghai Scientific and Technological Press, Shanghai 524 p.

Leshem, B., E. Werker and D.P. Shalev. 1988. The effect of cytokinins on vitrification in melon and carnation. **Ann. Bot.** 62: 271-276.

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

Nuanla R. and P. Sruamsiri. 2000. Insecticidal activity of bat flower plant crude extraction against common cutworm, pp. 200-209. *In* **Seminar Report on Tendency of the Medicinal Plant Improvement in Thailand.** 13-14 September 2000. Office of the National Research Council of Thailand. Bangkok.

Sudha, C. G., P. N. Krishnan, P. Pushpangadan and S. Seeni. 2005. *In vitro* propagation of *Decalepis arayalpathra*, a critically endangered ethnomedicinal plant. **In Vitro Cell. Dev. Biol.-Plant** 41: 648-654.

Tom Dawn Communication Ltd. 2007. **The Seed Germination Resource**. http://www.dawn-comms.co.uk/plants/default.htm, May 21, 2008.

Wutthithamawet W. 1997. Bat flower plant, pp. 315. *In* **The Encyclopedia of Thai Medicinal Plant and Folk Medicine**. The O-dian Store Press, Bangkok.

Yokosuka, A., Y. Mimaki, C. Sakuma and Y. Sashida. 2005. New glycosides of the campesterol derivative from the rhizomes of *Tacca chantrieri*. **Steroids** 70: 257-265.

Yokosuka, A., Y. Mimaki and Y. Sashida. 2002. Spirostanol saponins from the rhizomes of *Tacca chantrieri* and their cytotoxic activity. **Phytochem.** 61: 73-78.