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.

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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 \textit{in vitro} propagation via callus induction (He \textit{et al.}, 2002). However, there are few reports on micropropagation of bat flower. This investigation intended to provide a protocol for micropropagation 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 \textit{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 µmol m\(^{-2}\) 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**

\textit{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 \(\alpha\)-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 \textit{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
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

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**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.

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

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

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**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 plantlets 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%.
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.

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LITERATURE CITED


