***IN VITRO* PROPAGATION OF THREATENED TERRESRTIAL ORCHID *ANOECTOCHILUS SETACEUS* BLUME**

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**Abstract**

*Anoectochilus setaceus* Blume is a terrestrial orchid which is having ornamental and medicinal value. It is declared threatened orchid in Vietnam due to overexploitation and also due to habitat loss. In the present study we have developed a simple and efficient *in vitro* propagation protocol for *A. setaceus*. Multiple adventitious shoots were regenerated from shoot tip explants on Murashige and Skoog (MS) medium supplemented with benzylamino purine (BAP) or kinetin (Kn; 0.1 – 2 mg/l). MS medium supplemented with 0.6 mg/l Kn induced 5.4 shoots per explants. Among the various levels of sucrose tested (0 - 7%) for shoot regeneration, 2% sucrose was found suitable for shoot regeneration and growth. Rooting of shoots was achieved on the shoot regeneration medium itself.

**Key words**: Jewel orchid, multiple shoots, micropropagation, plant regeneration, ornamental plant

**INTRODUCTION**

*Anoectochilus* is an important tropical terrestrial orchid distributed throughout Southeast Asia and it is popularly called as “Jewel orchid” because of its beautiful foliage. Many species of *Anoectochilus* are used in Chinese folk medicine for many years in the treatment of hypertension, diabetes, heart, lung and liver diseases (Mak et al. 1990). *A. setaceus* is one such species which was once widely distributed in rain forests Vietnam and now it is considered as rare and threatened plant in Vietnam and listed in the Red data book because of overexploitation and habitat destruction (Anonymous, 2007). Propagation of this plant is by seeds; however, germination rate is very low because of mychorrhizal requirement. Mircorpropagtion is the suitable means for such rare and threatened plants and therefore, in the present study we attempted *in vitro* propagation of *A. setaceus* using shoot tip explants and developed a simple, rapid and efficient protocol.

**MATERIALS AND METHODS**

Plants of *Anoectochilus setaceus* Blume were collected from Tam Dao National Park, Vinh Prhuc Province, Vietnam. Shoot tips (1 – 2 mm in length) were disinfected with 70% ethanol for 10 s followed by surface sterilization with 2% sodium hypochlorite for 10 min, then washed thoroughly in sterile water and were cultured on Murashige and Skoog medium (1962, MS) supplemented with different concentrations of BAP (0.1, 0.3, 0.6, 1.0, 1.5 and 2.0 mg/l) kinetin (0.1, 0.3, 0.6, 1.0, 1.5 and 2.0 mg/l), sucrose (0, 1, 2, 3, 5, and 7%). The pH of the medium was adjusted to 5.8 before sterilization. All media used in the present experiment were solidified with 0.7% agar and were autoclaved at 121 oC for 20 min. Explants were cultured in 250 ml bottles containing 80 ml medium. All cultures were incubated 25 oC for 16-h photoperiod provided by cool white fluorescent lamps with a photon flux density of 40 µmol m-2 S-1. Developing shoots (1 - 2 cm in length) were separated and sub-cultured onto solid MS medium for further growth and rooting. Six explants were cultured per treatments and each experiment was repeated at least once. Observations on number of shoots, shoot length were recorded. Data were subjected to Duncan’s multiple range test.

**RESULTS AND DISCUSSION**

Shoot tip which were cultured on MS medium supplemented with cytokinins involved in swelling within two weeks and developed adventitious shoots in another two weeks of culture. Optimal regeneration was achieved on medium supplemented with 0.6 mg/l BAP (Table 1; Fig. 1a) and an average of 5.4 shoots per explant was developed on this medium. Similarly BAP has been widely used for micropropagation of several orchid species (Sheelavnathmath et al. 2005; Murthy and Pyati 2001). Different sucrose levels (0, 1, 2, 3, 5 and 7%) were used tested on shoot regeneration and results showed that 2% sucrose was best in induction shoots. 5-8 shoots were regenerated on medium supplemented with 2% sucrose and this concentration is also responsible for shoot elongation (average shoot length was 6.6 cm; Fig. 1b), however, higher concentrations of sucrose was not beneficial on regeneration and growth of the shoots. These results are in agreement with reported results that growth and development increased with increase in sugar concentration until optimum and then decreased at very high concentration (Ket et al. 2004).

The shoots which developed on MS medium supplemented with different concentration of sucrose (0, 1, 2, 3, 5, and 7%) were sub-cultured on to the same media where they have come from. The shoots involved in root regeneration at the base of shoots within four weeks of culture (Fig. 1C). In conclusion, a propagation method was developed in this investigation for the threatened orchid *A. setaceus*. Multiple adventitious shoots were induced from shoot tip explants on MS medium supplemented with BAP or Kn and rooting of shoot was achieved on the same medium after one subculture. This simple and efficient procedure could be used for large scale propagation and *ex situ* conservation of this orchid species

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**Legend to Figures**

Fig. 1. Adventitious shoots developed from shoot tip of *Anoectochilus setaceus* on MS medium supplemented with 0.6 mg/l BAP after 4 weeks of culture (1a) and after 8 weeks of culture (Fig. 1b). Growth of *A. setaceus* plantlets on MS medium with different sucrose concentrations after 8 weeks of culture (1c).

Table 1. Effect of BAP and Kinetin supplemented to full strength of MS medium on *in vitro* shoot proliferation of *Anoectochilus setaceus* shoots after eight weeks of culturea.

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Cytokinin Concentration Number of shoots/explant Length of shoots

(mg/l) (cm)

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Control 0 1.0 ± 0.2f 2.0 ± 0.3e

BAP 0.1 1.7 ± 02d 3.5 ± 0.3d

0.3 3.3 ± 0.3b 4.9 ± 0.3c

0.6 5.4 ± 0.2a 6.6 ± 0.3a

1.0 3.6 ± 0.2b 6.6 ± 0.5a

1.5 3.3 ± 0.2b 6.3 ± 0.4b

2.0 2.8 ± 0.3c 6.4 ± 0.4a

Kn 0.1 1.7 ± 0.3d 2.5 ± 0.3e

0.3 2.1 ± 0.1d 3.5 ± 0.3d

0.6 2.5 ± 0.2c 4.3 ±0.5c

1.0 3.3 ± 0.2b 6.1 ± 0.3 b

1.5 3.2 ± 0.2b 6.3 ± 0.2 b

2.0 3.3 ± 0.2 b 5.5 ± 0.4 c

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aMean values are with SE and different letters within the columns are significantly different according Duncan’s multiple range test at 5% level.

TO:

The Editor

Propagation of Ornamental Plants

Sub: Submission of revised manuscript entitled “*IN VITRO* PROPAGATION OF THREATENED TERRESRTIAL ORCHID *ANOECTOCHILUS SETACEUS* BLUME” for publication in Propagation of Ornamental Plants.

Dear Sir,

I am submitting herewith revised manuscript entitled *IN VITRO* PROPAGATION OF THREATENED TERRESRTIAL ORCHID *ANOECTOCHILUS SETACEUS* BLUME” for publication in Propagation of Ornamental Plants. We have revised the manuscript based on comments of reviewers as well as editor and incorporated all the suggestions made by them. Following are the specific corrections made in the revised manuscript.

1. Standard error values are given in the table/s.
2. We have used shoot tip explants for regeneration of *A. setaceus* and this information is written in the materials and methods section.
3. Adventitious shoots were regenerated from shoot tip explants of *A. setaceus* and this information is given in results and discussion section.
4. We have used six explants per treatment and experiment was repeated at least once and this information is written in materials and methods section.
5. The results of acclimatization have been removed as per the suggestion of editor.
6. Family names of authors have been given in small letters in the reference section.
7. The entire manuscript has been revised and reduced to six pages including text, table and figure (excluding author information first page).

I request you to accept the manuscript for evaluation and acknowledge the receipt ofthe same.

Thanking you,

Yours sincerely, 30th October 2013

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