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IN VITRO CONSERVATION OF *ORCHIS LATIFOLIA* : A THREATENED, MEDICINAL TERRESTRIAL ORCHID

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In vitro shoot multiplication of *Orchis latifolia*, a threatened medicinal orchid has been achieved on MS medium supplemented with BAP (1.0 mg/l) and NAA (0.1 mg/l) using sprouting buds as explants. Occasionally "protocorm" like structures were also produced on the same medium. The *in vitro* raised shoots could be successfully conserved for more than 10 months at 10°C without intermittent subculture.

Key words : Salam Panja, *Orchis latifolia*, orchid, *in vitro* multiplication, *in vitro* conservation

INTRODUCTION

Orchis latifolia L. (Orchidaceae), commonly known as "Salam panja" is a terrestrial orchid which yields "Salep" of commerce. It grows wild as herb in damp places in the Himalayas from Kashmir to Nepal at an altitude of 2,500-5,000 m. The tuberous roots of the plant are used as aphrodisiac and are extensively used in Ayurvedic and Unani Systems as nervine tonic (Anonymous, 1966). An infusion of the tubers is used to relieve hoarseness. Tubers are also used medicinally as astringent, expectorant and for bone fracture (Jain 1991). The tubers contain a bitter principle and a volatile oil and the leaves contain a glucoside, loroglossin.

The plant is facing extinction due to over-exploitation and lack of cultivation efforts (Hussain, 1982; Gupta, 1993). The loss of valuable germplasm may be mitigated by conservation of this species through *in vitro* methods. To our knowledge, there are no reports on micropropagation of this threatened species. The present paper is the first report on *in vitro* multiplication and short-term conservation of *O. latifolia*.

MATERIAL AND METHODS

Source and preparation of explants

Plants of *O. latifolia* collected from Lahaul Spiti region during 1992 were planted in soil to allow sprouting of buds. Rhizome pieces with sprouting buds were washed in 'Tween 80' or 15 min. at slow speed on a magnetic stirrer and later washed thoroughly under running tap water for 2 h and subsequently surface disinfected with 0.1% mercuric chloride (BDH) for 10, 15 or 20 min. and rinsed several times with sterile distilled water. The outer leaves were removed aseptically and explants were implanted vertically on nutrient medium.

In vitro multiplication

The basal medium comprised of the mineral salts and organic nutrients of Murashige and Skoog (1962) (hereafterward referred to as MS), 3% sucrose and 0.8% bacteriological agar (Hi-Media).

Different concentrations of 6-benzyladenine (BA) singly and in combination with α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) were added for initiation and further multiplication of cultures.

All the adjuvants were added to molten agar and the pH of the medium was adjusted to 5.8 before autoclaving at 106 kPa (121°C) for 15 min. in culture tubes (150 × 25 mm, rimless) closed with polypropylene caps. The cultures were maintained at 25° ± 3°C under 10 h photoperiod provided by cool white fluorescent tubes. Each treatment was replicated six times and all experiments were repeated twice.

Regenerated shoots were subcultured after 4-6 weeks. At each subculture, The individual shoots were separated and transferred to fresh medium.

Low temperature storage

Shoot cultures were transferred to either culture tubes or wide mouth culture vessel (Bioplast) containing 15 and 40 ml of culture medium, respectively. The tubes were closed with either cotton plugs or polypropylene caps. All the cultures were kept at culture room conditions for two weeks to detect and eliminate contamination. For cold storage the cultures were incubated at 10°C and monitored periodically.

RESULTS AND DISCUSSION

Sprouted buds and basal part of rhizome with bud obtained from *ex situ* were cultured on MS medium supplemented with 0.2-2.0 mg/1 BA alone

or in combination with either NAA, IAA or IBA (0.1-0.5 mg/1) to initiate the cultures. Responses of the explants to selected treatments are described briefly. Disinfection of explant with 0.1% mercuric chloride for 10 min. was sufficient to establish aseptic cultures. At low concentrations of BA (< 0.5 mg/1) no growth of explant was observed. In all other combinations of growth regulators tested, the explants remained green and leaves started expanding after two weeks from culture. Single bud sprouting was observed in most of the combinations tested after 4 weeks. Multiple shoot formation was observed only on MS medium supplemented with BA (1.0 mg/l) and NAA (0.1 mg/1). Higher concentrations of growth regulators lead to basal callus proliferation, hence its use was avoided in further experiments.

Data obtained after 4 and 8 weeks of culture revealed that the same medium i.e. MS + 1.0 mg/l BA + 0.1 mg/1 NAA gave an optimal response with regard to overall shoot development as assessed in terms of number of shoots/culture and shoot growth (Fig. 1A, B). Shoot emergence was observed between 2 - 3 weeks and 50% explants produced multiple shoots. On an average 3-4 shoots/culture were formed after 2 - 2.5 months and medium started browning. However, if on the same medium the number of shoots increased upto 10 (Fig. 1B). Inconsistent rooting was observed on the same medium after 10 weeks. Occasionally, small white "protocom like" structures were also observed on the same medium, which produced shoots on fresh medium of the same composition. This medium has supported the persistence of the regeneration for more than 3 years so far.

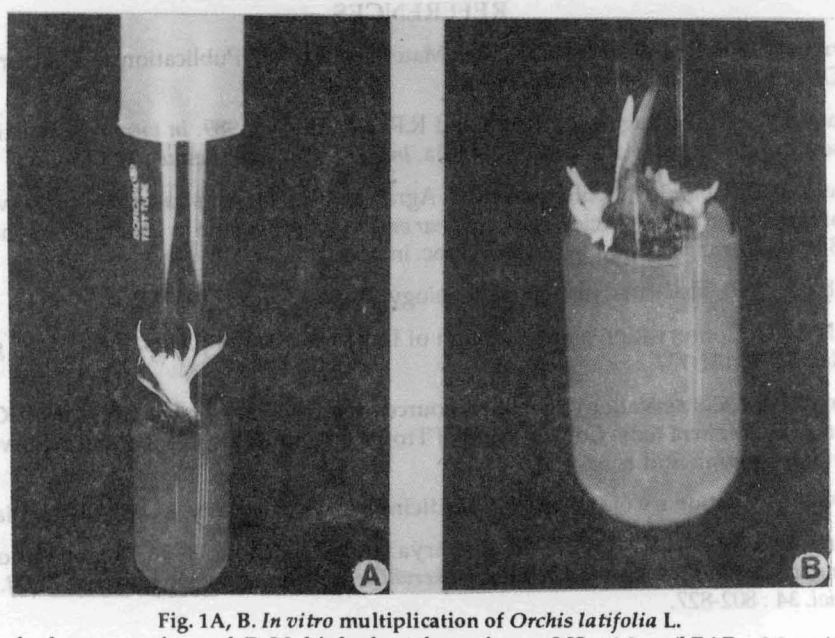

Fig. 1A, B. *In vitro* multiplication of *Orchis latifolia* L.
A. Single shoot sprouting and B. Multiple shoot formation on MS + 1.0 mg/l BAP + 0.1 mg/1 NAA after eight weeks

A preliminary study was undertaken on storage of available *Orchis* germplasm. The routine subculture period is 4-6 weeks as the medium turns brown the cultures can be maintained in viable condition at 25°C for 12 weeks with cotton plug as enclosure and for 20-22 weeks with polypropylene caps as enclosures. Maintenance of shoot cultures at low temperature (10°C) improved the survival and reduced the growth rate. The cultures could be successfully conserved for over 10 months.

Tissue culture studies are being increasingly exploited for *in vitro* multiplication and conservation of threatened plants of potential medicinal value including orchids (Chandel *et al.*, 1995; Constable, 1990; Malemnganba *et al.*, 1996; Sagawa and Kunisaki, 1984). Expectations are high about tissue culture methods providing sound strategy for conservation specially for the species in which roots or rhizome contain the active compound. In the present system, adventitious shoots regenerated without callus formation. Short-term conservation of shoot cultures for over 10 months was also achieved. The results compare favourably well with earlier published reports for medicinally important threatened plant species (Bhojwani *et al.*, 1989, Sharma and Chandel, 1992, Sharma *et al.*, 1995).

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REFERENCES

Anonymous. 1966. *The Wealth of India*, Raw Materials, Vol. VII, Publication and Information Directorate, CSIR, New Delhi, India.

Bhojwani S.S., N. Arumugam, R. Arora and R.P. Upadhyay. 1989. *In vitro* conservation of some endangered plant species of India. *Indian J. Pl. Genet. Resour.* **2** : 103-113.

Chandel K.P.S., R. Pandey, N. Sharma and A. Agrawal. 1995. *In vitro* strategy for conservation of threatened plant species. *Natl. Seminar on Changing Scenario in Plant Sciences*, held at B.H.U., Varanasi, Oct. 13-15, 1995. (Proc. in press).

Constable F. 1990. Medicinal plant biotechnology. *Planta Med.* **56** : 421-425.

Gupta R. 1993. Conservation and utilization of Indian medicinal plants. *Indian J. Pl. genet. Resour.* **6** : 131-137.

Hussain A. 1982. Conservation of genetic resources of medicinal plants in India. *In* : S.K. Jain and K.L. Mehrra (eds) Conservation of Tropical Plant Resources, Botanical Survey of India, Howrah, India.

Jain. S.K. 1991. Dictionary of Indian Fold Medicine and Ethnobotany. Deep Publ., India.

Malemnganba H., B.K. Ray, S. Bhattachrya and P.C. Deka. 1996. Regeneration of encapsulated protocorms of *Phaius tankervilliae* stored at low temperature. *Ind. J. Exp. Biol.* **34** : 802-827.

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

Sagawa Y. and J.T. Kunisaki. 1984. Clonal propagation : Orchids. *In* : I.K. Vasil (Ed.) *Cell Culture and Somatic Cell Genetics of Plants*, Vol I, p. 61-67, Academic Press, New York.

Sharma N. and Chandel K.P.S. 1992. Low temperature storage of *Rauvolfia serpentina* benth. ex Kurz - An endangered, endemic medicinal plant. *Plant Cell Rep.* **11** : 200-203.

Sharma N., Chandel K.P.S. and Paul A.K. 1995. *In vitro* conservation of threatened plants of medicinal importance. *Indian J. Pl. Genet. Resour.* **8** : 107-112.