SHORT COMMUNICATION

In Vitro Conservation of *Gentiana kurroo* Royle: An Indigenous Threatened Medicinal Plant

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In vitro conservation of shoot cultures of *Gentiana kurroo* Royle, a threatened medicinal plant, was achieved. On a standard shoot culture medium, cultures could be successfully maintained for seven months at 25°C by replacing cotton plugs with polypropylene caps as enclosures for culture tubes. Low temperature storage at 4°C, successfully extended shelf life of cultures for over 30 months. The cultures grew normally on multiplication medium.

Key Words: *Gentiana kurroo*, *In Vitro* Conservation, Low Temperature Storage, Threatened Plant

Conservation of biodiversity of medicinal plants is a shared commitment at global as well as regional level. A holistic approach to conservation is to amalgamate both *in situ* and *ex situ* methods and new emerging technologies to ensure sustained utilization. *Gentiana kurroo* Royle (Indian Gentian), commonly known as 'Karu' or 'Kutki' has been used in the indigenous system of medicine for a long time (Kirtikar and Basu, 1975, Chandel *et al.*, 1996). Dried rhizomes are not only used as a substitute for true gentian but also for treating syphilis and leucoderma.

Though a perennial herb, plants are still gathered from the natural stands by uprooting its roots and rhizome and have thus become threatened due to over exploitation (Gupta, 1988). Realizing the threat of extinction, coupled with limited documented information regarding mode of propagation and storage potential of propagules, storage of germplasm in the form of *in vitro* cultures appears to be the most promising option to ensure safe conservation of endangered plants especially those, such as *Gentiana,* in which the roots or rhizome contain the active compound.

Tissue culture techniques are invaluable to complement other conservation strategies particularly for vegetatively propagated and threatened medicinal plants. The major advantages of *in vitro* techniques are rapid multiplication and storage of relatively large number of propagules in small space, away from natural vagaries. *In vitro* multiplication of *G. kurroo* (Sharma *et al.*, 1993) and four other *Gentiana* species, namely *G. lutea, G. cruciata, G. purpurea* and *G. acaulis* (Momcilovic *et al.*, 1997) have been reported. The present paper demonstrates the feasibility of successful storage of shoot cultures of *G. kurroo* at low temperature.

Cultures of plant material obtained from All India Co-ordinated Research Project on Medicinal and Aromatic Plants Centre located at Solan, H.P., India, were initiated and multiplied using the procedure of Sharma *et al.*, (1993). Shoots were proliferated on Murashige and Skoog's (1962) medium (MS) containing 8.9 μm 6-benzyladenine (BA) and 1.1 μM 1-naphthaleneacetic acid (NAA) and 100 mg/l streptomycin. After 8 subcultures the proliferated shoots provided sufficient material for storage experiments.

Single node explants were transferred to glass tubes (25x150 mm) containing 15 ml of culture medium solidified with 0.8% agar. The tubes were closed with either cotton plugs or polypropylene caps. All the cultures were incubated at culture room conditions (Sharma *et al.*, 1993) for 2 weeks to detect and eliminate contamination. Healthy cultures were then transferred to cold module at 4° C in dark. Twenty-four replicates were used and the experiment was replicated twice. Survival of cultures was assessed by visual examination and by the ability of explant to resume growth on fresh medium.

Nodal cultures on multiplication medium produced 3-5 shoots/culture in 6 weeks from culture. High mortality was observed in cultures maintained at 25° C and covered with cotton plugs after 6 months mainly due to dessication and nutrient depletion. In contrast, cultures covered with polypropylene caps remained green and viable (100%) till 9 months. Thus, polypropylene caps were better than cotton plugs for storage of cultures. A similar response has been earlier reported for another threatened medicinally important taxa, *Rauvolfia serpentina* (Sharma and Chandel, 1992).

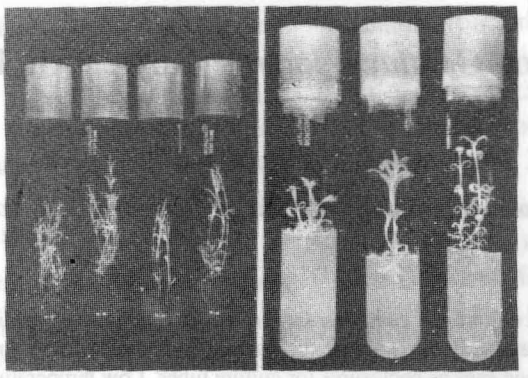

Fig. 1. In vitro *conservation of* Gentiana kurroo *(1A) Shoot cultures in vitro conserved for 30 months at 4° C; (1B) Revival of growth after 30 months of storage. Four-week-old cultures with multiple shoots*

Low temperature incubation was effective in reducing growth rate and enhancing subculture interval. Cultures remained healthy and continued to grow in dark, though slowly. Interestingly, when the shoots started senescing, new axillary sprouts were observed even after 20 months of storage. More than 50% cultures were viable and green up to 24 months. At the termination of experiment, over 40% cultures were green and viable, but only 33% were healthy after 30 months (Fig.1A). Even after this period, an average of six explants could be obtained from each surviving culture and all explants grew. Death of cultures at 4°C was probably due to depletion of nutrients and partly due to contamination. Cultures transferred to fresh medium for rejuvenation and maintained under standard growth conditions resumed normal growth and continued to produce multiple shoots (Fig. 1B).

The shoot cultures of this species have been maintained for 7 years through periodic subculturing at 25° C and 4°C, so far. The results obtained in the present investigations compare well with earlier published reports on medicinally important threatened plant species (Arora and Bhojwani 1989, Sharma and Chandel, 1992, 1996).

In vitro techniques are now successfully and routinely applied to a range of threatened species for multiplication (Chandel *et al.,* 1996). There is paucity of information regarding application of *in vitro* techniques for conservation and optimal methods still need to be developed for threatened medicinal plants which have received little scientific attention.

The present work demonstrates the feasibility of conserving threatened germplasm of *Gentiana* using *in vitro* techniques. Both short- and medium-term conservation has been achieved by incubating the cultures under normal/low temperature.

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