Plant Cell Reports (1992) 11:200–203

Plant Cell Reports

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Low-temperature storage of *Rauvolfia serpentina* Benth. ex Kurz.: An endangered, endemic medicinal plant

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Received August 20, 1991/Revised version received January 28, 1992 – Communicated by G. C. Phillips

ABSTRACT

On a standard shoot culture medium, nodal cultures of Sarpagandha (*Rauvolfia serpentina*) could be maintained for nine months at 25° C by replacing cotton plugs with polypropylene caps as enclosures for culture tubes. Low temperature incubation of *in vitro* cultures appeared highly promising because cultures exhibited normal health even after 15 months of storage at 15° C; while 10°C and 5°C were found deleterious to growth of the cultures of *R. serpentina*.

INTRODUCTION

Rauvolfia serpentina Benth. ex Kurz., commonly known as Sarpagandha (Apocyanaceae), has been a valuable source of the alkaloid reserpine. It grows as an undershrub in the sub- himalayan tract, Karnataka, Goa and Eastern and Western Ghats up to an altitude of 1200 m. Roots contain 0.15% reserpine- rescinnamine group of alkaloids (Anonymous 1969). Dried roots of 2-3 year old plants are widely used in the Ayurvedic and Unani systems of medicines in India and in South Asian region for treatment of cardiovascular diseases, hypertension and as a sedative or tranquilizer in allopathy.

Rauvolfia is threatened with extinction in India due to indiscriminate collection and over exploitation of natural resources for commercial purposes to meet the requirements of the pharmaceutical industry, coupled with limited cultivation (Nayar and Sastry 1987 ; Gupta 1989) . In view of this, there is an urgent need to apply *in vitro* culture methods for the micropropagation and conservation of this valuable threatened plant species.

While germplasm conservation of orthodox seed species is quite feasible in gene banks using low temperature (-20° C), species with poor seed viability, such as *R. serpentina*, are seriously constrained (Anonymous 1969). Field maintenance is not only expensive but also risky as serious losses can occur with time. For endangered plant species, tissue culture offers an alternative method for conserving germplasm because it serves the dual purpose of storing a relatively large number of propagules in a small space under artifical conditions, and when the need arises, for their rapid multiplication. Tissue culture also facilitates the exchange of germplasm within and across countries. The present paper reports the successful storage of nodal cultures of *R. serpentina* at reduced temperature.

MATERIALS AND METHODS

Establishment of shoot cultures

Cultures were initiated and multiplied from nodal cuttings (1.0- 1.5 cm long) taken from field grown plants using the procedure of Mathur *et al.* (1987). The shoots were proliferated on standard Murashige and Skoog (1962) medium supplemented with 1.0 $mg.l^{-1}$ 6- benzylaminopurine (BAP) and 0.1 $mg.l^{-1}$ 1-naphthaleneacetic acid (NAA). After 5-6 subcultures the proliferated shoots provided sufficient material for storage experiments.

Low temperature storage

Two strains of *Rauvolfia* (Delhi local and Indore local) were used to test the effect of reduced temperature. Single node explants with two axillary

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buds (nodal culture) were transferred to glass culture tubes (25 x 150 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 kept at culture room conditions (25 ± 3° C, 16 h photoperiod, 2800 - 3000 lux) for one week to detect and eliminate contamination. The cultures were then transferred to incubators maintained at 5° C, 10° C and 15° C with 16 h photoperiod (1400-1600 lux). Seventy two replicates were used for each treatment and the experiments were repeated twice. One set of cultures maintained at 25° C served as controls.

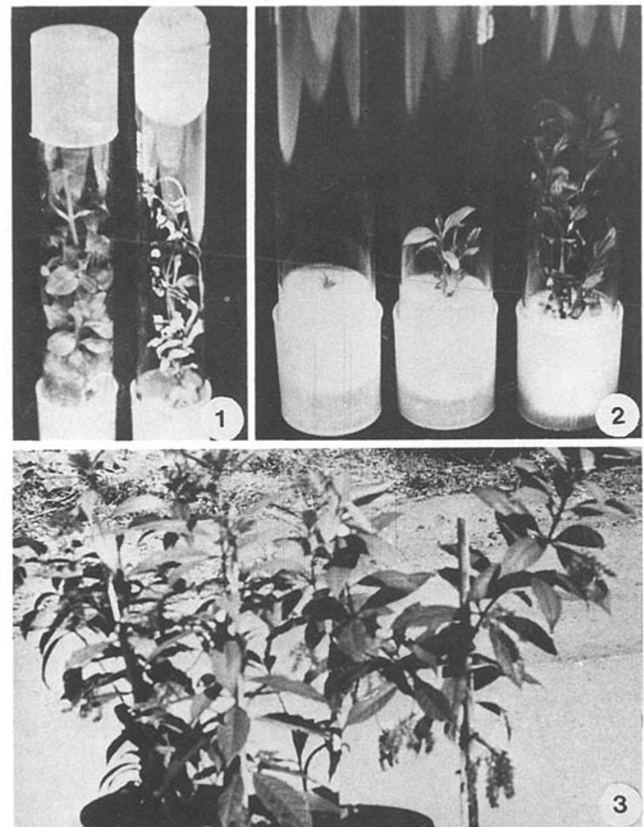
In the second set of experiments, the effect of basal medium and half - strength medium on storage of cultures covered with plastic caps at 25 ± 3^oC with 16 h photoperiod (2800 - 3000 lux) was tested. Twenty - four replicates were used for each treatment and the experiments were repeated twice.

Regrowth and establishment of plants

At three month intervals, six cultures from each treatment were transferred to fresh medium under standard culture room conditions. Survival of *in vitro* cultures was assessed by the ability of explants to resume growth on fresh medium.

Proliferated shoots were rooted upon transfer to NAA (1.5 $mg.l^{-1}$) medium.

Six to eight week old regenerated plantlets with well developed roots were washed free of agar, dipped in 0.2% Bavistin fungicide for 5-10 min, and potted in small plastic pots containing sterilized soilrite. The plantlets were covered with polythene bags to maintain high humidity. These were acclimatised at 25 ± 3^oC under 16 h photoperiod (2800-3000 lux) and watered regularly. After 3-4 weeks, the polythene bags were removed and established plantlets were transplanted to earthen pots in a greenhouse.



Figs 1-3. *In vitro* conservation of *R. serpentina* cultures.

Fig. 1. After three months of storage with cotton plugs (right) and six months of storage with polypropylene caps (left)

Fig. 2. Stages of *in vitro* multiplication during rejuvenation. Cultures after 1 wk, 2 wk and 4 wk of growth (L to R)

Fig. 3. *In vitro* generated plants established in soil

RESULTS AND DISCUSSION

Nodal cultures of *R. serpentina* were exposed to series of controlled temperature regimes of 5^oC, 10° C, 15° C and 25° C. Cultures maintained at 25° C and covered with cotton plugs suffered high mortality. Within six months the cultures with cotton plugs browned and died (Fig. 1), with survival being 16.6 % at five months. In comparison, those covered with polypropylene caps remained healthy for a longer period. After six months the cultures at 25^oC with plastic caps started yellowing and very few were alive after nine months. The survival was 66.6% at six months. Thus, the plastic caps were better than cotton plugs for storage of cultures. The death of the cultures covered with cotton plugs was mainly due to desiccation and nutrient depletion. The subculture period could be prolonged by changing the enclosure to polypropylene caps. A similar response was earlier reported in ginger and turmeric from this laboratory (Balachandran et al. 1989).

Table1. Percent survival of nodal cultures of *R. serpentina* covered with plastic caps following storage at different temperatures

(Six cultures were sampled at each time period and grown in standard conditions for four weeks)

Period	Growth temperatures			
(Months)	5°C	10°C	15°C	25°C
3	0	0	100.0	100.0
6	0	0	100.0	66.6
9	--	--	100.0	33.3
12	--	--	83.3	16.6
15	--	--	66.6	0.0

-- = Not tested

At 5° C and 10° C all cultures died within three months, without showing any further growth. Maintenance of *in vitro* cultures at 15° C improved survival considerably (almost three times after nine months) and depressed the growth rate. Cultures stored at 15°C exhibited excellent health even after 15 months of storage.

At regular intervals, six cultures were randomly withdrawn from different conditions and subcultured on fresh medium to assess their survival (Table 1). Cultures rejuvenated after nine months of cold storage with caps at 15°C, showed 100% survival. After this period some decline in the survival was observed. After 15 months only the cultures maintained at 15°C were viable, survival being about 70%. Decline in survival rate was due to exhaustion of medium and possibly due to latent infection as has been observed by Wanas et al. (1985). The results obtained in the present investigations compare favourably well with earlier published reports for medicinally important endangered plant species (Arora and Bhojwani 1989; Bhojwani et al. 1989).

Cultures transferred from all treatments to standard growth conditions resumed normal growth (Fig. 2). The viable cultures showed shoot multiplication comparable to the cultures maintained under culture room conditions (25 ± 3° C). Rooting was readily achieved upon transferring shoots to rooting medium. The plantlets thus formed were transferred to soil without any difficulty (Fig. 3). No morphological differences were observed in these plants.

In the second set of experiments, half - strength medium and full - strength medium without hormones were used to test their effect on survival of cultures at 25°C. None of these were effective in improving the shelf life of cultures in comparison to low temperature storage, with survival rates of only 33 - 50% after six months of storage.

In vitro techniques play a very important and crucial role in the conservation of plant germplasm (Kartha 1985; Withers and Williams 1986).In many cases a

simple reduction in temperature has been adequate to reduce growth rate and increase shelf life (Withers 1980; Wanas *et al.* 1985; El- Gizawy and Ford-Lloyd 1987).In the present system *in vitro* conservation using nodal cultures at reduced temperature has an additional advantage to earlier findings (Chaturvedi and Sharma 1986) in that plants can be seen to be alive and any loss of viability can be spotted easily.

The simple outlined procedure for *in vitro* conservation for *Rauvolfia* at 15°C offers a potential alternative system for conserving endangered medicinal plants, especially those in which the roots or rhizomes contain the active compound.

ACKNOWLEDGEMENT

We thank Dr. R.S. Rana, Director, National Bureau of Plant Genetic Resources for the necessary facilities. We also thank Mr. D.K. Nerwal for technical assistance and Ms. Latha Devdas for help in typesetting of manuscript. Financial support provided by the Department of Biotechnology, Ministry of Science & Technology, New Delhi is gratefully acknowledged.

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