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Research note

# Storage and plant regeneration from encapsulated shoot tips of *Rauvolfia serpentina* — An effective way of conservation and mass propagation

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**Abstract**

Encapsulated shoot tips of *Rauvolfia serpentina* were stored at 3 different temperatures (20 °C, 12 °C and 4 °C). Synseeds converted randomly after 4 weeks of storage at 12 °C and 20 °C. However, they demonstrated the most promising result at 4 °C where storage achieved up to 14 weeks with high percentage of regrowth throughout the period (68.5–100%). Extending the storage period beyond 14 weeks resulted in drastic loss of viability of the explants. The synseed-derived plantlets after root induction hardened, acclimatized and survived with 80% success. This study elucidates an effective *in vitro* conservation technique that can also be implemented for easy propagation and exchange of the plant species.


*Keywords:* Encapsulated shoot tips; *In vitro* conservation; *Rauvolfia serpentina*; Regrowth; Synseed

## 1. Introduction

*Rauvolfia serpentina* is among the high priority medicinal plants of India. It has been widely utilized by ayurvedic system for centuries and its usage in allopathic medicine is only for a few decades primarily in hypertension, insomnia, anxiety and other disorders of central epilepsy (Ghani, 1998). The root of the plant is a store house of therapeutically active alkaloids, e.g. reserpine, serpentine, ajmaline, ajmalicine, yohimbine etc. As a result of high commercial exploitation for a long time, the natural reserve of this plant has been dwindling alarmingly. As a consequence, it is included in the endangered category as well as in CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) list. Coupled with this, poor seed germination rate of the plant limits its natural propagation as well as cultivation and thereby magnifies the problem further.

Application of *in vitro* strategies in conservation and management of important plant species has been given priority when genetic resources are getting depleted rapidly from natural flora. *In vitro* conservation through the encapsulation of embryogenic and non-embryogenic plant parts i.e. synseeds provides a cost-effective and simplified option for germplasm conservation (Redenbaugh, 1990; Dodds, 1991; Danso and Ford-Lloyd, 2003). Besides, it facilitates distribution and easy exchange of the elite plant germplasm (Rao et al., 1998; Naik and Chand, 2006).

The synseed provides two fold advantages, the regenerable meristematic parts within the synseeds can be conserved for different time periods and simultaneously it secures ready availability of the germplasms for mass propagation. The present study emphasized on *in vitro* conservation of *R. serpentina* by encapsulating regenerated shoot buds. It primarily investigated optimization of storage condition (temperature) and subsequent regrowth of the synseeds. The study also included rooting of regenerated shoots and hardening of the resulting plantlets.

## 2. Materials and methods

*In vitro* culture of *R. serpentina* was established using apical bud excised from *in vivo* mature plant from Bose Institute experimental farm and maintained for 4 consecutive weeks at 25±2 °C and 16/8 h (light/dark) photoperiod (light intensity 30.76 μmol $m^{-2}$ $s^{-1}$) on MS medium (Murashige and Skoog, 1962) containing 3% sucrose and gelled with 0.8% agar. Axenic

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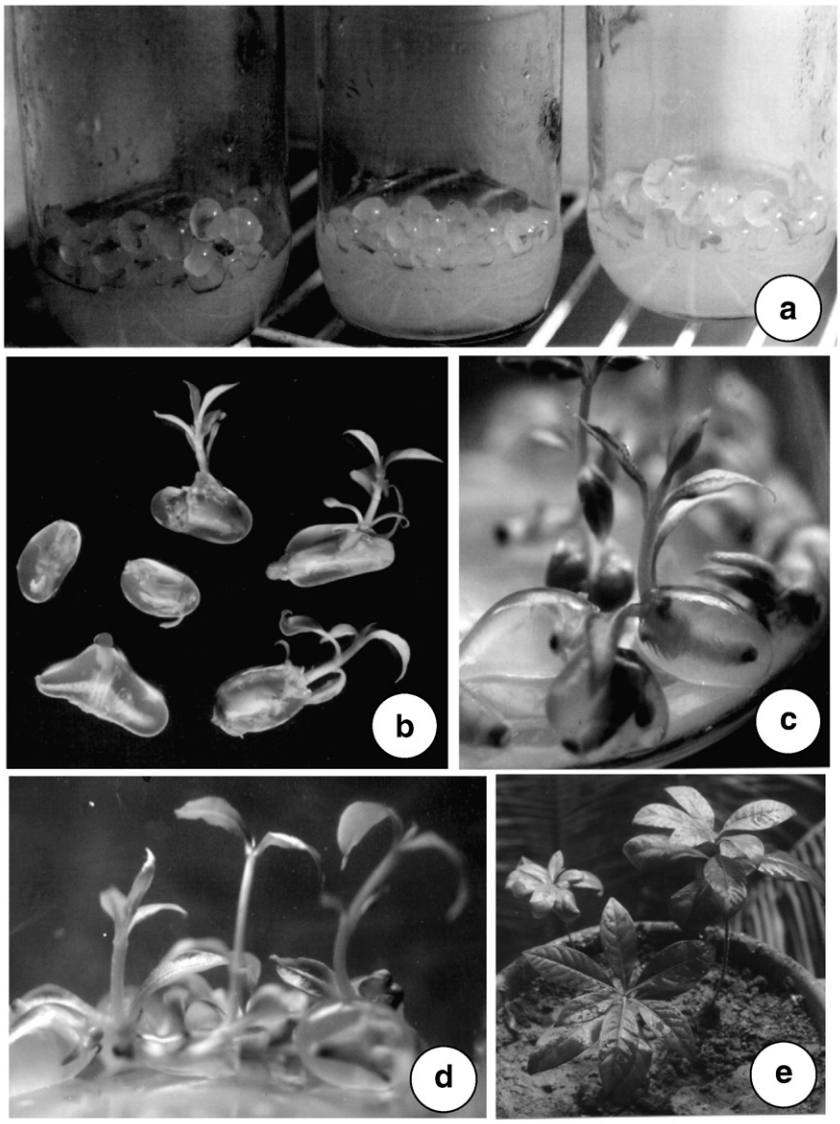


Fig. 1. (a) Storage of synseeds of *R. serpentina* at 4 °C; (b) different stages of germination of synseeds; (c) early phase of *in vitro* shoot formation; (d) growing plantlets derived from synseeds; (e) synseed-derived acclimatized plantlets.

shoot tips and nodal segments (3–5 mm) were dissected from *in vitro* raised *R. serpentina* plantlets and were used as explants for encapsulation.

Explants mixed with 3% sodium alginate were suspended drop wise in 100 mM $CaCl_2$ solution so that each drop contained single explant. They were kept for 45 min in a magnetic stirrer for complete polymerization of alginate. The beads, hence formed, were washed thrice with sterile distilled water, soaked in filter paper. Finally, encapsulated propagules were transferred to culture vessels containing MS with 3% sucrose without growth hormones and incubated under different temperatures (4 °C, 12 °C and 20 °C), under 1.5 μmol $m^{-2}$ $s^{-1}$ light intensity for various time span. The entire process of encapsulation was carried out in sterile conditions.

Synseeds were taken out to normal culture condition at an interval of 2 weeks for testing regrowth [25 °C and 16/8 h (light/dark) photoperiod (light intensity 30.76 μmol $m^{-2}$ $s^{-1}$) on MS with 3% sucrose]. Regrowth was expressed as percentage (%) of synseeds that had performed shoot formation by piercing the matrix wall. Shoots derived from synseeds were treated with auxins, IAA, IBA and NAA (0.5–2.0 mg/l) for root induction. Plantlets with well grown root and shoots maintained at culture before hardening.

Synseed-derived plantlets obtained from any of the experimental sets were transferred to *ex vitro* condition in small plastic pots containing soilrite: loamy soil and loamy soil stepwise depending on their steadiness. Finally, well grown plantlets were transplanted to soil in earthen pots, maintained in green house.

Experiments repeated at least thrice and each treatment consisted of 25 synseeds. Data represented as means ± standard errors (S.E.). All data were subjected to analysis of variance (ANOVA) and comparisons of means were made with least significant difference test at the 5% level of probability.

## 3. Results and discussion

On the basis of some preliminary findings on growth response of encapsulated nodal segments and shoot tips of *R. serpentina* in culture, only the latter were selected for further studies since encapsulated nodal segments of *R. serpentina* did not form shoots at all.

Synseeds resumed growth within 10–12 days after being taken out from storage condition (Fig. 1a) to *in vitro* culture condition. Fig. 2 shows the regrowth response of the synseeds at different temperature. After 2 weeks, synseeds exhibited 100% regrowth in all the sets and similar high regrowth percentage (92.7±3.81–100%) was also noted after 4 weeks of storage (Fig. 1b). No significant difference ($P$=0.05) was observed in regrowth frequencies among the sets during this period. However, the encapsulated shoot tips incubated at 12 °C and 20 °C were not conservable after 4 weeks due to random shoot formation during storage (Fig. 1c). Similar phenomenon during storage was earlier reported in *Jaccaranda mimosaefolia* (Maruyama et al., 1997) and in cassava (Danso and Ford-Lloyd, 2003) and it was attributed to the rapid resumption of metabolic activities in encapsulated explants, thereby restricting the storage potential of the synseeds. However, this enables the encapsulated shoot tips very attractive materials for easy germplasm exchange as growth can resume immediately after culture (Danso and Ford-Lloyd, 2003).

On the other hand, 4 °C induced enhanced storage potential of the synseeds up to 18 weeks, beyond this they failed to form shoots. Moderate (68.5±4.62%) to high (87.5±4.04%) regrowth was achieved during the period of 6 to 14 weeks of storage (Fig. 1d). But thereafter (after 14 weeks) a drastic reduction (14.5±3.18–39.8±2.77%) was noticed which was manifested through high rate of viability loss. The study clearly revealed that low temperature above freezing (around 4 °C) was suitable for storage and subsequent high regrowth of *R. serpentina* synthetic seeds and it was also supported by reports in other plants like pineapple (Soneji et al., 2002). The decline

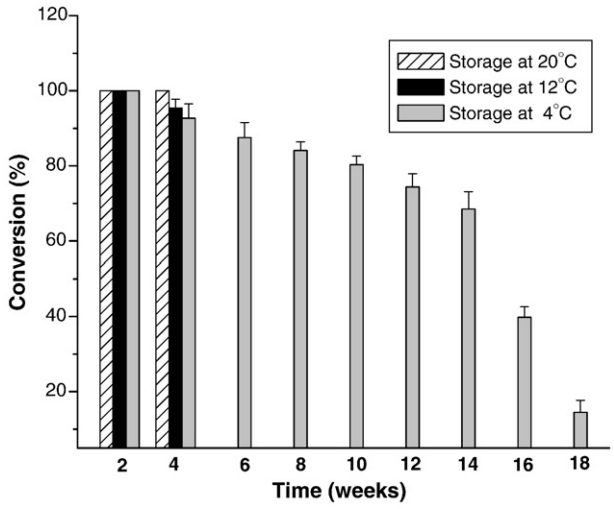


Fig. 2. Regrowth (%) of *R. serpentina* synseeds stored at different temperatures for various time periods.

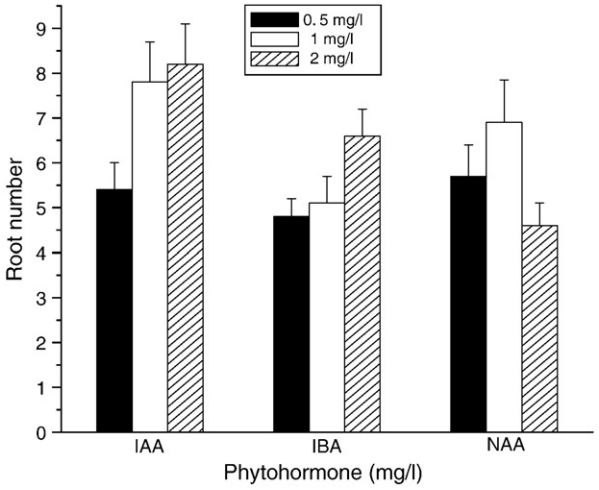


Fig. 3. Effect of different auxins on rooting (root number) of shoots derived from synseeds.

in regrowth frequency as a result of prolonged storage was also demonstrated by encapsulated nodal segments of *Dalbergia sissoo* (Chand and Singh, 2004). The decline in morphogenesis i.e. shoot forming capacity as a result of prolonged storage could be due to inhibited respiration of tissues or a loss of moisture due to partial desiccation (Danso and Ford-Lloyd, 2003).

Among three auxins, IAA (2 mg/l) produced roots at higher number (8.2±0.9) than IBA (6.6±0.6) and NAA (6.9±0.95) (significant at $P$=0.05) (Fig. 3). Rooting was associated with profuse callusing at the shoot base in the presence of NAA and shoot growth was inhibited.

The synseed-derived plantlets grew well in the plastic pots with the considerable increase in shoot and root length. Eight–ten week old plantlets were transferred from plastic pot to earthen pots in initially controlled condition and then finally in a glass house where they were observed to be growing well in the natural environment and survived successfully (Fig. 1e). Plant growth continued and almost 80% *ex vitro* survival was achieved. After one month of transferring plants to soil mixed with low quantity organic manure, the plants became stouter, showing normal growth, branching and flowering and looked morphologically similar with the donor plants.

The advent of synthetic seed technology has led the seed science to a new direction. Application of this technology has recently been made for plants ranging from herb to tree to solve problems arising out of storage, viability and germination of plants' natural germplasms represented by seeds or vegetative parts (viz., corm, bulb, rhizome etc.) functioning as seeds. The alginate coat-protected somatic embryos (Malabadi and Van Staden, 2005), shoot tips/nodal segments (Danso and Ford-Lloyd, 2003; Chand and Singh, 2004; Naik and Chand, 2006), axillary buds (Refouvelet et al., 1998), micro-plants/cuttings (Tsvetkov and Hausman, 2005), and roots having regeneration potential (Brischia et al., 2002) are all included under the category of synthetic or artificial seeds. As because somatic embryogenesis does not occur in all of the culturable plant species, encapsulation of *in vitro* derived tissues and organs, in particular use of shoot tips and nodal segments as beading

material has gained more popularity and ensured more widespread use of this technology in storage, exchange and mass propagation of plant species.

The present study describes a procedure for effective *in vitro* conservation of *R. serpentina* germplasm through encapsulation of shoot tips. Synseeds can be optimally storable up to 14 weeks with high regrowth frequency. Ready conversion of synseed may bypass inherent low seed germination problem. Therefore, the technique can open up the possibility of easy mass propagation of this important medicinal plant.

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