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# *In vitro* Germination of Ovules and Plant Regeneration for Cloning and Conservation of *Rauwolfia serpentina*

Devla [1], Md. Shahnawaz [2], Raageeva Bimal [2*]

1. Department of Chemistry, Delhi University, Delhi, India.
2. Biotech lab, University Department of Botany, B.R.A. Bihar university, Muzaffarpur-842001 (India), [E-mail: raageeva@gmail.com]

In an era of species extinction, conservation of biological diversity has assumed global importance. Consequently, biodiversity has become a hotly debated topic in the recent years. The Red Data Books of various regions exemplified the magnitude of vanishing genetic diversity of the biosphere and the rates of species extinction that may threaten mankind. *Rauwolfia serpentina*, a highly significant medicinal plant is one of the 20 endangered plants for which National Gene Bank at NBPGR, IARI, New Delhi has been standardizing protocol for *in vitro* conservation (Annual report 1997-1998, Department of Biotechnology, Government of India, New Delhi, p.39). In the present paper, a simple biotechnological protocol for plant regeneration from green fertilized ovary pieces cultured on MS basal medium supplemented with CW(10%) both in culture room at $25^{0C} \pm 3^{0}C$ and at room temperature has been reported which may be helpful in developing tissue culture nursery for supply of plantlets to herbal farmers. The tissue culture protocol may be used for conservation of *R. serpentina*.

*Keyword*: *In vitro*, Plant Regeneration, Cloning, Conservation, *Rauwolfia serpentina*.
Abbreviation: MS – Murashige & Skoog's medium; CW – coconut water.

## 1. Introduction

Plants are a major source of bioactive substances used as pharmaceuticals, agrichemicals, colour, flavor and fragrance ingredients, food additives etc[1,2,3,]. The use of medicinal plants will steadily increase in the coming years because of a shift in attitude of people towards 'natural drugs'. The preference for 'natural' implies an adverse impact on biodiversity and many important herbal drug yielding plants have become endangered. *R. serpentina* is one such plants which figures in the list of plants prepared by Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)[4,5,6] . The ecological plunder as well as difficulties associated with seed germination in this biotechnologically significant plant has led *R. serpentina* to figure in the Red Data Book.

*In vitro* biology and technology have been proposed for micropropagation of *R. serpentina*[7,8,9,10,11,12,13] as well as in *R. tetraphylla*[14,15,16,17] using shoot tip, leaf, nodal and root explants. In the present paper *in vitro* plant regeneration from cultured fertilized ovary pieces have been reported.

## 2. Material and Methods

A few plants of *R. serpentina* Benth. grows as a wild herb in the vicinity of my house at Muzaffarpur (Bihar), India. Fertilized red, green coloured ovary explants were collected washed (i) in running tap water (10 min) (ii) with detergent (5 min) and by detergent and surface sterilized by immersion in 0.1% mercuric chloride for 5 min followed by 2-3 washings in glass distilled water.

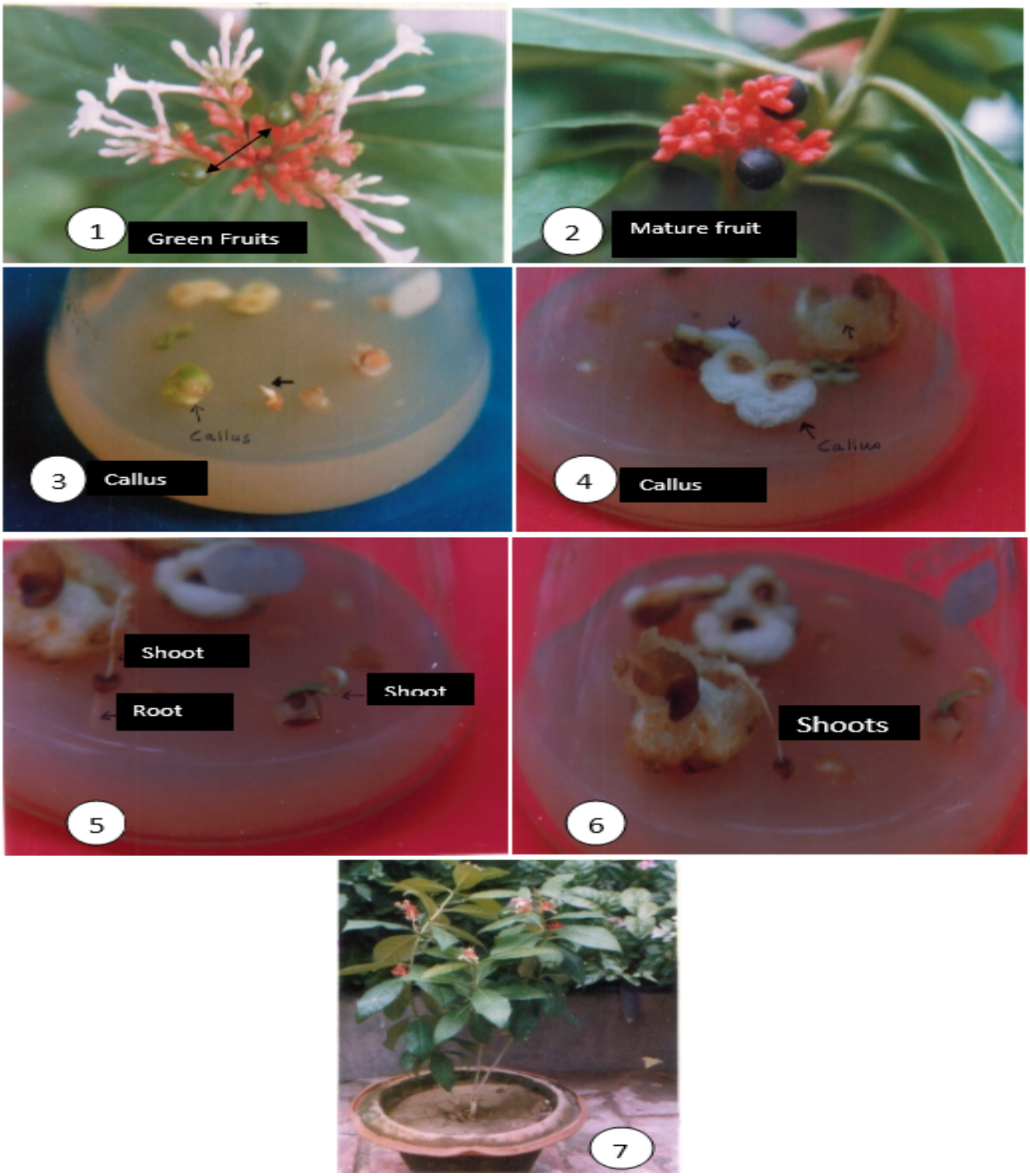


**Fig 1-7:** *In vitro* plant regeneration in *Rauwolfia serpentina*. Fig.1-2 - Photographs of R. serpentia showing flowers, green and black fruits. Fig. 3 – Photograph shows transversely cut green ovary pieces cultured on MS medium supplemented with CW (10% v/v) showing callus and a tiny shoot. Fig. 4 – Photograph shows white callus proliferation from cut ovary pieces cultured on MS+CW (10% v/v). Figs.5-6 – photographs showing callus proliferation and shoot as well as plant regeneration from cut ovary pieces cultured on MS+CW (10% v/v). Fig. 7 - *R. sepentina* plant growing in pot.

Each fertilized ovary was cut into 3-4 transverse pieces with the ovules attached to placenta. The cut ovary pieces were cultured on Murashige and Skoog's[18] (MS) powder medium (product purchased from High Media) supplemented with 5%, 10%, 15% CW (Coconut Water) or without CW. The cultures were incubated in the culture room at $25^{0C} \pm 3^{0}C$ under cool fluorescent light and 16 h photoperiod (3000 lux). The cultures were also kept at room temperature (months of April to May) under white fluorescent light (40 watt) and a table fan. The experiment was repeated 3-4 times to confirm the process of plant regeneration both in culture room and at room temperature.

## 3. Results and Discussion

Figures 1-2 show a fully grown plant showing flowers green fruits and black mature fruit of *R. serpentina* respectively. In about 4-6 days of culture, cut ovary pieces started callusing in the medium supplemented with CW (10% v/v) and a small white protuberance indicating emergence of shoot (Fig.3). Finally, a white fragile callus was produced in 12-15 days of culture (Fig.4). The shoot as well as plant regeneration have also been observed in 12-15 days of culture ( Figs. 5-6). MS basal medium as well as basal medium supplemented with CW (5%,15% v/v) did not have any morphogenetic effect on cut green ovary explants. No response was observed in cut ovary explants obtained from the mature, black fruits and cultured on MS medium without CW or with CW (5% , 10 % and 15%). Figure 7 shows a completely regenerated plant (18 months old) in pot.

The main problem associated with herbal farming of *R. serpentina* is that the seeds do not germinate even after one year of harvest and conventional promoters as well as physical, chemical or hormonal agents fail to reverse the inhibitory effect of seed coat[16,19]. The direct showing of seeds in the field has not been successful and hence seedlings are raised in nursery which too have low percentage of germination.

Mature seeds of many plants have been found to germinate in cultures but success in obtaining viable and germinable seeds from ovules excised immediately after fertilization has been limited[20,21,22]. Such studies are highly significant as ovule culture offers potentialities for the recovery of novel sexual hybrids. *R. serpentina* has been propagated through tissue culture using root, stem, leaf and shoot tip explants[23,24]. In the present experimental system the plants of *R. serpentina* have been regenerated from cut ovary pieces cultured on MS medium supplemented with CW ( 10% v/v ).

The significance of the work lies in the fact that the experiment was also completed strictly at room temperature using a table fan in the summer season while table fan was not used during the winter season and showing plant regeneration which is comparable to that in the culture room. The protocol mentioned above offers a very convenient and highly efficient method in terms of seed to plant regeneration, thereby solving the problem associated with seed germination and plant regeneration.

## 4. Conclusion

The present study describes a simple, well documented and reliable protocol for a high frequency seed to plant development using young green fertilized ovary pieces as explants for tissue culture.

## 5. Conflict of interests

Authors have declared that no competing interest exists.

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