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Ex situ conservation of endemic orchids of Western Ghats, Tamilnadu, India *via* asymbiotic seed germination

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ABSTRACT

The loss of plant genetic resources has necessitated the development of many ex situ conservation techniques. Micropropagation is one of the ex situ techniques that has been used increasingly for the conservation of endangered plants. This in vitro technique aids in mass propagation of the plant species facing risk of extinction. The application of plant tissue culture techniques in orchid conservation and propagation requires an efficient in vitro regeneration protocol. This study reports the development of such highly efficient protocols for the in vitro asymbiotic seed germination of three threatened orchids namely Coelogyne nervosa A.Rich., Eria pseudoclavicaulis Blatt. and Porpax reticulata Lindl. The application of in vitro seed propagation technique for orchid conservation is a powerful tool for ex situ conservation of biodiversity. With asymbiotic seed germination technology, thousands of seedlings could be raised to maturity from a single seed capsule.

Keywords: Asymbiotic seed germination, orchids, *in vitro* propagation, *ex situ* conservation.

INTRODUCTION

Ex situ conservation involves preservation and maintenance of samples of living organisms outside their natural habitat, in the form of whole plants, seeds, pollen, vegetative propagules, tissue or cell cultures. *Ex situ* techniques are generally used to complement *in situ* methods but in some cases they are the only possible techniques to conserve certain species [1]. Among *ex situ* conservation methods the most common are cultivation in botanic gardens, seed storage, and *in vitro* cultivation. *In vitro* regeneration systems are viable alternatives to conventional vegetative propagation methods and it is useful for large scale production of selected genotypes and commercialization of clonal plants [2, 3]. The regeneration of plants is the first step for introduction of genetic variation by genetic transformation techniques for development of cultivars with improved characteristics [4]

Orchidaceae is one of the largest plant families which contain more than 20,000 species [5]. They exhibit incredible range of diversity in size, shape, and color of flowers. They are well known for their long-lasting and bewitchingly beautiful flowers which fetch a high price in both national and international markets [6]. One of the special features of this family is the production of a large number of minute seeds with only minimal reserves of nutrients [7]. Because of this feature, orchids depend upon mycorrhizal fungi for the carbon resources necessary for the germination and subsequent growth [8]. Many orchids are threatened by extinction because of environmental disruption, human succession of natural habitats, medicinal properties and overexploitation for horticultural purposes. This necessitates the development of rapid propagation techniques for conservation of orchids. *In vivo* vegetative propagation is undesirable due to the heterozygosity of seed, minute seed size, presence of reduced endosperm, and the requirement of an association with mycorrhizal fungi [6]. The concept of *in situ* conservation of orchids is wrought with many difficulties. This necessitates the application of *in vitro* seed propagation technique for orchid conservation [9, 10].

According to the IUCN criteria, the orchids selected for conservation are classified as:
(i) *Coelogyne nervosa* A.Rich.: endangered [11],
(ii) *Eria pseudoclavicaulis* Blatt.: Vulnerable [11] and
(iii) *Porpax reticulata* Lindl.: status yet to be ascertained.

The main objective of this study is to mass propagate these important orchid species using asymbiotic seed germination technique.

MATERIALS AND METHODS

Plant materials

The immature capsules of *Coelogyne nervosa, Eria pseudoclavicaulis,* and *Porpax reticulata* were obtained from the National Orchidarium and Associated Garden, Botanical Survey of India (Southern Circle), Yercaud, Tamil Nadu.

Media preparation

Five different basal media namely Schenk and Hildebrandt medium (1972) (SH), Linsmaier and Skoog medium (1965) (LS), Lindemann orchid medium (1970) (LOM), Knudson C medium (1946) (KC) and Murashige and Skoog medium (1962) (MS) at 0.088M sucrose and 0.8% agar were prepared and the pH was adjusted to 5.6-5.8 with 1N NaOH or HCl.

Asymbiotic seed germination

Each capsule was surface sterilized using 70% alcohol (30 seconds) and 0.1% mercuric chloride (4 minutes) and then rinsed thoroughly in sterile distilled water. The seeds from the surface sterilized capsules were transferred to the basal medium. The cultures were maintained at 25 ± 2°C under 12hours photoperiod. Observations were made at weekly intervals with 10 replicates per treatment.

Statistical analysis

Bursting of the seed coat and emergence of the enlarged embryo was considered as germination. Germination frequency (%) was calculated by counting the total number of seeds germinated with that of total number of seeds observed.

$$\% \text{ of germination} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds observed}} \times 100$$

RESULTS

The immature pods of *Coelogyne nervosa*, *Eria pseudoclavicaulis* and *Porpax reticulata* showed differential response (Table 1) when cultured on different basal media. Greening and swelling of seeds was found to be the first significant change after 9 weeks of culture for *Coelogyne nervosa* and *Porpax reticulata* and 12 weeks for *Eria pseudoclavicaulis*.

Table 1: Effect of various basal media on the asymbiotic seed germination

***Coelogyne nervosa* A.Rich.**					
No. of days taken for seed germination	SH	LS	LOM	KC	MS
	65	65	65	65	65
Frequency of germination [%]	60	80	80	95	60
***Eria pseudoclavicaulis* Blatt.**					
No. of days taken for seed germination	SH	LS	LOM	KC	MS
	90	90	90	90	90
Frequency of germination [%]	100	36	60	28	3
***Porpax reticulata* Lindl.**					
No. of days taken for seed germination	SH	LS	LOM	KC	MS
	65	65	65	65	65
Frequency of germination [%]	30	75	80	30	60

Among the five different basal media used, KC medium was found to be most effective in inducing a germination frequency of 95% in *Coelogyne nervosa* (Figure 1), where as LS and LOM induced 80% seed germination. 100%

seed germination frequency for *Eria pseudoclavicaulis* was observed on SH medium (Figure 2) and 60% on LOM. 80% of the seeds of *Porpax reticulata* germinated (Figure 3) on LOM and 75% on LS medium.
Figure1.

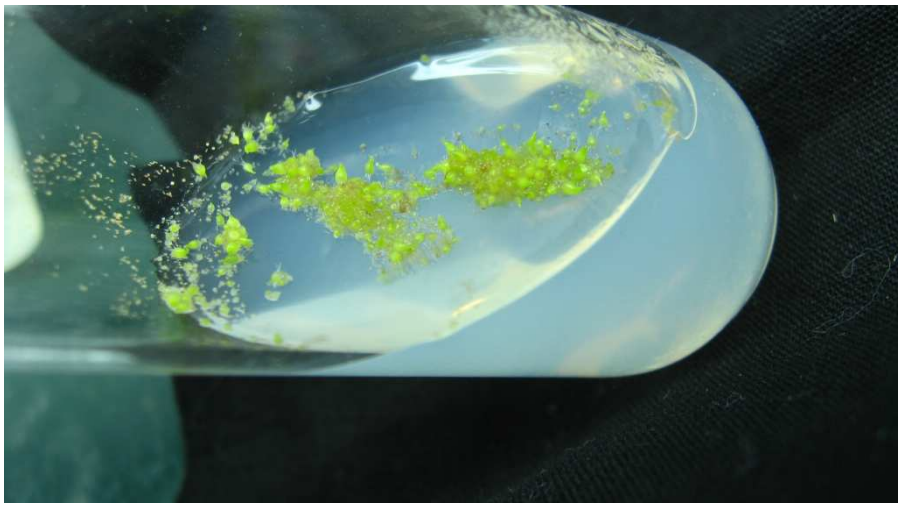

Figure 1. Seed germination of *Coelogyne nervosa* A. Rich on Knudson C medium.

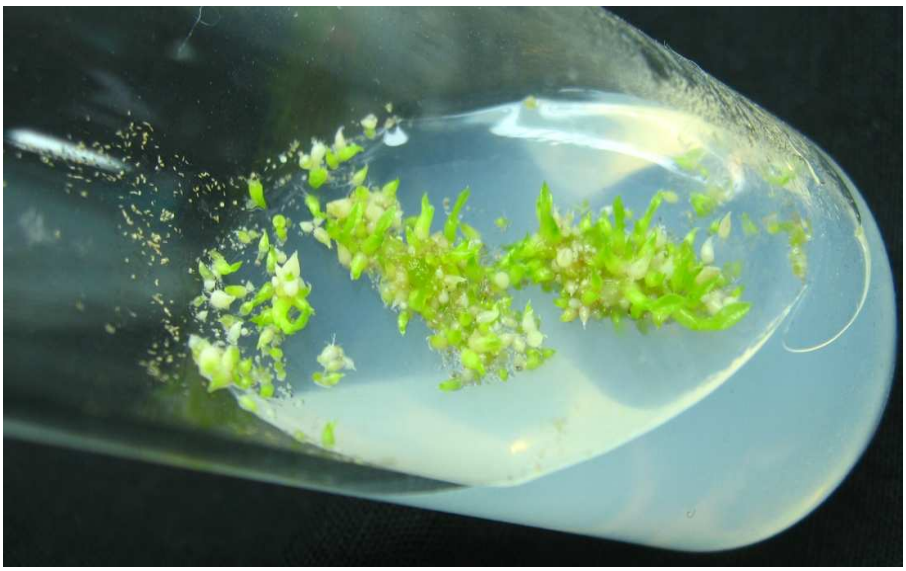

Figure 2. Seed germination of Eria *pseudoclavicaulis* Blatt. on Schenk and Hildebrandt medium.

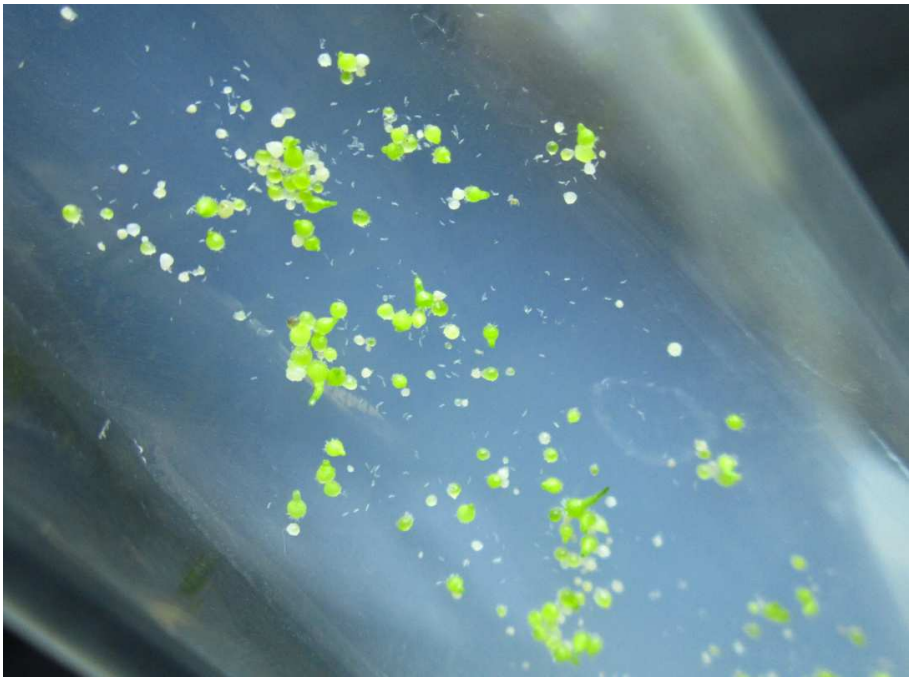

Figure 3. Seed germination of *Porpax reticulata* Lindl. on Lindemann orchid medium.

DISCUSSION

Many terrestrial and epiphytic orchids have been successfully propagated using *in vitro* asymbiotic seed germination techniques [12, 13, 14, 15, 16]. Species specific media for germination of seeds have been reported in many orchids [16, 17, 18]. This study reports the standardization of suitable seed germination medium for the selected orchid species. In the present study, KC medium was found to be the best suitable medium for the germination of *Coelogyne nervosa*. Similar results were observed with the early findings in *Calopogon tuberosus* [19] and *Habenaria macroceratitis* [15].

Media composition used in tissue culture studies accounts to a large proportion of any *in vitro* mass propagation protocol [20].The nutrient requirement for orchid seed germination differs from species to species as well as there is no universal medium for all the orchid species [21, 22, 15, 16, 23, 24, 25]. In the present study *Coelogyne nervosa*, *Eria pseudoclavicaulis* and *Porpax reticulata* showed higher germination rate in three different media KC, SH and LOM respectively. The germinated seeds continued to develop into complete plants on the same respective seed germination medium.

The organic nitrogen provided in nature by the symbiotic association with the fungus can be replaced with an organic nutrient source during the germination of *Platanthera ciliaris* seeds[26]. The difference between five different basal media used in the present study is in the form and the concentration of nitrogen source. While KC, MS and LS media contain inorganic nitrogen source (ammonium nitrate), LOM and SH contain amino acids as nitrogen sources. Malmgren [12, 27] and Van Waes and Debergh [28] found that the asymbiotic seed germination of terrestrial orchids were higher on media containing amino acids because this form of nitrogen can more readily be assimilated by the germinating seeds than inorganic nitrogen due to its simplified form. However, glycine, an aminoacid added to MS medium was reported to inhibit germination compared to ammonium nitrate [29, 30]. Hence, in the present study, it is inferred that the low germination frequencies observed for *Coelogyne nervosa* (60%), *Eria pseudoclavicaulis* (3%) and *Porpax reticulata* (60%) on MS medium may be due to the inhibitory effects of glycine (2mg/L) present in the medium. Effects of various amino acids on germination frequencies differ among orchid species [19]. Nitrogen in the form of ammonium was found to be more effective than nitrate or nitrite for the germination of orchid seeds [31, 32]. Niacin has also been found to be beneficial for orchid seed germination [33]. Niacin and its derivatives are essential for metabolism as components of NAD+ and NADP+ [19]. LOM and SH medium contain nicotinic acid, a derivative of niacin. This fact may account for the best results obtained (*Porpax reticulata*-80% on LOM and *Eria pseudoclavicaulis*-100% on SH medium) in the present study.
The results obtained in this study are highly significant for augmenting the conservation efforts of the selected orchid species.

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