IN VIVO GERMINATION OF ENCAPSULATED ORCHID SEEDS OF (*Epidendrum radicans*)

ABSTRACT

The orchid seeds are naked embryos without any nutritive tissue to support them for germination *in vivo.* In nature they germinate symbiotically when they come in contact with mycorrhiza found in the mother root tufts. Orchid capsule consists of hundreds or thousands of seeds which go waste without germination. The role of mycorrhiza is the conversion of starch into simple glucose which provides nutrition for the germinating seeds. Orchid cultivars and breeders use *in vitro* technique to culture the orchid seeds with various growth hormones. An attempt was made to grow orchid seeds *in vivo* by encapsulating them with calcium alginate similar to the production of synthetic seeds. *Epidendrum radicans* seeds were encapsulated with calcium alginate. The sodium alginate gel was prepared using Vacin and Went medium supplemented with 100ml L^{-1} CW, 2mg L^{-1} NAA and 20g L^{-1} sucrose. The encapsulation was carried out aseptically and the seeds were successfully germinated in sterilized soilrite medium.

Key words: calcium alginate, Epidendrum, iIn vivo, mycorrhiza, Soilrite, symbiotic.

INTRODUCTION

The orchid capsules contain numerous seeds, which are strongly reduced structures (Beer, 1983; Burgeff, 1911, 1936), without nutritive tissue **which** to help them for germination in vivo. A seed weighs between 6.3 and 0.3 micrograms. A small germ is surrounded by a single layered seed coat which has a very loose mantle like structure. However, a few seeds will germinate on the tufts of the mother plant by symbiotic association with mycorrhiza. Though most of the seeds are found viable only few seeds are noticed to survive in nature as they are wind dispersed. The literature survey revealed that, the orchid seeds were cultured *in vitro* either symbiotically or non- symbiotically (Bopaiah & Jorapur **S M** 1986; Sagaya **Mary B** & **K M** Divakar 2015; Jyothsna **B S** & Purushothama **K B** 2013). *In vitro* symbiotic method of seed culture was adopted by orchid breeders for intergeneric hybrids since many years. This method was found laborious as the mycorrhiza was found to be specific and difficult to isolate. However, Noel Bernard (1909) made the first attempt to grow these orchid seeds in controlled condition *in vitro*. Bernard (1909) believed that the orchids were obligate symbionts with specific nutrients necessary for the normal germination of seeds. On the other hand, Burgeff (1909) concluded that the fungus converts the starch in the embryo into glucose and increases the osmotic concentration of the cells and supply necessary carbohydrates for the seeds. In the present investigation an attempt was made to germinate the orchid seeds by encapsulating them with sodium alginate and calcium chloride solution, to convert them into calcium alginate beads to germinate, similar to technique followed in the preparation of synthetic seeds. These seeds can be raised in the nursery beds directly into seedlings.

MATERIAL AND METHODS

Sodium alginate gel was prepared in Vacin and Went medium supplemented with 100ml L^{-1} coconut water, 20g L^{-1} sucrose, 2mg L^{-1} NAA maintained at pH 5.8 (Table 1). The sodium alginate was mixed to warm VW medium with constant stirring till it formed a jelly. The jelly was autoclaved at 15Pb pressure for 15min in an autoclave and cooled. 100 µM of calcium chloride solution was prepared by dissolving 1.036 g of cCalcium chloride in 150 ml of pre sterilized DDH_2O, inside the laminar air flow. Mature fruit capsule, before dehiscence was collected from *Epidendrum radicans* (Fig 1). They were surface sterilized with 70% ethanol and gently flamed within the LAF. The capsules were cut open with sterile scalpel and the seeds scooped out of the capsule into the sodium alginate gel. Clumps of gel with the seeds were transferred to calcium chloride solution (Table 2). The encapsulated seeds (Fig 2) were packed in polythene sachets and preserved them in the refrigerator at 7-8^0 C till it was sown (for about 10 days).

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Sterilized soilrite (mixture of perlite and peat moss) was mixed with sterilized processed cocopith (Tannin content was removed by soaking cocopith in water for 48 hours) and sun dried. The entire mixture was treated with 2% bevestin (systemic fungicide) and filled to net pots. The encapsulated seeds were sown in net pots and transferred them to port treys. The port treys were kept in a hardening tunnel used for hardening tissue cultured plantlets, where 80-90% humidity and 25-30 ^{0}C temperature and natural illumination were provided.

Table 1: Vacin and Went medium + Additional supplements

No.**1.**	Component**Potassium dihydrogen orthophosphate**	mg/L**250mg/L**
1	Potassium dihydrogen orthophosphate	250
2.	Magnesium sulphate	250 **mg/L**
3.	Potassium nitrate	525 **mg/L**
4.	Ammonium sulphate	500**mg/L**
5.	Tricalcium phosphate	200**mg/L**
6.	Ferric tartarate	28.5**mg/L**
7.	Manganous sulphate supplements	7.5**mg/L**
8.	NAA	2**mg/L**
9.	Coconut water	100**ml/L**
10.	Sucrose	20**g/L**

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Table 2: Preparation of calcium alginate gel

1. Weigh 1 g of sodium alginate.
2. Dissolve in 50 ml of warm VW medium.
3. Weigh 1.036 g of calcium chloride

4. Dissolve calcium chloride in 150 ml of distilled water.

OBSERVATIONS

The observations were made at regular intervals of every 10 days. The seeds responded for the condition provided and started germination within a period of 45-50 days (Fig. 3). The seedlings obtained were allowed to grow in the same conditions till plantlets were observed (Fig.4). After 60 days NPK (20:20:20) solution diluted 10,000 times was sprayed daily to get better growth of the seedling. After 120 days of germination the seedlings started producing good rooting. Such seedlings were segregated and transferred to individual pots, containing broken pot pieces charcoal and moss for further growth, a method normally followed for orchid cultivation.

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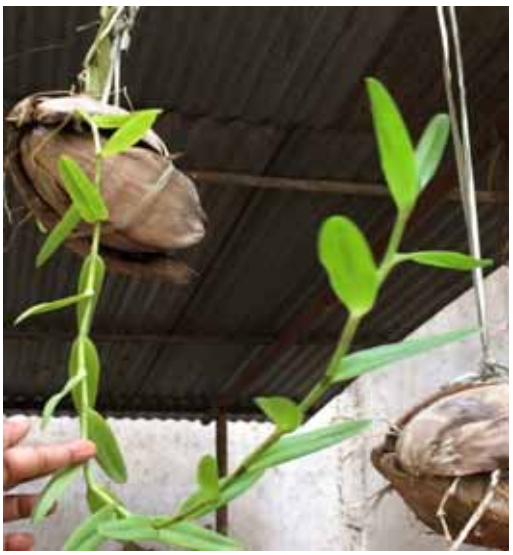

Fig 1- Mother plant- *Epidendrum radicans*

Fig 2 – Sodium alginate beads with *Epidendrum radicans* seeds

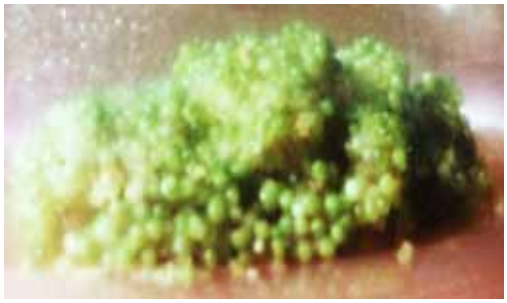

Fig 3 – Initiation of germination of seeds after 40 days

Fig 4 – Plantlet formation after 60 days

RESULTS AND DISCUSSION

Orchid seeds are microscopic in structure and devoid of nutrients to germinate on its own. However, the mycorrhiza found in the mother root tufts help in converting the available starch into glucose which help in germination. Only few seeds succeed in this process and hundreds of seeds go waste without germination. Orchid breeders now a days use *in vitro* culture technique for orchid seed germination to produce new intergeneric hybrid varieties. This process is time consuming and expensive. The mortality rate is very high during hardening. Since zygotic embryos are similar to somatic embryos in the morphological features, an attempt was made to encapsulate the orchid seed, which are devoid of nutritive tissue. Same technique used for synthetic seed preparation was followed. The results obtained indicate that the orchid seeds can be successfully germinated by using this simple method, which will help in saving time and money for the breeders. The present investigation was carried out for the first time. The literature survey indicates that the earlier works were mainly on symbiotic to non-

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symbiotic methods (Bopaiah & Jorapur **S M** 1986; Sagaya **Mary B** & **K M** Divakar 2015; Jyothsna **B S** & Purushothama **K B** 2013).

CONCLUSION

Orchids produce thousands of seeds both in wild species and hybrids, which has to be cultured *in vitro*. Intergeneric hybridization is possible in orchids to produce new varieties. The seeds obtained after hybridization were usually cultured in vitro, this takes several years (5-6 years) for the plants to flower and mortality is very high during hardening. This process is time consuming and laborious. Present investigation will help the breeders to minimize the time of flowering **and** and reduce mortality. This will help in bringing out new varieties of orchids to the marketed at a faster rate. This simplified method also helps to multiply the endangered wild orchids and their conservation in natural habitat.

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AUTHORS' CONTRIBUTIONS

Author 1- Prepared sStudy dDesign, cCollected the dData and references, performed the experiments, wrote the protocol, interpreted the dData and wrote the draft of the manuscript.

Author 2- Assisted in finalizing the manuscript and its preparation.

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ABBREVIATIONS

VW- Vacin and Went medium

CW- Coconut Water

NAA- Naphthalene Acetic Acid

LAF- Laminar Air Flow

NPK- Nitrogen, Phosphorous, Potassium

DDH_2O- Double Distilled Water

NAA- Naphthalene Acetic Acid

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