



In vivo* Germination of Encapsulated Orchid Seeds of- *Epidendrum radicans

Grace Leena Crasta^{1*} and A. K. Bopaiah^{1*}

¹Department of Botany, St. Joseph's College and Research Centre, Langford Road, Bangalore, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author GLC prepared the study design, collected the data and references, performed the experiments, wrote the protocol, interpreted the data and wrote the draft of the manuscript. Author AKB assisted in finalizing the manuscript and its preparation. Both authors read and approved the final manuscript.

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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 to convert starch into simple glucose which provides nutrition for the germinating seeds. Orchid 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 100 ml L⁻¹ CW, 2 mg L⁻¹ NAA, and 20 g L⁻¹ sucrose. The encapsulation was carried out aseptically and the seeds were successfully germinated in the sterilized soilrite medium.

*Corresponding author: E-mail: graceleena11@gmail.com, bopaiah59@yahoo.co.in;

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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
 DDH₂O : Double Distilled Water
 NAA : Naphthalene Acetic Acid

1. INTRODUCTION

The orchid capsules contain numerous seeds, which are strongly reduced structures [1] they do not contain nutritive tissue to help them for germination *in vivo*. However, a few seeds will germinate on the tufts of the mother plant by symbiotic association with mycorrhiza. An orchid seed weighs between 0.3 and 6.3 micrograms. A small germ is surrounded by a single-layered seed coat which has a very loose mantle like structure. 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 [2,3,4,5,6,7,8,9,10,11,12]. *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 the 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.

2. MATERIALS AND METHODS

Sodium alginate gel was prepared in Vacin and Went (VW) medium supplemented with

100 ml L⁻¹ coconut water, 20 g L⁻¹ sucrose, 2 mg 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 15 min in an autoclave and cooled. 100 µM of calcium chloride solution was prepared by dissolving 1.036 g of calcium chloride in 150 ml of pre-sterilized DDH₂O, 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 (do not burn the fruit capsule) over a spirit lamp using forceps, within the LAF. The capsules were cut open with a 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°C till it was sown (for about 10 days).

Table 1. Vacin and went medium + additional supplements

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

Sterilized soilrite (a 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 trays. The port trays were kept in a hardening tunnel that is normally used for hardening tissue cultured plantlets, where 80-90% humidity and 25-30°C temperature and natural illumination were provided.

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.



Fig. 1. Mother plant- *Epidendrum radicans*

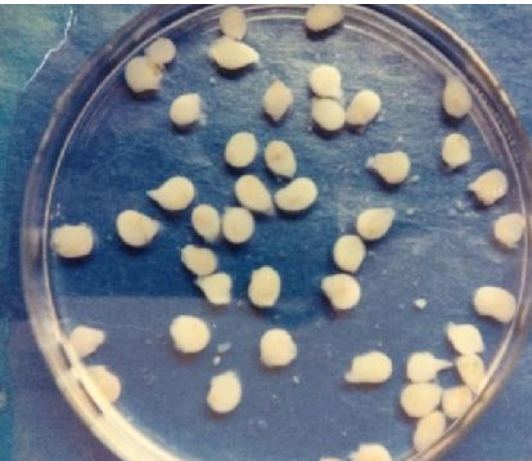


Fig. 2. Sodium alginate beads with *Epidendrum radicans* seeds

2.1 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 separated and transferred to individual pots, containing broken pot pieces charcoal and moss for further growth, a method normally followed for orchid cultivation.

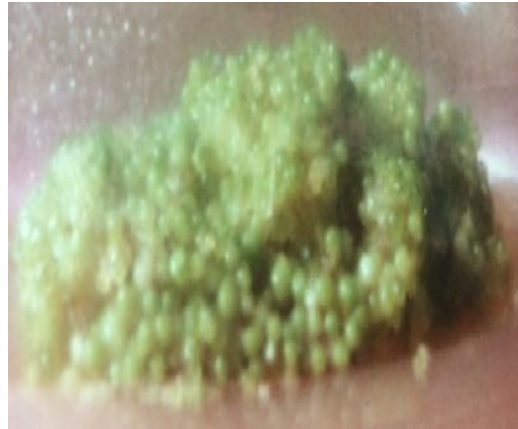


Fig. 3. Initiation of germination of seeds after 40 days



Fig. 4. Plantlet formation after 60 days

3. 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 a few seeds succeed in this process and hundreds of seeds go waste without germination. Orchid breeders nowadays 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-symbiotic methods [2,3,4,5,6,7,8,9,10,11,12].

4. CONCLUSION

Orchids produce thousands of seeds both in wild species and hybrids, which have 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 reduce mortality. This will help in bringing out new varieties of orchids to the market 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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