

1 **IN VIVO GERMINATION OF ENCAPSULATED ORCHID SEEDS**

2 3 4 **ABSTRACT**

5
6 The orchid seeds are naked embryos without any nutritive tissue to support them for
7 germination *in vivo*. In nature they germinate symbiotically when they come in contact with
8 mycorrhiza found in the mother root tufts. Orchid capsule consists of hundreds or thousands
9 of seeds which go waste without germination. The role of mycorrhiza is the conversion of
10 starch into simple glucose which provides nutrition for the germinating seeds. Orchid
11 cultivars and breeders use *in vitro* technique to culture the orchid seeds with various growth
12 hormones. An attempt was made to grow orchid seeds *in vivo* by encapsulating them with
13 calcium alginate similar to the production of synthetic seeds. *Epidendrum radicans* seeds
14 were encapsulated with calcium alginate. The sodium alginate gel was prepared using Vacin
15 and Went medium supplemented with 100ml L⁻¹ CW, 2mg L⁻¹ NAA and 20g L⁻¹ sucrose. The
16 encapsulation was carried out aseptically and the seeds were successfully germinated in
17 sterilized soilrite medium.

18 **Key words** : *In vivo, symbiotic, mycorrhiza, calcium alginate, Epidendrum, Soilrite.*

19 20 **INTRODUCTION**

21 The orchid capsules contain numerous seeds, which are strongly reduced structures (Beer,
22 1983; Burgeff, 1911, 1936). A seed weighs between 6.3 and 0.3 micrograms. They lack
23 nutrient tissue entirely. A small germ is surrounded by a single layered seed coat which has
24 a very loose structure. However, a few seeds will germinate on the tufts of the mother plant
25 by symbiotic association with mycorrhiza. Though most of the seeds are found viable only
26 few seeds are noticed to survive in nature. *In vitro* symbiotic method of seed culture was
27 adopted by orchid breeders for many years. This method was found laborious as the
28 mycorrhiza was found to be specific. However, Noel Bernard (1909) made the first attempt to
29 grow these orchid seeds in controlled condition. Bernard (1909) believed that the orchids
30 were obligate symbionts with specific nutrients necessary for the normal germination of
31 seeds. On the other hand, Burgeff (1909) concluded that the fungus converts the starch in
32 the embryo into glucose and increases the osmotic concentration of the cells. In the present
33 work an attempt is made to grow the orchid seeds by encapsulating them with sodium
34 alginate and calcium chloride solution, similar to the preparation of synthetic seeds in the
35 nursery beds.

36 MATERIAL AND METHODS

37 5% of sodium alginate gel was prepared in Vacin and Went medium supplemented with
38 100ml L⁻¹ coconut water, 20g L⁻¹ sucrose, 2mg L⁻¹ NAA. The sodium alginate was mixed to
39 warm VW medium with constant stirring till it formed a jelly. The jelly was autoclaved at 15Pb
40 pressure for 15min in an autoclave and cooled. 100µM of calcium chloride solution was
41 prepared by dissolving 100mg Calcium chloride in 100ml of pre sterilized DDH₂O, in the
42 laminar air flow. Mature fruit capsule before dehiscence were collected from *Epidendrum*
43 *radicans*. They were surface sterilized with 70% ethanol and gently flamed within the LAF.
44 The capsules were cut open with sterile scalpel and the seeds scooped out of the capsule
45 into the sodium alginate gel. Clumps of gel with the seeds were transferred to calcium
46 chloride solution. The encapsulated seeds were packed in polythene sachets and preserved
47 them in the refrigerator till it was sown.

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

54

55 OBSERVATIONS

56 The seeds responded for the condition provided and started germination within a period of
57 45-50 days. The seedlings obtained were allowed to grow in the same conditions. After 60
58 days NPK (20:20:20) solution diluted 10,000 times was sprayed daily to get better growth of
59 the seedling. After 120 days of incubation the seedling started producing good root
60 formation. Such seedlings were segregated and transferred to individual pots, containing
61 broken pot pieces charcoal and moss.

62

63 RESULTS AND DISCUSSION

64 Orchid seeds are microscopic in structure and devoid of nutrients to germinate on its own.
65 However, the mycorrhiza found in the mother root tufts help in converting the available
66 starch into glucose which help in germination. Only few seeds succeed in this process and
67 hundreds of seeds go waste without germination. Orchid breeders now a days use *in vitro*
68 culture technique for orchid seed germination to produce new varieties. This process is time
69 consuming and expensive. The mortality rate is very high during hardening. Since zygotic

70 embryos are similar to somatic embryos in the morphological features, an attempt was made
71 to encapsulate the orchid seed, which are naked embryos, with the same technique used for
72 synthetic seed preparation. The results obtained indicate that the orchid seeds can be
73 successfully germinated by using this simple method, which will help in saving time and
74 money for the breeders.

75 **CONCLUSION**

76
77
78 Intergeneric hybridization is possible in orchids to produce new varieties. The seeds
79 obtained after hybridization were usually cultured in vitro. this takes several years (5-6 years
80 sometimes) for the plants to flower. This process is time consuming and laborious. present
81 investigation will help the breeders to minimize the time of flowering, this will help in bringing
82 out new varieties of orchids to the market at a faster rate.

83
84
85

86 **REFERENCES:**

- 87
88 Arditti, J. 1967. Factors affecting germination of orchid seeds. Bot. rev 33:1-97.
89
90 Arditti, J. 1977. Orchid Biology – review and presrpectives. Comstock publishing associates,
91 Cornell university press. London.
92
93 Burgeff, H. 1936. Samenkeimung and kultur Europaicher Endorchideen. G. Fischer Verlag,
94 Stuttgart, 48.
95
96 Bopaiah A.K. Bopaiah and Jorapur S.M. 1986. Studies on growth and development of
97 Cymbidium aloifolium Sw. Seedlings in vitro. Biology, conservation, and culture orchids.
98 Affiliated East – west Pvt Ltd.
99
100 Knudson, L. 1922. Non symbiotic germination of orchid seeds Bot. Gaz; 73: 1-25.
101
102 Knudson, L. 1946. A new nutrient solution for the germination of orchid seeds. AM. Orchid
103 Soc. Bull., 15:214-17.
104
105 Withner, C.L. 1959. The orchids: A scientific survey. Ronald press, New York.

106
107

108 **ABBREVIATIONS**

- 109 VW- Vacin and Went medium
110 CW- Coconut Water
111 NAA- Naphthaline Aceyic Acid

- 112 LAF- Laminar Air Flow
- 113 NPK- Nitrogen, Phosphorous, Potassium
- 114 DDH₂O- Double Distilled Water
- 115
- 116
- 117