IN VIVO GERMINATION OF ENCAPSULATED ORCHID SEEDS

ABSTRACT

The orchid seeds are **naked** coated embryos without any nutritive tissue to support them for *in vivo* 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** collectors and breeders use *in vitro* technique to culture **the** orchid seeds with various growth hormones. An attempt was made to grow *Epidendrum radicans* 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 : *In vivo, symbiotic, mycorrhiza, calcium alginate, Epidendrum, Soilrite.*

Comment [RD1]: Alphabetic order

INTRODUCTION

The orchid capsules contain numerous seeds, which are strongly reduced structures (Beer, 1983; Burgeff, 1911, 1936). A seed weighs between 6.3 and 0.3 micrograms. They lack nutrient tissue entirely. A small germ is surrounded by a single layered seed coat which has a very loose structure. **However, a few seeds will germinate on the tufts of the mother plant by symbiotic association with mycorrhiza**. **Though** Despite most of the seeds are found viable only few **seeds** of them are noticed to germinate on the tufts of the mother plant by symbiotic association with mycorrhiza and survive in nature. *In vitro* symbiotic method of seed culture was adopted by orchid breeders for many years (Kulus, 2015). Unfortunatelly, **T**this method was found laborious as the mycorrhiza was found to be species-specific. **However,** Noel Bernard (1909) made the first attempt to grow **these** orchid seeds in controlled condition. 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. The synthetic seeds technology developed by Murashige (1977) for potato can be applied for the reproduction of ornamental plant species (Kulus and Zalewska, 2014)

Comment [RD2]: Not present in the reference list.

Comment [RD3]: Not present in the reference list

Comment [RD4]: Not present in the references

Comment [RD5]: Not present in the References

In the present work an attempt is made to grow the orchid seeds by encapsulating them with sodium alginate and calcium chloride solution, similar to the preparation of synthetic seeds in the nursery beds.

MATERIAL AND METHODS

5% of sodium Sodium alginate gel (5%) was prepared in Vacin and Went medium supplemented with 100ml L^{-1} coconut water, 20g L^{-1} sucrose, 2mg L^{-1} NAA. **The sodium alginate was mixed to warm VW medium with constant stirring till it formed a jelly.** The **jelly** matrix was autoclaved at 15Pb pressure for 15min in an autoclave and cooled. **100µM of** Ccalcium chloride solution (100µM) was prepared **by dissolving 100mg Calcium chloride** in **100ml of** pre sterilized DDH_2O, in the laminar air flow. Mature fruit capsules before dehiscence were collected from *Epidendrum radicans*. They were surface **sterilized** disinfected 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. The encapsulated seeds were packed in polythene sachets and preserved them in the refrigerator till it was sown.

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). 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 illumination were provided.

The seedlings obtained were allowed to grow in the same conditions. After 60 days NPK (20:20:20) solution diluted 10,000 times was sprayed daily to get better growth of the seedling. Seedlings with roots were segregated and transferred to individual pots, containing broken pot pieces charcoal and moss.

OBSERVATIONSRESULTS

The seeds responded for the condition provided and started germination within a period of 45-50 days. ~~The seedlings obtained were allowed to grow in the same conditions. 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 incubation the seedling started producing good roots **formation**. **Such seedlings were segregated and transferred to individual pots, containing broken pot pieces charcoal and moss.**

Comment [RD6]: Reference needed

Comment [RD7]: pH?

Comment [RD8]: How many?

Comment [RD9]: For how long? Did you washed with water afterwards?

Comment [RD10]: At what temperature?

Comment [RD11]: For how long?

Comment [RD12]: What illumination? What intensity, type? Photoperiod?

Comment [RD13]: For how long.

Comment [RD14]: What was analysed? Where is the statistical analysis? What about the control?

Comment [RD15]: Results should be described in more detail.

Comment [RD16]: How many of them?

Comment [RD17]: How many?

Comment [RD18]: What was the final survival and regrowth of the synthetic seeds?

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 varieties. This process is time consuming and expensive. The mortality rate is very high during orchid hardening. Since zygotic embryos are similar to somatic embryos in the morphological features, an attempt was made to encapsulate the orchid seed, which are naked embryos, with the same technique used for synthetic seed preparation. 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.

CONCLUSION

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 sometimes) for the plants to flower. This process is time consuming and laborious. The present investigation will help the breeders to minimize the time of flowering required, **this will**and help in bringing out new **varieties** cultivars of orchids to the market at a faster rate.

REFERENCES:

Arditti, J. 1967. Factors affecting germination of orchid seeds. Bot. Rrev. 33:1-97.

Arditti, J. 1977. Orchid Biology – review and presprctives. Comstock publishing associates, Cornell university press. London.

Burgeff, H. 1936. Samenkeimung and kultur Europaicher Endorchideen. G. Fischer Verleg, Stuttgart, 48.

Bopaiah A.K. Bopaiah and Jorapur S.M. 1986. Studies on growth and development of Cymbidium aloifolium Sw. Seedlings *in vitro*. Biology, conservation, and culture orchids. Affiliated East – west Pvt Ltd.

Knudson, L. 1922. Non symbiotic germination of orchid seeds Bot. Gaz; 73: 1-25.

Comment [RD19]: The discussion is very poor. You should discuss and compare your results with the findings of other Authors.

Comment [RD20]: All of this was in the Introduction.

Formatted: Font: Italic

Comment [RD21]: This was not a part of your research so it is not a conclusion. Transfer this sentence to the Introduction or Discussion.

Comment [RD22]: A deeper conclusion is required.

Formatted: Font: Italic

Knudson, L. 1946. A new nutrient solution for the germination of orchid seeds. AM. Orchid Soc. Bull., 15:214-17.

Withner, C.L. 1959. The orchids: A scientific survey. Ronald press, New York.

Murashige T., 1977. Plant cell and organ cultures as horticultural practices. ISHS Acta Horticulturae 78: 17-30.

Kulus D., Zalewska M., 2014b. *In vitro* plant recovery from alginate encapsulated *Chrysanthemum* × *grandiflorum* /Ramat./ Kitam. shoot tips. Propagation of Ornamental Plants 14(1): 3-12.

Kulus D., 2015. Selected aspects of ornamental plants micropropagation in Poland and worldwide. Nauki Przyrodnicze 4(10): 10-25. DOI: 10.13140/RG.2.1.5086.8082

Comment [RD23]: Not cited in the text!

Comment [RD24]: Not cited in the text

Formatted: No bullets or numbering

Formatted: Indent: Left: 0.39", No bullets or numbering

Formatted: Styl jaro_styl + Z lewej: 0 cm Pierwszy wiersz: 0 cm, No bullets or numbering

ABBREVIATIONS

VW- Vacin and Went medium

CW- Coconut Water

NAA- Naphthaline Aceyic Acid

LAF- Laminar Air Flow

NPK- Nitrogen, Phosphorous, Potassium

DDH_2O- Double Distilled Water