

IN VITRO GERMINATION CAPACITY AND PLANT RECOVERY OF SOME NATIVE AND RARE ORCHIDS

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ABSTRACT

Seeds of *Aerides multiflorum*, *Aerides odorata*, *Cymbidium* sp., *Dendrobium supperbum*, *Dendrobium wardianum*, *Dendrobium nobile*, *Dendrobium primulinum*, *Doritis pulcherrima*, *Paphiopedilum delenatii*, *Paphiopedilum callosum*, *Phaius tankervilleae*, *Vanda parishii* and *Vanda denisoniana* were tested for germination in different culture media (MS, modified MS, VW, Knudson C or Hyponex medium). The results obtained in these experiments showed that all seeds of these orchids germinated *in vitro* after 3 months of culture with different morphogenesis. Seeds developed plants, embryos-like structures, protocorm-like bodies (PLBs), and shoots which depended on the culture media. These shoots and PLBs proliferated to get a large amount of shoots and then transferred to plant-formed medium. All *in vitro* seed-derived plants were vigorous and well acclimatized in greenhouse conditions. This study was very important for the preservation of genetic resources and basic researches of plant breeding.

Keywords: Orchid, seed, germination

INTRODUCTION

Many plants have their devotees, but few give rise to such passion as orchids. Why is it that people become obsessed? Their beauty, strangeness and mystique must all play a part but perhaps most of all, their sheer variety appeals (la Croix, 2000). Orchids belonging to the family Orchidaceae comprise the largest family of flowering plants. Taxonomically they represent the most highly evolved family among the monocotyledons, with approximately 700 to 800 genera and 25.000 to 35.000 species (Arditti, 1979; Garay, 1960). The uniqueness of the family is reflected in its huge amount of diversity coupled with peculiar pollination contrivances and wide natural hybridization. Artificial hybridizations have also been made successfully between different species and genera under same or different sub-tribes. Today there are more than 90.000 hybrids registered with the Royal Horticultural Society in England.

The orchid flowers show an incredible range of diversity in size, shape, color, structure, number and fragrance of flowers. They are unique in that the range from microscopic to several inches in size. They have contributed significantly to the international trade in

cut-flower and ornamental potted plants. The flowers in some modern orchid hybrids have a shelf-life of 8-12 weeks and surpass all other flowers in this respect. Orchid cut-flower industry in the world today is a highly developed trade for local market and export. Development of new hybrids and commercial cultivation of orchids have become a lucrative industry in Europe, Hawaii, Australia, Thailand, Japan, Singapore, Sri Lanka and Kenya. Orchids are also used for a variety of therapeutic purposes. Some of the orchids are used in the Ayurvedic system of medicine as tonic.

Propagation of orchids through conventional means is a very slow process. Vegetative propagation through division of clumps or rhizomes, cuttings and separation of offshoots and keikis produced from the stem or pseudobulbs is very slow and one may not get more than a few plants after 2-3 years. The orchid seeds although produced in a very large number i.e. 2-3 million per capsule, are non-endospermic and do not contain nutrient. They require a mycorrhizal association for their germination, which may be provided under natural undisturbed conditions. Fungal endophytes in such associations are believed to provide simple sugar and other nutrients required for seed germination by breaking down starch (Arditi, 1967). Therefore, only 2-5% of seeds germinate in their natural environment.

Propagation and cultivation of orchid was revolutionized after the discovery by Knudson (1922) that orchid seeds can be germinated on a simple sugar containing medium. His work showed for the first time that germination of orchid was possible *in vitro* without fungal association. Subsequently, he proposed a new nutrient solution for the germination of orchid seeds in 1946. Since then many species have been successfully raised *in vitro* from seeds. In this report, different germination media and plantlet regeneration of some native and rare orchids of Vietnam will be investigated in order to store *in vitro* plantlets and reserve the native and rare orchid in Vietnam.

MATERIALS AND METHODS

Green pods of several native orchids (*Aerides multiflorum*, *Aerides odorata*, *Cymbidium* sp., *Dendrobium supperbum*, *Dendrobium wardianum*, *Dendrobium nobile*, *Dendrobium primulinum*, *Doritis pulcherrima*, *Paphiopedilum delenatii*, *Paphiopedilum*

callosum, *Phaius tankervilleae*, *Vanda parishii*, *Vanda denisoniana*) were obtained in Dalat. These pods were collected after pollination 7 – 10 months. They were rinsed with soap and washed with water. And then they were washed with ethanol 70% for 30 seconds and rinsed several times with sterile distilled water. After the wash green pods were dipped in HgCl_2 1‰ for 15 minutes and rinsed several times with sterile distilled water.

Orchid seeds were cultured on different media [MS (Murashige and Skoog, 1962), modified MS, VW (Vacin and Went, 1949), Knudson C (1922, 1946) or Hyponex medium]. Germinated seeds were cultured on plant-formed medium and transferred into greenhouse for acclimatization.

Orchid seeds and plantlets or different organs derived from seeds were placed at $25 \pm 1^\circ\text{C}$ and 70 – 80% relative humidity, with a light intensity of $45 \text{ } \mu\text{mol m}^{-2}\text{s}^{-1}$ provided by cool white fluorescent lamps for a light period of 10 hours per day.

RESULTS AND DISCUSSION

Aerides multiflorum, *Aerides odorata*

Seeds of *Aerides multiflorum*, *Aerides odorata* were cultured on MS medium containing 1 g.l^{-1} activated charcoal, 30 g.l^{-1} sucrose. The pH was adjusted to 5.8 before autoclaving at 121°C , 1 atm for 30 minutes. Germination and embryogenesis rate were very high, well-developed shoots with yellow thin leaves. The lower germination rate obtained when seeds were cultured in MS medium supplemented with 0.5 mg.l^{-1} BA, 0.5 mg.l^{-1} NAA, 20% coconut water (CW), 1 g.l^{-1} active charcoal (AC) and 30 g.l^{-1} sucrose. Shoots regenerated in this medium, however, were vigorous with dark green leaves. These shoots were subcultured onto VW medium containing 0.5 mg.l^{-1} NAA, 0.5 mg.l^{-1} BA, 15% CW, 1 g.l^{-1} AC and 30 g.l^{-1} sucrose, the optimal pH of this medium was 5.3. Plantlets were transferred to greenhouse with high survival rate (90%).

Cymbidium sp.

Seeds of *Cymbidium* sp. were placed on MS medium supplemented with 0.5 mg.l^{-1} BA, 0.5 mg.l^{-1} NAA, 1 g.l^{-1} AC and 30 g.l^{-1} sucrose. Results showed high germination and PLB formation in this medium. These PLBs were subcultured on proliferation medium containing 0.5 mg.l^{-1} NAA, 2.0 mg.l^{-1} BA, 20% CW, 1 g.l^{-1} AC and 30 g.l^{-1} sucrose. After 3 months of culture, vigorous developed-shoots were transferred to rooting-induced medium with 0.5 mg.l^{-1} NAA, 0.5 mg.l^{-1} BA, 15% CW and 30 g.l^{-1} sucrose. Plantlets were grown in nursery trays with high survival rate (90%).

Dendrobium supperbum, *Dendrobium wardianum*, *Dendrobium nobile*, *Dendrobium primulinum*

Seeds of *Dendrobium* sp. were cultured on various media. Results showed that high germination rate obtained in 1/2 MS medium containing 0.5 mg.l^{-1} NAA, 20% CW and 30 g.l^{-1} sucrose; MS medium with 20% CW, 1 g.l^{-1} AC and 30 g.l^{-1} sucrose; Hyponex medium containing vitamins, 30 % potato extract, 1 g.l^{-1} AC and 30 g.l^{-1} sucrose; VW medium supplemented with 0.5 mg.l^{-1} BA, 0.5 mg.l^{-1} NAA, 15% CW, 1 g.l^{-1} AC and 30 g.l^{-1} sucrose. Well-developed shoots, however, obtained in last medium. On MS medium supplemented with 0.5 mg.l^{-1} NAA, 0.5 mg.l^{-1} BA, 20% CW and 30 g.l^{-1} sucrose with or without 1 g.l^{-1} AC, germination and survival rate was lower.

Doritis pulcherrima

Seeds of *Doritis pulcherrima* germinated with high frequency on MS medium containing 0.5 mg.l^{-1} BA, 0.5 mg.l^{-1} NAA, 1 g.l^{-1} AC and 30 g.l^{-1} sucrose.

Paphiopedilum delenatii, *Paphiopedilum callosum*

Seeds of *P. delenatii* and *P. callosum* were cultured on Knudson C medium. Shoots derived from this medium were subcultured on MS medium supplemented with 2 mg.l^{-1} BA, 1 g.l^{-1} AC and 30 g.l^{-1} sucrose (pH 5.3). Survival rate of plantlets when transferred to greenhouse was high (90%).

Phaius tankervilleae

Seeds of *Phaius tankervilleae* were cultured on 1/2 MS medium containing 0.5 mg.l^{-1} NAA, 15% CW and 30 g.l^{-1} sucrose (pH 5.3). Results showed that this medium was not suitable for seed germination. Thus, further studies for this species are required.

Vanda parishii, *Vanda denisoniana*

Seeds of *V. parishii* and *V. denisoniana* were cultured on various media. The high germination rate obtained in Hyponex medium containing vitamins, 30% potato extract, 1 g.l^{-1} AC and 30 g.l^{-1} sucrose (pH 5.3); the lower rate obtained in MS medium supplemented with 0.5 mg.l^{-1} BA, 20% CW, 1 g.l^{-1} AC and 30 g.l^{-1} sucrose 0.5 or 2.0 mg.l^{-1} NAA (pH 5.3 or 5.8, respectively). Embryos formed on 1/2 MS medium containing 0.5 mg.l^{-1} NAA, 20% CW and 30 g.l^{-1} sucrose (pH 5.3). Shoots were subcultured on two different media: Hyponex medium containing vitamins, 30 % potato extract, 1 g.l^{-1} AC and 30 g.l^{-1} sucrose (pH 5.3) and VW medium supplemented with 0.5 mg.l^{-1} NAA, 0.5 mg.l^{-1} BA, 20% CW, 1 g.l^{-1} AC and 30 g.l^{-1} sucrose (pH 5.3). Plantlets developed from these media showed high survival rate (90%) after transferring to greenhouse.

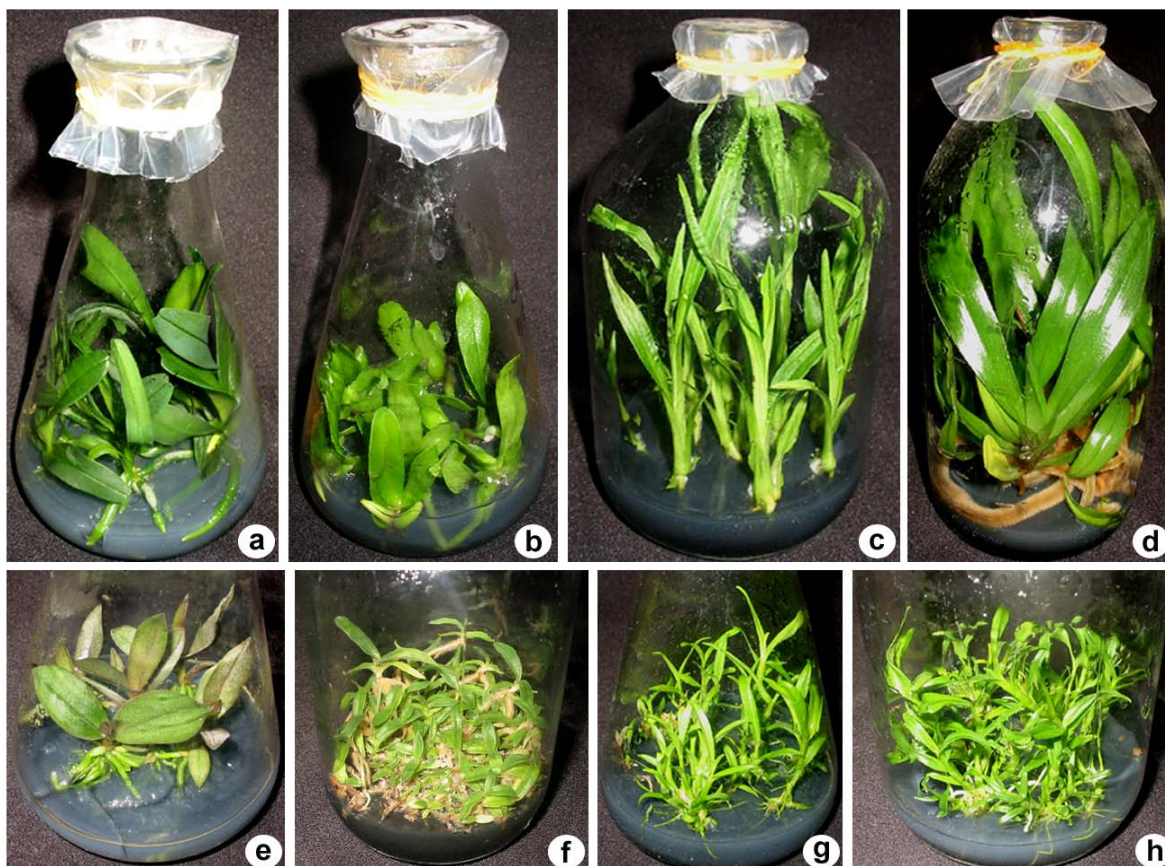


Figure 1. Plantlets of several orchids derived from their seeds germinated *in vitro*
 a. *Aerides odorata*, b. *Vanda parishii*, c. *Phaius tankervilleae*, d. *Paphiopedilum callosum*, e. *Doritis pulcherrima*, f. *Paphiopedilum delenatii*, g. *Dendrobium nobile*, h. *Dendrobium wardianum*

CONCLUSION

All seeds of *Aerides multiflorum*, *Aerides odorata*, *Cymbidium sp.*, *Dendrobium superbum*, *D. wardianum*, *D. nobile*, *D. primulinum*, *Doritis pulcherrima*, *Paphiopedilum delenatii*, *Paphiopedilum callosum*, *Phaius tankervilleae*, *Rhynchostylis gygantea*, *Vanda parishii*, *Vanda denisoniana* orchids germinated *in vitro* with different morphogenesis after 3 months of culture. Seeds developed plants, embryonic-like structures, PLBs, and shoots which depended on the culture media. All *in vitro* seed-derived plants were vigorous and well acclimatized in greenhouse conditions. This study was very important for the preservation of genetic resources and basic researches of plant breeding.

REFERENCES

- ARDITTI J., 1967. Factors affecting the germination of orchid seeds. *Bot. Rev.* 33:1–97.
- ARDITTI J., 1979. Aspects of the physiology of orchids. In: *Advances in botanical research* (Ed: Woolhouse H.W.) 7: 422–697.
- GARAY L.A., 1960. On the origin of the Orchidaceae. *Bot. Mus. Leaflets* (Harvard University). 19: 57–59.
- KNUDSON L., 1946. A new nutrient solution for orchid seed germination. *Am. O. Soc. Bull.*, 15: 214-17.
- KNUDSON L., 1922. Nonsymbiotic germination of orchid seeds. *Bot. Gaz.* 73: 1-25.
- LA CROIX I., 2000. Introduction. In *Orchid basics: selection, hybridization, propagation*. Bounty Books (Great Britain), 2000, pp. 6-10.
- MURASHIGE T. and SKOOG F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Plant. Physiol.* 15: 473-477.
- VACIN F. and WENT F.W., 1949. Some pH changes in nutrient solutions. *Bot. Gaz.* 110: 605-613.