

Asymbiotic Germination of *Dendrobium fimbriatum* var. *oculatum* Hk. f. Seeds on Different Media

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Seed germination of *Dendrobium fimbriatum* var. *oculatum* was tried on six different culture media. While the best germination was recorded on Nitsch's medium, the protocorm development was better in Murashige and Skoog's medium.

Key Words: *Dendrobium fimbriatum* var. *oculatum*, Seed germination, Culture media

Introduction

Orchid seeds are produced in large numbers. They are very minute and possess little or no endosperm. Only in association with a particular fungus, about 5% of the seeds germinate in nature (Rao 1977). On the other hand, a very high percentage of *in vitro* seed germination could be achieved asymbiotically. Using different media and nutrients, seed germination in a large number of orchid species has been accomplished (Stoutamire 1981, Arditti 1982, Arditti & Ernst 1984, Yam & Weatherhead 1988). Although medium concentration has been proved to be an important factor for orchid seed germination, no generalizations can be made due to the limited available information. This communication deals with the germination response of *Dendrobium fimbriatum* var. *oculatum* seeds to different media.

Materials and Methods

About four months old capsules of *Dendrobium fimbriatum* var. *oculatum* Hk. f. were collected from the plants growing in University Botanical Garden. These were surface-sterilized with sodium hypochlorite solution (1.25% available chlorine) for about 15 min and slit open under aseptic conditions to expose the seeds. The seeds were germinated aseptically on six different nutrient media

viz. Knudson C (1946), Vacin and Went (1949), Murashige and Skoog (MS 1962), White (1963), Nitsch (1969) and Mitra et al. (1976). The culture tubes were incubated at $25 \pm 2^\circ\text{C}$ under 8 hr of 1500 lux light.

After two months, percentage germination in different media was determined by examining the seeds microscopically. The seeds were considered to have germinated upon emergence of the embryo from the testa. The protocorm volume was determined using the formula given by Stoutamire (1981) for an oblate spheroid ($\frac{4}{3} \pi a^2b$, where a and b are minor and major axes, respectively). Ten replicates were taken for each medium and the experiments were repeated twice.

Results and Discussion

The best germination was recorded on Nitsch medium (91%), followed by MS (85%) and Mitra et al. (67%) (table 1). Ammonium nitrate, present in the former two media, is considered to be the most suitable source of nitrogen for orchid seed germination (Arditti and Ernst 1984). Mitra et al., Vacin and Went and Knudson C media contain both calcium nitrate and ammonium sulphate which did not support good germination in the present study. The difference in response to nitrogen sources used may be due to varied requirements of this species.

Table 1 Effect of different media on seed germination of *Dendrobium fimbriatum* var. *oculatum*

| Media | % Germination | Remarks |
|---------------------|---------------|---|
| Murashige and Skoog | 85 | Protocorms were round and green and exhibited differentiation into healthy root and shoot |
| Nitsch | 91 | Protocorms were small and green without any differentiation |
| Mitra et al. | 67 | The germinating protocorms formed a yellow callus-like mass |
| Vacin and Went | 48 | The developing protocorms showed signs of differentiation |
| Knudson C | 35 | The protocorms turned brown and did not differentiate |
| White | 20 | Very small green protocorms were produced |

Mixtures of vitamins in the above media improved germination and growth. The seeds of *D. fimbriatum* showed poor germination on Vacin and Went, Knudson C and White media as these do not contain sufficient amounts of nutrients and vitamins. Yam and Weatherhead (1988) reported that the nutritional requirement of orchid seeds for germination varies due to their physiological state.

The time required for germination on various media was different (table 2). The protocorm stage was attained within the first 4-5 weeks on MS, Nitsch, and Vacin and Went media; but this was delayed in other media. The largest protocorm volume was recorded in MS medium. Healthy growth of orchid protocorms in medium containing balanced supply of organic and inorganic nutrients has been reported (Arditti & Ernst 1984). The protocorms developing in Vacin and Went medium were quite large as compared to the ones growing in other media except MS. This could be due to availability of more nutrients to the few developing protocorms. The development of protocorms to the next stage with pointed vegetative apex was

Table 2 Effect of different media on the development of protocorms of *Dendrobium fimbriatum* var. *oculatum*

| Media | Protocorms | | | | | | | |
|---------------------|--------------------|----|----------------|----|--------------|----------------|---------------|------------------------------|
| | Development stage* | | | | Colour | Dimensions** | | |
| | I | II | III (weeks) | IV | | Length (mm) | Width (mm) | Volume (mm ³) |
| Murashige and Skoog | 2 | 4 | 8 | 12 | Dark green | 0.079 | 0.090 | 26.0 × 10 ⁻⁴ |
| Nitsch | 2 | 4 | 10 | 15 | Green | 0.0548 | 0.064 | 9.4 × 10 ⁻⁴ |
| Mitra et al. | 12 | 15 | — | — | Light yellow | 0.045 | 0.049 | 4.2 × 10 ⁻⁴ |
| Vacin and Went | 3 | 5 | 12 | 15 | Green | 0.058 | 0.071 | 12.0 × 10 ⁻⁴ |
| Knudson C | 15 | — | — | — | Brown | 0.024 | 0.032 | 1.2 × 10 ⁻⁴ |
| White | 2 | 12 | — | — | Green | 0.016 | 0.012 | 0.001 × 10 ⁻⁴ |

*I, Non-germinated seeds, embryo slightly swollen and white but still covered with its seed coat or testa;

II, Germinating seeds, embryo greatly swollen forming an ovoid tear-drop shaped protocorm without seed coat or testa;

III, Young protocorms showing pointed vegetative apex;

IV, Protocorms enlarged having root and leaf initials.

**Mean of ten values.

observed in case of MS, Nitsch and Vacin and Went media only. It has been reported that mortality rate is quite high at protocorm stage (Arditti et al. 1981). However, it could be reduced under suitable nutritional and environmental conditions enabling further development of protocorms to the next stage.

The present study reveals that the seeds of *Dendrobium fimbriatum* var. *oculatum* require a rich med-

ium (containing higher concentrations of nutrient salts and vitamins) for both germination and further development.

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References

- Arditti J 1982 Orchid seed germination and seedling culture—A manual; in *Orchid Biology: Reviews and Perspectives*. II. pp 245-370 ed. J Arditti (Ithaca: Cornell University Press)
- and Ernst R 1984 Physiology of germinating orchid seeds; in *Orchid Biology: Reviews and Perspectives*. III. pp 177-222 ed J Arditti (Ithaca: Cornell University Press)
- , Michaud J D and Oliva A P 1981 Seed germination of North American Orchids I. Native California and related species of *Calypto*, *Epipactis*, *Goodyera*, *Piperia* and *Platanthera*; *Bot. Gaz.* **142** 442-453
- Knudson L 1946 A new nutrient solution for the germination of orchid seeds; *Amer. Orch. Soc. Bull.* **15** 214-217
- Mitra G C, Prasad R N and Roychoudhury A 1976 Inorganic salts and differentiation of protocorms in seed callus of an orchid (*Dendrobium fimbriatum*) and correlated changes in its free amino acid content; *Indian J. Exp. Biol.* **14** 350-351
- Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures; *Physiol. Pl.* **15** 473-497
- Nitsch J P 1969 Experimental androgenesis in *Nicotiana*; *Phytomorphology* **19** 389-404
- Rao A N 1977 Tissue culture in orchid industry; in *Plant Cell Tissue and Organ Culture* pp. 44-69 eds J Reinert and Y P S Bajaj (Berlin: Springer-Verlag)
- Stoutamire W P 1981 Early growth in North American terrestrial orchid seedlings; in *Proc. Symp. II and Lectures, North American Terrestrial Orchids* pp. 14-24 ed. E H Planton (Southfield: Michigan Orchid Society)
- Vacin E and Went F W 1949 Culture solution for orchid seedlings; *Bot. Gaz.* **110** 605-613
- White P R 1963 *The Cultivation of Animal and Plant Cells* (New York: The Ronal Press) 228 pp
- Yam T W and Weatherhead M A 1988 Germination and seedling development of some Hongkong orchids I; *Lindleyana* **3** 156-160