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AXENIC GERMINATION OF SOME EPIPHYTIC ORCHIDS OF MEGHALAYA, INDIA.

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Abstract

Axenic seed germination of eight epiphytic orchid species was tried on Knudson C, Vacin and Went, and Pfeffer's media. The Knudson C medium was found better for germination of all the species. The maximum germination percentage was recorded in *Aerides multiflorum* (80%) and the lowest in *Coelogyne prolifera* (14%). The time required for germination and protocorm development ranged from 10 to 15 weeks. The volume of protocorm was largest in *A. multiflorum* and smallest in *C. prolifera*. Top-shaped protocorms were either white or green (even in dark) and developed rhizoids.

Introduction

Orchid seeds, produced in large numbers per capsule, are minute and non-endospermic with reduced embryos. Their germination in nature is dependent upon a suitable mycorrhizal fungus which is believed to provide physico-chemical stimulus required for growth initiation. Several interesting papers have appeared on orchid germination (Arditti, 1967, 1982 Harrison and Arditti, 1970; Stoutamire, 1974; Ernst, 1975; Arditti *et al.*, 1981, 1982; Mitra, 1986). Many media, particularly those proposed by Pfeffer, Barnes, Knudson, Thomale, Heller, Burgeff, and White have been used for the axenic germination of terrestrial and epiphytic orchids (Henrich *et al.*, 1981). None of these media is, however, universal. Orchid germination and subsequent development also varies depending on the light/dark and temperature requirements of the species (Stimart and Ascher, 1981).

The orchids of North-East India are fast disappearing due to ruthless exploitation and habitat destruction. The requirements for their germination and development are little understood for non-availability of seeds and cultural difficulties arising through decreased viability and/or onset of dormancy factors in the mature seeds. *In vitro* germination methods successful for one species are not always applicable to others and also procedures for orchids from one region may not be suitable for those from another (Arditti *et al.*, 1981). The knowledge of germination in axenic conditions is a pre-requisite to the study of the influence of symbiotic microbes. Therefore, the work on axenic seed germination of some orchids of this region was initiated in order to develop practical methods useful to preserve threatened and/or endangered species.

Materials and methods

Cymbidium elegans, *Coelogyne prolifera*, *C. cristata*, *C. porrecta*, *Aerides multiflorum*, *Sarcanthus pellidus*, *Bulbophyllum cosmosus*, and *Thunia alba* procured during August 1982 from different forested areas of Meghalaya (see Table 1) were maintained in net house at the Botany Department, North-Eastern Hill University, Shillong. About four months old unripe capsules of these species

Table 1 Source and per cent germination of orchid species in different media.

Species	Source	Per cent germination in medium		
		Knudson C	Pfeffer	Vacin & Went
Aerides multiflorum	Ranikor (Tropical deciduous forest)	80±2 50	61±2.85	67±2.18
Cymbidium elegans	Mawphlang (Sub-tropical forest)	73±2.30	59±2.44	64±5.44
Sarcanthus pellidus	Cherrapunjee (Sub-tropical forest)	24±1.18	0	0
Thunia alba	Balphakram (Tropical evergreen forest)	46±2.15	0	0
Bulbophyllum cosmosus	Khanjoy (Deciduous forest)	40±2.84	0	24±1.29
Coelogyne cristata	Cherrapunjee (Sub-tropical forest)	56±3.00	0	34±2.15
Coelogyne porrecta	Upper Shillong (Sub-temperate forest)	53±3.25	23±1.24	31±2.65
Coelogyne prolifera	Maheshkhola (Deciduous forest)	14±1.15	0	0

± S.E.

were surface sterilised with 7% calcium hypochlorite solution for 15 min and then washed with sterilised distilled water repeatedly to ensure complete removal of the disinfectant. The capsules were slit-opened and seeds removed under aseptic condition. Seeds were germinated in culture tubes on three nutrient media viz., Knudson C (1946), Pfeffer's as modified by Harvais and Hadley (1967), and Vacin and Went (1949). The cultures were incubated in a seed germinator at 25±2°C in dark for two months and later transferred to continuous light of 3000 lux obtained from a combination of fluorescent and incandescent light. Approximate embryo volume was determined as done for an oblete spheroid ($4/3\ \pi\ a^2b$) where a and b are minor and major semi axes, respectively (Stoutamire, 1981).

Results

The orchid seeds were considered germinated upon the emergence of the embryo from the testa. The germinaton in dark was recorded after two months. Knudson C medium was found better for germination as compared to Pfeffer's, and Vacin and Went media (See Table 1). While in *Aerides multiflorum* and *Cymbidium elegans* a higher percentage of germination was recorded, it was moderate in *Thunia alba*, *Bulbophyllum cosmosus*, *Coelogyne cristata*, *C. porrecta*, and poor in *Sarcanthus pellidus* and *Coelogyne prolifera* when germinated on

Knudson C medium. The seeds of *S. pellidus*, *T. alba*, and *C. prolifera* did not germinate in Pfeffer's, and Vacin nnd Went media. On the other hand *B. cosmosus* and *C. cristata* did not germinate in Pfeffer's medium.

The time required for seed germination varied from 5 to 10 weeks in different species in Knudson C medium (Table 2). The germination occurred first in *A. multiflorum* and *S. pellidus* and last in *T. alba*. The final stages of protocorm development having either root initials or rhizoids were observed in about 10 to 12 weeks time in most of the species. Even in dark the protocorms of *Cymbidium elegans* and *Coelogyne cristata* were green. The protocorms of different species varied in size (Table 2), and some of these are illustrated (Fig 1).

Table 2 Protocorm development in Knudson C medium.

Species	Protocorms							
	Developmental stage*				Colour	Dimensions**		
	I	II	III	IV		Length (mm)	Width (mm)	Volume (mm^3)
	(Weeks required for development)							
Aerides multiflorum	2	5	7	10	White	0.975	0.360	0.363
Cymbidium elegans	2	6	8	10	Green	0.527	0.330	0.237
Sarcanthus pellidus	2	5	10	12	White	0.270	0.270	0.049
Thunia alba	5	10	12	14	White	0.347	0.195	0.042
Bulbophyllum cosmosus	4	7	9	12	White	0.300	0.180	0.040
Coelogyne cristata	3	6	9	11	White	0.360	0.270	0.078
Coelogyne porrecta	2	7	10	12	White	0.375	0.225	0.078
Coelogyne prolifera	5	8	13	15	White	0.340	0.150	0.031

*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-shape protocorm, seed coat or testa; III, Young protocorm showing pointed vegetative apex; IV, Protocorm enlarged having root initials or rhizoids.

**Mean of five values.

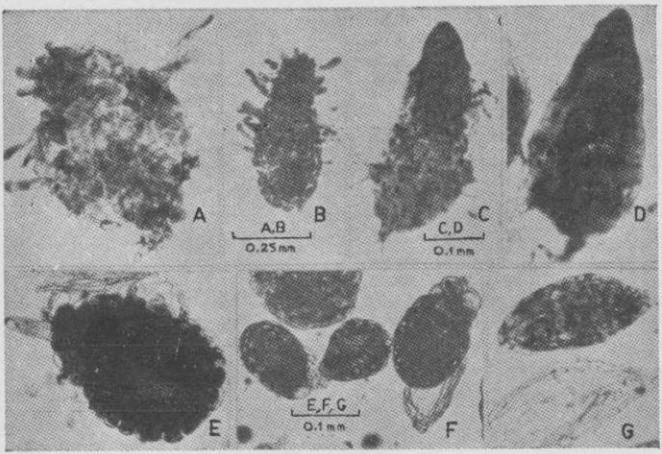

Fig. 1. Twelve week old orchid protocorms : *A, Aerides multiflorum; B, Cymbidium elegans:* C, *Coelogyne cristata;* D, *Coelogyne porrecta* E, *Sarcanthus pellidus;* F, *Thunia alba;* G,*Coelogyne prolifera.*

Discussion

Orchid seeds being smallest produced by flowering plants possess extremely reduced embryo and lack endosperm. These also lack radicle and leaf rudiment during germination. During germination, the embryo enlarges, testa ruptures, and an ovoid top-shaped protocorm is produced (Arditti *et al.*, 1981). Germination in orchid seeds is defined as the formation of green or white protocorms and their subseqent development results by the formation of rhizoids, shoots, and roots.

In the present investigation, the immature seeds were used because it has been reported that upon maturation the seeds of many orchid species show decrease in viability or become dormant (Arditti *et al.*, 1981). Mature seeds also take longer time to germinate and development of their seedlings is slower. The reasons for this reduced germination of mature seeds are not known.

The light may promote or inhibit germination of orchid seeds. Most of the epiphytic orchids germinate in both light and dark but require illumination for further development (Arditti 1979). The present results show that light did not inhibit germination in all the species tested. The protocorms of *A. multiflorum*, *S. pellidus*. *T. alba*, *B. cosmosus*, *C. porrecta*, and *C. prolifera* were insensitive to light during the course of the investigations. They were initially white

and remained white upon illumination. On the other hand, protocorms of *Cymbidium elegans* and *Coelogyne cristata* were green even in dark but showed pronounced growth when illuminated.

In the present investigations, Knudson C medium was found better for seed germination as compared to Pfeffer's, and Vacin and Went media (Table 1). This could be due to the fact that Knudson C is quite rich in macro- and micronutrients and it aiso contains sucrose. The germination of *A. multiflorum* from immature seeds was highest (80%), whereas lowest germination was observed in *C. prolifera* (14%). The literature on orchid seed germination has many reports on the suitability of basal Knudson C medium. In many instances, however, a modified version of this medium variously supplemented with peptone, tomato juice, fish emulsion, protein hydrolysate, orange juice, cotton seed meal, carbohydrates, vegetable charcoal, ripe banana homogenate, bark substrate, and amino acids has been found suitable (Ernst, 1976; Arditti, 1982), thereby suggesting that the requirements of orchid seeds vary from one another during germination.

We are continuing with our experiments to assess the effect of various physico-chemical factors on germination and growth of orchids indigenous to North-East India. The knowledge gained by these studies would be useful to manage existing populations and re-establish some which have disappeared.

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