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Studies on Asymbiotic Germination of Orchid Seeds

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ABSTRACT

Asymbiotic germination of seeds of eight epiphytic orchid species viz., ***Cymbidium elegans, Dendrobium nobile, Sarcanthus pallidus, Bulbophyllum comosum, Thunia alba, Coelogyne porrecta, C. prolifera*** and ***C. cristata*** was studied. The differentiation of upper and lower part of embryo in germinated orchid seeds has been observed. The seeds developed protocorm after 8 10 weeks of sowing. Maximum seed germination was observed in ***Cymbidium elegans*** (75.56%) and minimum in ***Coelogyne prolifera*** (5.55%). The quantitative variation in embryo in different orchids was also noted.

INTRODUCTION

Orchids grow luxuriantly and form a dominant constituent of epiphytic community in natural subtropical forests of north eastern India. But due to prevailing practice of shifting cultivation in N. E. Region, majority of the orchid species are either endangered or have become extinct. No work on their multiplication is available from this Region. Therefore, studies on orchid seed germination may be useful in managing the existing populations and multiplication of threatened species. Though the seeds of orchid are produced in large numbers in each capsule, they have very less food reserve and some of them may even be devoid of it. They possess a minute embryo, which contains gemma-like simple constriction. Studies on asymbiotic seed germination have been carried out by several workers (Ardittietal, 1981 ; Ernst, 1974 ; Knudson, 1950 ; Nishimura, 1981 ; Nakamura, 1982). Seed germination of orchids of N.E. region has not been studied. The present investigation was carried out to test the germination ability of orchid seeds in asymbiotic conditions.

MATERIALS AND METHODS

The green pods were collected from orchid plants maintained in the orchidarium of North-Eastern Hill University, Shillong (Meghalaya). The green pods were rinsed in calcium hypochlorite solution (7% W/V) for 15 minutes. The pods were washed several times in sterilized distilled water. One side of the pod was cut off with sterilized scalpel. The seeds were taken out by long, pointed and hooked sterilized needle. Knudson's medium was prepared and pH was adjusted to 5.00 after autoclaving (Knudson, 1946). The medium was poured in culture tubes. The seeds collected in sterilized condition from pods were sown on the surface of the medium. The seeds were incubated at 20 $\pm$ 2°C, in BOD incubator. Observations on seed germination were made after 8 week. Germination of seeds was observed by recognising the spliting of the testa, followed by the emergence of the protocorm. Minimum 100 seeds of each species were taken for calculating the germination percentage.

RESULTS AND DISCUSSION

The maximum secd germination was observed in *Cymbidium elegans* and minimum in *Coelogyne prolifera* (Table 1), while maximum embryogenous seeds were found in *C. elegans*. Seeds of *C. elegans, Thunia alba* and *Sarcanthus pallidus* germinated after 64 days, while *C. prolifera, C. cristata* and *Bulbophyllum comosum* took 80 days for their germination (Table 2). Majority of the seeds had brownish and fusiform ellipsoidal pro-embryo (Plate I). The seed coat was composed of hyaline, elongated cells. Following the sowing of seeds in nutrient agar medium, the embryo swelled and emerged out by rupturing the seed coat. The swollen embryo assumed an oval to pear shaped structure (Plate I). Various stages of germination were observed (Table 2 and Plate I). The embryo became green after attaining an appropriate size. Gorminated seeds of *C. elegans, S. pallidus* and *T. alba* species produced elongated absorbing root hairs or rhizoides along the lower sides of the protocorm (Plate I), while in *C. prolifera, C. cristata* and *Dendrobium nobile* very less number of rhizoids were produced (Plate I). The protocorms of *C. elegans,* and *S. pallidus* are characterised by much longer rhizoids, while in *T. alba* and *C. porrecta* the rhizoids were short (Plate I). *C. prolifera* and *B. comosum* also showed less and short rhizoids than *C. cristata* and *D. nobile* (Plate I).

The time for initiation and emergence of root and leaf primordia differed. The protocorm tissue, composed of dense mass of small cells at apical end, gave rise to the shoot and leaf primordia, whereas, the tissue at the distal end was the site for root formation (Plate I). The size of embryo varies and thus selection of seeds with proper size of embryo may be helpful in the regulation of orchid seed germination. The variation in percentage of seed germination and different stages of their germination could be accounted for physiological activity and nutriant status of seeds. Enhanced orchid germination is achieved by symbiotic fungi (Clements and Ellyard, 1979 ; Hadley, 1970; Knudson, 1925 ; Worcup, 1973). Necessity of fungal symbionts has been emphasised in germination, but on the other hand the nutrient medium enriched with vitamins, growth hormones and amino acids could supplement the role of fungal symbiont (Arditti, 1967 ; Ernst, 1967a, 1974 ; Fonnesbech, 1972 ; Hadley and Harvais, 1968).

The species which showed the early and fast seed germination, could be exploited for fast multiplication (Mitra, 1979:. Seeds also varied in the structure of testa, embryo and the pigmentation. These factors may also contribute to the variability of orchid seed germination. Leaf, stem and root primordia developed rapidly in *C. elegans, S. pallidus,* and *C. porrecta.* The adult plants of these species usually grow in wet and bright light conditions whereas, other species grow in relatively dry habitati, Such ecological environment may also affect to the physiological activity of seeds.

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Table 1. Percentage of seeds with embryo and their germination (%) on Knudson's medium.

Orchid species	Germination (%)	Seeds with embryo (%)
Cymbidium elegans	75.56	78.23
Sarcanthus pallidus	64.37	67.21
Thunia alba	49.72	60.54
Dendrobium nobile	10.61	70.29
Bulbophyllum comosum	31.25	55.55
Coelogyne porrecta	24.51	34.71
C. prolifera	5.55	73.14
C. cristata	30.18	34.27

Table 2. Different stages of seedling growth in various orchid species.

Orchid species	Stages of seedling growth			Time taken for seed germination (Days)
	Protocorm (%)	Protocorm with less than 5 primordia (%)	Protocorm with more than 5 primordia (%)	
Cymbidium elegans	62	23	15	64
Sarcanthus pallidus	66	22	12	64
Thunia alba	68	23	9	64
Dendrobium nobile	92	8	0	80
Bulbophyllum comosum	95	5	0	80
Coelogyne porrecta	46	37	17	80
C. prolifera	98	2	0	80
C. cristata	84	16	0	80

Legends of Plate I

Fig. 1. Germinated seed of *Cymbidium elegans* showing the roots primordia (rp) in bunch arising from the both side and leaves primodia (lp) from the apical end (X 40).

Fig. 2. *Coelogyne cristata* showing leaf primordia from apical end (X 400).

Fig. 3. *Sarcanthus pallidus* protocorm showing roots from both side and initial stage of leaves from apical end (X 100).

Fig. 4. Protocorm of *Bulbophyllum comosum* showing root primordia from each side and leaves from apical end (X 100).

Fig. 5. *Coelogyne porrecta* showing roots primordia from both side and initial stage of leaves at apical end (X 100).

Fig. 6. Dendrobium nobile protocorm showing three root primordia at lower end (X 400).

Fig. 7. Germinated seed of *Thunia alba* where the leaves primordia formed from apical end and roots from only one side (X 100).

Fig. 8. *Coelogyne prolifera* protocorm showing leaves from apical end and roots from both side (X 250).

PLATE I