

## **In vitro germination of seeds of some rare tropical orchids**

Lyudmila Buyun\*, Alla Lavrentyeva, Lyudmila Kovalska,  
Roman Ivannikov

N.N. Grishko National Botanical Garden, National Academy of Sciences of Ukraine,  
Timiriazevskaya 1, Kiev 01014, Ukraine

\*Corresponding author, E-mail: lbuyun@i.com.ua

### **Abstract**

The objective of the study was to develop an appropriate method for *in vitro* seed propagation of *Cattleya aelandiae* Lindl., *Cattleya bowringiana* Veitch, *Cattleya granulosa* Lindl., *Cattleya percivaliana* O'Brien, *Cattleyopsis lindenii* (Lindl.) Cogn., and *Dendrobium parishii* Rchb.f. species, members of the *Epidendroideae* subfamily of the *Orchidaceae* family. Special attention was paid to studying the morphology of seeds, different stages of protocorms, and seedlings produced *in vitro*. Two seed types were been recognized according to the classification of Dressler. The highest percent of seed germination (about 100 %) was obtained on the standard Knudson medium supplemented by 2 g l<sup>-1</sup> peptone, 50 mg l<sup>-1</sup> potassium hummate and 1 g l<sup>-1</sup> activated charcoal. It was shown that *Dendrobium parishii* had the shortest (about 300 days) period of *in vitro* cultivation, and *Cattleyopsis lindenii* had the longest (about 600 days). The formation of *Cattleya* seedlings, on average, took about 500 days. By this time, the most advanced seedlings had been transferred to glasshouse. The use of immature seeds capsules as a seed source shortened the reproductive cycle by approximately 2 to 2.5 months. The results of these experiments showed that all of the studied species can be effectively propagated by *in vitro* seed culture with the aim of *ex situ* biodiversity conservation.

**Key words:** *in vitro* culture, orchids, propagation, seed germination.

### **Introduction**

The application of *in vitro* seed propagation technique to rare tropical orchid species, which are suffering from over-collecting and continuous loss of their natural habitats, undoubtedly is a powerful tool for *ex situ* biodiversity conservation (Stenberg, Kane 1998; Gangaprasad et al. 1999). Many tropical native orchid species have been propagated in the National Botanic Garden of National Academy of Sciences of Ukraine through a range of asymbiotic seed germination techniques and tissue culture procedures aimed to preserve a number of individuals under artificial conditions in glasshouses in the temperate zone, with the aim to protect these species from complete extinction.

The objectives of this study were (i) to elaborate a method of seed propagation for four *Cattleya* species – *C. aelandiae* Lindl., *C. bowringiana* Veitch, *C. granulosa* Lindl., *C. percivaliana* O'Brien, as well as *Cattleyopsis lindenii* (Lindl.) Cogn. and *Dendrobium parishii* Rchb.f.; (ii) to describe the seed morphology; (iii) and to study the development of protocorms and seedlings *in vitro*.

## Materials and methods

To obtain seeds, flowers of the studied species were self-pollinated by hand under glasshouse conditions in the National Botanic Garden. Seeds and young seedlings were grown on Knudson (1992) medium modified by addition of 2 g l<sup>-1</sup> peptone, 50 mg l<sup>-1</sup> potassium hummate, 1 g l<sup>-1</sup> activated charcoal. For proliferation of protocorms, Murashige and Skoog (1962; MS) medium supplemented by 5 mg l<sup>-1</sup> benzylaminopurine (BAP) and 2 mg l<sup>-1</sup> naphthalenacetic acid (NAA) was used.

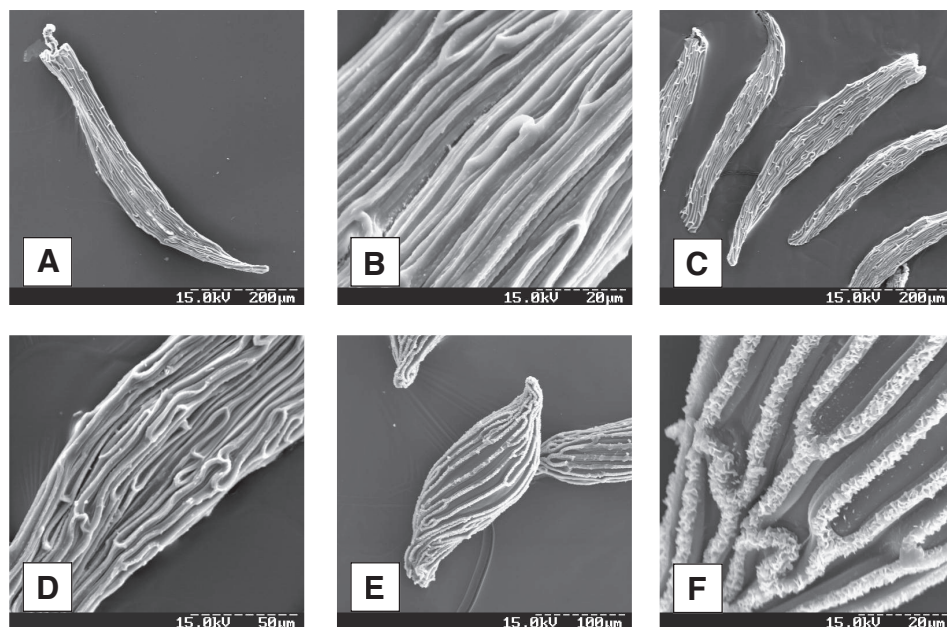
Seeds from dehisced capsules were sterilized in 10 % Clorox for 15 to 20 min, in 15 % H<sub>2</sub>O<sub>2</sub> for 10 min, then rinsed two times with sterile distilled water. Undehisced immature capsules were surface-sterilized as follows: rinsed with tap water for few minutes, then flamed after spraying with 96 % ethanol. Capsules were cut open and seeds were transferred to cultivation media.

The cultures were incubated in 250-ml Erlenmeyer glass flasks in the laboratory at 25 - 26 °C, photoperiod 16 h and relative moisture of air 70 %. After sowing of seeds, flasks were inspected for seed germination every seven days. The examination of seeds under a SELMI REMMA-102 scanning electron microscope was carried out in the secondary electron emission regime. Prior to examination, dry seeds were mounted with double-sided adhesive tape on aluminium stubs. Then they were carbon-copper-coated using a rotation and tilting specimen stage. For uniform coating of carbon and copper a thermal vacuum evaporator was used. The size of the seeds were measured on the scanning electron micrographs. Micrography was undertaken at an accelerating voltage of 15 kV and working distance of 19 - 22 mm. General classification of seed types followed Dressler (1993).

## Results and discussion

The seeds of all species studied were minute, dust-like, ranging from 280 µm (*Dendrobium parishii*) to 830 µm (*Cattleya bowringiana*) in length. The width of seeds did not differ significantly and ranged from 70 µm (*Cattleya percivaliana*) to 100 µm (*Cattleya granulosa*). The seeds of *Cattleya* spp. and *Cattleyopsis lindenii* usually were yellowish, elongate, oblong or slightly fusiform or narrow spindle-shaped. The general morphology of the seeds of all *Cattleya* spp. and *C. lindenii* were almost identical (Fig. 1), and belonged to the *Epidendrum*-type (Dressler 1993). Seeds of *D. parishii* were brightly-yellow, seed testa was intransparent, covered by very fine warts (Fig. 1F), corresponding to the *Dendrobium*-type (Dressler 1993). The seeds usually had the largest diameter near the middle, narrowing toward the polar ends. All seeds had an aperture, the micropylar pole, in one of the ends.

Seed germination of *Cattleya* species on average began after 2 or 3 weeks of culture. By this time embryos had enlarged by two times and occupied the whole seed coat. The seed coat split and the embryos developed into protocorms of 1 to 1.8 mm in length one month after sowing. Initially developing protocorms were elliptic or elongate, becoming clavate or pearshaped with a blunt apex. The colouration of protocorms varied from milk-white at the beginning of germination to bright-green some time later. As a rule, three to four or more epidermal hairs were produced at this stage. It was observed that the shape of protocorms was species-specific. The protocorms were formed by undifferentiated highly



**Fig. 1.** Scanning electron micrographs of seed specimens studied. A, *Cattleyopsis lindenii*, general view of seed. B, *Cattleyopsis lindenii*, testa cells. C, *Cattleya percivaliana*, general view of seed. D, *Cattleya percivaliana*, testa cells. E, *Dendrobium parishii*, general view of seed. F, *Dendrobium parishii*, testa cells.

vacuolated parenchymal cells, which were surrounded by a single layer of epidermal cells. Later, in apical zone of protocorms the formation of apex and leaf primordia were observed. This was accompanied by the differentiation of procambial and vascular bundles. The formation of protocorms with many meristematic apices began.

In general, during the *in vitro* germination of seeds of *Cattleyopsis lindenii* and *D. parishii*, similar developmental stages of protocorms and seedling were observed. Newly flasked seed of *D. parishii* were noticeably swollen and began greening after 15 to 20 days on nutrient medium. By approximately 45 to 50 days the protocorm stage was reached. At that time the apical meristem with a single leaf primordium had formed on the upper part of the protocorm. One-year-old seedlings of *D. parishii* generally consisted of 3 or 4 leaves and 2 or 3 axillary buds, with a well developed root system formed by adventitious roots 20 mm in length. It is interesting to note that seedlings of *D. parishii* underwent the same annual cycle of development *in vitro* as adult plants under greenhouse conditions. During a 1- to 1.5-months period (November–December) they rested in the flasks: leaves fell and no symptoms of growth were visible. This can be explained by the fact that in nature *D. parishii* grows in deciduous forest in South-East Asia, where the climate is strongly seasonal with successive dry and wet periods (Lavarak et al. 2000).

In comparison with the other species studied, *D. parishii* had the shortest (about 300-day) period of cultivation *in vitro* – from seed sowing to transferring plants into glasshouse conditions; *Cattleyopsis lindenii* had the longest cultivation period (about 600 days). The formation of *Cattleya* seedlings, on average, took about 500 days. By this time

the most advanced seedlings had been transferred to glasshouse culture conditions. It was shown that, for successful acclimatization, the substrate for seedlings must provide sufficient aeration while holding enough moisture for root development.

Orchid capsules are dehiscent, and seeds for *in vitro* germination may be obtained after dehiscence or even before when the capsule is still unripe. Several studies have shown that the seeds from immature capsules can germinate *in vitro* much earlier (Arditti et al. 1981; Stancato et al. 1998). Our results showed that the capsules of all species studied ripened by 7 to 7.5 months (*C. aclandiae*, *C. bowringiana*, *Cattleyopsis lindenii*, *D. parishii*) or by 9 to 10 months (*C. granulosa*, *C. percivaliana*) after pollination, but seeds from unripe capsules germinated *in vitro* much earlier. Therefore the risk of seed loss and its contamination can be eliminated and the length of the reproductive cycle sufficiently shortened. Also, the utilization of seeds from unripe capsules allows to avoid the negative influences of sterilized substances.

The use of immature seed capsules as a seed source shortened the reproductive cycle by approximately 2 to 2.5 months. The results of these experiments showed that all of the species studied could be effectively propagated by *in vitro* seed culture.

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