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IN VITRO PROPAGATION OF *CATTLEYA* LINDL. AND *LAELIA* LINDL. SPECIES

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Nowadays many wild species of South American's orchids are under threat of extinction from over-collection and habitat destruction. Many tropical native orchid species were propagated in the National Botanical 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. Our orchid collection includes plants of *Cattleya* and *Laelia* species. Some of these species are rare in the wild. To protect them from extinction the methods of propagation should be developed.

Thus the objective of this study was to elaborate a methods for mass rapid seed and clonal propagation *in vitro* of five *Cattleya* species (*C. aclandiae* Lindl., *C. bowringiana* Veitch., *C. granulosa* Lindl., *C. intermedia* Graham. ex Hooker., *C. percivaliana* O'Brien.), and seven *Laelia* species (*L. anceps* Lindl., *L. lobata* (Lindl.) Veitch., *L. lundii* Rchb. f. et Warm., *L. mantiqueirae* Pabst., *L. purpurata* Lindl., L. *rubescens* Lindl., *L. sincorana* Schltr.), to study the development of protocorms and seedlings in vitro[a, b].

To obtain seeds, flowers of the studied species were self-pollinated by hand under glasshouse conditions in the National Botanical Garden. The seeds of *L. rubescens* were received from the Main Botanical Garden (Moscow, Russia) in January 2001.Our preliminary results showed that the capsules of orchid studied usually, ripened about 9-10 months after pollination, but seeds from unripe capsules can germinate *in vitro* much more earlier. Therefore the seeds from immature capsules about halfway of maturation were used for sowing on Knudson medium modified by addition of 2 mg/l peptone, 50 mg/l potassium hummate, 1 mg/l activated charcoal.

Seeds from dehisced capsules were sterilized in 10% Clorox for 15 to 20 min, in 15% H_2O_2 for 10 min, and then rinsed two times with sterile distilled water. Undehisced immature capsules were surface-sterilized as follows: rinsed with tap water for five 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^{0}C, photoperiod 16h and relative moisture of air 70%. After sowing of seeds, flasks were inspected for seed germination and pathogen infection every seven days.

Seed germination of Cattleya and Laelia species on average began after 2 or 4 weeks of culture (tab. 1). Developing embryo exceeded initial size of embryo in 2 or 4 times, forming protocorms which shape is species-specific.

The protocorms were formed by undifferentiated highly vacuolated parenchyma cells, which are surrounded by a single layer of epidermal cells. For proliferation of protocorms the MS medium supplemented by the addition of 5 mg/1 BAP and 2 mg/1 NAA was used. Process of protocorm formation with many meristematic apices was highly influenced by the

[a] NBG's collection of tropical orchids was registered in Administrative organ CITES in Ukraine (notification ? 6939/19/1-10 from 23.06.2004).

[b] The name of species are given according to C. Withner 1990, 1991.

TABLE 1. The terms of seeds germination and seedlings propagation of *Cattleya* and *Laelia* species.

Nº	Species	Start germination	Seedlings formation	Ex vitro transplantation
			days	
1	*Cattleya acladiae* Lindl.	86	260	350
2	*Cattleya bowringiana* Veitch	16	134	300
3	*Cattleya granulosa* Lindl.	10	120	400
4	*Cattleya intermedia* Graham ex Hook	97	323	871
5	*Cattleya percivaliana* O'Brien	40	260	539
6	*Laelia anceps* Lindl.	95	287	1458
7	*Laelia lobata* (Lindl.) Veitch	11	240	1080
8	*Laelia lundii* Rchb.f. & Warm.	60	135	300
9	*Laelia mantiqueirae* Pabst	5-7	90	915
10	*Laelia purpurata* Lind.	27	191	394
11	*Laelia rubescens* Lindl.	17	270	930
12	*Laelia sincorana* Schltr.	80-90	270	400

level and distribution of exogenic hormones in cultural media. Later, in apical zone of protocorms the formation of apex and leaf primordia of shoot was observed. This was accompanied by the differentiation of procambial and conducting bundles.

Seedling formation, in average, took about 700 days (tab. 1). By the time seedling were transferred to glasshouse culture conditions. More over, seedling (and plantlets) also may be use as a secondary explants to enlarge the coefficient of propagation.

It should be noted that the process of ontogenesis of *Laelia* and *Cattleya* seedlings are quite similar. The differences are only in terms and details of development. The development of individuals in juvenile population of seedlings is not similar in vitro. The investigation has shown, that the seedlings have some pathways of ontogenesis in vitro. It was established, that at the initial stages of seedlings ontogenesis in vitro go through two basic patterns of development. For majority of species studied formation of secondary protocorms on primary protocorms are typical. The number of the seedlings, which have been developing through each of patterns, depends not only on abiotic factors complex. It is defined by interaction: a genotype ↔ a nutrient medium composition.

Different methods of clonal plant micropropagation of *Cattleya* and *Laelia* cultivars genotypes were developed in vitro culture. For propagation young growing shoots of 10-15 cm in height were selected. Apical and lateral shoots were used as initial explants. The buds of basal part of shoot had the highest morphogenetic potencies. Buds of some species after 6 months of cultivation on nutrient medium formed about 70 protocorms, while the middle part of shoot formed not more than 10-15 protocorms. So, we determined that apical meristems of young shoots of all genotypes studied are inert under cultivation in vitro. It doesn't develop later. Explants ability to regenerate depends on phase of plant-donor development. Buds found in May-June form protocorms more intensively and quickly, that is provoked by increasing of phytohormonal complex activity of plants in this period. We used different modifications of nutrient media for cultivation of Cattleya's and Laelia`s explants. Optimal medium for protocorm proliferation was MS with 5 mg/1 BAP, 2 mg/1 NAA, 100 mg/1 peptone, 15% coconut milk, 1.5 g/1 activated charcoal. More intensively protocorms were formed in darkness. The most active zones of protocorm formation are bases of leaf primordiums and bud squamules. As a rule 4-5 meristematic centers with lots of protocorms form simultaneously, they can be divided and cultivated.

Our research was carried out to examine the suitability of the basal and lateral buds of young shoots as explants for mass rapid clonal propagation of

species studied. The size of these explants did not exceed 0,5-1,0 cm. It was established that basal buds of shoot have the highest morphogenetic potencies.

Thus, combining some methods of seed and micropropagation in vitro we can get planting material of these beautiful ornamental plants. Effective method of Cattleya and Laelia plants micropropagation is induction of protocorm formations on leaves of plantlets and seedlings. Leaves were carefully separated from stem and cultivated on MS with 2 mg/1 BAP, 0.3 mg/1 NAA, 15% coconut milk. After one month at the base of leaves, at first from epidermal tissues form numerous of protocorms, followed by shoots formation.

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