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POST-ASEPTIC CULTIVATION OF SEEDLINGSOF SEVERAL TROPICAL SPECIES OF *LAELIA* LINDL. (*ORCHIDACEAE* JUSS.)

K e y w o r d s: Laelia Lindl., Orchidaceae, in vitro cultivation, post-aseptic cultivation.

Introduction

At present, propagation of the species of *Orchidaceae* Juss. (orchids) under sterile growing conditions is the most efficient way of obtaining large numbers of good-quality planting material. There is a sufficient experience of the seed and clonal propagation of tropical orchids *in vitro* culture available from the literature; however, the data on the search of mechanisms for increasing the adaptability of seedlings and regenerant plants after transferring them from sterile conditions to the conditions of autotrophic nutrition are fragmental.

It is known that the central aspect of phenotypic expression is a plastic reaction of orchids from various ecotypes to changes of external (environmental) conditions. An adaptive value of the plastic characters of epiphytes or overground plants depends on the functional state of specific peculiarities and their natural ecological conditions because they influence the adaptation and expression of plastic characteristic features. Having the close cycles of development, species of the same genus sometimes differ essentially in their phenotypic reaction on light, water, and heat. Thus, before elaborating post-aseptic cultivation methods, it is necessary to assess their theoretic base and to determine the mechanisms of growth and development control of plants by means of the thorough analysis of climatic and edaphic peculiarities within the natural ranges of plants, determination of their life forms and studies of their anatomical, physiological and biochemical peculiarities.

The main environmental factors are important for cultivation of orchids in greenhouses. The life forms and the rhythms of growth and development of orchids are important for evaluating their biotechnology.

According to the modified version of Raunkiaer's system of life forms [12], the majority of orchids belong to aerophytes, overground plants, and epiphytes; however, many orchid species are intermediate between the epiphytes and overground plants. The orchids occurring in the tropical and subtropical zones are mainly epiphytes and so-called «intermediate» plants; meanwhile, overground orchids occur in the tropics, subtropics, and the temperate zone.

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In the past, orchids were cultivated in greenhouses mainly on the rather complicated nutritious mixtures imitating the natural conditions [8], and at present there is a tendency of standardization of soil substrata [9]. In most cases, the nutritious mixtures are developed based on natural conditions, but without taking into consideration anatomical and physiological peculiarities of orchids; therefore, quite frequently plants acclimatize badly or even die.

The overground orchids are cultivated on nutritious mixtures containing large amounts of forest litter; epiphytes require for their cultivation another substratum (e.g., bark, charcoal, etc.), and the most difficult problem is to cultivate aerophytes as a rather large group of orchids growing mainly in the regions with xerophytic climate types, on rocks, or on tree trunks. If using for aerophytes the classical approaches of non-sterile cultivating, their seedlings and regenerants perish or grow weakly without flowering. Therefore, only a «block-culture» [10] is suitable for cultivating of the aerophytes *in vivo*.

It is evident from the above that development of a post-aseptic cultivation technology of tropical orchids in the temperate climate is important and topical. Therefore, our goal was to make a comparative analysis of natural ecological conditions of four species of *Laelia* Lindl. as the taxa most interesting because of their ornamental value, to study their anatomical and physiological peculiarities, and to work out proposals for optimizing the transfer of seedlings from the sterile conditions to the conditions of autotrophic nutrition (*in vivo*).

Material and methods

Our objects were *L. lobata* (Lindl.) Veitch. *L. lundii* Rchb. f. et Warm. (both epiphytes or lithophytes), *L. purpurata* Lindl. (epiphyte) and *L. sincorana* Schltr. (aerophyte). We studied 3–4-year old plants, and observed them during the year in a peculiar climatic chamber under 19–25 °C, 8–10 hours of light, 70–90 % humidity, and 3000–4000 ‹Š of artificial light. The structure of various substrata used for cultivation of *Laelia* species seedlings are presented in Table 1.

The content of photosynthetic pigments was studied using a spectrophotometric method (SF-2) of Pochinok [4]. The measuring was made under the wavelength 440 (carotinoides), 644, and 662 nm (chlorophylls). We used 90 % acetone for the extraction of pigments, and then determined the content of photosynthetic pigments in mg per 100 g of wet mass. We used a microscope MBI-15 for the calculation of chloroplasts, mesophyll cells, and for studying stomata. In addition to that, we

Table 1. **Structure of various substrata**

Variants	Components of substrata	Proportion
1	peat moss	—
2	mixture of polyacryl-nitril and basaltic fibers	—
3	peat moss + pine bark	1:2
4	peat moss + oak bark	1:2
5	granite crumb	—
6	pine bark + peat moss + sand	1:1:1
7	pine bark + peat moss + sand	1:2:1
8	pine bark + peat moss + sand	2:1:2

performed anatomical studies using generally accepted methods. For studying the physico-chemical characters of soil, we used traditional approaches of physical analysis. The results were processed according to the standard statistic methods [1].

Results and discussion

All studied species occur in Brazil, which ischaracterized by diverse climatic conditions; from equatorial in the northern part to temperate in the southern part [13]. The main climatic characteristics of the species ranges are given in Table 2.

Table 2. **Average annual climatic indices within the ranges of the studied species**

Species	Temperature, °C		Precipitation mm/month		Air moisture, %		Altitude a.s.l., m
	max	min	max	min	max	min	
L. purpurata	27 XII—II	12 V—VIII	600 XII—II	200 V—IX	90 XII—I	70 VII	10—100
L. sincorana	23 I—III	17 VII—VII	170 XII—III	50 VII—XI	78 I	50 VIII	1000—1100
L. lobata	26 I	20 VII	160 I	50 VII	80 I	75 VI	200—800
L. lundii	25 II—III	12 VI	2000 X—IV	100 VI—VII	84 XII—II	50 IV	100—500

As seen from Table 2, the plants of *L. lobata* and *L. sincorana* occur in rather xerophytic regions (with average precipitation within 50—170 mm), meanwhile, *L. purpurata* and *L. lundii* occur mainly in rather damp forests (with precipitation 100—2000 mm). It is remarkable that the maximum and minimum annual temperatures almost everywhere within their ranges are similar, but their limits during the daytime and nighttime are approximately 7 °C. We have to note that the environmental conditions of the high-mountain habitats of *L. sincorana* are harsh, or even extreme.

Precipitation is much lower at higher altitudes; e.g., within the range of *L. lundii* (lowlands) it is approximately 2000 mm and within the range of *L. sincorana* (highlands), 150 mm per month. This phenomenon is reflected in the anatomical structure of leaves of the orchids and in peculiarities of their photosynthesis (Table 3).

According to the data of the comparative quantitative-anatomical analysis, the studied species differ essentially in the structure of their leaf blades. We noted their distinctive features in the peculiarities of stomata (number, length and width) (Fig. 1), thickness of cuticle (from 17.4 to 26 mkm on the upper side of leaves, and from 10 to 26 mkm on the lower side), the mesophyll cell number (on both upper and lower sides; on the lower side their number is higher), and in the number of chloroplasts. Besides, we observed in *L. sincorana* a deep position of the stomata cells and their position at the level of the cuticle cells in other species. Meanwhile, the shape and size of

Table 3. **Some peculiarities of a leaf anatomical structure of the studied species**

Characters	Species			
	L. lobata	*L. lundi*	*L.purpurata*	*L. sincorana*
Stomata				
length, mkm	36.5±0.3	35.3±0.1	46.2±0.1	42.3±0.4
width, mkm	26.7±0.1	27.3±0.2	34.3±0.1	32.6±0.2
number per mm^2	78.3±0.2	37.8±0.4	54.0±05	40.5±0.2
Thickness of cuticula, mkm				
upper leaf side	26.0±0.3	17.4±0.5	20.1±0.4	22.3±0.4
lower leaf side	21.7±0.3	10.4±0.1	10.0±0.1	26.5±0.2
Mesophyll cell* number per mm^2				
upper leaf side	389.3±0.1	206.3±0.3	156.3±0.4	303.1±0.3
lower leaf side	519.0±0.4	260.1±0.1	237.4±0.2	432.5±0.3
Chloroplasts				
length, mkm	5.2±0.2	5.0±0.1	6.0±0.2	5.2±0.4
width, mkm	5.2±0.3	5.1±0.3	8.2±0.4	5.4±0.3
number per cell	35.6±0.1	35.2±0.3	27.1±0.4	30.4±0.4
number per 1 mm	16167±10	8208±10	5305±10	11033±10

*Mesophyll cell number was calculated on a leaf cross section

chloroplasts are closely similar in all species (except *L. purpurata*, which has slightly larger chloroplasts).

The Table 4 presents our data on the content of photosynthetic pigments in leaves of the studied species.

Table 4. **Content of the photosynthetic pigments in leaves of Laelia species (mg/100 g of fresh weight)**

Species	1	2	3	4	5	6	7
L. lobata	24.3±3.9	1.2±0.2	25.5	20.6	13.4±1.9	38.9	1.9
L. lundii	18.4±2.4	1.5±0.1	19.9	12.3	7.6±0.8	27.5	2.6
L. purpurata	28.7±4.7	6.6±0.7	35.3	4.3	8.3±1.0	43.6	4.2
L. sincorana	13.8±2.1	0.6±0.01	14.4	23.0	11.4±1.2	25.8	1.3

Note on horizontal pigments: 1 — chlorophyll «*a*», 2 — chlorophyll «*b*», 3 — chlorophylls «*a*»+»*b*», 4 — index «*a*»/»*b*», 5 — carotenoids (K), 6 — «*a*»+»*b*»+K, 7 — index «*a*»+»*b*»/K.

According to our data, *L. purpurata* is characterized by the highest content of all photosynthetic pigments and both chlorophylls (43.6 and 35.3 mg), and *L. sincorana* is characterized by their lowest content (25.8 and 14.4 mg). Meanwhile, the highest content of carotenoids was noted in *L. lobata* (13.4 mg) and the lowest one, in *L.lundii* (7.6). By indices «*a*»/»*b*» and «*a*»+»*b*»/K *L. purpurata* essentially

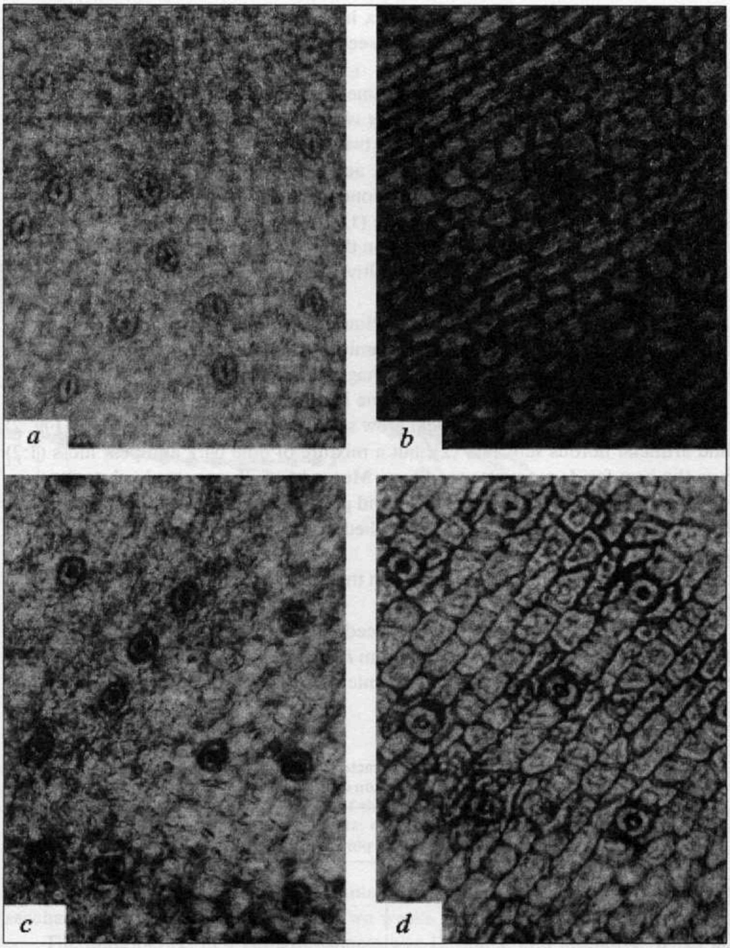

Fig. 1. External view of *Laelia* species stomata (9 ×16). Symbols indicate: *a* — *L. lobata*, *b* — *L. lundii*, *c* — *L. purpurata*, *d* — *L. sincorana*

differs from other species: the first index in that species is lowest (because of the high content of chlorophyll *b*) and the second one is highest (because of the low content of carotenoids).

As a result of our experiments we came to conclusion about the need of drying the plants before transferring them to *in vivo* culture. After washing off roots, we kept the plants on the filter paper during twenty-four hours (at natural illumination and room temperature), and afterwards, according to the experiment scheme, we transplanted seedlings into plastic cubic containers (40 × 40 × 60 mm), and arranged a crock drainage at the bottom of them (1/3 of a content height).

We performed several experiments on the study and selection of the substratum content to optimize the post-aseptic cultivation of seedlings of *Laelia* species (*in vivo*) (Table 5).

Our results demonstrated that the optimum substratum for seedlings of *L. lundii* is a mixture of polyacryl-nitril and basaltic fibers (variant 2), for seedlings of *L. purpurata* and *L. lobata*, peat moss (sphagnum, bog moss) + pine bark 1:2 (variant 3), and for seedlings of *L. sincorana*, pine bark (variant 8).

Plants of *L. lundii* and *L. lobata* grow satisfactorily on both peat moss (Fig. 2) and artificial fibrous substrate (2), but a mixture of pine bark and peat moss (1:2) was the best for *L. purpurata* seedlings. Meanwhile, all examined substrates were not so good for *L. sincorana* seedlings, and plants perished quickly after adding oak bark to the substrate (Fig. 2). We established that *L. sincorana* seedlings were deeply rooting on blocks of pine bark only.

If to make the horizontal incisions on the bark before transplanting the seedling, 70–75 % of seedlings survived.

We used an extra-root nutrition of seedlings by Ca $(NO_3)_2$ (0.5 g/l) during the first 3–4 weeks after taking out plants from *in vitro* culture. Every week the seedlings were fed by MS solution (macro- and microelements, twice dilution). Beginning

Table 5. Agricultural-physical characteristics of the various substrata for cultivation of *Laelia* species

N/N	Density, kg/m^2	Content of solid part, %	Total porosity, %	Humidity, %	pH %
1	87	6	94	94	4.0
2	105	7	93	78	7.0
3	121	13	87	51	4.7
4	127	15	85	45	5.0
5	189	29	71	23	7.1
6	143	21	79	38	4.8
7	130	18	82	42	4.7
8	151	24	76	31	5.2

Note: on vertical: variants of the substrata as in Table 1.

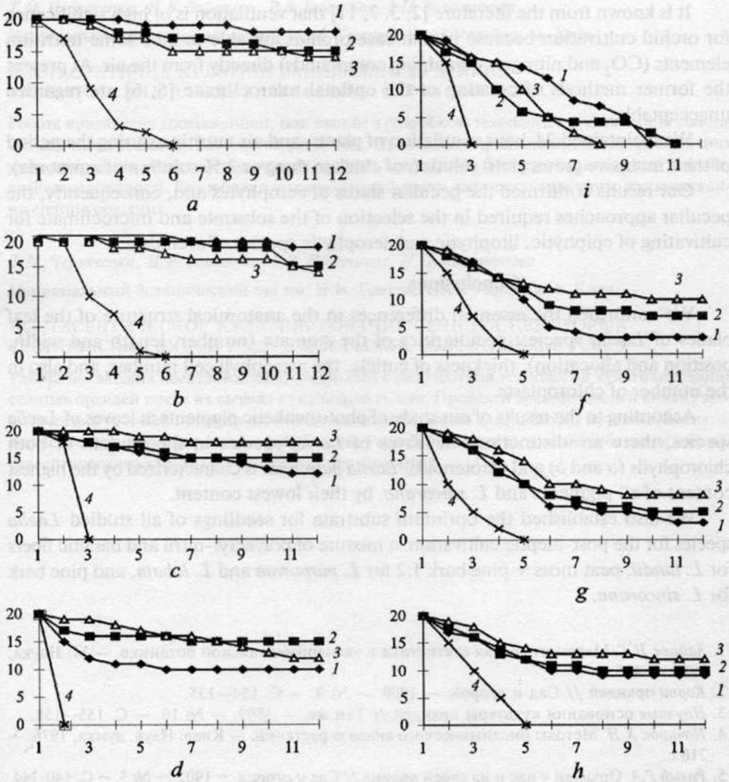

Fig. 2. Dynamics of surviving of seedlings of the studied species on the variants of substrata. Symbols indicate: 1–8 — variants of substrata; *a* — *L. lobata*, *b* — *L. lundii*, *c* — *L. purpurata*, *d* — *L. sincorana*; axis X — time (months); axis Y — number

from the second month, we fed the plants once a week with a mixture $Ca(NO_3)_2$: saccharose (1:3, 2 g/l), and once per two weeks, with superphosphate (1 g/l).

The photoperiod in a climatic camera was 12 hours, and humidity 70–95 %. Watering was done whenever necessary. For *L. sincorana* seedlings we used feeding initially and watering afterwards.

We used plants having CO_2 as a source of C_3. We also used different illumination modes for seedlings according to their physiological peculiarities; viz., *L. sincorana* seedling were placed at 5–10 cm distance from the light source, and *L. purpurata* and *L. lundii*, at the camera bottom.

It is known from the literature [2, 3, 7, 11] that ventilation is of high significance for orchid cultivation because in that case orchids are able to take some nutrition elements (CO_2 and nitrogen-containing compounds) directly from the air. At present the former methods of creating of the optimal microclimate [5, 6] are regarded unacceptable.

We maintained 24-hour ventilation of plants, and air nutrition during the period of their intensive grows (1:10 solution of chicken dung or 3 % solution of ammonia).

Our results confirmed the peculiar status of aerophytes and, consequently, the peculiar approaches required in the selection of the substrate and microclimate for cultivating of epiphytic, litophytic and aerophytic species of orchids.

Conclusions

We confirmed the essential differences in the anatomical structure of the leaf blades of *Laelia* species: peculiarities of the stomata (number, length and width, position and allocation), thickness of cuticle, the mesophyll cell number, and also in the number of chloroplasts.

According to the results of our study of photosynthetic pigments in leaves of *Laelia* species, there are distinctions in leaves of *Laelia* species in the content of both chlorophylls (*a* and *b*) and carotenoids. *Laelia purpurata* is characterized by the highest content of all pigments and *L. sincorana*, by their lowest content.

We also established the optimum substrata for seedlings of all studied *Laelia* species for the post-aseptic cultivation: a mixture of polyacryl-nitril and basaltic fibers for *L. lundii*; peat moss + pine bark 1:2 for *L. purpurata* and *L. lobata*, and pine bark for *L. sincorana*.

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Recommended for publication
By Ye.L. Kordyum

Submitted 04.01.2003

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ПОСТАСЕПТИЧНЕ КУЛЬТИВУВАННЯ СІЯНЦІВ ДЕЯКИХ ТРОПІЧНИХ ВИДІВ РОДУ *LAELIA* LINDL. (*ORCHIDACEAE* JUSS.)

Робота присвячена дослідженням, пов'язаним з розробкою технології культивування сіянців орхідей після їх виведення з культури *in vitro*. Проведено порівняльний аналіз екологічних умов природних місцезростань чотирьох видів *Laelia* Lindl., вивчено їх анатомічні та фізіологічні особливості. Розроблено оптимальні субстрати для постасептичного культивування сіянців орхідних.

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ПОСТАСЕПТИЧЕСКОЕ КУЛЬТИВИРОВАНИЕ СЕЯНЦЕВ НЕКОТОРЫХ ТРОПИЧЕСКИХ ВИДОВ РОДА *LAELIA* LINDL. (*ORCHIDACEAE* JUSS.)

Работа посвящена исследованиям, связанным с разработкой технологии культивирования сеянцев орхидей после их вывода из культуры *in vitro*. Проведен сравнительный анализ экологических условий природного обитания четырех видов *Laelia* Lindl., изучены их анатомические и физиологические особенности. Разработаны оптимальные субстраты для постасептического культивирования сеянцев орхидных.