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Anatomy

Seed structure and *in vitro* seedling development of certain Laeliinae species (Orchidaceae)

Estructura de semillas y desarrollo de plántulas in vitro *de ciertas especies de Laeliinae (Orchidaceae)*

Fabiana R. Gallo [a], Luiz A. Souza [a,*], Maria A. Milaneze-Gutierre [a], Odair J.G. Almeida [a,b]

[a] *Departamento de Biologia, Universidade Estadual de Maringá, Avenida Colombo, 5790, CEP 87020-900 Maringá, Paraná, Brazil*
[b] *Universidade Estadual Paulista, Unidade do Litoral Paulista, 11330-900 São Vicente, SP, Brazil*

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Abstract

Seeds and seedlings of neotropical Laeliinae species (*Cattleya loddigesii*, *Cattleya tigrina*, *Hadrolaelia purpurata*, *Laelia anceps*, *Schomburgkia gloriosa*, and *Sophronitis cernua*) were studied. The seed germination process and the seedling grown *in vitro* are described. Seeds of the studied species are unitegmic and have conspicuous thickening in the anticlinal and inner periclinal cell walls. The embryo consists of a relatively long and multicellular suspensor. At first stage of seedling development, the embryo differentiates into a protocorm with rhizoids and meristematic tissue. The budding occurs on the protocorms. The endogenous taproot is produced after the first leaves have emerged. The root is triarch or tetrarch and it possesses velamen. Structural characters related to seed coat, suspensor, ontogeny of leaves, and vascular system of the root are significant indicators for the separation of Laeliinae species.

Keywords: Embryo; Leaf; Protocorm; Root; Seed coat

Resumen

En este trabajo se realizó un análisis comparativo de semillas y plántulas en especies neotropicales de la subtribu Laeliinae (*Cattleya loddigesii*, *C. tigrina*, *Hadrolaelia purpurata*, *Laelia anceps*, *Schomburgkia gloriosa*, y *Sophronitis cernua*). Se describe el proceso de germinación de semillas y el desarrollo de plántulas cultivadas *in vitro*. Las semillas presentan un tegumento con un engrosamiento visible en las paredes. El embrión tiene un suspensor multicelular alargado. En la primera etapa del desarrollo de la plántula, el embrión se transforma en un protocormo con rizoides y tejido meristemático en el que aparecen los brotes primordiales. La raíz primaria se produce después de las hojas, es de tipo triarca o tetrarca con velamen. En conclusión, las características estructurales asociadas con la cubierta seminal, el desarrollo ontogenético de la hoja y el sistema vascular de la raíz, son indicadores significativos para la identificación de las especies estudiadas de Laeliinae.

Palabras clave: Embrión; Hoja; Protocormo; Raíz; Cubierta seminal

Introduction

Laeliinae species (Orchidaceae) are strictly neotropical and comprise about 50 genera with 1,500 species (Dressler, 1981, 1993), it is the third largest subtribe in the family after the Pleurothallidinae and Oncidiinae (Van den Berg et al., 2009). Laeliinae species show high morphological diversity and great

* Corresponding author.
E-mail address: lasouza@uem.br (L.A. Souza).
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taxonomic divergence (Van den Berg et al., 2009). Dressler (1981) included in this subtribe the genera *Cattleya* Lindl., *Laelia* Lindl., *Schomburgkia* Lindl. and *Sophronitis* Lindl.; Van den Berg et al. (2005, 2009) in a broad phylogenetic analysis within Laeliinae, supported the inclusion of *Schomburgkia* and *Sophronitis* species in *Laelia* and *Cattleya*, respectively. For the present study of Laeliinae genera, we followed the system according to the List of Species of the Brazil Flora (Lista de espécies da Flora do Brasil, 2012).

Van den Berg et al. (2009) pointed out the main factors of diversification within Laeliinae remain a rich field for research, due to the great variation of morphological features, the large number of species, and generic and infrageneric groupings. In addition, both the seed, as well as the fruit structure have been neglected in the Orchidaceae (Dressler, 1993); Barthlott (1976) stated that seed structures are especially useful at the tribal and subtribal levels.

Reproductive structures of orchids have wide morphogenetic potential, conditioned by the high totipotency of their cells which contributes to a complex reproductive system (Batygina, Bragina, & Vasileyeva, 2003). Furthermore, those authors pointed out that data set, the advance of theoretical knowledge on reproductive structures, and the methods of plant micropropagation may be useful for conservation programs, in addition to benefit the introduction of secondary populations under natural conditions.

In this scope, a comparative study of seed structure in 6 species of Laeliinae orchids: *Cattleya loddigesii* Lindl., *Cattleya tigrina* A.Rich, *Hadrolaelia purpurata* (Lindl.) Chiron & V.P.Castro, *Laelia anceps* Lindl., *Schomburgkia gloriosa* Rchb. and *Sophronitis cernua* Lindl. was accomplished. Furthermore, additional observations on the seedling anatomy were performed for *H. purpurata*, *S. gloriosa* and *S. cernua*. This study contributes to the structural knowledge of this group of flowering plants, which can be useful for future taxonomic treatments for Laeliinae orchids and may help to clarify the nature and structural relations about the seedling organs.

Materials and methods

Seeds from 6 species of orchids (*C. loddigesii*, *C. tigrina*, *H. purpurata*, *L. anceps*, *S. gloriosa* and *S. cernua*) from different origins were sterilized by soaking in 15% sodium hypochlorite solution (NaClO). In order to assure seed viability, samples were analyzed through the tetrazolium test for seed viability, using 2,3,5 triphenyl tetrazolium – 1% solution (Singh, 1981). Only seeds that achieved at least 50% of viability were selected for the study.

The viable seeds were placed on a sterilized (autoclaved for 20 min at 1 atm) nutrient medium Knudson "C" inside of culture flasks (Knudson, 1946). However, this was modified by the addition of coconut water (100 ml/L) or banana (100 g/L), with 3.5 g of agar (Himédia®). After 30 days in the culture medium, only 3 species (*H. purpurata*, *S. gloriosa* and *S. cernua*) were successful. Germinating seeds were maintained in chambers at 26 ± 3 °C, under 24 h light (40-W fluorescent lamp) and followed for 105 days.

The seeds of all 6 species and seedlings of the 3 germinated species (as mentioned above) were previously observed under a stereoscopic microscope, fixed in glutaraldehyde (1% in 0.1 M phosphate buffer, pH 7.2, maintained at 4 °C) (Karnovsky, 1965) and then preserved in 70% ethanol. Samples from the seeds and seedlings were dehydrated in ethanol series, embedded in historesin (Guerrits, 1991), sectioned (cross- and longitudinally) in a rotate microtome, and stained with toluidine blue (O'Brien, Feder, & Maccully, 1965) and toluidine blue/basic fucsin (Junqueira, 1990). Anatomical features were analyzed through optical microscopy using a Leica ICC50 microscope suite, through the Leica LAS EZ software, version 1.8.1.

Specific microchemical tests were carried out for detecting lipids (using Sudan IV and Sudan Black dyes) (Ruzin, 1999), starch (iodine-potassium iodide test), lignin (phloroglucinol test) and cellulose (chloriodide of zinc test) (Berlyn & Miksche, 1976). Seeds from all 6 studied species were mounted on aluminum stubs, coated with gold, and then examined using a scanning electron microscope (Shimadzu SS-550 Superscan) to obtain digital images.

Results

All analyzed species presented a minute, fusiform, unitegmic, and exalbuminous seeds (Fig. 1A and D), with an immature ellipsoidal embryo. There is an intercellular space relatively developed between the seed coat and the embryo (Fig. 1D). The translucid and lignified seed coat consists usually of 1 layer of elongated and narrow cells (Fig. 1B) with tapered and sometimes straight ends. The cells of the seed coat show differences concerning their cell wall thickening. All species have thin outer periclinal cell wall, and much thicker anticlinal cell wall (Fig. 1C), the inner periclinal cell wall is thickened in almost all species studied, except in *C. loddigesii* and *H. purpurata*, that have thin-walled inner periclinal cell (Table 1).

Table 1
Anatomical features of seed and seedling in studied Laeliinae species. *Species that seed had no germination success.

Species	Inner periclinal cell wall (seed coat)	Cell-layers of the suspensor	First leaf of the protocorm	Vascular system of the first root
Cattleya loddigesii	Thin-walled	2 cells thick	*	*
C. tigrina	Thickened	2 cells thick	*	*
Hadrolaelia purpurata	Thin-walled	2–3 cells thick	Lanceolate	Triarch
Laelia anceps	Thickened	2 cells thick	*	*
Schomburgkia gloriosa	Thickened	2 cells thick	Wide pointed sheathlike	Tetrarch
Sophronitis cernua	Thickened	2 cells thick	Lanceolate	Triarch