Flora 206 (2011) 601–609

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.de/flora

Anatomical development of the pericarp and seed of *Oncidium flexuosum* Sims (ORCHIDACEAE)

Juliana Lischka Sampaio Mayer[a], Sandra Maria Carmello-Guerreiro[b], Beatriz Appezzato-da-Glória[a,*]

[a] *Biological Sciences Department, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, PO Box 09, 13418-900, Piracicaba, São Paulo, Brazil*
[b] *Department of Plant Biology, Institute of Biology CP 6109, State University of Campinas – UNICAMP – 13083-970, Campinas, SP, Brazil*

ARTICLE INFO

Article history:
Received 26 June 2010
Accepted 14 October 2010

Keywords:
Embryology
Orchid
Pericarp
Reproductive anatomy
Zygotic embryo

ABSTRACT

Interpretation of the anatomical structure of the ovary and fruit of the Orchidaceae family is still controversial, which makes it difficult to understand the development and dehiscence of the fruit. The genus *Oncidium* is polyphyletic and is currently the subject of taxonomic studies. In this study, we have investigated the anatomical development of the pericarp and seed of *Oncidium flexuosum* Sims to determine important diagnostic characters that, along with molecular data, can assist in defining this group. We have found a new anatomical characteristic of the family: the presence of precursor cells for fruit dehiscence, which were visible from the beginning of development and located on the outer walls of the sterile valves. In contrast with what has been observed by different authors with other species, in the mature fruit of *O. flexuosum*, only the endocarp of the fertile valves and a few cells near the exocarp and the vascular bundle in the sterile valves show parietal thickening, while the rest remains parenchymatous. During the development of the ovule and embryo, we have shown that the embryonic sac of this species has eight nuclei and that the embryo has a long and elaborate suspensor.

Introduction

Structural and developmental studies of Orchidaceae fruits are scarce, although these organs vary greatly in shape, size, texture and ornamentation (Dressler, 1993; Rasmussen and Johansen, 2006). Most of the ontogenetic studies are concentrated on ovules and seeds and little is known about the structure and physiology of the fruits (Rasmussen and Johansen, 2006).

The flowers of the Orchidaceae are epigynous and the floral parts are adnate with the ovary in its full extension (Dressler, 1993). It is currently accepted that the ovary of orchids is composed of three carpels, although this organ has been interpreted in different ways over the years. For Lindley (1830–1840, 1847), Saunders (1923) and Arber (1925), the ovary of orchids was composed of six carpels, three with a placenta and three without a placenta. Brown (1831), apud Rasmussen and Johansen (2006), interpreted the ovary as being composed of only three carpels. Duncan and Curtis (1943), Swamy (1949a), Cribb (1999) and Rasmussen and Johansen (2006) supported the interpretation of Brown (1831), stating that the ovary in the family would be syncarpous and tricarpellate, showing a pattern of six valves in cross section: three fertile and three sterile. The sterile valves correspond to the bases of the sepals and the fertile valves correspond to petal base and two carpel-halves, carrying one marginal placenta from each (Rasmussen and Johansen, 2006).

The fruit is a capsule and is rarely described in studies, which is likely due to either a lack of knowledge or the belief of researchers that the fruits have little diagnostic value (Cribb, 1999). The capsules are generally dehiscent, and dehiscence usually occurs preferentially as a rupture along the midline of each carpel, and later between the carpels, generating three wide, fertile valves and three narrow, sterile valves. The six half-carpels remain joined at the apex in most species, but in some *Maxillaria* Ruiz & Pavón and *Lockhartia* Hooker taxa the carpels separate completely at the apex (Cribb, 1999; Dressler, 1993).

In this family, proliferation of the placenta and formation of the ovules generally occur only after pollination. The periods of time between pollination, fertilization and formation of the seeds are varying among species, even within the same genus (Duncan and Curtis, 1942; Swamy, 1949a). Pollination in Orchidaceae can be considered to have a dual effect: the first is to stimulate the enlargement of the ovary and the maturation of the ovules, and the second is to promote fertilization (Hildebrand, 1863; apud Duncan and Curtis, 1942).

Orchids produce large amounts of seeds, and each capsule can contain up to four million seeds (Arditti and Ghani, 2000). However, in natural conditions, few seeds successfully germinate because of the lack of both an endosperm and the ability to directly use nat-

* Corresponding author.
E-mail address: bagloria@esalq.usp.br (B. Appezzato-Da-Glória).

0367-2530/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.flora.2011.01.009

ural substrates thus requiring mycorrhizal associations (Withner, 1974). Most orchid species have small seeds with undifferentiated embryos, that is, a mass of undifferentiated cells. These can be compared to the globular stage of the embryos from dicotyledons. However, most orchid seeds exhibit acotyledonous embryos (Arditti, 1992; Veyret, 1974).

The genus *Oncidium* Sw. *sensu lato* contains more than 400 species (Chase et al., 2009), and its delimitation is controversial, as demonstrated by studies of chromosome numbers (Chase, 1986, 1987; Félix and Guerra, 2000) and of molecular systematics (Chase and Palmer, 1992). Studies of the molecular phylogeny show that the genus *Oncidium* is clearly polyphyletic, and several changes of the intrageneric relationships are currently being proposed (Chase, 1986; Chase et al., 2009).

Based on the few available studies of ovary and fruit structures of Orchidaceae the interpretation of the anatomy of the carpels is therefore still controversial, making it difficult to elucidate the development and dehiscence of the fruits of orchids. Moreover, there are few studies about the ontogeny of fruit, ovule and seed in this family. The present study investigated the anatomical development of the fruit and seed of *Oncidium flexuosum* Sims to identify important diagnostic characteristics that, along with molecular data, can assist in characterizing this genus. The species belongs to the subfamily Epidendroideae and occurs naturally in Brazil in the remaining fragments of the Atlantic Forest (Pabst and Dungs, 1977).

Materials and methods

For light microscopy and scanning electron microscopy (SEM), we collected samples of flower buds, flowers during anthesis, and fruits in different developmental stages from *O. flexuosum*. Artificial pollination was performed in ten individual cultures. A voucher of the investigated species (# 150303) was deposited in the UEC Herbarium, Brazil.

For light microscopy analysis, samples were fixed in Karnovsky (Karnovsky, 1965; modified by preparation in phosphate buffer pH 7.2) for 24 h, dehydrated in a graded ethanol series and embedded in Leica Historesin® (Heraeus Kulzer, Hanau, Germany). Serial sections (5 μm thick) were cut on a rotary microtome, stained with toluidine blue O (Sakai, 1973), and mounted in Entellan® synthetic resin (Merck, Darmstadt, Germany).

The chemical natures of the substances found in the embryos were determined using the following histochemical tests: Lugol's iodine solution to identify starch (Berlyn and Miksche, 1976), Sudan IV to identify lipid compounds (Pearse, 1985) and Aniline blue black (Fisher, 1968) to identify total protein. Photomicrographs were taken with a Leica® DM LB photomicroscope equipped with a Leica® DC 300F camera (Leitz, Wetzlar, Germany).

For scanning electron microscope analysis, samples of the *O. flexuosum* fruits were fixed in Karnovsky (Karnovsky, 1965; modified by preparation in phosphate buffer pH 7.2) for 24 h, dehydrated in a graded ethanol series and critical point-dried with CO_2 (Horridge and Tamm, 1969). The samples were attached to aluminum stubs and coated with gold (30–40 nm). Finally, the samples were examined under a LEO VP435 (Zeiss, Oberkochen, Germany) scanning electron microscope at 20 kV.

Results

Fruit morphology

The development of the *O. flexuosum* fruit from the 3rd to the 110th day after the artificial pollination can be seen in Fig. 1 and Table 1. Five days after the pollination of the flowers, the petals and sepals wither and the region of the inferior ovary begins to stretch. This expansion of the ovary continues until 40–45 d after pollination, when the fruit starts to increase in diameter. At 90–110 d after pollination, while the capsule is still green, the valves separate longitudinally, initially at the distal end. However, they remain attached at the apex and base when the seeds are released.

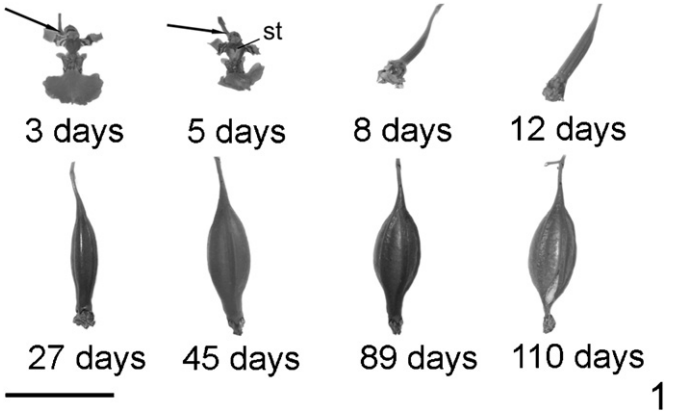

Fig. 1. Development of the fruit of *Oncidium flexuosum* Sims. Age refers to the period after the artificial pollination of the flowers. The arrows indicate the fruit. Region of the stigma: st. Scale bar = 2 cm.

Anatomical structure of the ovary

In a cross section of the ovary of the flower during anthesis, there are three carpels divided into six valves: three are fertile, with the presence of the placenta region, and three are sterile (Fig. 2). The outer epidermis of the ovary that contains stomata is single layered with cells that are elongated in the radial direction, in both cross and longitudinal sections, each with an obvious central nucleus and dense cytoplasm. The fundamental tissue has compact isodiametric parenchyma cells, and the cells near the outer epidermis are larger than those near the inner epidermis (Figs. 2 and 3). Each fertile valve has 14–16 layers of parenchyma cells (Fig. 2), and each sterile valve has 12–14 layers (Fig. 2). The fertile valves have one vascular bundle, and the sterile valves have two vascular bundles. The bundles in both the fertile and sterile valves are located near the inner epidermis, and idioblasts containing raphides are observed (Fig. 2). In the fertile valves, the placenta does not present fully developed ovules, only small projections composed by cells containing very dense cytoplasm (Figs. 2 and 3).

Anatomical development of the pericarp

Young fruit I: 23 d after pollination (Figs. 4–6)

Fertile valve: The exocarp is single layered, with small cells slightly elongated in the longitudinal direction (Fig. 6). The meso-

Table 1
Morphological and anatomical changes observed during the development of the *Oncidium flexuosum* Sims fruit after pollination.

3–5 d	Petals and sepals wither Growth of the margin of the column, sealing the stigmatic cavity Proliferation of the placenta Germination of the pollen grains
5–45 d	Growth in the length of the fruit Elongation of the pollen tubes
35–50 d	Enlargement of the fruit diameter Differentiation of the ovules
50–65 d	Fertilization of the ovules Degeneration of the pollen tubes
90–110 d	Dehiscence of the fruit