Ripening and dehiscence of the anther in some *Crocus* (Iridaceae) species

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Abstract — The anther development was examined in some *Crocus* species, by light and scanning electron microscopy. Epidermal cells develop a longitudinal stomium which is connected to the interlocular septum by cells with wide intercellular spaces. Endothecial cells are mono or bilayered, and they develop annular wall thickenings. Middle layer cells degenerate shortly after the microspore release. Tapetal cells are secretory with orbicules, and they form the tapetal membrane. The stomium disjoins from the interlocular septum after the lysis of the middle lamellae in the abscission region. The stomial cells break long before the flower opening, and in *C. boryithey* show signs of programmed cell death. Following the flower opening, the locular walls shrink tangentially, with flattening of epidermal cells and shortening of endothecial cells, and they bend up to turn inside out, exposing the pollen grains. The results are discussed in relation to the dehiscence mechanism.

Key words: anther, *Crocus*, dehiscence

INTRODUCTION

The anther is the organ which ensures the development of pollen and its release in the ripening stage, the developmental programs of anther and pollen are synchronised (GOLDBERG *et al.* 1993). In most angiosperms the anther is tetrasporangiate, and its wall consists of 4 cell layers: epidermis, endothecium, middle layer and tapetum. The middle layer and, in particular, the tapetal cells are programmed for sustaining the pollen development, and they degenerate after fulfilling this function (BHANDARI 1984; PACINI 1990). The epidermis, besides protecting the underlying tissues, develops the stomium which acts as the site for the anther dehiscence; in some species, the epidermis develops cell-wall thickenings which have a mechanical function as in the endothecial cells (BHANDARI and NANDA 1968; BIANCHINI and PACINI 1996). Endothecial cells have the characteristic of developing wall thickenings which may be so variable as to be a systematic character in some families (FRENCH 1985; GERENDAY and FRENCH 1988); the thickenings play a key role in the pattern of anther dehiscence. The mechanisms of anther dehiscence vary between and within families, and they are based on the detachment and the breaking of the stomium by a lytic or mechanical activity, and the contraction of drying anther walls that is mechanically controlled by cell wall thickenings (KEIJZER 1987; KEIJZER *et al.* 1987, 1996; BONNER and DICKINSON 1989, 1990; PACINI 1994).

The present report deals with the ripening and dehiscence of the anther in some *Crocus* species.

MATERIAL AND METHODS

Plants consisted of *Crocus* species with autumnal *(C. boryiGay, C. thomasii.,*Ten *C. sativusL., C. cartwrightianus* Herb.), winter *(C. variegatus* Hoppe and Hornsch), and spring *(C. vernus* Hill ssp. *vernus)* flowering; for their origin see CHICHIRICCO (1993, 1996). The plants were grown in pots in the Botanical Garden of the University of L'Aquila, and at the flowering time they were housed in our department at 17-20°C and RH 50-65%. Flowers dehisced naturally.

Light and fluorescence microscopy

Anthers at different developmental stages were fixed in 3 % glutaraldehyde in cacodylate buffer, dehydrated in an ethanol series and embedded in Technovit 7100 resin (Kulzer). Sections were stained with: a) PAS reagent for total insoluble polysaccharides (O'BRIEN and McCULLY 1981); 2) auramine 0 for cuticle (HESLOP-HARRISON 1977); 3) ethidium bromide for lignin (O'BRIEN and McCULLY 1981); and 4) toluidine blue (0.05% in water) for general cell staining.

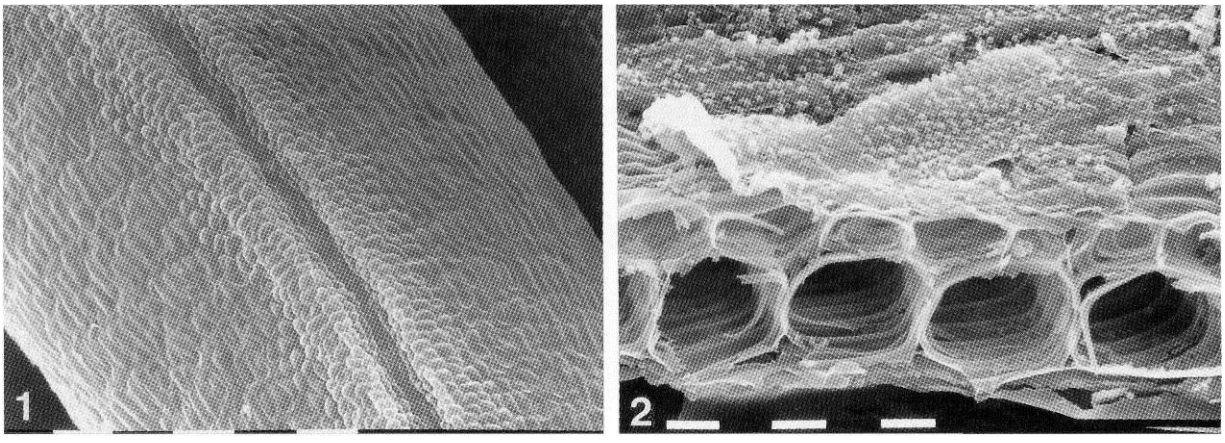
Scanning electron microscopy (SEM)

Anthers were fixed in acetic alcohol (1:3), dehydrated in ethanol and critical point dried in CO_2. They were then gold-coated using a Balzer's SCD 040 sputtering, and observed using a Philips 505 scanning electron microscope.

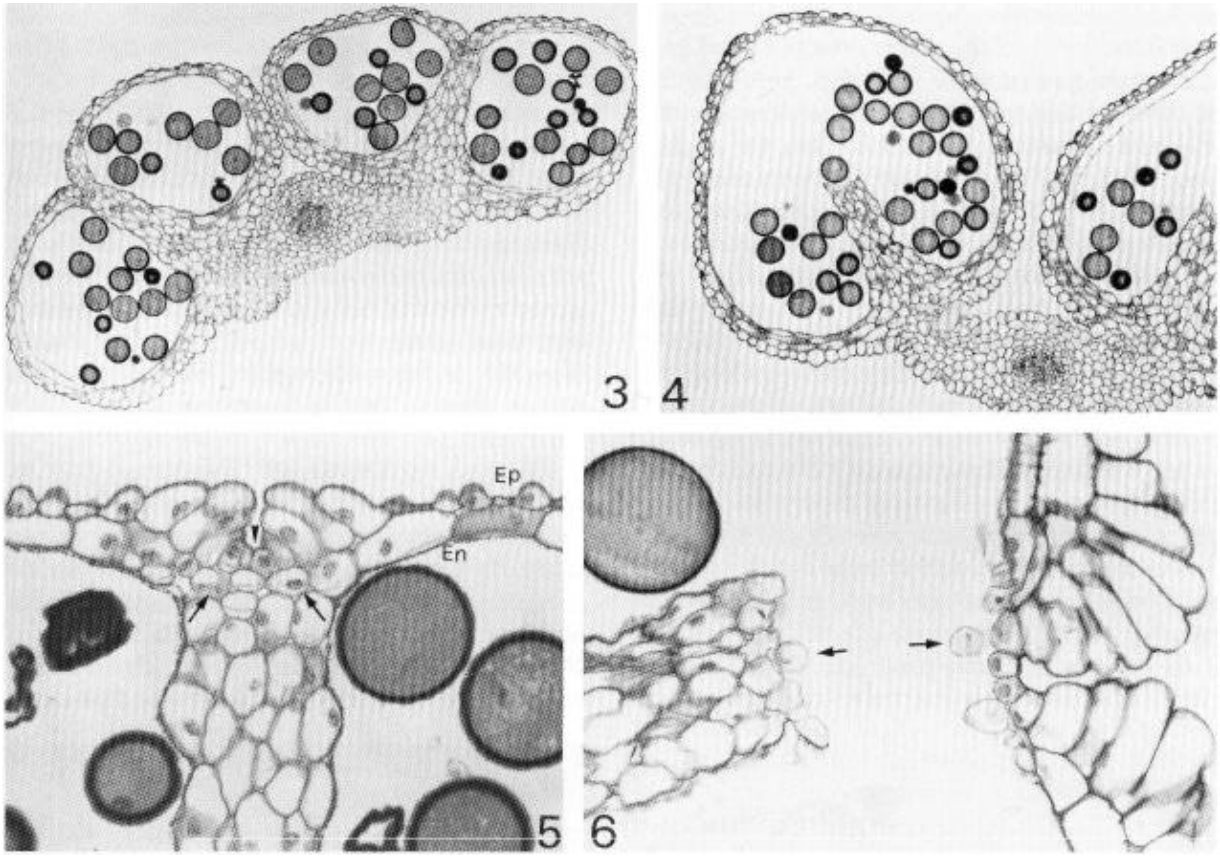
RESULTS

The examined species showed no marked differences as regards the pattern of anther development.

The anther of *Crocus* (Figs. 1,3) consists of a central column of connective tissue containing a vascular bundle, with two branches each of which bears a theca with two locules, the latter separated by a connective septum. The wall surrounding the locules of a young anther consists of four layers of polyhedral cells: epidermis, endothecium, middle layer, and tapetum. Epidermal cells are longitudinally elongated; along the distal region of the theca they are radially elongated forming two parallel crests delimiting the stomium (Figs. 1,5,6); this consists of 3-5 rows of small polyhedral epidermal cells which are connected to the interlocular septum by the inner tangential walls (Fig. 5). During gametogen-esis, the stomial cells do not show noticeable changes in size or shape, while their bordering cells expand; the other epidermal cells become periclinally flat. The epidermis is covered with a layer of cuticle which is thick on the cells bordering the stomium, and very thin or absent along the stomium (Fig. 8), and it lacks stomata. The endothecium consists of transversally elongated cells, and is monolayered in *C. cartwrightianus*, *C. sativus*, *C. variegatus*, *C. boryi*, and *C. thomasii* (Figs. 4, 5), and bilayered in *C. vernus* ssp. *vernus* (Figs. 2, 10). Next to the base of the septum, the endothecium is 2-4 cell-layered, close to the stomium it is most frequently bilayered, and below the stomium it is lacking (Figs. 4, 8). During gametogenesis, endothecial cells grow in length according to the loculus expansion, followed by a radial growth which is synchronised with the degeneration of the tapetum. Thickenings develop both on the tangential and transverse cell walls, forming a regular succession of continuous rings (Figs. 2, 8); the cells next to the stomium generally lack wall thickenings (Figs. 7, 8). The thickenings are PAS-positive, they are brightly fluorescent with auramine 0 (Fig. 8) and weakly fluorescent with ethidium bromide (not shown). The middle layer cells are tangentially elongated and shortlived; they are crushed between the endothecium and tapetum and disappear shortly



Figs. 1-2 — Anthers of *C. vernus* ssp. *vernus* in SEM. Fig. 1 — Frontal view of a theca showing the stomial groove delimited by radially elongated epidermal cells. Bar=100 m. Fig. 2 — Inner surface of locular wall after longitudinal dissection. See orbicules on the tapetal membrane and, below this, the bilayered endothecial cells with annular wall thickenings. Bar=10 m.



Figs. 3-10 — Cross sections of anthers before and after dehiscence. Fig. 3 — 4-locule stage in *C. boryi* after the tapetum degeneration, with bicellular pollen grains, x 92. Fig. 4 — 2-locule stage in C. *boryi*, note the dissociated septum, x 92. Fig. 5 — Stomial region of *C. thomasii* at a stage as in Fig. 3. Note radially elongated epidermal cells alongside of stomium (arrowhead), and just below this the small connective cells (arrows) with large intercellular spaces. X 370. Ep=epidermis, En=endothecium. Fig. 6 — Stomial region of *C. vernus* ssp. *vernus* just after opening of septum. Note that dissociated connective cells (arrows) are intact, x 370.

after the microspores are released from the tetrad callose. The interlocular septum consists of 4-6 layers of connective cells (Figs. 5, 6). Its cells are flattened by the locule expansion, becoming increasingly more flat up to the time when tapetal cells are degenerating; afterwards, these flattened cells expand to an ovoidal shape. The cells neighbouring the stomium are smaller than the other connective cells, and their interconnections are limited by intercellular spaces (Fig. 5). The tapetum is of the secretory type with orbicules (CHICHIRICCO 1999) and it nourishes the pollen by the locular fluid. Its cells are compressed and deformed during pollen grain development, and degenerate and disappear before the detachment of the stomium from the interlocular septum (Fig. 3). Following tapetum degeneration, the tapetal membrane becomes evident around the inner tangential faces of endothecium and interlocular septum, forming a locular lining on which the orbicules are deposited (Fig. 2); both the tapetal membrane and orbicules are fluorescent after auramine treatment (Fig. 8).

Anther dehiscence

The main steps for the anther dehiscence may be summarized as follows. At the time of tapetal cell degeneration, the cellular connection between the interlocular septum and the stomium becomes so loose that, while the flower bud is still tightly enclosed within the sheaths, the stomium disjoins from the septum, and the anther wall is pushed outward to form a unique locule which is heart-shaped in cross section (Fig. 4). Dissociation does not damage the connective cells involved, which remain integral and joined either to the stomium or to the detached septum (Fig. 6) by residual middle la-

mellae. By this time the distal tract of the anther wall is reduced to the stomial cell-layer. These cells undergo shrinkage, and they appear to be pressed by enlarging adjacent epidermal cells, and at the time of pollen maturation, the middle ones show a progressive degeneration and breakage. In *C. boryi,* the observations on this stage were characterized by the condensation of the middle stomial cells and darkening of the nucleus (Fig. 7, and the insert) followed by cell breakage. As a result of the cellular breakage a progressive longitudinal slit develops along the axis of the stomium, with the separation of the locular wall into two halves, each one with remnants of stomial cells on the rim. The splitting of the stomium occurs after the flower bud emergence, but before disclosure. Soon after the flower opening, the locular walls shrink tangentially, with epidermal and endothecial cells becoming anticlinally flat (Fig. 9). The locular walls diverge, and they progressively bend their extremities outwards and up to turn inside out (Fig. 9). As a result, pollen grains are exposed, and they are held together on the dry anther walls by the sticky pollenKitt.

Sometimes, in *C. vernus* ssp. *vernus* both the cells of the septum and stomial cells undergo protoplast degeneration, and their residual walls are closely packed between the loculi, turning into a thin septum. This acellular septum remains intact and connected with the enlarged epidermal cells, preventing both the locules from fusing and the anther from opening (Fig. 10), so that pollen grains are not released.

DISCUSSION

The process of anther ripening and dehiscence in some *Crocus* species may be distinguished in three main successive stages: 1) separation of the stomium from the connective cells, 2) breakage of the middle stomial cells, 3) bending outwards of the locular walls. The first stage

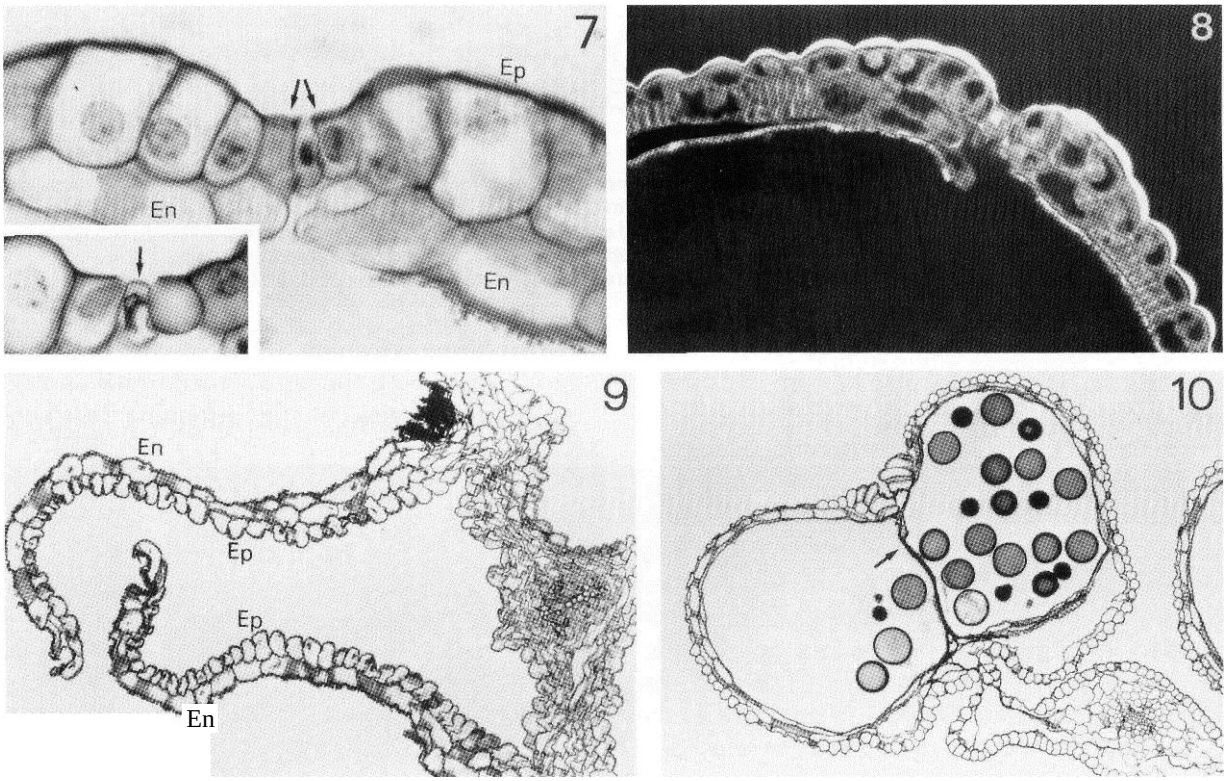


Fig. 7 — Particular of stomial cells (arrows) of *C. boryi* just before splitting. The middle stomial cell shows a pycnotic nucleus and it lacks the outer tangential wall; in the insert the middle cell (arrow) is pycnotic, with integral cell walls, x 920. Ep=epidermis, En=endothecium. Fig. 8 — The same as in Fig.7 with auramine 0 induced fluorescence, showing a very thin and discontinuous cuticle over the middle stomial cells. Note fluorescence of the endothecial wall thickenings and of underlying tapetal membrane and orbicules. X 370. Fig. 9. — Opened locular walls of two adjacent thecae of *C. cartwrightianus,* showing both epidermal (Ep) and endothecial (En) cells anticlinally flat. X 180. Fig. 10 — Unsuccessful anther dehiscence in *C. vernus* ssp. *vernus,* due to the persistence of the packed cell walls (arrow) of stomium -and septum cells. Note bilayered endothecium. x 88.

seems to occur by a mechanism which is typical of abscission processes (ADDICOTT and LYNCH 1995), namely the enzymatic lysis of the middle lamellae between the cells of the abscission zone. These cells which are small and contain intercellular spaces, are released from the middle lamellae without being damaged; if the dissociation was mechanical, due to stretching of expanding locules, it would damage the cells involved (see KEIJZER 1987). On the other hand, in anthers where the septum undergoes protoplast dissolution without damage to the apoplast, the expansion of pollen in itself is not enough to detach the stomium (Fig. 10). This indirect evidence, in the absence of data on enzymatic activities, supports the lytic detachment of the stomium, as already reported for other longitudinally dehiscing anthers (KEIJZER 1987).

Concerning the stomial break, the expansion of locular walls and, in particular, that of the distal epidermal cells, plays a role in subjecting the stomial cells to stress. The lack of a cuticular shield on the middle stomial cells may expose these to dehydration and shrinkage, but this cannot explain the precocious stomium opening. These observations are consistent with a mechanical break of the stomium, as suggested for some longitudinally dehiscing anthers (KEIJZER 1987). In *C. boryi*, however, the cell breakage follows some morphological changes, such as nuclear pycnosis and cell collapse, which are typical signs of programmed cell death (PCD) (SCHWARTZ 1992; HAVEL and DURZAN 1996; PENNELL and LAMB 1997), suggesting a PCD pathway for stomial disruption. Destruction of anther tissues is strictly coordinated with anther development so that it might be genetically programmed (see GOLDBERG *et al.* 1993). Recent studies on the PCD in plants (see BEER 1997) report that as the anther matures, its tissues such as the stomium, have increased levels of ubiquitin, as well as mRNAs and enzymes involved in hydrolytic activities. All these observations make the mere mechanical break of stomium unlikely, at least in *C. boryi*, and suggest a PCD mechanism combined with the mechanical stress which could act as the starter for the PCD. We will ascertain this hypothesis by future ultrastructural studies.

The opening of the locular walls is due to the dehydration-contraction of epidermal and endothecial cells, the latter limited by its wall thickenings. The major water loss is most probably due to reabsorption, as the evaporation is prevented by both the tapetal membrane and the cuticle layer enveloping the anther walls, as well as by the absence of stomata. The cell shrinkage proceeds from the distal to the proximal region of the locular wall, and it is controlled mechanically by the endothecial wall rings. These allow shrinkage in tangential direction, with shortening of endothecial cells and anticlinal flattening of epidermal cells, the two cell layers being arranged crosswise. As a result, the locular walls diverge, and they are pulled outwards by the progressive contraction of epidermal cells, so they bend up to turn inside out, exposing the pollen grains. In longitudinally dehiscing anthers of Liliaceae (KEIJZER 1987), the outward bending of the locular walls is controlled by the U-shaped endothecial thickenings which allow the shrinkage of the outer tangential wall.

In summary, the anther development includes a series of stages coordinated with pollen development, and developmental deviations such as the precocious degeneration of the septum, may preclude the pollen release. The process of anther dehiscence, on the basis of the anatomical and cytological observations, shows similarities with that reported for some Liliaceae (KEIJZER 1987). Major differences concern the cytological pattern of the stomium breakage in *C. boryi*, which could be worthy of further studies, and the pattern of endothecial wall thickenings which control the dynamics of the locular wall opening.

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