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Anther wall development and structure in wild tomatoes (*Solanum* sect. *Lycopersicon*): functional inferences

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Abstract. Development of the anther wall and its structure at maturity in wild tomatoes (*Solanum* sect. *Lycopersicon*) are described, and the features are discussed in relation to anther dehiscence and the buzz-pollination mechanism. The anther wall formation follows two different patterns in the same microsporangia and a high number of cells divisions may occur. The number of layers formed varies across the ventral, dorsal and lateral surfaces of each theca. Large epidermal cells develop, lining the stomium, and they could possibly be involved in stomium opening. Cells with thickenings are formed in the apical fifth of the anther, where the tissues seem to degenerate after the stomium opening, forming a wider aperture through which the pollen can be shed. The multilayered dorsal wall remains swollen and could act as an attractant to pollinators and as mechanical support. The apparently disordered anther wall development sets up different structures across and along the anther, which can be interpreted as histological adaptations to the buzz-pollination mechanism.

Introduction

The androecium in the tomatoes is well adapted to the buzz-pollination mechanism (Buchmann 1983; Endress 1994; Cocucci 1999). In this form of pollination, bees grasp the anther cone and transmit vibrations through their bodies to the anthers, thus producing pollen expulsion from the anther apices (Buchmann 1983; Endress 1994). In tomatoes, the united anthers are functionally equivalent to one poricidal anther (Cocucci 1999). The androecium morphology was described in detail in the cultivated tomato, *Solanum lycopersicum* L. (Chandra Sekhar and Sawhney 1984), and the same features were later observed in all wild-tomato species (Carrizo García 2003*a*). Therefore, the morphological adaptations of the androecium (e.g. anthers united by trichomes to form a solid antheral cone and apical appendages forming a unique exit for the pollen) are uniform within this group of species.

In anther histology, most of the attention has usually been focused on the locular content in the cultivated tomato (e.g. microsporogenesis, Sawhney and Bhadula 1988; male sterility, Rick 1948; Mazzucato *et al*. 1998; and tapetum development, Polowick and Sawhney 1993). Nevertheless, the anther wall has also been studied in this species, revealing interesting characteristics (Oryol and Zhakova 1976; Bonner and Dickinson 1989). Some of the anther-wall features have been considered histological specialisations to the buzz-pollination mechanism (Bonner and Dickinson 1989).

Anther wall development and structure has been only briefly studied in two wild tomatoes, *S. habrochaites* and *S. neorickii* (Carrizo García 2002*b*), whose features coincide with those observed in *S. lycopersicum* (e.g. distribution of cells with thickenings, epidermal ridges on the sides of the stomium). The histological structure is heterogeneous along the anther in the tomato species studied so far, and this peculiarity seems related to the mechanisms of anther opening and pollination. Thus, a study of the histological development and mature structure of the anther in the wild tomatoes was conducted to examine whether the histological features of the mature anthers are uniform among all tomatoes (as it occurs at the morphological level) and how they are set up during development, and to analyse how the different histological features can be related to anther opening and the pollination mechanism.

Materials and methods

A total of 10 species of wild tomatoes (*Solanum* sect. *Lycopersicon*; Table 1) was studied. Although *Solanum pennellii* Correll is usually considered a tomato, this species has been excluded here because its anther structure is different from the rest of the tomato clade. Apart from the conspicuous morphological differences (curved, unequal anthers without apical appendages, opening through a pore and a line), anatomical differences were also observed (e.g. distribution of thickened

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Table 1. Species studied and collection data
BGN, Botanical and Experimental Garden of Nijmegen, The Netherlands; TGRC, C.M. Rick Tomato Genetics Resource Center, USA. The *Lycopersicon* synonyms are given in parentheses

Species	Collector; sample source
Solanum arcanum Peralta (= *L. peruvianum* var. *humifusum*)	Carrizo García; BGN, No. 974750061
Solanum cheesmaniae (Riley) Fosberg (= *L. cheesmanii*)	Chetelat; TGRC, LA 0166
Solanum chilense (Dunal) Reiche (= *L. chilense*)	Chetelat; TGRC, LA 1970
Solanum chmielewskii (C.M.Rick, E.Kesicki, J.E.Fobes et M.Holle) D.M.Spooner, G.J.Anderson et R.K.Jansen (= *L. chmielweskii*)	Chetelat; TGRC, LA 1330
Solanum corneliomulleri J.F.Macbr. (= *L. peruvianum* f. *glandulosum*)	Carrizo García, BGN, No. 974750064
Solanum galapagense S.Darwin et Peralta (= *L. cheesmanii* f. *minor*)	Chetelat; TGRC, LA 0317
Solanum habrochaites S.Knapp et D.M.Spooner (= *L. hirsutum* and *L. hirsutum* f. *glabratum*)	Carrizo García; BGN, No. 944750111 Chetelat; TGRC, LA 2099
Solanum neorickii D.M.Spooner, G.J.Anderson et R.K.Jansen (= *L. parviflorum*)	Hunziker; BGN, No. 974750067
Solanum peruvianum L. (= *L. peruvianum*)	Carrizo García; BGN, No. 914750145
Solanum pimpinellifolium L. (= *L. pimpinellifolium*)	Hunziker 25483; Perú, Dpto. La Libertad Carrizo García; BGN, No. 934750008

cells, dehiscence-zone structure)[1]. The samples analysed are deposited at CORD herbarium and spirit collection (Cordoba, Argentina).

Buds and flowers were fixed in 70% ethanol or in FAA (10% formalin–50% ethanol–5% acetic acid) for 48 h, subsequently washed and stored in 70% ethanol. Flowers and flower buds of different stages (= sizes) were dehydrated in an ethanol–xylene series and infiltrated in paraplast (Sigma, St Louis, MO, USA). These materials were serially cut in cross-sections, the youngest buds 6–10 µm thick and the oldest buds and flowers 12 µm thick. For each species, 8–12 flower buds between 0.5 and 3 mm were sectioned, whereas approximately 10 flower buds were sectioned from 4 mm to the flower stage. The sections were stained for 30 s with activated haematoxylin (Biopur, Buenos Aires, Argentina) and observed under a light microscope. The distribution of cells with thickenings was outlined under polarised light. The photographs were taken with an Axiophot microscope (Zeiss, Göttingen, Germany).

Results

The anthers are dithecal, tetrasporangiate and slightly dorsiventrally asymmetrical because of the smaller size of the ventral microsporangia.

Anther-wall formation

When the four microsporangia are clearly noticeable in a cross-section of the anther, two secondary parietal layers, the outer and the inner one, are found between the epidermis and the sporogenous tissue. Different patterns of periclinal cell divisions are observed from these two layers, which give rise to all subepidermal layers present later in the mature anther wall. These layers are well defined early in the anther development, before the meiosis in the pollen mother cells has begun.

In all taxa observed, the initial sequence of cell divisions follows the dicotyledonous type (Davis 1966), where the cells of the outer secondary parietal layer divide periclinally, forming two new layers, the prospective endothecium and a middle layer, whereas the inner layer develops directly as tapetum (Fig. 1*A*, *B*). In all taxa, in some parts of the same microsporangia, there are also occasional cell divisions that follow the basic type (Davis 1966), where the cells of the inner secondary parietal layer also divide periclinally, forming another middle layer (Fig. 1*A*, *B*, arrows) and the tapetum.

From this point of development, in each theca it is possible to observe three general zones, where different patterns of subsequent periclinal cell divisions can occur. These zones, although not well defined, are the ventral surface of the ventral microsporangium, the dorsal surface of the dorsal microsporangium and the lateral surface on each side of the stomium, which comprises the lateral sides of both microsporangia (Fig. 1*C*).

New periclinal cell divisions occur in the cells formed in ventral and dorsal zones, except in the tapetum, which continues its differentiation. Since the dicotyledonous type of wall formation is the most frequent pattern, there are usually one (in the middle layer) or two (in the middle layer and the endothecium) cell divisions that originate up to four subepidermal layers (Fig. 1*D*). Thereafter, it is common that some of the cells formed divide periclinally in turn in the dorsal surface. Particularly, this happens near the connective tissue where 1–6 subsequent cell divisions occur, producing from 5 (most species) to 8–10 (*S. habrochaites* and *S. corneliomulleri*) subepidermal layers (Fig. 1*E*). The outermost layer is usually referred as endothecium (although only part of its cells develops thickenings), whereas the remaining ones are called middle layers. In contrast, usually

[1]Material studied: *Solanum pennellii* Correll: Hunziker, Cult. BGN, No. 964750063; *Solanum pennellii* var. *puberulum* Correll: Carrizo García, Cult. BGN, LA 1926.

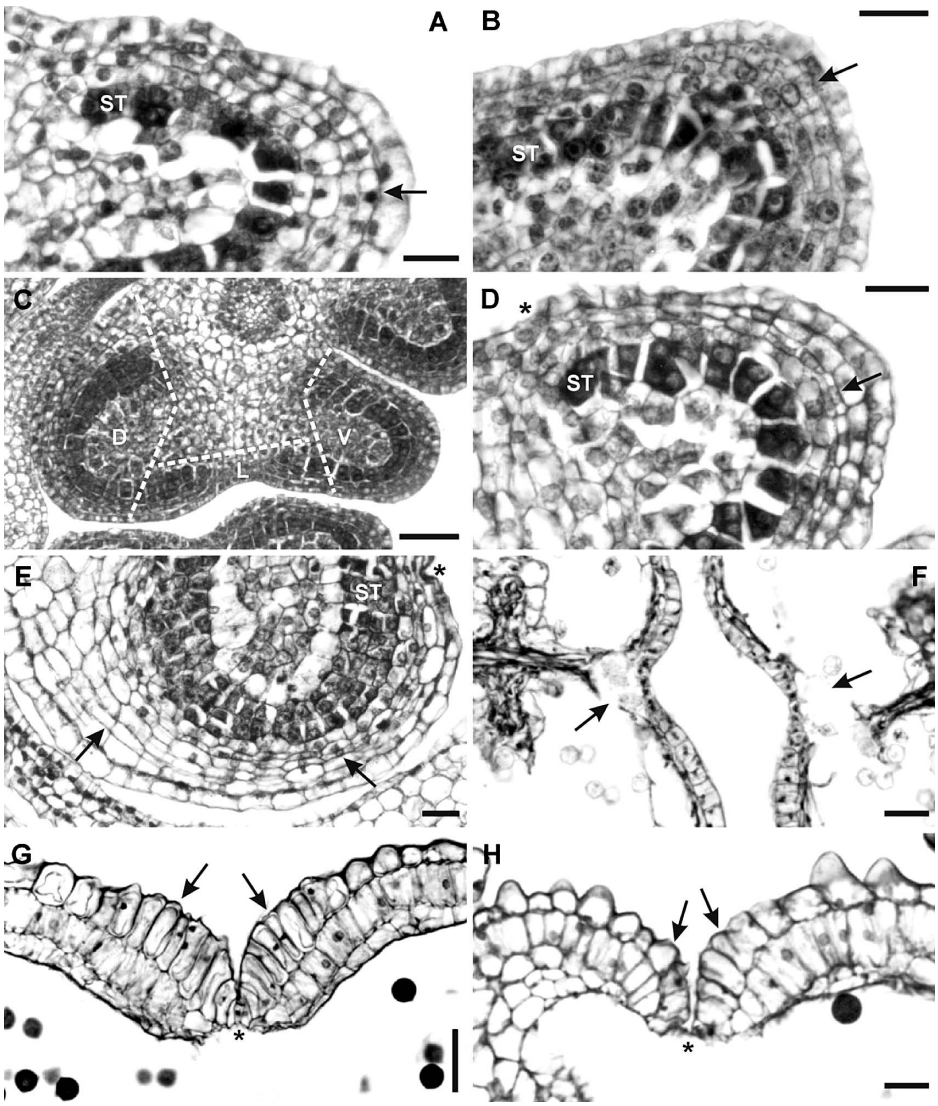

Fig. 1. Histological features of the anther wall in wild tomatoes (*Solanum* sect. *Lycopersicon*). The images correspond to approximately the middle part of the anther, except (*H*). (*A*, *B*) Dicotyledonous (majority of cell divisions) and basic (arrows) types of wall formation in (*A*) *S. galapagense* and (*B*) *S. habrochaites*. (*C*) Theca cross-section of *S. habrochaites*; the zones marked by lines are the ventral (V), dorsal (D), and lateral (L) surfaces. (*D*, *E*) Subsequent cell divisions (some marked with arrows) in the anther wall formation in the ventral theca of *S. arcanum* (*D*), and in the dorsal theca of *S. habrochaites* (*E*). Observe the higher number of cell divisions on the dorsal surface (*E*), and the absence of subsequent divisions on the lateral surface of both thecae (*; *D*, *E*). (*F*) Resorption tissue disrupted (arrows) in neighbour anthers of *S. galapagense*; partially broken septum on the left, completely broken septum on the right. (*G*) Large epidermal ridges (arrows) lining the stomium (*) in the middle part of the theca of *S. habrochaites*. (*H*) Small epidermal ridges (arrows) lining the stomium (*) in the apical end of the theca of *S. habrochaites*. ST, sporogenous tissue. Scale bars = 50 µm (*A*, *G*), 20 µm (*B–E*), 25 µm (*F*, *H*).

none or sometimes only one subsequent periclinal cell division (in the middle layer) occurs in the lateral surface of the anther; thus, the wall remains thin (Fig. 1*D*, *E*). The dehiscence region differentiates afterwards in this side of the anther.

Dehiscence region

The dehiscence region of each anther consists of a stomium, other specialised epidermal cells lining the stomium and the subepidermal resorption tissue formed in the septum. These structures are fully differentiated when the microspores are released from the tetrads and all the wall layers are formed.

The resorption tissue is first differentiated as a group of subepidermic cells of the septum (arranged in one or two layers), characterised by a dense cytoplasm that is gradually filled with crystal sands. Subsequently, the cells of the resorption tissue disintegrate, forming a cavity in the septum where the crystals remain enclosed (Fig. 1*F*). Afterwards, the thin walls of the septum and the rest of the tapetum that form the cavity are broken, and

thus, the two microsporangia of the theca become unified (Fig. 1*F*).

While the resorption tissue is formed and destroyed, the stomium differentiates in the epidermis facing the septum. The stomium consists of two small cells that become the only cells in the anther wall after the rupture of the resorption tissue and septum (Fig. 1*G*). The epidermal cells lining the stomium gradually enlarge radially, forming some sort of ridges (Figs 1*G*, *H*, 2*C*). The cells of the ridges can be up to 4–5 times larger than the cells of the stomium (the largest cells were observed in *S. cheesmaniae* (Fig. 2*C*) *S. galapagense* and *S. peruvianum*), with the cytoplasm highly vacuolated and swelled (Figs 1*G*, 2*C*). The ridges in mature anthers are formed by a variable number of cells among species; the number of cells also varies along the anther since there are always fewer cells in the apical region (Fig. 1*H*), where cells with thickenings differentiate (see below). The smallest ridges (of 4–6 cells) were observed in *S. neorickii* and *S. chmielewskii*, whereas *S. galapagense* had the largest ridges (of about 20 cells). However, most species have intermediate ridges of 8–12 (14) cells (Figs 1*G*, 2*C*).

Mature anther wall structure

The subepidermal layers formed in the early development remain swollen until maturity. The exception is the tapetum that degenerates during pollen formation. The ventral surface is formed by 2–4 layers (Fig. 2*A*). The dorsal surface is formed by 4–6 layers in most species (Fig. 2*B*), although it can be up to 8–10 layers in *S. habrochaites* and *S. corneliomulleri*. On the lateral surface some cells degenerate; near the stomium, sometimes only the epidermal

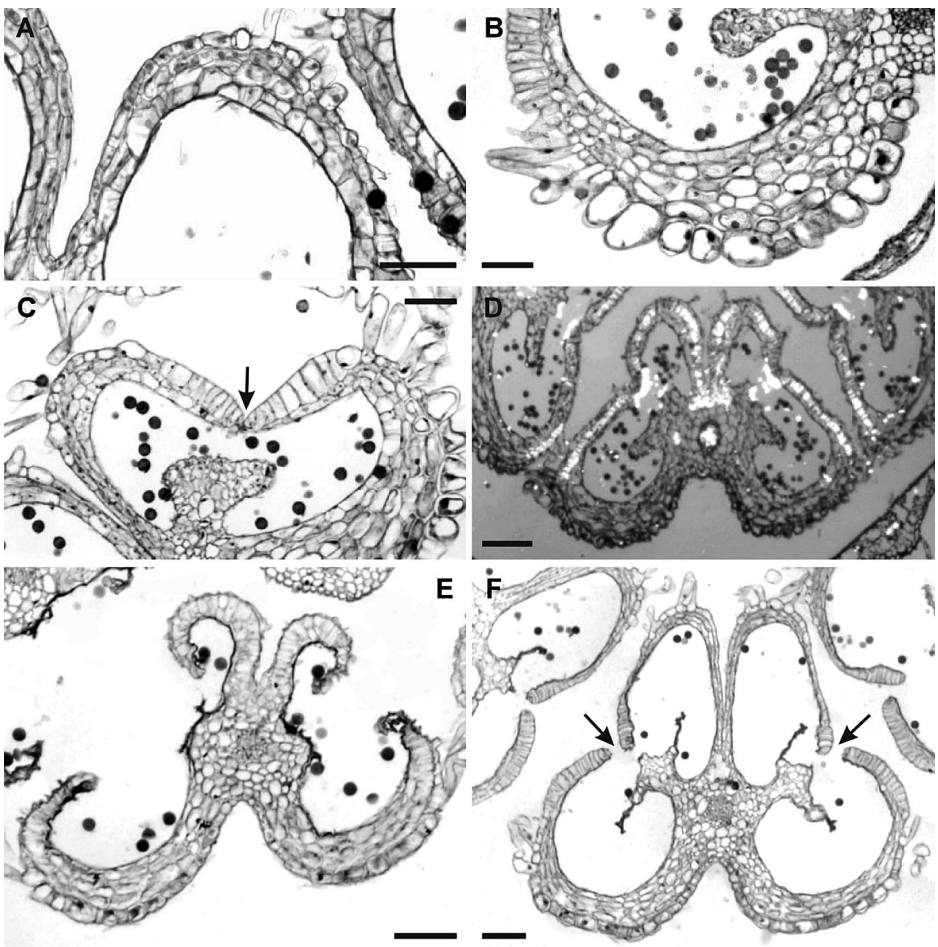

Fig. 2. Histological features of the anther wall in wild tomatoes (*Solanum* sect. *Lycopersicon*). (*A*, *B*) Wall structure in the middle part of the mature anther: (*A*) ventral theca of *S. peruvianum*, (*B*) dorsal theca of *S. galapagense*. Observe the higher number of cell layers on the dorsal theca, the papillaceous dorsal epidermis, and the lack of thickenings in both thecae. (*C*) Theca previous to the stomium opening in *S. cheesmaniae*. Observe the wall bending towards the locule interior (arrow) and the large epidermal ridges lining the stomium. (*D*) Distribution of cell with thickenings (bright cells) in a cross-section of the anther apex before dehiscence of *S. corneliomulleri*. (*E*, *F*) Open stomium in *S. pimpinellifolium*. Observe the wider aperture in the upper part of the anther (*E*) and the inconspicuous aperture in the middle part of the anther (*F*, arrows). (*A–C*, *E*, *F*) Brightfield, (*D*) polarised light. Scale bars (*A–C*) = 75 μm, (*D–F*) = 150 μm.

ridges form the wall. Then, up to three layers may constitute the lateral surface of the theca (Fig. 2*C*).

In the apical tip (approximately one-fifth) of the anther wall, reticulate thickenings are formed in the endothecial cells and in a few cells of the outermost middle layer of the ventral and lateral surfaces of each theca (Fig. 2*D*).

Before dehiscence, the lateral walls of the anther bend slightly inwards, pushing the stomium inside the locule (Fig. 2*C*). Afterwards, the two cells of the stomium separate from each other, so that the stomium opens, apparently in a basipetal sense. In the lateral surfaces of the wall where thickened cells are present, the tissues degenerate after dehiscence (Fig. 2*E*). These parts of the wall (including the epidermal ridges) shrink laterally and form a wide stomium (Fig. 2*E*), whereas the thickened ventral wall and the swollen dorsal wall remain almost intact (Fig. 2*E*). Where thickenings are absent, neither the epidermal ridges nor the subepidermal cells seem to degenerate in the lateral surface, so the stomium hardly opens in this part of the anther (Fig. 2*F*).

The dorsal surface of the anther is wide in cross-section all along, and it has a turgid aspect even in old flowers because of its large swollen cells (Fig. 2*B*, *D–F*), including large papillous epidermal cells in several species (Fig. 2*B*).

Discussion

Two types of anther wall formation (after Davis 1966) appear simultaneously in wild-tomato species; the dicotyledonous type predominates, whereas the basic type is observed sporadically in some parts of the same microsporangia. The anther wall formation in *S. lycopersicum* coincides with this pattern (data not shown). Only these two types of anther wall formation have been observed among the Solanaceae species (Carrizo García 2002*a*, and references therein). In a few species of the family, a combination of two types in one species, even in the same microsporangium, has been observed (Bhandari and Sharma 1987; Carrizo García 2003*b*). Another peculiar feature is the occurrence of periclinal cell divisions subsequently to those that characterise the type of anther wall formation, a character that has also been reported in many Solanaceae species (Carrizo García 2002*a*, 2002*b*, 2003*b*). Therefore, wild tomatoes conform to characteristics already described for Solanaceae. However, tomatoes are particular because the number of subsequent cell divisions is different between the dorsal, ventral and lateral sides of each theca, setting up different wall structures on each surface of the anther. The different structure observed between surfaces is not due only to the different number of periclinal cell divisions in each region, but also to the persistence until maturity of almost all, if not all, middle layers formed. Usually, the middle layers disappear during development (Davis 1966); thus, the atypical persistence of the numerous middle layers in the tomatoes gives a particular meaning to the patterns of cell divisions observed.

Some features of the dehiscence region in wild tomatoes agree with characters already observed in Solanaceae, such as the stomium formed by two small cells (Carrizo García 2002*b*) and the presence of a resorption tissue with crystal sands (D'Arcy *et al*. 1996; Carrizo García 2002*b*), whose development follows the general sequence described for other species of the family with a similar structure (Carrizo García 2002*b*). A distinctive feature of the dehiscence region in the tomatoes is the differentiation of the epidermal ridges lining the stomium. The ridges have been related to anther opening in *S. lycopersicum*, by generating and transmitting a mechanical force over the cells of the stomium (Oryol and Zhakova 1976; Bonner and Dickinson 1989), possibly working together with the endothecium (Bonner and Dickinson 1989). Because wild tomatoes have the same kind of epidermal ridges, a similar function could be suspected in relation to anther opening. With regard to the specific mechanism of anther opening, Bonner and Dickinson (1989, 1990) suggested that the driving force to open the stomium could not be tissue dehydration because most tissues remain swollen and only a small percentage of water is lost from the anthers during anthesis. Keijzer (1987) suggested that the highly turgescent epidermis and endothecium promote mechanical stomium opening in *Gasteria verrucosa* (Liliaceae). Then, the high hydration of the ridges in tomatoes (i.e. ridges formed by large, vacuolated and turgid cells) could be the reason of the increasing pressure over the cells of the stomium which promotes its opening. The ridges would work together with the thickened cells in the apical part of the anther, whereas the larger ridges would work by themselves to open the stomium where thickened cells are absent.

It has been suggested for species of other families that thickened cells participate in the tangential shrinkage of the anther wall by dehydration, thus widening the stomium aperture (Venkatesh 1956, 1957; Keijzer *et al*. 1996; Matsui *et al*. 1999, 2000). In wild tomatoes, once the stomium is open, the aperture of its apical end widens apparently by dehydration of the thickened cells and surrounding tissues, as described for *S. lycopersicum* (Oryol and Zhakova 1976; Bonner and Dickinson 1989). Since the rest of the anther tissue remains swollen, the stomium hardly opens where thickened cells are absent. As a consequence of this localised dehydration, a sort of elongated pore is formed in the apical part of the anther, immediately below the apical appendages. The pollen can be shed through these apertures when the pollinators vibrate the anthers. Then, the pollen is supposed to move outside through the unique aperture of the antheral cone, formed by the united appendages (see Rick and Robinson 1951 for details of the pollen movements). The purpose of having an open slit downwards the wide apical opening is unclear, when

the slit aperture is almost inconspicuous and possibly non-functional, i.e. not participating in pollen shedding. Maybe it is only a remnant of a longitudinal anther that has evolved to mimic a poricidal one.

The dorsal surface of the anther does not seem to be directly involved in anther dehiscence. However, its high number of swollen layers gives certain strength and rigidity to the anther cone, which is useful as a support for the pollinators, according to the behaviour described by Buchmann (1983). Besides, the swollen cells give to the anther the aspect of being full of pollen, which is an attractant to pollinators (Buchmann 1983).

To summarise, in tomatoes, the anther wall development shows a combination of some particular features that suggest that although it seems to follow a disordered pattern, the apparent disorder has a meaning when the final histological structure of the anther is analysed. Particular developmental pathways on each surface that set up different structures were revealed, concerning mainly the number of wall layers, the differential enlargement of the epidermis near the stomium and the deposition of fibrous thickenings in part of the endothecium. The histological features of the anther at maturity, which are uniform among the tomato species (wild and cultivated), can be interpreted as a group of adaptations to the following:

(1) possibly opening the anther without the need of tissue dehydration, because the anthers remain swollen (see Points 3 and 4 below); the pressure of highly hydrated epidermal ridges could help to produce stomium opening;
(2) widening the apical end of the stomium because of tangential wall shrinkage caused by dehydration and degeneration of thickened cells restricted to that area, forming the main opening of the anther near the unique aperture of the antheral cone; each anther could work as a poricidal anther inside the antheral cone, which as a whole also functions as a poricidal anther;
(3) giving an attractive aspect to pollinators, with anthers apparently full of pollen, owing to the turgid dorsal wall; and
(4) keeping a solid structure to support pollinators when they grasp the anthers, by having a multilayered dorsal wall formed after numerous periclinal cell divisions during wall formation.

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