DENDROBIOLOGY
2009, vol. 62, 11–16

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Development of the multilayered epidermis covering fruit of *Sorbus torminalis* (Rosaceae)

Received: 9 April 2009; Accepted: 2 July 2009

Abstract: In this paper we focus on anatomic structure of the outer cellular layers of the fruit of *Sorbus torminalis*. We have confirmed the theory of existing of multilayered epidermis providing developmental, histogenetic evidence. The studies were made from blooming time (ovules) to fully ripen fruits, using various histological techniques and microscope types. The multilayered epidermis is derived from successive tangential divisions of cells of initially single-layered epidermis commencing about two weeks after full bloom. The subsequent epidermal layers result from division of cells of epidermal meristem. The mature fruits – about 12 weeks after full bloom – are covered with four – to five-layered epidermis; each layer with its own cuticle. The lenticels developed beneath stomata from phellogen which is not in continuity with the epidermal meristem. Measurements of cuticular membranes thicknesses during development were also made.

Additional key words: trees, anatomy, pome, epidermis.

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Introduction

The genus *Sorbus* belongs to subfamily Maloideae, family Rosaceae. The entire subfamily is characterised by pome fruit – a pulpous fruit with peculiar morphology and anatomy. Pome anatomy of *Sorbus* species and subfamily Maloideae in general was described and discussed widely (Kovanda 1961; Gabrielian 1978; Rohrer et al. 1991; Jankun 1993, Aldasoro et al. 1998a, b). According to Rohrer et al. (1991), the skin of the pomes of subfamily Maloideae consists of a single epidermal layer of anticlinally flattened, rectangular cells covered with cuticle. However, Miller (1984) found and described a multilayered epidermis in *Mespilus germanica*. In a multivariate morphometric study of the genus *Sorbus* and the survey of pome anatomy of subfamily Maloideae, Aldasoro and others (1998a, b) detected that also the fruits of *Pyrus* sect. *Pashia* and *Sorbus torminalis* have a multilayered epidermis. The peculiar tissue covering fruits of *S. torminalis* has previously been reported by Gabrielian (1978). However, this author, described it as a periderm – a protective tissue of secondary origin, which is an exception in entire genus *Sorbus*. Neither Gabrielian nor Aldasoro and others, however, provided histogenetic evidence of their discoveries.

The objective of our study was to give a precise description of a tissue covering fruit of *S. torminalis* and to show the successive stages of development of the tissue. Providing developmental, histogenetic evidence, we wanted to definitely decide whether multilayered epidermis or periderm is a tissue covering fruits of *S. torminalis*.

Methods

Random samples of pomes were obtained from three *S. torminalis* trees growing in Dendrological Garden of Poznań University of Life Sciences. Fruits were collected at different intervals from May (blooming) until October (fully ripen) and fixed in mixture of 2% paraformaldehyde and 2% glutaraldehyde (1:1) at room temperature, for 2h. After rinsing three times in the 0.05 M cacodylic buffer (pH 6.8) material was dehydrated in graded ethanol series (5–70%) and then the samples were stored.

For histological analysis, both paraplast and semi-thin sections were prepared. Specimens were cut transversely. The fixed fruits for paraplast procedure, were dehydrated in graded ethanol series (80–100%), cleared in butanol treatments and infiltrated in paraplast. The paraplast sections (12 μm) were fixed on glass slides, double stained with safranin and fast green or Heidenhein's iron hematoxylin and fast green (Jensen 1962). Finally, slides were mounted in entellan.

For semi-thin sections, the fixed fruits were dehydrated in ethanol followed by acetone and embedded in Spurr's low-viscosity epoxy resins (Spurr 1969). The samples were cut into 1.5 μm sections, fixed on glass slides, and stained with methylene blue and basic fuchsin (Humprey and Pittman 1974).

The slides were examined both under white and UV light in a Zeiss Axioscop Microscope. Images were photographed on Fujifilm 100.

For scanning electron microscopy material was dehydrated according to procedure applied for material preparation for semi-thin sections. Dehydrated samples were dried in liquid carbon dioxide, coated with gold and examined under a Phillips SEM-515 with an accelerating voltage of 7.5 kV. Images were stored digitally.

Measurements of cuticle thickness were made on transverse semi-thin section using LSM Image Browser Program (Carl Zeiss Jena). The measurements of cuticle thickness were done on digitally saved images under Light Microscope Axiovert 200M. The cuticle thickness were calculated for 50 cells from each stage of epidermis development.

Results

The young epidermis

The epidermis of an ovary during the blooming and one week later is composed of one layer of cells (Fig. 1 A). After two weeks of full bloom, the epidermis consists of one and in some places of two layers of cells. Numerous unicellular trichomes (hairs) arise as outgrowths of the epidermal cells (Fig. 1 B). The hairs are ephemeral and only a few of them persist until fruit ripening. Stomata are elevated above the epidermal surface and they are visible only in young epidermis (Fig. 1 C). Beneath the guard cells the substomatal chamber is formed by parenchymatous cells (Fig. 1 C).

The young epidermal cells are dense in cytoplasmic content (Fig. 1 D). Cells forming a single-layered epidermis are more or less isodiametric, thin-walled and are covered by a cuticle about 0.4 μm in thickness (Ta-

Table 1. The cuticle thickness of multiseriate epidermis during *Sorbus torminalis* fruit development

Collection period after blooming (week)	The number of epidermal layers	Cuticle thickness range in μm (mean)	Mean cuticle thickness of all layers in μm
1	1	0.2–0.9 (0.4)	0.4
5	2	0.8–2.3 (1.5)	1.7
		1.1– 2.8 (1.9)	
7	3	1.1–3.9 (1.6)	2.5
		1.0–4.2 (2.3)	
		2.1–5.0 (3.6)	
10	4	1.0–2.6 (1.5)	2.5
		1.6–3.7 (2.8)	
		2.7–4.3 (3.1)	
		1.6–4.8 (2.7)	
12	5	1.7–2.8 (1.6)	3.5
		2.0–4.2 (3.1)	
		2.4–3.2 (2.8)	
		2.1–5.7 (3.9)	
		2.1–6.4 (4.1)	

ble 1; Fig. 1 E). Locally, these epidermal cells divide tangentially forming two cells which are together of the same size as parent ones (cuticle is still only on outer fruit surface) (Fig. 1 F).

The multilayered epidermis

A distinct two-layered epidermis is prevalent about three weeks after blooming (Fig. 2 A). The cells of both epidermal layers assume more or less radial arrangement. In intensively growing fruits some of epidermal cells divide anticlinally to increase the diameter of epidermal layer (Fig. 2 A). Below epidermal cells two layered hypodermis is formed and usually the cells are tightly packed (Fig. 2 A).

About 4–5 weeks after full bloom the cells of a second layer of epidermis develop a cuticle about 1.9 μm in thickness on their outer periclinal wall (Table 1; Fig. 2 B). A single layer of meristematic cells is visible below the cells of second epidermis layer (Fig. 2 B). The sequence of cell differentiation after periclinal epidermal cell divisions is following: the outer cells differentiate and form the epidermal layer while the inner cells become merystematic and undergo sequent periclinal division to form the next epidermal layer of cells. The three-layered epidermis are formed two weeks later. In each succeeding layer the outer tangential cell walls of epidermis are covered by cuticle layer which overlapping on antyclinal walls (Fig. 2 C). The inner tangential cell walls of epidermis are thin as well as anticlinal ones. In ten week of blooming four-layered epidermis is observed (Table 1). The cuticle layers are well recognized under UV Microscope (Fig. 2 D).

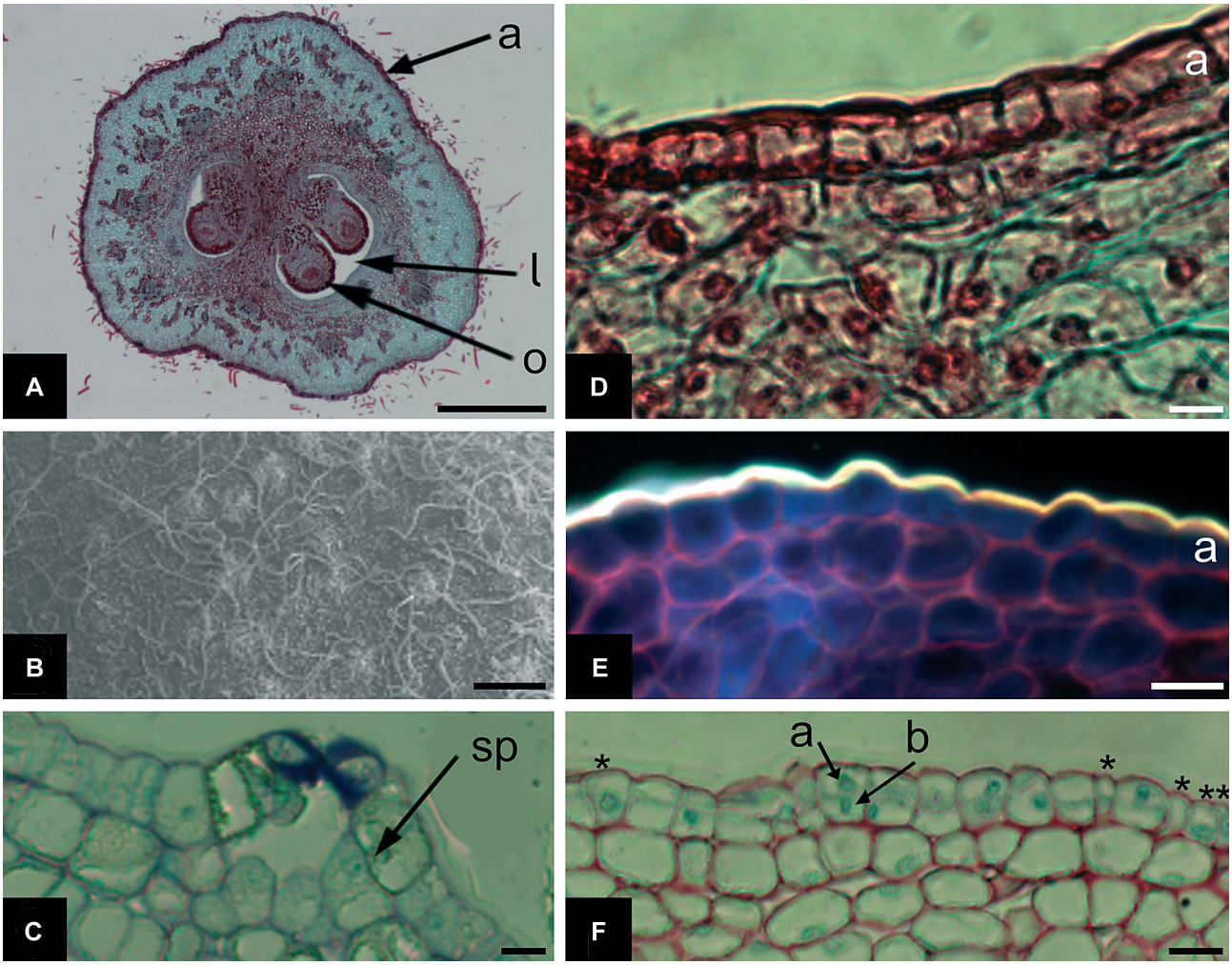

Fig. 1. Images of epidermis of *Sorbus torminalis* ovary and young fruits: A – the transverse section through ovary with ovules (o) in loculus (l) at the time of blooming; one-layered epidermis (a) with trichomes intensively stained by safranin; B – bushy trichomes on the surface of young fruit, 2 weeks after blooming – the SEM image; C – stomata in one-layered epidermis, 1 week after blooming; parenchymatous cells (sp) forming substomatal chamber with nuclei and dense cytoplasm undergoing redifferentiation; D – isodiametric cells of epidermis (a) with nuclei and thin layer of cuticle on outer cell walls, 1 week after blooming; E – autofluorescence of cuticle layer on outer surface of epidermal cells, 1 week after blooming – the UV image; F – periclinally divided cells forming two-layered epidermis (a, b), 2 weeks after blooming; some epidermal cells have not been divided (*) yet; A – bar = 1 mm, B – bar = 200 μm, C–E – bar =10 μm

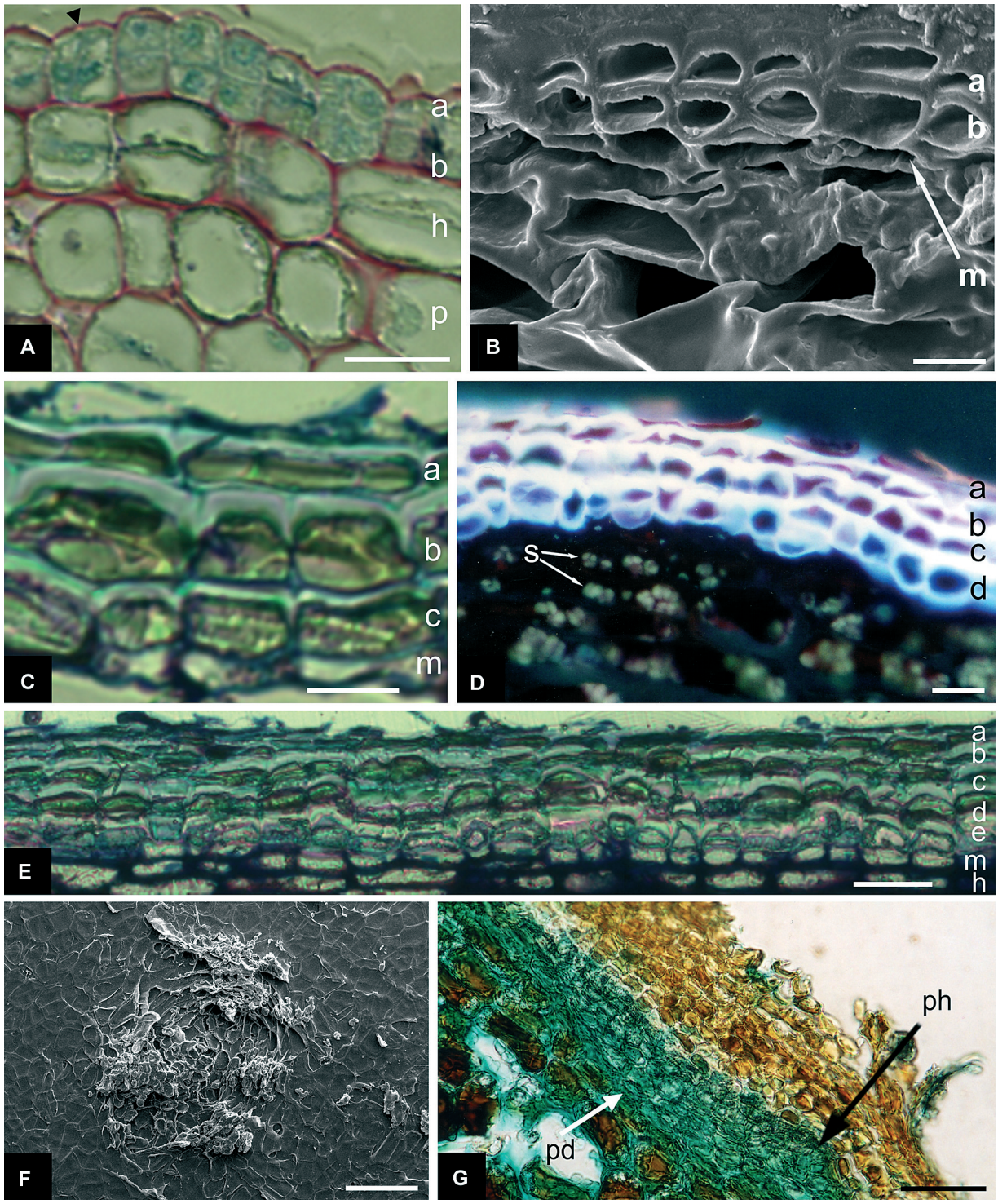

Fig. 2. Images of multilayered epidermis of *Sorbus torminalis* fruits; A – two-layered epidermis (a, b) with cuticle present only on the outer cell walls of first epidermal layer, 3 weeks after blooming – notice epidermal cells divided antyclinally (?) and periclinaly divided subepidermal cells forming hypodermis (h); under hypodermis isodiametric highly vacuolated cells of parenchyma (p) are visible; B – two-layered epidermis with swellings on outer peryclinal cell walls of both layers (a, b) and tangentially expanded meristematic cells (m), 5 weeks after blooming; C – three- layered epidermis (a, b, c) on the semi thin-section, 7 weeks after blooming; the cuticle layers visible on tangential outer wall of cells; D – autofluorescence of cuticle in four-layered epidermis, 9 weeks after blooming – the UV image; numerous starch grains (s) are present in parenchymatous cells of core; E – five-layered epidermis (a, b, c, d, e) tangentially compressed and meristematic cells are still visible, 12 weeks after blooming; F – lenticels with loosely packed cells protruding on the surface of fruit epidermis, 4 weeks after blooming – the SEM image; G – the anatomical arrangement of lenticels on the transverse section, 12 weeks after blooming; loosely arranged cells forming lenticels, phellogen (ph), and phelloderm (pd) are visible; A–E – bar = 10 μm, F – bar = 100 μm, G – bar = 50 μm

In circumference, the outermost epidermal layers become tangentially compressed so their former distinct entities are only recognized by the persistence of their cuticles (Fig. 2 E). With time portions of the crushed outer epidermal layers commence to gradually slough. The process continues through fruit maturation (Fig. 2 D, E). The number of epidermal layers in some parts increases to five about 12 weeks after full bloom. Pome with four to five epidermal layers persists through fruit maturation (Fig. 2 E). The mature epidermal cells of all layers appear to remain similar in size. They are tangentially compressed cells, each layer with cuticular membrane. The mean thickness of five-layered epidermis cuticle is about 3.5 μm and it ranges between 1.67–6.38 μm (Table 1). The epidermal meristem continues to be present when the fruit reaches maturity and is still present in fully ripen fruit (Fig. 2 E). In parenchymatous cells large number of starch grains are present (Fig. 2 E). Brachysclereids with thick cell walls are spread in parenchyma tissue, singly or in small clusters.

Lenticels are observed on the surface of fruits after four weeks of blooming (Fig. 2 F). They develope beneath stomata. The parenchymatous cells surrounding substomatal chamber redifferentiate. Their cytoplasm become dense and the nuclei are well visible (Fig. 1 C). The phellogen is formed by periclinal division of parenchymatous cells and it is not in continuity with the epidermal meristem. Moreover, the cells forming lenticels do not posses swellings on periclinal outer wall of cells (Fig. 2 G). The epidermal meristem is visible around the circumference of the fruit with the exception of lenticels presence. The meristem of lenticels lies deeper and produces a few layers of phelloderm cells (Fig. 2 G).

Discussion

Numerous plants have been described as developing a multilayered (=multiple, multiseriate) epidermis, mainly in stems and leaves, but rather seldom in fruits. The occurrence of the multilayered pome epidermis was detected in *Sorbus torminalis*, *Pyrus* (section *Pashia*) and *Mespilus germanica* so far (Miller 1984; Aldasoro et al. 1998a, b). According to the cited authors the multilayered epidermis has a few layers of tangentially compressed cells, each layer with cuticular membrane. The cells develop from a tangential subepidermal meristem layer which is somewhat similar to the phellogen. The current paper documents the development of a multiple epidermis in *Sorbus torminalis* consisting of four to five layers, with each epidermal layer developing its own distinct cuticular membrane. The process of development of multi epidermis-cuticle complex is very similar to that described by Miller (1984) in *Mespilus germanica*. In *Pyrus* fruits (Aldasoro et al. 1998b) the numbers of 3–6 layers of epidermal cells were formed in *Mespilus germanica* 4–6 (Miller 1984).

The occurrence of the multilayered pome epidermis in three not closely related taxa of subfamily Maloideae – *Mespilus*, *Pyrus* and *Sorbus* subg. *Torminaria* (Phipps et al. 1991; Campbel et al. 1995) are most probably related to their seed dispersal by mammals. All studied pomes with multilayered epidermis show traits associated with mammalian zoochory syndromes: green or brown skin, copious lenticels permitting scent to emanate, seeds protected by many sclereids, high tannins and fiber content (Herrera 1987, 1989; Aldasoro et al. 1998b). The multilayered pome epidermis was probably derived independently in these genera.

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