Anatomical study on the developing pericarp of selected Rosa species (Rosaceae)

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Abstract: Results of anatomical studies on the developing pericarp of selected wild roses are presented. Using SEM and CLSM, the changes in the pericarp structure of 5 species have been observed during its formation, from the flowering stage to fully ripe achenes. In the morphological development of the pericarp of Rosa species two main phases can be distinguished: the phase of intensive growth of the pericarp during which the fruit achieves its final shape and volume, and the subsequent phase of pericarp ripening when no significant morphological changes in the pericarp occur. Similarly, in the process of the anatomical development of the pericarp two phases are noticeable, however, during both stages, great internal changes proceed in the fruit. The first phase consists of intensive cell divisions and enlargement, gradual thickening of cell walls and formation of all pericarp layers. Due to these changes, the pericarp achieves its final anatomical structure. The second phase, involving the pericarp ripening, is manifested in the modification of cell walls, mainly by their quick thickening, but first of all by their lignification. The lignification of pericarp cell walls begins in the inner endocarp; it proceeds in the outer endocarp, later in mesocarp and finishes in the hypodermal cells of the exocarp. The epidermal cells remain alive the longest and their walls do not (or hardly) become lignified. The death of all cells finishes the pericarp ripening.

Additional key words: achenes, anatomy, SEM, lignin autofluorescence

Introduction

Within the family Rosaceae the species of the genus Rosa are easily recognisable almost in all stages of the development, and their flowers and fruits are especially characteristic. In each flower there are many styles which are densely set inside the urceolate hypanthium and usually separated from each other by long, stiff, unicellular hairs (Fig. 1).

With time the styles transform into hard achenes, while the walls of the hypanthium become thick, fleshy and colourful. Altogether the fleshy receptacle and the achenes form an aggregate fruit (Lawrence 1958) commonly known as a hip. Ovaries and young achenes are protected from unfavourable environmental conditions by the young hypanthia. Later, when the mature hypanthia become attractive as food for animals or disintegrate spontaneously, the seeds are protected by the already hard pericarp.
Anatomy of the fruit was the subject of many studies (many textbooks eg. Esau 1977, Gillaspy et al. 1993, Toma 2002, Lindström et al. 2007, Yang et al. 2010, Mayer et al. 2011, Pabón-Mora N., Litt A. 2011, Romanov et al. 2011, Gagliardi et al. 2012). However, only few studies concerned fruits of roses. The anatomical structure of the rose pericarp was studied with the use of a light microscope by Starikova (1973, 1975, 1977, 1983) and Khrzhanovskii et al. (1985). The results of the SEM work on the anatomy of the pericarp have been recently published by Zieliński et al. (2010).

The pericarp has a similar anatomical structure in all species of the genus *Rosa* and consists of endocarp, mesocarp and exocarp. The endocarp is composed of 2 layers: the inner and outer ones. The mesocarp is built of sclereids of different shape and size, from distinctly elongated to almost isodiametric cells. Their walls are often thinner than those of endocarp fibres, so their lumina are larger. The orientation of cells is less regular, however radially arranged long sclereids dominate. The exocarp is composed of 1- to 2-cell thick epidermis and hypodermis (Zieliński et al. 2010).

Research on the developmental anatomy of roses’ pericarp was conducted so far by Starikova (1975) on *Rosa rugosa* Thunb., however, as it appears from the recent studies by Zieliński et al. (2010), *R. rugosa* has a unique structure of pericarp and is not representative in this respect for the genus *Rosa* as a whole. It has a spongy mesocarp composed of isodiametric, thin-walled cells, therefore the question arises as to how the pericarp changes proceed in *Rosa* species having mesocarp formed by thick-walled cells with small lumina. We do not agree with the final conclusions drawn by Starikova (l.c.) Some of them seemed to be controversial (e.g. differences in pericarp structure may be of taxonomic value at the species level) and needed verification on richer, more representative material. The aim of the studies was to follow the changes of pericarp structure of selected wild roses starting from the early stage of their development to full maturity. Special attention was paid to the formation and degree of lignification of particular pericarp layers.

**Material and methods**

The development of the pericarp has been observed on 5 species belonging to different systematic groups:

- subgenus Platyrhodon: *R. roxburghii* Tratt.

The material was collected from plants growing in similar conditions in the Kórnik Arboretum in western Poland. The first samples were gathered just after the fall of petals, and the next ones every 7 days until the full ripeness of the achenes. The consecutive stages of fruit development were, out of necessity, analysed on different achenes, growing in changeable weather conditions, hence the total time of pericarp formation and of its individual phases can be only roughly described.

A scanning electron microscope (SEM), LM (light microscope) and confocal laser scanning microscope (CLSM) were used for histological analysis. Fully developed achenes are very hard and especially difficult to cut precisely. Thus they first had to be macer-
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Achenes taken directly from fresh, living hypanths were first preserved in FAA (formalin 5%, acetic acid 5%, ethyl alcohol 90%), and then macerated in glycerol and 70% ethyl alcohol mixture (1:1 vol.). Sections (about 12 µm thick) through the achenes were obtained using a cryomicrotome, vibrotome (Leica VT 1200 S) and also hand sections were prepared. Cross- and longitudinal-slices were coated with gold and viewed with Hitachi S300N SEM belonging to the Institute of Plant Protection in Poznań. Selected objects were photographed. The ovaries taken from several living plants were observed in LM after staining with safranin and fast green (safranin appears as a brilliant red in lignified cell walls, while the fast green should be equally brilliant in the cellu-

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cases between them and of local weather conditions during the vegetation period. However, the period of the fruit formation in the whole group of individuals, from the fall of petals to the full ripeness of pericarp, lasted in total about 100 days. It started at the end of May, the earliest in *Rosa spinosissima* and finished at the end of August, the latest in *R. arvensis*.

In the beginning of the first phase, intensive cells divisions were observed in the pericarp of all studied species, but it is very probable that this process already takes place in the flower buds. In the very young pericarp (Figs. 4 D, 5A), its cells are undifferentiated, thin-walled, and it has the character of parenchymatic tissue.

At the end of June, that is about 10 days after the fall of petals, the cells of each layer get their final shape, however they are still relatively thin-walled (Figs. 3A,B,C, 4 A, E, G, 5D). The pericarp layers are easily recognisable and for the first time also inner and outer endocarp can be distinguished. Due to the cells’ enlargement the achenes reach their final size but cell walls are still un lignified. At this time the differences in the anatomical structure of pericarp of some taxa become well visible. The achenes of *Rosa arvensis* (Fig. 5) with their mesocarp composed of strongly radially elongated sclereids are particularly characteristic. In contrast to the above mentioned species, the mesocarp of *R. rugosa* (Figs. 3, 6) is built of almost isodiametric cells typical for the parenchyma. During the last 7–10 days of the first phase, gradual suppression of cell divisions takes place and, as a result, further anatomical changes in the pericarp are practically indiscernible in SEM. This relatively short but distinct slowdown of the pericarp development is probably connected with the seed formation.

As it was mentioned above, the second phase of the pericarp development involves the modification of cell walls. In most of the species, the thickening of walls is continued (compare the Figs. 4, 7 and 8); however, lignification is the main process which distinguishes the second phase. In all the species the lignification of pericarp cell walls begins in the inner endocarp, it proceeds in the outer endocarp, later in mesocarp and finishes in the hypodermal cells of the exocarp (Figs. 6, 7).

The mature endocarp has a similar structure in mature achenes of all taxa, walls of this tissue cells being evenly, strongly thickened, but the mesocarp cells change differ in individual species. In *Rosa roxburghii* (Fig. 4 D–F) and *R. spinosissima* (Figs. 4 A–C, 7, 8) the cell walls thicken very strongly and in the end, the sclereids form a very compact layer in which individual cells are difficult to distinguish. In *R. arvensis* (Fig. 5) and *R. virginiana* (Fig. 4 G–I) this process is less intensive, while in *R. rugosa* (Fig. 3) the walls of mesocarp cells seem to be only slightly modified or not changed at all. Nevertheless they are distinctly lignified (Fig. 6).

The exocarp is a layer in which the transformations are the least dramatic and occur the latest. The walls of the hypodermal cells are rather weakly thickened in all studied species, while changes in the epidermis, which remains alive the longest, are practically unnoticeable (Fig. 7 H, I). In the epidermal cells of *Rosa*
Fig. 2. Macro- and microscopic changes in pericarp
A. The increase in pericarp volume (black line): intense growth and development phase (I) when the achene maximum size is reached, stagnation phase (II) when no further changes are observed on morphological level. Anatomical changes seen in SEM (grey lines): A – intense development, pericarp layers formation, in each layer the final number of cells is reached; dashed line – no changes in SEM are seen; thickening of cell walls in: B – endocarp, C – mesocarp and D – exocarp layers; B. The length of achenes (time of material collection: 30.05; 14.06; 21.06; 28.06; 5.07; 12.07; 25.07; 2.08; 16.08; and 31.08 respectively) ROX – R. roxburghii; VIR – R. virginiana; ARV – R. arvensis; SPI – R. spinosissima; RUG – R. rugosa; C. Achenes of R. spinosissima (30.05; 14.06; 21.06; 28.06; 12.07; 2.08; 16.08; and 31.08 respectively)
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The remnants of the protoplast were observed in the mature achenes (Fig. 5 C, E). The death of all cells finishes the pericarp ripening.

The distinct slowdown in pericarp development at the end of the first phase is probably connected to the expenditure of energy on seed formation. Our observations suggest that the seeds are already fully developed just before or at the beginning of pericarp lignification. At this time, the seed entirely fills the inside of the pericarp. The above statements seem to agree with the observations of rose breeders and gardener, who advise collecting and sowing achenes of roses before their full maturity. Such achenes germinate usually much better than those collected later, because the seeds have not yet entered physiological dormancy, besides the immature, not fully lignified pericarp more easily undergoes destruction in soil and does not hinder seed germination.

The two-phase period of morphological development of rose pericarp contrasts clearly with three phases distinguished during the pericarp formation of Prunus and Rubus species. The basis for the distinguishing of the third, most spectacular phase in the above genera, are intensive changes in the mesocarp. During this phase the fruit grows very quickly, becomes fleshy and usually changes its colour (Tukey and Young 1939; Sterling 1953; Reeve 1954a, 1954b; Boynton and Wilde 1959). This phase is absent in Rosa species.

Starikova (1975), influenced probably by the data published by the abovementioned authors, recognised also three phases of pericarp development of Rosa rugosa. She defined them as follows: 1) intensive cell divisions and cell enlargement resulting in intensive growth of the fruit and establishment of pericarp structure (14–20 days), 2) differentiation of the fruit tissues and beginning of cell wall lignification and cu-

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Fig. 3. The cross-section through the pericarp of R. rugosa (A, C – LM, sections after safranin and fast green staining; red colour characteristic for lignin; B, D – SEM); En1 – inner endocarp; En2 – outer endocarp; Me – mesocarp; e – epidermis; sd – seed A–C. Arrangement of pericarp layers well visible, the final breadth and number of cell layers in the inner and outer endocarp have been achieved; cells of all layers are thin-walled; D. The final stage in the pericarp development; very strong thickening of endocarp cell walls is visible, the boundary between inner and outer endocarp is impossible to distinguish, and cell walls of mesocarp remain thin-walled.
Fig. 4. Sections through the pericarp of *Rosa spinosissima*, *R. roxburghii* and *R. virginiana* in SEM; scalebars = 100 μm; En1 – inner endocarp; En2 – outer endocarp; Me – mesocarp; e – epidermis; sd – seed; A, C, E, F, I – cross-section; B, G, H – longitudinal section.

A–C. *R. spinosissima*: A. The final arrangement and breadth of pericarp layers are clearly visible, but cells are thin-walled; B. Thickening of endocarp and mesocarp cell walls; C. Fully developed pericarp; strongly thickened cell walls of endocarp and mesocarp; lumina of the cells strongly reduced, almost invisible; the boundary between inner and outer endocarp is difficult to distinguish; D. One of the young achenes of *R. roxburghii* (magnification from the Fig. 1 B), pericarp layers are already clearly visible; E–F. *R. roxburghii*: E. All pericarp cells are still thin-walled. F. The final stage of the pericarp development, cell walls very strongly thickened, boundary between the layers is indistinct; G–I. *R. virginiana*: G. Thin-walled cells of inner and outer endocarp, and mesocarp. H. Strongly thickened fibres of outer endocarp; I. The final stage of the pericarp development.
Fig. 5. Sections through the pericarp of *Rosa arvensis*, A, B, C, E, F – SEM; D – LM; En1 – inner endocarp; En2 – outer endocarp; Me – mesocarp; e – epidermis; sd – seed; A – D – cross-section; E, F – longitudinal section

A, B. Very young achenes; C, E, F. The final stage of the pericarp development; D. Layers are clearly visible, inner and outer endocarp cells with lignin presence in the cell walls, very thin-walled cells of mesocarp
tinisation (14 days) and 3) full lignification of the pericarp (7 days). The drawings illustrating the anatomical changes in the fruit presented by Starikova (l.c.) and her commentaries allow identification of the second stage distinguished by the author with the period of slowdown of changes observed at the end of the first phase.

Results of present research generally confirm the earlier data on the anatomical structure of the pericarp of Rosa species. Analysis of the pericarp cross-section through the achenes of Rosa rugosa in the phases of the pericarp development; blue – autofluorescence of lignin (A–H) and cuticle (B–H) (confocal microscope, UV excitation); En1 – inner endocarp; En2 – outer endocarp; Me – mesocarp; h – hypodermis; e – epidermis

Fig. 6. One of the first phases of the pericarp lignification: all pericarp layers (inner and outer endocarp, mesocarp and exocarp) are clearly formed, the final number of cell layers has been achieved; autofluorescence of lignin in cell walls of the inner and outer endocarp is visible, but all the cells are thin-walled; B. Subsequent phase of the pericarp lignification; centrifugal direction of lignification is observed; autofluorescence of lignin can also be observed within the mesocarp; C. Image B merged with the photograph of the same tissue fragment observed in transmitted light; D. Increase of intensity of the lignin autofluorescence, especially within the endocarp cell walls; E. Image D merged with the photograph of the same tissue fragment observed in transmitted light; F. Autofluorescence of the lignin in the mesocarp and hypodermis cell walls; no lignin fluorescence in epidermis cells; G. Increase of the intensity of lignin autofluorescence in hypodermis in the subsequent phase, for the first time the autofluorescence of lignin in the epidermis is stated; H. Autofluorescence of the lignin in the cell walls of all pericarp layers. In subsequent phase cell walls within the endocarp thickened, within mesocarp and exocarp no further changes were observed.
Fig. 7. The cross-sections through the achenes of *Rosa spinosissima* in different stages of the pericarp development; blue – autofluorescence of lignin (A–I), of cuticle (B–I) (confocal microscope, UV induction); En – endocarp; En1 – inner endocarp (=); En2 – outer endocarp; Me – mesocarp; h – hypodermis; e – epidermis; arrow ⇒ cuticle

A. An initial stage of the pericarp lignification; lignin autofluorescence in cell walls of inner and outer endocarp; in other pericarp layers the lignin autofluorescence was not observed; B. Autofluorescence of the pericarp is centrifugal; it is visible within the endocarp and neighbouring parts of mesocarp; a part of mesocarp cells remains un lignified; C. Pericarp lignification proceeds and in the subsequent phase it spreads through the whole mesocarp (D). Intensity of lignin autofluorescence also increases, but endocarp and mesocarp cells (E) are still thin-walled (E – magnified fragments of D); F, G. A very intensive increase of cell wall thickness and of fluorescence intensity occurs (The picture G was taken using the same parameters as for A–E). The intensity of autofluorescence was artificially limited in the pictures F–I. F. Image G after diminishing the fluorescence intensity: very thick, lignified cell walls, in most cells of endocarp and of mesocarp lumina are very small; H. Development of mesocarp is finished and the walls of its cells have their final thickness; cells of hypodermis are still rather thin-walled, within epidermis only weak fluorescence is observed. I. In the final stage of pericarp ripening cell walls of hypodermis thicken considerably and fluorescence of epidermis cell walls is clearly visible.
and longitudinal sections reveal again that literature data which say that the outer endocarp is composed of isodiametric cells, are erroneous (Starikova 1975). Like the inner endocarp it is formed by strongly elongated fibres and sclereids, but both endocarp layers are perpendicularly or diagonally oriented in respect to each other, at least in the middle part of the pericarp (Figs. 3A–C, 4A–C, H, I, 5C–F).

In light of data showing the differences between *Rosa* species having woody mesocarp and taxa of *Rubus* and *Prunus* s.l. with fleshy mesocarp, the different positions of vascular bundles in the fruit of roses and the latter genera becomes clear. In the fleshy mesocarp, mesocarp being the living tissue until the fruit ripening, they are distributed throughout this layer (Tukey and Young 1939; Archibald and Melton 1987). In roses, the mesocarp becomes woody rather quickly and probably for this reason the vascular bundles are situated at its outer parts, just under the hypodermis, the cells of which are alive the longest. Conclusions:

1. The changes observed during pericarp development are realised according to the same plan in all studied species. The general scheme of pericarp structure in roses is also similar; however, there are some important differences between species due probably to their genetic identity.

2. The growth of the pericarp of roses from the fall of petals to full ripeness of the fruit consists of two main phases: the phase of the volume enlargement and the phase of pericarp ripening, when no substantial changes in the morphology of achenes occur.

3. The anatomical development of the pericarp also occurs in two phases. The first of them consists of cell divisions and enlargement, cell wall thickening and differentiation of all pericarp layers which result in the pericarp achieves its final characteristic structure. The second stage is manifested in the modification of cell walls, mainly lignification.

4. The process of lignification begins in the inner endocarp, proceeds to the outer endocarp, later in the mesocarp and finishes in the hypodermal cells of the exocarp.

5. The mesocarp is the most differentiated layer of the pericarp, both in the shape of sclereids and thickness of their walls, however in mature fruits its cells are strongly lignified. It also concerns *Rosa rugosa*, the mesocarp of which is built of relatively thin-walled cells.

6. The phases of the pericarp formation are correlated with seed development. The 7–10-day period of the slowdown in the pericarp development observed at the end of the first phase is probably connected with the seed development and ripening. It seems that it finishes just before or at the beginning of the phase of the pericarp lignification.

7. The two-phase period of the morphological development of the rose pericarp contrasts with the three-phases of the pericarp formation of *Prunus* and *Rubus* species. However, the intensive changes manifested in the quick enlargement and ripening of the fleshy mesocarp are the basis for distinguishing the third phase in the above genera.

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