SEED MORPHOLOGY OF THE POLISH NATIVE SPECIES OF THE GENUS *RIBES* L. PART 1. GENERAL CHARACTERISTIC

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ABSTRACT

The article contains general morphological and anatomical characteristics and analysis of the chemical composition of seeds of Polish *Ribes* species: *R. uva-crispa* L., *R. nigrum* L., *R. alpinum* L., *R. petraeum* Wulfen, *R. rubrum* L. and *R. spicatum* Robson. The investigations have been based on the different types of LM and SEM.

KEY WORDS: Grossulariaceae, *Ribes*, seed morphology, seed anatomy, fruit morphology, SEM, LM.

INTRODUCTION

The five species of currants, naturally occurring in Poland: *R. alpinum* L., *R. uva-crispa* L., *R. nigrum* L., *R. petraeum* Wulfen i *R. spicatum* Robson were investigated. The *R. rubrum* L., commonly cultivated and often running wild, was also observed. This species, with a range within west Europe, looks alike *R. spicatum*. That is way both species are often classified as collective species or aggregate – *R. rubrum* L. agg.

The monograph of the genus *Ribes* was worked out by Janczewski (1907). He divided this taxon into the 6 subgenera. Native species are included among four of them: *Ribes* (= *Ribesia* Berl.) to which belong *R. petraeum*, *R. rubrum* and *R. spicatum*; *Coreosma* Jancz. represented by *R. nigrum*; *Berisia* (Spach) Jancz. with *R. alpinum* and *Grossularia* (Mill.) A. Rich. with *R. uva-crispa*.

The seed morphology of the genus *Ribes* has not been, so far, the subject of systematic studies. Superficial characteristics of currant seeds can be found in few atlases of seeds and in the manuals of seed production for praticians or for students of forestry and of horticulture. The extrac-tion of parynchymal layers of testa is very difficult. For this reason those descriptions give average dimensions, shape and colour of the seed-coat, but they refer only to the seeds covered with dry exotesta, sarcotesta and with parynchymal layer (Brouwer and Stählin 1955; Schermann 1967; Anderberg 1994). Such seeds are of different colour, polish and sculpture, in comparison with the seeds that have been cleaned. This is why all measurements and investigations on sculpture, colour and polish of seeds and the features of hilum and of micropyle trace were carried out after the parynchymal layer of exotesta has been removed, that is after exposure of sclerotesta.

The seed anatomy of the genus *Ribes* is relatively well recognized. Multilayer testa, crystalline layer, small embryos, callosity of cell walls of endosperm are considered to be the anatomical features specific for the genus *Ribes*. The above characteristics, as well as the presenece of cellulose, proteins and fats in endosperm, indicate the early begining and independent development of the family Saxifragaceae (Krach 1976).

The seed anatomy of *Ribes* was of great importance in expression of the hypotheses referring to the evolutionary trend of development and of the origin of the genus. According to Krach (1976), it is probable that the Saxifragaceae family and the genus *Ribes* had a common ancestor.

According to Corner (1976) the seed anatomy of *Ribes* is similar to the structure of specialized seeds of the families Crassulaceae and Saxifragaceae. The presence of sarcotesta and arils in the seeds of *Ribes* suggests, however, that this genus is filogenetically older. For that reason Corner (l.c.) puts the families Grossulariaceae between the families Dilleniaceae and Polygalaceae, which have arils too. The seeds of those three families are also connected by the location of mechanical layer in endotesta and for this reason Corner (l.c.) includes them into endotestal seeds.

In this work the general morphological and anatomical description and analysis of chemical composition of the seeds of the genus *Ribes* are presented. The investigations were based on the 6 following native species: *R. uva-crispa* L., *R. nigrum* L., *R. alpinum* L., *R. petraeum* Wulfen, *R. rubrum* L. and *R. spicatum* Robson.

The aim of the work was the exact recognition of the seed and fruit morphology of the native species of the genus *Ribes* L. and then to distinguish all the seed features, which would allow to differentiate individual currant

species. The author wanted also to verify whether the mentioned features confirm the traditional systematic divisions of the genus *Ribes*, based mainly on flower morphology and building of the vegetative organs.

MATERIAL AND METHODS

Forty nine samples of fruits were collected between 1992 and 1994. The fruits were obtained from natural localities within the whole country. For comparison fruits from cultivated plants were also used (with the exception of *Ribes petraeum*). For each species 8-10 samples were examined. In the case of *R. petraeum*, that has a limited range of natural occurrence in Poland, only 5 samples were investigated. Fifty well developed seeds were selected from every analysed sample and in general 2450 seeds were examined. Herbal material is collected in the Department of Forest Botany of the Agricultural University in Poznań – POZN (Mirek et al. 1997).

The structure, colour and consistency of sarcotesta, arils and raphe of fresh seeds were observed. In the case of seeds with removed parenchymal layers of exotesta 7 quantitative and qualitative features, such as seed dimensions (length, width and thickness), outline, colour, polish, sculpture of surface, were analysed. The outline and colour of hilum and of micropyle trace were described too. The endosperm structure and the structure and sculpture of the layer, described by me as "cellulose-pectinous layer" were exactly examined.

To expose the sclerotesta the seeds were soaked for 24 hours in water solution of lemon acid to remove fresh or dried up parenchyma layers. If this method was not effective, then they were cooked in 10% solution of KOH (about 10-15 minutes). Finally the softened parenchymal layers of testa were rubbed off by means of blotting-paper.

The sclerotesta was also removed to examine the cellulose-pectinous layer situated below it. The lignified sarcotesta was removed after the cooking of seeds for 30 minutes. Chemical composition was analysed to explain the structure of the controversial cellulose-pectinous layer.

The colour reactions were carried out to analyse the pectin, storage proteins, fats and starch content. The following reagents were used: red ruthenium, bromophenol blue, Sudan IV, J in KJ. The polarization microscope was used to detect starch and cellulose. Autofluorescence and reaction with 4'6 diamidino-2-phenylindole (DAPI), disclosing DNA in nucleus and plastids were carried out. The analyses were based on the methods described by Młodzianowski and Woźny (1982) and Harris and Oparek (1994).

Morphological descriptions were based on the measurements and observations with the stereoscope microscope PZO type MST-130, light microscope OLYMPUS (SZ 6045) and scanning microscopes JOEL JSM-5300, ISI 60, PHILIPS 515.

The elaborations by Janczewski (1907), Dostal, Futak and Novak (1966), Artyushenko (1990) and Stace (1993) were helpful in describing the seed features.

The colours of selected elements of fruits and seeds were marked according to the colour scale of the Royal Horticultural Society – RHS, worked out by Synge (1966). The names of colour classes were given with the descrip-

tions of the genus, while the characteristics of particular species, besides the name of group, contained the number of colour (in brackets) and a letter mark of their tinges.

MORPHOLOGICAL CHARACTERISTIC OF SEEDS OF NATIVE SPECIES OF GENUS *RIBES* L.

The fruit parenchyma in *Ribes* is formed both by pericarp and to a lesser or greater extent by arils. The first type of this tissue is sappy and mostly sour, while the second is thick, sticky and often insipid or sweet in taste. The parenchyma may be yellow-white, orange-yellow, orange, orange-red or red (Synge 1966).

The outer part of exotesta, named sarcotesta, takes a shape of a thick, sappy layer of mucilaginous and tremellose consistency. It is formed by large, elongated, parenchymous cells, holding the mucilage (Figs 1, 2A-C, 3-6). Some species of currants can be distinguished on the basis of sarcotesta thickness and mucilage colour.

Fig. 1. Seed anatomy of *Ribes uva-crispa* L. 1 – sarcotesta, 2 – parenchymal layer, 3 – sclerotesta, 4 – cellulose-pectinous layer, 5 – endosperm (orig.).

Fig. 2. Aril types of investigated species of genus *Ribes* L.: A – *R. nigrum*, *R. alpinum* type, B – *R. uva-crispa* type, C – *R. petraeum*, *R. rubrum*, *R. spicatum* type. S – sarcotesta, Ar – aril, H – hilum, M – micropyle trace, R – raphe (orig.).

The sarcotesta has a differentiated thickness. Sometimes it is thin, but it can also attain dimensions equal to the seed width. Intensely coloured, thickened walls of parenchymal cells of sarcotesta form a characteristic network-like pattern, clearly visible in some species only. This reticulate

structure is also completely or partly preserved after drying up the sarcotesta. The strongly colouring of mucilage determines the sarcotesta colour. It fills the parenchymal cells of sarcotesta and is of the same colour: yellow-white, orange-white, orange, orange-red and red (Synge 1966).

Fig. 3. (LM) – Seed of *Ribes nigrum* L. covered with the aril, sarcotesta visible (×25).

Fig. 4. (LM) – Seed of *Ribes alpinum* L. covered with the aril, sarcotesta and raphe visible (×34).

Fig. 5. (LM) – Seed of *Ribes uva-crispa* L. covered with the aril, sarcotesta and raphe visible ($\times 19$).

Fig. 6. (LM) – Seed of *Ribes petraeum* Wulfen covered with the aril, sarcotesta and raphe visible ($\times 34$).

The raphe of seeds of the genus *Ribes* arises due to partial fusion of funicle with the integuments of anatropous ovule. It ranges along the lateral edges of the seed or along the ventral edge (Figs 2A-C, 4-6). The raphe of currants partly and usually marginally accretes to sarcotesta cells in the zone of hilum; rarely it is free. It is well developed, long, narrow, cylindrical, sometimes striped. It is bright, yellow-white, rarely white. Since the raphe of *Ribes* is completely sunken in testa, it is only visible on fresh seeds.

The arils of currants develop from growing funicle or from placenta. They can enclose the whole seed, or a smaller or greater part of it. Arils surrounding the whole seed are large and shapeless, often deeply lobed or parted (Figs 2, 3, 4).

There are two types of arils, partly encircling the seeds. The arils surrounding the greater part of the seed and resembling in structure the arils, which entirely enclose the seed, are included in the first type (Figs 2B, 5). The second type is determined by arils in the form of small hood-like or saucer-like outgrowths occurring in the area of hilum (most often over hilum) (Figs 2C, 6). The arils usually extend from hilum toward the micropyle trace. They have the greatest dimensions in the zone of micropyle trace or in

the area of hilum. Arils are white, rarely yellow-orange or orange. They can also take the colour of fruit parenchyma or of seed sarcotesta.

As mentioned above, sarcotesta is a fleshy part of the seed, so it will affect the seed appearance only when it is in a fresh state and is not covered with arils. After seed drying the sclerotesta and cellulose-pectinous layer will determine its form and first of all its shape. In practice all descriptions of seeds relate to fresh ones or with dry parenchymal layers of exotesta. However dry parenchymal layers of exotesta "deform" the seeds, which makes their comparison difficult.

I decided for the first time to examine morphological features of sclerotesta of currants, which are visible after the removal of parenchymal cell-layers and sarcotesta (placed below the parenchymal layer) (Figs 1, 7). With the naked eye the sclerotesta surface of the investigated seeds of currants seems to be smooth. Observed under a larger magnification it appears to be reticulate. Such type of microsculpture results from the structure of sclerotesta cells. This tissue is created by the layer of thick-walled, tightly adjacent cells with small lights. The sclerotesta of *Ribes* is usually grey-orange, rarely grey-brown, brown, grey-yellow or grey (Synge 1966).

Fig. 7. (SEM) – Close up of seed surface of *Ribes uva-crispa* L.; outside the reticulate sclerotesta with the elongated cells of cellulose-pectinous layer below ($\times 470$).

Fig. 8. (LM) – Transversal section of the seed of *Ribes uva-crispa* L., exposed to the reaction of red ruthenium; cellulose-pectinous layer (pink) with the uncoloured sclerotesta above ($\times 1000$).

Fig. 9. (LM) – Transversal section of the seed of *Ribes rubrum* L. The luminosity of cellulose in the cell walls of cellulose-pectinous layer and endosperm in the polarized light visible ($\times 150$).

After the extraction of sarcotesta and placed below it parenchymal cell-layers, the seeds are (1.5-)2.0-2.7(+3.5) mm long, (0.8-)1.0-2.0(-2.5) mm wide and (0.2-)0.8-1.3(-1.8) mm thick. The average value of particular dimensions are as follows: 2.3 mm, 1.5 mm and 1.1 mm. The length to width ratio, which ranges from 1.0 to 2.8 (average 1.54), appears to be the important feature. The seed outline is mostly elliptical, broadly ovate or broadly elliptical. The apex is rounded, truncate or acute and the base is rounded, truncate or retuse.

The micropyle trace and the hilum are situated on the opposite poles of seeds (Fig. 2C). The hilum is wide, placed near the seed base. Its outline is very variable (transversely elliptical, transversely obovate and transversely triangular), as well as its form (crater-like and concave, surrounded by a cylinder, crater-like and concave without a cylinder or flat). The hilum is of the same colour as the seed, rarely it is a little darker. As regards the colour, outline and form, the micropyle trace is very similar to hilum, but it is usually smaller. Sometimes it is placed on the pointed apex of the seed and it is covered with wrinkled outgrowths, which are probably the remains of testa cells or of arils.

All analysed taxa have seeds with dorso-ventral symmetry and at the same time they are usually 2-3-angular in outline, rarely multiangular with blunted apexes.

During the chemical cleaning of the outer seed-coat layers it turned out, that a thick layer, composed of strongly elongated and compressed cells is the most resistant to chemical treatment. This layer is located between the seed-coat and the endosperm and it probably protects the latter

Fig. 10. (LM) – Transversal section of the seed of *Ribes uva-crispa* L. Autofluorescence of lignin under the influence of high energetic light; the lignin, contained in the sclerotesta and in the outside layer of endosperm, emits willow green light ($\times 400$).

Fig. 11. (LM) – Transversal section of the seed of *Ribes uva-crispa* L. exposed to the reaction of bromophenol blue; storage proteins (violet colour) only observed in the endosperm cells ($\times 125$).

Fig. 12. (LM) – Transversal section of the seed of *Ribes uva-crispa* L. exposed to the reaction of J in KJ. The lack of violet colour indicated the shortage of starch ($\times 250$).

Fig. 13. (LM) – Transversal section of the seed of *Ribes rubrum* L. exposed to the reaction of Sudan IV; the fats (red colour) are only in the outside layer of the endosperm ($\times 250$).

Fig. 14. (LM) – Transversal section of the seed of *Ribes uva-crispa* L. exposed to the reaction of 4'6 diamidino-2-fhenylindole (DAPI). There are no cell nucleus in the cellulose-pectinous layer. The cell nucleus of endosperm is visible as undersized, bright spot (×400).

Fig. 15. (SEM) – The seed of *Ribes spicatum* Robson; elongated cells of cellulose-pectinous layer visible (×180).

Fig. 16. (SEM) – The seed of *Ribes rubrum* L.; elongated cells of cellulose-pectinous layer visible (×180).

one. At the same time it is of great importance for the development of the whole seed. It seems to take over the function of the protection of endosperm and embryo from the poorly developed, one-stratified sclerotesta.

There are not many data about the origin and structure of the described layer in the anatomic characteristics of the

currant seeds. Nobody but Netolitzky (1926) determines it as a separate layer – the internal cuticle. Pohl (1923) considers it the outer areola of endosperm. Corner (1976) and Krach (1976), who investigated the anatomical structure of currant seeds in detail, do not express their opinion on the mentioned layer.

The colour reactions were carried out to examine the chemical composition of this interesting layer. It turned out, that cell walls consisted of pectin (reaction with ruthenium red) and of cellulose (shining of walls in polarized light). Thus they have the chemical composition typical of primitive cell walls (Figs 8, 9). The analysed layer contains neither lignin (no reaction to high-energy light in fluorescent microscope), nor nutrients (no reaction with bromophenol blue, Sudan IV and J in KJ) (Figs 10-13). The investigations proved, that the cells are devoid of nuclei and so they are dead (no reaction with 4'6 diamidino-2-phenylindole – DAPI) (Fig. 14).

The entire lack of aliphatic compounds in the cell protoplast and a distinctly marked cell structure univocally indicate, that the described layer has nothing to do with Netolitzky's, traditionally understood term of internal cuticle (Netolitzky 1926) (Figs 13, 15, 16). Although no one can completely exclude Pohl's hypothesis (1923), that the analysed layer belongs to endosperm, I would suggest to regard it as a separate layer. The distinct difference of the characterized layer, both from seed-coat and from endosperm, confirms this statement. The analysed layer has reduced cell lights in comparison with the endosperm. It is also devoid of intercellular spaces and is characterized by a less diverse chemical composition.

Similarly, as observed by Essau (1973) in *Asparagus officinalis* L., this layer at seeds of currants probably develops from nucellus cells of the ovule. It is not unlikely that from the outer side it may be also formed by cells of the inner integument of the ovule.

The cells of this layer keep only the skeleton in the form of original cell walls, built of cellulose and of pectin. I called this layer, referring to its origin, "cellulose-pectinous". It has a diversified, often complicated microsculpture. This layer is less or more deeply striate, most distinctly in the area of hilum and of micropyle trace. The striae are probably created by walls of the cells of cellulose-pectinous layer. They are usually arranged along the seed axis, rectilinearly or slightly undulately (Figs 15, 16). Sometimes there are striae running perpendicularly to the seed axis. To some extent the sclerotesta and endosperm can also influence the microsculpture of cellulose-pectinous layer. The sclerotesta cells leave their traces in the outer zone, adjoining the seed-coat. The endosperm acts through the intercellular spaces, under which the depressions may develop, increasing the undulation of the cellulose-pectinous layer (Figs 7, 8).

The endosperm consists of large, elongated, thick-walled, loosely arranged cells (Fig. 1). The endosperm includes nutrients, such as proteins (reaction with bromophenol blue), fats (reaction with reagent Sudan IV) and starch (reaction with J in KJ) (Figs 11-13). It has primitive cell walls, composed of pectins (reaction with ruthenium red) and cellulose, shining in polarized light (Figs 8, 9).

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MORFOLOGIA NASION KRAJOWYCH GATUNKÓW Z RODZAJU *RIBES* L.
CZĘŚĆ I. OGÓLNA CHARAKTERYSTYKA

STRESZCZENIE

W pracy przedstawiono wyniki badań morfologicznych, anatomicznych oraz analizy składu chemicznego nasion, uzyskane w oparciu o 6 krajowych gatunków z rodzaju *Ribes* L. (Grossulariaceae): *R. uva-crispa* L., *R. nigrum* L., *R. alpinum* L., *R. petraeum* Wulfen, *R. rubrum* L. and *R. spicatum* Robson. Obserwacje przeprowadzono przy użyciu różnych typów mikroskopów: stereoskopowego, świetlnego oraz skaningowego. Opisy morfologiczne nasion zostały zilustrowane seriami zdjęć spod tych mikroskopów. Nasiono porzeczek składa się z dwuwarstwowej łupiny nasiennej, mięsistego bielma i słabo zróżnicowanego, bardzo małego zarodka. Łupina nasienna zróżnicowana jest na dwie warstwy, zewnętrzną testę oraz wewnętrzny tegmen. Testa zbudowana jest z trzech warstw, sarkotesty, warstwy komórek miękiszowych oraz sklerotesty. Pod wymienionymi warstwami, a więc pomiędzy łupiną nasienną a bielmem, występuje gruba, najbardziej trwała tkanka nasion porzeczek. Warstwa ta powstaje prawdopodobnie ze strawionych komórek ośrodka załążka. Pełni ona funkcję zabezpieczającą bielmo i zarodek przed uszkodzeniem. Ze względu na skład chemiczny nazwałam ją warstwą celulozowo-pektynową. Najokazalszą częścią nasion *Ribes* jest bielmo.

SŁOWA KLUCZOWE: Grossulariaceae, *Ribes*, morfologia i anatomia nasion, morfologia owoców, SEM, LM.