

## COMPARATIVE HISTOLOGICAL EVALUATION OF THE FRUIT OF *RIBES NIGRUM* AND *RIBES RUBRUM*

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**Abstract:** To complete the wide range of studies on chemical composition and pharmacological potential of the fruit of *Ribes nigrum* and *Ribes rubrum*, this work aimed to perform a comparative morpho-anatomical characterization of the fruits of both species and complete previously records of black currant as frequently studied species. Microslides of fruits were made with rotation microtome, and then cross sections were stained with toluidine blue. Documentation and evaluation were carried out in microphotos. In *R. rubrum*, the epidermis cells are ovoid to round, while those of *R. nigrum* are rectangular to almost round. Under the epidermis, in both fruits, there are two layers of oval hypodermal cells. Underneath the hypodermal layers, parenchyma cells of different size with thin walls and scattered collateral closed bundles were detected. The seeds are embedded in a gelatinous sheath having large thin-walled cells. Endocarp separates the arillar tissue from parenchyma cells, in addition, a gap can be found among these layers, endocarp, arillar tissue, and seeds. The seed coat consists of sclerenchyma cells in both species. In conclusion, histological differences and similarities were described in the fruits of the selected *Ribes* species, highlighted the first morpho-anatomical description of the berry of *Ribes rubrum*.

**Keywords:** *Ribes nigrum*, *Ribes rubrum*, fruit, microscopic examination.

### 1. Introduction

Grossulariaceae family is composed of approximately 195 species (The Plant List, 2018). According to Plant Database (USDA, 2018) there are 65 species of *Ribes* genus including native and introduced plants in the USA. In Europe 16 *Ribes* species are known (Marhold, 2011; EU-NOMEN) involving 8 taxa in Romania namely *R. uva-crispa* L., *R. nigrum* L., *R. alpinum* L., *R. aureum* Pursh, *R. sanguineum* Pursh, *R. petraeum* Wulfen in Jacq., *R. spicatum* Robson, and *R. rubrum* L.

(Ciocarlan, 2000). *Ribes nigrum* (black currant) is a perennial shrub which grows in bushy places, groves (Ciocarlan, 2000). It is also widely cultivated for berry production. The species has a wide variety of secondary metabolites. Leaves contain lignoids (Sasaki et al. 2013), glycosides of quercetin and kaempferol as major phenolics (Liu et al., 2014), accompanied by significant amounts of salicylic acid, p-coumaric acid, caffeic and gallic acids (Nour et al., 2014). Mineral and

trace elements also were measured in the leaves of different cultivars of the plant (Nour et al., 2014). Fruits contain phenolics: they are especially rich in anthocyanins, e.g. glycosides of delphinidin and cyanidin (Gavrilova et al. 2011). The level of anthocyanins varies depending on the season, degree of maturity, environmental conditions, type of cultivation, and genetic characters (Milivojevic et al., 2012; Zheng et al., 2012; Vagiri et al., 2013; Mikulic-Petkovsek et al., 2015). Among organic acids, citric, fumaric and malic acids are dominant (Mikulic-Petkovsek et al., 2012; Woznicki et al., 2017). The fruits are rich in vitamin C (Häkkinen et al. 1999).

*Ribes rubrum* (red currant) is cultivated in Romania, which is not the member of the spontaneous flora (Ciocarlan, 2000). In the literature it is known under synonymies *R. vulgare* Lam. and *R. sylvestre* Mert. et Koch (Ciocarlan, 2000; Marhold, 2011; EU-NOMEN). In phytochemical aspect, anthocyanin content – mostly glycosides of cyanidin (Gavrilova et al., 2011; Mikulic-Petkovsek et al., 2015) – is about 10% in the fruit compared to that of black currant (Gavrilova et al., 2011; Mikulic-Petkovsek et al., 2015). Among flavonoids, glycosides of quercetin, myricetin and kaempferol (Mikulic-Petkovsek et al. 2015), as well as citric, fumaric and malic acids were detected in the plant in similar, but shikimic acid in 13 times higher quantity compared to the fruit of black currant (Mikulic-Petkovsek et al. 2012).

In the fruit of both species, fructose and glucose are predominant among sugars (Mikulic-Petkovsek et al., 2012; Woznicki et al., 2017). The seed oil is rich in tocopherol, unsaturated fatty acids, linoleic,  $\alpha$ -linolenic, and  $\gamma$ -linolenic acids (Traitler et al., 1984; Goffman and Galetti, 2001; Leskinen et al., 2009; Vuorinen et al., 2016).

In pharmacological aspect, currants were studied for the effect in dietary management of

various diseases (Zágoni, 2005; Zdunić et al., 2016). Polyphenolic substances of the fruit and leaf were earlier examined for the anti-inflammatory, antioxidant, antifungal, and anticancer effect (Knekt et al., 1997; Šavikin et al., 2009; Bishayee et al., 2010; Slavin and Lloyd, 2012). The polyphenolic composition of the fruit and the leaf of black currant plays a role in the structure of biological and lipid membranes, highlighted flavonols in the leaf, and anthocyanins in the fruit as dominant antioxidant compounds. The studied extracts protected the membranes against the oxidation and modified the properties of the hydrophilic regions of the membranes (Cyboran et al. 2014). The fruits of black and red currant were tested *in vitro* for potential management of type 2 diabetes and hypertension resulted high  $\alpha$ -glucosidase,  $\alpha$ -amylase and ACE inhibitor activities in red currant (Da Silva Pinto et al., 2010).

Despite the described phytochemical and pharmacological potential of the selected species, there are few studies on the morpho-anatomical description of the fruits as medicinally used parts in *Ribes* species (Wrońska-Pilarek, 2001; Wrońska-Pilarek, 2002; Kendir et al., 2015). In an earlier report arillar tissue was observed in several species of the genus. Among them, arils cover the seeds in the slightly fleshy pericarps in *R. nigrum*, which is absent in *R. rubrum* (Corner, 1976).

In the last two decades, only one histological report was published on the fruit of black currant (Glidewell et al., 1999), and according to our best knowledge no other recent study is available concerning the histology of red currant. Thus, this work aimed to perform a comparative histological characterization of the fruit of *R. nigrum* and *R. rubrum* collected in Romania.

## 2. Materials and Methods

### 2.1 Plant material

Fruits of *R. nigrum* and *R. rubrum* (10 pieces of each species) were harvested at full maturity from a local farmer's garden in July and August 2016 (Cornești, Mureș County, Romania). Collected samples were frozen and stored at -20°C until analyses.

### 2.2 Morphological and anatomical study

For microscopic examination, whole fruits were preserved in the mixture of ethanol : glycerine : distilled water (1:1:1). Fruits were dehydrated in 50%, 70% and 96% ethanol (Békési-Kallenberger et al. 2016), then they were embedded in Technovit® 7100 resin (Realtrade Ltd., Hungary). 15 µm thick cross-sections were obtained with a rotary microtome (Anglia Scientific 0325) (Papp et al., 2013). The slides were dried at 50°C for 2 hours, stained in toluidine blue (0.02%) for 5 min, and soaked twice in 96% ethanol (3 min each), isopropanol (4 min), and twice in xylol (3 and 4 min). Sections were studied with a NIKON Eclipse 80i microscope and micrographs were taken with SPOT Basic 4.0 image analysis system.

## 3. Results and discussions

### 3.1 Histological features of the fruit of *Ribes nigrum*

In the fruit of *R. nigrum*, the epidermis is formed of rectangular to almost round shape cells covered by cuticle layer. Under the epidermis (epicarp), two layers of oval hypodermal cells can be found (Fig. 1 A,B). Under the hypodermal layers the mesocarp parenchyma is composed of different size of thin-walled cells and scattered vascular bundles (Fig. 1 A,C). The collateral closed vascular bundles consists of xylem and phloem that are surrounded by sclerenchyma (Fig. 1 D). Seeds are embedded in a gelatinous sheath which is constructed from large thin-walled cells (Fig. 2

A,B,C), accordingly to a previous study (Glidewell et al. 1999). The arillar tissue is separated from the endocarp by a space (Fig. 2 B). The seed coat (testa) consists of sclerenchyma cells (Fig. 2 D).

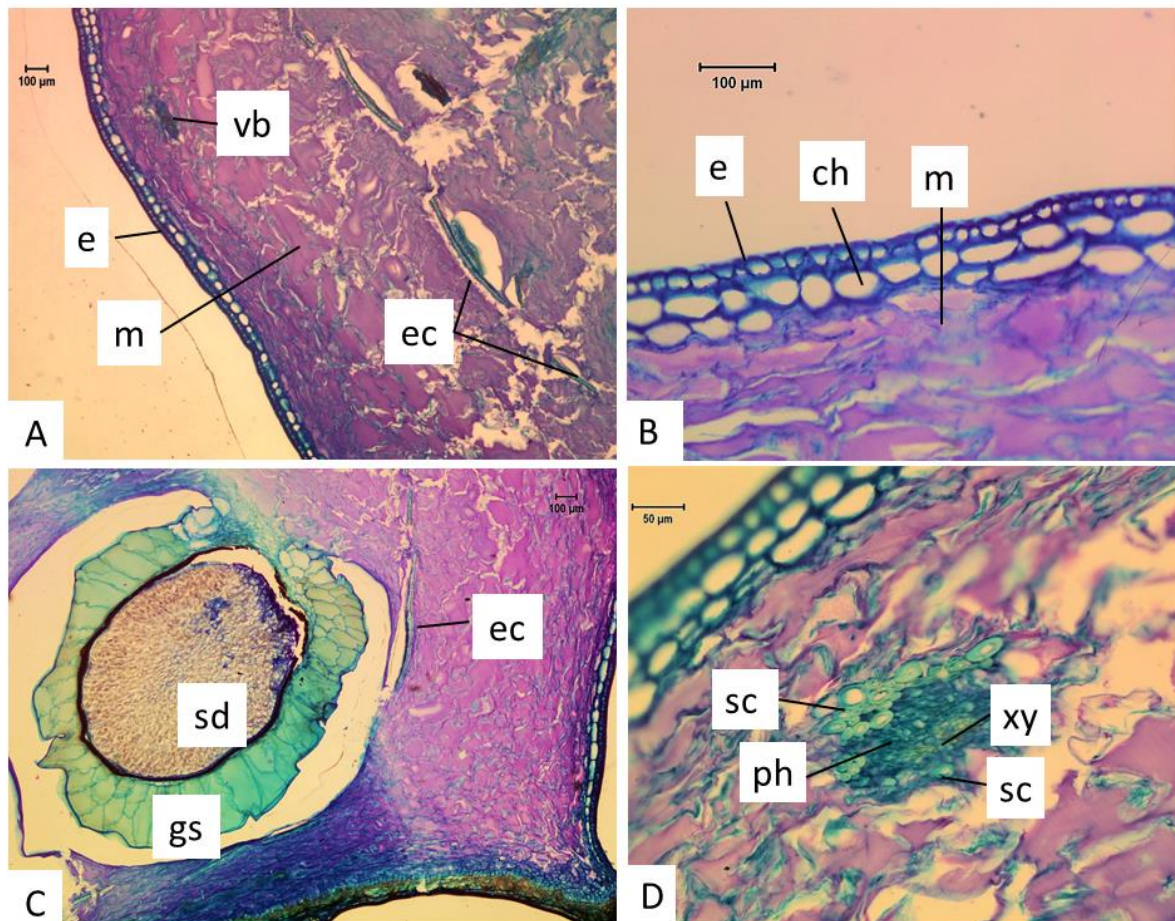
### 3.2 Histological features of the fruit of *Ribes rubrum*

In *R. rubrum*, the epidermis of the fruit is formed from more ovoid to round cells than those of *R. nigrum*. The double-layered hypodermis contains horizontally thick-walled and elongated cells (Fig. 3 A, 3 D). Parenchyma is composed of thin-walled cells in various sizes. The scattered collateral closed vascular bundles (Fig. 3 A) have only xylem and phloem elements (Fig. 3 B). Endocarp separates the arillar tissue from parenchyma cells; between arillar tissue and seeds a gap was observed (Fig. 3 C). Similar to black currant, seeds are embedded in a gelatinous sheath (Fig. 3 C, Fig. 4 A) consisting of large cells with slightly sinuous cell walls (Fig. 4 C). The seed coat includes sclerenchyma cells (Fig. 4 B), while the storage ground tissue consists of thick-walled cells in the seed (Fig. 4 D).

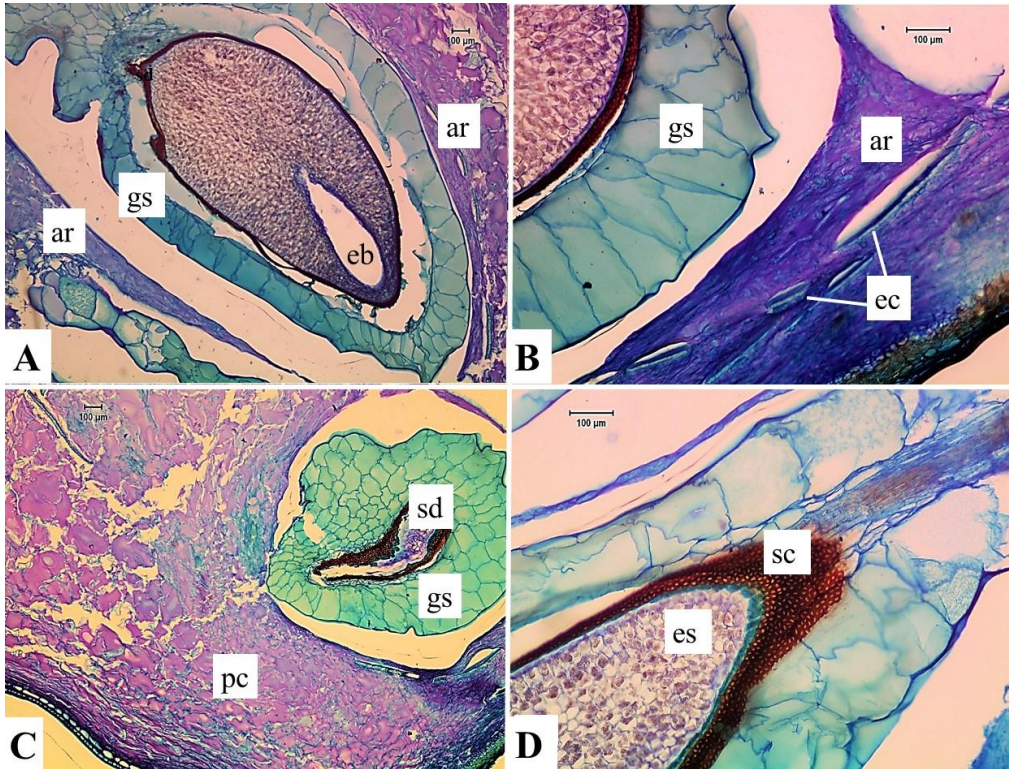
Under histological aspect, the stem of some *Ribes* species were described in wood anatomical atlases (Metcalfe and Chalk, 1957; Schweingruber et al. 2011), e.g. a diffuse-porous xylem in *Ribes nigrum* (Schweingruber et al. 2011). In another work, the whole fruit of *Ribes nigrum* was analysed in different developmental stages (Glidewell et al. 1999). Morphological and histological characters of the seed were reported for native species like *R. uva-crispa*, *R. alpinum*, *R. petraeum*, and *R. spicatum* in Poland (Wrońska-Pilarek, 2001; Wrońska-Pilarek, 2002), as well as a surface analysis by scanning microscope on the fruit, seed and pollen of *R. biebersteinii* Berl. ex DC, *R. uva-crispa*, *R. alpinum*, *R. orientale* Desf., *R. multiflorum* Kit. ex Romer & Schultes, and *R. anatolica* Behçet which are native to

Turkey (Kendir et al., 2015), without detailed histological description of all parts of the fruits. Based on these studies, our work describes the histological features of the whole fruit of *Ribes nigrum* and *R. rubrum* including the seeds from the collected samples from Romania. In this work the fruit of red currant is described in all details here for the first time. Similarities and differences were observed in microscopic preparations of the fruit of both currant species. Among similar features, a thin layer of cuticle, lack of stomata, two layers of hypodermal cells (Fig. 1 B, 2 A), scattered collateral closed bundles (Fig. 1 D, 3 B), and arillar tissue (Fig. 2 B) can be mentioned. The seed coats of both

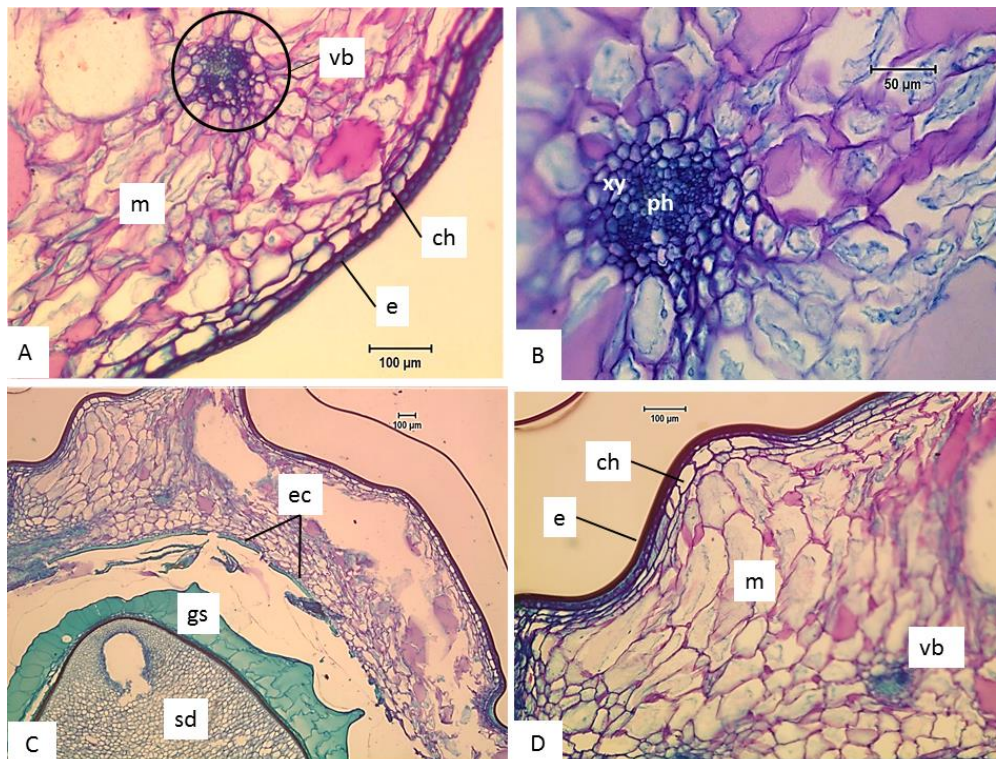
species consist of sclerenchyma cells surrounded by a gelatinous sheath (Fig. 1 C, 2 C) which is in accordance with earlier literature data (Glidewell et al. 1999). This sheath, also called sarcotesta, is different in thickness in *Ribes* species. Sometimes it is equal to the width of the seeds (Wrońska-Pilarek, 2001), but it is less than half of those in *R. nigrum* and *R. rubrum* described in this study. In both fruits, major part of the pericarp and the placental region is occupied by medium and large parenchyma cells (Fig. 1 A,C and Fig. 3 A,C,D), as it was previously described in black currant (Glidewell et al. 1999).



**Fig. 1.** Histological features of the fruit of *Ribes nigrum*. A-B) fruit wall; C) seed embedded in gelatinous sheath; D) collateral closed vascular bundle; e: epicarp (epidermis), ec: endocarp, ch: collenchymatic hypodermis, gs: gelatinous sheath, m: mesocarp, xy: xylem, ph: phloem, sc: sclerenchyma, sd: seed, vb: vascular bundle



**Fig. 2.** Histological features of the seed of *Ribes nigrum*. A) seed; B) aril; C) gelatinous cells; D) testa; ar: aril, eb: the place of embryo (tissue is missing), ec: endocarp, es: endosperm, gs: gelatinous sheath, pc: parenchyma cells, sc: sclerenchyma cells, sd: seed

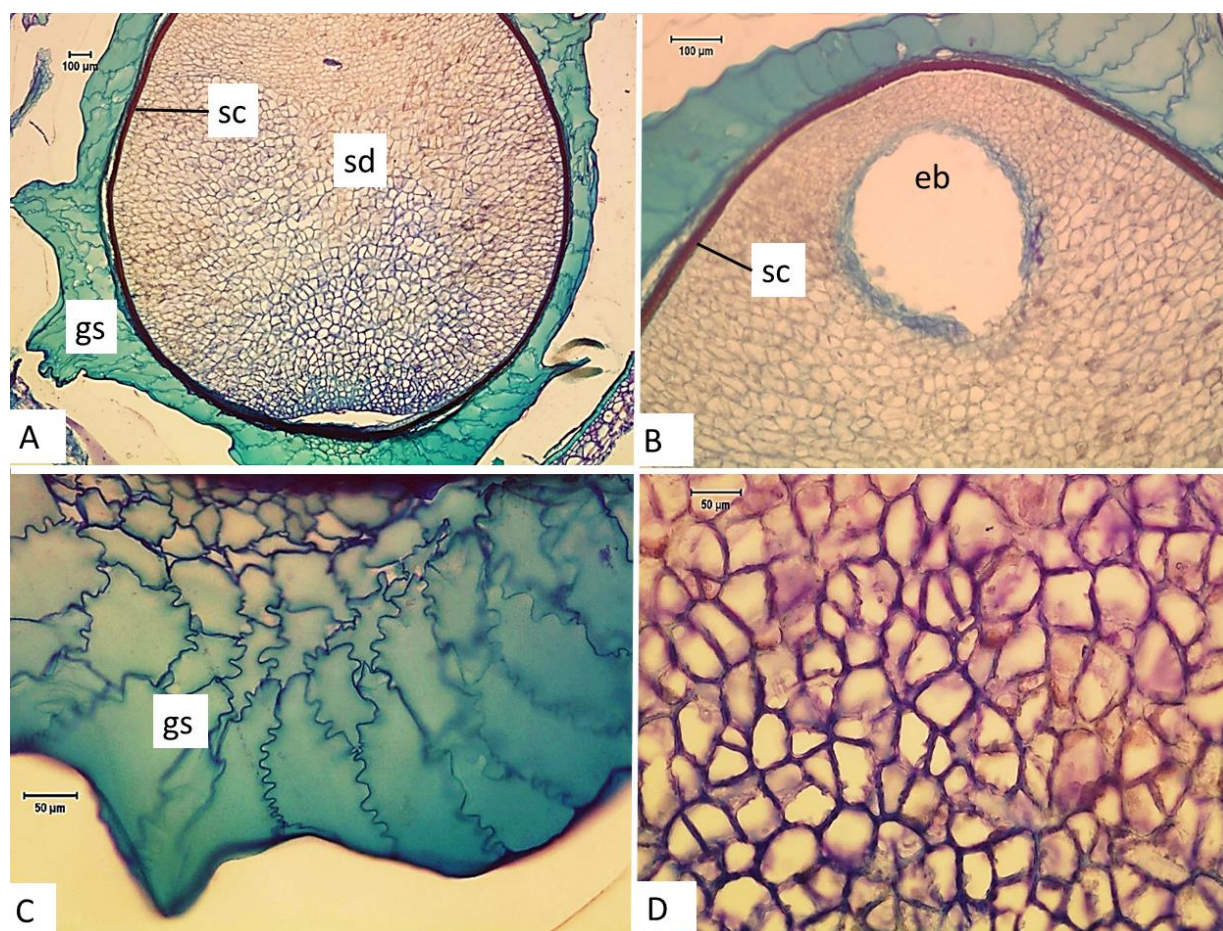


**Fig. 3.** Histological features of the fruit of *Ribes rubrum*. A) pericarp; B) vascular bundle; C) seed embedded in gelatinous sheath; D) pericarp; e: epicarp (epidermis), ec: endocarp, ch: collenchymatic hypodermis, gs: gelatinous sheath, m: mesocarp, xy: xylem, ph: phloem, sd: seed, vb: vascular bundle

As differences, various shapes of epidermis cells (**Fig. 1 B, 3 A**) and different intensity of dyeing of parenchymal cells were observed: stronger coloration of the pericarp and placenta refer to a higher polysaccharide content in black currant compared to red currant (**Fig. 1 A, 3 A,C**). Contrary to gooseberry's fruit (Williamson et al., 1993), the gelatinous sheath around the seeds is not single-layered, but multi-layered up to six cell layers in the studied species (**Fig. 1 C, 4 A**). In the endosperm, fatty oil drops were detected in *R. nigrum* (**Fig. 2 D**), which does not appear in *R. rubrum* (**Fig. 4 D**). This is in accordance with earlier findings using Sudan IV:

coloration was found only in the external layer of the endosperm in red currant (Wrońska-Pilarek 2001).

Generally, in berries (Giongo et al. 2013), as in other fruits such as tomato (Saladie et al. 2007), the texture of the fruit is related to the cell wall structure and to change in ripening phases (Giongo et al. 2013). The structure of the pericarp has a great influence on the storage ability of the ripe fruit and the postharvest management. As expected, epidermal cells in the berry of black and red currant are covered by cuticle which can explain the relative long maintenance of their freshness.



**Fig. 4.** Histological features of the seed of *Ribes rubrum*. A) Seed embedded in gelatinous sheath; B) seed; C) gelatinous sheath; D) endosperm; eb: the place of embryo (tissue is missing), gs: gelatinous sheath, sc: sclerenchyma cells, sd: seed

## Conclusions

The histological determination of the fruit of *Ribes nigrum* and *R. rubrum* complete the reported data of the species. In the fruits of the selected *Ribes* species differences and similarities are described, which highlight the first morpho-anatomic study of the fruit of *Ribes rubrum*. The observed features play an important role in the characterisation, which will be useful providing a reliable basis for identification of the fruits.

## Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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