“Structure and the female gametophyte development in some representatives of the genus *Sedum* (Crassulaceae)”

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The ovule of angiosperm plants develops within the ovary of the pistil. The mature, usually seven-celled female gametophyte (embryo sac) develops in the nucellus of the ovule as a result of two consecutive processes, i.e., megasporogenesis and megagametogenesis. Depending on the course and result of these processes, types of development of the female gametophyte are distinguished, which are divided into three main groups (mono-, bi- and tetrasporic embryo sacs). This division depends on the number of nuclei involved in the construction of the functional megaspore formed from the megasporocyte during megasporogenesis. The different outcome of this process is related to the presence/absence of cytokinesis after the first and second meiotic division [1]. During megagametogenesis, the functional megaspore (the embryo sac mother cell) undergoes a certain number of mitotic cycles, leading to the formation of the coenocytic and, after cytokinesis, the cellular female gametophyte, which plays an important role in plant reproduction. Studies on the development, structure, and ultrastructure of the embryo sac are widespread in angiosperm plants, but questions still remain regarding, among others, the function of the cells of the female gametophyte, including the antipodal cells, which constitute the most variable, often ephemeral component of the structure of the female gametophyte [2].

Studies on the ultrastructural level combined with cytochemical methods have not been performed so far during megasporogenesis and megagametogenesis within Crassulaceae. So far, studies using the above-mentioned techniques have been carried out among selected representatives of this family, but they were focused on the processes observed after fertilization, i.e., embryo development, haustorial suspensor and endosperm. These data constitute, among others, the first report in the world literature among angiosperms on the presence of plasmodesmata with adherent electron-dense material in the embryo suspensor of species of the genera *Sempervivum, Jovibarba, Graptopetalum, Aeonium, Monanthes, Aichryson* and the majority of *Sedum* (Crassulaceae) [3-8]. The analyses performed provided an additional, important argument for the rationale of continuing embryological studies on Crassulaceae species.

In light of the available embryological data on Crassulaceae species, it has become necessary to apply new, unused so far research techniques to expand our knowledge of the structure and development of the female gametophyte within this family. The main objective of this dissertation was to conduct comparative analyses on anatomy, ultrastructure and cytochemistry of cells forming during megasporogenesis and megagametogenesis in three species of the genus *Sedum* (*S. hispanicum* L., *S. sediforme* (Jacq.) Pau and *S. rupestre* L.) from this family. The implementation of the planned research was aimed at tracing, describing and determining the type of megasporogenesis and megagametogenesis in these species, while observing and analyzing the anatomical structure of the ovule. The results presented in

the dissertation were subjected to comparative analysis in order to determine the common and different characteristics for the studied *Sedum* species.

The species studied belong to the largest within the Crassulaceae family genus *Sedum* (comprising about 420 species), which is strongly polyphyletic [9-10]. Moreover, *S. sediform*e and *S. rupestre* are species included in the series *Rupestria*, distinguished within the *Sedum* genus, which is sometimes also elevated to the rank of a genus - *Petrosedum* Grulich [10-11]. The morphological features of the species from ser. *Rupestria* (in relation to other representatives of the genus) and the results of molecular analyses reveal a different character of this group of plants [10-11]. The analyzed species from ser. *Rupestria* and *S. hispanicum* are included in different clades i.e., *Sempervivum* and *Leucosedum*, respectively [10]. Therefore, an additional aim of this study was to check and determine if there is a relationship between the results obtained during the planned study and the systematic position of the selected species. The issue taken up seemed interesting in the context of the embryological observations described so far. Only among the examined species from ser. *Rupestria* (*S. sediforme* and *S. rupestre*) no presence of plasmodesmata unique to Crassulaceae with adherent electron-dense material was found, which were observed in previous works in the embryo suspensor. Furthermore, only *Sedum* species of ser. *Rupestria* are characterized by filamentous suspensor formation among Crassulaceae [3,8,12]. Differences in cell structure and the course of the processes studied were also expected during the formation of the female gametophyte in the species selected for analysis. Thus, the hypothesis that *Sedum* species show differentiation in the course of formation and structure of cells formed during megasporogenesis and megagametogenesis was verified in this study.

The monosporic type of megasporogenesis and the *Polygonum* type embryo sac are the most commonly described in angiosperm plants, including most members of the family Crassulaceae. According to previous literature, *Polygonum* and *Allium* types are present in *Sedum* [11-15]. However, the presence of bisporous embryo sacs of *Scilla/Endymion* type is questioned [15]. Moreover, among some species of Crassulaceae, including mainly the genus *Sedum*, the occurrence of synergid, antipodal and megaspores haustoria has been described [16]. These structures have not been previously studied from ultrastructural and cytochemical aspects within the family. In light of the above, an additional objective of this dissertation was to verify the presence of haustoria during megasporogenesis and megagametogenesis.

The research material consisted of ovules at different stages of development, isolated from flower buds and freshly opened flowers of selected *Sedum* species. The following techniques were used for the analysis of development and anatomical structure: ovule clearing technique, semi-thin sections stained with cytochemical methods, and observation under light microscope, including Nomarski contrast. Observation of the distribution and changes in the content of major groups of organic compounds was carried out after previous selective staining of lipids, proteins and insoluble polysaccharides. Transmission electron microscopy was used to study the submicroscopic structure of cells forming during megasporogenesis and

megagametogenesis. The procedures for fixation and preparation of plant material for analysis depended on the research method used.

The study undertaken as part of the dissertation contributed to a comprehensive presentation of the development of the female gametophyte of the species *S. hispanicum* [**17**] and to the first description and determination of the type of embryo sac development of *S. sediforme* [**18**] and *S. rupestre* [**19**]. The publications present successively the results obtained for each species.

Regardless of the course and outcome of megasporogenesis, a monosporic type of megasporogenesis was observed among all *Sedum* studied. The polar structure of the meiocyte is manifested by the distribution of plasmodesmata, which are located in the walls in the chalazal part of the cell. The functional megaspore is always a mononuclear cell that is formed after meiosis and cytokinesis at the chalazal pole of a linear triad or tetrad. In ovules, only one embryo sac was always observed, whose development is consistent with the type of megagametogenesis - *Polygonum* type. The ovule in all studied species is anatropous, bitegmic and crassinucellate (*Sedum* type of nucellus). Microscopic analyses carried out at particular stages of development of megaspores and female gametophyte allow concluding that the studied species do not show haustoria formation both during megasporogenesis and megagametogenesis.

The course of megaspores formation and the structure of cells formed during megasporogenesis differ in the studied representatives of the genus *Sedum*, which is also reflected in the systematic position of these plants. The species of the genus *Sedum* ser. *Rupestria* are characterized by the formation of a triad of cells of which only one, located closest to the micropyle is a binucleate cell. The result obtained in this study is related to the inhibition of the cytokinesis mechanism within the non-functional triad cell. So far, this type of triad structure has been described as an additional possible result of megasporogenesis among Crassulaceae species. I therefore conclude that this is the first such observation within the family, made exclusively among species of *Sedum* ser. *Rupestria*, but further studies are necessary to conclude that this is a characteristic and common feature of this plant group. A different result of megasporogenesis was observed in *S. hispanicum*. In the ovules of this species, always one linear megaspore tetrad forms in the micropylar part of the nucellus.

The process of megagametogenesis produces a female gametophyte, which in the *Sedum* studied is initially composed of seven cells. The three antipodals located at the chalazal pole of the embryo sac are ephemeral structures. The finally formed, mature female gametophyte is built of cells of the egg apparatus (two synergids and an egg cell) and a central cell. Cytochemical staining performed determined that proteins, lipids, and insoluble polysaccharides are present within the cells of the developing *Sedum* female gametophyte. At the stage of coenocytic embryo sac, an intensive process of accumulation of spare materials in the female gametophyte is clearly visible, which is connected with simultaneous degeneration of non-functional megaspore and surrounding cells of the nucellus.

During maturation, *Sedum* female gametophyte cells become rich in cell organelles. The ultrastructure of the synergids and the central cell indicates their relatively higher metabolic activity compared to the egg cell. Synergids become more active cells with the progressive disappearance of antipodals. The results indicate that antipodal cells (*S. sediforme, S. rupestre*) and synergids of the studied *Sedum* are transfer cells. The wall growths in the micropylar portion of the synergids form a filiform apparatus that facilitates the secretion and absorption of substances. Moreover, the occurrence of well-developed organelles (e.g. active dictyosomes, profiles of the endoplasmic reticulum, mitochondria often located near wall ingrowths) in the cytoplasm of these cells indicates their high metabolic activity. Ultrastructural results indicate that the micropylar and chalazal poles of the *Sedum* embryo sac are predisposed to intense substance transport. Both antipodals and synergids are involved in nutrition and nutrient transport for the developing megagametophyte. In addition, all the cells of the embryo sac are connected to each other by plasmodesmata, which allows intensive nutrient flow within the embryo sac.

Ultrastructural studies planned within the framework of my dissertation enabled to verify and confirm the hypothesis that during megasporogenesis and megagametophytogenesis of selected species of the genus *Sedum* (Crassulaceae), characteristic plasmodesmata with electron-dense material unique for this family are formed. Moreover, on the example of species from ser. *Rupestria*, a hypothesis was confirmed that the lack of plasmodesmata formation during embryogenesis does not exclude the possibility of their formation at earlier (in relation to embryogenesis) stages of development, i.e., megasporogenesis and megagametogenesis. The results obtained by me reveal features of the cell structure of the developing female gametophyte which have not been described so far in other plants. The walls of megaspores of *S. hispanicum* show the presence of simple plasmodesmata to which electron-dense material adheres. Intercellular connections constructed in this way are also seen in the walls of the functional megaspore and coenocytic embryo sac of this species. In the ovules of *S. sediforme* and *S. rupestre*, plasmodesmata with electron-dense material were found only at the stages of megagametophytogenesis (coenocytic embryo sac, outer walls of antipodals). Antipodal cells of species from ser. *Rupestria* show similarity in structure (presence of wall ingrowths and plasmodesmata with electron-dense material in chalazal walls of these cells). Based on the available literature [20] and the observations made, it can be assumed that the described plasmodesmata with electron-dense material in the outer walls of the megaspores/coenocytic gametophyte/antipodals are functional and their presence may affect symplasmic communication between the cells of the developing female gametophyte and the surrounding cells of the nucellus in *Sedum*.

In conclusion, the results obtained in this dissertation complement and provide new knowledge in the field of cytoembryology of three species of the genus *Sedum*. On the basis of detailed analyses of the development and structure of the cells of the female gametophyte, similarity of embryological features was demonstrated among the species of *Sedum* ser. *Rupestria* (clade *Sempervivum*), distinguishing them from the species *S. hispanicum* (clade

Leucosedum). In my work I proved that *Sedum* species show differentiation in the development of the female gametophyte, which concerns the course of megasporogenesis, the structure of cells formed during megasporogenesis and the formation of the embryo sac. The study made it possible to conclude that the presence of plasmodesmata with electron-dense material is a feature unique to all Crassulaceae species examined to date at the ultrastructural level (including *S. sediforme* and *S. rupestre*). The research results presented in this dissertation can be used as support for solving current taxonomic problems within the genus *Sedum*. Moreover, my results provide a basis for further research in the field of experimental embryology on symplasmic communication during megasporogenesis and megagametogenesis.

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