# The development of female gametophyte and antipodal embryo formation in *Sedum fabaria*

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## Abstract

In *Sedum fabaria,* the ovule is anantropus, bitegmic and crassinucellate. The development of the nucellus conforms to the *Sedum* type. The development of the embryo sac is of the *Allium* type. The antipodal cells in unfertilized embryo sac occasionally divide and one of them forms four-celled structures resembling embryos and remaining once elongate in the form of haustoria. The entry of the pollen tube is porogamous. After division the primary endosperm nucleus forms two cells: the apical one develops into cellular endosperm according to the *Acre* type and the basal one acts as the endosperm haustorium of the *Sempervivum* type. The embryogeny corresponds to the *Caryophyllad* type.

*Additional key words*: archesporial cell, *Crassulaceae,* embryo sac, endosperm, megasporogenesis.

## Introduction

Many plant species belonging to the *Crassulaceae* family including the *Sedum, Sempervivum, Crassula, Kalanchoë,* and *Bryophyllum* genera were investigated at the turn of the 19th century. In 1933, Mauritzon published the results of his own embryological observations conducted on varius species of this family and gave the classification of developmental types for the nucellus, endosperm, and endosperm haustorium. These types are used only with respect to the species of the *Crassulaceae* family. Of interest to the researchers is the formation of antipodal embryos observed by Mauritzon (1933) in *Sedum fabaria.* This fact, often quoted in numerous embryological studies remains controversial and requires verification by other researchers (Johri 1992).

So far, the antipodal embryos have been described to develop in embryo sacs of *Allium odorum* (Tretjakow 1895, Hegelmaier 1897, Haberlandt 1925, Modilewski

*Received* 4 March 1997, *accepted* 12 June 1997.
*Acknowledgement:* The authors would like to thank Professor M. Zenkteler for critical reading of the manuscript and Ms G. Winiecka for helpful technical assistance particularly her contribution to the preparation of illustrations.
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1930), *Ulmus americana* (Shattuck 1905), *Ulmus glabra* (Edahl 1941), *Alangium lamarckii* (Gopinath 1943), *Elatostema* (Fagerlind 1944), *Paspalum scrobiculatum* (Narayanaswami 1954), *Rudbeckia sulivantii* (Battaglia 1955), *Stipa* sp. (Yakovlev and Solntseva 1965), *Ulmus campestris* (Guignard and Mestre 1966), *Solidago canadensis* (Pullaiah 1978), *Allium tuberosum* (Kojima and Nagato 1992).

The aim of this work was to investigate megasporogenesis, female gametophytogenesis, and embryogenesis and to reinvestigate formation of the antipodal embryos in *Sedum fabaria*.

## Materials and methods

Flower buds of *Sedum fabaria* in various developmental stages were collected from plants growing in the Botanical Garden of A. Mickiewicz University in the period 1991 - 1992. The material used in the present study included 1600 ovules. For cytoembryological studies whole flower buds were fixed successively in acetic-alcohol for paraffin sections and in $FPA_{50}$ fluid (formalin 5 $cm^3$, propionic acid 5 $cm^3$, 50 % ethanol 90 $cm^3$) for the clearing technique (Herr 1971). Paraffin microtome sections (3, 7 and 12 μm) were stained with safranin and counterstained with fast green. Photographs of these sections were taken with *Amplival Optiphot Microscope (Carl Zeiss,* Jena, Germany).

300 ovules out of the 1600 ovules, were cleared with methyl salicylate (Young *et al.* 1979) and later on observed on Raj's glasses in clearing fluid. Photographs were taken with an interference microscope (*Biolar*, Poland).

## Results

The ovule of *Sedum fabaria* is crassinucellate, anatropus and bitegmic. Only in one case, two ovules were surrounded by a common integument at the chalazal region (Fig. 1). The archesporial cell (Fig. 2) cuts off a primary parietal cell (Fig. 3) which divided and formed a parietal layer. The sporogenous cell enlarged considerably before becoming the megasporocyte (Fig. 3). Meiosis in the megaspore mother cell was normal, but after the second meiotic division the cell-wall was not formed, leading to the formation of a dyad of which each cell contained two nuclei (Fig. 4). The chalazal functional dyad with two nuclei transformed into a binucleate embryo sac (Fig. 5). A vacuole developed between the two nuclei, pushing them to the opposite poles where they went through further mitotic divisions resulting in tetra- and octonucleate megagametophytes (Figs. 6,7). In *Sedum fabaria,* the development of the female gametophyte was bisporic and conformed to the *Allium* type.

The immature embryo sac of *Sedum fabaria* was small and occupied one-eighth of the length of the nucellus. During maturation, the embryo sac grew intensively towards the chalaza end, destroying the nucellus and accumulating a large amount of starch (Figs. 8,9). The starch grains were diminishing gradually following fertilization.

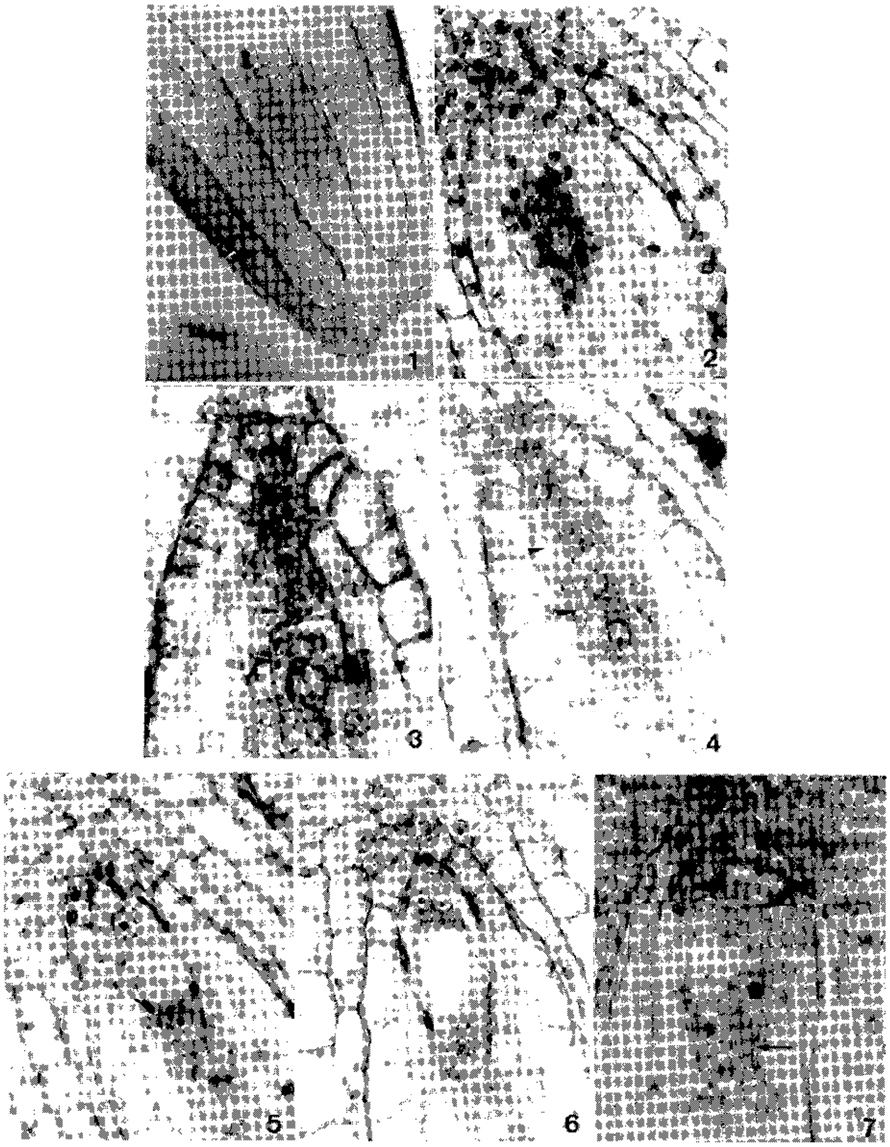

Fig. 1. Two ovules surrounded by a common integument at the chalazal part (240 ×).

Fig. 2. The archesporial cell in nucellus tissue (450 ×).

Fig. 3. The archesporial cell after mitotic division (1000 ×); megasporocyte - *bigger arrow*, parietal cell - *small arrow*.

Fig. 4. The functional chalazal dyad (*big arrow*)and the degenerating micropylar one (*small arrow*) (550 ×).

Fig. 5. The two-nucleate embryo sac (1000 ×).

Fig. 6. The four-nucleate embryo sac (1000 ×).

Fig. 7. The eight-nucleate embryo sac (*arrows*) (470 ×).

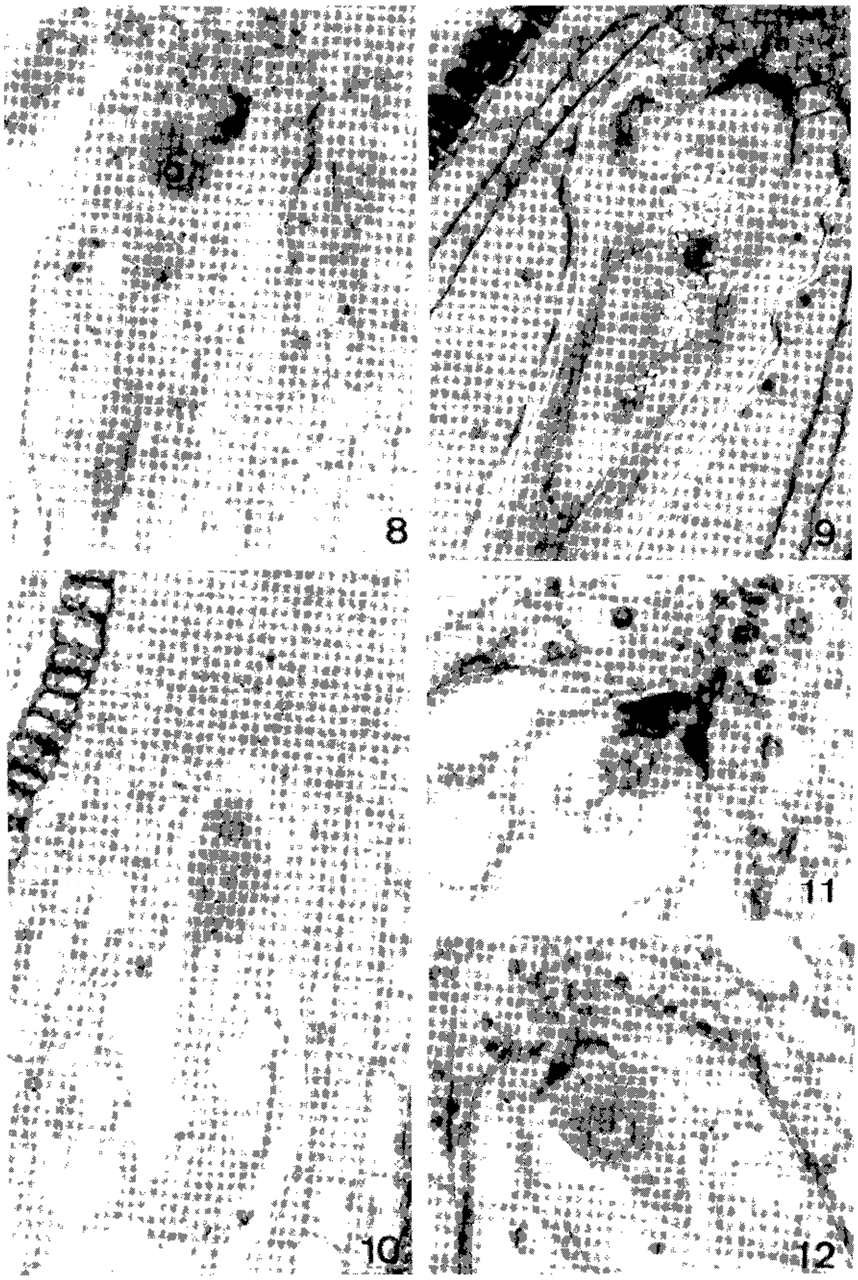

Fig. 8. The embryo sac with storage material (585 ×).
Fig. 9. Large amount of starch grains presented in an embryo sac under a polarized microscope (790 ×).
Fig. 10. The egg aparatus (600 ×).
Fig. 11. The polar nuclei placed below the egg (880 ×).
Fig. 12. The secondary nucleus situated just below the egg cell (890 ×).

The mature embryo sac of *Sedum fabaria* was eight-nucleate and seven-celled. The cells of the egg apparatus formed a triangular group (Fig. 10). The egg cell was broad at the chalazal end, where the nucleus is located, and tapered towards the micropylar end. The synergids were also pyriform, but considerably smaller than the egg. The nuclei of synergid cells were centrally positioned.

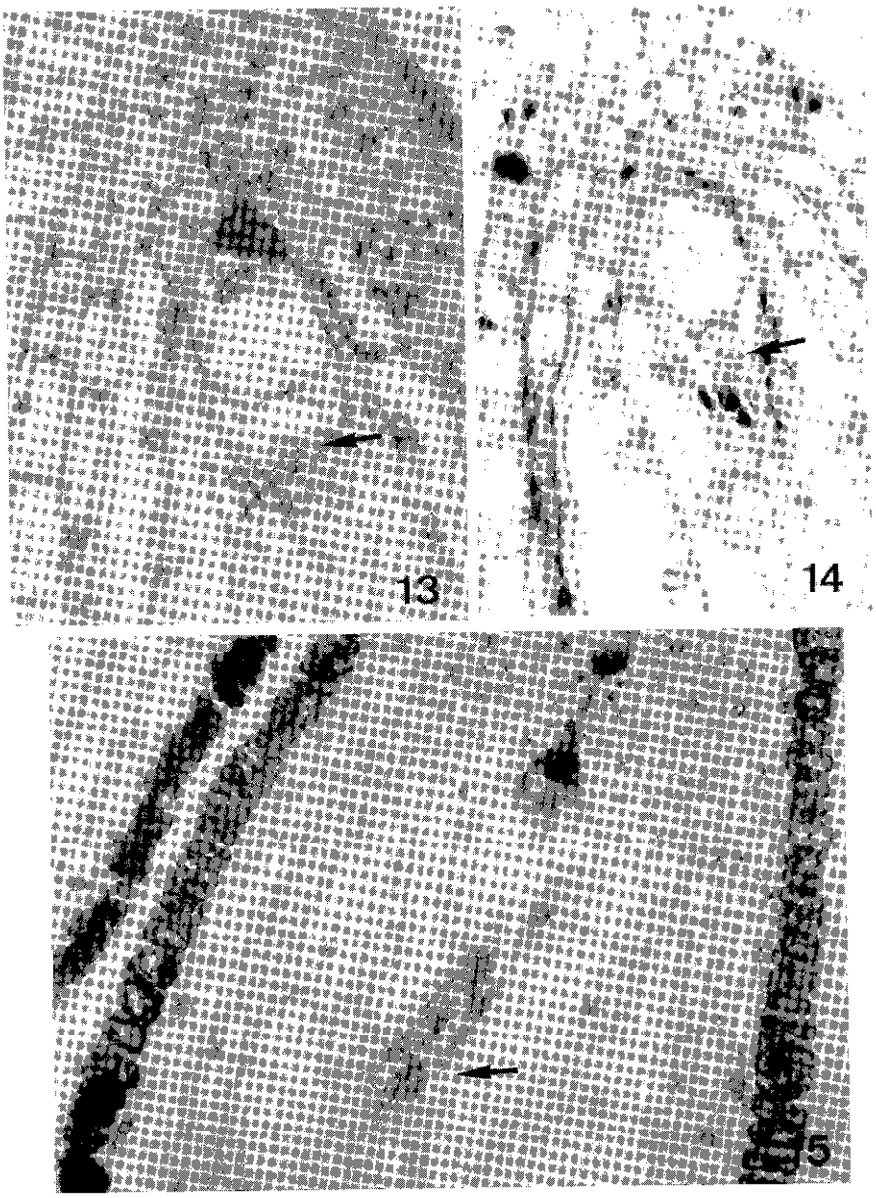

Fig. 13. The antipodal cells in linear arrangement (*arrow*) (700 ×).
Fig. 14. One increased antipodal cell resambling the egg cell (*arrow*) (580 ×).
Fig. 15. The antipodal embryo (*arrow*) and the antipodal haustoria (470 ×).

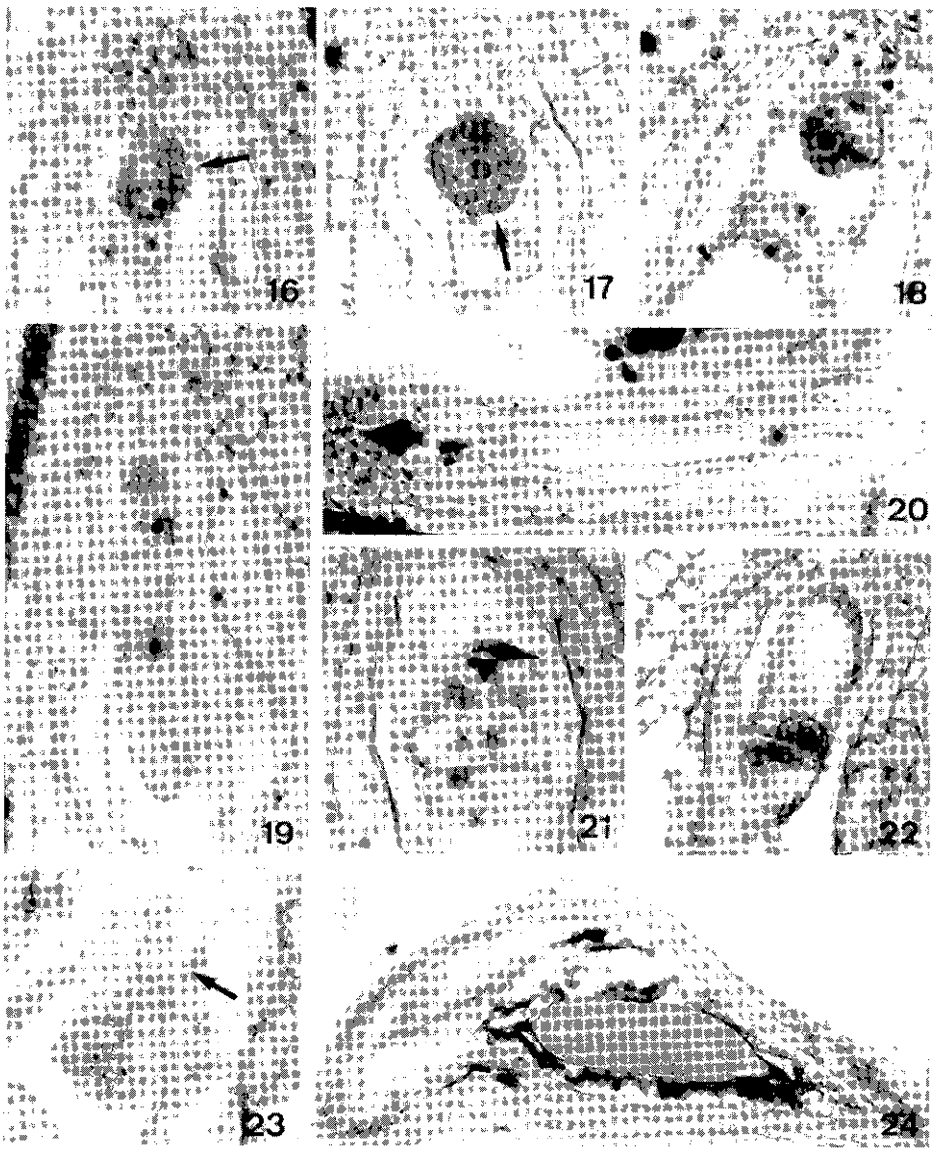


Fig. 16. The pollen tube with a sperm cells (*arrow*) in the embryo sac (560 ×).
Fig. 17. The sperm cell (*arrow*) adhered the egg cell (700 ×).
Fig. 18. The primary endosperm nucleus after transversal division (620 ×).
Fig. 19. The formation of endosperm haustorium - the basal cell and the division of the apical endosperm cell (700 ×).
Fig. 20. The growth of antipodal haustorium (300 ×).
Fig. 21. The division of endosperm cells (470 ×).
Fig. 22. The division of zygote (510 ×).
Fig. 23. The proembryo with haustorial cell (*arrow*) (570 ×).
Fig. 24. The fully development dicotyledoneus embryo (47 ×).

The central cell containing 2 nuclei occupied the largest portion of the embryo sac. At the beginning, the nuclei lay in the middle of the cell, and subsequently they move towards the micropylar end and in the mature embryo sac, they are situated just below the egg (Fig. 11). The polar nuclei fuse early before fertilization (Fig. 12).

At the chalazal end of a mature embryo sac, there are three antipodal cells. These three lineary arranged cells were the smallest cells of the embryo sac (Fig. 13). Out of the whole number of 210 analyzed embryo sacs only in 11 embryo sacs one of the antipodal cells increased in size and resembled the egg cell (Fig. 14).

Occasionally, it was observed that one of the antipodal cells in the unfertilized embryo sacs divided twice forming a four-celled structure resembling an embryo (Fig. 15). The other antipodal cells divided ones and later on they formed antipodal haustoria (Fig. 15). The antipodal haustoria always elongated towards the micropylar end of the embryo sac but they never reached the length of the endosperm haustorium.

In *Sedum fabaria*, the pollen tube entered the ovules through the micropyle and moved into the embryo sac (Fig. 16). It was observed that one of the two sperm cells adhered at the basal end of the egg cell (Fig. 17).

The division of the primary endosperm nucleus was accompanied by wall formation it started to divide before the first division of the zygote nucleus (Fig. 18). After the first division of the endosperm the basal endosperm cell did not divide any longer, but it extended longitudinally as far as the chalaza end of the nucellus and acted as a haustorium (Figs. 19,20). The apical cell of the endosperm underwent two longitudinal and two transversal divisions (Fig. 21). Further divisions of the endosperm cells were irregular. In *Sedum fabaria* the development of the endosperm is of the cellular type. The zygote remained quiescent for a period of time, and then elongated and divided by transversal wall (Fig. 22). The apical cell extended and acted as a haustorium, as the basal one developed into an embryo (Fig. 23). The development of the embryo in *Sedum fabaria* was typically dicotyledoneus (Fig. 24) and conformed to the Caryophyllad type.

## Discussion

The present embryological study of *Sedum fabaria* deals with megasporogenesis, gametophytogenesis, and early stages of embryo development. Mauritzon's classification (1933) was used to describe the nucellus and the development of endosperm and endosperm haustoria. Our investigation confirm Mauritzon's classification that the nucellus of *Sedum fabaria* develops according to the *Sedum* type and cellular endosperm according to the *Acre* type. With respect to the development of endosperm haustoria, Mauritzon described two types: *Sempervivum* and *Echeveria*. In *Sedum fabaria* we observed that the endosperm haustorium is formed according to the *Sempervivum* type.

The development of the female gametophyte in *Sedum fabaria* is of the *Allium* type as in *Sedum populifolium* and *S. populifolium* var. *notarjanni* (Mauritzon 1933).

However, in the other species of the *Crassulaceae* family, the embryo sac develops according to the *Polygonum* type (Johri *et al.* 1992).

On the whole number of 1600 analyzed ovules only in one case two ovules were surrounded by a common bitegmic integument at the chalazal end. Similar cases were recorded by Mauritzon (1933) in two other species of *Sedum*: *Sedum populifolium* and *S. hillebrandii*.

According to Mauritzon (1933) in unfertilized embryo sacs of *Sedum fabaria* the egg apparatus degenerates, while one or two antipodal cells divide and form antipodal embryos. In our observations, it was established that one of the antipodal cells of the unfertilized embryo sacs proliferated occasionally and always developed a four-celled structure resembling an embryo. Similar six-celled antipodal embryos were observed in *Paspalum scrobiculatum* (Narayanaswami 1954) and 4-6-celled antipodal embryos in *Alangium lamarckii* (Gopinath 1943).

Mauritzon (1933) claimed that hormones released from the degenerating egg apparatus induce cellular divisions of an antipodal cell and consequently lead to the formation of antipodal embryos. Similarly, the formation of antipodal embryos in unfertilized embryo sacs were observed in *Solidago canadensis* (Pullaiah 1978). However, as it ensue from the literature (Johri *et al.* 1992) the antipodal embryos were mainly noticed in those species where zygotic embryos were present at the same time. Our study demonstrated unequivocally that exclusively zygote embryos developed in the fertilized embryo sacs of *Sedum fabaria*.

According to Mauritzon (1933) antipodal embryos in *Sedum fabaria* degenerated due to the lack of endosperm in unfertilized embryo sacs. Quite different opinions are expressed by Modilewski (1930) concerning *Allium odorum* and Kojima and Nagato (1992) concerning *A. tuberosum*. Those authors revealed that antipodal embryos developed until the globular stage and their subsequent growth was arrested irrespectively of whether endosperm is present or not.

Due to a low percentage of the formation of antipodal embryos in *Sedum fabaria* (3 %) as well as in *Ulmus campestris* (Guignard and Mestre 1966), *Paspalum scrobiculatum* (Narayanaswami 1954) and *Solidago canadensis* (Pullaiah 1978), it was not possible to conduct a more detailed analysis of these structures.

It is reported that in *Allium odorum* (Haberlandt 1925, Modilewski 1930), *A. tuberosum* (Kojima and Nagato 1992), and *Ulmus glabra* (Edahl 1941), those antipodal cells which formed antipodal embryos usually resemble egg cell. In *Sedum fabaria*, as in *Ulmus americana* (Shattuck 1905), the formation of antipodal like eggs was observed only infrequently.

It is important to note that the process of fertilization of *Sedum fabaria* is hard to observe and this fact was also emphasized by Mauritzon (1933). In this species Mauritzon (1933) did not observe the process of fertilization but he only concluded from the endosperm and the embryo development that the double fertilization occured. In presented paper evident examples of the entrance of the pollen tube into the embryo sac (porogamy) were recorded as well as the adherence of the sperm cell to the egg cell at its chalazal end.

According to Johri *et al.* (1992) another interesting but questionable aspect concerning embryology of *Sedum fabaria* (1933) is the growth of the embryo sac.

Mauritzon (1933) described that the embryo sac of *Sedum fabaria* intensively grew towards the chalazal end, becoming haustorial and accumulated a large quantity of starch. Indeed in our work, an analysis of histological slides revealed that during maturation embryo sacs did intensively grow towards the chalazal part, with numerous starch grains accumulating inside the embryo sac.

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