ACTA BIOLOGICA CRACOVIENSIA Series Botanica 48/2: 67–72, 2006

DEVELOPMENT AND CYTOCHEMISTRY OF THE EMBRYO SUSPENSOR IN *SEDUM*

MAŁGORZATA KOZIERADZKA-KISZKURNO* AND JERZY BOHDANOWICZ

Department of Genetics and Cytology, University of Gdańsk, ul. Kładki 24, 80–822 Gdańsk, Poland

Received April 28, 2006; revision accepted August 5, 2006

The development of the suspensor in *Sedum acre* L. and *S. hispanicum* L. was investigated using cytochemical methods and light microscopy. After the first division of the zygote, two cells of unequal size are formed: the large basal cell and the smaller apical one. The basal cell grows enormously and produces haustorial branches invading ovular tissues. The mature differentiated suspensor consists of a large basal cell and 3–4 chalazal cells. Proteins, insoluble polysaccharides, nucleic acids and lipids are localized in the suspensor during different phases of embryo growth. Cytochemical tests showed the presence of high amounts of macromolecules in the suspensor cells, especially during the globular and torpedo-shaped stages of embryo development. The present data indicate that in *Sedum* the suspensor is involved mainly in absorption and transport of metabolites from the ovular tissues to the developing embryo proper.

Keywords: *Sedum acre* L., *S. hispanicum* L., suspensor differentiation, basal cell, cytochemistry.

INTRODUCTION

The suspensor is the first differentiated structure produced during plant embryogenesis. The zygote in angiosperms usually divides into the apical cell and the basal cell, the micropylar basal cell of the suspensor. The apical cell produces mainly a few chalazal cells and the embryo proper. Angiosperm suspensors vary widely in size and morphology, from a single cell to a massive structure composed of hundreds of cells (Mauritzon, 1933; Maheshwari, 1950; Yeung, 1980; Lersten, 1983). A few suspensors produce elaborate outgrowths (haustoria) that invade surrounding endosperm or maternal tissues (for review: Yeung and Meinke, 1993). In most cases the suspensor functions early in embryogenesis and degenerates during later stages of development. Classically, the suspensor was thought to maintain the embryo proper in a suitable position and to push it into the interior of the female gametophyte (Maheshwari, 1950). However, extensive cytochemical (Pritchard, 1964; Pritchard and Bergstresser, 1969; Newcomb and Fowke, 1974; Malik et al., 1976; Bohdanowicz, 1987), ultrastructural (Clutter and Sussex, 1968; Schulz and Jensen, 1969; Nagl, 1976; Bohdanowicz, 1987) and physiological studies (Clutter et al., 1972; Singh et al., 1980; Picciarelli et al., 1991) have shown that suspensors synthesize essential growth factors and transport nutrients to the embryo proper to stimulate its growth and development.

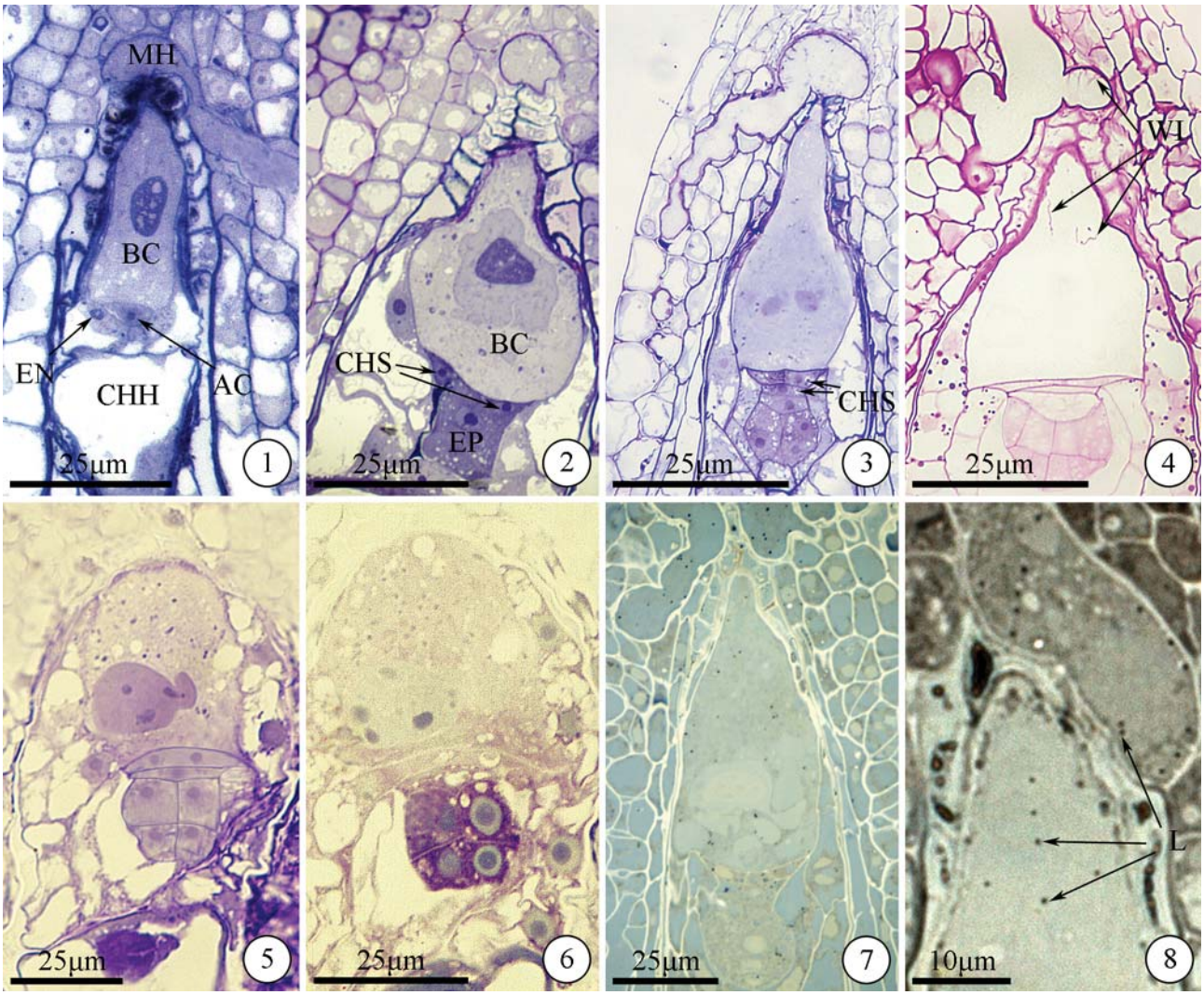
This paper reports observations of the development and cytochemistry of this embryonic organ in *Sedum acre* L. and *S. hispanicum* L., and discusses its possible roles in embryogenesis.

MATERIALS AND METHODS

The materials were collected from *Sedum acre* and *S. hispanicum* (Crassulaceae) growing in natural habitats of Gdańsk and Gdynia in northern Poland. Ovules in various developmental stages were fixed in 2.5% glutaraldehyde and 2.5% paraformaldehyde in 0.05 M cacodylate buffer (pH 7.0) for 4 h at room temperature. The material was rinsed with buffer, dehydrated with acetone and embedded in Spurr's resin. Sections 1 μm thick were cut with glass knives and mounted on glass slides. For light microscopy, sections were stained with 0.05% Toluidine Blue 0 for 1 min at 60°C on a hot plate. Cytochemical tests included periodic acid-Schiff (PAS) reaction for insoluble polysaccharides (Jensen, 1962), Coomassie Brilliant Blue R–250 (Fisher, 1968; Cawood et al., 1978) and Aniline Blue Black (Jensen, 1962) for proteins, Azure-B bromide (Flax

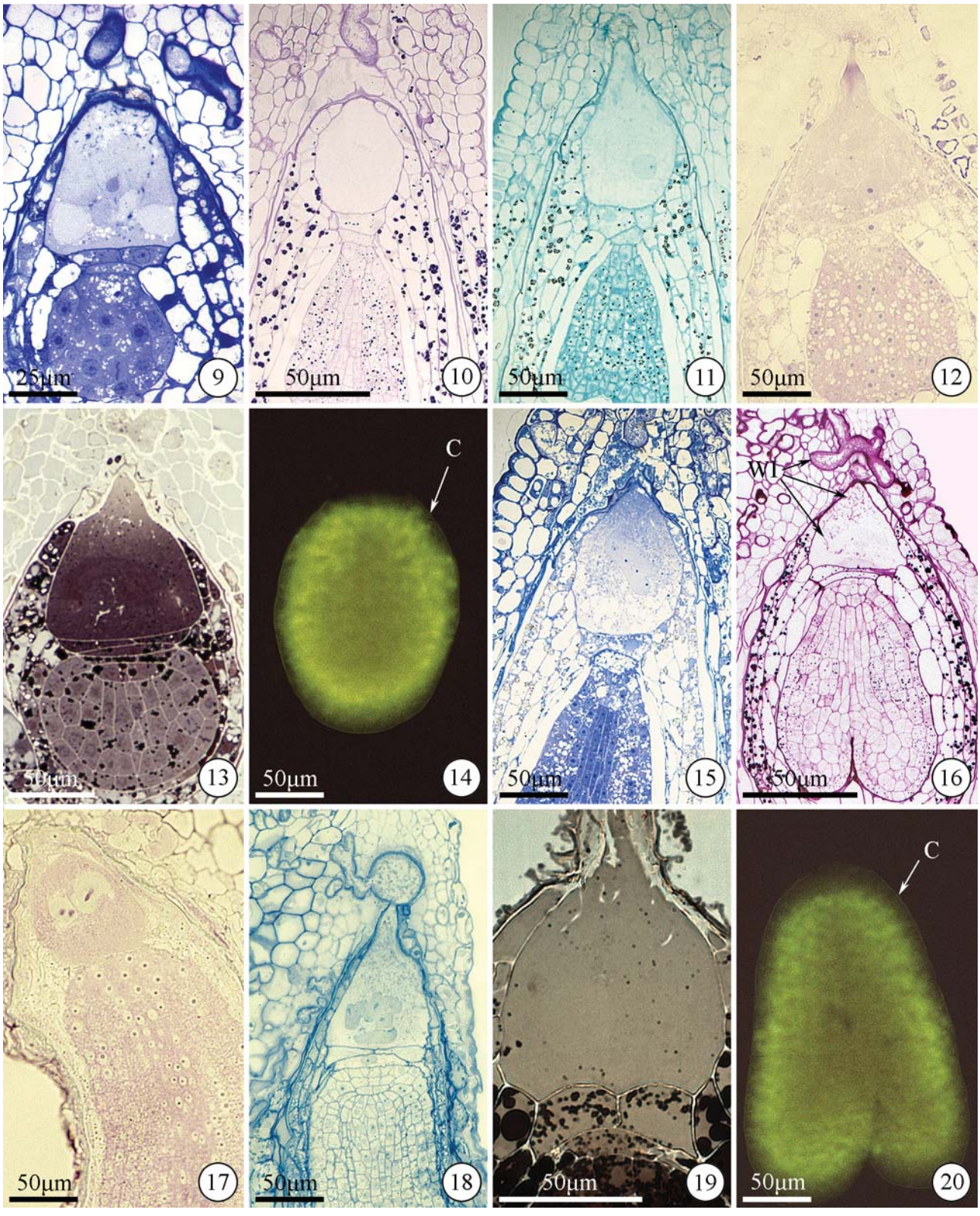
*e-mail: stokrota@biotech.univ.gda.pl

PL ISSN 0001-5296



Figs. 1–8. Development of *Sedum acre* (**Figs. 1–7**) and *S. hispanicum* (**Fig. 8**) embryo. Results of cytochemical tests; Spurr sections. **Fig. 1**. Two-celled embryo. **Figs. 2–8**. 5–10-celled embryo. **Fig. 1**. The large basal cell (BC) with the micropylar haustorium (MH) and the smaller apical cell (AC). At the chalazal pole, uninucleate haustorium (CHH) and endosperm (EN). **Fig. 2**. Semithin section showing the large basal cell (BC) with nucleus and nucleolus, one layer of chalazal suspensor cells (CHS) and a few cells of the embryo proper (EP). **Fig. 3**. Semithin section showing a pear-shaped basal cell, two layers of chalazal suspensor cells (CHS) and a few cells of the embryo proper. **Fig. 4**. Embryo stained for polysaccharides. Note the PAS-positive ingrowths of the basal cell and the micropylar haustorium (WI). **Fig. 5**. Embryo stained with Coomassie Brilliant Blue showing protein distribution. **Fig. 6**. Section stained with Azure-B bromide showing localization of nucleic acids. **Fig. 7**. Section stained with Sudan Black B showing lipid distribution. **Fig. 8**. High magnification of the basal cell and the micropylar haustorium. Arrows indicate lipid droplets (L).

Figs. 9–20. Development of *Sedum acre* (**Figs. 9, 12–14, 16–18, 20**) and *S. hispanicum* (**Figs. 10, 11, 15, 19**) embryo. Results of cytochemical tests; Spurr sections (**Figs. 9–13, 15–19**), isolated embryos – Auramine 0 (**Figs. 14 and 20**). **Figs. 9–14**. Late globular and heart embryo. **Figs. 15–20**. Torpedo embryo. **Fig. 9**. Semithin section showing the large basal cell, two layers of chalazal suspensor cells and the embryo proper. **Fig. 10**. Embryo stained for polysaccharides. The micropylar part of the basal cell wall and the micropylar haustorium forms PAS-positive ingrowths. **Fig. 11**. Embryo stained with Aniline Blue Black showing localization of proteins. **Fig. 12**. Embryo stained with Azure-B bromide showing distribution of DNA and RNA. **Fig. 13**. Section stained for lipids showing localized lipids (L). **Fig. 14**. Embryo with fluorescing cuticle after Auramine 0 treatment. **Fig. 15**. Stage of senescence of the suspensor showing the large basal cell, two layers of chalazal suspensor cells and the torpedo embryo proper. **Fig. 16**. Section stained for polysaccharides showing PAS-positive ingrowths of the basal cell and the micropylar haustorium. **Fig. 17**. Embryo stained with Azure-B showing DNA and RNA. **Fig. 18**. Section stained with Aniline Blue Black showing protein distribution. **Fig. 19**. Embryo stained with Sudan Black B showing localization of lipid droplets. (L). **Fig. 20**. Cuticle fluorescence on surface of embryo.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and Himes, 1952) for nucleic acids and Sudan Black B for lipids (Bronner, 1975). Fresh embryos at different stages of development were dissected from living ovules and placed in a drop of fluorochrome Auramine 0 solution. The presence of cuticle was checked with Auramine 0 staining (Heslop-Harrison, 1977). Sections were examined and photographed with a Nikon Eclipse 800 microscope.

RESULTS

Sedum acre and *S. hispanicum* undergo the Caryophyllad type of embryonic development. The development and cytochemistry of the embryo suspensor in the two species are very similar, so the results will be described together.

Three main stages in the development of the embryo suspensor can be distinguished:

Stage 1. Differentiation of the basal cell (proembryo).

Stage 2. Full development and function (late globular, heart embryo proper).

Stage 3. Senescence of the suspensor (torpedo embryo proper).

TWO-CELLED EMBRYO

The zygote divides into the apical cell (~13 × 10 μm) and the basal cell (~47 × 21 μm). The basal cell undergoes no division, becomes much enlarged, produces haustorial branches invading the micropyle and adjacent tissues, and protrudes out of the ovule. The apical cell develops into the embryo proper and chalazal suspensor. Simultaneously the endosperm forms a uninucleate haustorium at the chalazal pole (Fig. 1). Differentiation of the basal cell begins shortly after the division. The basal cell is anchored to the micropylar end of the embryo sac. The wall surrounding the basal cell is thicker than the apical cell wall. A single huge nucleus containing one dense nucleolus is situated in the central part of the basal cell (Fig. 1). The nucleus gradually grows to a considerable size.

At this stage of development, the micropylar anucleate haustorium of the basal cell is already strongly developed and ramifies in the integumentary tissue (Fig. 1).

5–10-CELLED PROEMBRYO

When the embryo proper reaches the early globular stage of development, the basal cell undergoes further differentiation. The early globular stage embryo consists of the large haustorial basal cell (~75 × 33 μm) and 2 smaller chalazal suspensor cells in one layer in the 5-celled proembryo (Fig. 2) or 3–4 chalazal suspensor cells in two layers in the 10-celled proembryo (Fig. 3). The cell walls of the suspensor and the embryo proper are distinctly PAS-positive. The micropylar part of the basal cell wall and the micropylar haustorium wall form small PAS-positive ingrowths (Fig. 4). At this stage of differentiation the cytoplasm of the basal cell and of the chalazal suspensor are less strongly stained for proteins than that of the embryo proper cells (Fig. 5). The elongated nucleus has 1–2 nucleoli and is located in the central part of the basal cell (Figs. 2, 3). The dense cytoplasm of the basal cell contains considerable quantities of DNA and RNA (Fig. 6). In addition, some lipid droplets are present in the cytoplasm of the whole suspensor, including the micropylar haustorium. Occasionally, lipids are present also in the embryo proper at this stage (Figs. 7, 8). There is no fluorescence after Auramine 0 treatment on the proembryo surface.

LATE GLOBULAR AND HEART-STAGE EMBRYOS

The morphology and cytochemistry of the late globular and heart-stage embryos are similar enough that these stages may be described together. The fully developed suspensor is built of a large pear-shaped basal cell (~25 × 46 μm) and 2–4 chalazal cells in two layers (Fig. 9). As compared with earlier stages, the number and size of wall ingrowths increase in *S. acre* but not in *S. hispanicum*. The ingrowths extend over the entire micropylar half of the cell wall but still maintain their highest concentration at the micropylar apex of the cell. They are branched and PAS-positive. The micropylar part of the basal cell wall in *S. hispanicum* forms very short ingrowths, but there are long PAS-positive ingrowths in the micropylar haustorium. The wall of the micropylar haustorium is thicker than the basal cell wall (Fig. 10). A single huge nucleus containing 3–4 nucleoli is situated in the central part of the basal cell (Figs. 9, 11, 12). The cytoplasm of the suspensor is less stained for proteins than that of the embryo proper cells (Fig. 11). In addition, the basal cell shows very intense staining for DNA and RNA (Fig. 12). Numerous lipid droplets are distributed throughout the basal cell, the micropylar haustorium, embryo proper and endosperm (Fig. 13). A thin cuticle over the surface of the embryo proper forms at this stage of development, but it is absent from the suspensor (Fig. 14).

TORPEDO-STAGE EMBRYOS

The senescing suspensor, composed of a large basal cell (~125 × 46 μm) and 3–4 chalazal cells, characterizes the torpedo embryo proper, which is ~200 μm long. The chalazal suspensor cells in both species are arranged in two layers (Figs. 15–19). The basal cell begins to degenerate. In the micropylar

part of the suspensor basal cell a covering of ingrowths has developed, showing a PAS-positive reaction which penetrates the cell to a distance of 40–50 μm (Fig. 16). In the micropylar haustorium, the ingrowths stain intensely with PAS and develop into an extensive network containing a system of cytoplasmic channels. The giant endopolyploid nucleus (512C–1024C; Kozieradzka-Kiszkurno and Bohdanowicz 2003) is larger than at the previous stage and usually has 3–4 nucleoli. The nucleus contains a huge quantity of DNA and RNA compared to the cells of the chalazal suspensor and embryo proper (Fig. 17). The protein content of the cytoplasm in the degenerating basal suspensor cell is distinctly lower than in the chalazal suspensor cells (Fig. 18). There is an increase in the number of lipid droplets, particularly in the embryo proper and endosperm (Fig. 19). At the torpedo stage, cuticle is still present only on the surface of the embryo proper (Fig. 20).

DISCUSSION

The haustorial suspensor in *Sedum*, in particular its basal cell, undergoes developmental changes during embryogenesis. It is suggested that some if not all of these changes are connected with the physiological requirements of the developing embryo. In particular, the basal cells contain prominent wall ingrowths that are covered by a plasma membrane, greatly increasing the ability of these cells to absorb nutrients from surrounding tissues (Pate and Gunning, 1972). Suspensor cell ultrastructure in many species is consistent with this proposed role in absorbing nutrients from maternal tissues and transporting them to the embryo proper (Schulz and Jensen, 1969; Raghavan, 1986). The wall ingrowths develop during the early stages of embryogenesis in *Sedum* and are most extensive by the torpedo stage. The occurrence of such ingrowths, greatly increasing the absorptive surface of the plasma membrane, seems to be a common feature of these cells and has been reported previously for several other species. This type of wall projection has been observed in many plant cells that are actively engaged in absorption and secretion ("transfer cells"; Gunning and Pate, 1969). Wall ingrowths have been found in the suspensor cells of many species: *Phaseolus coccineus* (Clutter and Sussex, 1968; Yeung and Clutter, 1978), *Capsella bursa-pastoris* (Schulz and Jensen, 1969), *Pisum sativum* (Marinos, 1970), *Stellaria media* (Newcomb and Fowke, 1974), *Epilobium, Antirrhinum, Vicia, Lathyrus, Scrophularia* (Gunning and Pate, 1974), *Diplotaxis erucoides* (Simoncioli, 1974), *Tropaeolum majus* (Nagl, 1976), *Alyssum maritimum* (Prabhakar and Vijayaraghavan, 1983), *Alisma plantago-aquatica* and *A. lanceolatum* (Bohdanowicz, 1987), *Triglochin palustre* (Kozieradzka-Kiszkurno and Bohdanowicz, 2000). The micropylar part of the suspensor basal cell and the micropylar haustorium in *Sedum* are covered with wall ingrowths typical of transfer cells (Kozieradzka-Kiszkurno, 2003). Cytochemical results on the composition and distribution of macromolecules (proteins, insoluble polysaccharides, nucleic acids and lipids) at various stages of the development of the embryo proper and suspensor, and analysis of the suspensor ultrastructure in *S. acre* (Kozieradzka-Kiszkurno, 2003) show that the basal cell is a site of intense metabolic activity.

Analysis of the development and cytochemistry of the suspensor basal cell in *Sedum* suggests that the basal cell is an active transfer cell absorbing nutrients from the nucellar tissue and endosperm, metabolizing and translocating them through the chalazal suspensor cells to the embryo proper.

ACKNOWLEDGEMENTS

This work was financed in part by University of Gdańsk research grant no. 1412–5–0329–6

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