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ULTRASTRUCTURAL AND CYTOCHEMICAL STUDIES OF THE EMBRYO SUSPENSOR OF *SEDUM REFLEXUM* L. (CRASSULACEAE)

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The Crassulaceae family mainly comprises herbaceous leaf succulents, some of which are used as ornamentals. The development of the embryo suspensor in *Sedum reflexum* L. was investigated using cytochemical methods, light and electron microscopy. The full development and functioning of the suspensor occurs during the late globular and heart-stage embryos. The suspensor consists of a large basal cell and a single row of 6–10 chalazal cells. The basal cell produces a branched haustorium which invades ovular tissues. The walls of the haustorium and the micropylar part of the basal cell form the wall ingrowths that are typical for transfer cells. The dense cytoplasm filling the basal cell is rich in profiles of endoplasmic reticulum, active dictyosomes, mitochondria, plastids, microtubules, bundles of microfilaments, microbodies and lipid droplets. The present work reveals that the suspensor structure in *S. reflexum* markedly differs from that found in other representatives of Crassulaceae. Ultrastructural analysis and cytochemical tests (including proteins, insoluble polysaccharides and lipids) indicate that in *S. reflexum* the embryo suspensor is involved mainly in absorption and transport of metabolites from the ovular tissues to the developing embryo proper via the chalazal suspensor cells.

Key words: Embryogenesis, suspensor differentiation, ultrastructure, cytochemistry, *Sedum*.

INTRODUCTION

In the embryogenesis of many angiosperms it is typical for the early embryo to differentiate into two parts, the suspensor and the embryo proper. The suspensor is a specialized structure which functions mainly to facilitate the continued development of the embryo proper within the seed. There is an enormous amount of diversity of suspensor morphology among angiosperms. The differentiated suspensor ranges from a reduced structure consisting of a single cell to a massive structure composed of hundreds of cells. Morphologically, a simple filamentous multicellular suspensor is the type described in *Capsella bursa-pastoris*, *Arabidopsis*, and members of the Brassicaceae (Raghavan, 2006). Others are spheroid masses of large cells and are much larger than the young embryo proper. Some suspensors develop haustoria that penetrate the endosperm and integuments. Suspensor diversity most likely reflects the different roles of the suspensor in complex interactions between maternal tissues and the endosperm in providing nutrition to the developing embryo proper (for review see Yeung and Meinke, 1993; Shwartz et al., 1997).

Classically, the function assigned to the suspensor has been that of holding the embryo in a fixed position in the seed. Hence this embryonic organ was thought to play a rather passive role in the development of the embryo. However, an increasing number of microscopy, physiological, biochemical and genetic studies have produced data suggesting that the suspensor plays an active role during early embryo development (Schulz and Jensen, 1969; Newcomb and Fowke, 1974; Yeung and Clutter, 1978; Singh et al., 1980; Kozieradzka-Kiszkurno and Bohdanowicz, 2006; Lee et al., 2006; Kozieradzka-Kiszkurno et al., 2011a,b; Kozieradzka-Kiszkurno and Płachno, 2013). Recently there has been significant progress in studies in *Arabidopsis* and tobacco in vivo as well as in microspore-derived embryos of *Brassica* in vitro (Panitz et al., 1999; Prem et al., 2012). In most species this rapidly developing short-lived organ undergoes programmed cell death (PCD) and is not present in the mature plant (Raghavan 2006). The *Arabidopsis* suspensor is an attractive

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system for studying the mechanisms of PCD in plants. The *Arabidopsis* suspensor and embryo proper together offer a good system for identifying molecular mechanisms involved in specification and maintenance of cell identity during plant embryogenesis (Kuriyama and Fukuda, 2002; Kawashima and Goldberg, 2010).

Crassulaceae is one of the families in which the suspensor develops haustorial branches that invade the micropyle and adjacent tissues. This family, which includes approximately 1500 species, is one of the groups of succulents that are widely cultivated as ornamentals. *Sedum*, the largest genus of this family, is cosmopolitan in its distribution and shows much of the morphological diversity present in Crassulaceae as a whole. The ultrastructural and cytochemical aspects of suspensor development in this family have been described in only two species of *Sedum*: *S. acre* and *S. hispanicum* (Kozieradzka-Kiszkurno and Bohdanowicz, 2006; Kozieradzka-Kiszkurno et al., 2011a; Kozieradzka-Kiszkurno and Płachno, 2013) and three other genera: *Sempervivum* (*S. arachnoideum*), *Jovibarba* (*J. sobolifera*) and *Graptopetalum* (*G. bellum*) (Kozieradzka-Kiszkurno et al., 2011b; Kozieradzka-Kiszkurno and Płachno, 2013). These studies clearly confirmed structural and functional differences between the suspensor and the embryo proper. They indicated that the suspensor basal cells function as a synthetically active transfer cell which absorbs nutrients from maternal tissue, and then metabolizes and translocates them through the chalazal suspensor cells to the embryo proper.

In this paper we report further research on suspensor development in Crassulaceae. We show its similarities and structural differences in this genus/family, and especially that the suspensor morphology of *S. reflexum* differs from that of two *Sedum* species previously studied. We describe the development, ultrastructure and cytochemistry of the embryo suspensor in *S. reflexum* and discuss the possible role of this embryonic organ in embryogenesis.

MATERIALS AND METHODS

PLANT MATERIAL

Plants of *Sedum reflexum* L. at different stages of development were obtained from a Polish company (http://kaktusiarnia.pl/index1.html).

ELECTRON MICROSCOPY

For ultrastructural studies, ovules at different stages of embryo development were fixed in 2.5% formaldehyde and 2.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.0) for 4 h at room temperature. Then the material was rinsed in the same buffer and post-fixed in 1% osmium tetroxide in cacodylate buffer at 4°C overnight. Specimens were treated with 1% uranyl acetate in distilled water for 1 h, dehydrated in an acetone series and embedded in Spurr's resin. Serial ultrathin (60–100 nm) sections were cut with a diamond knife on a Sorvall MT 2B microtome. Material on the grids was post-stained with a saturated solution of uranyl acetate in 50% ethanol and 0.04% lead citrate. Observations were made using a Phillips CM 100 transmission electron microscope operating at 80 kV.

LIGHT MICROSCOPY

For light microscopy, semithin sections (0.5–1.5 μm) were cut with glass knives and mounted on glass slides. Sections were stained with 0.05% Toluidine Blue O in 1% sodium tetraborate for 1 min at 60°C on a hot plate. For cytochemical studies, sections were stained with periodic acid-Schiff (PAS) for localization of insoluble polysaccharides (Jensen, 1962), with Aniline Blue Black for proteins (Jensen, 1962) and with 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 O solution. Cuticle was localized using Auramine O staining (Heslop-Harrison, 1977). Sections were examined and photographed with a Nikon Eclipse E 800 microscope equipped with a Nikon cooled CCD camera.

RESULTS

Embryo development in *Sedum reflexum* is of the Caryophyllad type. After fertilization the zygote divides transversely into two unequal-size cells: a large basal cell (~54 × 24 μm) and a smaller apical one (~11 × 7 μm) (Fig. 1a). The basal cell is anchored to the micropylar end of the embryo sac. This cell produces haustorial branches that invade the micropyle and adjacent integumentary tissue. At this stage the basal cell is filled with dense cytoplasm. The nucleus is the most conspicuous organelle within the cell and is located at the chalazal pole (Fig. 1a). The basal cell undergoes no further division and becomes the basal suspensor cell, while the apical cell develops into the embryo proper and the chalazal suspensor. Differentiation of the suspensor was compared with the development of the embryo proper.

DIFFERENTIATION OF THE BASAL CELL – GLOBULAR-STAGE EMBRYO

The basal cell is anchored to the micropylar end of the embryo sac. The suspensor of the globular-stage embryo is morphologically simple and filamentous,

consisting of a large pear-shaped basal cell (~70 × 33 μm) and a few chalazal cells. The micropylar anucleate haustorium of the basal cell is already strongly developed and ramifies in the integumentary tissue (Fig. 1b). At this stage of development the basal cell undergoes further differentiation. A single nucleus is located in the peripheral cytoplasm in the middle of the cell (Fig. 1b–d). The cytoplasm of suspensor cells is highly vacuolated, more so than the embryo proper (Fig. 1b). Aniline Blue Black staining indicates that the protein content of the cytoplasm of the basal cell and of the chalazal suspensor is lower than in the cells of the embryo proper (Fig. 1c). Numerous lipid droplets are visible in the basal cell at the beginning of the globular stage. Occasionally lipids are also present in the chalazal suspensor cells and in the micropylar haustorium (Fig. 1d), in an amount comparable with that in the embryo proper. The cell walls of the suspensor and the embryo proper are clearly PAS-positive. The basal cell wall is visibly thicker than the chalazal cell wall. Delicate PAS-positive ingrowths are produced in the micropylar part of the basal cell and in the micropylar haustorium cell (Fig. 1e). After staining with Auramine O, microscopy indicated the presence of a fluorescent cuticle on the surface of the embryo proper but not on the suspensor (not shown).

FULL DEVELOPMENT AND FUNCTION OF THE SUSPENSOR – LATE GLOBULAR AND HEART-STAGE EMBRYOS

The morphology and cytochemistry of the late globular and heart-stage embryos are similar enough that these stages can be described together. The fully developed filamentous suspensor contains an enlarged basal cell (~70 × 28 μm) which is attached to the maternal tissues and a single row of 6–10 chalazal cells. The cytoplasm of the basal cell becomes denser than in the previous stage of development. As development progresses the nucleus gradually enlarges. The chalazal suspensor, linking the basal cell with the embryo proper, consists of a few strongly elongated and vacuolated cells (Fig. 2a). Aniline Blue Black staining indicates similar protein content in the cytoplasm of the basal suspensor cell, the micropylar haustorium and chalazal suspensor cells (Fig. 2b). Numerous lipid droplets of various size are visible throughout the suspensor and the micropylar haustorium (Fig. 2c,d). A fluorescing cuticle envelops the embryo proper but does not extend to the suspensor as in the previous stage of development (Fig. 2e). Ultrastructural investigations indicate that an electron-dense cuticular layer accumulates on the surface of the walls of the embryo proper (Fig. 2f).

ULTRASTRUCTURE OF THE SUSPENSOR

The ultrastructure of the fully differentiated suspensor was observed in late globular and heart-stage embryos. The dense cytoplasm of the basal cell contains an irregularly shaped nucleus. The nucleus is the most prominent organelle within the cell (Fig. 3a). It grows to a considerable size and the amount of chromatin and the size of the nucleolus also increase. There are many pores in the folded nuclear envelope and the interior of the nucleus is filled with patches of condensed chromatin interspersed with decondensed chromatin (Fig. 3a). At higher magnification, bundles of actin filaments are seen in the cytoplasm near the nucleus (Fig. 3 c). The cytoplasm of the basal cell is rich in different organelles: mitochondria, plastids, dictyosomes, profiles of endoplasmic reticulum, microbodies and vacuoles (Figs. 3a, 4a,b). Many mitochondria of different size (both spherical and ellipsoidal) are distributed throughout the cytoplasm; they have a few tubular cristae (Figs. 3a, 4a,b). At the micropylar end of the basal suspensor cell, wall ingrowths appear as small papillae along the cytoplasmic face of the cell wall. Mitochondria are abundant and are located near the wall ingrowths (Fig. 3b). There are fewer plastids than mitochondria. They are also generally larger than the mitochondria, and sometimes are irregular in shape. These organelles are situated mainly near the nucleus and are poorly differentiated. The plastids are filled with a low-density matrix with very small tubules, and sometimes a few internal membranes (Figs. 3a, 4a,b). Occasionally there are also plastids that have a spherical body consisting of intertwined bundles of tubules (Fig. 5a). During this stage there are a few dictyosomes which produce different kinds of dictyosomal vesicles distributed throughout the basal cell cytoplasm (Fig. 4b). In the transverse cell, which separates the basal cell from the chalazal cells, there are many plasmodesmata. Ribosome density is lower in the basal cell than in the chalazal suspensor cells. A group of vacuoles containing fine fibrillar material appears in the chalazal region of the cell (Fig. 4a). Free ribosomes are numerous and occur singly or are aggregated in small polysomes which may have a helical arrangement (Fig. 5a). The profiles of the endoplasmic reticulum are rather long and may be scattered singly throughout the cytoplasm. The ER frequently runs parallel to the plasmalemma (Fig. 3b) and to the surface of the plastids (Fig. 5a). Some rough ER cisternae appear to be stacked in three to four layers parallel to the cell or the nuclear surface (Fig. 4b). At this stage the cytoplasm of the basal cell has a well-developed smooth endoplasmic reticulum (SER) (Fig. 4b). Microbodies occur less frequently in the cytoplasm than other organelles. Various types of vesicles are

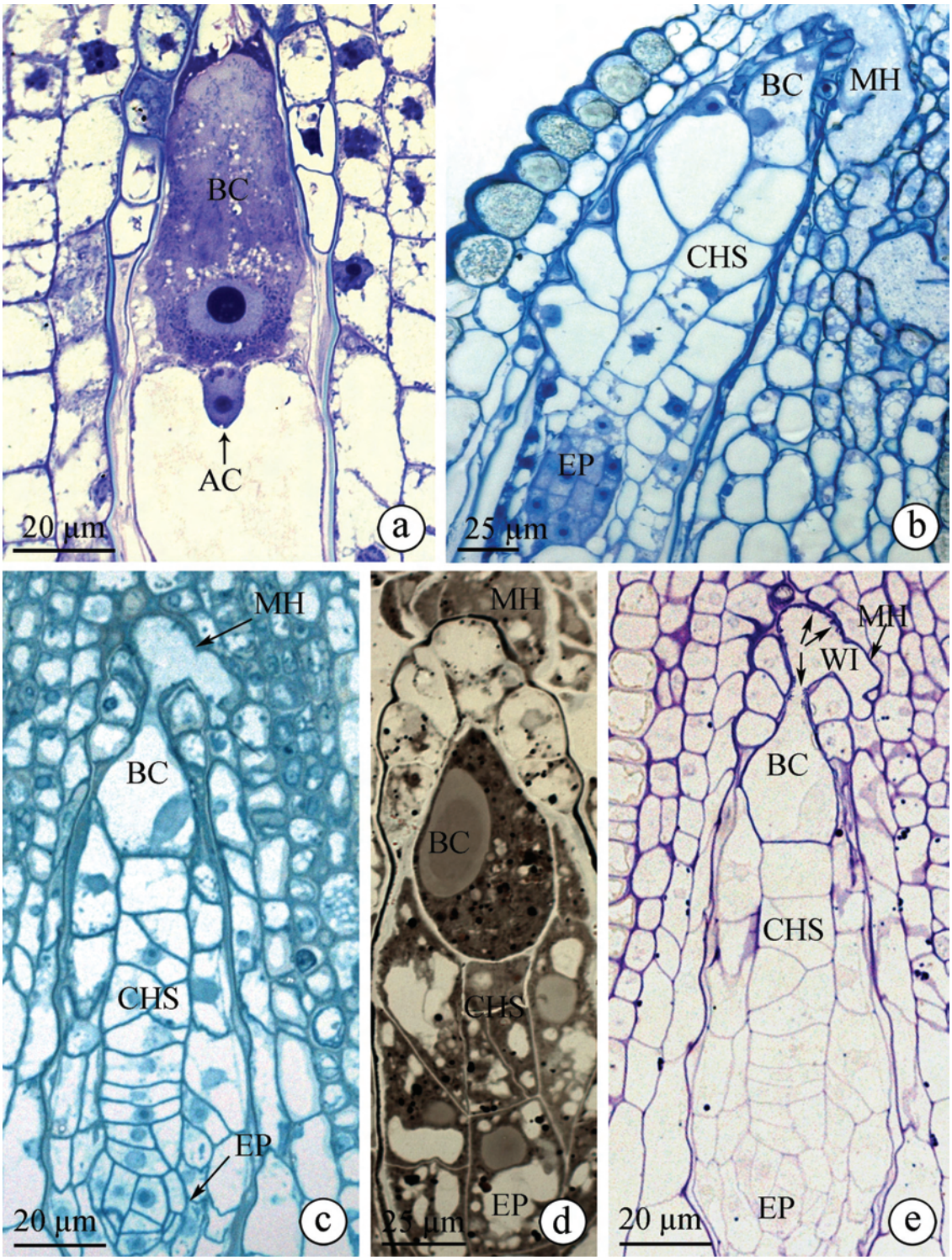

Fig. 1. Development of the *Sedum reflexum* L. embryo. Results of cytochemical tests. (**a**) Two-celled proembryo. Longitudinal section showing large basal cell and smaller apical cell, (**b–e**) Globular stage embryos: (**b**) Semithin section showing basal cell with large uninucleate micropylar haustorium, a few chalazal suspensor cells and embryo proper, (**c**) Embryo stained with Aniline Blue Black showing protein distribution, (**d**) Section stained with Sudan Black B showing lipid distribution, (**e**) Embryo stained for polysaccharides. Note PAS-positive wall ingrowths of basal cell and micropylar haustorium. AC – apical cell; BC – basal cell; CHS – chalazal suspensor cells; EP – embryo proper; MH – micropylar haustorium, WI – wall ingrowths.

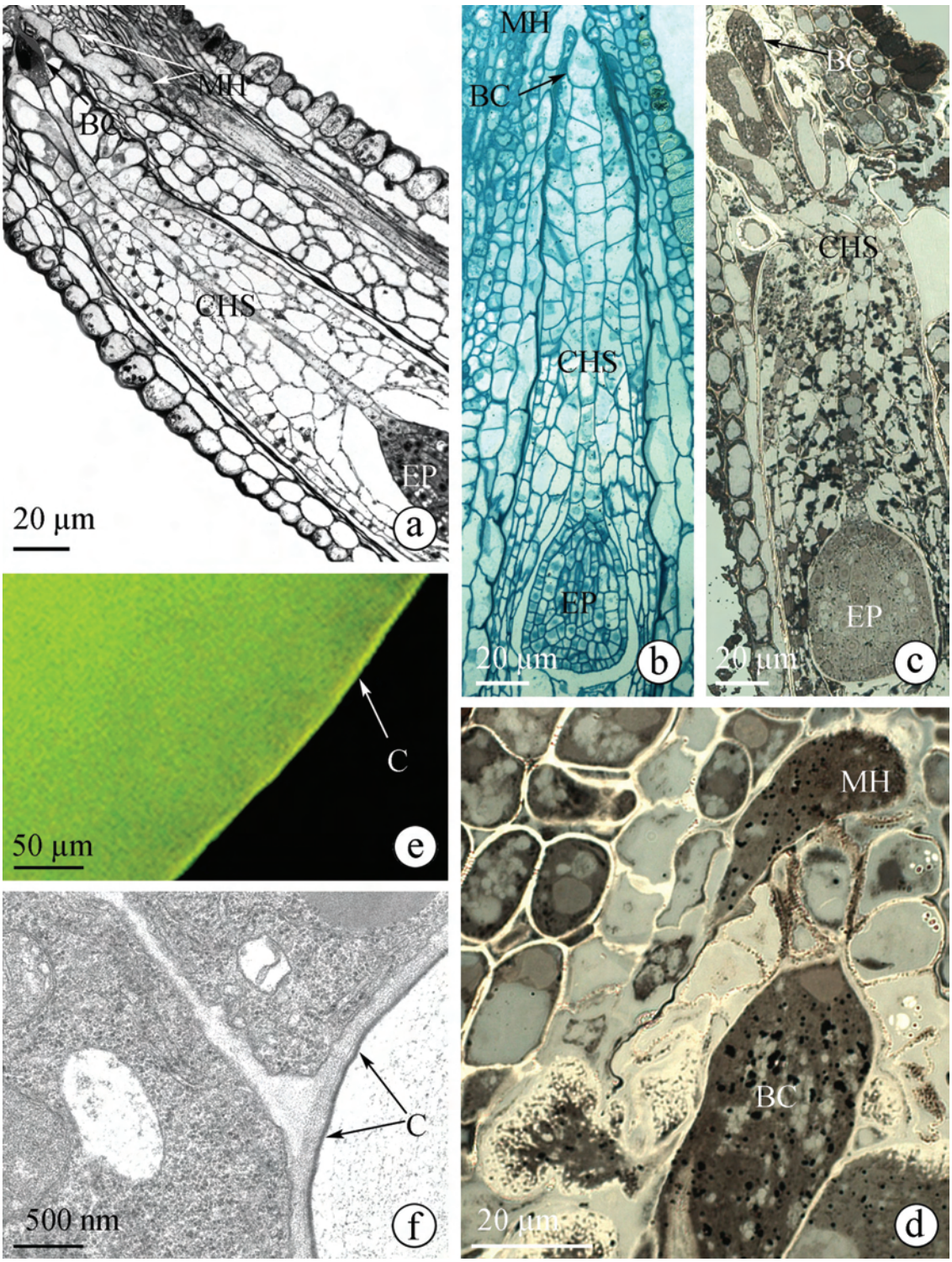

Fig. 2. Late globular and heart-stage embryos. Results of cytochemical tests. (**a**) Semithin section showing fully developed suspensor consisting of large basal cell with micropylar haustorium, a few chalazal suspensor cells; the embryo proper, (**b**) Section stained with Aniline Blue Black showing the location of proteins; basal cell, micropylar haustorium, chalazal suspensor cells, embryo proper, (**c–d**) Sections stained with Sudan Black B showing lipid distribution, (**c**) basal cell, chalazal suspensor cells, embryo proper, (**d**) basal cell, micropylar haustorium, (**e**) Portion of embryo proper. Fluorescing cuticle visible on surface of embryo proper, (**f**) Fragment of embryo proper with electron-dense cuticle layer. BC – basal cell; MH – micropylar haustorium; CHS – chalazal suspensor cells; EP – embryo proper; C – cuticle.

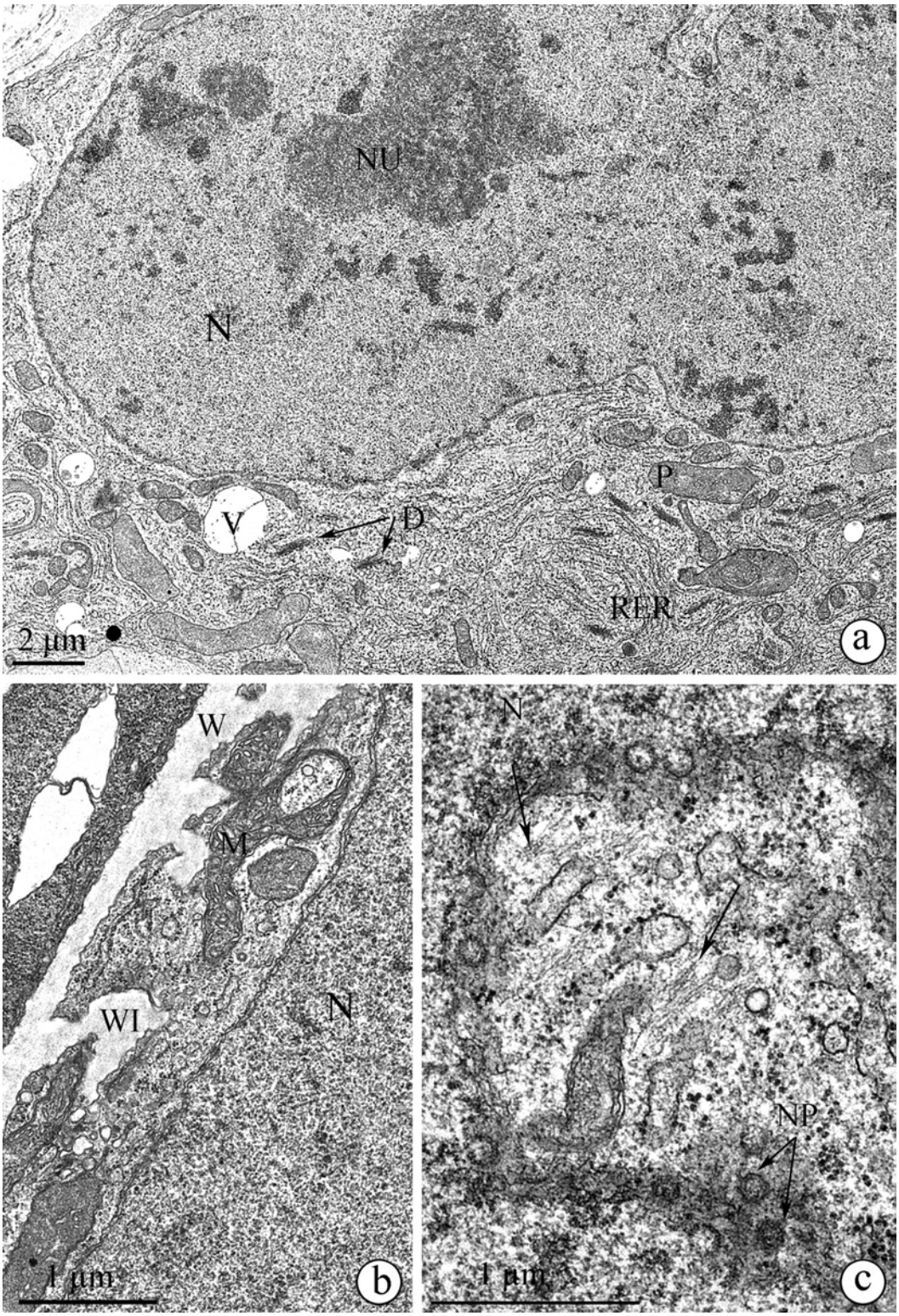

Fig. 3. Suspensor development in late globular and heart-stage embryos. Electron micrographs. (**a**) Fragment of basal cell contains large nucleus (N) and nucleolus (NU). Cytoplasm is filled with rough endoplasmic reticulum (RER) cisternae and dictyosomes (D); plastid (P), small vacuole (V), (**b**) Fragment of micropylar part of basal cell. Mitochondria (M) near wall ingrowths (WI); cell wall (W), nucleus (N), (**c**) Many nuclear pores (NP) visible in folded nuclear envelope. Note bundles of microfilaments (arrows) near nucleus.

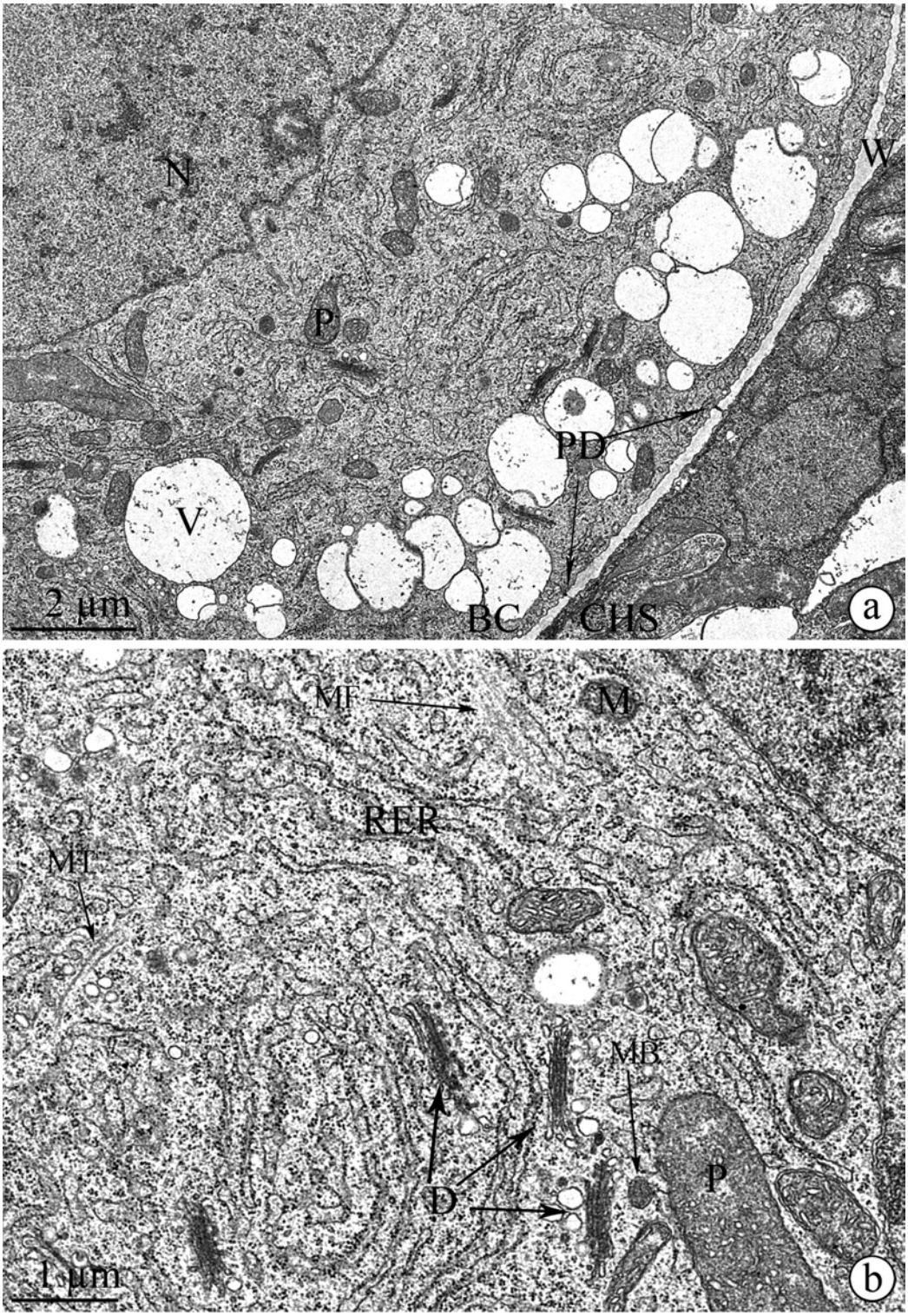

Fig. 4. Suspensor development in late globular and heart-stage embryos. Electron micrographs. (**a**) Part of cell wall (W) between basal cell (BC) and first cell of chalazal suspensor cells (CHS) with plasmodesmata (PD). Ribosome density is lower in basal cell than in chalazal suspensor cells. In chalazal region of basal cell, a group of vacuoles (V) containing fibrillar material appears; plastid (P), nucleus (N), (**b**) Higher magnification of portion of basal cell in vicinity of nucleus. Cytoplasm is rich in mitochondria (M), profiles of rough endoplasmic reticulum (RER), dictyosomes (D). Occasionally, microbodies (MB), bundles of microfilaments (MF) and microtubules (MT) were observed; plastid (P).

found in the cytoplasm, both electron-dark and electron-bright (Fig. 4b). Microfilaments and microtubules near the nucleus are also observed occasionally (Figs. 3c, 4b).

The chalazal suspensor consists of 6–9 highly vacuolated cells (Fig. 5b). The size and structure of the nucleus of the chalazal suspensor cells do not differ from those of the nuclei of the embryo proper. Many free ribosomes are visible in the cytoplasm but there are few RER cisternae and dictyosomes. Mitochondria are not very numerous; they are spherical or ellipsoidal in shape. The few plastids generally are smaller than the basal cell plastids. The wall separating the basal cell from the chalazal suspensor contains numerous plasmodesmata. There are a large number of plasmodesmata in the thin inner walls of the chalazal suspensor and embryo proper, but they are absent from the outer walls of the entire embryo proper (Fig. 5b).

ULTRASTRUCTURE OF THE MICROPYLAR HAUSTORIUM

The basal cell forms an anucleate micropylar haustorium. Together with the enlargement of the basal cell, its micropylar haustorium grows and gradually penetrates deeper into the integument tissue (Fig. 6a). Delicate ingrowths are formed on the micropylar haustorium. There are numerous mitochondria and rough endoplasmic reticulum profiles in close proximity to the ingrowths (Fig. 6b). The haustorium has very dense cytoplasm. Many dictyosomes are distributed throughout the cytoplasm of the micropylar haustorium. The cytoplasm is filled with many active dictyosomes which produce dictyosomal vesicles. Vacuoles, plastids and lipid droplets of various sizes are also visible (Fig. 6b).

MATURE EMBRYO

In the next stage of senescence, the suspensor consists of a basal cell (~57 × 29 μm) and a few elongated chalazal cells. The embryo proper is ~700 μm long (Fig. 7a). The cytoplasm is filled with a very small number of small vacuoles. The cytoplasm of the micropylar haustorium also contains vacuoles but they are larger than in the basal cell. The chalazal cells of the suspensor are strongly vacuolized (Fig. 7b). The nucleus of the basal cell becomes lobed, loses its regular shape and becomes centrally located in the cell (Fig. 7b,d). As compared with the earlier stages of embryo development, the number and size of wall ingrowths increases slightly, especially in the micropylar part of the basal cell (Fig. 7c). Aniline Blue Black staining suggests slightly lower protein content in the cytoplasm of the basal cell, in chalazal cells, and in the micropylar haustorium than in earlier stages (Fig. 7d). The number of lipid droplets decreases in both the basal cell and the micropylar haustorium as compared with earlier stages (Fig. 7e). There is a thin layer of cuticle on the outer surfaces of the embryo proper (not shown).

DISCUSSION

The diversity of size, shape, longevity and cytological features of the suspensor observed among different taxa is related to the mechanism of suspensor functioning in the nutrition of the embryo. In Crassulaceae the suspensor forms aggressive haustoria which penetrate the surrounding ovular tissue and are thought to be involved in translocation of nutrients from somatic cells of the ovule to the developing embryo (Raghavan, 1986). The haustorial suspensor in *S. reflexum* significantly differs in structure from previously investigated suspensors of Crassulaceae taxa. One difference is in the structure of chalazal suspensor cells. The suspensor of *S. reflexum* is built of 5–10 single cells in a filament-like arrangement. These cells are very strongly vacuolated. In the suspensor of *S. acre*, *S. hispanicum*, *Jovibarba sobolifera* or *Sempervivum arachnoideum* the chalazal suspensor cells are few (2–4) and arranged in two levels. Another feature distinguishing this suspensor is the presence of very specific, linearly arranged endosperm cells adjacent to the suspensor along its entire length. These cells are highly vacuolated and have wall ingrowths in the micropylar fragment of the wall. The endosperm cells surrounding the linear portion of the suspensor most likely play an important role in embryo development, especially when the linear suspensor cells are more vacuolated. In the suspensor of both *S. acre* and *S. hispanicum* the cellular endosperm was very poorly developed.

In *Sedum reflexum* the role of the suspensor in nutrient transport to the embryo is confirmed by basal cell structure. This cell undergoes developmental changes during embryogenesis. In terms of the possible physiological functions of the suspensor, perhaps the most salient feature of its subcellular morphology is the presence of wall ingrowths (Raghavan, 2006). The walls of the haustorium and the micropylar part of the basal cell form prominent wall ingrowths which are covered by a plasma membrane, which greatly increases the ability of these cells to absorb nutrients from the surrounding tissues. Numerous studies on the occurrence of transfer cells indicate morphological, temporal and spatial correlations between the degree of activity of transfer cells and the extent of their wall ingrowth development (Gunning and Pate, 1969). In *S. reflexum* the wall ingrowths of transfer cells develop during early embryogenesis and are most extensive by the late globular and early heart-shaped stage. The

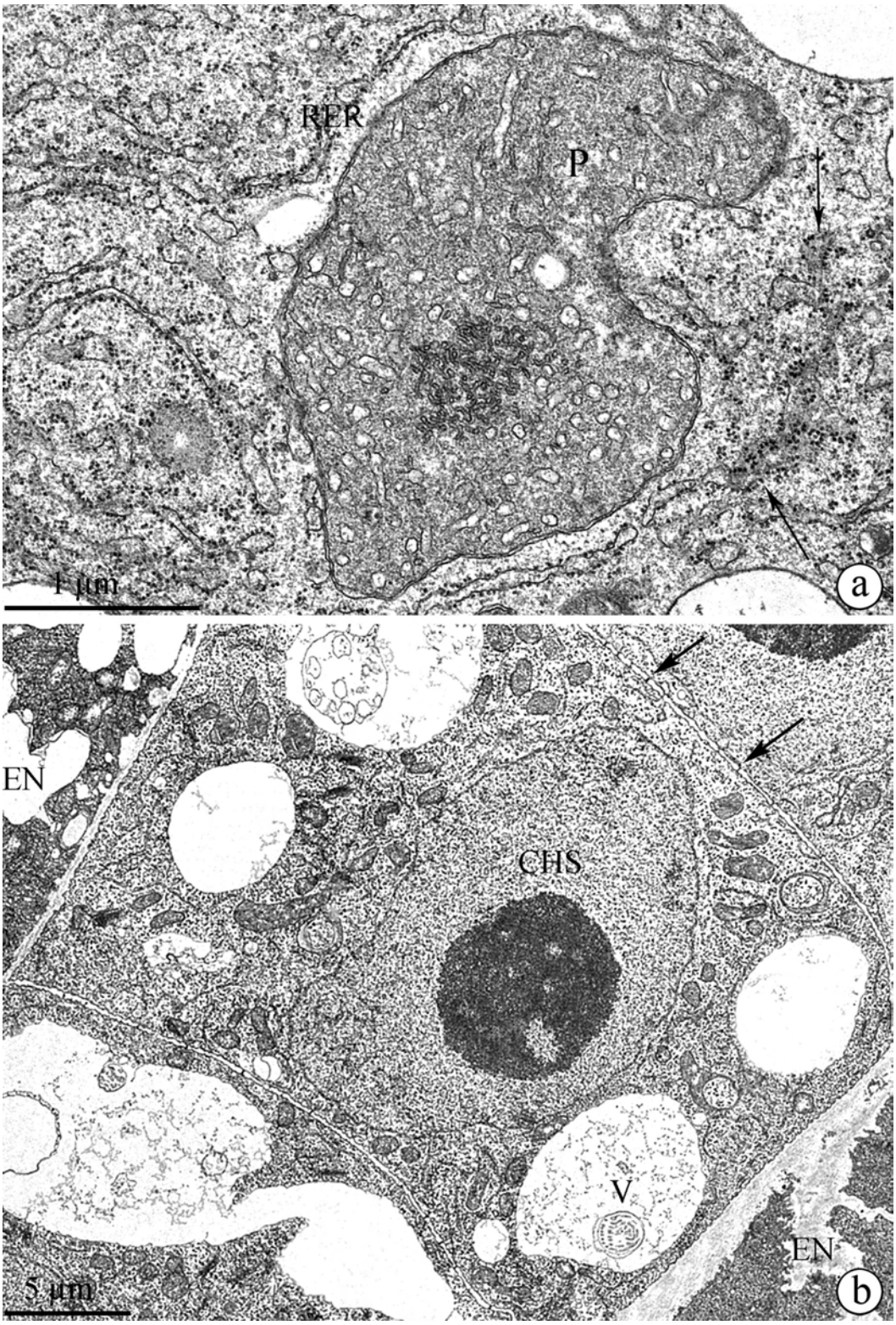

Fig. 5. Suspensor development in late globular and heart-stage embryos. Electron micrographs. (**a**) Magnification of plastid (P) with spherical body containing intertwined bundles of tubules. Rough endoplasmic reticulum (RER) profiles frequently run parallel to surface of plastids. Arrows indicate polysomes, (**b**) Portion of chalazal suspensor cells (CHS). Plasmodesmata (arrows) present in cell wall separating chalazal suspensor cells; vacuole (V), endosperm (EN).

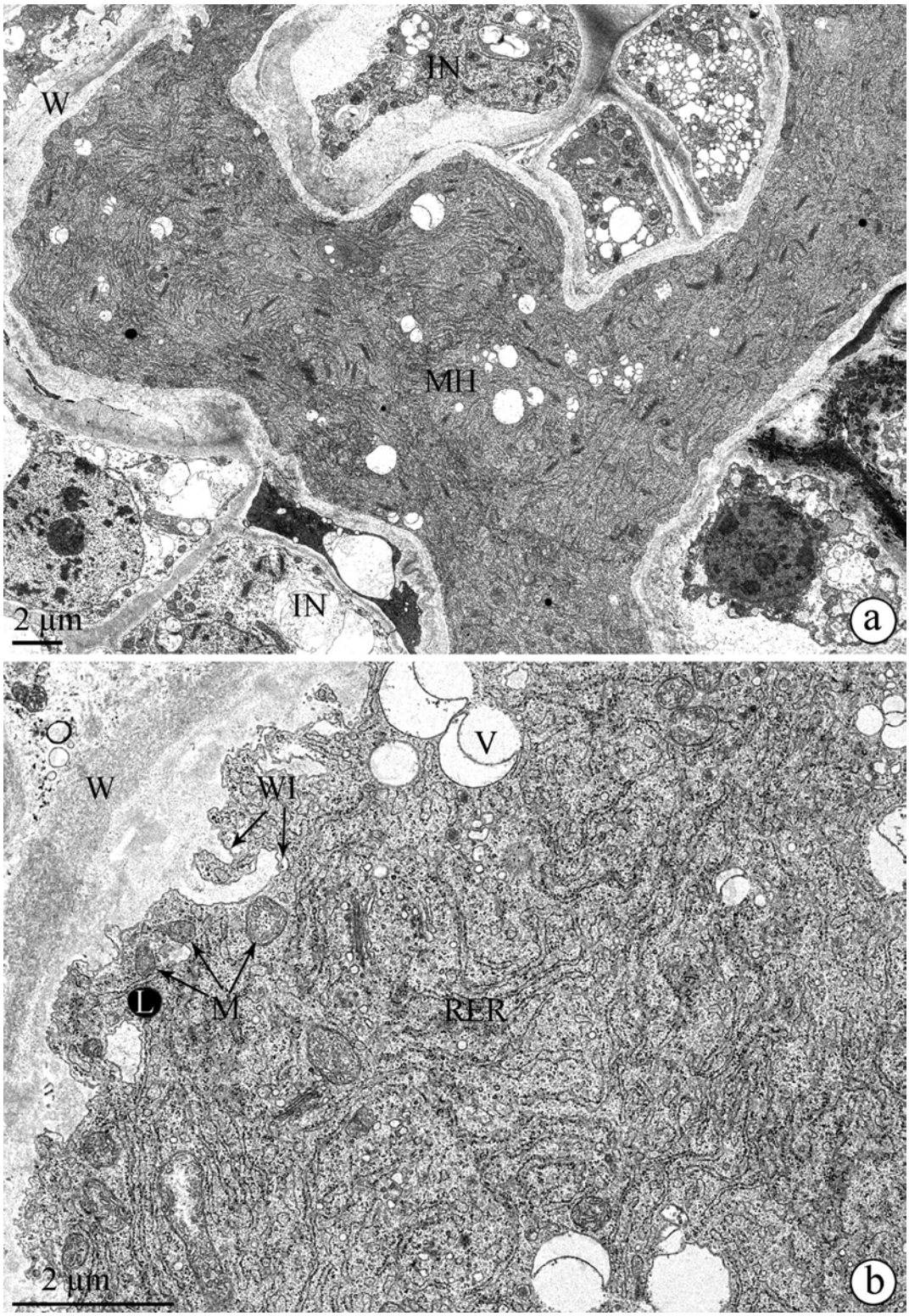

Fig. 6. Uninucleate micropylar haustorium of basal cell in fully developed suspensor. (**a**) Micropylar haustorium (MH) branching into integumentary tissues (IN); cell wall (W), (**b**) Higher magnification of fragment of haustorium showing wall ingrowths (WI) and haustorium wall (W). Note the numerous mitochondria (M) near the wall ingrowths; profiles of rough endoplasmic reticulum (RER); vacuole (V), lipid droplet (L).

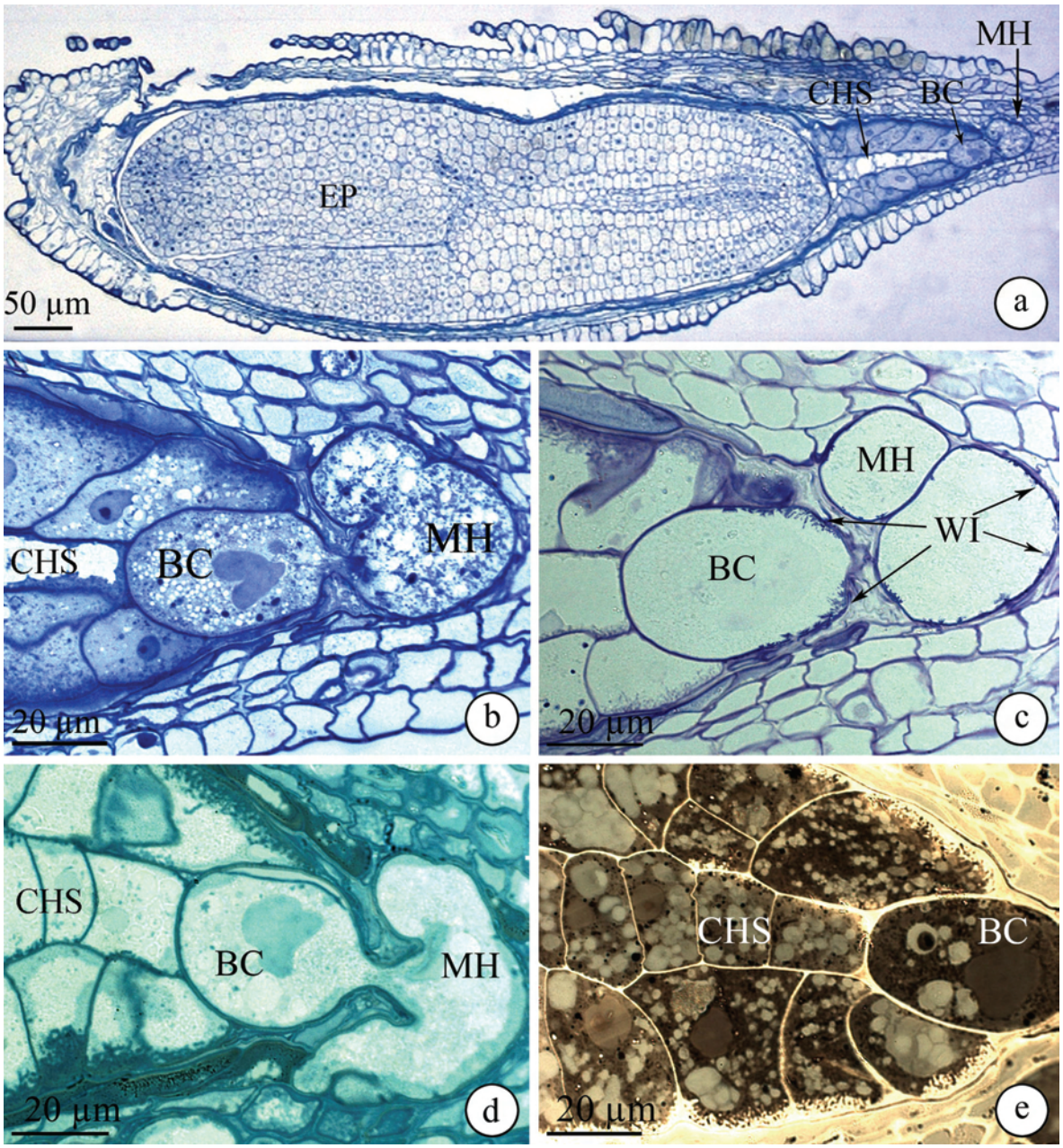

Fig. 7. Mature embryo proper. Results of cytochemical tests. (**a**) Stage of suspensor senescence showing basal cell (BC) with micropyle haustorium and a few chalazal suspensor cells, embryo proper, (**b**) Higher magnification of basal cell, micropylar haustorium and fragment of chalazal cells, (**c**) Micropylar haustorium and micropylar part of basal cell wall form PAS-positive ingrowths, (**d**) Section stained with Aniline Blue Black showing protein distribution. Protein distribution in haustorial basal cell and chalazal cells; micropylar haustorium, (**e**) Section stained with Sudan Black B showing lipid distribution; basal cell, chalazal cells. BC – basal cell; MH – micropylar haustorium; CHS – chalazal suspensor cells; EP – embryo proper; WI – wall ingrowths.

wall ingrowths that develop at the micropylar end of the basal cell and haustorium are involved in the intensive transport of metabolites. Similar suggestions have been made for the wall ingrowths that occur in *Capsella bursa-pastoris* (Schulz and Jensen, 1969), *Phaseolus coccineus* (Yeung and Clutter, 1978), *Alisma* (Bohdanowicz, 1987); *Arabidopsis thaliana* (Mansfield and Briarty, 1991), *Paphiopedilum delenatii* (Lee et al., 2006), *Sedum acre* and *S. hispanicum* (Kozieradzka-Kiszkurno and Bohdanowicz, 2006), *Sempervivum arachnoideum*, *Jovibarba sobolifera* and *Graptopetalum*

bellum (Kozieradzka-Kiszkurno et al., 2011b). According to Pate and Gunning (1972), any cell that contains wall ingrowths and is thought to be involved in short-distance transport of solutes can be termed a transfer cell. The numerous organelles that may support active transport of nutrients across the plasma membrane and translocation of the absorbed materials to the embryo proper are closely associated with these wall projections. The abundance of mitochondria adjacent to the ingrowths suggests high energy requirements for absorption and movement of nutrients across the cell membrane by the cells in question. The occurrence of such mitochondria has been found in the suspensor cells of many species: *Phaseolus coccineus* (Yeung and Clutter, 1978), *Capsella bursa-pastoris* (Schulz and Jensen, 1969), *Pisum sativum* (Marinos, 1970a), *Stellaria media* (Newcomb and Fowke, 1974), *Tropaeolum majus* (Nagl, 1976), *Medicago* (Sangduen et al., 1983), *Alisma plantago-aquatica* and *A. lanceolatum* (Bohdanowicz, 1987), *Paphiopedilum delenatii* (Lee et al., 2006), *Sempervivum arachnoideum* and *Jovibarba sobolifera* (Kozieradzka-Kiszkurno et al., 2011b). Wall ingrowths similar to those in *S. reflexum* have also been observed in two *Sedum* species: *S. acre* and *S. hispanicum* (Kozieradzka-Kiszkurno and Bohdanowicz, 2006). In the *Sedum* species studied here, the structural specializations along with the formation of wall ingrowths occur in the suspensor already at the proembryo stage. Their presence, which covers most of the micropylar basal cell during the developmental stages, suggests that this region requires a higher level of transport because it is metabolically dynamic. Further evidence that the suspensor functions as a channel for transport of nutrients to the developing embryo proper from the surrounding maternal tissues is the presence of plasmodesmata in the chalazal-end wall of the basal cell and in the end walls of the suspensor, while plasmodesmata are absent from the outer walls of the embryo proper. A distribution of simple plasmodesmata similar to that in *S. reflexum* was reported in suspensors of sunflower (Newcomb, 1973), *Capsella bursa-pastoris* (Schulz and Jensen, 1969), *Alisma plantago-aquatica* and *A. lanceolatum* (Bohdanowicz, 1987). However, the structure of plasmodesmata in *S. reflexum* differs from that of suspensor plasmodesmata recorded in several Crassulaceae species: *Sedum acre* and *S. hispanicum* (Kozieradzka-Kiszkurno and Bohdanowicz, 2010), *Sempervivum arachnoideum* and *Jovibarba sobolifera* (Kozieradzka-Kiszkurno et al., 2011a,b, 2011c). There are branched plasmodesmata with an unusual dome of electron-dense material on the side of the cell in those species. In *S. reflexum*, as in most angiosperm species, the suspensor does not have a cuticle layer to facilitate direct communication with adjacent seed tissues, unlike the embryo proper, which is surrounded by a cuticle that covers the protoderm surface (Szczuka and Szczuka, 2003).

In *Sedum reflexum* the basal cell is the site of intense metabolic activity. This was reflected in the composition and distribution of proteins (Aniline Blue Black staining), lipids (Sudan Black B staining), insoluble polysaccharides (PAS-reaction) and cuticular materials at various stages of development of the embryo proper and suspensor. Our analysis of suspensor ultrastructure also confirmed it. The various organelles found in the suspensor cells suggest that this embryonic organ may be the site of metabolic processes that do not occur within the embryo proper. The suspensor basal cell of *S. reflexum* undergoes developmental changes during embryogenesis. The presence of polysomes in the haustorial suspensor of *S. reflexum* suggests active synthesis of protein, which would be needed for rapid growth of the suspensor basal cell. The profiles of endoplasmic reticulum (ER), which are well developed in the *S. reflexum* basal cell, often run from the vicinity of the micropylar transfer wall to the chalazal end of the cell and may well play an active part in absorption and intracellular transport of nutritive substances. In *S. reflexum* the endoplasmic reticulum cisternae of the basal cell are covered with ribosomes and probably participate not only in transport but also in synthesis of various substances. Well-developed RER normally is associated with intensive protein synthesis (Gunning and Steer, 1975). The considerable amount of cytochemically detected protein in the cytoplasm of the basal cell would appear to confirm this interpretation of RER function. Transport functions have also been proposed for the suspensor endoplasmic reticulum in *Capsella* (Schulz and Jensen, 1969), *Helianthus* (Newcomb, 1973), *Stellaria* (Newcomb and Fowke, 1974), *Phaseolus* (Yeung and Clutter, 1978), *Alisma* (Bohdanowicz, 1987), *Jovibarba sobolifera* and *Sempervivum arachnoideum* (Kozieradzka-Kiszkurno et al. 2011b). The occurrence of lipid droplets in the cytoplasm of both the haustorial suspensor and embryo proper of *S. reflexum* probably is due to the abundant tubular smooth endoplasmic reticulum (SER) in late globular and heart-stage embryos. SER participates in synthesis and secretion of lipids. The endoplasmic reticulum is also equipped with enzymes and structural proteins involved in biogenesis of oil bodies and in storage of lipids. Microbodies are not very numerous in the suspensor and micropylar haustorium of *S. reflexum*. They are not found in the embryo proper. Most microbodies contain at least a part of either the glycolate pathway or the glyoxylate cycle. Thus it seems likely that these organelles are important metabolically. Important metabolic enzymes have been found in other plant microbod-

ies (Gunning and Steer, 1975; Tolbert and Essner, 1981). In most angiosperm species a unique structural feature of suspensor cells is the presence of large differentiated plastids (Yeung and Meinke, 1993). According to Kozieradzka-Kiszkurno and Płachno (2013), Crassulaceae plastids are highly variable in shape and size. The shape of these organelles varies from spherical to more or less oval, irregular, and sometimes cup-shaped. A dense stroma is a typical feature of most of the plastids in angiosperms (Raghavan, 1986) but in *S. reflexum* the plastids are not so intensely electron-dense as in the other Crassulaceae examined. The plastids in *S. reflexum* most resemble those in the suspensor of *Pisum sativum* (Marinos, 1970b). They have a centrally located body consisting of intertwining tubules surrounded by a stroma and a double membrane. In the Crassulaceae suspensor the plastids are in close contact with the mitochondria, endoplasmic reticulum profiles and the nucleus. This applies to *S. reflexum* as well. Such close contact between these organelles has also been observed in the suspensor cells of many species of, for example, *Stellaria* (Newcomb and Fowke, 1974), *Phaseolus* (Yeung and Clutter, 1978) and *Medicago* (Sangduen et al., 1983). The close contact between plastids and the nuclei and plasma membranes of plant cells suggests that this physical interaction may enhance the functional interactions between the organelles. The plastid-nucleus complex can be considered a semicell, that is, a structure capable of metabolism, photosynthesis, protein and RNA synthesis but which is not covered by a plasmalemma (Selga et al., 2010).

In many plant species, differentiation of suspensor cells is accompanied by endopolyploidization of their nuclei (for review see D'Amato, 1984). Large endopolyploid nuclei are also present in the basal cells of *Alisma* (Bohdanowicz, 1987), *Triglochin palustre* (Kozieradzka-Kiszkurno et al., 2002), *Sedum acre* (Kozieradzka-Kiszkurno and Bohdanowicz, 2003) and a number of other angiosperms (Raghavan, 1986). In *S. reflexum*, enlargement of the basal cell nucleus is one of the first indications of its specialization. Multiplication of the genome number in the nucleus of an endopolyploid cell usually leads to a proportionate increase in its synthetic activity (D'Amato, 1984).

The diversity of suspensor morphology suggests diversity of suspensor function. For example, the massive suspensors of *Phaseolus coccineus* and *Tropaeolum majus* may be more involved in macromolecular synthesis than smaller suspensors are, and they may serve as storage tissue that provides nutritional support for embryogenesis of the developing late-stage embryo (Raghavan, 1986), while more reduced filamentous suspensors (an example of which is *S. reflexum*) may function primarily in absorbing nutrients from maternal tissues and transporting materials to the embryo proper (Yeung and Meinke, 1993). Nagl (1973) aptly compared the embryonal suspensor in plants to the mammalian trophoblast, which acts as a supply line for nutrition of the fetus.

Rapidly progressing genomic technologies have opened up new opportunities to study the role and evolution of the suspensor in the plant kingdom. It is now possible to investigate gene activity in the suspensor of almost any plant species and uncover novel genes and pathways that play important roles in establishing suspensor form and function (Kawashima and Goldberg, 2010). In recent years, *Brassica napus* L. has emerged as a model system for studying microspore embryogenesis. Isolated microspore cultures in vitro provide a window for studying the cellular events that mark reprogramming and totipotency at single-cell level (Supena et al., 2008; Soriano et al., 2013). A new microspore embryogenesis system under low temperature mimics zygotic embryogenesis initials and efficiently regenerates doubled-haploid plants in *B. napus* (Prem, 2012).

Here we showed that suspensor structure in *S. reflexum* markedly differs from that found at other representatives of the Crassulaceae family. It is a type of suspensor development not found heretofore in *Sedum*. All of our observations, both cytochemical and ultrastructural, support the suggestion that in *Sedum reflexum* the suspensor plays an important physiological role in the early stages of embryogenesis. Our results and those of others on the structure-function relationships of the suspensor make it clear that this organ is a dynamic part of the embryo complex, functioning in absorption, short-distance translocation and exchange of metabolites needed for growth of the embryo.

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