

# SUSPENSOR DEVELOPMENT IN *GAGEA LUTEA* (L.) KER GAWL., WITH EMPHASIS ON THE CYTOSKELETON

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The study used fluorescence microscopy to examine changes in cytoskeleton configuration during development of the embryo suspensor in *Gagea lutea* and to describe them in tandem with the development of the embryo proper. During the early phase of embryo suspensor development, tubulin and actin filaments were observed in the cytoplasm of the basal cell from the micropylar to the chalazal ends of the cell. Around the nucleus of the basal cell were clusters of numerous microtubules. These accumulations of tubulin arrays congregated near the nucleus surface; numerous bundles of microtubules radiated from the nucleus envelope. At this time, microfilaments formed a delicate network in the cytoplasm of the basal cell. In the fully differentiated embryo suspensor, microtubules were observed at the chalazal end of the basal cell. Numerous bundles of microtubules were visualized in the cytoplasm adjacent to the wall separating the basal cell from the embryo proper. Microfilaments formed a dense network which uniformly filled the basal cell cytoplasm. There were some foci of F-actin material in the vicinity of the nucleus surface and at the chalazal end of the basal cell. In all studied phases of embryo suspensor development a prominent cortical network of actin and tubulin skeleton was observed in embryo proper cells.

**Key words:** Cytoskeleton, embryo suspensor, *Gagea lutea*, immunoassay.

## INTRODUCTION

The cytoplasmic skeleton (cytoskeleton) is an essential component of the cytoplasm of living cells. It participates in many processes involved in its functioning: for example, it allows cells to assume different shapes, to perform coordinated movements, to effect organized transport, to transfer nuclei, chromosomes and large cellular organelles such as mitochondria, and to shift small objects such as dictyosomes. The structure of cytoskeleton elements in somatic cells, their protein composition, and configurations of the cytoskeleton undergoing changes in successive phases of the cell cycle, are quite well recognized (Derksen et al., 1990; Goddard et al., 1994; Baskin, 2000; Staiger et al., 2000; Hasezawa and Kumagai, 2002; Kost et al., 2002). Numerous studies have addressed the configuration of the cytoskeleton during endosperm cell differentiation (Brown et al., 2003; Nguyen et al., 2001, 2002; Olsen, 2001) and during microsporogenesis (Brown and Lemmon, 2000; Bohdanowicz et al., 2005). There is much less information on the cytoskeleton configuration in processes of plant reproduction.

Changes in the cytoskeleton configuration during megasporogenesis, embryo sac development or embryogenesis have been described for only a few plant species (e.g., Heslop-Harrison and Heslop-Harrison, 1989; Huang and Russel, 1994; Russel, 1996; Ye et al., 1997; Fu et al., 2000; Tung et al., 2000; Ying et al., 2000; Nguyen et al., 2001; Płachno and Świątek, 2012). There have been many studies on the cytochemistry, ultrastructure and metabolic activity of suspensors in many species of flowering plants (for reviews see Yeung and Meinke, 1993; Raghavan, 2006) but there is still little information on the cytoskeleton during the formation and development of endopolyploid cells of the embryo suspensor (Kozieradzka-Kiszkurno et al., 2011). In *G. lutea* the first division of the zygote is uneven and leads to the formation of the proembryo, the larger cell of which differentiates into a large basal cell. Karyological assays indicate that differentiation of the suspensor basal cell in *G. lutea* is accompanied by endoreduplication. The suspensor obviously precedes the development of the embryo and endosperm proper. The basal cell nucleus increases in size and nuclear DNA content, and reaches maxi-

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mum degree of ploidy (128C) during normal development (Kozieradzka-Kiszkurno et al., 2007). Endoreduplication is a form of nuclear polyploidization by which cells gain additional copies of genomic DNA (Joubes and Chevalier, 2000). This process usually occurs in tissues and organs that actively function for only a short period of ontogenetic development (Brodsky and Uryvaeva, 1985).

Previous observations of the cytoskeleton in polyploid cells are limited to studies of the cytoskeleton during differentiation of polyploid trichomes in *Arabidopsis thaliana* (Mathur et al., 1999; Szymanski, 2000; Mathur and Chua, 2000), the endopolyploid basal cell of the suspensor in *Alisma plantago-aquatica* (Świerczyńska and Bohdanowicz, 2007, 2009) and in *Sedum acre* (Kozieradzka-Kiszkurno et al., 2011), the highly endopolyploid cell of the chalazal endosperm haustorium of *Rhinanthus serotinus* (Świerczyńska and Bohdanowicz, 2003; Świerczyńska et al., 2013), chalazal endosperm haustoria in *Utricularia* (Płachno et al., 2012) and the heterokaryotic syncytia of the endosperm placenta in *Utricularia* (Płachno et al., 2011, 2012). Our earlier ultrastructural and cytochemical studies indicated that the basal cell of the suspensor in *G. lutea* is a highly specialized, metabolically active cell which participates in the uptake and transport of nutrients from maternal tissues to the developing embryo proper (Bohdanowicz, 2001). This makes the suspensor of *G. lutea* a good subject for study of both cytoskeletal domains in highly endopolyploid plant cells. Here we describe the configurations of the actin and tubulin cytoskeleton during differentiation of the embryo suspensor in *Gagea lutea*, using fluorescence and immunofluorescence detection.

## MATERIALS AND METHODS

Plants of *Gagea lutea* were obtained from natural habitats of Gdańsk in northern Poland. Flowers in various developmental stages were collected in spring months (March, April).

### FLUORESCENCE ASSAY OF F-ACTIN

Ovules were excised from ovaries and pretreated for 15–60 min in 400  $\mu$ M m-maleimidobenzoic acid N-hydroxysuccinimide ester (Sonobe and Shibaoka, 1989; Huang and Russell, 1994) in piperazine buffer to which 5% dimethyl sulfoxide (DMSO) was added to permeate the cells (Traas et al., 1987). Then the ovules were fixed in 4% formaldehyde freshly prepared from paraformaldehyde in piperazine buffer containing 5% DMSO for 4 h at room temperature. Next, the ovules were rinsed in piperazine buffer and phosphate-buffered saline (PBS). Then the F-actin

was stained with Alexa Fluor 488-phalloidin (Invitrogen A12379) in PBS containing 5% DMSO for 1.5 h. Following several rinses in PBS, nuclei were stained with 4', 6'-diamidino-2-phenylindole dihydrochloride (DAPI, 1  $\mu$ g/ml, Sigma). The suspensor and embryo proper were isolated manually from ovules under a stereomicroscope and placed on a microscope slide.

### FIXATION FOR IMMUNOSTAINING

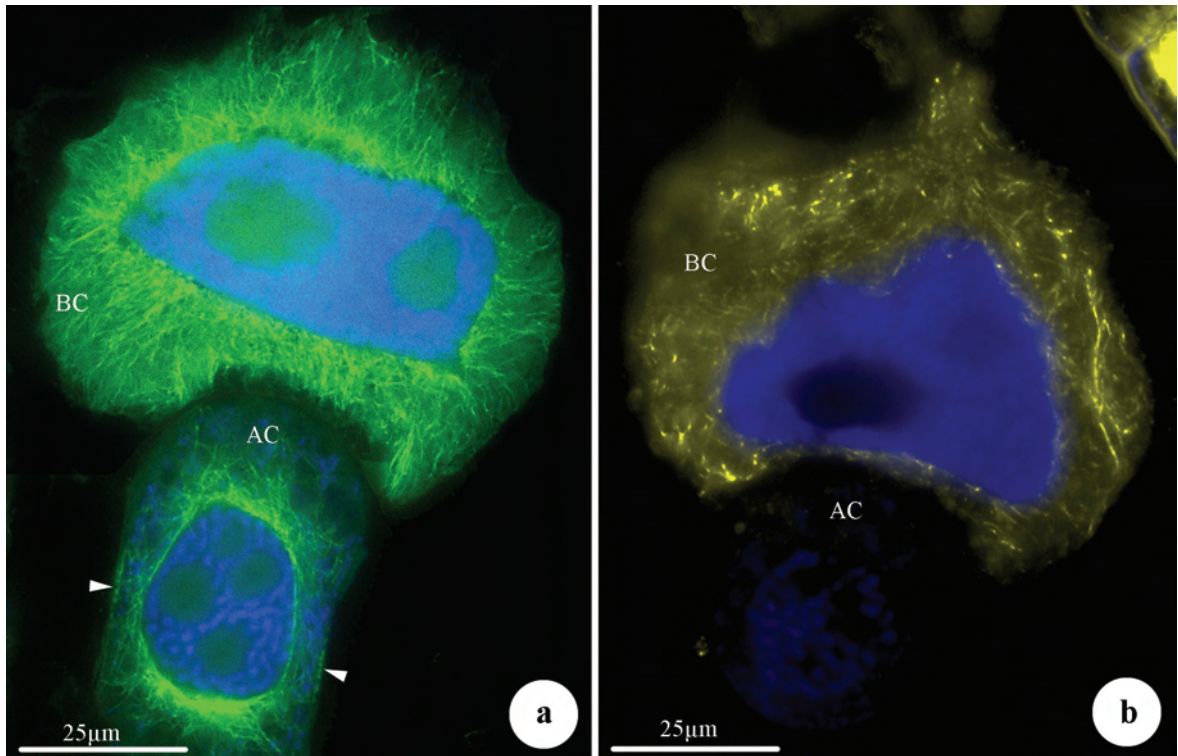
To visualize microfilaments, ovules were fixed as described above and then treated according to the procedure of Świerczyńska and Bohdanowicz (2003). For visualization of microtubules, ovules were fixed in 4% formaldehyde (freshly prepared from paraformaldehyde) and 0.25% glutaraldehyde in piperazine buffer for 4 h at room temperature. Then the ovules were prepared using the procedure described by Bohdanowicz et al., (2005). After fixation and several washes in piperazine buffer they were dehydrated in a graded ethanol series, each step containing 10 mM dithiothreitol (Brown et al., 1989) to minimize the background of the cytoplasm. Then the plant material was infiltrated with Steedman's Wax (Vitha et al., 2000). After polymerization of the wax, 5  $\mu$ m thick sections were cut and placed on microscope slides coated with Mayer's egg albumen. The sections were dried overnight, dewaxed in ethanol, rehydrated in an ethanol-PBS series and rinsed in PBS.

### IMMUNOLABELLING OF MICROFILAMENTS AND MICROTUBULES

Tissue slides were preincubated in PBS containing 0.1% bovine serum albumin (BSA) for 45 min at room temperature. For single visualization of microfilaments and microtubules, specimens were incubated overnight at 4°C with mouse anti-actin monoclonal antibody (clone C4, ICN; diluted 1:1000) and mouse anti- $\beta$ -tubulin monoclonal antibody (Amersham N357, diluted 1:200) respectively. The sections were washed in PBS and incubated for 4 h in secondary Alexa 488-conjugated anti-mouse antibody (Molecular Probes; diluted 1:800). Then the sections were rinsed in PBS and the nuclei were stained with 4', 6'-diamidino-2-phenylindole dihydrochloride (DAPI, 1  $\mu$ g/ml, Sigma). Next, the slides were treated with 0.01% toluidine blue to diminish cell wall autofluorescence and mounted in antifading solution (Citifluor, Agar). Control experiments omitting the primary antibody followed the same protocol.

### FLUORESCENCE MICROSCOPY

Fluorescence was observed with a Nikon Eclipse E 800 epifluorescence microscope fitted with a CCD



**Fig. 1.** Bicellular proembryo of *Gagea lutea*. **(a)** Immunostained microtubules (green), **(b)** Immunostained microfilaments (yellow), nuclei stained with DAPI (blue). **(a)** Longitudinal section depicting huge basal cell (BC) and apical cell (AC). In basal cell, microtubules congregate around nucleus, forming characteristic radial perinuclear systems. In apical cell, preprophase band visible (arrowheads), **(b)** Microfilament network visible in basal cell (BC) cytoplasm. A few clusters of actin material present in apical cell (AC) cytoplasm.

cooled camera using a B-1E filter (EX 470–490 nm, DM 505, BA 520–560) to observe actin stained with Alexa Fluor 488-phalloidin and tubulin and actin immunolabelled with Alexa 488, and a UV-2A filter (EX 330–380, DM 400, BA 420) to observe DAPI-stained nuclei. Colored image processing was done with Adobe Photoshop.

## RESULTS

### BICELLULAR PROEMBRYO

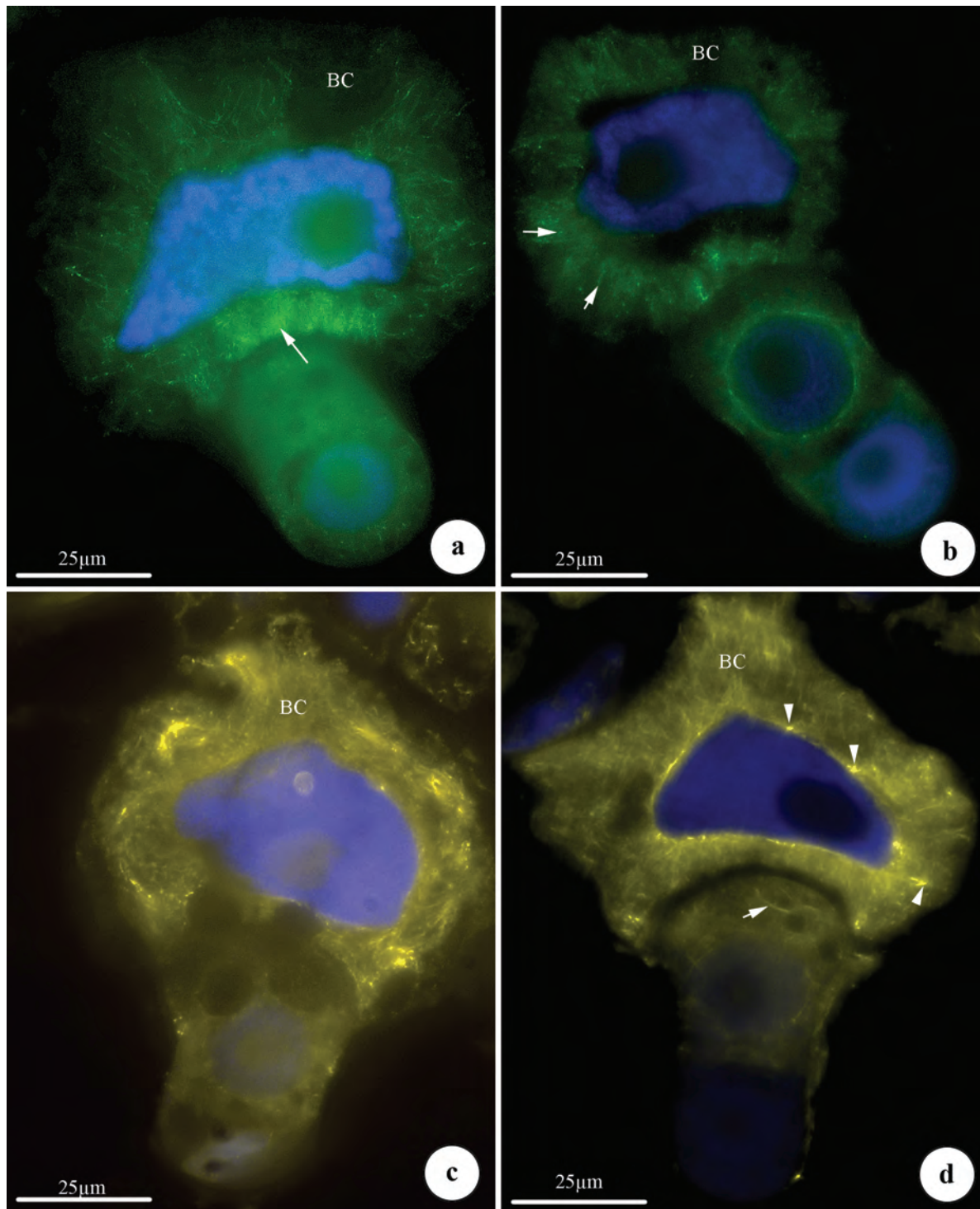
At the bicellular proembryo stage the basal cell is clearly enlarged and fills the micropylar area of the embryo sac. The nucleus of the basal cell is much larger than the apical cell nucleus. The enlarged elliptical nucleus is in the center of the basal cell. Enlarged nucleoli are visible in the nucleus. Microtubules can be seen near the basal cell nucleus, arranged radially, and most of them appear to radiate from the nuclear envelope to form a very characteristic radial perinuclear system (Fig. 1a). Numerous microtubule bundles arranged more loosely than the near-nucleus clusters are visible at the micropylar end and in the

cytoplasm of the basal cell. The preprophase band (PPB) is found in the apical cell (Fig. 1a). During that stage of development, actin filaments are present in the basal cell cytoplasm in the form of a number of bundles oriented in different directions. Some F-actin material is also visible as a bright cluster in the basal cell cytoplasm. Unlike the microtubules, the microfilaments do not form obvious clumps around the polyploid nucleus of the basal cell (Fig. 1b). The fluorescence of microfilaments seems weaker in the apical cell than in the basal cell. Only a few foci of actin material are visible in the apical cell (Fig. 1b).

### 3–9-CELL PROEMBRYO

In the 3–9-cell proembryo the surface of the basal cell nucleus is more wrinkled than in the previous stage of basal cell development. Its nucleus is further enlarged and is flattened along the micropylar-chalazal axis (Fig. 2a). Microtubules in the basal cell are still abundantly distributed around the nucleus, forming a radial arrangement. Some microtubule bundles are arranged obliquely and transversely to the micropylar-chalazal axis of the basal cell





**Fig. 2.** Three-cell proembryo of *Gagea lutea*. **(a, b)** Immunostained microtubules (green), **(c, d)** Immunostained microfilaments (yellow), nuclei stained with DAPI (blue). **(a)** Basal cell containing dense microtubule network radiating from nucleus surface. Microtubule bundles denser at chalazal end of basal cell (BC). Numerous bundles of microtubules concentrated near wall separating basal cell from proembryo cells (arrow), **(b)** Radial arrangement of microtubules (arrows) around basal cell nucleus. Microtubule network visible in proembryo cells, **(c)** F-actin skeleton forming abundant network in basal cell (BC) cytoplasm. Fluorescence of F-actin significantly weaker in proembryo cells, **(d)** Fine network of microfilaments in basal cell (BC) cytoplasm. Some microfilaments forming bright arrangements (arrowheads). A few microfilaments present in cytoplasm of proembryo cells (arrow).

(Fig. 2a,b). The microtubular skeleton is extremely dense at the chalazal end of the basal cell. Microtubule bundles run parallel to the micropylar-chalazal axis of cell and extend from the wall separating the basal cell from embryo cells towards the nucleus (Fig. 2a,b). The configuration of the tubulin cytoskeleton in embryo cells is not changed very much in comparison to the bicellular proembryo stage (Fig. 2a,b). The microfilament distribution patterns differ from the topography of microtubules. The actin elements in the basal cell cytoplasm form a fine network. This F-actin pattern seems to be the dominant feature in the basal cell cytoplasm (Fig. 2c,d). However, some concentrations of actin material, visible as bright dots, occur locally in the basal cell. Unlike the microtubules, the microfilaments do not form conspicuous radial perinuclear systems (Fig. 2c,d).

A dense microfilament network which locally forms a compaction of actin material is also present in older basal cells (4-cell proembryo stage, Fig. 3a,b). Microfilament bundles arranged circumferentially around the nucleus are visible in the basal cell cytoplasm. The actin skeleton elements are oriented transversely to the micropylar-chalazal axis of the basal cell (Fig. 3c). A concentration of actin material also appears near the nuclear surface. Arrangements of microfilaments are visible near the wall separating the basal cell from the embryo cells (Fig. 3c). In the embryo cells a small amount of actin skeleton elements appears as single bright points in the cell cytoplasm or as a few clusters of actin at the surface of the nuclear envelope (Fig. 3a,c). At that development stage a dense network of microfilaments is observed in basal cell cytoplasm after staining with Alexa Fluor 488-phalloidin. At the micropylar and chalazal ends of the basal cell are clusters of intensely fluorescing microfilaments. Concentrations of F-actin material are also visible near the basal cell nucleus (Fig. 3d).

The next stage of suspensor basal cell development corresponds to the 6–9-cell proembryo. At that stage, actin filaments form a dense network filling the basal cell cytoplasm. Numerous microfilaments are compacted, forming intensely fluorescing aggregates (Fig. 4a). The basal cell nucleus, located centrally in the cell, is significantly enlarged and flattened. Radially oriented microtubules are still present around the nucleus and DAPI staining reveals the polytene character of the nucleus. Microtubule compactations are still visible at the chalazal end of the basal cell (Fig. 4b). Fluorescent staining of F-actin reveals intensely glowing microfilaments arranged mainly transversely and obliquely to the micropylar-chalazal axis of the basal cell, and a cortical network of actin filaments in proembryo cells (Fig. 4c).

#### 10–20-CELL EMBRYO (FULL DEVELOPMENT OF SUSPENSOR BASAL CELL)

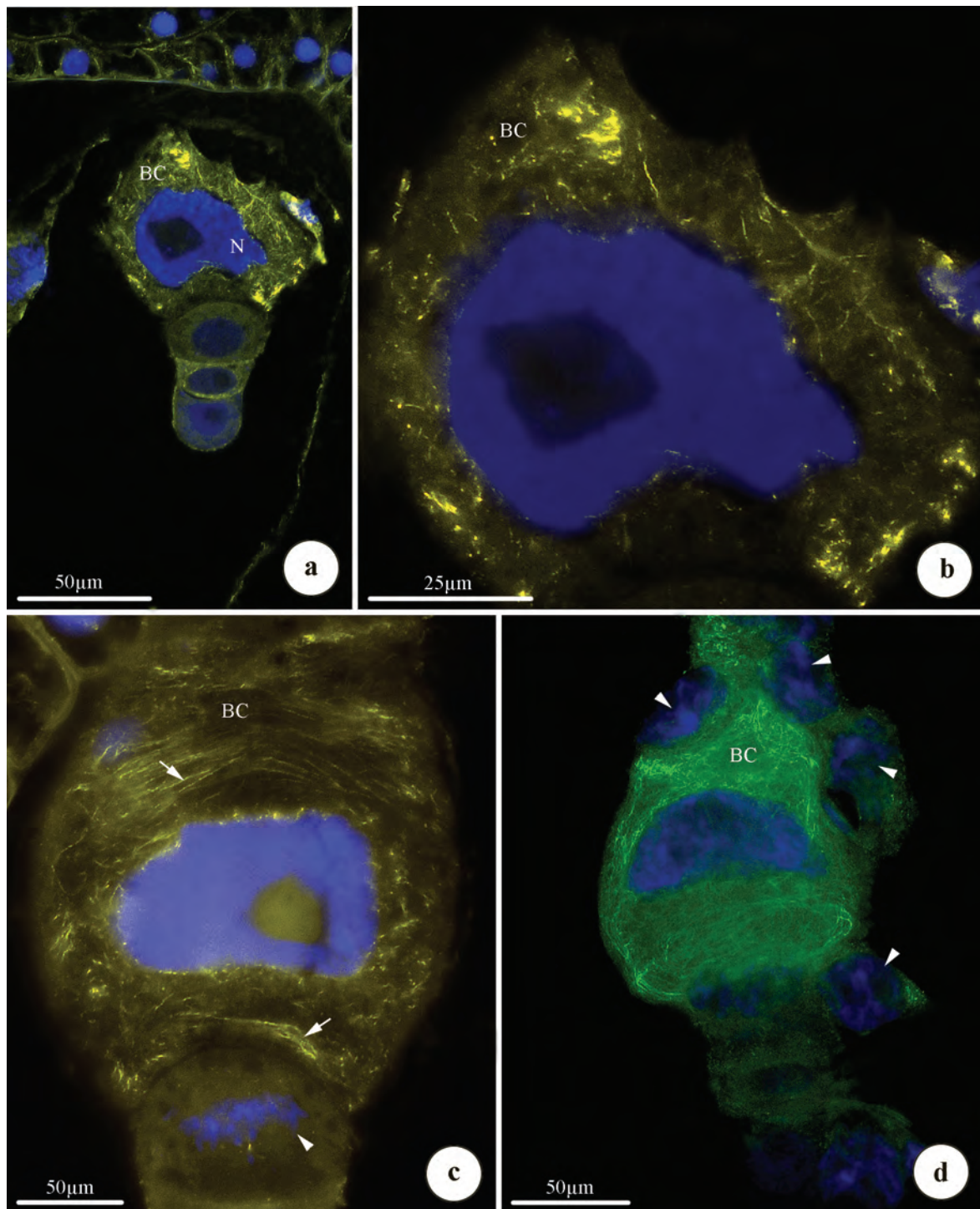
In the 10–20-cell embryo the suspensor basal cell is no longer enlarging and the embryo proper consists of 10 to 20 cells. The fully formed suspensor consists of the basal cell and several chalazal suspensor cells (Fig. 5a). Immunolocalization of the tubulin skeleton in the suspensor basal cell reveals a few microtubules irregularly distributed in the cell cytoplasm. As in the previous stage, microtubules are present mainly at the chalazal end of the basal cell as weakly glowing foci of tubulin material. Increased cytoplasmic vacuolization is noted in the suspensor basal cell. The chalazal suspensor cells show numerous microtubules (Fig. 5b). After Alexa Fluor 488-phalloidin staining the actin skeleton shows abundant microfilaments forming a network uniformly filling the basal cell cytoplasm (Fig. 5c). F-actin concentrations are localized near the nuclear envelope and in the cytoplasm (Fig. 5c). There are numerous microfilaments in the cytoplasm, oriented transversely to the micropylar-chalazal axis of the basal cell (Fig. 5c,d). Some microfilament bundles form wide rings arranged around the micropylar-chalazal axis of the basal cells (Fig. 5c). Microfilament foci also appear at the chalazal end of the basal cell (Fig. 5c,d). The actin cytoskeleton in the chalazal suspensor and embryo proper cells takes the form of a cortical network (Fig. 5c,d).

## DISCUSSION

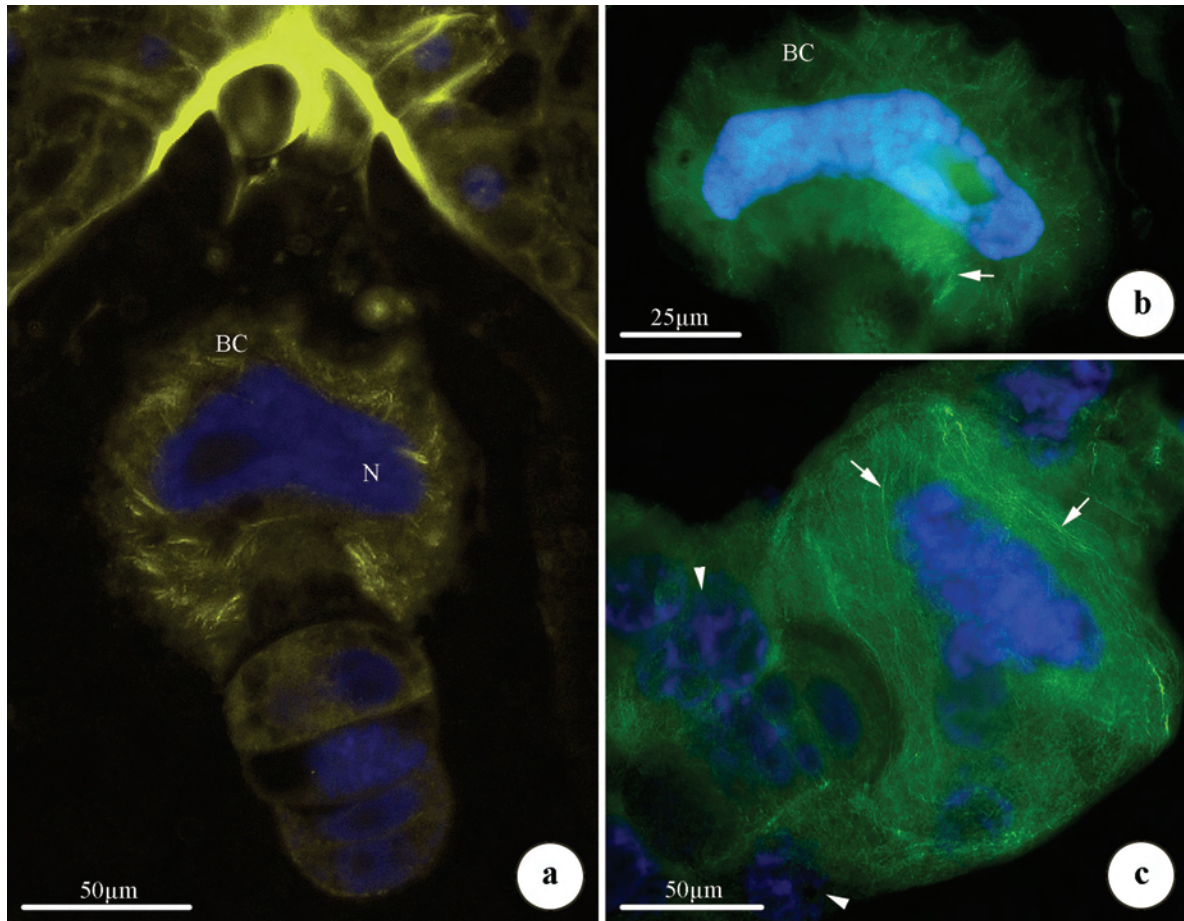
The embryo in many flowering plant species is differentiated into two parts showing different development patterns: one is the embryo proper, in which organogenesis occurs, and the other is the embryo suspensor, a fast-growing, short-lived organ which holds the embryo inside the embryo sac (for review see Yeung and Meinke, 1993). The suspensor differs in size and structure between species. There are suspenders consisting of only a single cell (e.g., in *Phaius tankervilleae*, Yeet et al., 1997) or hundreds of cells (e.g., in *Phaseolus coccineus*, Yeung and Meinke, 1993). In *Gagea lutea* the suspensor consists of a large endopolyploid basal cell and several chalazal suspensor cells (Kozieradzka-Kiszkurno et al., 2007).

In many species, DNA endoreduplication takes place during differentiation of the suspensor cells, leading to high ploidy (Brodsky and Uryvaeva, 1985; Kozieradzka-Kiszkurno and Bohdanowicz, 2003). Endoreduplication is a form of nuclear polyploidization by which cells gain additional copies of genomic DNA. It usually occurs in tissues and organs that actively function for only a short period of ontogenetic development, such as antipodals, the embryo





**Fig. 3.** Four-cell proembryo of *Gagea lutea*. **(a, b, c)** Immunostained microfilaments (yellow), **(d)** F-actin localized with Alexa Fluor 488-phalloidin assay (green), nuclei stained with DAPI (blue). **(a)** Longitudinal section showing 4-cell proembryo. Enlarged nucleus (N) and network of microfilaments visible in basal cell (BC). Fine microfilament network visible in proembryo cells, **(b)** Magnified fragment of Fig. 3a showing enlarged basal cell (BC). Immunolocalization of F-actin skeleton reveals compactness of actin filaments forming an abundant network, **(c)** Basal cell (BC), microfilament bundles arranged around nucleus. Some microfilaments oriented transversely to micropylar-chalazal axis of cell (arrows). Mitotic division (arrowhead) visible in proembryo cell, **(d)** Clusters of intensely fluorescing microfilaments in basal cell (BC) cytoplasm. At micropylar and chalazal ends, microfilaments oriented in different directions with respect to micropylar-chalazal axis of basal cell. Microfilament network dominates in proembryo cells, and nuclei of the endosperm also visible (arrowheads).



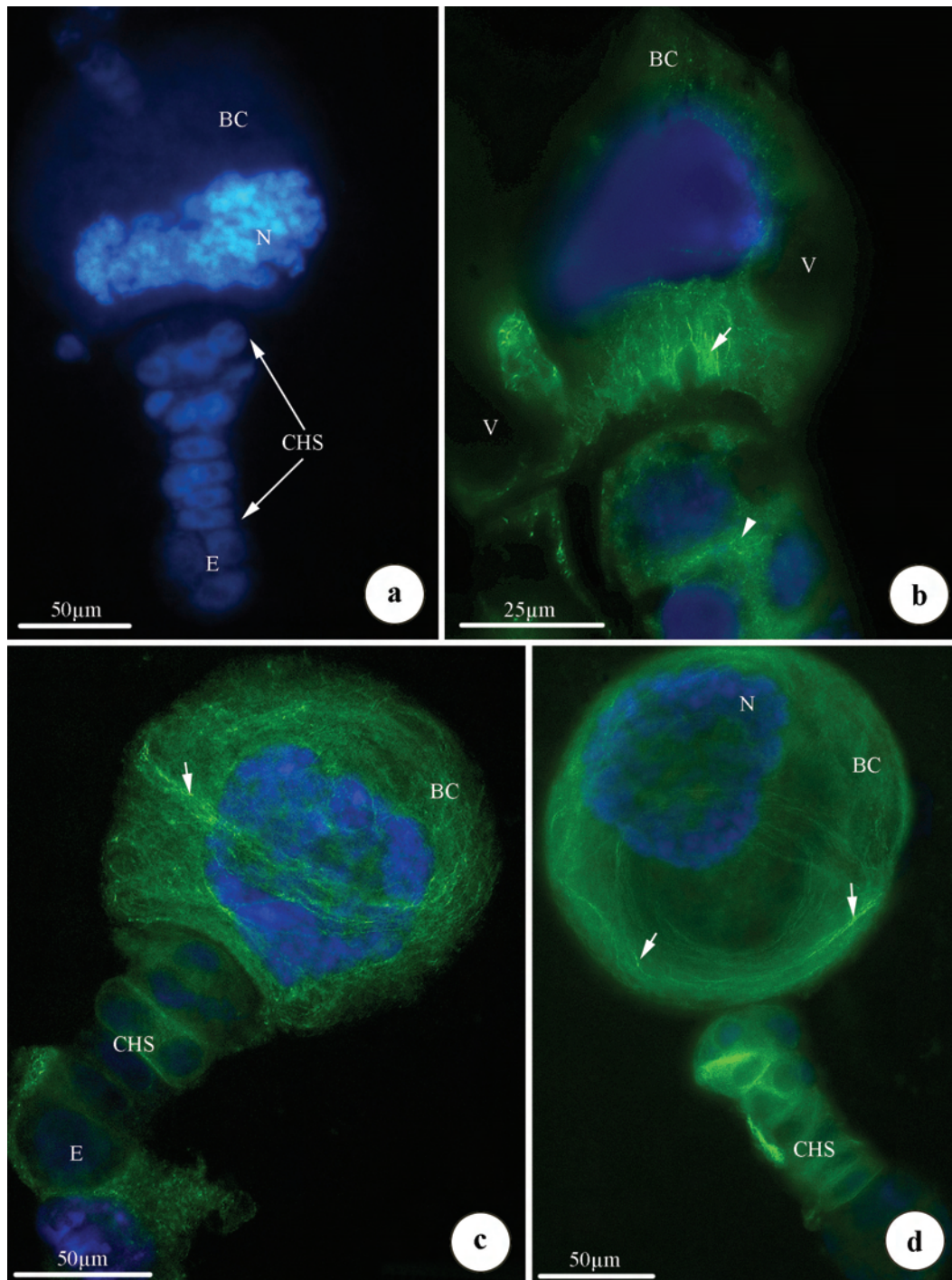
**Fig. 4.** Six-cell proembryo of *Gagea lutea*. **(a)** Immunostained microfilaments (yellow), **(b)** Immunostained microtubules (green), **(c)** F-actin localized with Alexa Fluor 488-phalloidin assay (green); nuclei stained with DAPI (blue). **(a)** Longitudinal section showing 6-cell proembryo. Enlarged nucleus (N) and microfilament network visible in basal cell (BC). Fine microfilament network visible in proembryo cells. **(b)** Radially oriented microtubules around nucleus of basal cell (BC). Intensely fluorescing microtubule bundles at chalazal end of basal cell (arrow), **(c)** F-actin forming numerous bundles transversely and obliquely oriented versus micropylar-chalazal axis of basal cell (arrows). Arrowheads indicate nuclei of endosperm.

suspensor, synergids, and also endosperm haustoria (Nagl, 1990). Reaching a high degree of endopolyploidy apparently increases the metabolic capacity of such cells. It is a normal and genetically determined mechanism of differentiation of these cells, and probably a better solution in the economy of a cell at a certain stage of development and differentiation (Joubes and Chevalier, 2000).

There have been many studies of the cytochemistry, ultrastructure and biochemistry of embryo suspensors in many plant species (Yeung and Clutter, 1979; Singh et al., 1980; Picciarelli et al., 1991; Lee et al., 2006; Kozieradzka-Kiszkurno et al., 2012; Kozieradzka-Kiszkurno and Płachno, 2013). They have shown that the suspensor is actively involved in the uptake and transport of nutrients and regulators from surrounding tissues

to the embryo proper, and that it is a source of signals or a mediator of signals affecting the course of embryogenesis (Yeung and Clutter, 1979; Bohdanowicz, 1987, 2001). There is not so much published work reporting observations of the cytoplasmic skeleton in differentiating cells of embryo suspensors (Webb and Gunning, 1991; Ye et al., 1997; Huang et al., 1998; Tung et al., 2000), and only a few reports describe the cytoskeleton in highly endopolyploid embryo suspensor cells (e.g., in *Alisma plantago-aquatica*, Świerczyńska and Bohdanowicz, 2007, 2009; in *Sedum acre*, Kozieradzka-Kiszkurno et al., 2011). To understand the role of the cytoskeleton in the development of highly endopolyploid plant cells it is necessary to know the distribution of the actin and tubulin skeleton during the differentiation of these cells.





**Fig. 5.** *Gagea lutea*, stage of full embryo suspensor development. (a) Embryo suspensor and embryo, (b) Immunostained microtubules (green), (c, d) F-actin localized with Alexa Fluor 488-phalloidin assay (green), nuclei stained with DAPI (blue). (a) DAPI staining shows ~20-cell embryo, basal cell (BC) with chalazal suspensor cells (CHS) and embryo proper (E). Enlarged and lobed nucleus (N) visible in basal cell, (b) Immunoassay of tubulin skeleton reveals microtubule clusters at chalazal end of basal cell (BC). Microtubules run parallel to micropylar-chalazal axis of basal cell (arrow). Large vacuoles (V) visible in basal cell (BC) cytoplasm. Network of microtubules present in chalazal suspensor cells (arrowhead), (c) Basal cell (BC) with chalazal suspensor cells (CHS) and embryo proper (E). Dense microfilament network visible in suspensor basal cell. F-actin bundles oriented mainly transversely and obliquely to micropylar-chalazal axis of basal cell (BC) (arrow), (d) Basal cell (BC) with chalazal suspensor cells (CHS). Large polytene nucleus (N) and numerous F-actin bundles becoming denser at chalazal end of basal cell (arrows). Actin filaments form cortical network in chalazal suspensor cells.



The suspensor, especially the suspensor basal cell in *G. lutea*, clearly precedes the development of the embryo proper. Basal cell development begins with accelerated growth, followed by nucleus endopolyploidization, cytoplasm expansion, and the formation of transfer walls, as well as the stage of full development. The occurrence of an abundant tubulin and actin cytoskeleton is observed in the micropylar region of the cell near the contact surface with nucellus cells throughout basal cell development. Cytoskeletal compactations at the micropylar end have been observed in the embryo suspensor in the Nun orchid (*Phaius tankervilleae*) (Ye et al., 1997), in endopolyploid suspensor basal cells in *Alisma plantago-aquatica* (Świerczyńska and Bohdanowicz, 2007, 2009), in *Sedum acre* (Kozieradzka-Kiszkurno et al., 2011), in the syncytium of *Utricularia* (Płachno et al., 2010, 2012), as well as at the chalazal end in the endosperm haustorium of *Rhinanthus serotinus* (Świerczyńska and Bohdanowicz, 2003; Świerczyńska et al., 2013). We observed numerous bundles of microtubules and microfilaments in the cytoplasm of the basal cell in *G. lutea* near the chalazal wall separating the basal cell from chalazal suspensor cells. Supporting this finding, ultrastructural studies (Bohdanowicz, 2001) revealed that both the micropylar and chalazal walls in the basal cell of *G. lutea* have a transfer function and produce ramifying wall ingrowths. A large amount of cytoskeleton material, particularly microtubules, has been observed at the chalazal end in the suspensor basal cell of *Sedum acre* (Kozieradzka-Kiszkurno et al., 2011), and F-actin foci in the endosperm haustorium of *Rhinanthus serotinus* (Świerczyńska and Bohdanowicz, 2003; Świerczyńska et al., 2013). Perhaps the presence of microtubule and microfilament compactations in the micropylar and chalazal regions of the basal cell in *G. lutea* are associated with intense short-distance transport of solutes in transfer cells (Gunning and Pate 1969, 1974; McCurdy et al., 2008). It is well known that the presence of the transfer wall increases the contact surface of the plasmalemma with the external environment, providing more efficient uptake and transport of nutrients from ovule tissues to the embryo proper, as previously shown in numerous species of flowering plants (Gunning and Pate, 1974; Yeung and Clutter, 1979; Talbot et al., 2002; Offler et al., 2003). The arrangement of cytoskeletal elements along the micropylar-chalazal axis of the suspensor basal cell in *G. lutea* may indicate that microtubules and microfilaments are intracellular transport paths for organelles, macromolecules and growth regulators; and the cytoskeleton, especially the actin filaments, may affect the location of vacuoles (Boevink et al., 1998; Kandasamy and Meagher, 1999; Higaki et al.,

2006; Dhonukshe et al., 2008; Kadota et al., 2009). Probably the cytoplasmic skeleton components also regulate the movement of secretory vesicles carrying material for building the transfer walls, playing a cleanup role in the organization of the cytoplasm in transfer cells such as the suspensor basal cell of *G. lutea* (Tegeder et al., 2000; Thompson et al., 2001). In the suspensor basal cell of *G. lutea* we observed numerous microtubules forming radially oriented bundles, apparently radiating from the nucleus surface, as well as microtubule compactations at the surface of the nucleus envelope near the nucleus. In a number of plant species the occurrence of perinuclear radial microtubule systems has been described in division cycles of free-nuclear endosperm (Brown et al., 2003; Nguyen et al., 2001, 2002; Olsen, 2001). The perinuclear radial microtubule system and the microfilament concentrations observed at the nuclear surface of the *G. lutea* basal cell apparently are able to position the huge endopolyploid nucleus that occupies the central part of that large cell. The described configurations of the cytoskeleton probably are also involved in maintenance of the characteristic shape of the basal cell nucleus. Similar relationships have been observed in angiosperms in the endosperm (Huang et al., 1998), syncytia (Płachno et al., 2010, 2012), large polyploid cells such as the embryo suspensor (Świerczyńska and Bohdanowicz, 2007, 2009; Kozieradzka-Kiszkurno et al., 2011) and endosperm haustoria (Płachno et al., 2012; Świerczyńska et al., 2013). Disturbances of radial perinuclear microtubule systems as well as increasing vacuolization of the cell were observed in older suspensor basal cells of *G. lutea*. There is also progressive development of the endosperm, which in a later stage of development can take over the nutritive functions of the developing embryo. We suggest that at that stage the suspensor basal cell probably is beginning to dismantle the tubulin skeleton, a sign of cell degeneration. Further observations are needed to confirm this in *G. lutea*. The organization of microtubules and microfilaments in the chalazal suspensor and embryo proper cells of *G. lutea* seems typical for intensively mitotically dividing cells, as previously described in flowering plant species (Zee et al., 1995; Fu et al., 2000; Ye et al., 2000; Olsen, 2001; Nguyen et al., 2001, 2002; Świerczyńska and Bohdanowicz, 2007, 2009; Kozieradzka-Kiszkurno et al., 2011; Świerczyńska et al., 2013).

Our observations revealed the presence of an abundant cytoplasmic skeleton in the endopolyploid suspensor basal cell of *Gagea lutea*. The cytoskeleton formed unusual configurations not described before in the highly endopolyploid embryonic suspensor cells of different angiosperms. Most

likely the cytoskeleton is involved in positioning the huge nucleus of the basal cell and probably mediates macromolecule transport from the nucleus to the developing embryo proper.

## AUTHORS' CONTRIBUTIONS

JŚ, JB study conception and design; JŚ acquisition of data; JŚ, JB analysis and interpretation of data, JŚ drafting of manuscript; JB critical revision of manuscript.

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