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ENDOREDUPLICATION IN THE SUSPENSOR OF *GAGEA LUTEA* (L.) KER GAWL. (LILIACEAE)

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Karyological processes of differentiation of the suspensor of *Gagea lutea* (L.) Ker Gawl. were compared with the development of the embryo proper. The zygote divides into the smaller apical cell and the bigger basal cell, which becomes the basal cell of the suspensor. The mature suspensor consists of a huge basal cell and a few chalazal cells. The nuclear DNA content of the suspensor basal cell attains a high degree of ploidy, up to 128C. Nuclei with the highest ploidy level of 128C were found only in fully differentiated basal cells of more than 20-celled embryos. During polyploidization, the volume of the nuclei increased, and changes in the chromatin structure of polyploid nuclei were noted. With increasing levels of ploidy, polytene chromosomes were observed in the suspensor nucleus. Changes in DNA content, nucleus size and chromatin structure point to endoreduplication as the mechanism of polyploidization of the suspensor in *Gagea lutea*.

Key words: *Gagea lutea*, DNA cytophotometry, endoreduplication, polytene chromosomes, suspensor, basal cell.

INTRODUCTION

In the majority of flowering plants, the zygote usually divides transversely to form an apical cell and a basal cell, which forms the body of the embryo suspensor. The suspensor is an embryonic organ and is essential to embryo development (for review: Yeung and Meinke, 1993). Earlier 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). Numerous investigations of the cytochemistry (Pritchard and Bergstresser, 1969; Avanzi et al., 1970; Kozieradzka-Kiszkurno and Bohdanowicz, 2006), ultrastructure (Cluter and Sussex, 1968; Bohdanowicz, 1987) and biochemistry of the suspensor (Brady, 1973; Cremonini and Cionini, 1977; Singh et al., 1980) have drawn attention to the probable function of the suspensor in absorption, synthesis and/or translocation of nutrients from maternal tissue to the embryo proper (Natesh and Rau, 1984). In a number of plants, multiplication of nuclear DNA content and polytenization of chromosomes are often associated with differentiation of the suspensor. The most common mode of polyploidization in the suspensor is endoreduplication. During endoreduplication, cells undergo repeated rounds of DNA synthesis without mitosis, resulting in cells with multiple ploidy levels. Most examples of endopolyploidy have been described in specific cell types such as endosperm cells (Kowles et al., 1990) and embryo suspensor cells (D'Amato, 1984). A high degree of ploidy during suspensor differentiation has been reported: *Phaseolus coccineus* 8192C (Brady, 1973), *Phaseolus hysterinus* 4096n (Nagl, 1974), *Tropaeolum majus* 2048C (Nagl, 1976), *Alisma plantago-aquatica* 1024n (Bohdanowicz, 1973) and *Sedum acre* 1024C (Kozieradzka-Kiszkurno and Bohdanowicz, 2003). Polytene chromosomes have been observed in highly specialized and synthetically active cells (Nagl, 1981; Brodsky and Uryvaeva, 1985). The presence of polytene chromosomes has been reported in only a few plant species (for review: Carvalheira, 2000). In this paper we present the results of karyological and cytophotometric studies on the participation of polyploidization processes in differentiation of the embryo suspensor in *Gagea lutea* (L). Ker Gawl.

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MATERIALS AND METHODS

The present work used several plants of *Gagea lutea* (Liliaceae) growing in natural habitats of Gdańsk in northern Poland. Flowers in various developmental stages were fixed for 4 h in 1:3 acetic ethanol at room temperature and stored in 75% ethanol at 4°C. Ovules were excised from ovaries and hydrolyzed for 1 h in 4 N HCl at 20°C. Then the ovules were stained by the Feulgen method. Basal cells of the suspensor and embryo proper were isolated from ovules and squashed in a drop of 45% (v/v) acetic acid. After freezing, the coverslips were quickly removed and the slides were air-dried and embedded in Euparal.

The nuclear DNA content of 100 nuclei from suspensor basal cells was measured using an Amplival Photometrie MFV 4001 cytophotometer. The wavelength used for the Feulgen absorption measurements was the green line (546 nm). Measurements were made at 100× magnification using a scan spot size of 2 (0.4 μm). The DNA content values were calculated based on cytophotometric measurements and expressed in arbitrary units. The 2C and 4C values were established based on measurement of DNA content in telophasic and prophasic nuclei of cells from the embryo proper.

The structure of nuclei was examined in preparations stained with acetocarmine and with the fluorochrome 4',6'-diamidino–2-phenylindole dihydrochloride (DAPI).

TABLE 1. Nuclear DNA content of suspensor basal cell of *Gagea lutea* (L.) Ker Gawl.

Nuclear DNA content min. – max. (arbitrary units)	Degree of ploidy	No. and % of nuclei
531 – 1002	4C	5
1081 – 1884	8C	8
1955 – 2251	8 – 16C	5
2435 – 3609	16C	21
3854 –4216	16 – 32C	6
4303 –7201	32C	24
7365 –7690	32 – 64C	6
8566 – 12935	64C	18
13332 – 15395	64 – 128C	3
19041 – 19893	128C	4
TOTAL		100

TABLE 2. Relation between developmental stage of embryo (number of cells) and of suspensor basal cell (degree of ploidy) of *Gagea lutea* (L.) Ker Gawl.

No. of embryo cells	2C	4C	8C	16C	32C	64C	128C
1–5	–	5	2	3	4	–	–
6–10	–	–	6	14	8	12	–
11–20	–	–	–	4	12	6	–
21–30	–	–	–	–	-	-	4

RESULTS

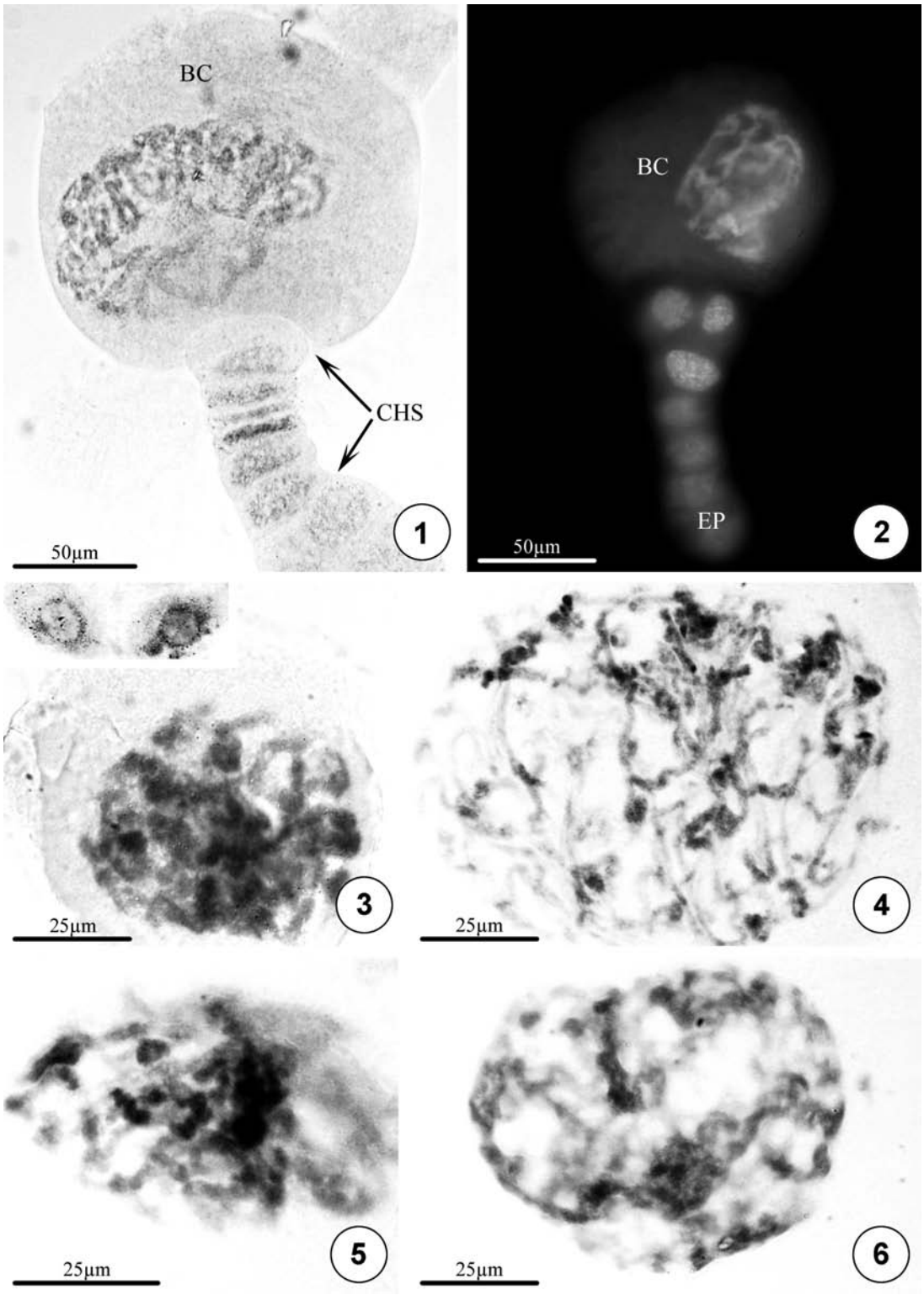
After the first division of the zygote in *Gagea lutea*, two cells are formed: a large basal cell and a smaller apical one. The apical cell divides several times and develops into the embryo proper and chalazal suspensor, whereas the basal cell undergoes no division, becomes much enlarged, and forms the basal suspensor cell.

The mature suspensor consists of a large basal cell and a few chalazal cells. The fully differentiated basal cell is nearly spherical and contains a conspicuous nucleus, situated centrally (Figs. 1, 2). The karyological differentiation of the suspensor was compared with the development of the embryo proper.

The results of nuclear DNA content measurements (Tab. 1) permitted us to establish six classes of nuclei (4C, 8C, 16C, 32C, 64C, 128C) and four intermediate ploidy levels (8C–16C, 16C–32C, 32C–64C, 64C–128C) in suspensor basal cells.

Ploidy level 4C was found in the basal cell of very young 1–5-celled embryos. Higher ploidy levels (from 8C to 64C) were noted in suspensor basal cells of 6–20-celled embryos. The highest ploidy level (128C) was observed only in the basal cell of more-than–20-celled embryos (Tab. 2). The most common were nuclei with DNA content of 32C (24%). In the examined material there were no nuclei with DNA content corresponding to 2C. This suggests that polyploidization started soon after the formation of the basal cell. The suspensor undergoes degeneration when the embryo exceeds 30 cells.

The chromatin structure in diploid cells of the *G. lutea* embryo proper was classified as chromomeric with small chromocenters (Fig. 3, inset). Changes in chromatin structure were observed during multiplication of the nuclear DNA content of the suspensor basal cells. Polytene chromosomes were formed in nuclei of basal cells with DNA content of 16C–32C (Figs. 3, 4). In nuclei at higher (64C) and the highest (128C) ploidy levels, polytene chromosomes were enlarged (Figs. 5, 6). The cells of the chalazal suspensor undergo no polyploidization and their nuclei remain similar to those of the embryo proper. All observed changes in nuclear DNA content, size of nuclei and the presence of polytene chromosomes point to endoreduplication as the mechanism of polyploidization in suspensor basal cells of *Gagea lutea*.



Figs. 1–6. *Gagea lutea*. **Fig. 1.** Acetocarmine-stained squash preparation of suspensor basal cell (BC) and chalazal suspensor (CHS). **Fig. 2.** DAPI staining shows embryo proper (EP) and enlarged basal cell (BC). **Figs. 3–6.** Polyploid nuclei from suspensor basal cells at different levels of ploidy. **Fig. 3.** 16C. Inset: chromocentric diploid nuclei of embryo proper. **Fig. 4.** 32C. Polytene chromosomes enlarge with increasing level of ploidy. **Fig. 5.** 64C. **Fig. 6.** The nucleus at the highest level of ploidy (128C).

DISCUSSION

The suspensor is the first differentiated structure produced by the developing embryo. In most species of angiosperms, the suspensor plays an active role in supporting early development of the embryo proper by providing nutrients and growth regulators (Yeung and Sussex, 1979; Yeung, 1980). The suspensor later undergoes programmed cell death (Natesh and Rau, 1984; Raghavan, 1986; Schwartz et al., 1997).

An interesting feature of many plant species is that suspensor basal cell differentiation is accompanied by polyploidization. Polyploid cells are characteristic for secretory and/or nutritional organs limited to ovary tissues (antipodals, synergids, endosperm, embryo suspensor) (Nagl, 1978; Brodsky and Uryvaeva, 1985). Endoreduplication is the most common mechanism leading to the highest level of ploidy in suspensor cells (for review: D'Amato, 1984). In various angiosperms, high levels of ploidy in the embryo suspensor have been observed: for example, 4096n in *Phaseolus vulgaris* (Nagl, 1974), 1024n in *Alisma plantago-aquatica* (Bohdanowicz, 1973) and 1024C in *Sedum acre* (Kozieradzka-Kiszkurno and Bohdanowicz, 2003). Multiplication of genome number in the nucleus of a highly endopolyploid cell usually leads to a proportionate increase in its physiological activity (D'Amato, 1989; Joubes and Chevalier, 2000).

The first karyological anatomy investigations in the suspensor of *G. lutea*, performed by Hasitschka-Jenschke, (1962), established that the cytodifferentiation of suspensor cells is accompanied by endopolyploidization. Our cytological observations in *G. lutea* confirm that the nuclear DNA content of the suspensor basal cell reaches a maximum level of 128C when the embryo proper has from 21 to 30 cells. Analysis of DNA content in suspensor nuclei of *G. lutea* revealed that they may undergo up to six cycles of endoreplication.

Numerous karyological investigations have established that in many plant taxa the differentiation of suspensor cells is accompanied by endopolyploidization of their nuclei, or much more rarely by the formation of restitution nuclei (for review: D'Amato, 1984). The highly endopolyploid nuclei resulting from suspensor differentiation are characteristic for many plant species. For example, in *Alisma lanceolatum*, *Potamogeton densus* (Hasitschka-Jenschke, 1959) the nucleus of the suspensor basal cell attains a ploidy level of 128n, in *Triglochin maritimum* 256C (Łuszczek et al., 2000), in *T. palustre* 256C (Kozieradzka-Kiszkurno et al., 2002), and in *Alisma plantago-aquatica* 1024n (Bohdanowicz, 1973). Endoreduplication may lead to the formation of polytene chromosomes in highly polyploid, terminally differentiated cells. This kind of chromosome has been reported in basal cells of the suspensor in *Potamogeton densus* (Hasitschka-Jenschke, 1959), *Gagea lutea* (Hasitschka-Jenschke, 1962), *Phaseolus coccineus*, *P. hysterinus* (Nagl, 1974) and *Tropaeolum majus* (Nagl, 1976, 1981; for review: Carvalheira, 2000). In suspensor nuclei of *G. lutea*, polytene chromosomes are formed. The formation of polytene chromosomes starts during early stages of development (16C). By contrast, Hasitschka-Jenschke (1962) observed polytene chromosomes in the suspensor of *G. lutea* only at higher ploidy levels (128C). In our study, polytene chromosomes were arranged evenly in the suspensor nuclei, as similarly observed by Hasitschka-Jenschke (1962). The chromatin structure of suspensor nuclei in *G. lutea* does not reveal puffs or clearly banded structures as has been observed in basal cells. Polytene chromosomes with puffs and a clearly banded structure have been observed in basal cells of the suspensor in *Potamogeton densus* (Hasitschka-Jenschke, 1959), *Phaseolus coccineus* and *P. hysterinus* (Nagl, 1974).

In *G. lutea* the suspensor precedes the embryo proper as well as the endosperm in development. In earlier stages of embryogenesis the suspensor probably takes part in nutrition of the embryo, supplanting in this function the poorly developed endosperm. A similar relationship between the suspensor and the endosperm was observed by Pritchard (1964) in *Stellaria media*, by Schulz and Jensen (1969) in *Capsella bursa-pastoris*, and by Kozieradzka-Kiszkurno and Bohdanowicz (2002) in *Triglochin palustre*. Extensive cytochemical and ultrastructural studies of the embryo suspensor suggest its role in synthesis and/or specialization in active transport (Nagl, 1990). The occurrence of wall labyrinths and protuberances in the suspensor has been described in numerous reports: for example, in *Phaseolus coccineus* (Clutter and Sussex, 1968), *Capsella bursa-pastoris* (Schulz and Jensen, 1969), *Stellaria media* (Newcomb and Fowke, 1974), *Tropaeolum majus* (Nagl, 1976), *Alisma plantago-aquatica* and *Alisma lanceolatum* (Bohdanowicz, 1987), and *Sedum acre* (Kozieradzka-Kiszkurno and Bohdanowicz, 2006). Wall ingrowths have been observed in cells presumed to have a role in absorption and translocation of metabolites from maternal tissues to the embryo proper. Gunning and Pate (1969, 1974) stated that the projections multiply the absorptive surface of the plasma membrane in transfer cells. Ultrastructurally, the micropylar part of the suspensor basal cell in *G. lutea* is covered with wall ingrowths typical of transfer cells (Bohdanowicz, 2001).

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