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SEDUM ACRE EMBRYOGENESIS: POLYPLOIDIZATION IN THE SUSPENSOR

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Cytological processes of differentiation in the embryo suspensor of *Sedum acre* L. were compared with the development of the embryo proper. The zygote undergoes an asymmetric division to produce an apical cell and a basal cell, which becomes the basal cell of the suspensor. The mature differentiated suspensor consists of a large haustorial basal cell and 3–4 chalazal cells. The basal cell nucleus gradually grows to a considerable size, and the amount of nuclear DNA also increases. The highest degree of ploidy (1024C) was observed in basal cells in large >100-celled embryos. Chromocenters at low (8C–16C) and middle (32C–64C) levels of ploidy, and endochromocenters at higher (128C–256C) and the highest (512C–1024C) levels of ploidy were observed. Changes in DNA content, nucleus size and chromatin structure point to endoreduplication as the mechanism of polyploidization of the suspensor in *Sedum acre*.

Key words: *Sedum acre*, DNA cytophotometry, endoreduplication, polyploidization, suspensor, basal cell.

INTRODUCTION

In the majority of flowering plants, the first division of the zygote is transverse and often asymmetrical, producing a large basal cell and a smaller apical cell. The apical cell produces mainly the embryo proper, whereas the basal cell usually forms the body of the embryo suspensor.

Angiosperm suspensors vary widely in size and morphology from a single cell to a massive column of over 100 cells (Maheshwari, 1950; Yeung, 1980; Lersten, 1983). In most cases the suspensor is short-lived and enters a process of degeneration during a predestined stage in embryo development.

The functional role of the suspensor was long thought to be limited to mechanically pushing the embryo into the nutrient endosperm (Maheshwari, 1950). It now appears that the suspensor in flowering plants is an embryonic organ essential to embryo development (for review: Yeung and Meinke, 1993).

Extensive cytochemical (Pritchard and Bergstresser, 1969; Avanzi et al., 1970; Bohdanowicz, 1987), ultrastructural (Clutter and Sussex, 1968; Bohdanowicz, 1987) and biochemical studies (Brady, 1973; Cremonini and Cionini, 1977; Singh et al., 1980) with a variety of angiosperms have shown the suspensor to play an active role early in development by promoting continued growth of the embryo proper.

In many angiosperms a high degree of ploidy during suspensor differentiation has been observed: *Phaseolus coccineus* 8192C (Brady, 1973), *Phaseolus hysterinus* 4096*n* (Nagl, 1974), *Tropaeolum majus* 2048C (Nagl, 1976), *Alisma plantago-aquatica* 1024*n* (Bohdanowicz, 1973), and *Triglochin palustre* 256C (Kozieradzka-Kiszkurno et al., 2002).

Multiplication of nuclear DNA content is one of the most common processes connected with cell differentiation in plants. Processes of polyploidization leading to particularly high levels of polyploidy are

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characteristic of trophic and secretory cells or tissues (Brodsky and Uryvaeva, 1985).

The formation of a huge haustorial suspensor also accompanies embryogenesis in *Sedum acre* and some other genera of Crassulaceae (Mauritzon, 1933).

The suspensor of *S. acre* was the main object of our recent developmental, ultrastructural and cytochemical studies (unpubl. data). This paper presents the results of karyological and cytophotometric studies on the participation of polyploidization processes in differentiation of the embryo suspensor in *Sedum acre*.

MATERIALS AND METHODS

The study materials were collected from plants of *Sedum acre* L. (Crassulaceae) growing in natural habitats of Gdańsk and Gdynia in northern Poland. Inflorescences 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 isolated from the pistils under a stereoscopic microscope were hydrolyzed for 1 h in 4 N HCl at 20°C and stained by the Feulgen method. The basal cells and embryo proper were isolated from the ovules. Squash preparations were made by the dry ice method, dehydrated in ethanol and embedded in Euparal.

Nuclear DNA content of 127 nuclei from basal cells was measured with an Amplival Photometric MFV 4001 cytophotometer. The 2C and 4C values were established from measurements of DNA content in telophasic and prophasic nuclei of cells from the embryo proper. Nucleus structure was examined in preparations stained with acetocarmine or with the fluorochrome 4',6-diamidino-2-phenylindole (DAPI).

RESULTS

After the first division of the zygote in *Sedum acre*, two cells of unequal size are formed: a large basal cell and a smaller apical one. The basal cell undergoes no division, becomes much enlarged, and produces haustorial branches invading the micropyle and adjacent tissues, and protruding out of the ovule. The apical cell develops into the embryo proper and chalazal suspensor.

The mature suspensor consists of a large pear-shaped basal cell and a few chalazal cells. A single huge and lobed nucleus is situated in the central part of the basal cell. The cytological differentiation of the suspensor was studied in comparison with the development of embryo proper, beginning with 5–10-celled ones; manual isolation of younger embryos was unsuccessful.

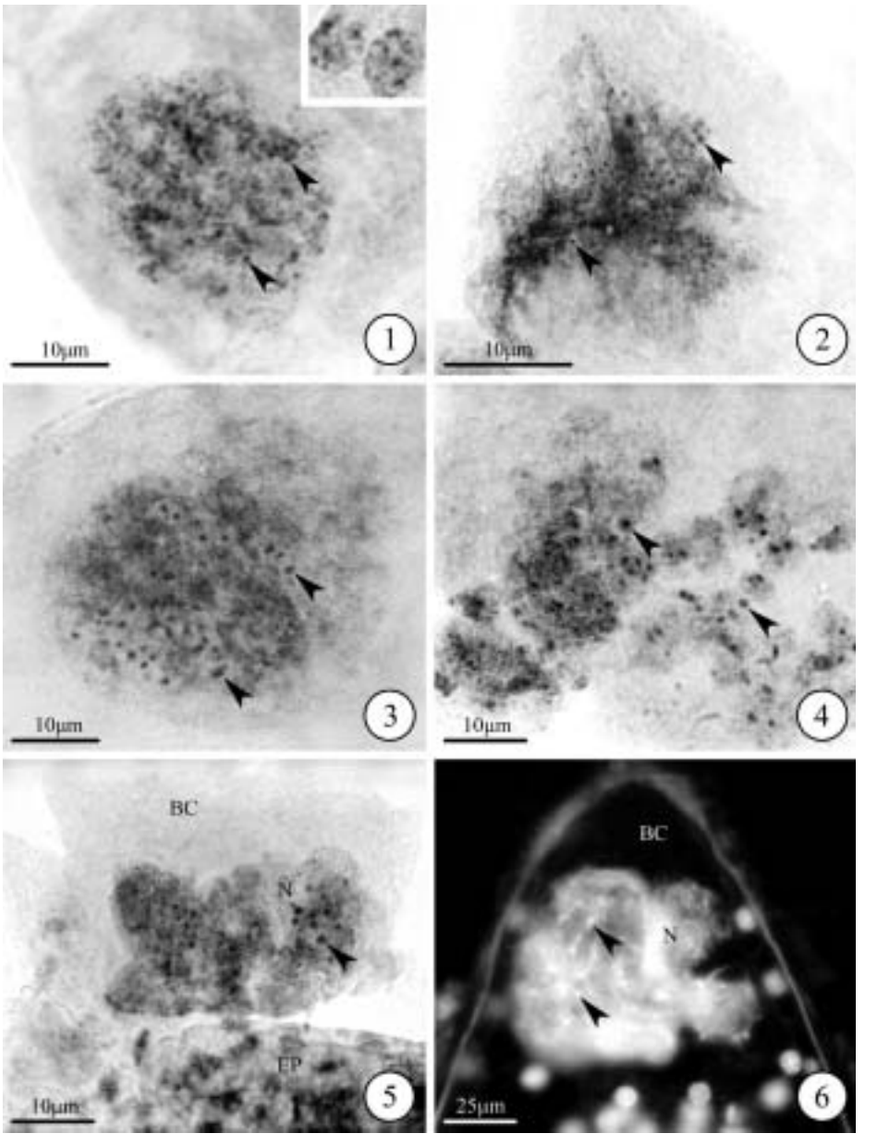
TABLE 1. Nuclear DNA content of suspensor basal cell in *Sedum acre* L.; (%)

Nuclear DNA content min. – max. (arbitrary units)	Degree of ploidy	No. of nuclei
298	8C	1 [0.8]
355	8C – 16C	1 [0.8]
444 – 622	16C	3 [2.4]
674 – 816	16C – 32C	4 [3.1]
883 – 1168	32C	2 [1.6]
1308 – 1687	32C – 64C	5 [3.9]
1952 – 2262	64C	7 [5.5]
2448 – 3326	64C – 128C	10 [7.9]
3529 – 4797	128C	12 [9.4]
5005 – 6708	128C – 256C	17 [13.4]
6853 – 9510	256C	15 [11.8]
9762 – 13512	256C – 512C	23 [18.1]
13780 – 19068	512C	16 [12.6]
19466 – 26424	512C – 1024C	9 [7.1]
28522	1024C	2 [1.6]
Total		127[100.0]

The nuclear DNA content of the suspensor basal cell was measured cytophotometrically after Feulgen staining. Analysis of the measurements established the degrees of polyploidy attained by the basal cell. Eight classes of nuclei (8C, 16C, 32C, 64C, 128C, 256C, 512C, 1024C) and 7 intermediate ploidy levels (8C–16C, 16C–32C, 32C–64C, 64C–128C, 128C–256C, 256C–512C, 512C–1024C) were found in suspensor basal cells (Tab. 1).

The development of the suspensor precedes that of the embryo proper (Tab. 2). Polyploidization of the basal cell proceeded very quickly: ploidy levels (from 8C to 256C) were observed in very young 5–10-celled embryos. The highest ploidy levels (512C and 1024C) were noted only in suspensor basal cells in large >100-celled embryos. The most common were nuclei with DNA content of 512C (12.6%).

The cells of the chalazal suspensor undergo no polyploidization and their nuclei remain similar to the embryo proper. Diploid nuclei of the embryo proper were classified as chromocentric (Fig. 1, inset). A very similar structure characterizes suspensor nuclei at lower (8C, 16C) and middle degrees (32C, 64C) of ploidy. The chromocenters in the nuclei in basal cells with DNA content of 128C–1024C were



Figs. 1–6. *Sedum acre*. Polyploid nuclei from suspensor basal cells at different levels of ploidy; some endochromocenters labelled with arrowheads. **Fig. 1.** 128C; endochromocenters visible. Inset: chromocentric diploid nuclei of an embryo proper. **Fig. 2.** 256C. **Fig. 3.** 512C. **Fig. 4.** Part of nucleus at the highest level of ploidy (1024C); note the increased size of endochromocenters, basically unchanged in number. **Fig. 5.** Acetocarmine-stained squash preparation of suspensor basal cell (BC) and part of embryo proper (EP). **Fig. 6.** DAPI staining shows a huge nucleus (N) located centrally in the basal cell (BC); endochromocenters visible inside the nucleus.

enlarged in length and diameter and formed endochromocenters (Figs. 1–4).

Analysis of the nuclei of suspensor basal cells stained with acetocarmine (Fig. 5) and with DAPI (Fig. 6) confirms the similarity to the general chromatin structure. During polyploidization the volume of the nucleus and nucleoli increased and endochromocenters were observed.

DISCUSSION

In the majority of angiosperm taxa the embryo suspensor is a fast-growing and short-lived organ. Differentiation of the suspensor cells is frequently accompanied by polyploidization of their nuclei (Nagl, 1962; for review: D'Amato, 1984). Polyploidization is associated with differentiation of some

TABLE 2. Relation between developmental stages of embryo (number of cells) and of suspensor basal cell (degree of ploidy) in *Sedum acre* L.

No. of embryo cells	2C	4C	8C	16C	32C	64C	128C	256C	512C	1024C
5 – 10	–	–	1	2	1	1	1	1	–	–
11 – 20	–	–	–	1	1	3	1	1	1	–
21 – 50	–	–	–	–	–	3	3	2	2	–
51 – 100	–	–	–	–	–	–	2	4	5	–
101 – 500	–	–	–	–	–	–	4	5	4	1
>500	–	–	–	–	–	–	1	2	4	1

secretory and/or nutritive cells and tissues inside the ovule (antipodals, endosperm, synergids, suspensor) (for review: Nagl, 1978). The nuclear DNA content of polyploid cells is sometimes a hundred or a thousand times higher than the DNA content of diploid cells of the same tissue (Nagl, 1978; Brodsky and Uryvaeva, 1985).

In *Sedum acre*, enlargement of the basal cell nucleus is one of the first indications of its specialization. The nucleus of the suspensor basal cell increases its nuclear DNA content and attains a maximum level of 1024C when the embryo proper consists of more than 100 cells. The rhythmic increase of DNA content and nucleus size in *S. acre* basal cells, and the structural changes in their chromatin, suggest that polyploidization of nuclei in suspensor basal cells is due to endoreduplication.

In many plant species, differentiation of the suspensor cells is accompanied by endoreduplication of their nuclei, or less frequently by the formation of restitution nuclei (Nagl, 1962; D'Amato, 1984). High levels of ploidy and endoreduplication as a means of suspensor differentiation are characteristic of many species, for example in Helobiae. In *Alisma lanceolatum*, *Potamogeton densus* (Hasitchka-Jenschke, 1959) and *Echinodorus tenellus* (Nagl, 1962) the nucleus of the basal cell attains a ploidy level of 128*n*, in *Triglochin maritimum* (Łuszczek et al., 2000), and *T. palustre* 256C (Kozieradzka-Kiszkurno et al., 2002), and in *Alisma plantago-aquatica* 1024*n* (Bohdanowicz, 1973).

Endoreduplication may lead to the formation of polytene chromosomes in the suspensors of many plants such as *Potamogeton densus* (Hasitchka-Jenschke, 1959), *Phaseolus coccineus* (Nagl, 1962), *Phaseolus hysterinus* (Nagl, 1974) or *Tropaeolum majus* (Nagl, 1976).

Analysis of nuclear DNA content in the basal cell of *S. acre* suggests that the nucleus may undergo nine cycles of DNA replication. In suspensor nuclei, the chromocenters enlarged and endochromocenters were formed at higher levels of ploidy. The chromatin structure of suspensor nuclei in *Sedum acre* does not reveal such various forms as have been observed in suspensors of other species, for example in *Potamogeton densus* (Hasitchka-Jenschke, 1959), *Alisma plantago-aquatica* (Bohdanowicz, 1973) or *Triglochin maritimum* (Łuszczek et al., 2000). The highly endoreduplicated nuclei of the *Potamogeton* suspensor frequently show polytene chromosomes with a clearly banded structure, while endoreduplicated nuclei of *Alisma* are most frequently characterized by more or less loose chromosome bundling, endochromocenters, or an overall granular structure of their chromatin.

Polyploid and polytene cells have a particular significance for the function of the tissues and organs of which they are an integral part.

Multiplication of genome number in the nucleus of an endopolyploid cell usually leads to a proportionate increase in its synthetic activity (Cremonini and Cionini, 1977; D'Amato, 1989; Nagl, 1990).

Cytochemical and ultrastructural investigations of the suspensor suggest its role in synthesis, or specialization in active transport (Nagl, 1990). The wall ingrowths characteristic of so-called transfer cells occur in the micropylar part of the suspensor basal cell of, for example, *Phaseolus coccineus* (Clutter and Sussex, 1968), *Stellaria media* (Newcomb and Fowke, 1974), *Tropaeolum majus* (Nagl, 1976), *Capsella bursa-pastoris* (Schulz and Jensen, 1969), *Alisma plantago-aquatica* and *Alisma lanceolatum* (Bohdanowicz, 1987), and *Triglochin palustre* (Kozieradzka-Kiszkurno and Bohdanowicz, 2000). Wall ingrowths are characteristic for *Sedum acre* also (unpubl. data).

In *Sedum acre* the suspensor precedes the embryo proper as well as the endosperm in development. This suggests that in the early stages of embryogenesis the suspensor functionally replaces the poorly developed endosperm in nutrition of the embryo proper. Similar observations have been

made in *Tropaeolum majus* (Nagl, 1962), *Capsella bursa-pastoris* (Schulz and Jensen, 1969), *Alisma plantago-aquatica* (Bohdanowicz, 1973) or *Triglochin palustre* (Kozieradzka-Kiszkurno et al., 2002) as well as in *Eruca sativa* embryos in culture in vitro (Corsi, 1972).

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