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POLYPLOIDIZATION OF ENDOSPERM CHALAZAL HAUSTORIUM OF *RHINANTHUS SEROTINUS* (SCROPHULARIACEAE)

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The paper reports a study of karyological differentiation of the chalazal endosperm haustorium of *Rhinanthus serotinus* (Schönheit) Oborny. Polyploidization began soon after the formation of the haustorium cell. The fully differentiated chalazal haustorium is a large elongated cell containing two huge nuclei. The nuclei are situated halfway along the length of the haustorium cell. Measurements of their nuclear DNA content revealed its degree of ploidy, which could attain a maximum 768C. Nuclei with higher (192C–384C) and the highest (768C) levels of ploidy were found in mature chalazal haustorium cells (from 100- to 500-celled endosperm proper). 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 haustorium nuclei. The rhythmical increase of DNA content and changes in nuclei size and chromatin structure point to endoreduplication as the mechanism of polyploidization of the haustorium cell.

Key words: *Rhinanthus serotinus*, DNA cytophotometry, endoreduplication, polyploidization, endosperm, chalazal haustorium.

INTRODUCTION

In flowering plants, the primary endosperm nucleus begins to divide after double fertilization, giving rise to an ephemeral tissue, the endosperm. Three general patterns of endosperm development are recognized: nuclear, cellular and helobial (Maheshwari, 1950; Bhatnagar and Sawhney, 1981; Vijayaraghavan and Prabhakar, 1984). The main function of the endosperm is to transfer nourishment to the embryo during its development (Raghavan, 1976, 1986). In many angiosperms the endosperm produces highly specialized endosperm haustoria, which penetrate the maternal tissues (Johri and Ambegaokar, 1984; Vijayaraghavan and Prabhakar, 1984). The development and function of these intriguing structures have been reported in several studies of seed development (Maheshwari, 1950; Davis, 1966; Chopra and Seth, 1977).

In a number of plants, the multiplication of nuclear DNA content and polytenization of chromosomes are associated with differentiation of endosperm cells. The most common mechanism leading to polyploidization in endosperm is endoreduplication, found in, for example, *Echinocystis lobata* (Turała, 1966) and *Vicia faba* subs. *minor* (Marciniak, 1991). A high degree of ploidy during endosperm haustoria differentiation, 1536n, has been described in *Plantago atrata* (Czapska-Dziekanowska, 1965). Polytene chromosomes have been observed in highly specialized and synthetically active cells which seemed to be restricted to cells of the ovule and immature seed tissue (Nagl, 1981; Brodsky and Uryvaeva, 1985). The formation of polytene chromosomes has been reported in only a few plant species: for example, in the suspensor in *Phaseolus coccineus* (Nagl, 1967) and *Phaseolus vulgaris* (Nagl, 1969), in endosperm in *Bryonia dioica* (Turała-Szybowska, 1974), in *Echinocystis lobata* (Wojciechowska and Olszewska, 2003), and in endosperm haustoria in the genus *Rhinanthus* (Tschermak-Woess, 1957).

In this paper we present observations of karyological processes during chalazal endosperm haustorium differentiation in *Rhinanthus serotinus*.

MATERIALS AND METHODS

The present study used several plants of *Rhinanthus serotinus* (Scrophulariaceae) growing in natural habi-

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tats of Rumia and Puck 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. The endosperm chalazal haustorium and endosperm proper were isolated from the ovules under a stereomicroscope. Squash preparations were made by the dry ice method, dehydrated in an ethanol series, and embedded in Euparal.

The nuclear DNA content of 156 nuclei from chalazal haustorium cells was measured with an Amplival Photometrie MFV 4001 cytophotometer. The 3C and 6C values were established based on measurements of DNA content in telophasic and prophasic nuclei of cells from the endosperm proper.

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

RESULTS

After the first division of the primary endosperm nucleus in *Rhinanthus serotinus*, two cells are formed: the initial chalazal haustorium cell and the micropylar cell. The micropylar cell divides several times and develops into the endosperm proper and the micropylar haustorium, whereas the initial chalazal haustorium cell forms the chalazal haustorium. The fully developed chalazal haustorium consists of a single huge kidney-shaped cell with two large and lobed nuclei. The nuclei are located halfway along the length of the haustorium cell. The enormous growth of the haustorium is no doubt connected with multiplication of the basic nuclear DNA content during its development. The karyological differentiation of the chalazal haustorium was studied at various stages of endosperm development. The results of nuclear DNA content measurements (Tab. 1) permitted us to establish six classes of nuclei (24C, 48C, 96C, 192C, 384C, 768C) and five intermediate ploidy levels (24C–48C, 48C–96C, 96C–192C, 192C–384C, 384C–768C) in chalazal haustorium cells. Ploidy levels of 24C and 48C were found in haustorium cells of 50- to 100-celled endosperm proper. Higher (96C, 192C and 384C) and the highest (768C) levels were noted only in chalazal haustorium cells of endosperm consisting of 100 to 500 cells (Tab. 2). The most common were nuclei with levels of 192C (16.1%) and 96C (14.1%). In the examined material there were no nuclei with nuclear DNA content corresponding to 6C and 12C. This suggests that polyploidization started soon after the formation of the haustorium cell. The maximum ploidy reached by chalazal haustorium cells corresponds to the level of 1536C (2×768C). In about 87% of all haustoria investigated, the two sister nuclei attained the same level of ploidy; in 13%, the two sister nuclei attained levels one step apart.

TABLE 1. DNA content of nuclei of chalazal endosperm haustoria of *Rhinanthus serotinus* [%]

Nuclear DNA content min.-max. (arbitrary units)	Degree of ploidy	No. of nuclei
1167 – 1290	24C	2 [1.3]
1329 – 1712	24 – 48C	5 [3.2]
1881 – 2523	48C	15 [9.6]
2589 – 3508	48 – 96C	21 [13.5]
3839 – 5147	96C	22 [14.1]
5248 – 7214	96 – 192C	26 [16.7]
7335 – 10282	192C	25 [16.1]
10580 – 14037	192 – 384C	14 [8.9]
14790 – 20321	384C	15 [9,6]
20909 – 27149	384 – 768C	10 [6.4]
29273	768C	1 [0.6]
TOTAL		156 [100]

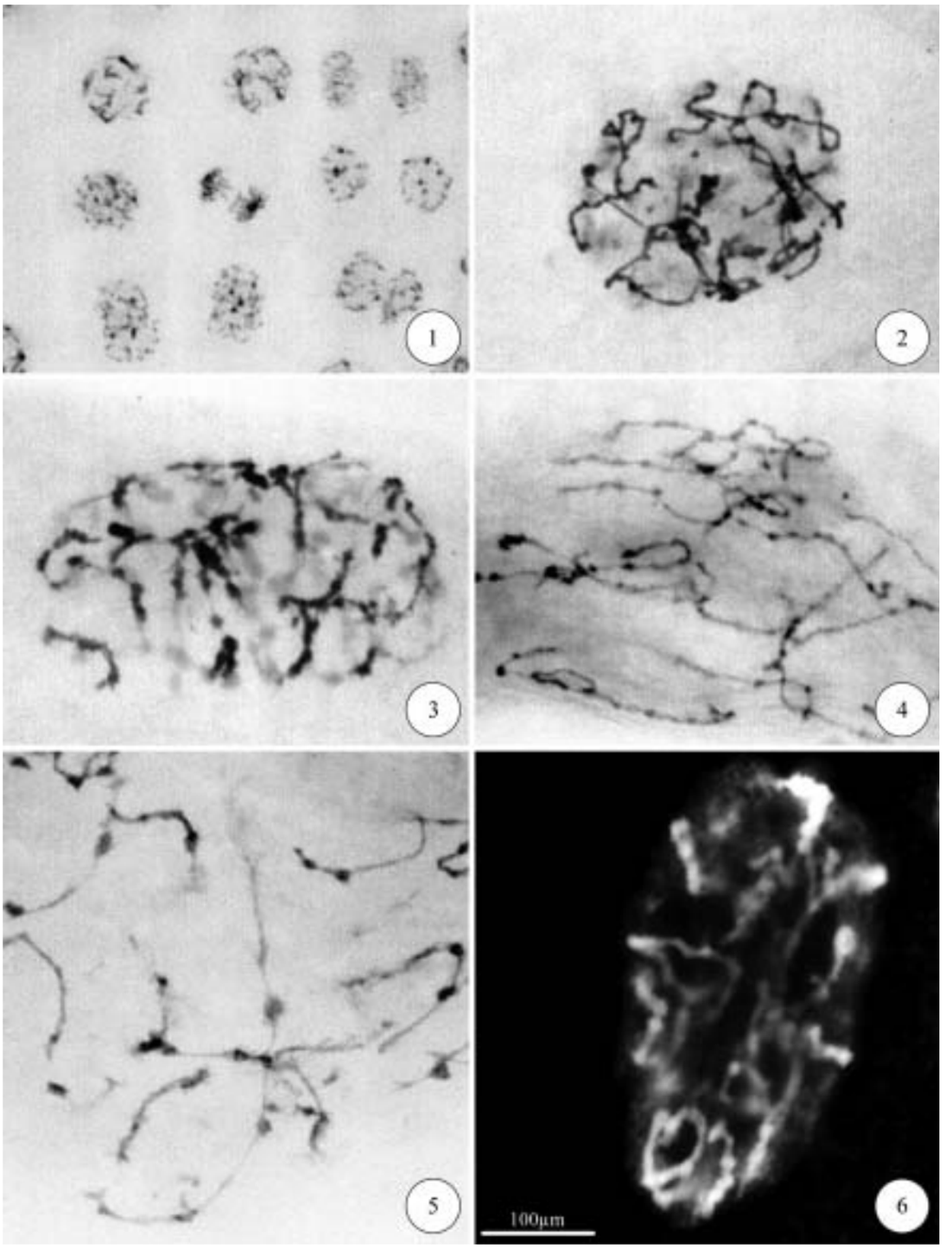
TABLE 2. Relation between developmental stages of endosperm (number of cells) and chalazal haustorium nuclei (degree of ploidy) of *Rhinanthus serotinus*

No. of endosperm cells	3C	6C	12C	24C	48C	96C	192C	384C	768C
50 – 100	-	-	-	2	15	16	-	-	-
101 – 500	-	-	-	-	-	4	16	5	-
> 500	-	-	-	-	-	2	9	10	1

The chromatin structure in cells of the endosperm proper was classified as chromocentric (Fig. 1). Changes in chromatin structure during multiplication of nuclear DNA content of chalazal haustorium were noted. Polytene chromosomes were formed in nuclei of haustorium cells with 24C–96C DNA content (Fig. 2). In nuclei at higher (192C and 384C) and the highest (768C) ploidy levels, polytene chromosomes were enlarged in diameter and length (Figs. 3, 4). Chalazal haustorium nuclei stained with acetocarmine (Fig. 5) and DAPI fluorochrome (Fig. 6) show polytene chromosomes also. 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 the chalazal haustorium of *R. serotinus*.

DISCUSSION

In a number of angiosperm genera, the endosperm produces micropylar and/or chalazal haustoria, which penetrate ovular tissues. Endosperm haustoria are fast-growing, highly specialized and synthetically active organs. In plants, polyploid cells with huge DNA content are characteristic for rapidly growing nutri-



Figs. 1–6. Endosperm chalazal haustorium of *Rhinanthus serotinus*. **Fig. 1.** Chromocentric nuclei of endosperm proper. **Figs. 2–4.** Polyploid nuclei at different levels of ploidy. **Fig. 2.** 96C. **Fig. 3.** 192C; polytene chromosomes enlarge with increasing level of ploidy. **Fig. 4.** The nucleus at a higher level of ploidy (384C). **Fig. 5.** Fragment of huge nucleus at high level of ploidy; acetocarmine-stained squash preparation. **Fig. 6.** DAPI staining shows nucleus of chalazal haustorium cell; polytene chromosomes visible inside nucleus. Scale bar in Fig. 6 also applies to Figs. 1–5.

tional organs limited to ovary tissues (antipodal cells, synergids, endosperm and embryo suspensor cells) (Nagl, 1978; Brodsky and Uryvaeva, 1985). The most common mechanism leading to the highest level of ploidy in endosperm cells is endoreduplication (for review: D'Amato, 1984). Karyological investigations by Tschermak-Woess (1957) and Erbrich (1965) confirm that in some plant species the cytodifferentiation of endosperm haustoria is frequently connected with endopolyploidization of their nuclei. High levels of ploidy in endosperm haustoria have been noted in several angiosperms: for example, 384n in *Phlomis viscosa* (Enzenberg, 1961), 768n in *Bartsia alpina* (Erbrich, 1965), 1536n in *Plantago atrata* (Czapska-Dziekanowska, 1965), and 24576n in *Arum maculatum* (Erbrich, 1965). Cytological investigations in *Rhinanthus serotinus* established that the nuclear DNA content of the haustorium cell reaches the maximum level of 2×768C when the endosperm proper consists of about 500 cells. Analysis of DNA content in endosperm haustorium nuclei of *R. serotinus* reveals that they may undergo up to eight cycles of endoreduplication, that is, one cycle more than in three other species of *Rhinanthus* (*R. alectorolophus*, *R. minor*, *R. aristatus*), which attain a maximum ploidy level of 384n (Tschermak-Woess, 1957). A comparable difference in levels of endomitotic polyploidization within one organ has been observed in, for example, suspensor basal cells of *Phaseolus*: 8192n in *P. coccineus* and 4096n in *P. hysterinus* and *P. vulgaris* (Nagl, 1974). In about 13% of *R. serotinus* haustoria the two sister nuclei exhibit levels of polyploidy one step apart, similarly to other species of *Rhinanthus* (Tschermak-Woess, 1957, 1967).

The occurrence of polytene chromosomes has been noted in highly polyploid, terminally differentiated cells. Polytene chromosomes with puffs and other functional structures have been observed in basal cells of the suspensor in *Phaseolus coccineus* and *P. hysterinus* (Nagl, 1974). In haustorium nuclei of *R. serotinus*, the chromocenters became enlarged and polytene chromosomes finally were formed. The huge haustorium cell in *R. serotinus* displays giant chromosomes generally similar in structure to those in *Tropaeolum majus* (Nagl, 1981) and other species of the genus *Rhinanthus* (Tschermak-Woess, 1957; Nagl, 1992; Bohdanowicz et al., 1993).

Multiplication of genome number in the nucleus of an endopolyploid cell usually leads to a proportionate increase in its physiological activity (D'Amato, 1989; Nagl, 1990; Joubes and Chevalier, 2000; Larkins et al., 2001). Cytochemical and ultrastructural observations of endosperm haustoria confirm their role in synthesis and/or specialization in active transport (Torosian, 1971; Pacini at al., 1975; Bhatnagar and Kallarackal, 1980; Dute and Peterson, 1992; Nagl, 1992; Bohdanowicz et al., 1993; Świerczyńska, 2004). The formation of wall labyrinths and protuberances has been described as an indication of the presence of transfer cells specializing in short-distance, active transport of solutes through the plasma membrane. Amplification of the plasma membrane area is accompanied by increased capacity for uptake of nutrients into the cell (Gunning and Pate, 1969, 1974). The wall ingrowths characteristic of so-called transfer cells have been reported to occur in endosperm haustoria in several plant species: for example, *Lobelia dunni* (Torosian, 1971), *Vaccinium macrocarpum* (Brisson and Peterson, 1975), *Iberis amara* (Vijayaraghavan and Prabhakar, 1984) and *Rhinanthus minor* (Nagl, 1992). Elaborate wall ingrowths and several important ultrastructural features of the chalazal haustorium of *Rhinanthus serotinus* suggest that the cell plays a role in absorption and translocation of metabolites from maternal tissues to the endosperm proper (Bohdanowicz et al., 1993; Świerczyńska, 2004).

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