ACTA BIOLOGICA CRACOVIENSIA Series Botanica 44: 189–193, 2002

POLYPLOIDIZATION IN THE SUSPENSOR OF *TRIGLOCHIN PALUSTRE* L. (JUNCAGINACEAE)

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Received February 19, 2002; revision accepted May 13, 2002

Differentiation of the suspensor basal cell was studied in *Triglochin palustre* (2n = 24). The zygote divides into the smaller apical cell and the bigger basal cell, which becomes the basal cell of the suspensor. The nuclear DNA content of the suspensor basal cell attains a high degree of ploidy, up to 256C. Nuclei with the highest ploidy levels of 128C and 256C were observed in mature basal cells (from 50- to 500-celled embryos). As a result of polyploidization the volume of the nucleus increased and changes in the chromatin structure of polyploid nuclei were noted. Endochromocenters at middle ploidy level and bundle-like aggregations of chromatin at the highest ploidy levels were found. Rhythmic enlargement of the DNA content and nuclear volume of the basal cell, as well as the characteristic structure of its chromatin, point to endoreduplication as the mechanism of polyploidization in the suspensor.

Key words: *Triglochin palustre*, DNA cytophotometry, endoreduplication, suspensor, basal cell.

INTRODUCTION

In many angiosperms the division of the zygote, usually transverse, forms the daughter cells; the chalazal cell is destined to produce the embryo proper, while the micropylar cell produces the basal cell.

Classically, suspensors are rapidly developing and short-lived organs which develop prematurely, but whose cells are not involved in the formation of the seedling (Maheshwari, 1950). Numerous studies suggest that the suspensor functions in synthesis of hormones for the control of embryogenesis and the transfer of nutritive substances from the surrounding tissues to the embryo proper (Maheshwari, 1950; Schulz and Jensen, 1969; Bohdanowicz, 1973; Nagl, 1974; Newcomb, 1974).

An interesting feature of many plant species is that suspensor basal cell differentiation is accompanied by polyploidization (Hasitchka-Jenschke, 1959; Bohdanowicz, 1973). Multiplication of nuclear DNA content and polytenization of chromosomes is often associated with suspensor development. The most common mechanism of polyploidization in the suspensor is endoreduplication; regular formation of restitution nuclei is less frequently observed (e.g., in four species of *Lathyrus*, Nagl, 1962). High degrees of ploidy during suspensor differentiation have been observed in several species of Helobiae. In *Potamogeton densus* the nucleus of the basal cell may attain a ploidy level of 128n (Hasitchka-Jenschke, 1959), in *Echinodorus tenellus* 128n (Nagl, 1962), in *Alisma plantago-aquatica* 1024n (Bohdanowicz, 1973), and in *Triglochin maritimum* 256C (Łuszczek et al., 2000).

This study investigated the karyological differentiation of the suspensor in *Triglochin palustre* L. and compared it with *Triglochin maritimum* L. belonging to the family Juncaginaceae.

MATERIALS AND METHODS

The studied plants originated from natural habitats on the Baltic Sea coast at Mrzezino near Puck.

PL ISSN 0001-5296

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 4N HCL at 20°C and stained by the Feulgen method. The basal cells of the suspensor and embryo proper were isolated from the ovules. Squash preparations were made by the dry ice method. The measurements were made with an Amplival Photometrie 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. The structure of nuclei was examined in preparations stained with acetocarmine and with the fluorochrome 4'6 diamino-2 phenylindole (DAPI).

RESULTS

Chromosome numbers of the plants were determined in squash preparations made from young acetocarmine-stained anthers. In each of these specimens the haploid chromosome number n = 12 (2n = 24) was found. The same chromosome number has been reported for *T. palustre* by Piotrowicz (in: Skalińska et al., 1961).

Transverse division of the zygote in *Triglochin palustre* L. gives rise to the large basal cell and the smaller apical cell. The apical cell develops into the embryo proper and the suspensor stem, whereas the basal cell forms the basal suspensor cell. Suspensor development was investigated in various stages of the embryo proper, beginning with ~20-celled; manual isolation of younger embryos was unsuccessful.

The mature suspensor, consisting of a basal cell and 1–3 cells of the stem, characterizes the embryo proper (> 100-celled). The fully differentiated basal cell is nearly spherical and contains a huge nucleus, located centrally (Figs. 1, 2).

The results of nuclear DNA content measurements (Tab. 1) permitted us to establish 5 classes of nuclei in suspensor basal cells and 3 intermediate ploidy levels. The classes correspond to ploidy levels from 16C to 256C.

A ploidy level of 16C was found in the basal cell of ~20-celled embryo proper. Higher (32C, 64C and 128C) and the highest (256C) levels were reached only in the suspensor basal cell in embryos formed of 20 to 500 cells (Tab. 2). The most common were nuclei with DNA content of 64C (17.7%) and 32C (14.4%). Ranges of 32C–64C, 64C–128C and 128C–256C were also found (Tab. 1).

Some changes in nuclear structure accompanied the multiplication of DNA content in the basal cells. Diploid nuclei of the embryo proper were classified as chromocentric (Fig. 3, inset). The chromocenters in the nuclei in basal cells with DNA content of 16C–64C were enlarged in length and diameter and formed endochromocenters (Figs. 3–5). In nuclei at the highest ploidy levels (128C–256C), bundle-like aggregations of chromatin structure were observed (Figs. 6,7). The number of endochromocenters did not change markedly with increasing ploidy level and endochromocenter size.

This study of the nuclear DNA content of polyploid suspensor basal cells reveals their cyclic growth, with the regular spherical or oval shapes of the nuclei retained. All observed changes – the absence of mitoses, rhythmical enlargement of DNA content and the size of the nuclei, and structural changes in chromatin – point to endoreduplication as the mechanism of polyploidization.

DISCUSSION

In many angiosperms the suspensor is an indispensable, rapidly developing, short-lived organ which disappears before the formation of the mature seed (Maheshwari, 1950). Polyploidization is the most common means of cytological differentiation in plant as well as animal cells and tissues during ontogenesis (Brodsky and Uryvaeva, 1985). Karyological investigations by Hasitschka-Jenschke (1959) and Nagl (1962, 1974) established that in many plant species the differentiation of the suspensor cells is accompanied by endopolyploidization. High levels of

Figs. 1–7. *Triglochin palustre*. **Fig. 1.** Acetocarmine-stained squash preparation of suspensor basal cell (BC) and 2-celled stem (S) with huge nucleus and nucleolus (Nu) situated centrally. **Fig. 2**. DAPI staining shows globular embryo proper (EP) with enlarged basal cell (BC). Inset: endochromocenters clearly visible. **Figs. 3–7**. Polyploid nuclei from suspensor basal cells at different levels of ploidy. **Fig. 3**. 16C; endochromocenters noticeable. Inset: chromocentric, diploid nuclei of embryo proper. **Fig. 4**. 32C. **Fig. 5**. 64C. **Fig. 6**. 128C; size of endochromocenters grows with increasing level of ploidy, whereas their number does not change markedly. **Fig. 7**. Nucleus at highest level of ploidy (256C), with bundle-like aggregations of chromatin. Bar in Figure 3 applies also to Figs. 4–7.

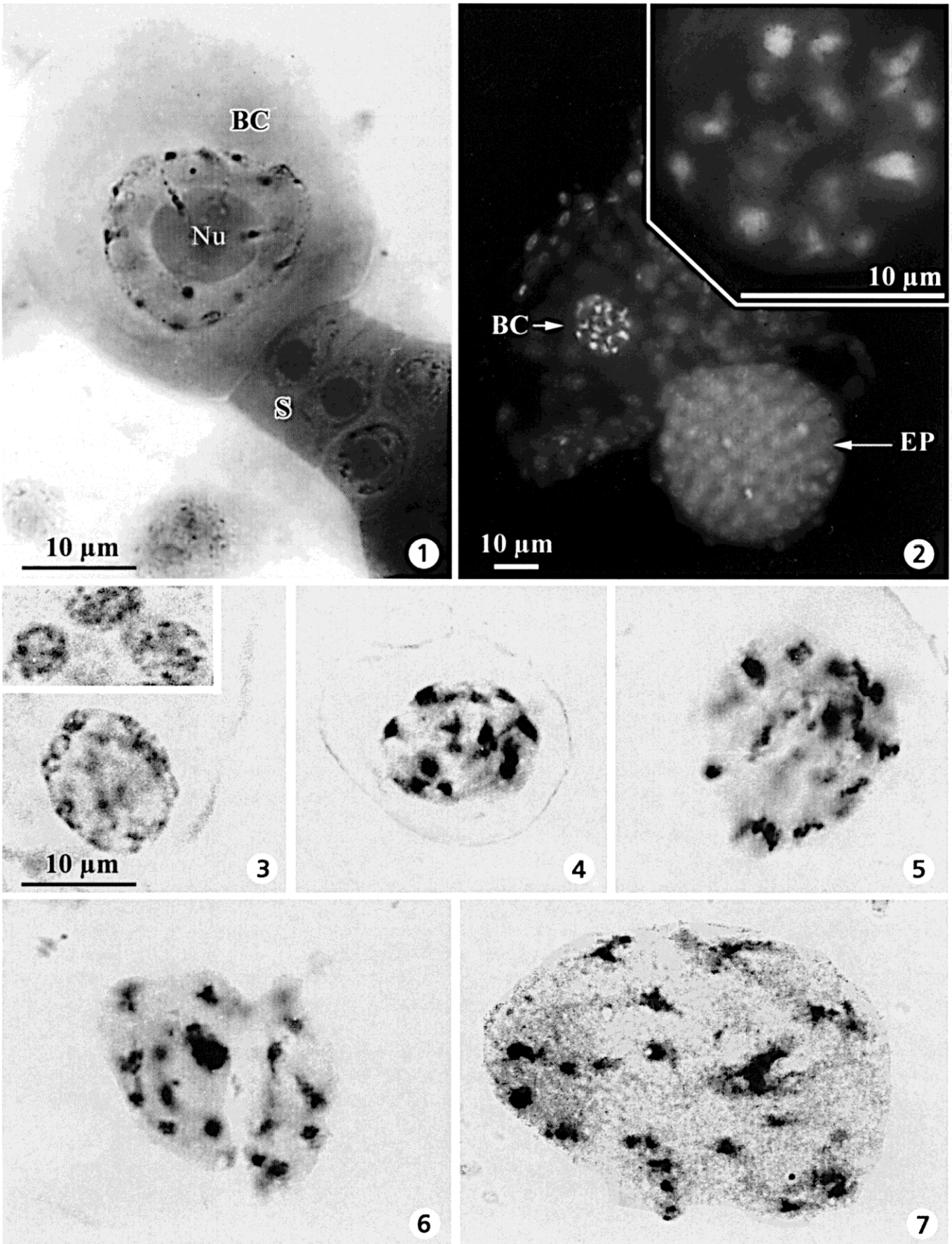
BC
Nu
S
10 µm
1
10 µm
BC
EP
10 µm
2
10 µm
3
4
5
6
7

TABLE 1. Nuclear DNA content of suspensor basal cells in *Triglochin palustre* L.

Nuclear DNA content		No. of nuclei	% of nuclei
min-max (arbitrary units)	C values		
238	16C	1	1.7
421–569	32C	8	14.4
578–790	32C–64C	10	17.7
835–1099	64C	10	17.7
1168–1609	64C–128C	8	14.4
1671–2285	128C	6	10.7
2396–3198	128C–256C	8	14.4
3320–4567	256C	4	7.1

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

No. of embryo cells	Degree of ploidy of suspensor basal cell				
	16C	32C	64C	128C	256C
20–50	1	3	1	–	–
50–100	–	–	2	1	–
100–500	–	3	2	1	1
> 500	–	2	5	4	3

ploidy in the suspensor have been observed in several angiosperms: for example, 4096n in *Phaseolus vulgaris* (Nagl, 1962, 1974), 1024n in *Alisma plantago-aquatica* (Bohdanowicz, 1973) and 256C in *Triglochin maritimum* (Łuszczek et al., 2000). Karyological studies in *Triglochin palustre* reveal that the nuclear DNA content of the suspensor basal cell reaches the maximum level of 256C when the embryo proper consist of at least 100 cells, similarly to the suspensor in *T. maritimum* (Łuszczek et al., 2000). Analysis of nuclear DNA content in the basal cell of *Triglochin* suggests that the nucleus may undergo seven cycles of endoreduplication in *T. palustre* (2n = 24) as well as in *T. maritimum* (2n = 48). Studies on somatic polyploidization in *Arenaria ciliata* L. s.l. (Titz, 1965) suggest that the quantity of endomitotic polyploidization within one tissue is the same in different cytotypes of the same species. On the other hand, different ploidy levels have been observed in basal cells of *Phaseolus*: 8192n in *P. coccineus* and 4096n in *P. hysterinus* and *P. vulgaris* (Nagl, 1974).

Chromatin structure changed during the process of suspensor polyploidization in *T. palustre*. In suspensor nuclei, the chromocenters enlarged and endochromocenters were formed. The presence of bundle-like aggregations of chromatin structure were observed in highly polyploid suspensor nuclei. The changes in chromatin structure in *T. palustre* suspensor nuclei do not present such numerous forms as have been noted in other species of Helobiae, for example in *Alisma lanceolatum* and *Potamogeton densus* (Hasitschka-Jenschke, 1959), *Echinodorus tenellus* (Nagl, 1962) and *A. plantago-aquatica* (Bohdanowicz, 1973). The highly endopolyploid suspensor nuclei in *Potamogeton densus* often reveal polytene chromosomes with a clearly banded structure, while the highly endoreduplicated nuclei of *A. plantago-aquatica* are most frequently presented by more or less loose chromosome bundles.

Ultrastructural and cytochemical studies have yielded possible explanations of the suspensor's role (Pritchard, 1964; Schulz and Jensen, 1968; Avanzi et al., 1970). The role of the suspensor in nutrient synthesis and/or transport to the embryo proper is confirmed by the structure of the basal cell. The lack of mitoses in the basal cell of *T. palustre*, the rhythmical enlargement of nuclear DNA content, and the regular spherical or oval shapes of the nuclei, and changes in chromatin structure, all point to endoreduplication as the mechanism of polyploidization. Multiplication of genome number in the nucleus at high ploidy levels usually leads to a proportionate increase in its synthetic activity (Cremonini and Cionini, 1977; D'Amato, 1989; Nagl, 1990). Comparison of the ploidy levels of the differentiating basal cell in *T. palustre* suggests that endoreduplication starts soon after the cell forms, and precedes the early development of the embryo proper. A relationship between suspensor and embryo development has been described in *Capsella bursa-pastoris* by Schulz and Jensen (1969) and in *A. plantago-aquatica* by Bohdanowicz (1973). Similar results have been obtained from crucifer (*Eruca sativa*) cultured in vitro (Corsi, 1972).

Wall ingrowths and several other structural and ultrastructural features occurring in the suspensor basal cell of *Capsella* (Schulz and Jensen, 1968), *Phaseolus* (Nagl, 1970, 1974), *Stellaria* (Newcomb and Fowke, 1974) and *Alisma* (Bohdanowicz, 1987) suggest that the suspensor may be involved in respiration and in transport of nutrients from maternal tissues to the embryo proper. Ultrastructurally the suspensor basal cell of *T. palustre* looks like a typical transfer cell (Kozieradzka-Kiszkurno and Bohdanowicz, 2000).

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