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POLYPLOIDIZATION OF SUSPENSOR BASAL CELL IN *TRIGLOCHIN MARITIMUM* L. (JUNCAGINACEAE)

DOROTA ŁUSZCZEK, JOANNA ŚWIERCZYŃSKA AND JERZY BOHDANOWICZ*

Department of Genetics and Cytology, University of Gdańsk, ul. Kładki 24, 80–822 Gdańsk, Poland

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Differentiation of the suspensor in *Triglochin maritimum* L. (2n = 48) was studied in comparison with the development of the embryo proper. The zygote divides into the apical cell and the basal cell, which becomes the basal cell of the suspensor. The fully differentiated suspensor consists of 2–3 stem cells and a large basal cell. A single, huge nucleus is situated in the central part of the cell. Measurements of the nuclear DNA content and nuclear volume of the suspensor basal cell indicated its degree of ploidy, which could reach a maximum 256C. Lower ploidy levels – 4C, 8C and 16C – characterize the basal cell of small, 3–10-celled embryos. Nuclei with the highest ploidy levels of 128C and 256C were found only in fully differentiated basal cells of more than 100-celled embryos. During polyploidization there were some changes in the chromatin structure of polyploid nuclei. Chromocenters at low levels of ploidy, endochromocenters at the middle levels, and bundle-like aggregations of chromatin at the highest levels of ploidy were observed. The lack of mitoses, rhythmic enlargement of DNA content and nuclear volume of the basal cell, as well as the characteristic structure of its chromatin point to endoreduplication as a mechanism of polyploidization in the suspensor.

Key words: *Triglochin maritimum* L., DNA cytophotometry, endoreduplication, polyploidization, suspensor, basal cell.

INTRODUCTION

The zygote in angiosperms usually divides transversely to form a basal cell and an apical cell, which gives rise to the embryo proper. The basal cell often creates a complete suspensor or only part of it (e.g., its basal cell). Angiosperm suspensors generally are rapidly developing and short-lived organs, varying widely in size and morphology from a single cell to a massive column of several hundred cells (Yeung and Meinke, 1993). Classically, the suspensor was thought to maintain the embryo proper in a suitable position and to push it into the interior of the embryo sac (Maheshwari, 1950). However, extensive cytochemical, ultrastructural and physiological studies have shown the suspensor to play an active role during development of the embryo proper (Pritchard, 1964; Schulz and Jensen, 1968; Avanzi et al., 1970; Picciarelli et al., 1984). Multiplication of nuclear DNA content and polytenization of chromosomes often accompanies suspensor development. The most common mechanism of polyploidization in the suspensor is endoreduplication. Regular formation of restitution nuclei is observed less frequently (Nagl, 1962, 1981).

High levels of ploidy and endoreduplication as a means of suspensor differentiation are characteristic of many species 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 128n, and in *Alisma plantago-aquatica* (Bohdanowicz, 1973) even as high as 1024n.

This paper presents the results of an investigation of cytological processes during suspensor differentiation in *Triglochin maritimum* L., which also belongs to Helobiae.

*e-mail: jurboh@biotech.univ.gda.pl

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MATERIALS AND METHODS

The examined materials were *T. maritimum* L. (Juncaginaceae) flowers at various stages of their development, originating from natural habitats on the Baltic Sea coast in Mrzezino near Puck. The young and overblown flowers were fixed for 4 h in 1:3 acetic ethanol at room temperature and stored in 75% ethanol at 4°C.

Two different methods were used to determine the ploidy levels of suspensor cells. The earlier stages of the embryo and suspensor development were studied by the volumetric method, and the older ones were examined by the cytophotometric method.

Most of the embryological preparations were made using the paraffin method. Microtome sections 25 μm thick, after deparaffinization and hydration, were stained with acetocarmine, dehydrated and embedded in Euparal. The volumes of nuclei were calculated according to the formula:

$$V = \frac{1}{6} \pi \cdot d_1 \cdot d_2 \cdot d_3$$

where d_1, d_2, d_3 represent the diameters of the nuclei.

Nuclear DNA content was determined after Feulgen staining. Ovules isolated from the pistils were hydrolysed for 1 h in 4N HCl at 20°C and stained with Schiff's reagent for 2 h. Squashed preparations were made from the ovules by the dry ice method, dehydrated in ethanol and embedded in Euparal. All the measurements were performed using an Amplival Photometrie MFV 4001 cytophotometer.

Analysis of nuclear DNA content or nuclear volume in more than fifty telophasic and prophasic nuclei of embryo cells permitted estimation of the 2C = 2n and 4C = 4n values. The nuclear volumes from the 138–248 μm^3 range correspond to the 2n level (telophasic nuclei), and from the 282–498 μm^3 range to the 4n level (prophasic nuclei). On the other hand, the values of DNA content of the telophasic nuclei (2C) ranged from 57 to 84 (in arbitrary units) and of the prophasic nuclei (4C) from 113 to 189 (in arbitrary units). The results from the two methods were consistent.

A few cytotypes of *T. maritimum* have been reported for Poland: 2n = 12, 24, 30 and 48 chromosomes (Piotrowicz in: Skalińska et al., 1961). To determine which cytotype characterizes the studied plants, squashed preparations from young acetocarmine-stained anthers were analyzed. In all these specimens the haploid chromosome number n=24 (2n=48) was found.

RESULTS

DEVELOPMENT OF THE SUSPENSOR

The zygote in *T. maritimum* L. shows the polarity typical for that kind of cell. The nucleus, surrounded by dense cytoplasm, occupies the chalazal pole, and a large vacuole occurs at the micropylar pole. After the first division of the zygote, two cells of unequal size are formed: the large basal cell and the smaller apical one (Fig. 1, inset). The basal cell does not divide further but enlarges considerably and becomes the suspensor basal cell, whereas the apical cell undergoes two more divisions, resulting in the formation of three new cells. The cell adjacent to the suspensor basal cell develops into the suspensor stem. The embryo proper originates from the other two cells (Figs. 1–3).

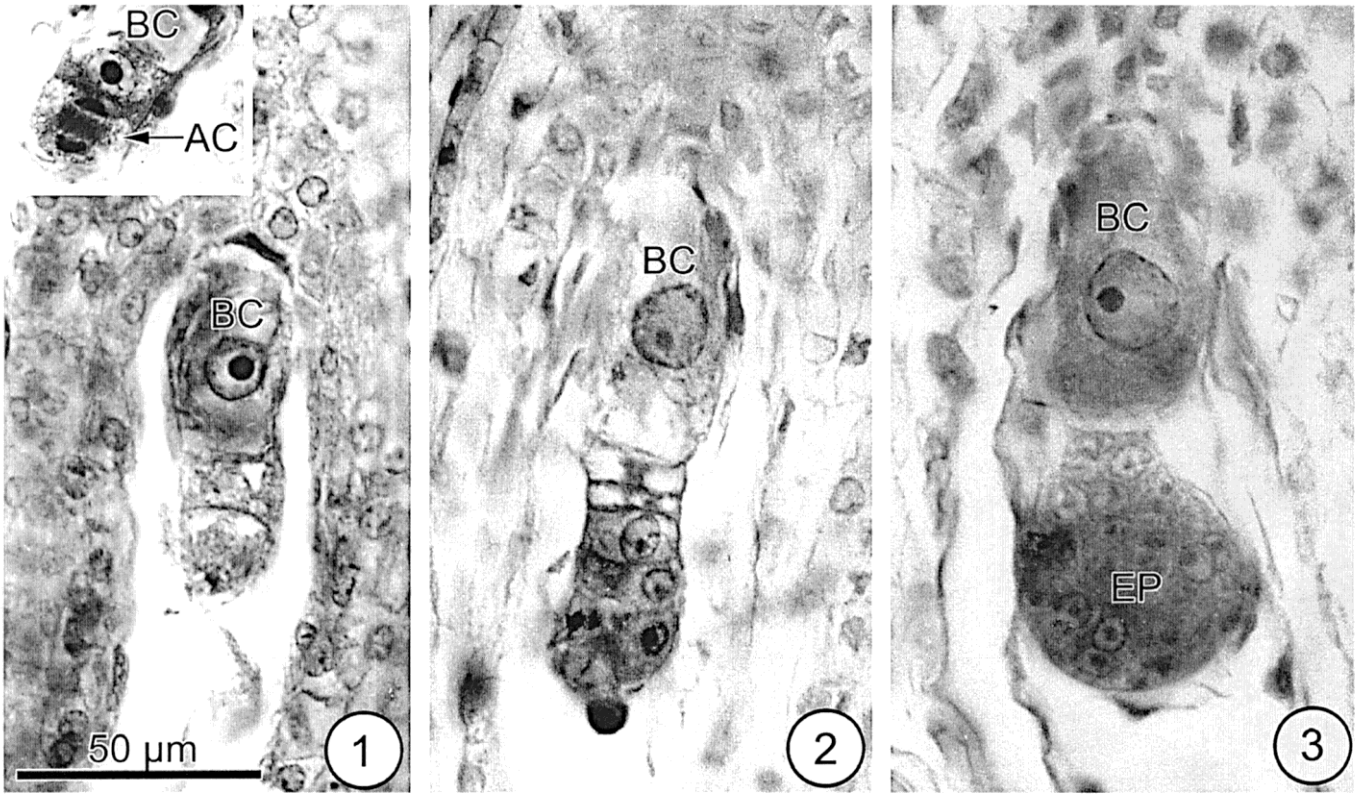
The fully differentiated suspensor consists of a very large basal cell and 2–3 cells of the stem. The suspensor basal cell is generally spherical or ellipsoidal, and its nucleus of appreciable size is situated in the central part (Figs. 2, 3).

POLYPLOIDIZATION OF THE SUSPENSOR BASAL CELL

The process of suspensor basal cell differentiation was investigated in various stages of embryo development. Over two hundred nuclei of suspensor basal cells were analyzed by two different methods – volumetric and cytophotometric. The two methods of ploidy determination produced similar results (see also: D'Amato, 1989).

The initial stages of development of the suspensor basal cell and the embryo proper (up to ~ 50-celled embryos) were studied on 25 μm thick paraffin sections, which preserved complete, undamaged embryos in one slice. For the same reason, further stages (from 50- to ~ 500-celled embryos) were examined mainly in squashed preparations after Feulgen staining. The results of nuclear volumes and DNA content measurements (Tab. 1) permitted seven classes of nuclei to be distinguished, corresponding to levels of ploidy from 4n to 256n.

Ploidy levels of 4n and 8n were found in the basal cells of very small, 2-celled embryos (Tab. 2). On the other hand, in the examined basal cells there were no nuclei with volumes corresponding to 2n. These two facts suggest that basal cell nuclei may undergo two cycles of endopolyploidization at the beginning of embryo development. Moreover, these observations indicate that polyploidization of the suspensor basal cell and the first mitotic division in the apical cell probably begin simultaneously (see



Figs. 1–3. Early development of *Triglochin maritimum* embryo; longitudinal sections of ovules. **Fig. 1.** 3-celled embryo with enlarged basal cell (BC). Inset: 2-celled embryo. Note basal cell (BC) and dividing apical cell (AC). **Fig. 2.** 10-celled embryo with suspensor composed of basal cell (BC) and 2-celled stem. **Fig. 3.** Globular embryo proper (EP) and ellipsoidal suspensor basal cell (BC) with huge nucleus situated in its central part.

Fig. 1). Octoploid nuclei of basal cells were also present in larger embryos, but not in those greater than 10-celled. The greatest variability in the number of cells (from 10 to ~ 100) constituting the embryo corresponds to ploidy levels of 16n and 32n. This suggests that the ploidy levels of 16n and 32n, reached by the basal cell in the early stages of embryogenesis are a prerequisite for further normal development of the embryo.

The higher (64n and 128n) and highest (256n) ploidy levels (Fig. 7) were reached only in suspensor basal cell in very large embryos (formed from 50 to ~ 500 cells). Some nuclei at intermediate ploidy levels (between 64n and 128n) were noted in these embryos. The continuous multiplication of nuclear DNA indicates that endoreduplication takes place in the differentiated basal cell as well.

During multiplication of DNA content in the suspensor basal cell, some changes in its chromatin structure were observed. The chromatin structure in diploid cells of the *T. maritimum* embryo could be classified as chromocentric (Fig. 7, inset). A very similar structure characterizes suspensor nuclei at lower degrees of ploidy (4n and 8n). Nuclei at the middle levels of ploidy (16n and 32n) reveal a distinct chromatin structure. The chromocenters have grown in length and diameter, forming endochromocenters with short aggregations of chromatin visible at their ends (Figs. 4, 5). In nuclei at the highest ploidy levels (from 64n to 256n), further enlargement of endochromocenters is observed (Figs. 6, 7). Evidently the endochromocenters have evolved into elongated, bundle-like aggregations of chromatin scattered throughout the nucleus. Numerous chromatin threads radiating out of these aggregations form a net regularly distributed inside the nuclei (Fig. 7). At the same time, with increasing ploidy levels and chromocenter size, the number of chromocenters does not change significantly. The cells of the suspensor stem undergo no polyploidization. Their nuclei remain similar to embryo proper nuclei.

Analysis of the data on nuclear volume and DNA content of polyploid suspensor nuclei reveals their rhythmical growth, with the regular spherical or oval shapes of the nuclei retained. All the de-

TABLE 1. Nuclear volume and DNA content of suspensor basal cells in *Triglochin maritimum* L.

Degree of ploidy established by:		Volume of nuclei min – max (μm^3)	Nuclear DNA content min – max (arbitrary units)	Number of nuclei analysed by:	
Volumetric method	Cytophotometric method			Volumetric method	Cytophotometric method
4n	4C	448 – 493		3	
8n	8C	568 – 980		19	
16n	16C	1118 – 1999	503 – 683	33	2
16n – 32n	16C – 32C	2014 – 2090	757 – 792	6	2
32n	32C	2248 – 3955	877	75	1
32n – 64n	32C – 64C	4062 – 4323	1542 – 1635	3	2
64n	64C	4502 – 7300	1893 – 2720	34	9
64n – 128n	64C – 128C		2810 – 3885		10
128n	128C	10804 – 13911	3902 – 5312	2	10
128n – 256n	128C – 256C		6541 – 7384		2
256n	256C		8006 – 8012		2

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

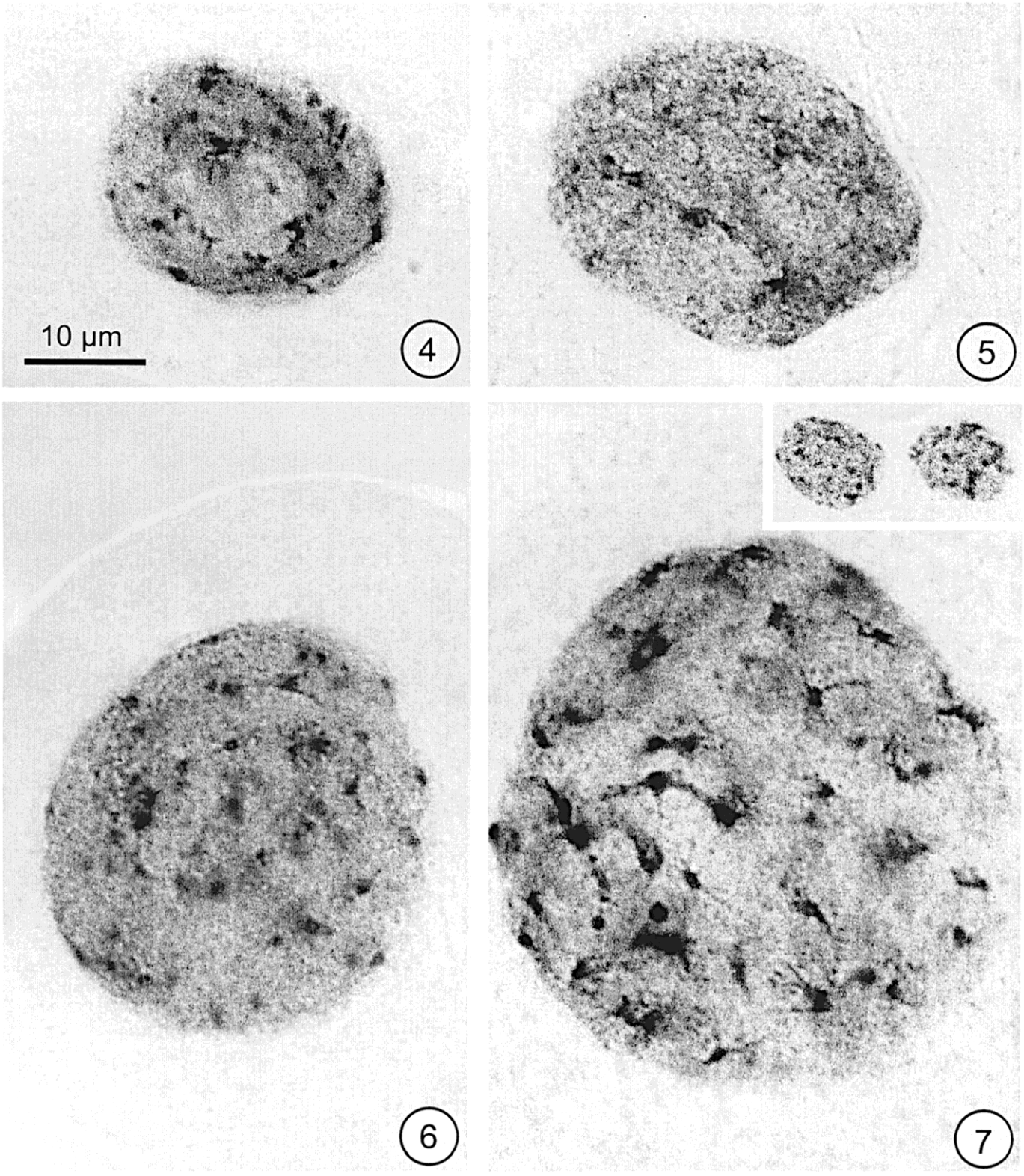
Number of embryo cells	Degree of ploidy of suspensor basal cell							
	2n	4n	8n	16n	32n	64n	128n	256n
2	–	1	2	–	–	–	–	–
3 – 4	–	2	3	–	–	–	–	–
5 – 10	–	–	11	10	1	–	–	–
11 – 20	–	–	–	10	2	–	–	–
21 – 50	–	–	–	9	2	–	–	–
50 – 100	–	–	–	1	69	34	2	–
100 – 500	–	–	–	1	1	6	2	–
>500	–	–	–	–	–	3	8	2

scribed changes in chromatin structure also indicate an increase in the amount of DNA in the nuclei. Neither endomitoses nor other processes causing similar effects were observed. These observations lead to the conclusion that polyploidization of nuclei in suspensor basal cell occurs due to endoreduplication.

DISCUSSION

Development of the suspensor, a short-living organ, in association with the embryo proper, is a very common though not universal feature of embryogenesis in angiosperms. Numerous karyological investigations have established that in many plant species the differentiation of suspensor cells is accompanied by endopolyploidization of their nuclei, or much more rarely by the formation of restitution nuclei (Nagl, 1962; for review: D'Amato, 1984). Endoreduplication also seems to be the most common mechanism of suspensor differentiation within Helobiae. In *Alisma lanceolatum* and *Potamogeton densus* (Hasitchka-Jenschke, 1959), *Echinodorus tenellus* (Nagl 1962) and *Alisma plantago-aquatica* (Bohdanowicz, 1973), the basal cell, which originates from unequal transverse division of the zygote, grows directly into a large suspensor basal cell. Its nucleus undergoes endoreduplications and reaches a maximum ploidy level of 128n in *A. lanceolatum* and *Potamogeton densus*, and even 1024n in *A. plantago-aquatica*. Suspensor development in *T. maritimum* L. proceeds very similarly to that in other species of Helobiae described earlier. After the first unequal zygotic division, the basal cell increases only in size and becomes the suspensor basal cell. At the same time, the apical cell divides twice more and forms the initial cells of suspensor stem and embryo proper. The suspensor basal cell nucleus rhythmically increases its nuclear volume and DNA content and attains a maximal ploidy level of 256n.

The highly polyploid nuclei of *Potamogeton densus* suspensor frequently show polytene chromosomes with a clearly banded structure (Hasitchka-Jensch-



Figs. 4–7. *Triglochin maritimum*. Polyploid nuclei from suspensor basal cells at different levels of ploidy. **Fig. 4.** 16C. Endochromocenters clearly visible. **Fig. 5.** 32C. **Fig. 6.** 64C. Size of endochromocenters increases with increasing ploidy level, whereas number of endochromocenters undergoes no significant changes. **Fig. 7.** Nucleus at the highest level of ploidy (256C), with bundle-like aggregations of chromatin. Inset: chromocentric, diploid nuclei of an embryo proper.

ke, 1959), whereas the higher ploidy nuclei of *Alisma plantago-aquatica* suspensor are characterized first of all by more or less compact chromatin bundles (Bohdanowicz, 1973). The chromatin structure of suspensor nuclei in *T. maritimum* does not reveal such various forms as have been observed in other species of Helobiae. Only the presence of endochromocenters and elongated, bundle-like aggregations of chromatin were noted, in polyploid and highly polyploid nuclei of the basal suspensor cell, respectively.

Both in *T. maritimum* or in other species of Helobiae studied up to now, the nuclei of the suspensor stem generally are similar to the embryo proper nuclei, and they undergo no polyploidization. In various angiosperms the degree of ploidy usually increases toward the base of the suspensor, reaching up to 8192C in the basal cells of *Phaseolus coccineus* (Brady, 1973) and 4096C in *Phaseolus vulgaris* (Nagl, 1974). 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, 1990a). Very extensive studies of the suspensor in *Phaseolus* by many researchers (Avanzi et al., 1970; Nagl, 1970; Cremonini and Cionini, 1977) using many different techniques reveal high synthetic activity in suspensor basal cells. In their polytene nuclei the rate of RNA synthesis is hundreds of times higher than in embryo cells, and transcriptional activity per amount of DNA is twice that of the embryo proper (Walbot et al., 1972). Cytochemical and ultrastructural studies of the suspensor provide evidence indicating either its role in synthesis or its specialization in active transport (Nagl, 1990b; Nagl et al., 1991). The wall ingrowths characteristic of so-called transfer cells occur in the micropylar part of the suspensor basal cells of, for example, *Capsella* (Schulz and Jensen, 1968, 1969), *Phaseolus* (Schnepf and Nagl, 1970), *Stellaria* (Newcomb and Fowke, 1974), *Tropaeolum* (Nagl, 1976), *Vigna sinensis* (Hu et al., 1983) and *Alisma* (Bohdanowicz, 1987).

These numerous investigations of the suspensor suggest that its role during embryogenesis is not restricted to pushing the embryo into the embryo sac where it would be exposed to the endosperm. The correlation between suspensor development and formation of the embryo proper confirms the suspensor's importance to normal embryogenesis.

In *T. maritimum*, fast progress of endoreduplication in the basal cell coincides with the first division of the embryo mother cell, and differentiation of the suspensor precedes that of the embryo proper. Further development of embryo very probably is possible only when the basal cell nucleus undergoes a few (3–4) rounds of endoreduplication. A similar relationship between suspensor and embryo development has been reported by Schulz and Jensen (1969) in *Capsella bursa-pastoris* and Bohdanowicz (1973) in *Alisma plantago-aquatica*. The important regulatory role of the suspensor (as a source of growth substances and nutrients) during early embryogenesis has been confirmed by many authors (Lorenzi et al., 1975; Yeung and Sussex, 1979; Yeung, 1980) in *Phaseolus*. This implies that the suspensor functionally replaces the endosperm in the first stages of embryogenesis. However, further multiplication of the nuclear DNA of suspensor nuclei in *T. maritimum* proceeds simultaneously with the growth of the embryo (to ~ 500 cells). The basal cell nuclei attain a ploidy level of 256C. This seems to indicate high physiological activity by the suspensor during late embryogenesis. This finding seems consistent with studies by Forino et al. (1992) and Andreucci et al. (1994), reporting extensive metabolic activity by the suspensor in the heart-shaped and cotyledon embryo stages.

The results obtained in this investigation of suspensor differentiation in *T. maritimum* support the suggestion that the suspensor plays an active role during early and late embryogenesis.

REFERENCES

Andreucci AC, Ruffini Castiglione M, and Tagliasacchi AM. 1994. RNA synthesis and immunogold localization of 5-methylcytosine rich regions in polytene chromosomes of *Phaseolus coccineus* embryo suspensor, at two stages of embryogenesis. *Cytobios* 78: 99–113.

Avanzi S, Cionini PG, and D'Amato F. 1970. Cytochemical and autoradiographic analyses on the embryo suspensor cells of *Phaseolus coccineus*. *Caryologia* 23: 605–638.

Bohdanowicz J. 1973. Karyological anatomy of the suspensor in *Alisma* L. I. *Alisma plantago-aquatica* L. *Acta Biologica Cracoviensia Series Botanica* 16: 235–248.

Bohdanowicz J. 1987. *Alisma* embryogenesis: the development and ultrastructure of the suspensor. *Protoplasma* 137: 71–83.

Brady T. 1973. Feulgen cytophotometric determination of the DNA content of the embryo proper and suspensor cells of *Phaseolus coccineus*. *Cell Differentiation* 2: 65–75.

Cremonini R, and Cionini PG. 1977. Extra DNA synthesis in embryo suspensor cells of *Phaseolus coccineus*. *Protoplasma* 91: 303–313.

D'Amato F. 1984. Role of polyploidy in reproductive organs and tissues. In: Johri [ed.], *Embryology of Angiosperms*. Springer-Verlag, Berlin, Heidelberg, NewYork, Tokyo.

D'AMATO F. 1989. Polyploidy in cell differentiation. *Caryologia* 42: 183–211.

FORINO LMC, TAGLIASACCHI AM, CAVALLINI A, CIONINI G, GIRALDI E, and CIONINI PG. 1992. RNA synthesis in the embryo suspensor of *Phaseolus coccineus* at two stages of embryogenesis, and the effect of supplied gibberellic acid. *Protoplasma* 167: 152–158.

HASITCHKA-JENSCHKE G. 1959. Bemerkenswerte Kernstrukturen im Endosperm und im Suspensor zweier Helobiae. *Österreichische Botanische Zeitschrift* 106: 301–314.

HU SY, ZHU CH, and ZEE SY. 1983. Transfer cells in suspensor and endosperm during early embryogeny of *Vigna sinensis*. *Acta Botanica Sinica* 25: 1–10.

LORENZI R, BENNICI A, CIONINI PG, ALPI A, and D'AMATO F. 1978. Embryo-suspensor relations in *Phaseolus coccineus*: Cytokinins during seed development. *Planta* 143: 59–62.

MAHESHWARI P. 1950. *An introduction to the embryology of Angiosperms*. McGraw-Hill, New York, Toronto, London.

NAGL W. 1962. Über Endopoliploidie, Restitutionskernbildung und Kernstrukturen im Suspensor einer Angiospermae und einer Gymnospermae. *Österreichische Botanische Zeitschrift* 109: 431–494.

NAGL W. 1974. The *Phaseolus* suspensor and its polytene chromosomes. *Zeitschrift für Pflanzenphysiologie* 73: 1–44.

NAGL W. 1976. Early embryogenesis in *Tropaeolum majus* L.: Ultrastructure of embryo-suspensor. *Biochemie und Physiologie den Pflanzen* 170: 253–260.

NAGL W. 1980. Polytene chromosomes of plants. *International Review of Cytology* 23: 21–51.

NAGL W. 1990a. Polyploidy in differentiation and evolution. *International Journal of Cell Cloning* 8: 216–223.

NAGL W. 1990b. Translocation of putrescine in the ovule, suspensor and embryo of *Phaseolus coccineus*. *Journal of Plant Physiology* 136: 587–591.

NAGL W, SEELINGER T, and HARTMANN A. 1991. Immunocytochemical localisation of the storage protein phaseolin in the embryo-suspensor of *Phaseolus coccineus*. *European Journal of Basic and Applied Histochemistry* 35: 175–183.

NEWCOMB W, and FOWKE LC. 1974. *Stellaria media* embryogenesis: the development and ultrastructure of the suspensor. *Canadian Journal of Botany* 52: 607–614.

PICCIARELLI P, ALPI A, PISTELLI L, and SCALET M. 1984. Gibberellin-like activity in suspensors of *Tropaeolum majus* L. and *Cytisus laburnum* L. *Planta* 162: 566–568.

PRITCHARD HN. 1964. A cytochemical study of embryo development in *Stellaria media*. *American Journal of Botany* 51: 472–479.

SCHNEPF E, and NAGL W. 1970. Über einige Strukturbesonderheiten der Suspensorzellen von *Phaseolus vulgaris*. *Protoplasma* 69: 133–143.

SCHULZ SR, and JENSEN WA. 1968. *Capsella* embryogenesis: the egg, zygote and young embryo. *American Journal of Botany* 55: 807–819.

SCHULZ SP, and JENSEN WA. 1969. *Capsella* embryogenesis: The suspensor and the basal cell. *Protoplasma* 67: 139–163.

SKALIŃSKA M, PIOTROWICZ M, and SOKOŁOWSKA-KULCZYCKA A. 1961. Further additions to chromosome number of Polish Angiosperms. *Acta Societatis Botanicorum Poloniae* 30: 463–489.

WALBOT V, BRADY T, CLUTTER M, and SUSSEX I. 1972. Macromolecular synthesis during plant embryogeny: rates of RNA synthesis in *Phaseolus coccineus* embryos and suspensor. *Developmental Biology* 29: 104–111.

YEUNG EC, and SUSSEX I. 1979. Embryogeny of *Phaseolus coccineus*: The suspensor and the growth of the embryo-proper *in vitro*. *Zeitschrift für Pflanzenphysiologie* 91: 423–433.

YEUNG EC. 1980. Embryogeny of *Phaseolus*: The role of the suspensor. *Zeitschrift für Pflanzenphysiologie* 96: 17–28.

YEUNG EC, and MEINKE DW. 1993. Embryogenesis in Angiosperms: Development of the suspensor. *Plant Cell* 5: 1371–1381.