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ENDOSPERM DEVELOPMENT IN SEEDS OF *ECHIUM VULGARE* L. (BORAGINACEAE) FROM POLLUTED SITES

ANITA BISKUP AND ROMANA IZMAIŁOW*

Department of Plant Cytology and Embryology, Jagiellonian University,
ul. Grodzka 52, 31-044 Cracow, Poland

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Analysis of endosperm development in plants from contaminated sites (vicinity of the Żelazny Most copper post-flotation reservoir in the Legnica-Głogów Copper District, and the zinc spoil in Katowice-Wełnowiec) showed general similarities in the pattern of endosperm formation in *Echium vulgare*, but also deviation from typical haustorium structure (~23% frequency), premature degeneration of the haustorium, or degeneration both of the haustorium and endosperm proper (~40%). The most significant irregularities of endosperm development included lower or higher number of nuclei in two cells of the lateral part of the endosperm, lower ploidy level of haustorium nuclei, and cellularization (instead of coenocytic structure) in the lateral cells of the endosperm. Irregularities and degenerative processes presumably resulted from the stress of environment conditions. Because of the nutritive function of those structures, degeneration or atypical structure of the endosperm and its haustorium in some maturing seeds may reduce the fertility of plants colonizing contaminated sites.

Key words: *Echium vulgare* L., endosperm, endospermal haustorium, disturbances in endosperm, polyploidization, heavy metals, pollution.

INTRODUCTION

Studies of the attenuating effects of pollutants on embryological processes in higher plants have been fewer than those on plants' tolerance of heavy metals. The latter research has focused mainly on mechanisms of heavy metal tolerance, compartmentalization and tolerance at the plant and cell level, and patterns of accumulation of heavy metals in cells, tissues and organs (e.g., Levitt, 1972; Ernst, 1999; Adriano, 2001). Apart from the heavy metals in environments polluted by industrial waste, a wide spectrum of other factors may exert various effects on processes in plants, depending on the tolerance level of individual plants. Fewer studies address the effects of extreme habitat conditions on processes in anthers and ovules of plants growing at polluted sites during the whole life cycle (Ostrolucká, 1989; Ostrolucká et al., 1995; Izmaiłow, 2000, 2002a,b; Kościńska-Pająk, 2000, 2002; Czapik and Kaźmierska, 2002; Czapik et al., 2002; Izmaiłow and Biskup, 2003). The present study continues our research on processes in the reproductive organs of *Echium vulgare* from two contaminated sites (Silesia, Poland) (Izmaiłow and Biskup, 2003). The plants of one population grew at the base of the Żelazny Most copper post-flotation reservoir located in the Legnica-Głogów Copper District, where the soil is highly contaminated with Pb, Cu, Cd, Co, Hg and As (Krajewski, 1993; Piątkowski and Skibicki, 1997; Kijewski, 1998). The second sample originated from the zinc spoil in Katowice-Wełnowiec, which has a high concentration of Zn, Pb and Cd (Tokarska-Guzik et al., 1991). The control plants for comparison grew in uncontaminated pastures near Rymanów (south-eastern Poland).

Our previous study revealed degenerative processes and disturbances in anthers and ovules at

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*e-mail: izmailow@iphils.uj.edu.pl

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different developmental stages, reducing the fertility of the plants colonizing the polluted sites. To enlarge the scope of results, we studied endosperm development in ovules transforming into seeds under the unfavorable conditions of the contaminated sites. The condition of this tissue determines seed development, as the endosperm nurses the embryo till it becomes self-sufficient, and provides growth regulators which control developmental processes. A specific feature of endosperm development in *Echium* is the formation of central and lateral parts; the lower cell of the latter is transformed into a coenocytic eight-nucleate haustorium. Its nuclei attain high levels of polyploidy (96n) (Małecka, 1975). The haustorium, invariably located close to the funiculus and invading the integument tissue, participates in nutrient transport to both the embryo and the developing endosperm proper.

MATERIALS AND METHODS

The materials were fixed in situ at two contaminated sites: (1) the base of the Żelazny Most copper post-flotation reservoir (Legnica-Głogów Copper District) and (2) the zinc spoil in Katowice-Wełnowiec. Plants from uncontaminated pastures near Rymanów (southeastern Poland) were the control material.

The studied plants were tetraploids (2n = 32). Seeds at different developmental stages were fixed in 96% ethanol:acetic acid (3:1). Paraffin-embedded material was cut to 8 µm thick sections and stained in Heidenhain's hematoxylin and alcian blue.

Endosperm development was studied in 260 ovules from plants growing at Żelazny Most, 330 ovules from Katowice-Wełnowiec, and 170 ovules from near Rymanów.

The ploidy level of endosperm nuclei was calculated as in Małecka (1975), from their volumes according to the formula:

$V = 1/6\, \pi\, d_1\, d_2\, d_3$

(d_n = diameter of nucleus in µm).

RESULTS

OUTLINE OF TYPICAL ENDOSPERM DEVELOPMENT

The pattern of endosperm development in the flowers from the control material and the majority of those from the plant populations at Żelazny Most and Katowice-Wełnowiec was in agreement with the pattern described for *Echium vulgare* by Małecka (1975). Two parts, central and lateral, form after the first mitosis of the primary endosperm nucleus followed by cytokinesis. The two cells, of unequal size, follow different courses of development. The larger, central cell gives rise to the endosperm proper (nuclear and then cellular type); the smaller, lateral cell divides, producing one cell lying near the zygote (higher lateral cell) and another one situated near the chalaza (lower lateral cell) (Fig. 1). After three mitoses without cytokinesis, these cells became coenocytes with eight nuclei.

Initially the nuclei in the lateral cells and those of the endosperm proper are of equal ploidy level (3n = 48). However, at the stage of the young proembryo, when lateral cells presumably take part in its nourishment, their nuclei enlarge, attaining higher levels of polyploidy. Analysis of the structure, rhythmic growth and volume calculations indicates endoreduplication as the mechanism of polyploidization. It is assumed that endopolyploidy increases the functional activity of cells, including their absorptive capacity. The nuclei in the higher lateral cell attain 24n, and a maximum 96n in the lower lateral cell. The same ploidy levels were reported by Małecka (1975).

The coenocyte neighboring the proembryo degenerates at its young globular stage. The nutritive function presumably is taken over by the lower lateral cell, which increases considerably, undergoes distinct vacuolization, and invades the adjoining integument tissue in the region of the funiculus, becoming funnel-shaped (Fig. 2). This cell, transformed into a haustorium, shows a specific cell wall structure which projects deep towards the central part of the coenocyte; such a structure presumably

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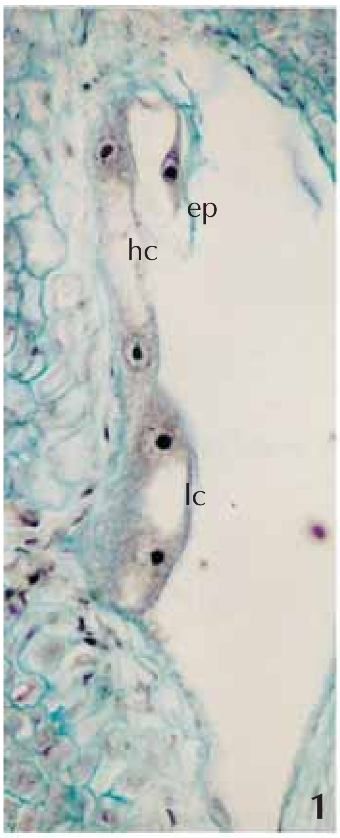
Figs. 1–6. Typical and abnormal endosperm development in maturing seeds of *Echium vulgare.* **Fig. 1.** Early stage of typical endosperm development: binucleate higher lateral cell (hc) and lower lateral cell (lc); ep – nucleus of endosperm proper (plant from Katowice-Wełnowiec). × 400. **Fig. 2.** Typical endosperm development: lower lateral cell being transformed into coenocytic haustorium (h) assuming the funnel shape and invading integument tissue (i); ep – cytoplasm of endosperm proper (plant from Katowice-Wełnowiec). × 400. **Fig. 3.** Fragment of haustorium (h) with high-polyploid nuclei and cells of endosperm proper (ep) presumably with triploid nuclei (3n) (plant from Katowice-Wełnowiec). × 400. **Fig. 4.** Fragment of embryo sac with precociously degenerated haustorium (h); aborted young proembryo (e) at micropylar pole (plant from Żelazny Most). × 400. **Fig. 5.** Micropylar part of embryo sac with globular proembryo (e) and higher lateral cell (hc) still alive, after cellularization; ep – endosperm proper (plant from Żelazny Most). × 400. **Fig. 6.** Chalazal part of embryo sac; atypical haustorium (h) with cellular structure, surrounded by cells of endosperm proper (ep) (plant from Katowice-Wełnowiec). × 200.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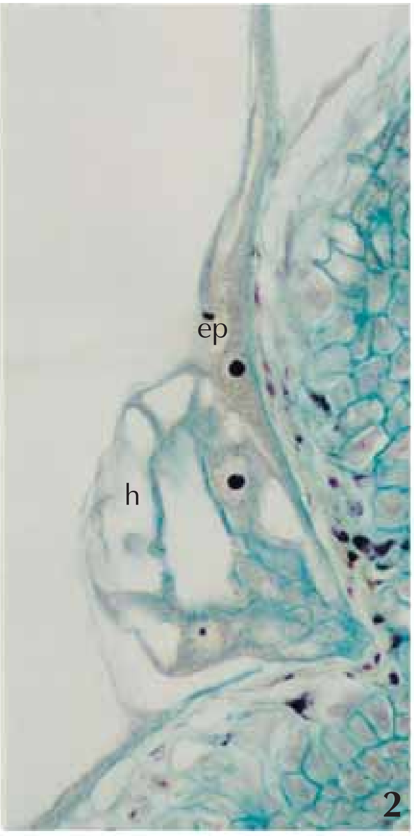
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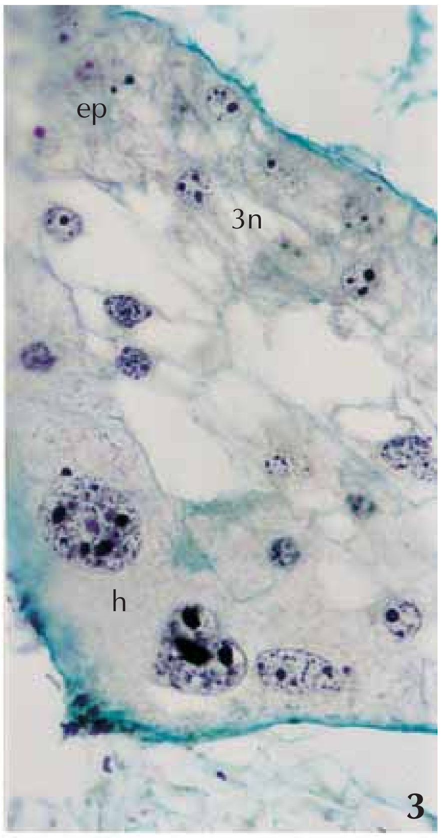
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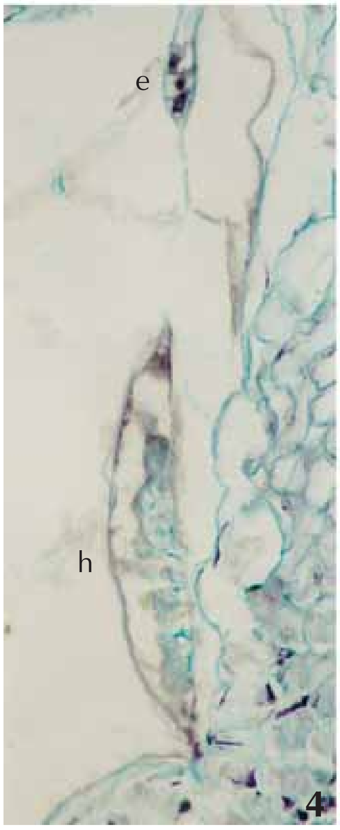
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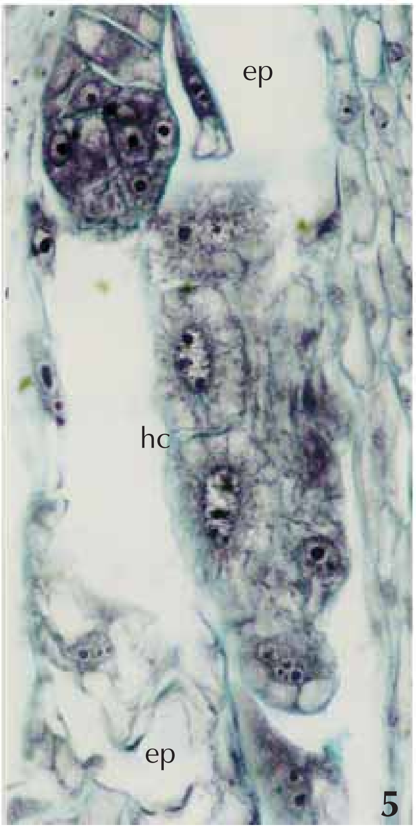
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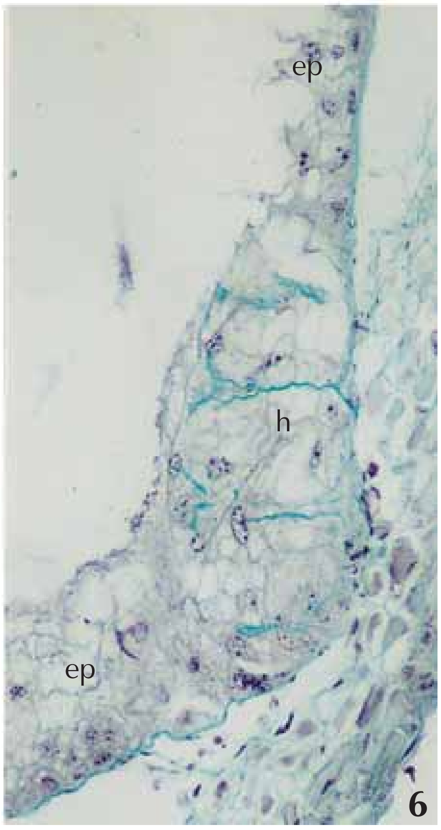
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is associated with the absorptive activity of the haustorium and functions as a transfer wall. At this stage of development the haustorium becomes closely surrounded by the endosperm proper, which undergoes cellularization (Fig. 3) and whose nuclei in this region may undergo one or two endoreduplication cycles. A coenocytic haustorium was observed in maturing seeds till the late torpedo-shaped embryo stage.

DEVIATIONS OF ENDOSPERM DEVELOPMENT AND DEGENERATION IN PLANTS FROM POLLUTED SITES

Analysis of endosperm development in seeds maturing in situ in polluted sites has shown general similarities to the pattern typical of the genus *Echium* (Echium-type), but deviation from typical haustorium structure was observed in ~23% of cases. Apart from atypical endosperm development in material from contaminated sites, degenerative processes in endosperm were found in ~40% of examined ovules. Both lateral cells frequently showed precocious degeneration at the early four-nucleate stage or already before polyploidization; precocious degeneration of the young haustorium also was observed (Fig. 4). In several ovules, simultaneous degeneration of the endosperm proper at the nuclear stage and abortion of the embryo were noted. These processes resulted in reduced fruiting of plants growing at the contaminated sites.

The most significant irregularities included deviations in the number of nuclei in both lateral cells at their mature stage. Variation of the number of mitoses (reduced or increased) in lateral cells resulted in 4, 6 or occasionally 12 nuclei in coenocytes instead of the typical 8 nuclei. In some ovules the mitotic divisions in the lower lateral cell were distinctly delayed versus those of the higher lateral cell and versus the stage of the proembryo.

Another type of development of the lateral part of the endosperm was found in several ovules: instead of maintaining a coenocytic structure, the higher and lower lateral cells underwent cellularization (Figs. 5, 6); this has not been reported hitherto for *Echium*, neither by Svensson (1925) who described various developmental types of endosperm in the family Boraginaceae, nor by Małecka (1975). The volume of nuclei in the multicelled lateral complex corresponded to 6n and 12n polyploidy levels. In ovules from polluted sites the degrees of ploidy attained by nuclei in lateral cells generally were lower than in the typical pattern of the control plants, particularly in the haustorium, whose nuclei reached a maximum 24n.

In later stages the polyploid nuclei of the haustorium could resume their mitotic activity. Analysis of the mitoses yielded approximate estimations of chromosome numbers (~190 and ~380), confirming the occurence of 2 and 3 endoreduplication cycles (12n and 24n, respectively). Postmitotic polyploid nuclei were irregularly shaped as a result of mitotic disturbances.

DISCUSSION

Uptake and transport of heavy metals within plants can be modified by other environmental factors such as water conditions, soil pH or mycorrhiza, depending on the species, metal, and stage of life cycle. Metal-tolerant plants of *Armeria muelleri, Thlaspi coerulescens* and *Silene vulgaris* from natural sites (Ernst et al., 1990), as well as *Mimulus guttatus* and *Silene dioica* cultivated under controlled laboratory conditions (Searcy and Mulcahy, 1985 a,b), showed small amounts of metals in the reproductive organs. Even low concentrations of Cu and Zn transported into flowers resulted in a lowered percentage of functioning pollen (Searcy and Mulcahy, 1985a).

Particularly significant results were obtained in PIXE microanalysis of element distributions in seeds of *Silene vulgaris* and *Gypsophila fastigiata* from calamine spoil (Mesjasz-Przybyłowicz et al., 1998, 1999). *Silene* seeds showed a relatively homogenous Zn distribution, with slightly higher amounts in the testa, hilum and perisperm. Seeds of *Gypsophila* accumulated much higher amounts of this element, mainly around the hilum and in the radicle; Cu and Pb were noted mainly in the embryo. In view of these results, one can suggest that the small amounts of heavy metals transported to the flower and discovered in some seed tissues could be active during seed maturation, affecting this process in low-tolerant organisms. If present in mature seeds, these elements may have played a role in embryogenesis and endosperm development, which might be affected by other extreme habitat conditions as well.

Our previous findings (Izmaiłow, 2002a) might support this suggestion: in *Ranunculus repens* cultivated on polluted soil, nearly 10% of the older ovules showed some atypical features of endosperm development (disturbances of cellularization, small amounts of storage materials). The present analysis of endosperm development in *Echium vulgare* from polluted sites showed general similarities to the Echium-type pattern (Svensson, 1925; Małecka, 1975), but also developmental irregularities, precocious degeneration of the lateral part of endosperm destined for haustorium formation, or degeneration of both the haustorium and endosperm proper.

Irregularities in the structure and development of the haustorium, or precocious degeneration of it, are of great significance on account of its specific functions. Any change in the pattern of haustorium formation may have an important impact on endosperm functions. The physiological role of polyploidy in reproductive organs and tissues has been discussed in many classic papers (e.g., Nagl, 1978; D'Amato, 1984; Brodsky and Uryvaeva, 1985). The pattern of ploidy levels in different regions of the endosperm indicates that the degrees of nuclear activity have a spatial distribution (Jankun, 1970; D'Amato, 1984). In *Echium*, endoreduplication in haustorium nuclei may be connected with an increase of its functional activity; on the other hand, the haustorium's metabolic activity and efficiency of transport may be lowered by reduction of the number of endoreduplication cycles, continuation of the initial triploid level, or cellularization.

Comparison of the results for *E. vulgare* from the two sites impacted by industrial activity with those for the control plants, and with the findings of Svensson (1925) and Małecka (1975), corroborate the suggestion that degeneration and irregular endosperm development are specific responses to the stress factors of the polluted environments, and were the cause of distinctly reduced fruiting in the plants growing at contaminated sites.

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