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CYTOLYSIS OF THE ENDOSPERM IN DIFFERENT TYPES OF CORRELATION IN ENDOSPERM AND EMBRYO DEVELOPMENT

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SUMMARY

Histochemical analysis of acid phosphatase and non-specific esterase indicated that the endosperm cell cytolysis may be observed in different periods and spatial successivity in connection with 3 types of correlation in embryo and endosperm development.

1. INTRODUCTION

Differences in the relative rate of the endosperm and embryo development in various Angiosperm species have led to a characterization of 3 types of correlations in embryo and endosperm development (ERDELSKÁ 1984, 1985a, b).

In different correlation types the endosperm cytolysis starts in different phases of the development. Histochemical detection of hydrolytic enzymes was used for characterization of this period and for the localization of cytolysis.

2. MATERIAL AND METHODS

Seeds of *Papaver somniferum, Nicotiana tabacum, Jasione montana, Campanula persicifolia, Linum usitatissimum, Capsella bursa-pastoris* and *Pisum sativum* were used as experimental material. The plants were cultivated under field conditions.

The young seeds were extirpated from the fruits and fixed in Navashin or Baker solution (4% formaldehyde with 1% $CaCl_2$).

Microtome sections from seeds fixed in Navashin solution were stained by haematoxylin (Heidenhain) or by PAS reaction. Sections from seeds fixed in Baker solution were used for the histochemical reactions of acid phosphatase and non-specific esterase. Both enzymes were identified by the azocopulation method. In the acid phosphatase identification α-naphtylphosphate was used as the substrate and the Fast Red TR as the stable diazonium salt. In the non-specific esterase identification, α-naphtylacetate and Fast Blue B were used, according to Gomori's method modified by LOJDA & PAPOUŠEK (1970): 10 mg of α-naphtylacetate were dissolved in 1 ml of acetone, mixed with 50 ml of 0.1M phospate buffer of pH 7,4 and 50 mg of Fast Blue B were added. After shaking and filtering, the deparaffinized sections were incubated in the solution for 20–30 minutes at room temperature, then washed in water and enclosed in the medium mixed with water (Aquamount, Gurr Ltd., London).

3. RESULTS

In species of Type I correlation, i.e. *Papaver somniferum, Nicotiana tabacum, Jasione montana* and *Campanula persicifolia*, histochemical analysis indicated the presence of acid phosphatase and non-specific esterase and the onset of cytolysis of the endosperm already occurring in the middle or late globular phase of the embryo development (*fig. 1*). The zone of the endosperm cell desintegration appears first around the apical pole of the embryo and spreads into the central part of the endosperm, i.e. the area which will be gradually occupied by the growing embryo (*fig. 2*). This prospective cytolytical zone cellularizes in species with nuclear type of the endosperm usually as the last one. It evidently contains the least amount of reserve substances. However, the cells of the cytolytical zone may also differ from cells of the outer peripheral endosperm layers in their size, form, content and structure (*fig. 3*).

Cells of the cytolytical zone are released and gradually desintegrate in advance, before the growing cotyledones of the embryo reach them (*Nicotiana, Papaver*). However, in species with a morphologically different cytolytical zone, the walls of some cells remain sometimes well preserved. It is only the cell content which desintegrates. The outer layers of endosperm cells full of reserve substances are depleted only in the course of germination.

In species of Type II correlation, it is only possible to detect the occurrence of hydrolytical enzymes in the endosperm much later, when the cellularized endosperm makes contact with the embryo. The desintegration begins in some areas when the embryo reaches the late heart-shaped or torpedo-phase of its development.

In *Capsella bursa-pastoris* the histochemical reaction is first evident around the inner curvature of cotyledones and then around the apical part of cotyledones.

In *Linum usitatissimum* the reaction appears first around the outer surface of cotyledones which come into contact with the cellularized endosperm in the central part of the embryo sac. In the contact zone the endosperm cell layers do not fully desintegrate during the cytolysis but are first compressed.

The occurrence of hydrolytical enzymes can be seen in the cells of the seed coat from which the embryo draws nutrients in the first phase of the embryogenesis.

In species of the Type III correlation (*Pisum sativum*) hydrolytical enzymes appear almost simultaneously with the degeneration of the massive suspensor. The desintegration of the endosperm occurs both in the layer of the free nuclear endosperm around the embryo and in the wall layer of the endosperm (*fig. 4*). The area of the cellularized endosperm around the radicule also gradually desintegrates.

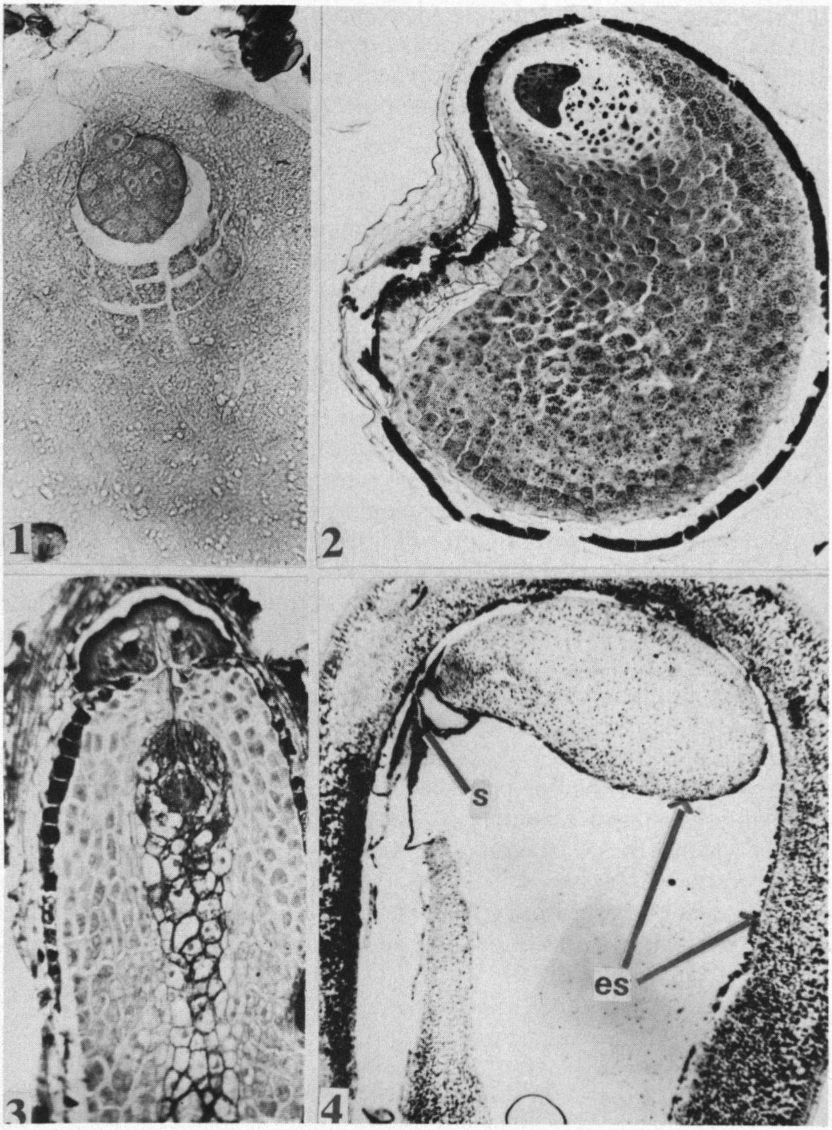

Fig. 1. The initial cytolytical zone round the globular embryo of *Papaver somniferum*, non specific esterase reaction (340 x).

Fig. 2. The cytolytical zone enlarging in the central part of the endosperm of *Papaver somniferum*, stained with haematoxylin (72 x).

Fig. 3. The perspectively cytolytical zone in the centre of the endosperm of *Campanula persicifolia*, PAS reaction (224 x).

Fig. 4. Occurrence of the non specific esterase in the disintegrating endosperm (es) and suspensor (s) as well as in the seed coat of *Pisum sativum* (32 x)

In the seed coat the hydrolytical enzymes appear already in the globular phase of the embryo development. The amount of hydrolytical enzymes in the seed coat increases substantially after the degeneration of the endosperm and suspensor, in connection with the degeneration of the seed coat cell content.

4. DISCUSSION

The developmental correlation between the embryo and the endosperm during embryogenesis may be characterized from different points of view. However, a close connection with the nutritive, regulatory and protective function of the endosperm can always be found (SARFATTI 1964, BRINK & COOPER 1947, MIANNAY et al. 1977 MESTRE & VANNEREAU 1980, SCHEL et al. 1984). Analysis of the occurrence of hydrolytical enzymes indicating endosperm cell cytolysis helps to define the period and the area of the nutrients release and determination of the space for further growth of the embryo.

In Type I correlation the embryo is in close contact with the cellular endosperm from the initial phase of its development. Cytolysis of the endosperm cells around the embryo starts after the proliferation of the endosperm has been accomplished (*Papaver, Campanula*).

In Type II correlation the endosperm cells desintegrate at the time and in the areas where the proliferating embryo and the cellularized endosperm come in contact.

In Type III correlation the endosperm development is very limited (Viciaceae). The endosperm desintegrates after the function of the suspensor has been completed. It desintegrates also in those areas, which are not in direct contact with the embryo surface. From the published data it is not clear whether the endosperm cytolysis is always an autolysis (JONES 1974, GEORGIEVA 1977, 1980, and others) or whether the desintegration is evoked by the digestive function of the embryo (OLSON 1981, IKUMA & THIMANN 1963, NAGY & KÉRI 1984 and others). The facts presented in our work refer to the possibility of the action of different mechanisms in the cytolysis of endosperm cells in different species of Angiosperms.

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