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# Cleavage polyembryony *in vivo* and *in vitro*

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## Abstract

The development of cleavage polyembryos of wheat, maize and radish *in vivo* and *in vitro* was compared. Some frequent features (such as common suspensors or suspensor-like structures, "concrescences", enlarged radicular meristems) suggest a similar origin of cleavage polyembryos induced *in vivo* and those induced *in vitro* from the proembryo. Cleavage of the original zygotic or somatic proembryo may occur either at a few-celled stage or later in the phase of radicular meristem establishment.

## Introduction

In plants only the zygote is regularly determined to show the total embryogenic competence. Any other cell has to be not only meristematic but also "decorrelated" *i.e.* released from the developmetal integrity of plant tissue for the expression of its totipotency. The term "decorrelation", in the sense of Dostál's school of experimental morphology (Dostál 1967), indicates those changes in gene expression which are responsible for this release and for determination of embryogenesis. It is an alternative to the frequently used term "cell isolation", the role of which has been reviewed *e.g.*, by Williams and Maheshwaran (1986). The "decorrelation" is typical for different types of polyembryony. For example, for nucellar or integumental polyembryony a cell of nucellus or integument has to be released from the role it plays as an integral component of nucellar or integumental tissue. The same is true also for the cleavage polyembryony which originates after individual cells or cell groups have been released from the integrity of the early proembryo. In this report we document the similar origin of cleavage polyembryos *in vivo* and *in vitro*.

## Materials and methods

**Treatment and culture of ears:** All experimental mother plants were grown in field conditions. Cleavage polyembryony *in vivo* was induced by treatment of wheat

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(*Triticum aestivum*, cv. Jubilejná and cv. Jarka) or maize ears (*Zea mays*, VIR 17) by 2,4-D (1 g $l^{-1}$ in 50 % acetone). The treatment was done 2 - 7 d after anthesis by dipping the whole wheat ear for 5 s into 2,4-D solution. 2,4-D solution was applied on one side of isolated ears of maize 2 d after pollination.

**Wheat:** Wheat stems bearing the ears were cut after treatment 3 cm below the peduncular node and after brief surface cleaning immersed in half strength Knop's solution with 2 % saccharose, 0.5 g $l^{-1}$ glutamin and 0.5 ml $l^{-1}$ sterilizing agents SAVO. The semisterile procedures were simplified versions of methods used by Donovan and Lee (1977) and Singh and Jenner (1983). Stems with ears were cultured at 25 °C and 60 μmol $m^{-2}$ $s^{-1}$ (photoperiod 16 h) for 3 - 4 weeks in 60 cm tall vessels of 4 cm diameter. The solution was changed twice in the first week and daily during the two following weeks.

**Maize:** The basal parts (2 - 3 cm) of isolated maize ears deprived of scales were placed in the same solution as described for wheat, after treatment with 2,4-D, and cultured for the same time in similar conditions to wheat ears.

**Somatic embryogenesis:** For induction of somatic embryogenesis, the immature semitransparent zygotic embryos of wheat and maize from mother plants grown in field conditions were cultivated in sterile conditions on agar solidified MS medium (Murashige and Skoog 1962) with 3 % saccharose and 2.8 mg $l^{-1}$ 2,4-D in Petri dishes. The axis of excised embryos was oriented to the agar medium. Embryogenic calli were transferred after 3-5 weeks of cultivation to MS medium with 3 % saccharose and 0.1 mg $l^{-1}$ kinetin.

**Somatic embryos of *Raphanus sativus* (var. *radicicola*)** were induced *in vitro* on the hypocotyl of zygotic embryos germinating on the medium after the method of Monnier (1973), with 5 % saccharose supplemented with 0.05 mg $l^{-1}$ 3,6-dichloro-2-methoxybenzoic acid and 200 mg $l^{-1}$ myoinositol (Erdelská *et al.* 1991).

**Histological method:** For histological study, isolated zygotic or somatic embryos were fixed with Navashin's solution and embedded in *Histoplast S* (*Serva*, Heidelberg, Germany). Sections were stained with haematoxylin after Ehrlich or with PAS reaction.

## Results and discussion

Cleavage polyembryony *in vivo* and somatic polyembryogenesis *in vitro* shows very often the same or similar processes of development. According to the terminology of Sharp *et al.* (1980), cells of zygotic or somatic proembryos are preembryogenically determined, being meristematic and developmentally integrated into one unit. "Decorrelation" (release from the integrity of the same developing embryo) of these cells can occur spontaneously or can be induced. In certain species (*e.g.* conifers)

cleavage polyembryony *in vivo* occurs spontaneously and regularly, and usually *in vitro* as well (Gupta and Durzan 1986).

In the majority of angiosperms the spontaneous occurrence of cleavage polyembryony is rarely observed. The induction of cleavage polyembryony *in vivo* in dicotyledons as a consequence of the effect of auxins or some other substances has been described by Haccius (1955). In some monocotyledons (*e.g.* maize, rice) it can be induced by irradiation (Morgan and Rappleye 1951, Osone and Oono 1970) or by influence of 2,4-D, in the first days after fertilization. Very similar events were observed but differently interpreted by Jakovlev and Snegirev (1954) and Fergusson *et al.* (1979) in wheat. We have described it in maize (Erdelská and Vidovencová 1992).

The application of 2,4-D to young caryopses a few days after fertilization may result in the cleavage of the proembryo and in the development of a polyembryonal unit (Fig. 1). In wheat it seems that not only individual cells of the proembryo (by application of 2,4-D, 2 d after anthesis) but also entire cell generations of the proembryo originating from the first proembryonal cells (by application of 2,4 D later) can give rise to the individual embryos of a polyembryonal unit.

Cleavage of the original zygotic or somatic proembryo of wheat can be realized in various developmental phases. If it occurs at the level of a few-celled proembryo, twins or triplets of relatively separate embryos are formed *in vivo* with a partly shared scutellum and a common suspensor (Fig. 1). Very similar development is characteristic also for the cleavage of few-celled zygotic proembryos of maize (Erdelská and Vidovencová 1992).

Another type of cleavage - "delayed cleavage" can be realized in the phase of radicular meristem establishment of wheat. Because of being larger at the start, the radicular meristem grows longer and later separates into two or more individual radicles of each expected embryo of the polyembryonal unit (Figs. 3, 4, 5). While a plumule of the main embryo in the polyembryonal unit is established earlier than the radicle, the adventitious parts of the polyembryonal unit are formed in the reverse sequence, with plumule formation following the already formed radicle (Figs. 4, 5). Sometimes only a radicular primordium without a plumular one can be developed.

Cleavage polyembryony may be observed *in vitro* as well, when somatic embryogenesis is induced directly on the explanted tissue or indirectly from the embryogenic callus, as may also be seen from the figures of Ozias-Akins and Vasil (1983) and Van der Valk *et al.* (1989). These formations, sometimes considered to be concrescent embryos, are actually polyembryos induced by successive cleavage of somatic proembryos (Fig. 2). The frequent focal arrangement of somatic embryos on explanted tissue or in callus suggests that more somatic embryos from one cluster arise from one proembryo or one proembryonal mass (Fig. 7).

Extended radicular areas suggesting the same type of "delayed cleavage" as *in vivo* may also be observed (Figs. 6, 7) during development of the somatic polyembryos *in vitro*.

Secondary somatic embryos are often formed from scutellum or cotyledons of primary somatic embryos (Fig. 7).

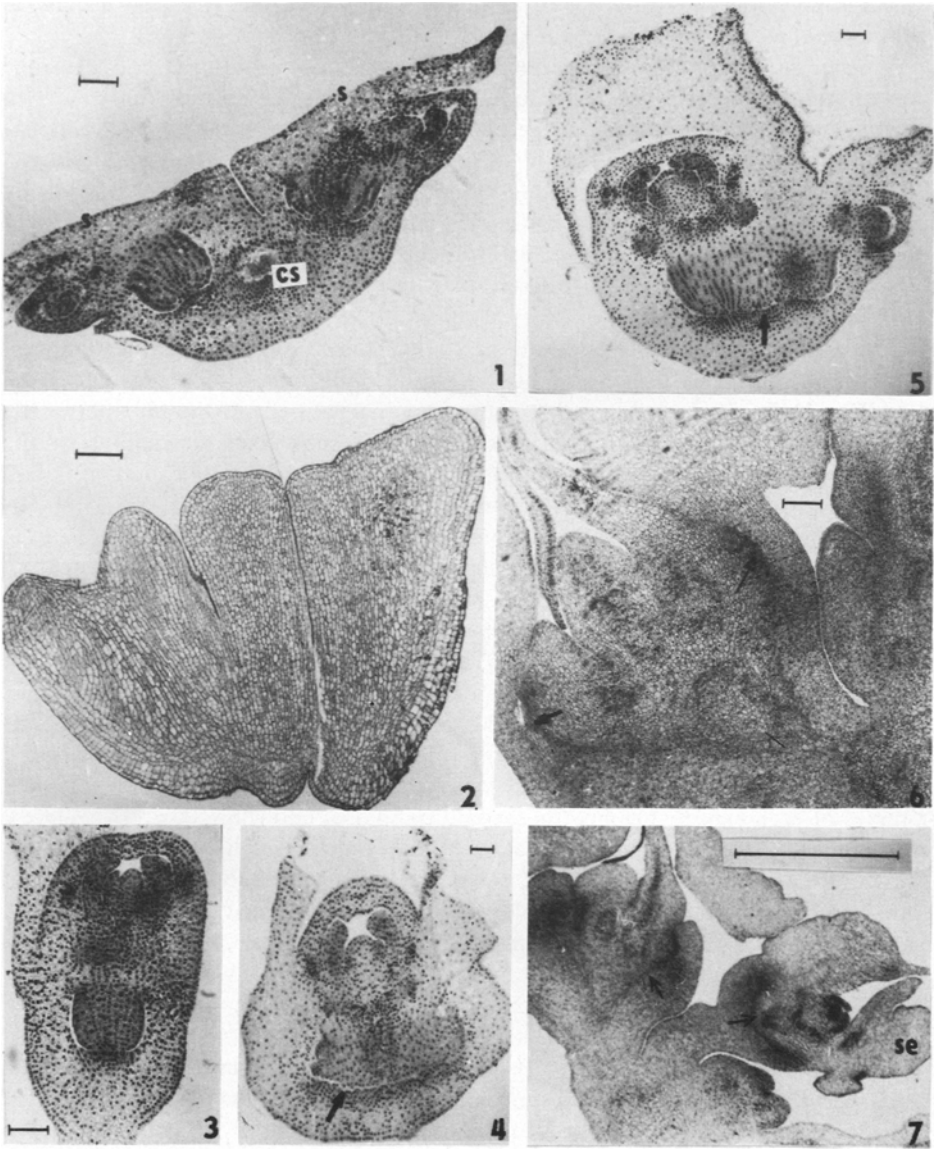
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Fig. 1. Transverse section of the basal area of wheat (cv. Jubilejná) polyembryo with two embryonal parts (*s* - scutellum, *cs* - common suspensor). Bar = 0.1 mm.

Fig. 2. Somatic polyembryo of *Raphanus sativus* formed directly on a hypocotyl of a zygotic embryo cultivated *in vitro*. Bar = 0.1 mm.

Fig. 3. Control (single) immature embryo axis of wheat cv. Jarka. Bar = 0.1 mm.

Fig. 4. Developing polyembryo of wheat with extended radicular meristem (*arrow*). Bar = 0.1 mm.

Fig. 5. Developing polyembryo of wheat with separating radicular parts (*arrow*). Bar = 0.1 mm.

Fig. 6. Somatic polyembryo of maize developing *in vitro* with extended radicular meristem (*arrows*). Bar = 0.1 mm.

Fig. 7. Cluster of somatic maize embryos with extended radicular meristems (*arrows*) and developing secondary embryos (*se*) from scutellum. Bar = 1 mm.

Relatively frequent occurrence of polyembryos *in vitro* as well as *in vivo* results from the fact that cells of proembryos have relatively "short way to return" to the full totipotency of the first induced or released cell. The epigenetic "distance" of proembryonal cells from the embryogenic state is very short (Merkle *et al.* 1990).

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