# Cleavage polyembryony in maize

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Summary. Two types of cleavage polyembryony are described in the inbred line VIR 17 of maize. Suspensorial embryony was observed to occur spontaneously. Typical cleavage of the zygotic proembryo occurred spontaneously, but could also be induced by treating the developing caryopses with 2,4-Dichlorophenoxyacetic acid (2,4-D) on the second day after pollination. 2,4-D was active as a decorelative factor also evoking the expression of totipotency in individual proembryonal cells.

**Key words:** Zea mays – Maize – Polyembryony

### Introduction

The spontaneous development of multiple embryos in maize caryopses was described long ago by Kieselbach (1926), Randolph (1936) and others. The frequency of multiple embryos depends on the genotype and normally does not exceed 0.1%. However, in some polyembryonal and apomictic genotypes of maize it reaches 25.3% (Pesev et al. 1976) or even 75% (Silva et al. 1971) and may be increased by selection (Judin and Chvatova 1965) or by delayed pollination (Tyrnov and Enaleeva 1983).

Multiple embryos are haploid, diploid, triploid or polyploid depending on their origin. They arise from egg cells in two or more embryo sacs in the ovule, from several fertilized or unfertilized cells of the single gametophyte (Kermicle 1971; Ustinova 1960; Zverzhanskaya et al. 1977) or by cleavage of the zygotic embryo (Morgan and Rappleye 1951).

The purpose of our paper is to report the occurence of two types of spontaneous cleavage polyembryony in maize and also the possibility of cleavage polyembryony induction on attached and isolated ears.

#### Material and methods

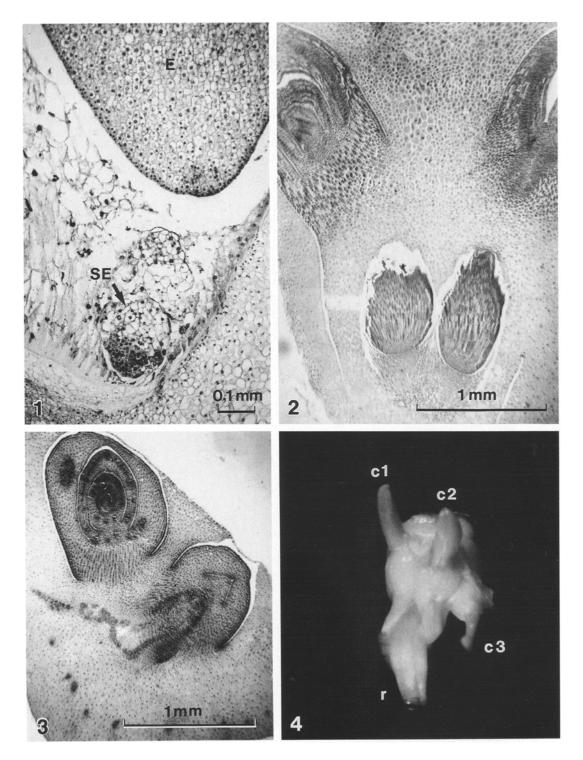
Cleavage embryony was studied in maize inbred line VIR 17. For histological and cytological analysis of caryopses and isolated embryos the paraffin (Histoplast – S, Serva) technique was used. Sections were stained by haematoxylin or by the periodic acid – Schiff reaction.

Polyembryony was induced on ears attached to the mother plant or on isolated ones. After removal of the husks the caryopses on one side of the ear were treated with 5 ml of 2,4-D (1 g/l in 50% acetone) on the second day after pollination. Earlier or later treatments were unsuccesful. The basal part, of the isolated ears was then dipped into half concentrated Knop's solution in a glass vessel and cultured for 1 month in the growth chamber at 25° C and 3000 lx. The solution was changed twice a week. The treated attached ears were recovered with their own husks and kept on the mother plant till maturity.

# Results and discussion

Suspensorial polyembryony was observed in two caryopses. In addition to a zygotic embryo already in the advanced phase of its development (about 20 days after fertilization), the development of another globular embryo in the suspensorial area of the embryo sac was clearly visible (Fig. 1). The globular embryo was polarized with smaller meristematic cells in its apical part and larger vacuolated cells in its basal part. Numerous diploid mitoses in the apical zone testify to additional recent meristemisation of some suspensor cells, perhaps as a result of precocious interruption of the embryosuspensor complex. Suspensorial polyembryony has been described mainly in dicotyledonous taxa (Haccius 1965) and may be considered to be a special type of cleavage polyembryony.

Typical cleavage polyembryony occurs in some genotypes of maize spontaneously (Fig. 2). Morgan and Rappleye (1951) also induced this state by X-irradiation of pollen. In our experiments the development of twins and triplets was induced by treating caryopses on the ear with 2,4-D on the second day after pollination (Fig. 3). The induction was successful on ears maintained on the



Figs. 1–4. Spontaneous and induced polyembryony in maize. Fig. 1. Suspensorial embryo (SE) in the micropylar region of the embryo sac. E Zygotic embryo. Fig. 2. Longitudinal section of spontane-

ously occurring twins of cleavage origin. **Fig. 3.** Transverse section of plumular part of twins induced by 2,4-D. **Fig. 4.** Embryonal triplet induced by 2,4-D. *c1*, *c2*, *c3* coleoptiles, *r* radicular complex

mother plants as well as on isolated ones. About 40% of the caryopses in the treated area of the ears (about 30 cm<sup>2</sup>) was polyembryonal, whereas some caryopses degenerated (10–30%) and the rest were normal. Values are only approximate because of the impossibility to specify the exact extent of the influenced area. Developing polyembryos have an enlarged scutellum and a susp-

ensor in common. Embryo axes are separated from each other to various extents. The number of embryos arising and the degree of their separation seem to depend on the number of proembryo cells (3–5) at the time of treatment. Polyembryonal caryopses germinate with two or three coleoptiles and multiple radicles (Fig. 4).

Polyembryonal caryopses are usually smaller than

normal ones due to their lower growth potential. Therefore a practical utilization of cleavage polyembryony in the improvement of the nutritional value of maize caryopses by the increase in nutritionally more valuable embryo tissues seems to be doubtful.

However, induced cleavage polyembryony is interesting from the standpoint of proembryo cell differentiation. 2,4-D was active as a decorelative factor, being capable of interrupting developmental correlations of cells in the proembryo and of evoking the expression of totipotency in each individual cell of a 3-, 4- or 5-celled proembryo, with probably the exception of the first suspensorial cell.

Acknowledgment. The skilful technical assistance of Alica Vargová is grateful acknowledged.

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