POLYEMBRYONY IN MAIZE : HISTOLOGICAL ANALYSIS

OLGA ERDELSKÁ

Institute of Botany, Slovak Academy of Sciences,
Dúbravská 14, 84223 Bratislava, Slovak Republic

ABSTRACT

The histological analysis enabled to distinguish the main types of polyembryony in maize according to the origin of embryos connected with their localization in caryopses, differences in their structure (presence of common tissues) and type of germination. Twin embryos from multiple embryo sacs are usually localized on opposite sides (or distant places) of the caryopsis, they have no common tissues and the germination is separate. Twins or triplets from individual cells of the egg apparatus or multiple egg cells are closely adherent, but strictly separated by epidermal layers. Endosperm is common. Plumules and radicles are independent. Cleavage polyembryos arising *in vivo* spontaneously or after induction share a common suspensor, part of scutellum and surface layers of radicles. Therefore they germinate with one radicular complex and with separated plumules.

KEY WORDS: maize, polyembryo, twins, triplets.

INTRODUCTION

The spontaneously occurring polyembryony was already observed in maize in the first half of our century (Kieselbach 1926, Randolph 1936). Later this phenomenon was studied in more details and some types of polyembryony were characterized according to the different origin of arising embryos in polyembryonal caryopses. With the different origin the development, ploidity, form and size of multiple embryos are usually connected. Besides the spontaneously occurring polyembryony the polyembryony induced *in vivo* and *in vitro* was also described.

The frequency of multiple embryos rises from 0,1% (in most normal genotypes) to 23,5% (Pesev et al. 1975) or even 75% (Silva et al. 1971) in some special polyembryonal and apomictic genotypes.

The aim of this paper is to distinguish the different types of polyembryony in maize according to the origin of embryos linked with the localization, structure and form of individual embryos and with the special typ of germination.

MATERIAL AND METHODS

The spontaneously occurring polyembryony was studied on genotypes VIR – 17, TAMv 310, HMV 404, CE 330 and Golden Bantam (from the Maize Research Institute in Trnava, Slovak Republik). Cleavage polyembryony *in vivo* was induced in caryopses of the imbred line VIR – 17 treated with 2,4 - D (1g l^{-1} in 50% acetone) on the second day after pollination (Erdelská, Vidovencová 1992). The development of polyembryos *in vitro* was observed in embryogenic callus cultures induced from immature zygotic embryos of VIR – 17 and CHI – 31 (Erdelská, Vidovencová 1994).

For histological analysis of caryopses the paraffin (Histo-plast S – Serva) technique was used. Sections were stained by haematoxylin according to Heidenhain or by the periodic acid – Schiff reaction (PAS).

RESULTS AND DISCUSSION

Three different types of polyembryony were observed in maize caryopses of studied maize genotypes:

a) Two or more embryos may arise from the egg cell of two or more embryo sacs in one ovule. Two embryo sacs may originate from two functioning megaspores instead of only one. Ustinova (1960) reported the occurrence of two very similar embryo sacs with antipodal complexes of both in close contact and the egg cells localized on opposite poles in one ovule. Exceptionally, she also observed the further development of four megaspores. Individual embryos from two embryo sacs are usually surrounded with their own endosperm and situated on opposite poles of the caryopsis.

Two or more embryo sacs in one ovule may also arise as a consequence of apomictic embryo sac formation from archesporial cells or somatic tissues of the ovule (Chebotar 1976, Zverzhanskaya et al. 1980). The tendency to apomixis is mainly connected with some genotypes. Embryos are usually diploid rarely haploid.

b) Two or more diploid and/or haploid embryos may originate from multiple egg cells, fertilized (in case of polyspermy) or unfertilized synergids besides the embryo from zygote or egg cell (Kermicle 1971, Ustinova 1960). Embryos from individual cells of the normal or multiple egg apparatus are closely adherent, but in all parts strictly separated by an epidermis (Fig. 1). Roots and shoots germinate separately. The scutellum forms are adjusted to the limited space conditions in the

Fig. 1. Embryos (e1, e2, e3) from triplet in the micropylar region of one embryo sac of Golden Bantam, bar = 0,5 mm.

Fig. 2. The developing cleavage polyembryo with common suspensor (s) and extended radicular region (VIR - 17), bar = 1 mm.

Fig. 3. Germinating cleavage polyembryo with radicular complex and separated plumules (VIR - 17).

Fig. 4. Suspensorial polyembryony (e - normal embryo 20 days after pollination, se - suspensorial embryo delayed in its development), bar = 0,1 mm.

embryo sac. In this type of polyembryony besides the most frequent diploids also haploids, aneuploids and triploids were described according to presence of fertilization, polyspermy and the occurrence of parthenogenetic or androgenetic development. The number of haploids and polyembryos may be increased by selection (Judin and Chvatova 1965) or by the artificially delayed pollination (Tyrtov and Enaleeva, 1983).

Chemically induced adventive embryony from the somatic tissues of the ovule in some maize hybrids was described by Hu et al. (1991).

c) The early zygotic proembryo may give rise to 2-3 cleavage embryos spontaneously or after treatment of caryopses with 2,4 - D (Erdelská, Vidovencová 1992). Morgan and Rappleye (1951) induced a very similar phenomenon by X-irradiation of pollen used for pollination. Cleavage embryos are diploid. They share a common suspensor, common parts of the scutellum and also common surface layers of the radicle (Fig. 2.). In consequence of this fact, the polyembryonal unit germinates with two or three individual shoots and with the root complex consisting of two or three partly concrescent primary roots (Fig. 3).

The additional meristemization of some suspensor cells and the delayed development of the second embryo from the suspensor as special type of cleavage polyembryony *in vivo* was observed in two caryopses of VIR - 17 (Erdelská and Vidovencová 1992). This phenomenon may arise as a result of precocious interruption of the embryo - suspensor connection (Fig. 4). The suspensorial embryo probably degenerates very soon, before its differentiation.

Cleavage polyembryony is relatively frequent also in somatic embryo development from immature zygotic embryos *in vitro* (Erdelská and Vidovencová 1994). Characteristic for the development of polyembryonal complexes are the widened radicular meristems (later radicular complex), common scutella and separated plumular poles.

The histological analysis enables to distinguish the different types of polyembryony in maize caryopses connected with the different origin of twins or triplets. The crucial differences are: a) in the localization of embryos in the caryopsis, b) in the structure of embryos, mainly in presence or absence of some common tissues or cell layers and c) in the type of germina-

tion. However, the analysis within the main types requires a chromosomal and detailed developmental characterization.

LITERATURE CITED

- CHEBOTAR A.A., 1976, Some cases of apomixis in maize In: S.S. Chochlov: Apomixis in breeding. Amerind Publ. Co. Pvt. Ltd. New Delhi, pp. 102-108.
- ERDELSKÁ O., VIDOVENCOVÁ Z., 1992, Cleavage polyembryony in maize. Sex. Plant Reprod. 5: 224-226
- ERDELSKÁ O., VIDOVENCOVÁ Z., 1994, Cleavage polyembryony *in vivo* and *in vitro*. Biologia plantarum 36: 329-334
- HU G., LIANG G.H., WASSOM C.E., 1991, Chemical induction of apomictic seed formation in maize. Euphytica 56: 97-105
- JUDIN B.F., CHVATOVA M. N., 1965, The cytogenetical study of twins in diploid and tetraploid maize. Genetika (Moskva) 4: 131-137 (in Russian).
- KERMICLE J.L., 1971, Pleiotropic effect on seed development of the indeterminate gametophyte gene in maize. Am. J. Bot. 58: 1-7
- KIESELBACH T.A., 1926, False polyembryony in maize. Am. J. Bot. 13: 33-36.
- MORGAN D.T., RAPPLEYE R.D., 1951, Polyembryony in maize and lily following X - irradiation of the pollen. J. Hered. 42: 91-93
- PESEV N., PETROVIC R., LECEVIC L., MILOSEVIC M., 1976, Study of possibility in raising maize inbred lines with two embryos Theor. Appl. Genet 47: 197-201
- RANDOLPH I. F., 1936, Developmental morphology of the caryopsis in maize. J. Agric. Res. 53 : 881 - 916
- SILVA J., MAGNAVACA R., BARROS FREIRE A., 1971, Poliembrione no milho "dentato composto". Pesqui Agropecu Bras Ser. Agron. 6: 103-107
- TYRNOV V. S., ENALEEVA N.CH., 1983, The autonomous development of embryo and endosperm in maize. Dokl. Akad. Nauk SSSR 272: 722-723 (in Russian).
- USTINOVA E.I., 1960, Peculiarities of the female gametophyte development and the polyembryony in maize. Bot. Zh. 45: 765-769 (in Russian).
- ZVERZHANSKAYA L. C., GRISHINA E. V., KOMAROVA P. I., The cytobryological study of haploidy and polyembryony in restitution lines of maize. Citologia i genetika (Kyev) 14: 3-9 (in Russian).

POLIEMBRIONIA KUKURYDZY - ANALIZA HISTOLOGICZNA

STRESZCZENIE

Histologiczna analiza umożliwiła rozróżnienie głównych typów poliembrii kukurydzy zgodnie z pochodzeniem zarodków, zależnym od ich położenia w ziarniaku, różnic struktury zarodków (obecność wspólnych tkanek) i sposobu kiełkowania.

Bliźniacze zarodki powstałe z dwóch woreczków zalążkowych znajdują się zwykle w odległych miejscach po przeciwnych stronach caryopsis, nie mają one wspólnych tkanek i kiełkują oddzielnie. Bliźnięta lub trojaczki powstałe z komórek aparatu jajowego lub wielokrotnych komórek jajowych ściśle przylegają ale są wyraźnie oddzielone warstwami epidermy. Bielmo jest wspólne; plumule i radicle są oddzielone.

Rozszczepieniowe poliembryony powstające spontanicznie *in vitro* lub po indukcji mają wspólny suspensor i częściowo wspólne scutellum i zewnętrzne części radicle, dlatego przy kiełkowaniu pojawia się jeden kompleks radikularny z oddzielnymi plumulami.

SŁOWA KLUCZOWE: kukurydza, wielozarodkowy, bliźniaczy, potrójny.