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BRIEF COMMUNICATION

Somatic embryogenesis of maize hybrids: histological analysis

O. ERDELSKÁ and B. SÝKOROVÁ*

Institute of Botany, Slovak Academy of Sciences, Dúbravská 14, 84223 Bratislava, Slovak Republic
*Zeainvest a.s., Trstínska 1, 91700 Trnava, Slovak Republic**

Abstract

The immature zygotic embryos of reciprocal maize hybrids (CHI-31 × GF_1 and CHI-31 × GE_2) were used as the initial material for induction of somatic embryogenesis *in vitro*. Histological analysis of somatic embryogenesis revealed high developmental variability. The arising formations were classified into 5 groups: *A*) somatic embryos phenotypically similar to zygotic embryos, *B*) polyembryos, *C*) formations with radicle but without meristematic plumule, *D*) formations with radicle without differentiated plumule, and *E*) formations with plumule without radicle. The formatioms *A* and *B* regenerated directly into plants. Plant regeneration from formations *E* required preculture on the rooting medium. Formations *C* and *D* failed to develope into plants possibly because of early loss of meristematic cell character during the embryo axis differentiation. The reverse sequence of radicle and plumule differentiation in somatic embryos in comparison with zygotic ones was noted. The epigenetic character of the scutellum, coleoptile, coleorhiza and leaves primordia development was discussed.

Additional key words: morphogenesis, plumule, polyembryos, radicle, scuttellum, *Zea mays*, zygotic embryos.

The competency for morphogenesis in maize embryos is strongly dependent on genotype (Hodges *et al.* 1986, Tomes and Smith 1985, Willman *et al.* 1989) and is cross-transmissible (Bruneau 1985). This assumption was the reason for experiments with the induction of somatic embryogenesis from zygotic embryos of reciprocal hybrids originated from responsive genotype CHI-31 with some other non-responsive

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but very valuable genotypes. The embryogenic callus formation in CHI-31 was observed on 96 % of cultivated zygotic embryos. In hybrids represented this value only 40 - 45 %. The number of plantlets arising from one embryogenic callus in all hybrids was evidently lower (0.3 - 0.5 in hybrids of CHI-31 × GF_1 and 0.8 - 0.9 in CHI-31 × GE_2) than that of the genotype CHI-31 (1.7 - 2). Therefore the histological analysis was used to elucidate developmental disturbances responsible for the reduction of phenotypically normal plantlets number arising from zygotic embryos of hybrids.

For induction of somatic embryogenesis excised zygotic embryos from reciprocal hybrids of the genotype CHI-31 with genotypes GF_1 and GE_2 were used. Mother plants were cultivated in the glasshouse.

Embryos (1 - 1.5 mm long) were asseptically excised from grains 14 d after pollination. They were placed on the nutrient medium in 100 cm^3 Erlenmayer flasks (7 embryos in one flask), with the scutellum exposed and the embryo axis embedded in the medium. For embryogenic callus initiation N6 medium (Chu 1976) with 120 g dm^{-3} saccharose and 5 mg dm^{-3} 2,4-dichlophenoxyacetic acid (2,4-D) was used. Embryos were cultivated at 24 - 26 °C and 16/8 light/dark period (irradiance of 60 μmol(PAR) m^{-2} s^{-1}). In two repeated experiments 140 embryos of each hybrid and also of control genotype (CHI-31) were used for the evaluation.

The explants were cubcultured in 15-d-intervals (4 - 5 times) on the same medium and than on the medium N6 without 2,4-D with the lowered content of saccharose (60 mg dm^{-3}). The young plantlets were continuously separated from the mother explants and subcultivated. Plantlets 4 - 7 cm high were subcultivated on sterile perlite with half-MS solution (Murashige and Skoog 1962) in closed flasks for 7 d. The plants were than cultivated 5 - 7 d on the same medium in opened flasks and finely in the non sterile soil in the glasshouse, to the maturity.

In some cases shoots have been developed without roots. They were subcultured on the rooting medium containing macro- and micro-elements of the medium B5 (Gamborg *et al.* 1968), with 1.25 mg dm^{-3} naphthaleneacetic acid (NAA) and 1.8 mg dm^{-3} indole-3-acetic acid (IAA). After root regeneration were these plants subcultivated in perlite and later in the soil.

Ten embryogenic calli of each hybrid in two repeated experiments were fixed in FAA [formaline (5 cm^3) + acetic acid (5 cm^3) + 50 % etanol (90 cm^3)] successively between 7 - 45 d of cultivation. They were embedded in *Histoplast S* (*Serva*) and sectioned at 8 - 12 μm. Sections were stained using periodic acid-Schiff (PAS) reaction or Heidenhain haematoxylin.

The development of somatic embryos was observed from tissues of radicular parts of scutella in all studied embryos. This part of the scutellum is delayed in its determination (Vasil *et al.* 1985). Very often was observed the formation of coherent meristematic zone in the subsuperficial area of the scutellum. The differentiation of somatic embryos from cells localizes outside this zone was observed more often.

In embryogenic explantates it was possible to distinguish the development of different embryonal formations, which may be divided into characteristic groups:

A) Phenotypically normal embryos with clearly defined plumule, radicle, scutellum, coleoptile and suspensor (Fig. 1). Leaf primordia (1 - 2) may be

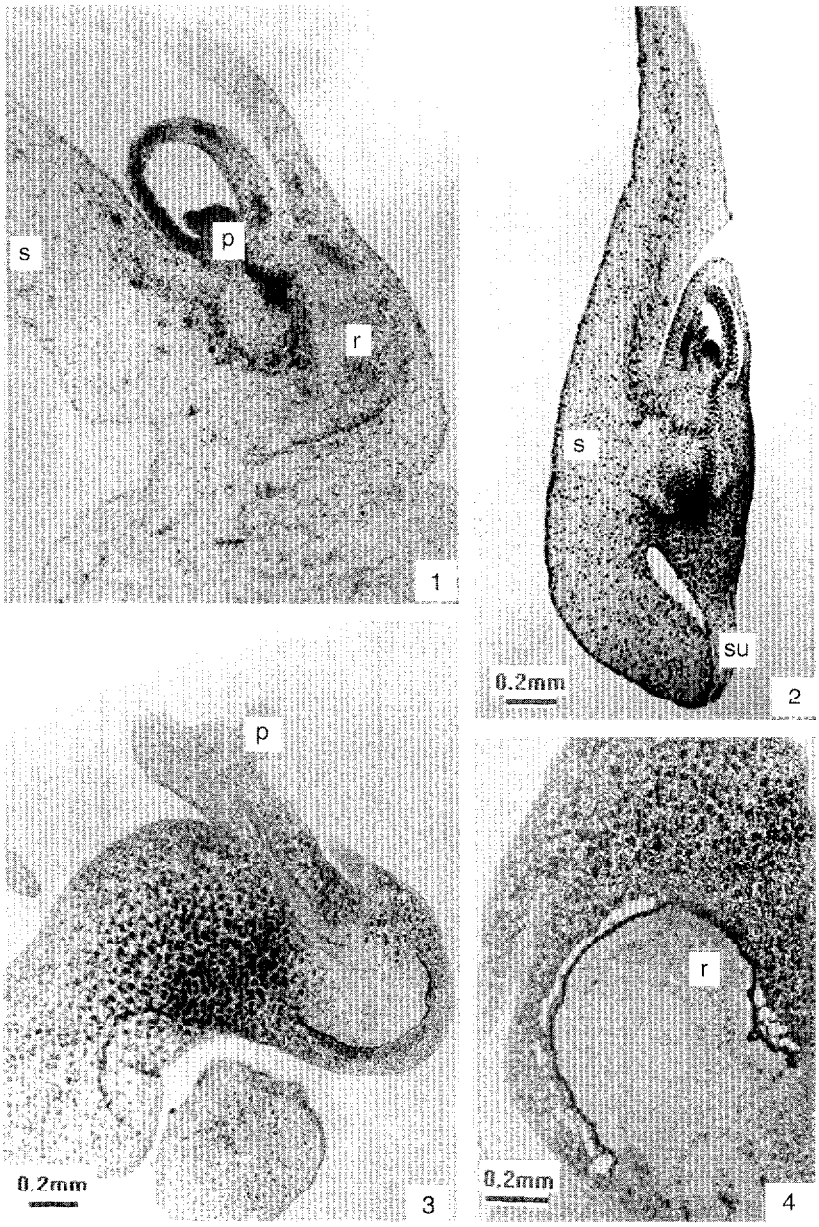

Fig. 1. Somatic embryo of the hybrid CHI-31 × GF_1 (group A of the development).
Fig. 2. Zygotic embryo of maize excised 20 d after pollination.
Fig. 3. Somatic embryo of the hybrid CHI-31 × GE_2 (group C). Plumule is not meristematic.
Fig. 4. Transversal section through the polyembryonal unit in the polyradicular area, showing only two vascular poles (*arrow*) in the radicle (GE_2 × CHI-31) (*p* - plumule, *r* - radicle, *s* - scutellum, *su* - suspensor).

differentiated on the plumule, too. These embryos are structurally very similar to the zygotic ones, but the form, size and position of the scutellum is variable. Whereas the scutellum in zygotic embryos closely embraces the embryo axis in its plumular part (Fig. 2), the scutellum of the somatic embryo is often irregular and declined from the embryo axis similarly to the scutella of germinating zygotic embryos. In the radicular part of somatic embryos is the scutellar tissue fused with the coleorhiza and suspensor-like callus tissue.

B) Very frequent are polyembryonal formations with enlarged plumular meristems and polyradicular poles. Their declined scutella have different form and size in connection with spatial conditions in the area of their occurrence.

C) Formations which have relatively normaly differentiated radicle, and scutellum, but the plumule is deprived of the meristematic character (Fig. 3).

D) Formations with clearly defined radicular or often polyradicular poles but without differentiated plumules. The tissue orientation and localization of these formations corresponds with that of completely differentiated somatic embryos (radicle to the centre of the explantate and plumule to its surface).

E) Relatively rare are formations with differentiated plumule, sometimes also with the apical part of the scutellum, but without radicle. Connection of these formations to vascular tissues of the callus is usually not evident. These formations usually give rise to complete plants on the rooting medium.

In all groups (*A* - *E*) were somatic embryos or embryo-like structures orientated with their apical (plumular) part to the surface of the explantate. In groups *A* - *D* are the radicular poles evidently separated from tissues of the suspensor or callus by the polysaccharide (slime) layer, intensively stained by PAS reaction. Very low number (2 - 6) of vascular tissues poles in radicles of the somatic embryos in comparison with that of zygotic embryos in the same hybrids has been recorded on the transversal sections (Fig. 4). The development of leaf primordia in somatic embryos was usually retarded and restricted to only two. Adventive root primordia were not observed.

Only normally developed plumulo-radicular axis of the embryo give rise to the phenotypically normal plant. Therefore it is possible to suppose the normal embryo and plant development only in groups *A* and *B*. The differentiation of somatic embryos in groups *A* and *B* corresponds with the results of Novák and Doleželová (1983), Vasil *et al.* (1985) and Emons with Kieft (1991) for other genotypes of maize.

The defects (deviations) of somatic embryo development in groups *C* and *D* may be caused by the fact, that the sequence of plumule and radicle differentiation in the explants is reverse in comparison with that of the zygotic embryo. While in zygotic embryos the plumule formation slightly preceeds that of the radicle, the sequence of these processes in the explants is opposite. The loss of the meristematic character of some tissues during embryo differentiation before completion of plumule differentiation (*C*) or already before the beginning of plumule differentiation (*D*) may be caused by undesirable change of microconditions in cultivation medium (late subcultivation, *etc.*).

Plantlets and later plants arising from somatic embryos were evidently smaler (70 - 80 cm high) in comparison with those cultivated from seed embryos (120 - 170 cm high).

Relatively high structural variability of somatic embryos, giving rise to phenotypically normal plants initiated the question about the influence of environmental conditions on the embryo development. Our observations contribute to the fact of the epigenetic control of the scutellum (size, form, localization) development. Coleoptiles are very often concrescent with tissues of the scutellum and lose their meristematic chatacter. It is problematic to distinguish tissues of the scutellum, coleorhiza and suspensor in the radicular area of the somatic embryo. The results of Emons and Kieft (1991) describing the absence of scutellum, leaves and coleoptile of somatic embryos in the suspension culture and their presence in the callus culture of the same genotype testify also the epigenetic control of scutellum, coleoptile and leaf primordia development. The epigenetic control of scutellum, coleoptile, coleorhiza and primordia of leaves and adventive roots development is similar for zygotic and somatic embryos. This phenomenon is possible to observe namely in the development of excised zygotic embryos *in vitro*.

The control of the suspensor development is also partially epigenetic. However, some differences in size (cell number) and the development of the suspensor between zygotic and somatic embryos may be caused by the influence of maternal genome templates acting in early zygotic embryogenesis but not in the early somatic embryogenesis.

In the callus culture it is possible to initiate and observe relatively high form (shape) variability of arising somatic embryos taking into account the variability of nutritional, spatial, microclimatic, *etc.*, conditions on different places of the explantate and in different time of their origin in relation to the intervals of subculturing. Therefore all comparative structural studies of somatic and zygotic embryos are problematic.

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