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Somatic Embryogenesis in *Zea mays* L.

C. Lu, I. K. Vasil and P. Ozias-Akins
Department of Botany, University of Florida, Gainesville, Fla. (USA)

Summary. Immature embryos of 12 genotypes of *Zea mays* L. were cultured on agar nutrient media containing various concentrations of 2,4-dichlorophenoxyacetic acid and sucrose. An opaque, white, compact and organized callus tissue was formed by the proliferation of the scutellar cells present in the vicinity of the scutellar node near the coleorhiza. Well-organized somatic embryos were formed on the surface of the proliferating scutellar callus within about three weeks. The embryos showed the typical organization of grass embryos with a scutellum, coleoptile and coleorhiza, and germinated to form normal green plants which were grown to maturity. An embryogenic cell suspension culture obtained from the scutellar callus is now being used for the isolation and culture of protoplasts.

Key words: *Zea mays* L. – Tissue culture – Somatic embryogenesis – Plant regeneration

Introduction

All of the major species of cereals and grasses can now be clonally propagated in vitro (Green 1978; Vasil 1981; Vasil and Vasil 1980). However, plant regeneration is often found to be sporadic and transient, and only a few genotypes of each species respond favorably to morphogenetic stimuli. The cultures tend to rapidly lose the potential for regeneration. Plants are thought to be formed by the derepression of presumptive shoot primordia which proliferate adventitiously in vitro by the process of micro-tillering (Dunstan et al. 1979; Thomas et al. 1979; Brettell et al. 1980).

Plant regeneration from tissue cultures of *Zea mays* (corn, maize) was first reported by Green and Phillips (1975). These results have been successfully repeated by many other workers, but the fact remains that plant regeneration is limited to only a few genotypes, with much of the published information confined to the inbred line A188. Plant regeneration in maize callus cultures takes place by the de novo organization of shoot meristems in scutellar callus (Springer et al. 1979).

The phenomenon of somatic embryogenesis in tissue cultures of cereal and grass species has been discovered only recently. We have provided extensive evidence of the formation of somatic embryos in callus, cell suspension and protoplast cultures initiated from immature embryos, young inflorescences or leaves of several species of the Gramineae (Vasil and Vasil 1980, 1981 a, b, 1982; Lu and Vasil 1981 a, b, 1982; Haydu and Vasil 1981; Lu et al. 1981, Ozias-Akins and Vasil 1982; Wang and Vasil 1982). We now describe the regeneration of plants through somatic embryogenesis from cultured immature embryos of *Zea mays* L.

Materials and Methods

Immature embryos (1–1.5 mm in length) were excised aseptically from open-pollinated plants of 12 randomly selected genotypes of maize (Table 1). Thirty embryos of each genotype were placed on MS (Murashige and Skoog 1962) or the N_6 (Chu et al. 1975) nutrient medium containing various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D; 0.25–5.0 mg/l) and sucrose (0–12%). The media were adjusted to a pH of 5.8 before autoclaving. All cultures were incubated in dark at 27 °C.

Embryoids formed in vitro and the associated tissues were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), rinsed with buffer and then post-fixed in 1% osmium tetroxide. After ethanol dehydration, the samples were critical point dried and coated with gold. The prepared specimens were examined and photographed in a Hitachi S-450 Scanning Electron Microscope operating at 20 KV.

Results and Discussion

In our initial experiments, conducted during the summers of 1979 and 1980, we cultured immature embryos

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Table 1. Number of immature embryos (in %) of *Zea mays* forming embryogenic callus tissue. Thirty embryos were cultured on each medium, and determination of the embryogenic/non-embryogenic nature of the callus produced was made 12 days after culture

Medium Genotype	MS + 0.25 mg/l 2,4-D + 6% sucrose	MS + 0.5 mg/l 2,4-D + 6% sucrose	MS + 0.5 mg/l 2,4-D + 9% sucrose	MS + 0.5 mg/l 2,4-D + 12% sucrose	N_6 + 0.5 mg/l 2,4-D + 9% sucrose
Asgrow Rx 112	6.7	10.0	6.7	10.0	10.0
Coker 16	3.3	10.0	3.3	13.3	0
Coker 22	3.3	10.0	10.0	26.7	3.3
Dekalb XL 80	3.3	3.3	3.3	10.0	0
Dekalb XL 82	20.0	13.3	13.3	30.0	6.7
Funks G 4864	20.0	16.7	26.7	23.3	0
Funks G 4507 A	0	0	10.0	10.0	10.0
Jac 247	3.3	6.7	10.0	16.7	6.7
McCurdy 8190	3.3	6.7	10.0	10.0	0
Pioneer 1360	0	0	0	3.3	0
Pioneer 3320	6.7	6.7	20.0	6.7	10.0
Silver Queen	–	30.0	40.0	33.0	–
Average	6.4	9.4	12.8	16.1	4.2

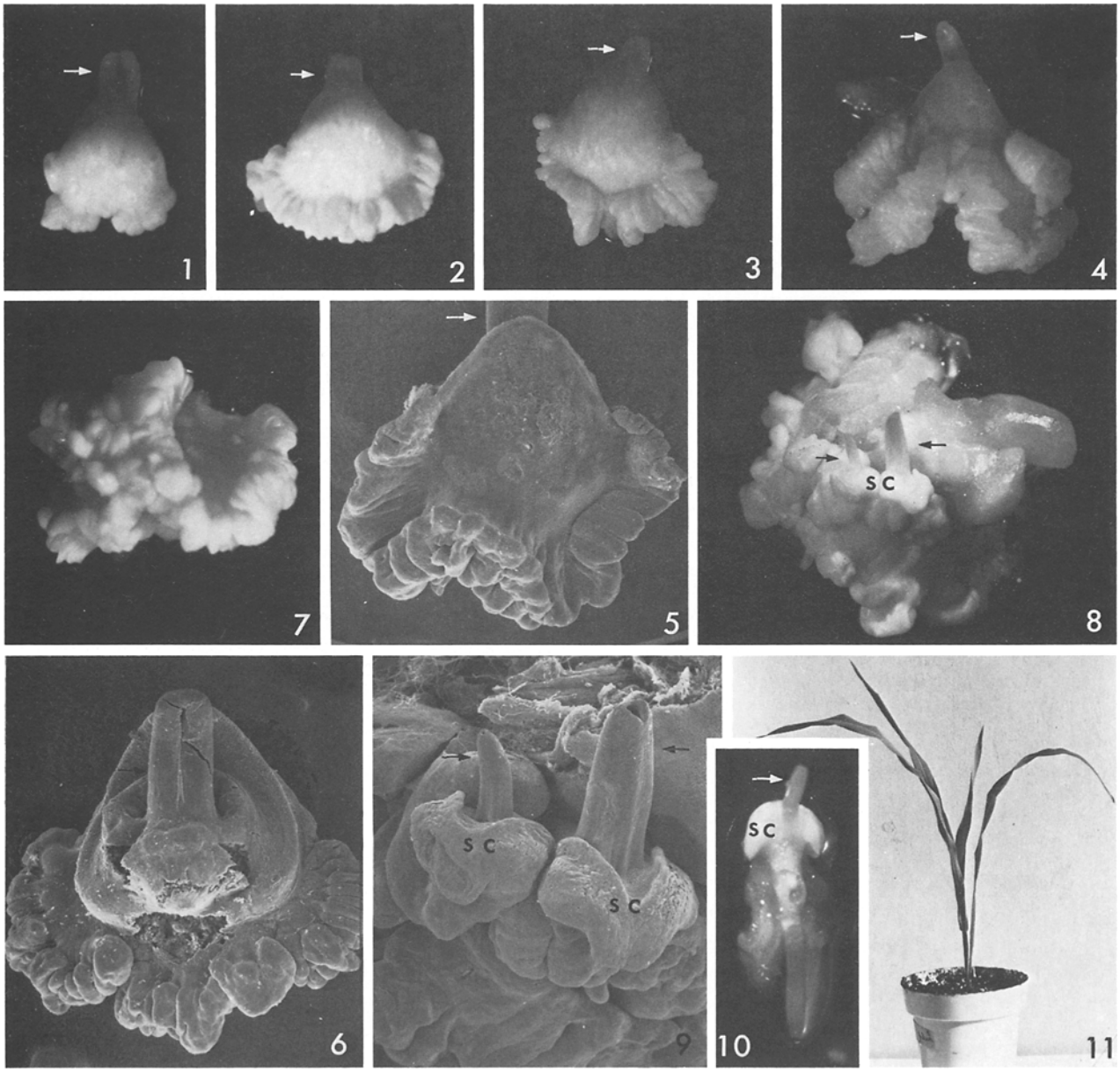
of maize (A188 and W64A) and obtained callus cultures which formed green leafy structures, shoots and some embryo-like structures. In tissue cultures of wheat and pearl millet similar green leafy structures formed before the development of shoots have been interpreted as the scutellum of precociously germinating embryos (Ozias-Akins and Vasil 1982; Vasil and Vasil 1982). This observation led us to a more detailed investigation of somatic embryogenesis in maize during the summers of 1980 and 1981. These experiments showed that a combination of 0.5 mg/l 2,4-D and 12% sucrose was most suitable for the formation of embryogenic callus tissues from the scutellum of immature embryos. Higher concentrations of 2,4-D were less effective, and no embryogenic callus was formed at 4 or 5 mg/l 2,4-D.

Five combinations of 2,4-D and sucrose in MS or N_6 medium were used (Table 1). The immature embryos were chosen by size (1–1.5 mm) rather than age because considerable variability was found in material selected on the basis of age of the embryos. Embryos were placed on the agar medium in a face-down position so that the embryo axis was in contact with the medium and the scutellum was exposed to air. Proliferating callus tissue could be seen on the coleorhizal end of the embryo one week after the initiation of cultures (Fig. 1). The coleoptile end of the scutellum and the embryo axis were not involved in callus formation (Fig. 6). The callus tissue formed was opaque, white, compact and with prominent ridges and furrows (Figs. 1–7), and similar to the embryogenic callus tissues described from immature embryos of *Pennisetum americanum* (Vasil and Vasil 1981a) and *Panicum maximum* (Lu and Vasil 1982). The scutellar embryogenic callus of maize proliferated slowly (Fig. 7). Embryogenic callus tissue was obtained from the scutellum of the immature embryos of all of the genotypes used (Table 1). Some genotypes were clearly better than others. However, no attempt was made to obtain mature embryoids or plants from all the genotypes. Plants were regenerated by somatic embryogenesis only from 'Silver Queen' which gave the best results (Table 1).

Well organized somatic embryos were formed on the surface of the proliferating scutellar callus within three weeks (Figs. 8, 9). The embryoids showed bilateral or radial symmetry. It is noteworthy that embryoids were formed on the same 2,4-D containing media where the initital proliferation of the scutellum was obtained. As is the case with most other cereals and grasses, it was not necessary to transfer the callus cultures to lower concentrations of 2,4-D. The embryogenic callus was sub-cultured three times and maintained for a little over 2 months. Prolonged culture was not possible because of extensive organization within the callus mass and the formation of green leafy structures which likely represent the scutellum of precociously germinated embryoids (see Vasil and Vasil 1982; Ozias-Akins and Vasil 1982; Wang and Vasil 1982).

The embryoids were transferred singly to MS medium without 2,4-D but containing 1 mg/l gibberellic acid to induce germination (Fig. 10). The germinating embryoids were then placed on half-strength MS medium and allowed to develop into plantlets. The latter, with several well developed green leaves and roots, were transplanted into soil in small pots, acclimatized for a few days in a growth chamber (Fig. 11), and finally moved to the greenhouse and grown to maturity.

Based on our earlier experience with *Pennisetum americanum* and *Panicum maximum* (Vasil and Vasil



Figs. 1 – 11. Somatic embryogenesis and plant regeneration from immature embryos of *Zea mays* (arrows indicate coleoptile; the coleorhiza is facing downward in all the photographs; SC = scutellum). **1** One week old cultured immature embryo. White and compact callus can be seen on the coleorhiza end of the scutellum (× 10); **2, 3, 5** Nine day old cultured immature embryos (Fig. 2. × 10; Fig. 3. × 10; Fig. 5. × 20); **4** Thirteen day old cultured immature embryo (× 10); **6** Thirteen day old cultured immature embryo in axis view showing that the embryo axis is not involved in callus formation (×20); **7** Proliferating immature embryo callus (× 10); **8** Three week old culture showing two embryoids (× 10); **9** Scanning electron micrograph of the two embryoids shown in Fig. 8. (× 20); **10** Germination of embryoid (× 10); **11** Plant regenerated via somatic embryogenesis (× 3.2)

1980, 1981b; Lu et al. 1981; Lu and Vasil 1981b), we have isolated stable embryogenic cell suspension cultures from the scutellar callus tissues of maize, and are using these as a source of protoplasts in attempts to develop a suitable single cell system for maize. These observations will be published elsewhere.

Somatic embryogenesis has also been recently observed in callus tissue cultures obtained from the scutellum of immature embryos of the inbred line A188 of maize (Green, personal communication). The embryoids showed the normal organization of grass embryos and germinated to give rise to normal green plants.

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Prof. Dr. I. K. Vasil
Department of Botany
University of Florida
Gainesville, Fla. 32611 (USA)