A Histological Examination of Tissue Culture Initiation From Immature Embryos of Maize

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Summary

The tissue origin, growth patterns and organization of in vitro tissue cultures initiated from immature maize (Zea mays L.) embryos were cytologically examined. Examination of thick sections from initiating cultures indicated that the scutellum is the tissue of origin. During culture initiation the scutellum, especially in the region of the epithelium, becomes highly meristematic and proliferates rapidly. These cultures were characterized by a cambial zone which provided for extended in vitro proliferation and by apical meristems developing on the culture surface. These meristems developed to the two leaf primordia stage under the cultural conditions of this study. Differentiation of root primordia associated with the apical meristems was not observed. The apical meristems appeared to arise de novo at the tissue surface and were probably multicellular in origin. The observations are discussed in relation to their significance concerning mutation and selection.

Keywords: Culture initiation; Embryo culture; In vitro proliferation; Zea mays L.

1. Introduction

The utility of cell and tissue culture techniques in the study of plant and cell biology (BARZ et al. 1977; SMITH 1974), as well as the potential utility in plant improvement (NELSON 1977, PHILLIPS 1977), has provided the impetus for identification of appropriate in vitro cultures of plant cells from a wide array of species and tissue sources. An important element in the usefulness of these systems is the ability to regenerate plants from the in vitro cultures. This has

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been achieved for many species. The cereals, however, have been rather recalcitrant to the establishment of cultures with consistent plant regenerative capacity. One notable exception has been the use of excised immature embryos of several cereals (maize—Green and Phillips 1975, oats—Cummings et al. 1976, sorghum—Gamborg et al. 1977, Thomas et al. 1977) to establish cultures from which plants can be regenerated.

Mott and Cure (1978) cytologically examined maize tissue cultures derived from four different somatic sources from which plant regeneration has not been achieved. Their observations indicate an aberrant root-like growth pattern for all tissues examined rather than an unorganized, undifferentiated callus growth pattern. They suggest that shoot regeneration may be precluded in instances where in vitro culturing simply results in the proliferation of differentiated root tissue. A similar situation was observed for in vitro tissue from wheat and oat seeds (Cure and Mott 1978). This is in contrast to the situation for rice where initial root-like growth can be altered to an undifferentiated growth form from which shoots could be regenerated (Lai 1971, Lai and Liu 1971).

These results raise developmental questions regarding the in vitro culturing of immature embryos. We have sectioned isolated embryos and cultures initiated from embryos to gain insight into the following questions: 1. Is the scutellum the source of in vitro cell proliferation? 2. What is the fate of the various tissues in the original explant during culture initiation? 3. To what degree is culture growth the result of organized tissue proliferation as opposed to unorganized cell proliferation (true callus growth)?

2. Materials and Methods

2.1. Culture

Immature embryos (1.0–1.5 mm in length) were excised aseptically from ears of inbred A188 which had been sib-pollinated. These embryos were placed on Murashige and Skoog (MS) medium containing 0.5 mg per liter 2,4-dichlorophenoxyacetic acid (2,4-D), 1 mM L-asparagine, and 0.7% Bacto agar for culture initiation as described earlier (Green and Phillips, 1975). Incubation was at 28 °C with a 16-hour photoperiod from cool-white fluorescent lights. Control embryos were fixed immediately after isolation from the ear. Embryos and

Fig. 1. Tangential longitudinal section from a control (1.0–1.5 mm) embryo excised from an immature ear of inbred A188. A Entire section showing the various tissues present in the young embryo. Note the single cell layer epithelium covering the scutellum (×200). B Enlargement of central portion of A. The initial signs of vascularization can be seen as a group of elongated cells originating in the region of the scutellar node. The degree of differentiation in the radicle also can be seen (×350). P plumule; R radicle; Su suspensor; Sc scutellum; V early signs of vascularization

Fig. 2. Longitudinal section through the radicle of a control embryo showing the state of differentiation. The calyptrogen (Ca) is clearly distinct from the plerome (Pl) and periblem (Pe). ×700