Morphogenesis from Cultured Leaf Tissue of *Sorghum bicolor*—
The Morphogenetic Pathways

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Abbreviations:

- 2,4-D = 2,4-Dichlorophenoxyacetic acid
- Kinetin = 6-Furfurylaminopurine
- 6-BAP = 6-Benzylaminopurine
- MS = Murashige and Skoog

Summary

Immature leaf explants of *Sorghum bicolor* (L.) Moench can be stimulated *in vitro* to form roots, shoots or embryos. When the cultures were maintained with the high 2,4-D level which was essential for optimal culture initiation, the organs or embryos proliferated as suppressed primordia, but they could always be identified by simple histological means. Perivascular cells of comparatively old but still immature leaf sheath regions appeared to be strongly determined to form adventitious roots or root-type "callus" cultures. We have evidence that the embryogenic tissue, and ultimately the embryos, are of multicellular origin. This ontogeny of the embryos appears to be contradictory to the often stated view that somatic embryos generally arise from single "committed" cells. The implications of these findings for basic and applied research on cereal tissue culture are discussed.

Keywords: Cell-lineage; Cereal tissue culture; Somatic embryogenesis; *Sorghum bicolor*.

1. Introduction

Certain cells in plant tissues are capable of sustained divisions, resulting in a proliferating, apparently disorganized mass of cells termed callus, when cultured *in vitro* on artificial culture media containing nutrients and an appropriate supply of growth regulators. In a large number of species new organs and eventually complete new plants may be regenerated on a large scale from such calluses. This has led to the often stated impression that the cells in a callus proliferate as a uniform population of "dedifferentiated, totipotent" cells from which plants can be "initiated" at will.

An alternative view which has been put forward is that, at least in many cases, organogenesis is already induced very early, namely during initiation of the culture. The resulting calluses are then nothing else but a mass of primordia of various sizes (e.g. pro-embryogenic masses, HAPRIN and JENSEN 1967; organized aggregates, THOMAS and STREET 1970) which are kept in a proliferating yet suppressed state by the exogenously supplied growth regulators. Such calluses may be interspersed with rapidly growing but non-morphogenic cell-line type cells. This approach has recently been summarized (THOMAS and WERNICKE 1978).

The significance of this view once again becomes evident as scientific interest is increasingly focused on tissue culture of cereals. This economically important plant group has proved to be extremely recalcitrant to *in vitro* culture techniques, particularly with regard to culture initiation and reliable plant regeneration. However, highly regenerative cell cultures would be extremely useful for studies on somatic cell genetics (KING et al. 1978).

In previous reports where *Sorghum bicolor* was used as a model cereal we have suggested that one reason for our difficulties may be the reluctance of mature cells to

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“dedifferentiate” in vitro, in contrast to most dicotyledonous species (Wernicke and Brettell 1980, 1981, see also Wernicke et al. 1981). The only reliable sources for tissue culture appear to be explants taken from regions which are still close to a meristematic state in vivo. In a preliminary study when using immature leaf explants we could demonstrate that Sorghum cells were also capable of forming somatic embryos in addition to proliferic root formation (Wernicke and Brettell 1980). It was proposed that the actual induction of embryogenesis occurred very early in culture. We now present further detailed histological studies on the initiation and maintenance of the organogenic and particularly the embryogenic potentialities during culture.

2. Materials and Methods

Culture Initiation and Maintenance

Cultures were initiated from immature leaf sections of Sorghum bicolor (L.) Moench as described previously (Wernicke and Brettell 1981). In the present study immature regions of the 3rd and the 4th foliage leaves of 10-day old plants were taken. The MS culture medium (Murashige and Skoog 1962) supplemented with 40 g l⁻¹ sucrose was used as standard basal medium throughout all experiments. Individual callus pieces were selected under a stereo microscope and were transferred every 5-6 weeks to fresh culture media. The cultures could be grown in the dark or in the light without significant difference in response, but green leaves could only be obtained in the light. In addition to the variety G 522 (seeds were a gift from Funk Seeds International) which was already studied extensively, the following varieties were included in the experiments: Oro (Funk Seeds), Plainsman, Caprock, WAC 692, FS 115, Regular Hegari (seeds kindly provided by Dr. O. J. Webster, University of Arizona, Tucson). The histological sections presented in this paper were derived from variety G 522, although unless otherwise stated the responses in the other varieties were similar.

Histology

Emphasis has been put on the spatial reconstruction of the calluses at the cellular level by means of serial microtome sections. For this purpose macroscopically identified samples were fixed in 10% acrolein and dehydrated according to the protocol of Feder and O’Brien (1968). After embedding in Histoplast S (Serva, Heidelberg) the samples were cut on a Meinototary microtome into serial sections of 6-7 μm thickness. In certain instances, in order to get a better cellular resolution, samples were fixed in glutaraldehyde/osmium tetroxide, embedded in an epoxy resin and cut with a glass knife into 2 μm thin sections as described recently (Wernicke and Brettell 1981). In both cases the sections were stained in an aqueous toluidine blue solution.

3. Results

3.1. Cultures Derived from the Third Foliage Leaf

As has previously been reported, cell proliferation in cultured leaf sheath regions of the 3rd foliage leaf occurred preferentially from cells that were close to the vascular bundles (bundle sheath cells?). Serial sections showed that the response was not limited to regions close to lesions caused by excision. On the contrary, in most explants nearly all perivascular cells shared in the response. After a few days in culture massive outgrowths disrupted the non-responding leaf regions (Fig. 1). The protuberances had a characteristic surface structure in that the outermost cells were only loosely attached and embedded in a mucilaginous liquid. Histological examination revealed the presence of a sub-superficial layer with small cytologically rich cells (Fig. 2). It appeared as if growth of the culture predominantly resulted from (periclinal) meristematic activity of this layer by shedding cells to the inside of the culture and to the surface. The surface cells became detached but remained entrapped in the mucilaginous slime (Fig. 3).

Upon subculture onto the same medium used for initiation (MS + 2 mg l⁻¹ 2,4-D) a nodular callus was obtained which again had a sub-superficial meristem-like region of growth. The cultures had a yellowish colour and were entirely covered by mucilaginous cells.