Cytodifferentiation under *in vitro* Conditions

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INTRODUCTION

The induction of xylogenesis in isolated mesophyll cells (1-6) and mesophyll protoplasts (7) of *Zinnia elegans* has stimulated interest in the enigmatic role of cell division in xylogenesis. The *Zinnia* system apparently consists of two cell populations; some cells directly differentiate (1-4), whereas other require cell cycle activity (5,6). The time of arrest in G1 may be a factor that separates the two populations (8,9). Although evidence exists for three mitotic cycles prior to xylogenesis in tuber explants of *Helianthus tuberosus* (10,11), the stage of development of the parent tuber is a decisive factor (12). Gamma irradiation of tubers, sufficient to prevent DNA synthesis and mitosis, completely blocked xylogenesis in explants from mature tubers but not from immature tubers (12'). Since direct xylogenesis occurred in *Zinnia* mesophyll from immature leaves as well as from explants of immature tubers, both systems may have something in common. Although cell arrest in G2 and polyploidy were associated with secondary vascular development in cultured roots of *Helianthus annuus* L. (13) and *Raphanus sativus* L. (14), a causal relationship was not suggested by the authors. The possible role of the cell cycle in xylogenesis has been reviewed (9,15,16).

Aloni (17) examined sieve and tracheary element (TE) differentiation in cal-

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lus cultures of *Daucus carota* L., *Syringa vulgaris* L., *Glycine max* (L.) Merr., *Helianthus annuus* L., *Hibiscus cannabinus* L., and *Pisum sativum* L. The differentiation of phloem invariably commenced before xylogenesis, and TE were never found in the absence of sieve elements. The type of vascular cell differentiated was unaffected either by the level of sucrose or by the auxin-sucrose ratio. The presence of a phloem-localized xylogenic factor was postulated (17), and Savidge and Wareing (18) have suggested that conifer needles may contain a tracheid-differentiation factor.

A positive correlation exists between xylogenesis and the induction of PAL activity in suspension cultures of *Phaseolus vulgaris* L. (19). During secondary wall thickening of TE, xylem synthetase induction was paralleled by the induction of PAL activity in bean callus (20). The same enzyme system played a role in xylem differentiation in the cambial region of *Acer pseudoplatanus* and *Populus robusta* (21, 22). Esterase activity may be used as an early marker of differentiation in *Helianthus tuberosus* explants (23), and, in a similar manner, hydrolase activity, in *Phaseolus lunatus* L. root tips (24). Reviews have appeared on the biochemical events occurring during TE differentiation (25, 26).

The induction of exogenesisis in cultured root parenchyma (*Pisum sativum* L.) consisted of two phases of cold sensitivity: an early cold-sensitive phase and a later cold-insensitive phase: The latter began after xylogenesis had been initiated (27).

Several publications have appeared on the hormonal requirements for xylogenesis. A reinvestigation of the effects of hormones on xylogenesis in explants of *Helianthus tuberosus* showed that GA3 inhibited both xylogenesis and cell proliferation, whereas abscisic acid increased cell number but strongly blocked xylogenesis (28). Pith parenchyma explants of *Coleus blumei* exhibited xylogenesis after 10 days on an IAA-sucrose medium (29). Cyclic AMP may play a role in TE formation in cultured carrot-root slices (30). A basal medium supplemented with citric acid initiated xylogenesis in isolated lemon fruit vesicles (31). Additional aspects of *in vitro* differentiation and phytohormones can be found in Gresshoff’s review (32).
A BIPOLAR GRADIENT TECHNIQUE
FOR STUDYING XYLOGENESIS

Mechanism governing the orientation of vascular differentiation are poorly understood, and tentative explanations involve the spatial distribution of diffusible morphogenes, e.g., auxin and sucrose (33, 34, 35). A study was undertaken to examine the induction of xylogenesis in cylindrical (5 x 10 mm) explants of lettuce (Lactuca sativa L. cv. Lakes) pith parenchyma positioned between two plates containing agar media (36). Various combinations of IAA, zeatin, and sucrose were applied to the opposite ends of the explants by this bipolar gradient technique. Callus proliferation and xylogenesis was limited to one or both ends of the explants; the central region never responded to any of the treatments. TE were found only in association with callus. Xylogenesis was invariably restricted to the explant ends receiving IAA, and, with a few exceptions, xylogenesis also required the presence of zeatin at the same end. Presumably sucrose or its metabolic products moved freely throughout the length of the explants. Xylogenesis was observed in the absence of exogenous carbohydrate in some experiments, and evidence indicated that 3% (w/v) sucrose may be inhibitory to xylogenesis. The developmental responses were influenced to some extent by an inherent tissue polarity and by gravity (36). Sachs (37) has reviewed the control of patterned vascular differentiation and offered an auxin-flux hypothesis. Alternative differentiation hypotheses are given by Mitchison (38) and Wodzicki and Cileagles (39).

CARBON SOURCES FOR XYLOGENESIS

Previous in vitro studies on the induction of xylogenesis have led to the conclusion that a source of exogenous carbohydrate is necessary (40), and there have been no investigations of the possibility that an alternative carbon source may serve this role. A recent study revealed that glocerol and myo-inositol, respectively, functioned as media supplements for the induction of TE formation in the absence of other major C sources (41). Both compounds were employed at a level of 2% (w/v) and the TE produced in lettuce pith explants cultured on such media were indistinguishable from TE produced under similar cultural con-
ditions in the presence of glucose (2% w/v) (41).

**EVIDENCE FOR ETHYLENE AS A HORMONE IN XYLOGENESIS**

The induction of xylogenesis in parenchymatous explants requires exogenous auxin, cytokinin, and a carbon source (40). The hypothesis that ethylene (E) is also a hormone in TE differentiation rests mainly on the following lines of evidence. Stress-induced E production was associated with altered secondary xylem formation (42, 43). The application of E gas or CEPA (ethrel) influenced secondary xylem formation (44, 45). A thigmomorphogenic response was an increased secondary xylem production, and ethrel treatment produced the same effect (46). Xylogenesis in lettuce pith explants was enhanced with exogenous L-methionine (47). In fact, the enrichment of the induction medium with ACC, the immediate precursor to E, greatly stimulated xylogenesis *in vitro* (48). Xylogenesis in callus derived from the Clark 63 cv. of soybean (*Glycine max*) revealed greater numbers of TE in response to exogenous L-methionine at 25°C but not at 20°C. This mutant has a temperature-dependent E biosynthetic pathway that operates only at 25°C. Inhibition of xylogenesis by the addition of AG⁺ to the induction medium was partially reversed by exogenous L-methionine (49).

Measurements (GC) of E evolved from lettuce pith explants grown on a xylogenic medium in sealed culture vessels indicated a peak in E production prior to any visible sings of xylem differentiation. Sampling for E after three days of culture showed that explants which had differentiated TE also had produced significantly greater quantities of E than explants lacking TE (48).

The induction of xylogenesis in lettuce pith explants cultured on an auxin-cytokinin medium was strongly inhibited by the presence of the E inhibitors aminothoxyvinyl glycine (AVG), AG⁺, Co⁺⁺, and sodium benzoate. Both AVG and Ag⁺ inhibition, respectively, were partially reversed by exogenous L-methionine. As indicated by staining of TE for the presence of lignin with the Maule reaction and safranin O, respectively, lignification was strongly inhibited by the AVG treatment (48). Our preliminary results support the view that E has a regulatory role in lignification during *in vitro* xylogenesis.
SOME COMMENTS ON TECHNIQUE

Xylogenesis in explants of lettuce pith (*Lactuca sativa* L. cv. Romana) was influenced by the relative amount of surface water adhering to the explant at the time of culture, i.e., the water film on the lower side adjacent to the medium (50). Other workers reported a relationship between xylogenesis and water stress in *Nicotiana* callus (51). The bactericidal antibiotic gentamicin was found to have inhibitory effects on both cell division and xylogenesis in explants of lettuce pith and Jerusalem artichoke tuber (52) and to block organogenesis in other systems (53).

REFERENCES


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