

DNA Levels in Differentiating Tracheary Elements

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The relative amount of nuclear DNA in recently differentiated tracheary elements of cultured pea root cortical cells was determined. Cells containing 2c, 4c, 8c, and 16c amounts of DNA were observed, and the distribution of the DNA classes was similar to that of the undifferentiated cell population.

INTRODUCTION

The formation of tracheary elements in tissue culture has been regarded for some time as a model system for the study of differentiation in plants (Torrey *et al.*, 1971). Tracheary elements are differentiated cells of the xylem characterized by distinctively patterned secondary cell walls. In the final cell maturation, the cell contents autolyze and the cell wall remains, forming the water-conducting channels within the plant. When pea root cortical explants are transferred to a nutrient medium containing auxin and cytokinin, DNA synthesis, cell division, and cell differentiation following according to a definite and predictable time course (Phillips and Torrey, 1973). Consequently, this system offers opportunity for the analysis of cellular events preceding cytodifferentiation.

Previous studies on this and similar systems have shown that all tracheary elements derive from cortical cells that have divided at least once, and that the cells destined to differentiate synthesize DNA within the 5-6 days before sculptured secondary walls are evident (Torrey and Fosket, 1970). While the cortical cells are initially diploid (Libbenga and Torrey, 1973), the first mitoses observed are largely

tetraploid with a subsequently increasing proportion of octaploids (Phillips and Torrey, 1973). These results suggest a developmental sequence involving hormone-stimulated endoreduplication of DNA followed by polyploid mitosis and terminal cell differentiation.

The involvement of endoreduplication in various kinds of differentiating plant tissues was discussed by D'Amato (1964a,b). Avanzi and co-workers recently concluded that the developmental sequence in metaxylem formation in *Allium* roots involved not only an early phase of endoreduplication, but also a subsequent period during which amplification of ribosomal cistrons associated with the nucleolar organizer took place (Innocenti and Avanzi, 1971; Avanzi *et al.*, 1973). In cultured tissues of *Cymbidium* Nagl and co-authors have reported both endoreduplication of DNA and replication of heterochromatic DNA preceding differentiation of hair cells and specialized storage cells (Nagl, 1972; Nagl and Rucker, 1972; Nagl *et al.*, 1972). A relationship between DNA replication and the differentiated state under hormonal control was suggested.

The objective of the present study was to determine the DNA content of nuclei in early differentiating tracheary elements whose identity could be established by the characteristic secondary wall pattern, but whose nuclei had not yet autolyzed in the final and terminal stage of differentiation.

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MATERIALS AND METHODS

Seeds of the garden pea, *Pisum sativum* cv. Little Marvel, were surface sterilized and germinated aseptically. Segments 1 mm thick were cut at 10–11 mm from the root tip and the central cylinders removed using the tissue punch described by Libbenga and Torrey (1973). The cortical explants were transferred aseptically to petri dishes containing S2M medium supplemented with 1.0 mg/liter kinetin and solidified with 0.8% agar and were maintained in darkness at 25°C.

After 6 days (when sampling first showed differentiated elements) the explants were fixed overnight in absolute ethyl alcohol:

glacial acetic acid (3:1), hydrolyzed for 45 min with 5 N HCl at 20°C, stained with Schiff's reagent for 60 min (Bosshard, 1964) and then squashed onto microscope slides coated with Haupt's adhesive. The cover slip was removed after freezing with dry ice, and the preparation was rinsed in alcohol and mounted in Euparal.

The relative amounts of DNA in nuclei of tracheary elements were determined using a Vickers M85 scanning microdensitometer. Only those elements containing a single, entire nucleus were included (Fig. 1). That the nuclei were actually within the elements was checked by focussing on the sculptured wall above and below each

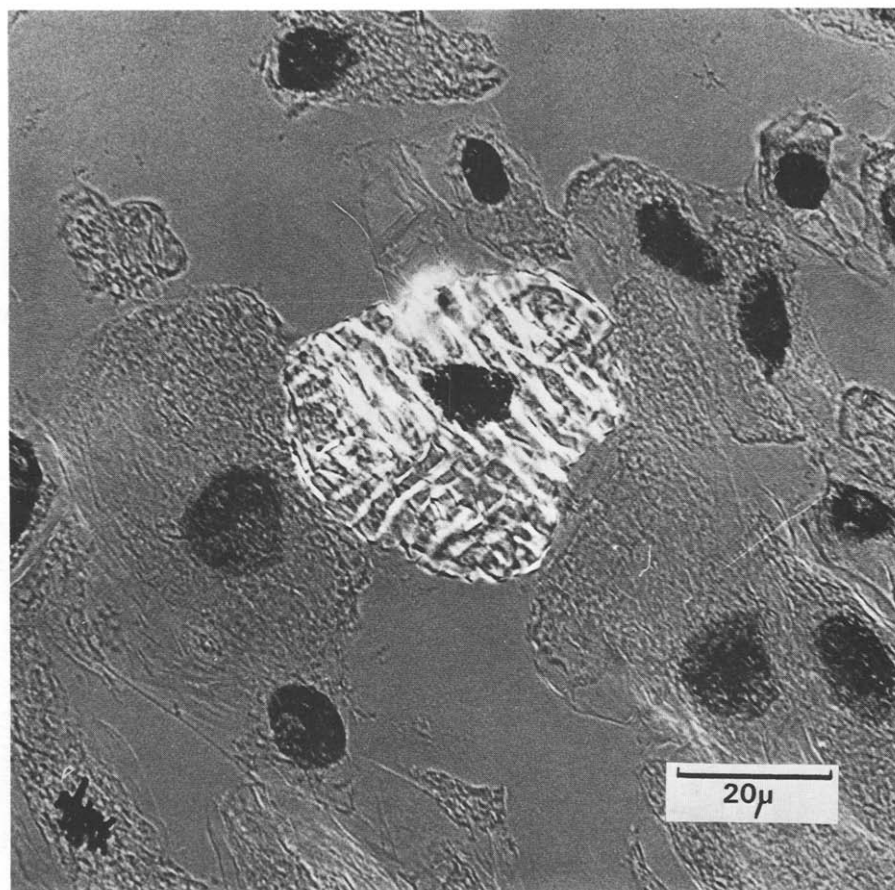


FIG. 1. Feulgen-stained squash preparation showing a single nucleated tracheary element and several undifferentiated cells. The photograph was taken in polarized light, showing the birefringence of the secondary cell wall.

nucleus. Elements containing fragments or obviously damaged nuclei were ignored, as were overlapping elements in the denser parts of the squash.

RESULTS AND DISCUSSION

The DNA values observed fell into four major and distinct classes, corresponding to the 2c, 4c, 8c, and 16c levels of DNA (Fig. 2), with the 4c and 8c values predominating. The distribution of DNA classes among the undifferentiated population of the same tissue squashes is shown in Table 1, together with that of the tracheary elements. With the exception of the small population of 2c nuclei among tracheary elements, these data correspond closely to those reported by Libbenga and Torrey (1973) for 7-day-old explants on the same medium determined by quite different methods. The data indicate that differentiation occurs in cells at several different DNA levels rather than being associated with any particular level of DNA as, for example, is the case in *Hydrocharis* trichoblasts (Cutter and Feldman, 1970). Differ-

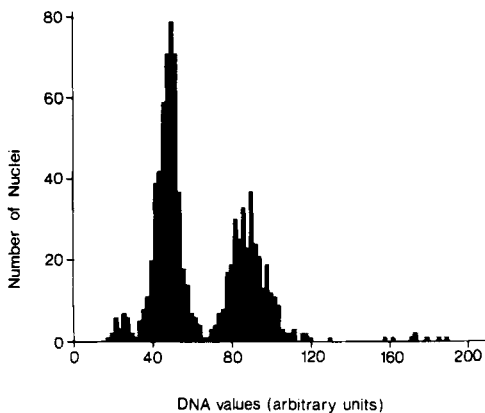


FIG. 2. Nuclear DNA contents of 881 differentiating tracheary elements from squash preparations of pea root cortical explants. The microdensitometer readings were calibrated in terms of DNA content by measurement of prophase nuclei from the same squashes, and were checked against prophase nuclei from pea root tips which were known to be 4c. The DNA values obtained expressed in arbitrary units were: 2c = 25; 4c = 48; 8c = 94; 16c = 185.

TABLE 1
DISTRIBUTION OF DNA CLASSES IN THE
UNDIFFERENTIATED CELL AND TRACHEARY ELEMENT
POPULATIONS OF CORTICAL EXPLANTS CULTURED FOR 6
DAYS ON S2M + 1 MG/LITER KINETIN^a

Cell type	DNA values (% of total)			
	2c	4c	8c	16c
Undifferentiated	6	50	38	6
Tracheary element	3	57	39	1

^a Microdensitometer measurements were made of 424 nondividing Feulgen-stained nuclei from undifferentiated cells, and 881 nuclei from differentiating tracheary elements.

entiating elements with nuclei at different levels of DNA occur in frequencies roughly in the same proportion as those of the cell population at large.

The tracheary elements containing the 2c amount of DNA must represent diploid cells in G1, and it seems unlikely that endoreduplication could be involved in the history of these cells. However, they would have come from cortical cells which had undergone DNA synthesis and cell division in culture before differentiating. The 4c, 8c, and 16c classes cannot be interpreted unequivocally at present, since 4c nuclei may be diploids in G2 or tetraploids in G1, and similar considerations apply to the 8c and 16c classes. However, it is known that tracheary elements arise only from recently divided cortical cells (Torrey and Fosket, 1970), and that the majority (>60%) of mitoses are tetraploid, i.e., 8c at metaphase. Consequently, it seems to us most likely that tracheary elements containing the 4c amount of DNA derive from the daughter cells of these tetraploid mitoses which have differentiated without another intervening period of DNA synthesis. By extension of this argument it seems likely that 8c tracheary elements derive from the daughter cells of octaploid (16c) mitoses, and that the 16c elements result from rare 16-ploid divisions. The validity of this argument may be checked experimentally; if it is correct, then the differentiating cells

all will be in G_1 , no matter what the DNA level.

Although DNA doubling by endoreduplication appears to be a usual concomitant in the differentiation of the vast majority (97%) of the tracheary elements in the pea root cortical explant system, there is no direct or indirect evidence obtained to date for the occurrence of DNA amplification in these cells such as that reported to occur in other systems (Avanzi *et al.*, 1973; Nagl *et al.*, 1972). The distinct features in the differentiation of tracheary elements in the pea explants are the following: hormone-induced DNA synthesis, typically by endoreduplication, mitosis, and cell division followed in the immediately succeeding G_1 period by specific cytodifferentiation. This sequence apparently can occur in cells with different nuclear DNA contents and must be presumed to occur independent of the DNA level per se.

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