Planta 135, 213–216 (1977)

Planta
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# DNA and Histone Content of Immature Tracheary Elements from Cultured Artichoke Explants

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**Abstract.** Relative amounts of DNA and histone were determined by Feulgen microdensitometry and alkaline fast-green microdensitometry in differentiating tracheary elements in cultured explants of Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. The absence of endopolyploidy in cultured artichoke tissue was confirmed, and the nuclei of tracheary elements were exclusively at the 2C level for both DNA and histones.

**Key words:** DNA – Helianthus – Histones – Tissue culture – Tracheary elements.

## Introduction

A relationship between the cell cycle and xylem differentiation has been suggested by several workers; Roberts (1976) has summarised much of the evidence. One aspect that remains unresolved is whether the xylem precursor cell becomes committed to the new pathway of cytodifferentiation at some particular stage of the cell cycle. An attempt to resolve this question by measurement of the relative amount of nuclear DNA in young tracheary elements formed in cultured pea root tissue was frustrated by the finding that several different levels of ploidy were present (Phillips and Torrey, 1974). Somatic polyploidy in xylem precursor cells occurs widely (Lai and Srivastava, 1976; Innocenti and Avanzi 1971; List, 1963); relatively few species appear to remain strictly diploid during development. However, Adamson (1963) reported that cells from dormant tubers of Jerusalem artichoke and their cultured derivatives do not become polyploid; furthermore, tracheary elements can be rapidly and consistently induced in cultured explants of Jerusalem artichoke (Phillips and Dodds, 1977).

The synthesis of histones in dividing cells is synchronised with that of nuclear DNA, so that the histone: DNA ratio remains constant (Antropova et al., 1970). Apparent changes in the proportions of histone to DNA have been reported in developing xylem of corn leaves (Lai and Srivastava, 1976) and onion roots (Innocenti, 1975), but interpretation of these results is difficult since polyploidy occurs in both systems.

The purpose of this investigation was threefold; to establish whether all cells in cultured artichoke explants undergoing xylogenesis remain diploid; to establish whether immature tracheary elements contain a characteristic DNA level, and to measure the relative proportions of DNA and histone in differentiating cells as compared with cycling cells.

## Materials and Methods

Explants 3 mm in diameter and 0.6 mm thick containing approximately $10^4$ cells were excised from dormant artichoke tubers and incubated at 30°C on filter paper discs moistened with 0.1 ml culture medium, as described previously (Phillips and Dodds, 1977). Rapidly growing root tips were obtained from tubers planted in moist vermiculite in the greenhouse. Root tips and explants cultured for 0, 48 or 72 h were fixed in 10% neutral buffered formalin for 24 h at room temperature, washed, and stored in 70% ethanol.

### *DNA Measurement*

Explants and root tips were hydrolysed in 5 N HCl at room temperature for 30 min, stained in Schiffs reagent for 1 h at 30°C, rinsed in freshly prepared $SO_2$ water, and squashed on a microscope slide in 45% acetic acid. Cover slips were removed after freezing on dry ice and the squash preparations air-dried at 30°C.

After mounting in DPX mountant the relative amounts of DNA in the nuclei of undifferentiated cells and tracheary elements was determined at 565 nm with a Vickers M 85/86 scanning microdensitometer. Only tracheary elements containing a single entire nucleus (Fig. 1) were included. Overlapping elements from the denser parts of the squash were ignored, as were more mature, lignified elements whose sculptured secondary walls took up stain.

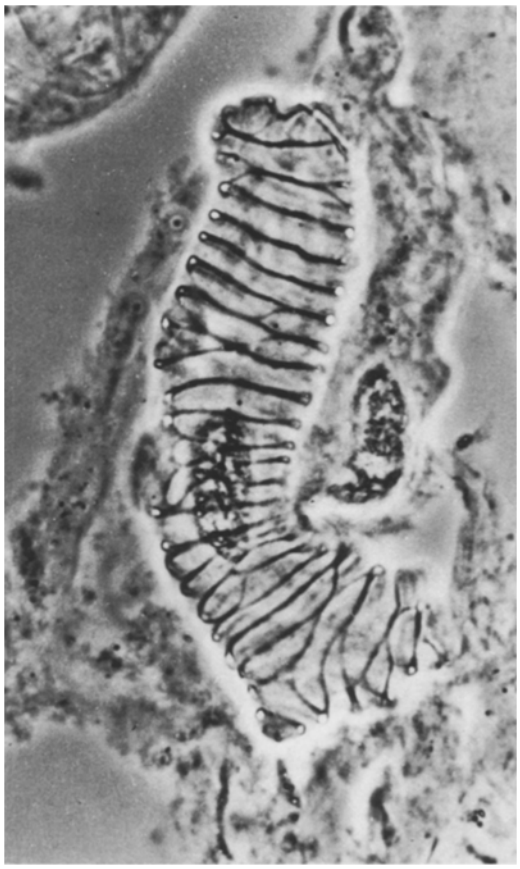

**Fig. 1.** Immature tracheary element of the type selected for DNA and histone measurement, Feulgen-stained and photographed in phase contrast to show the developing secondary wall. When mounted for microdensitometry and viewed in transmitted light, only the stained nuclei were visible; tracheary elements were then located by the birefringence of the secondary wall in polarised light

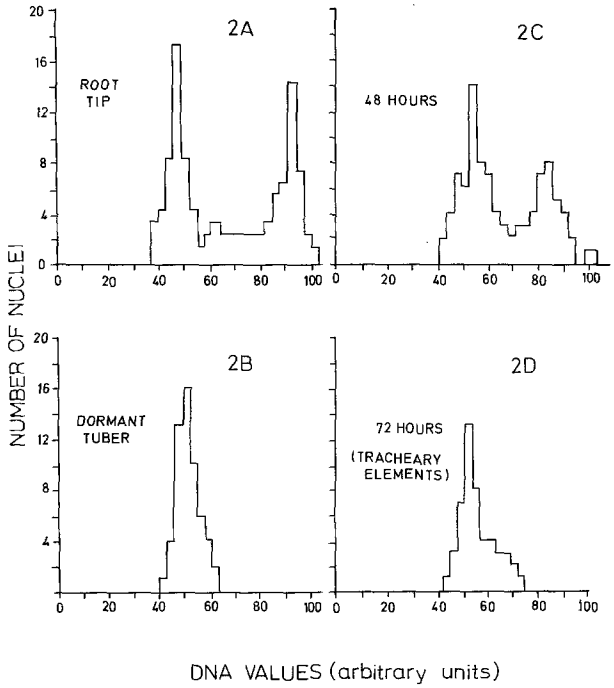


**Fig. 2A–D.** Histograms of the relative amount of DNA per interphase nucleus in squash preparations of Jerusalem artichoke measured by Feulgen-stain microdensitrometry. **A** Root tips. **B** Tissue freshly excised from dormant tubers. **C** Undifferentiated cells from tuber explants cultured for 48 h. **D** Immature tracheary elements from explants cultured for 72 h

*Histone Measurement*

Air-dried, Feulgen-stained squash preparations were treated with 5% TCA at 90–100°C to remove nucleic acids and Schiffs stain. Effective removal of DNA was demonstrated by the failure of TCA – treated nuclei to re-stain with Schiffs reagent. After rinsing in 70% ethanol and water (pH 8.1) histones were stained with freshly prepared 0.1% Fast Green at pH 8.1 for 10 min at 4°C (Alfert and Geschwind, 1953). Slides were then rinsed for 1 min in water (pH 8.1), dehydrated with absolute ethanol and mounted in DPX. The relative amount of histone in the nuclei of undifferentiated cells and tracheary elements was determined using a Vickers M 85/86 scanning microdensitometer at a wavelength of 635 nm.

## Results

### *DNA*

The DNA content of cycling cells from rapidly growing root tips fell into two classes (Fig. 2A). The DNA content of prophase mitotic nuclei corresponded with the higher peak, confirming the upper and lower peaks as equivalent to the 4C and 2C DNA levels respectively. The nuclei of freshly excised tuber tissue were exclusively at the 2C level (Fig. 1B); cells of the developing tuber thus arrest in G1.

The pattern of cell proliferation in artichoke explants transferred to culture medium was known from previous studies (Phillips and Dodds, 1977). The first, partially synchronous, mitosis occurred after a lag period of 18–22 h and was followed by a second at 30–34 h. By 48 h the cell population was dividing actively and without synchrony; by 72 h the proliferation rate was declining, and about 5% of the population had differentiated as immature tracheary elements.

After 48 h, approximately 60% of the cells were at the 2C level, and 40% at 4C; no nuclei with DNA contents above 4C were observed (Fig. 2C). Of the nuclei of undifferentiated cells at 72 h, approximately 80% were at 2C, 10% at 4C and 10% at intermediate levels. No nuclei above the 4C level were observed. Nuclei of immature tracheary elements at 72 h were exclusively at the 2C level (Fig. 2D), and were thus in the G1 phase of the cell cycle.

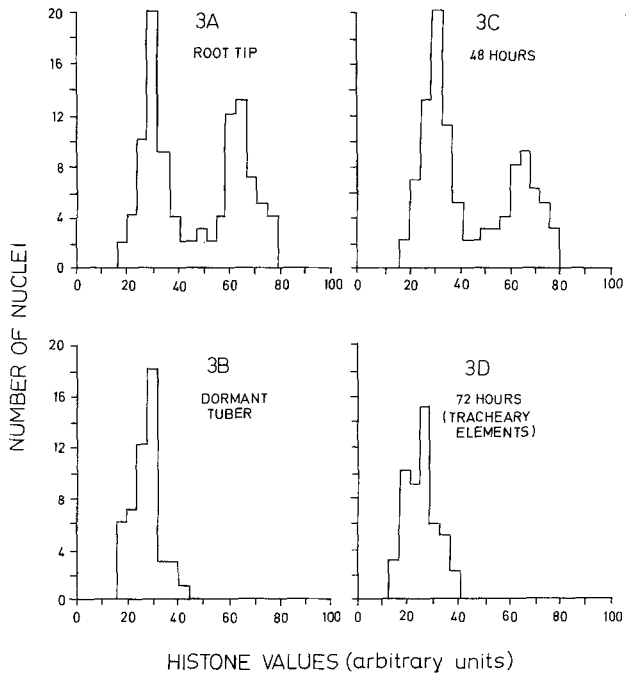


**Fig. 3A–D.** Histograms of the relative amount of histone per interphase nucleus in squash preparations of Jerusalem artichoke, measured by alkaline fast-green stain microdensitometry. **A** Root tips. **B** Tissue freshly excised from dormant tubers. **C** Undifferentiated cells from tuber explants cultured for 48 h. **D** Immature tracheary elements from explants cultured for 72 h

*Histones*

The histone content of root tip cells fell into two classes corresponding to the 2C and 4C values for DNA (Fig. 3A). All nuclei from freshly excised tuber tissue were at the 2C histone level (Fig. 3B) while the asynchronously dividing cultured cells at 48 h were distributed between the two histone levels (Fig. 3C) in the same way as root tip cells. Nuclei of immature tracheary elements at 72 h were exclusively at the 2C histone level (Fig. 1D).

## Discussion

The findings of this study confirm that cultured cells derived from dormant artichoke tubers remain strictly diploid for at least 72 h, during which period the cells undergo at least 3 rounds of mitosis, and enter a phase of rapid tracheary element differentiation (Phillips and Dodds, 1977).

Immature tracheary elements were exclusively at the 2C DNA level. Previous studies on the DNA content of developing xylem cells have reported higher levels, of up to 32C in roots of *Zea mays* and *Arisaema triphyllum* (List, 1963) and 16C in *Allium cepa* root metaxylem (Avanzi et al., 1973). In leaves of *Zea mays* the DNA content of young vessel elements was double that of procambial cells; whether this represents 4C or some higher level is not clear as the measurements were not calibrated against diploid mitotic nuclei. Tracheary elements differentiating in cultured pea root explants were mostly at the 4C and 8C levels, although smaller proportions of both 2C and 16C elements were observed (Phillips and Torrey, 1974). The distribution of DNA values in the undifferentiated cell population was similar to that of tracheary elements, indicating that the ploidy level was not an important causal factor in the induction of differentiation. However, wound vessel members differentiating in cultured lettuce pith explants in the presence of $10^{-5}$ M fluorodeoxyuridine were exclusively at the 2C level of nuclear DNA (Turgeon, 1975).

In onion root metaxylem cell lines, differentiation was preceeded by a decrease in the histone: DNA ratio (Innocenti, 1975), apparently due to repeated cycles of DNA endoreduplication without accompanying histone synthesis. Lai and Srivastava (1975) on the other hand, reported a doubling in the histone: DNA ratio in corn leaf vessel elements with secondary walls as compared with their immediate precursor cells. Our results show that in cultured artichoke tubers, the synthesis of DNA and histones remain closely linked during xylem differentiation.

The nuclear content of both DNA and histone is characteristic of cycling cells in G1. One important consequence of this finding for further studies on the relationship between the cell cycle and cytodifferentiation in this system is that unequivocal interpretation of data on DNA synthesis is possible; any precursor cells synthesising DNA must divide prior to overt differentiation.

One of us (J.H.D.) was supported during this investigation by an Elliot-Blake studentship from University College.

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*Received 11 March; accepted 4 April 1977*