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# Nuclear DNA Content and Number of Chloroplasts in *Elodea canadensis* after Treatment with Growth Regulators

By

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With 1 Figure

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Key words: Nuclear DNA content, chloroplast number, growth regulators, *Elodea canadensis*.

## Summary

GUTTENBERGER H. 1987. Nuclear DNA content and number of chloroplasts in *Elodea canadensis* after treatment with growth regulators. – Phyton (Austria) 27 (1): 47–54, with 1 figure. – English with German summary.

Cells of the upper epidermis of young and full-grown leaves of water-weed *(Elodea canadensis* RICH.) treated with 2,4-dichlorophenoxyacetic acid (2,4-D), gibberellic acid ($GA_3$), and (2-chloroethyl)-trimethylammoniumchloride (CCC) showed an increase of the number of chloroplasts. This effect is accompanied by changes of the diameters of the chloroplasts and of the nuclear DNA content. The relationships between DNA content, number of chloroplasts per cell, and cell size are discussed.

## Zusammenfassung

GUTTENBERGER H. 1987. Chloroplastenzahl und Kern-DNA-Gehalt von *Elodea canadensis* nach Beeinflussung durch Wachstumsregulatoren. – Phyton (Austria) 27 (1): 47–54, mit 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Die Chloroplastenzahl der Zellen der oberen Epidermis von jungen und ausgewachsenen Blättern von *Elodea canadensis* RICH. nimmt nach Behandlung mit 2,4-Dichlorphenoxyessigsäure (2,4-D), Gibberellinsäure ($GA_3$) und (2-Chloräthyl)-trimethylammoniumchlorid (CCC) zu. Durch den Einfluß wird auch der Chloroplastendurchmesser verändert. Zusammenhänge zwischen Kern-DNA-Gehalt, Anzahl der Chloroplasten pro Zelle und Zellgröße werden diskutiert.

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## Introduction

Growth regulators may cause a change in the number of chloroplasts per cell. Abscisic acid, cytokinine, gibberelline, and ethylene may enhance their number. This is possibly caused by endopolyploidy – auxins themselves have little influence on the number of chloroplasts (BUTTERFASS 1979).

The DNA amount per genome produces the basic determination of chloroplast numbers; on top of this prevailing effect, influences of single genes or groups of genes or control processes independent of DNA amounts can appear throughout growth and differentiation (BUTTERFASS 1983). A relationship between nuclear DNA content per cell and cell size is evident; thus POSSINGHAM & LAWRENCE 1983 think that the nuclear DNA amount probably has more direct effect on the final plastid number via its effect on cell size.

This investigation is concerned with the alternatives of the parameters cell size, number and size of the chloroplasts, and DNA content of the nucleus after treatment with 2,4-D, $GA_3$, and CCC.

## Material and Methods

4 pieces of the shoot tip of water-weed (*Elodea canadensis* RICH.), each of them 20 cm long, without any root or lateral shoot, were simultaneously cultivated in a vessel of 1000 $cm^3$ content. For illumination 16 fluorescent tubes, Osram 165 W/19 daylight 500 De Luxe, were used. Plants were cultivated in a 12 hour day-night-rhythm, intensity of light beeing 6000 lx. The temperature was kept at 25° ± 2° C throughout.

2,4-D (Serva 19410), $GA_3$ (Fluka 48880), and CCC (Merck 2838) were dissolved in tap-water in a concentration of $10^{-6}$ mol. $l^{-1}$. Using this concentration, LENZI 1982 found the highest increase of chloroplast number in the upper epidermis of young leaves of water-weed. Tap-water was used in order to avoid harms caused by deioniced water (cp. MODER 1932). The solutions were exchanged every third day.

14 days after beginning the experiment plants were evaluated. Leaves 1 cm off the growing point were used. Cells from the upper epidermis of the middle area of the leaf blade between midrip and margin were investigated. Cells from full-grown leaves were examined in the same way. These leaves were taken 5 cm away from the growing point. The area of the upper epidermis cells, number of chloroplasts, diameters of the chloroplasts were examined. The measurements were carried out using a drawing apparatus in connection with the research microscope Zetopan (Reichert Co.).

The values were treated statistically (mean, standard deviation, t-test – WEBER 1967, tables in GEIGY 1968). As random sample the plants investigated are taken, i. e. 24 control plants and 24 plants treated with growth regulators. 3 leaves (young and full-grown respectively) were taken from

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each investigated plant. 10 cells from each leaf (therefore 1440 cells altogether) resp. their number of chloroplasts were counted. The size of 30 chloroplasts per leaf (i. e. 1440 chloroplasts altogether) was determined.

After fixation in ethyl alcohol – glacial acetic acid 3 : 1 (v : v) for 24 hours at 4° C – Feulgen-reaction was used to determine nuclear DNA content. Leaves or root tips were hydrolized at 60° C in 1 N HCl. In order to ascertain which hydrolysis time allowed maximum DNA-Feulgen staining, spectral absorption curves were generated for each hydrolysis time. Reaction with freshly prepared Schiff's reagent for 120 min (23° C) in darkness followed. Stained preparations were washed out carefully and applied to absolute ethanol. By using the „quick freeze" method (cp. NAGL 1976) alcohol series was avoided. Preparations were embedded in Euparal (Chroma 3 C 239).

The nuclear DNA content was measured with a microscope photometer (Reichert) equipped with a continuous interference filter in connection with the research microscope Zetopan (Reichert Co.). The double wavelength method (ORNSTEIN 1952; PATAU 1952) was used. The reference values 2 C and 4 C were determined by analysis of 51 telophases and 59 prophases of root tips.

The nuclear DNA content is expressed in arbitrary units (au). Pro- and telophases from onion *(Allium cepa)* root tips were used as internal standard.

## Results

### Young Leaves

The number of chloroplasts per epidermis cell of 2,4-D-, and CCC-treated plants increases with high significance – see Table 1. 2,4-D influence causes an increase of the size of the chloroplasts by 20% ($P<0.001$); no significant difference exists between the control and $GA_3$- or CCC-influence. Neither 2,4-D, nor $GA_3$, nor CCC causes a significant increase of the area of the cells of the upper epidermis in young leaves of *Elodea canadensis*. Correlation between cell area and number of chloroplasts is positive and significant ($r = + 0.75$, $P<0.001$).

The frequency histogram of the nuclear DNA content of the epidermis cells of the control plants shows a defined 2 C peak with a more flat decline to 4 C. This means that the most nuclei are in $G_1$-phase, other nuclei are in S or $G_2$. Nuclear DNA content of treated plants is not much different from that of the control plants (see Fig. 1): errors excepted, less nuclei have reached $G_2$- or S-phase. According to the formula given by NOSOV & al. 1983 the ratio of conditional probabilities of the nuclear DNA content to definite control classes has been calculated. In this way more of the nuclei of the growth regulator-treated plants show a DNA content corresponding to 2 C,

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Table 1

Number and size of the chloroplasts of the upper epidermis cells resp. epidermis cell area from young and full-grown leaves of water-weed, treated with 2,4-D, $GA_3$, and CCC.

| | | CONTROL | 2,4-D | $GA_3$ | CCC |
|---|---|---|---|---|---|
| Number of chloroplasts per cell | young leaves | 20.6±2.2 | 25.2±4.2*** | 22.8±3.8° | 25.9±4.1*** |
| | full-grown leaves | 21.5±4.0 | 27.5±5.7*** | 27.6±5.4*** | 29.1±5.8*** |
| Diameter of the choroplasts (μm) | young leaves | 4.5±0.8 | 5.4±0.9*** | 4.5±1.3° | 4.5±0.9° |
| | full-grown leaves | 5.4±1.0 | 6.2±1.5* | 4.9±0.8° | 5.7±0.8° |
| Epidermis-cell area ($\mu m^2$) | young leaves | 1660±600 | 1010±700° | 1800±630° | 1790±490° |
| | full-grown leaves | 2130±500 | 3460±890*** | 2760±590*** | 2170±600° |

Symbols: *** = P<0.001; ** = 0.025>P>0.001; * = 0.05>P>0.025; ° = P>0.05.

less nuclei show values between 2 C and 4 C, or have reached DNA contents corresponding to 4 C, than at the control.

## Full-Grown Leaves

Full-grown leaves have much more chloroplasts in their epidermis cells when treated with 2,4-D, $GA_3$, or CCC. Size of the chloroplasts increases with 2,4-D treatment, decreases with $GA_3$-treatment, but the significance of the difference is low (0.05 >P<0.025) or is not significant. 2,4-D and $GA_3$ cause larger epidermis cells (P<0.001), – see Tab. 1. Number of chloroplasts per cell and cell size are positive but weakly correlated (r = 0.45; P>0.05). Most nuclei of the control plants have a DNA content of 4 C in their epidermis cells. With the influence of 2,4-D many nuclei persist in a DNA value of 2 C. As at the control, a 4 C peak is present. Some nuclei appear with DNA values of more than 4 C, but not associated with determined C values (Fig. 1). With the influence of $GA_3$ a 2 C peak is not present. Contrary to the control more nuclei have 8 C, some nuclei have reached DNA values of 16 C; often nuclei appear with DNA contents not associated with determined C-values. CCC causes a persisting of many nuclei at the 2 C-state; like at the control the most nuclei have a DNA content of 4 C.

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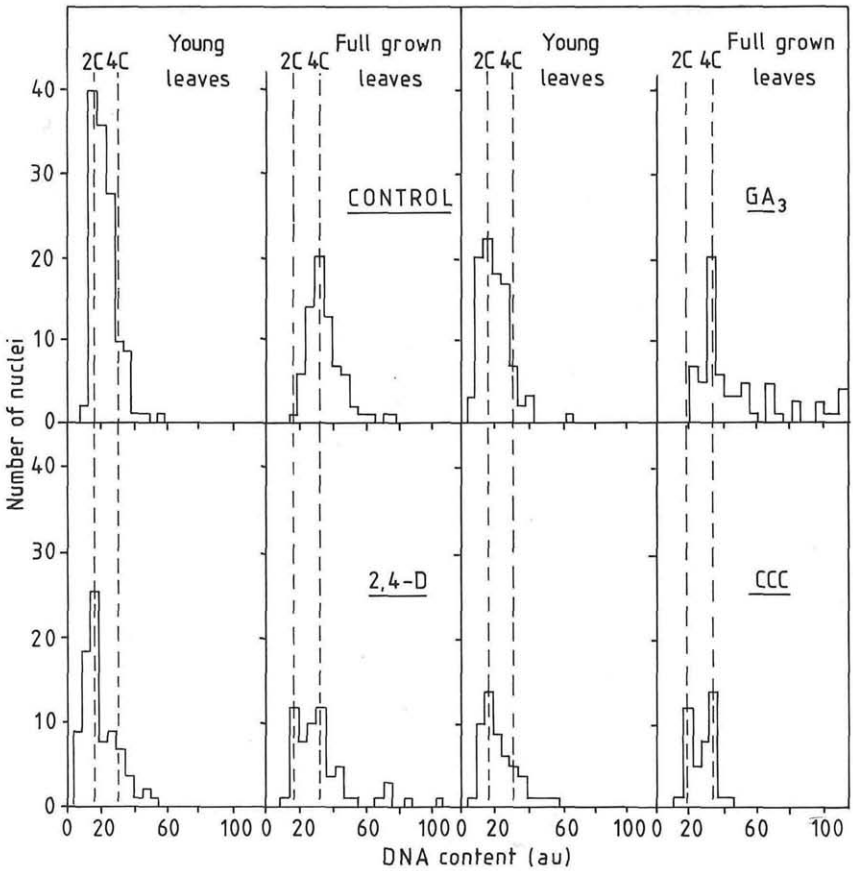


Fig. 1. Histograms of the frequency distribution of the nuclear DNA content in cells of the upper epidermis of *Elodea canadensis*. 2 C resp. 4 C values determined by analysis of telo- and prophases of the root tips.

## Discussion

The kind of the growth regulator, its concentration, its application, the plant species used, the kind of influence (e. g. continuous or not continuous), all these and more facts may produce different influences on the plastid number (cp. e.g. MACCHINI 1975, BUTTERFASS 1979, LENZI 1982). In order to produce a higher number of chloroplasts the own experiments were applied – the results confirm the correct application: the growth regulators used caused a significant increase of the chloroplast number per cell in full-grown *Elodea* leaves.

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The nuclear DNA content of young leaves is not very different from unterated control plants. Errors excepted, less nuclei have reached higher DNA values than 2 C. Possibly the growth regulators disturb DNA synthesis in developing *Elodea* leaves. The results with full-grown leaves are different. After 2,4-D- and $GA_3$-treatment more nuclei exist with DNA values higher than 4 C. Often these nuclei are not coordinated with determined C values. Besides this effect, many nuclei remain in a DNA value of 2 C treated with 2,4-D. These results correspond with the findings of NAGL & RÜCKER 1972 in *Cymbidium* tissue culture for the most parts. They concluded from their results that 2,4-D obstructs DNA replication in the mitotic and perhaps even in the endomitotic cycle, but promotes the increase of the heterochromatic parts of the genom (i. e. DNA-amplification), and $GA_3$ shows the opposite: promotion of mitosis and endomitosis, obstruction of amplification. However, my own results show many nuclei not coordinated with determined C values as well after $GA_3$-treatment. Possibly $GA_3$ is even able to cause DNA-amplification or underreplication. CCC, a substance which inhibits $GA_3$ synthesis (cp. GOODWIN & al. 1978), produces many nuclei which remain in a value of 2 C. MACCHINI 1975 found a not significant but lower DNA level after CCC-treatment in epidermis cells of *Helianthus* leaves.

Therefore, in the case of long-time influence of 2,4-D, $GA_3$, and CCC on *Elodea,* the higher number of chloroplasts does not proceed higher nuclear DNA values and vice versa, no correlation between nuclear DNA content and number of chloroplasts per cell is evident.

The diameter of chloroplasts increases under long-time influence of 2,4-D (this phenomenon is more obvious in young leaves); CCC, and $GA_3$ cause no significant difference. Simultaneously with the diameter the content of autochthonous starch grows in these chloroplasts. WOZNY & al. 1973 treated cells of callus developing on the phloem explants of chicory roots with indolylacetic acid (IAA). They found an increasing starch content of the plastids, depending on IAA concentration. Possibly 2,4-D is able to cause the same effect in appropriate concentrations.

The area of epidermis cells increases with 2,4-D and $GA_3$. Cell enlargement is one of the best known effects of auxins. After application of gibberellins generally the enlargement of plants or plant organs is caused by an increasing in cell number, and only a small increasing in cell length (cp. GOODWIN & al. 1978).

POSSINGHAM & LAWRENCE 1983 pointed out that possibly the nuclear DNA content has more direct influence on the final plastid number via its effect on the cell size. Giant guard cells contain 50 to 100% more chloroplasts and exceed the regular ones by 50 to 60% in lenghth and by 30 to 45% in breadth, but have the same nuclear DNA content (GUTTENBERGER 1985). Investigation of individual cells of diploid and autotetraploid *Triticum monococcum* indicate that the larger number of chloroplasts is more funda-

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mentally related to their bigger size than to their higher nuclear ploidy (ELLIS & LEECH 1985). Of the developing mesophyll cells of *Triticum aestivum* chloroplast number is also independent of increases in the nuclear DNA-content (ELLIS & al. 1983), cellular growth and plastid reproduction may be correlated in developing tissues (BOASSON & al. 1971).

The results presented here indicate that chloroplast number, chloroplast- and cell size respond indepently to DNA-contents of the nuclei, after application of 2,4-D, $GA_3$, and CCC.

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# Recensio

**BOPP, M(artin) 1986. Plant Growth Substances.** Proceedings of the 12th International Conference on Plant Growth Substances, held at Heidelberg August 26–31, 1985. – Gr. –8°, XIV + 520 Seiten mit 135 Figuren, Leinen gebunden. – Springer Verlag Berlin, Heidelberg, New York, Tokyo. – DM 118,–. – ISBN 3-540-16267-4.

Der vorliegende Band ist nach den 1976 und 1979 erschienenen (vgl. Recensionen in Phyton Vol. 18: 246 und 22: 166) der dritte dieser Art. Von den auf dem im Titel genannten Kongreß gehaltenen 207 Vorträgen und 300 Postern sind die eingeladenen Vorträge sowie die in den 5 workshops gehaltenen ausgenommen. Der größte Teil der 52 Beiträge stammt aus den USA und aus Großbritannien (15 bzw. 14 Beiträge), gefolgt von der Bundesrepublik Deutschland (11); aus Australien, Belgien, der DDR, aus Frankreich, Israel, Japan, Niederlande, Schweden, der Schweiz und der UdSSR kommen jeweils 1 oder 2 Beiträge. – Einleitend gibt WAREING einen Überblick über die anstehenden Probleme, wobei er besonders die Überschätzung der Wuchsstoffmengen als Kriterium für die Reaktion der Pflanze die Bedeutung der (allerdings schwer untersuchbaren unterschiedlichen Empfindlichkeit der Zellen gegenüber den Wuchsstoffen (Wst.) hervorhebt; die Kompetenz der Zellen gegenüber den Wst. und der Determinationsgrad sind weitere nicht zu übersehende Faktoren. Damit erscheint der Weg für künftige Forschungen vorgezeichnet. Die weiteren Beiträge sind in 5, allerdings nicht scharf abgegrenzten Generalthemen gruppiert. Im Methodischen Teil (5 Beiträge) stehen immunologische Techniken im Vordergrund, 11 Beiträge befassen sich mit der Biosynthese und der Biochemie der Wst. Im umfangreichsten Teil, Mechanismus der Wst.-Wirkungen (14 Titel) werden Permeation, Transport, Genexpression und Altern behandelt; hier findet sich auch ein Beitrag MULKEY-VAUGHANS über den gegenwärtigen Stand des Problems Auxin und Gravitropismus in der Wurzel. Eine bunte Palette von Problemen bietet die Abteilung „Hormone effects" (14 Beiträge), die letzte faßt Darstellungen über Anwendungen von Wst. in Landwirtschaft und Gartenbau zusammen. Der Band gibt damit einen guten Überblick über den gegenwärtigen Stand (genauer gesagt: über den Stand von August 1985) der Wuchsstofforschung wieder, er wird den auf diesem Gebiet Arbeitenden eine wertvolle Informationsquelle sein.

O. HÄRTEL

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