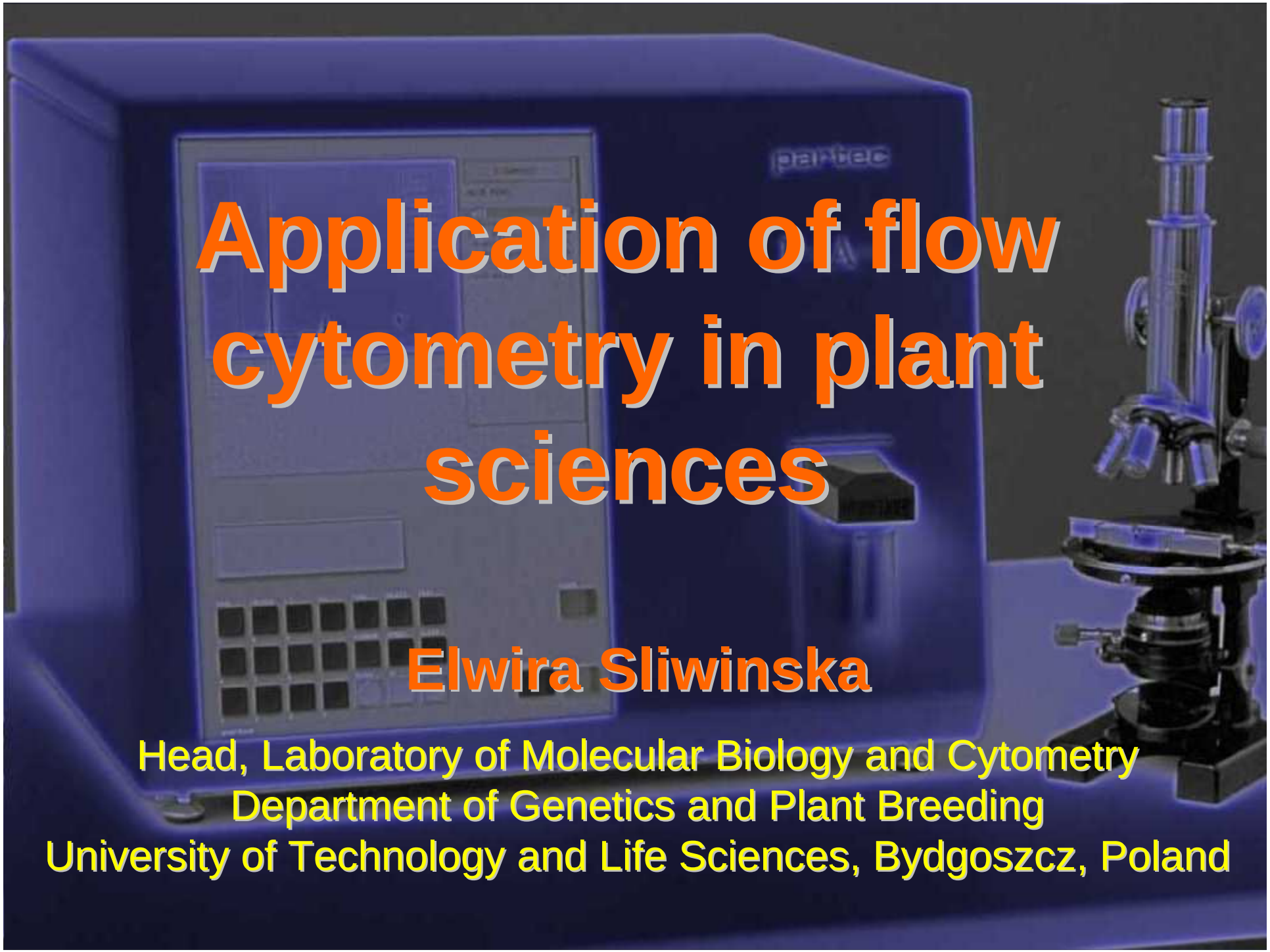

Application of flow cytometry in plant sciences

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im. Jana i Jędrzeja Śniadeckich
w Bydgoszczy

University of Technology and Life Sciences in Bydgoszcz

- **The biggest technical school in our region**
- **60 years of teaching tradition** (**1951 –** Establishment of the Evening School of Engineering with two faculties: Mechanics and Chemistry)
- **7 faculties**
- **Almost 700 academic teachers**
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Programes:

- **BSc – 21 programs**
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Faculty of Agriculture and Biotechnology

Programes:

Agriculure

Biotechnology

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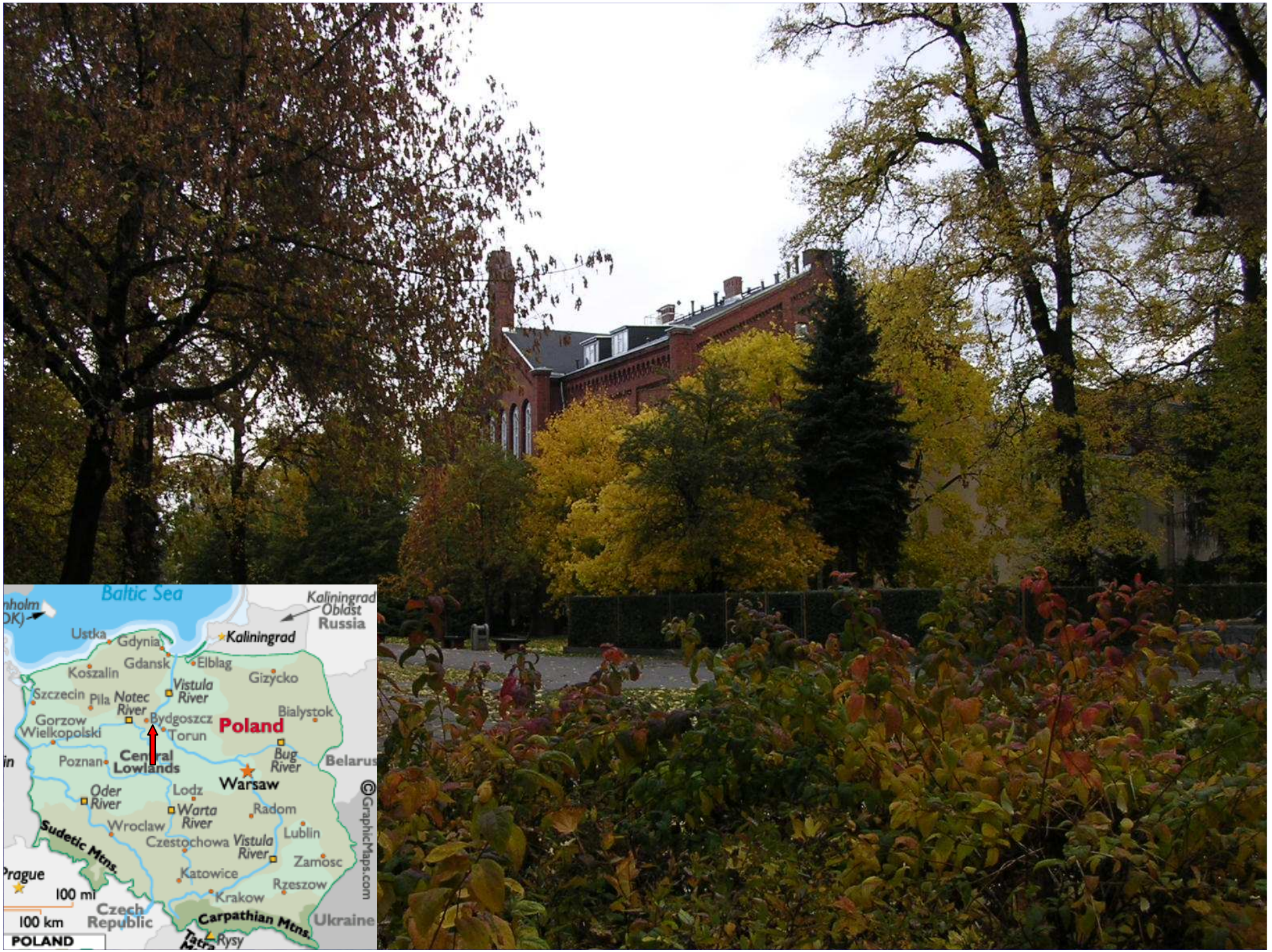
Baltic Sea
Kaliningrad Oblast Russia
Ustka
Gdynia
Kaliningrad
Gdansk
Elblag
Koszalin
Gizycko
Szczecin
Pila
Notec River
Vistula River
Bialystok
Gorzow Wielkopolski
Bydgoszcz
Torun
Poland
Poznan
Central Lowlands
Bug River
Belarus
Warsaw
Oder River
Lodz
Warta River
Radom
Wroclaw
Lublin
Sudetic Mtns.
Czestochowa
Vistula River
Zamosc
Katowice
Rzeszow
Krakow
100 mi
100 km
Czech Republic
Carpathian Mtns.
Ukraine
Rysy
POLAND
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POCZTA

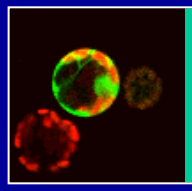

Orange
Lee
DELIKATEST WIEDEŃSKIE

Laboratory of Molecular Biology and Cytometry

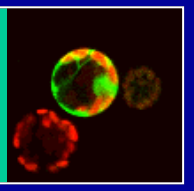
Research

1. Seed enhancement* and testing.
2. Functional and molecular basis of endoreduplication in different organs/parts of seed and seedling of species of families Chenopodiaceae and Fabaceae.
3. Ploidy and genome size of different plant species and their hybrids (including plant material from *in vitro* cultures).

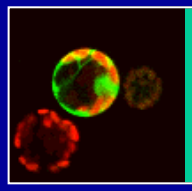
* = seed priming; post-harvest treatments that improve germination or seedling growth, or facilitate the sowing of seeds



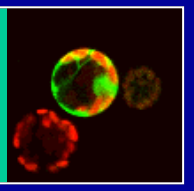
Flow cytometry



- Involves analysis of populations of single cells (nuclei) within suspensions.
- Is very rapid (>20,000 cells/sec can be analyzed and sorted).
- Is based on the analysis of the optical properties of cells.
- Was originally developed for characterization of lymphoid cells (particularly white blood cells).

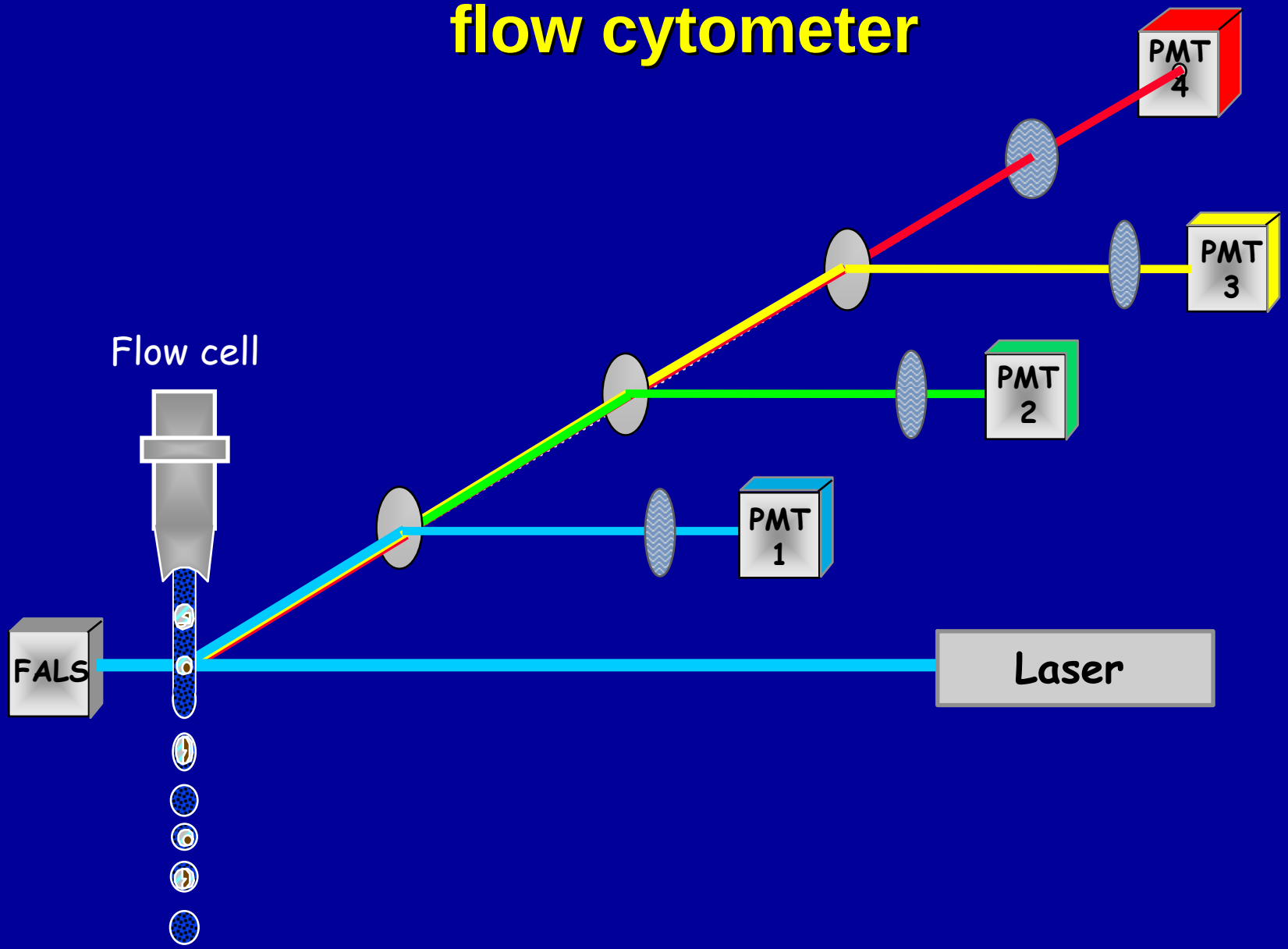


Flow cytometry



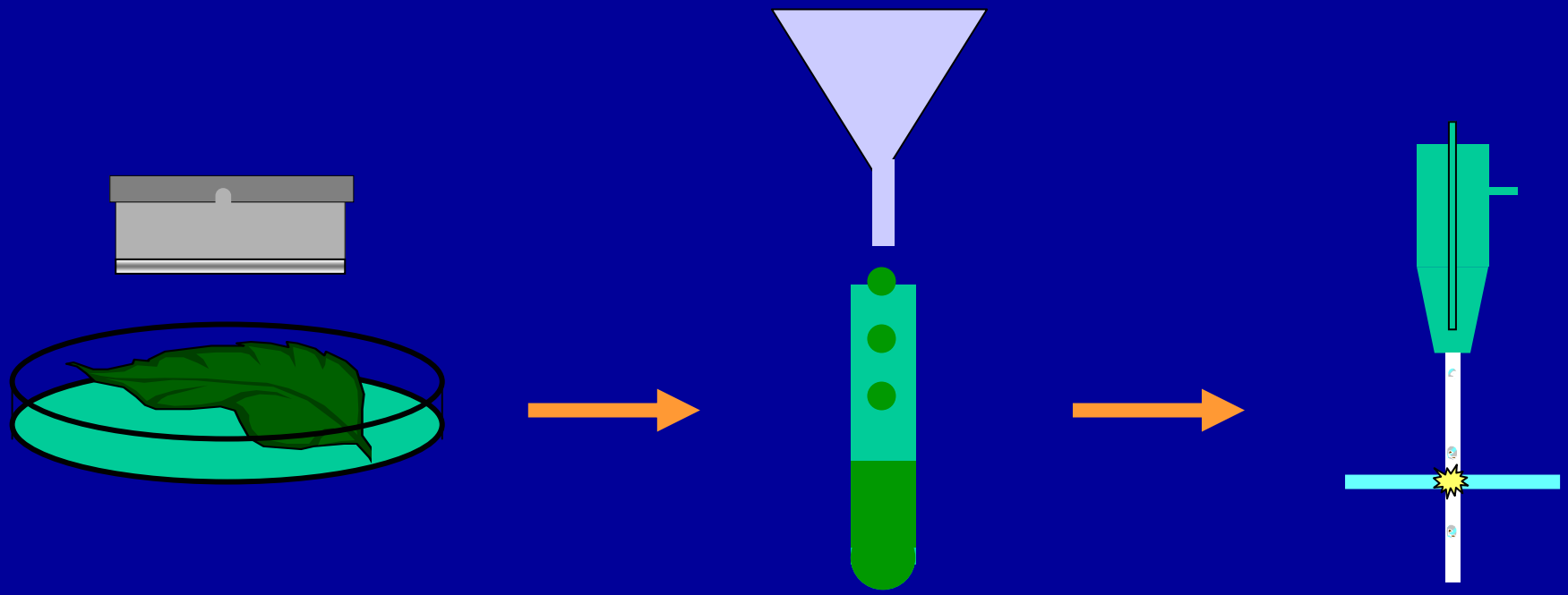
- The cells/nuclei in suspension stained with fluorescent dye (e.g. PI, DAPI for DNA content estimation) are forced to pass in a fluid stream through a flow cell.
- The fluid stream intersects the focus of an intense light source, typically a laser or an HBO lamp.
- Each cell as it passes through the laser beam absorbs light and emits fluorescence.
- This information is accumulated for analysis.

Typical layout for a laser-based flow cytometer

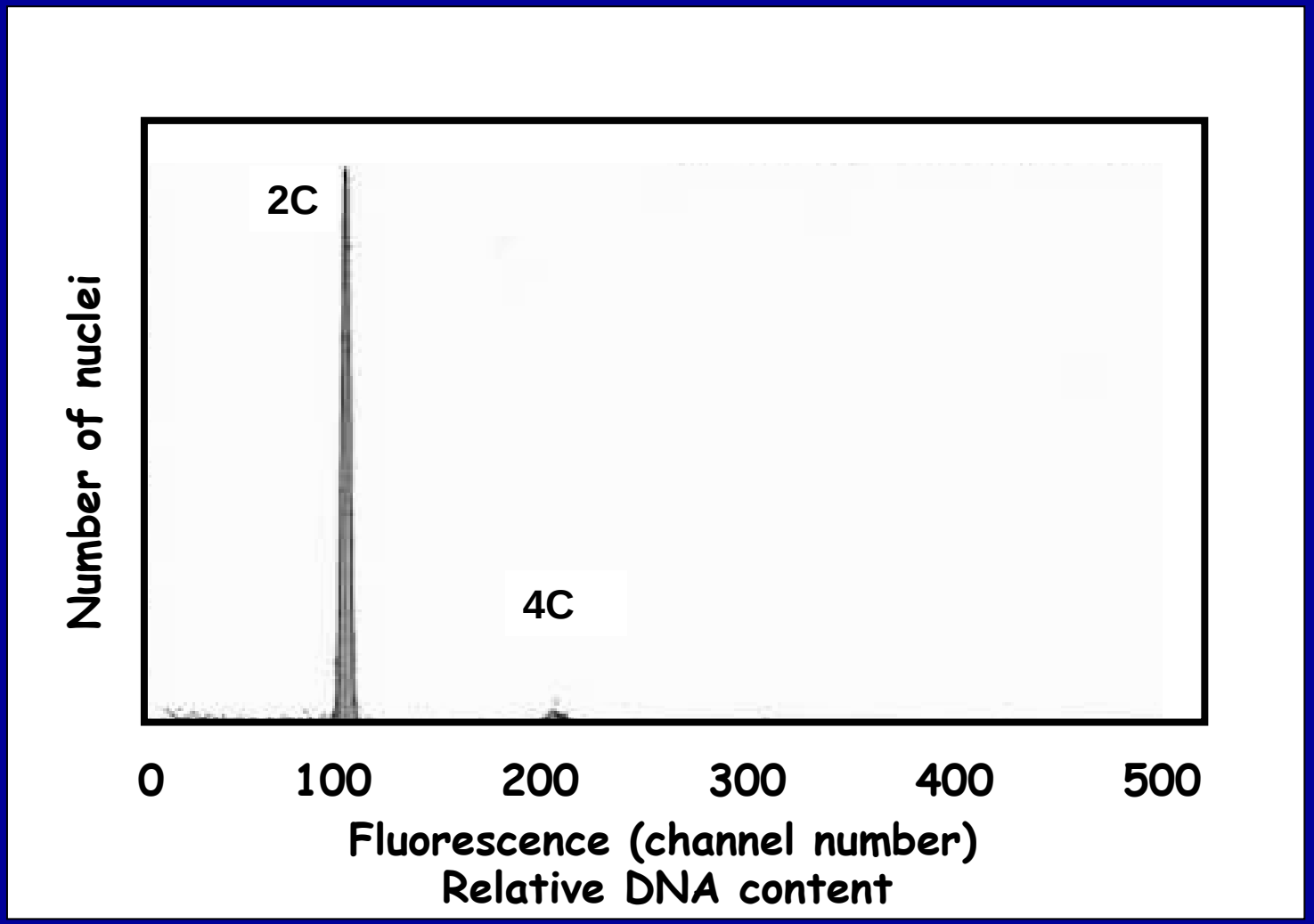


Method for DNA content and ploidy estimation

1. Select tissue of interest.
2. Place tissue in Petri dish, in cold “chopping” medium.
3. Chop tissue using a single-edge razor blade, for approximately 1 min.
4. Filter tissue through nylon mesh (pore size 15-50 mm).
5. Add appropriate fluorochrome to desired concentration.
6. Analyze fluorescence emission using flow cytometry.



Galbraith *et al.*, *Science* 220:1049-1052 (1983).



Histogram of DNA content in nuclei isolated from Onion leaf

C – DNA content of a holoploid genome with chromosome number *n* (meiotically reduced chromosome number)


partec
Ploidy Analyser

Applications of flow cytometry to plant sciences

- Ploidy estimation.
- Establishing nuclear DNA content.
- Studying cell cycle activity.
- Studying DNA endoreduplication.
- Selection of interspecific hybrids.
- Sorting of cells/chromosomes.

Applications of flow cytometry to plant sciences

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Ploidy* control

- Breeding of polyploid crops (sugar beet, banana).
- *In vitro* culture (haploid production, protoplast fusion, somaclonal variation).
- Growing plants under stress conditions.

*The nuclear DNA content of the 2C nucleus reflects the ploidy of a cell.

Production of triploid hybrids of Sugar beet

P. maternal line MS × pollinator

2x

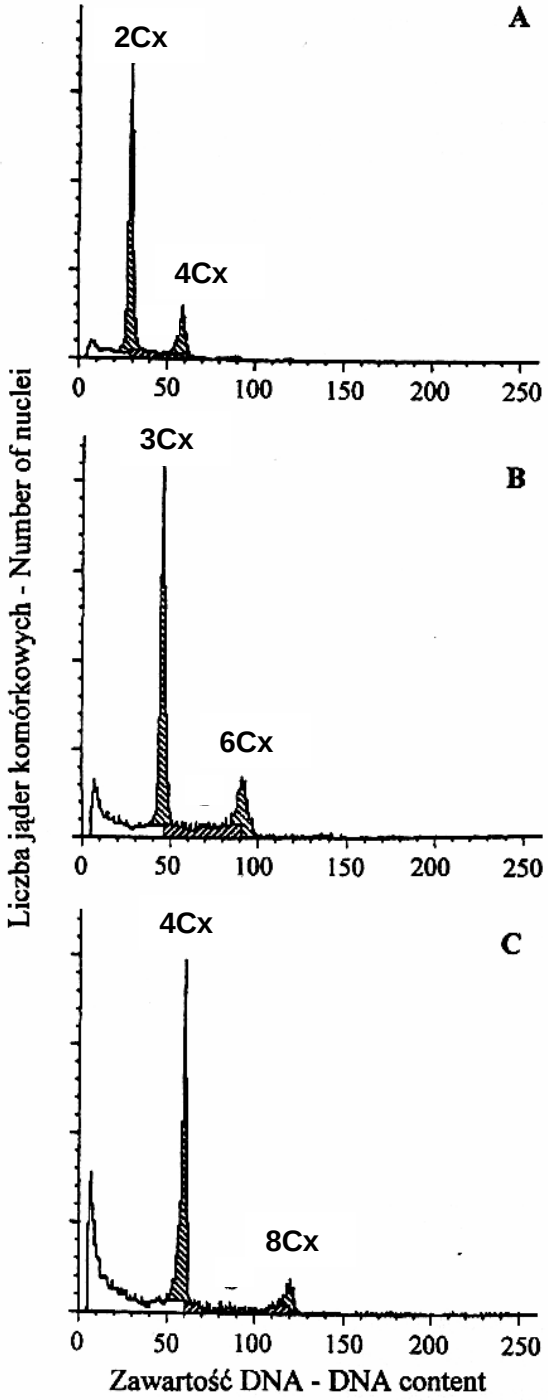
4x

gametes 1x 2x

Double fertilization

egg cell 1x + sperm nucleus 2x → embryo 3x

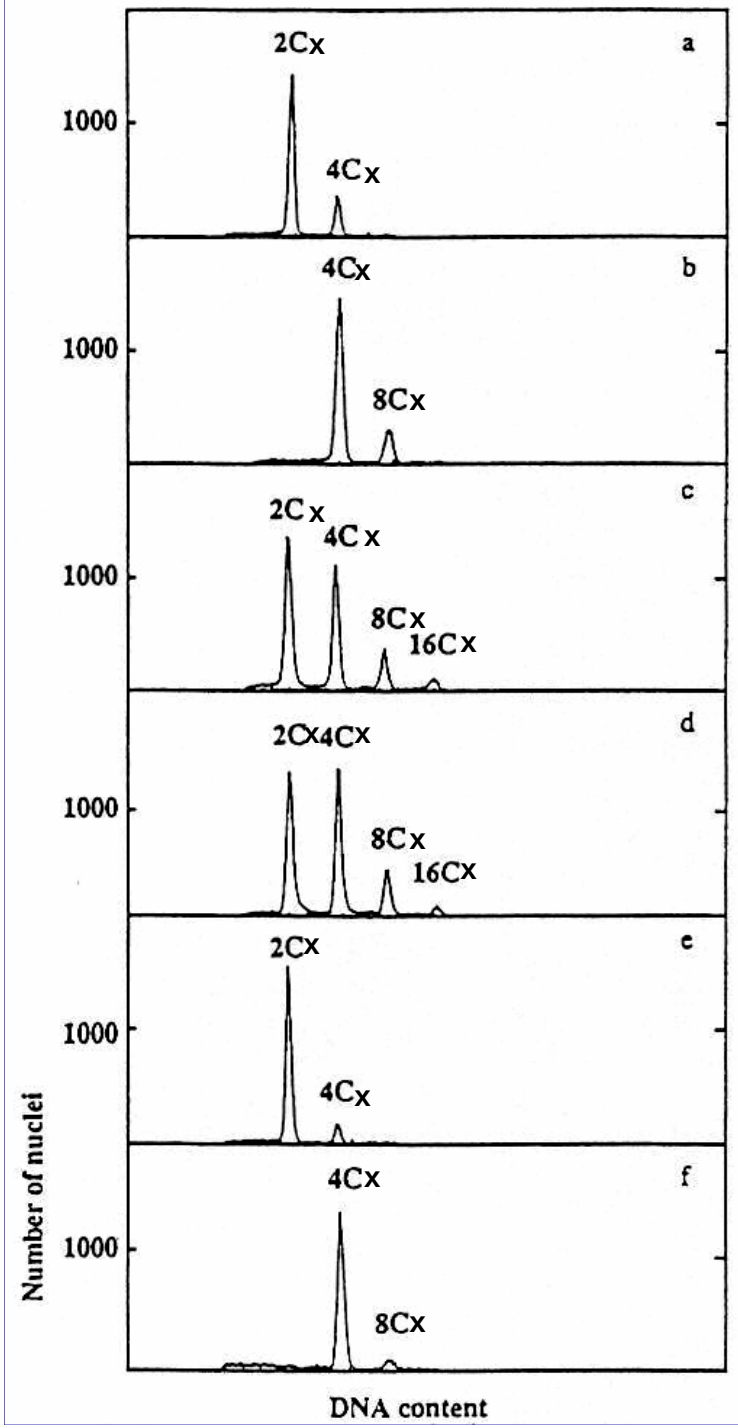
polar nuclei 2x + sperm nucleus 2x → endosperm 4x



Flow cytometric histograms obtained from Sugar-beet leaves (DAPI staining)

A – diploid (2C=2Cx)
B – triploid (2C=3Cx)
C – tetraploid (2C=4Cx)

Cx-value: DNA content of a monoploid genome with chromosome base number *x*; abbreviation for monoploid genome size



Sugar beet micropropagation (maternal 2x plant)

diploid control plant

tetraploid control plant

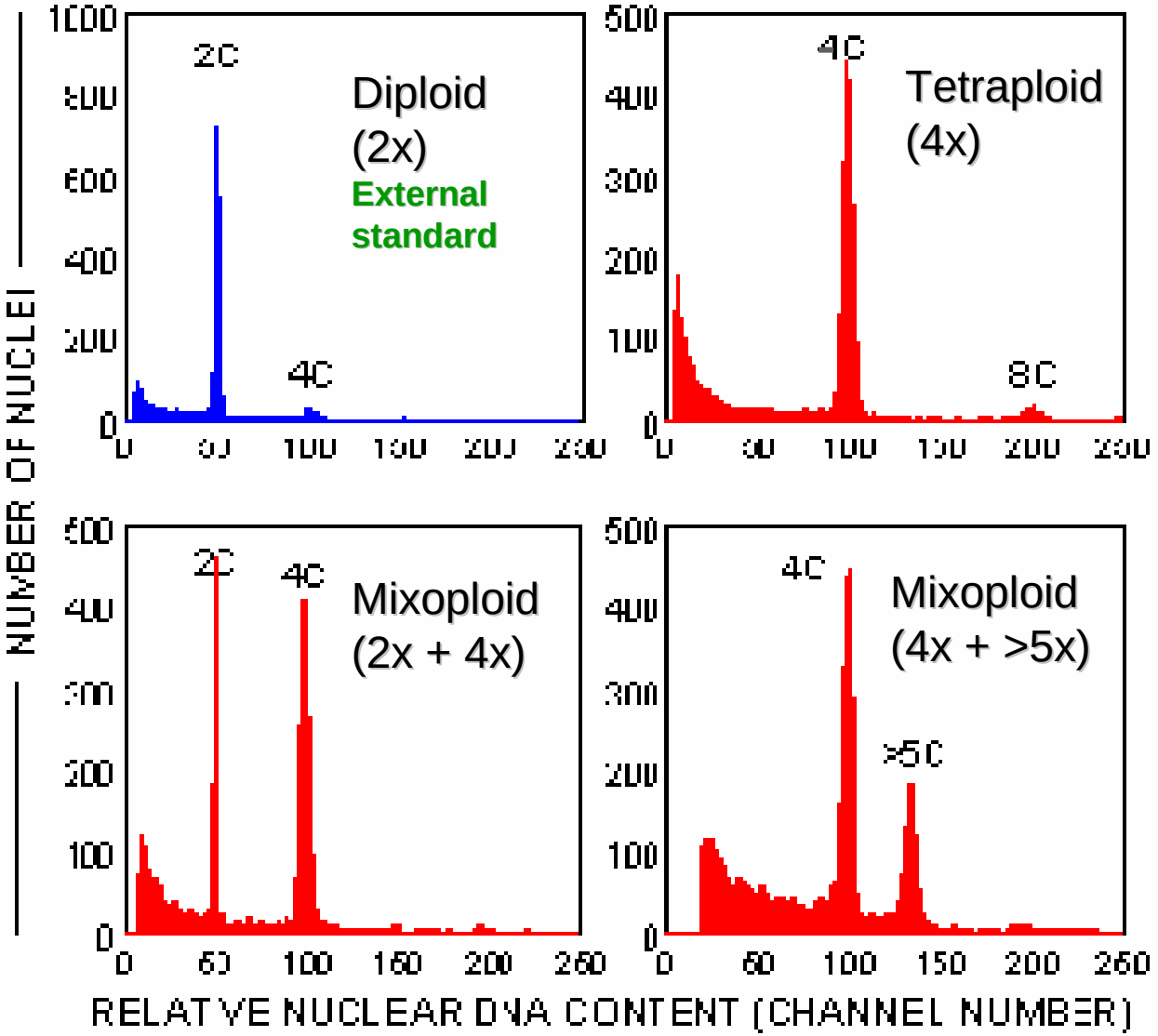
hypocotyl

callus

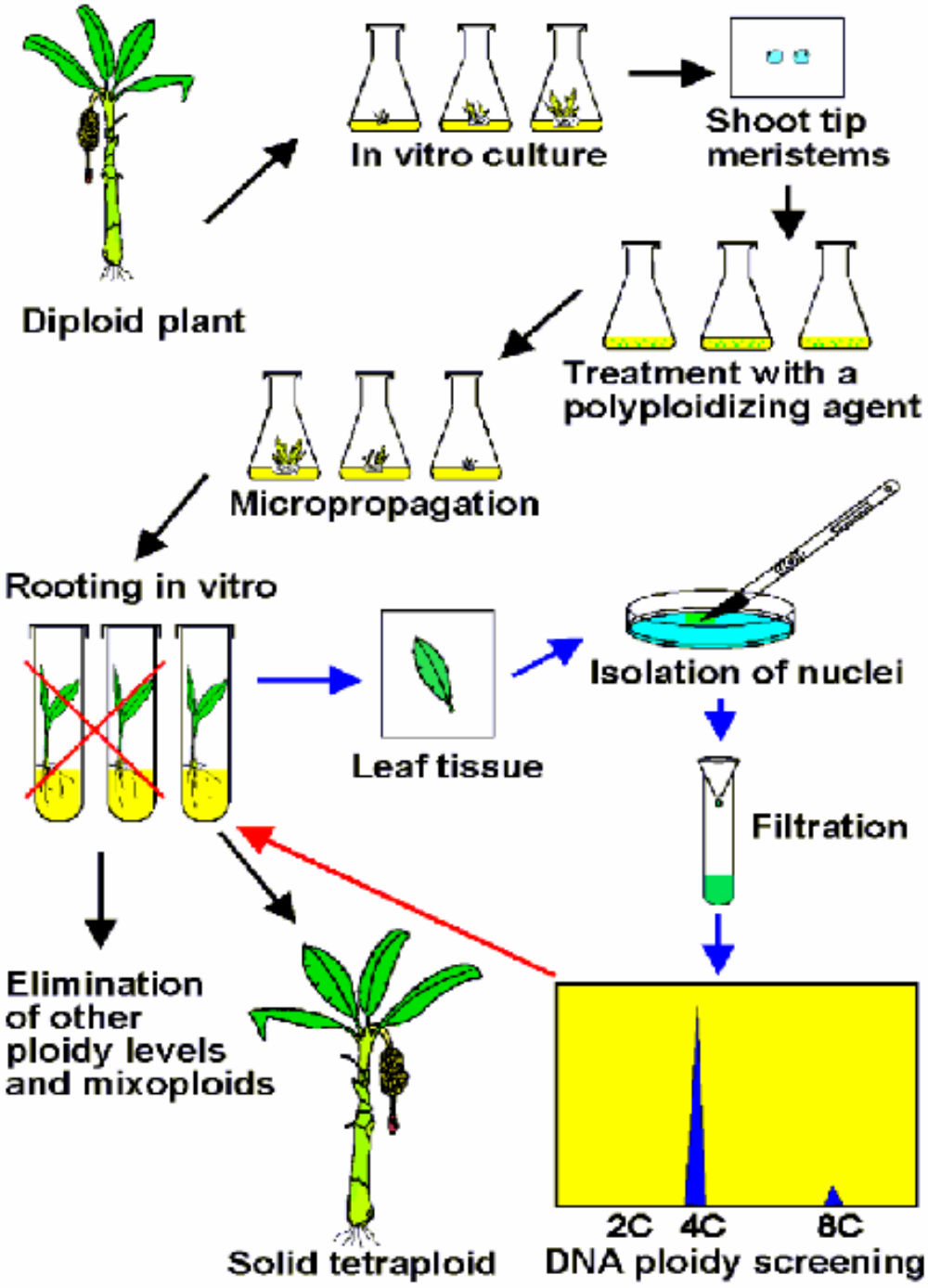
regenerated diploid plantlets (majority)

regenerated tetraploid plantlets

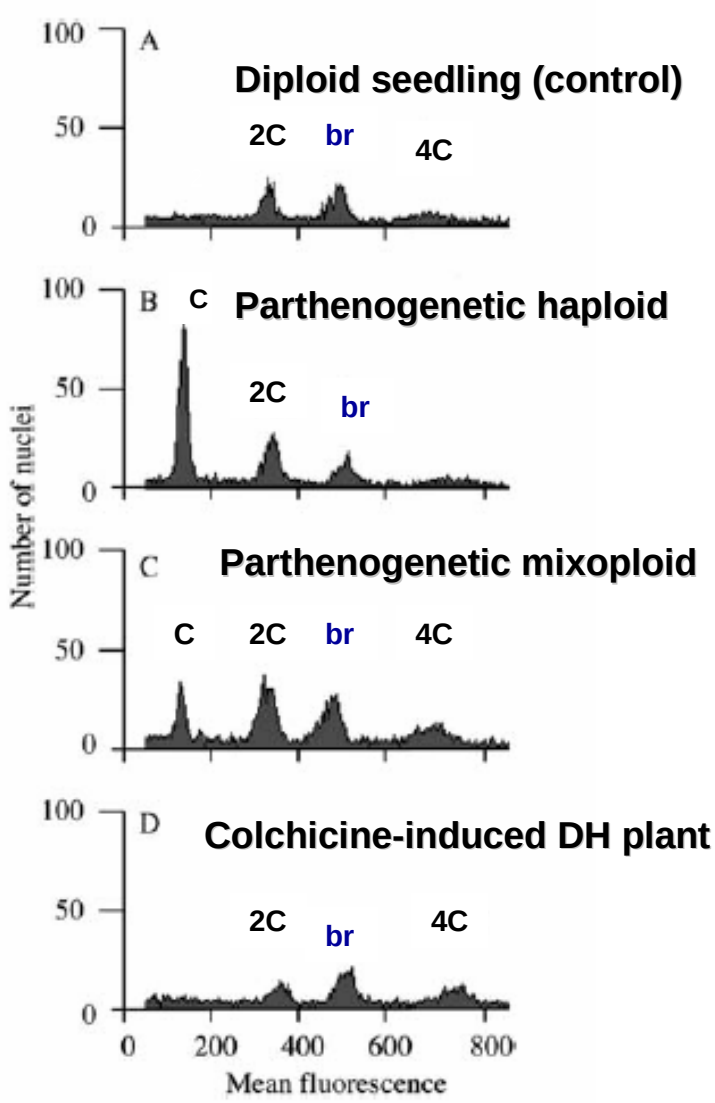
Histograms of DNA content in nuclei isolated from young casava leaves (in vitro culture after colchicine treatment)



Production of tetraploids of *Musa* sp.



Production of haploid and double haploid (DH) plants of Melon



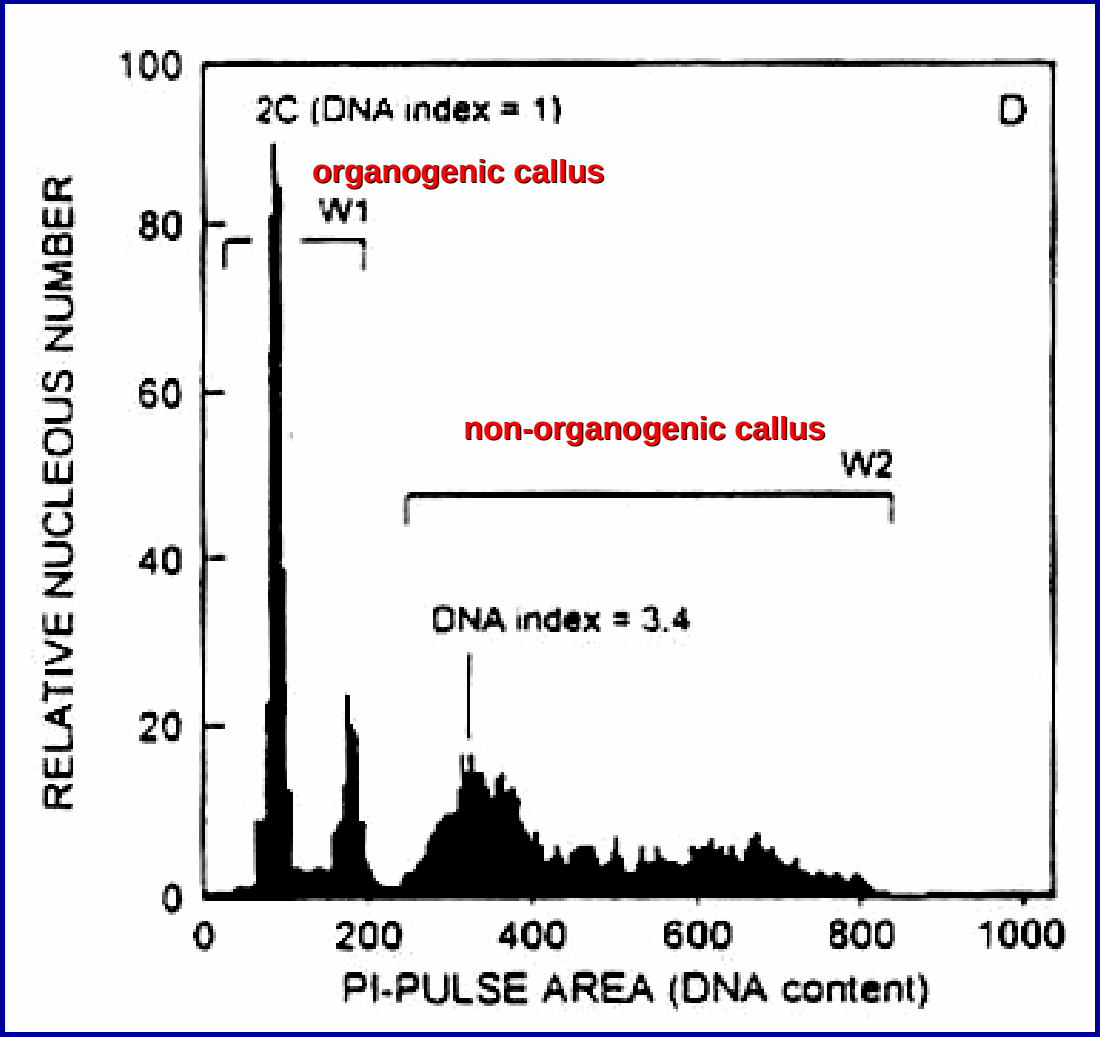
Micropropagated parthenogenetic plantlets

Spontaneous mixoploid plant - fruit

br – broccoli internal standard

Lotfi et al., *Plant Cell Rep*. 2003, 21, 1121-1128

DNA content in organogenic and non-organogenic callus of Sugar beet



Kevers et al. 1999, *Biol. Plant.* 42, 321-332

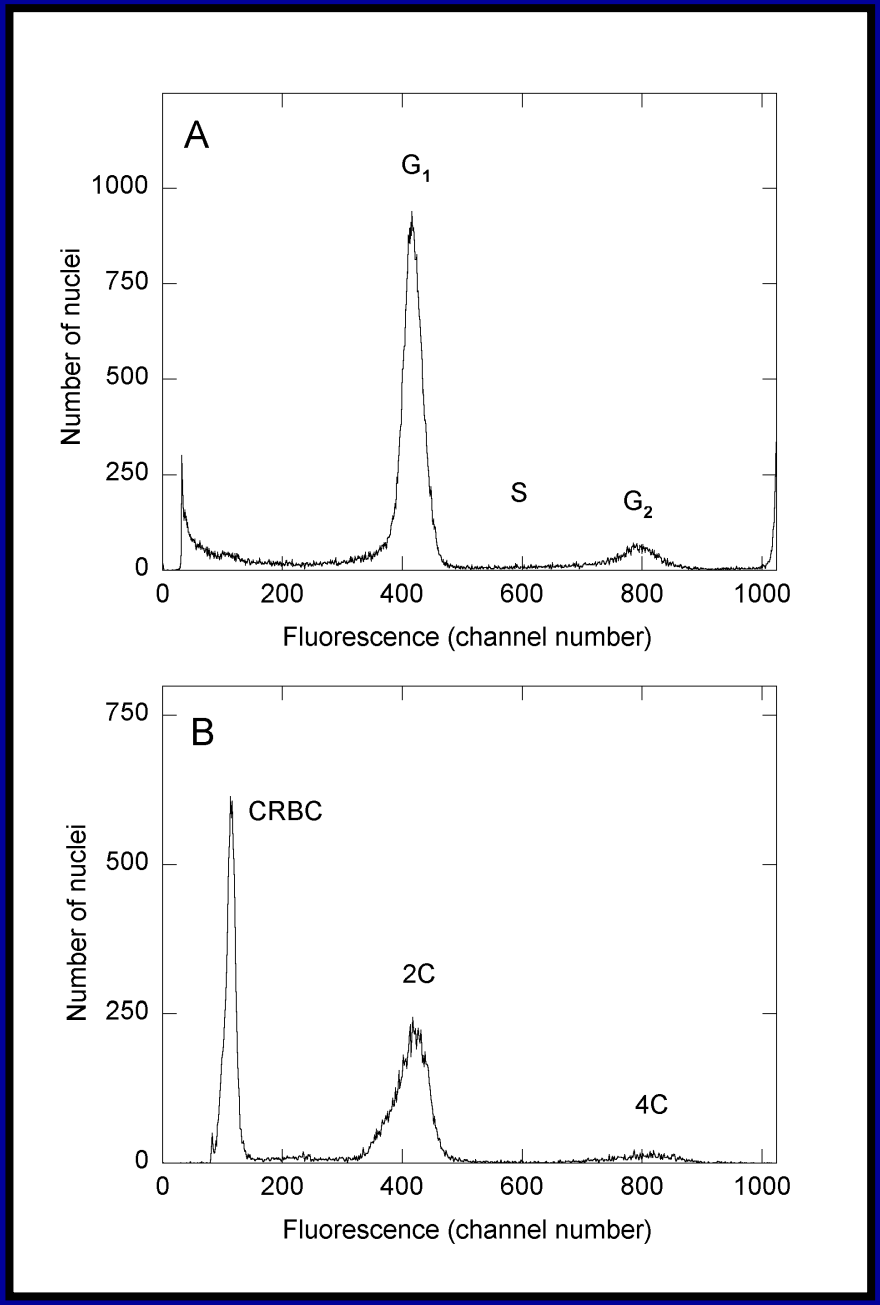
Applications of flow cytometry to plant sciences

- Ploidy estimation.
- Establishing nuclear DNA content.
- Studying cell cycle activity.
- Studying DNA endoreplication.
- Selection of interspecific hybrids.
- Sorting of cells/chromosomes.

Determination of nuclear genome size

Comparison of the relative positions of G_1 peaks in the sample plant (unknown) nuclei with those in nuclei isolated from a plant (control) with known DNA content permits accurate determination of the unknown DNA content (pg/nucleus).

Absolute values in pg DNA can be converted to the number of base pairs. The conversion factor is 1 pg = 978 Mbp.



Analysis of DNA content in the leaf of *Nicotiana tabacum*

Note use of CRBCs as a standard (2.33 pg/2C DNA content)

Why to measure DNA content?

- Identification of species, verification of their taxonomic position,
- Starting point for projects involving genome sequencing,
- Optimizing molecular biology methods,
- Identification of plant material cultured *in vitro*, the genome of which has been changed by somaclonal variation,
- Studying the role of the C-value in plant growth and development, and responses to environmental stresses.

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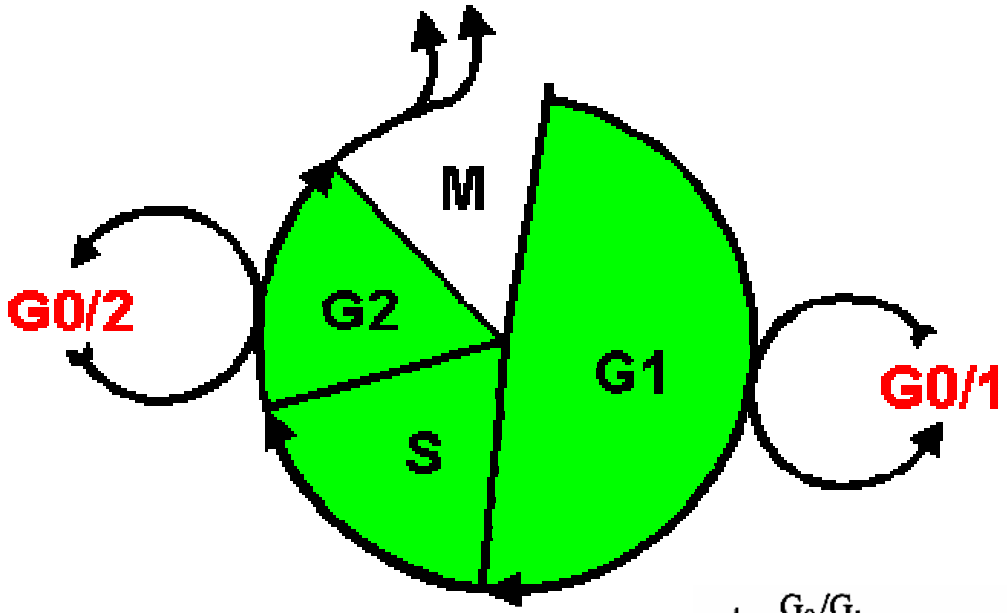
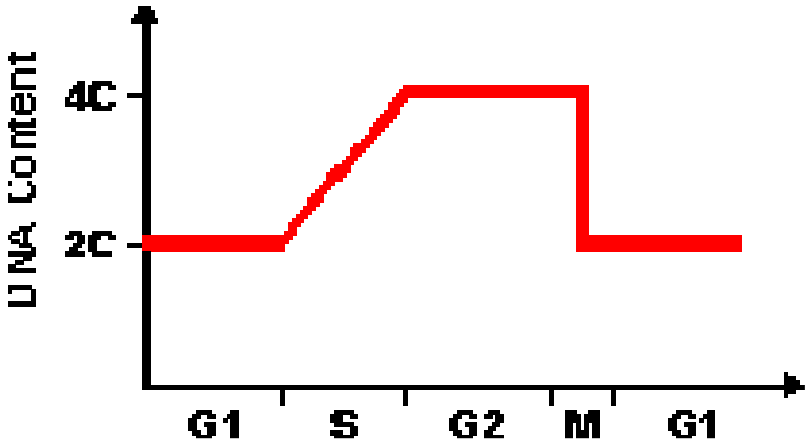
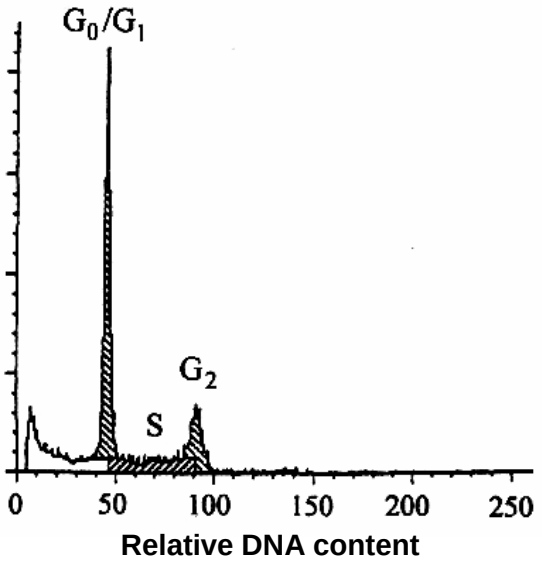
Cell cycle


M
G0/2
G2
G1
S
G0/1

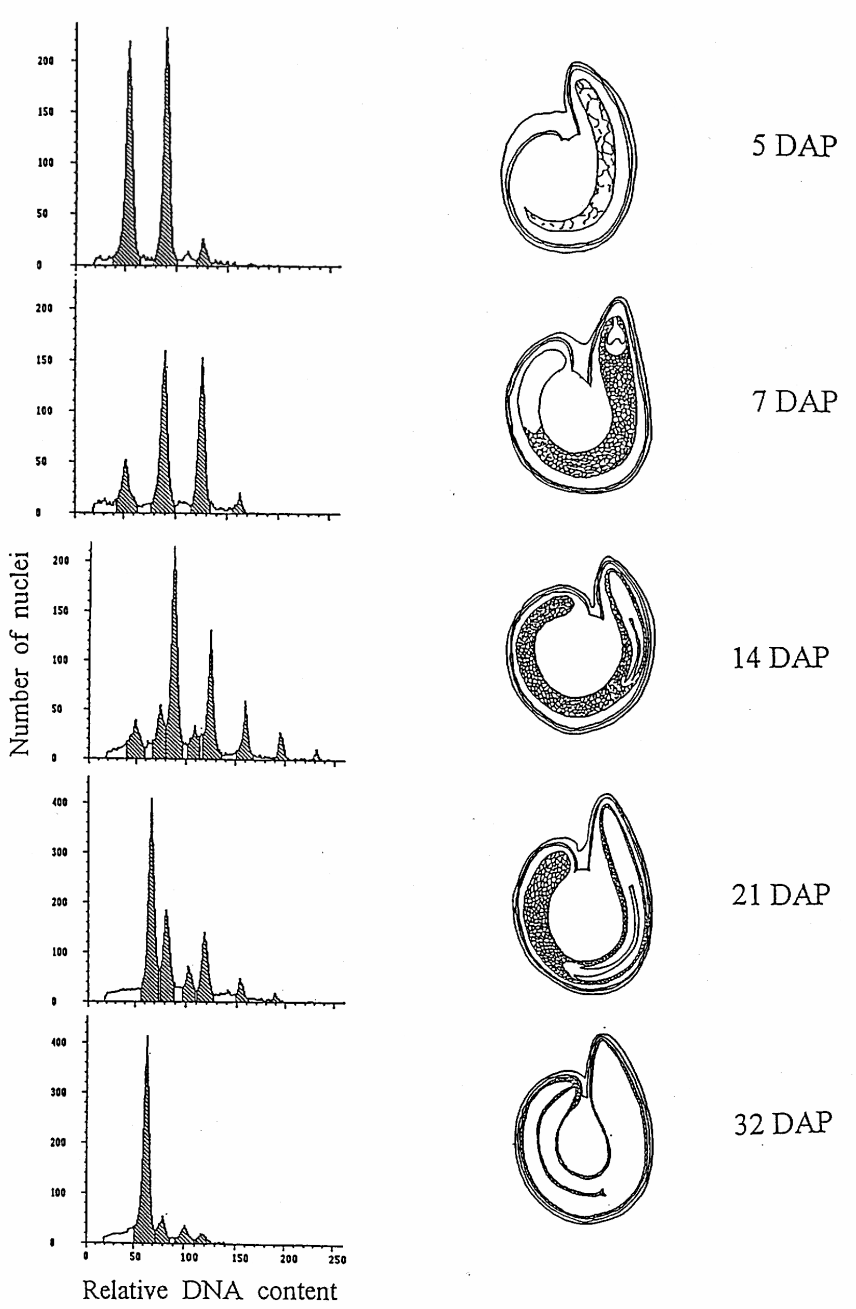

DNA Content
4C
2C
G1
S
G2
M
G1

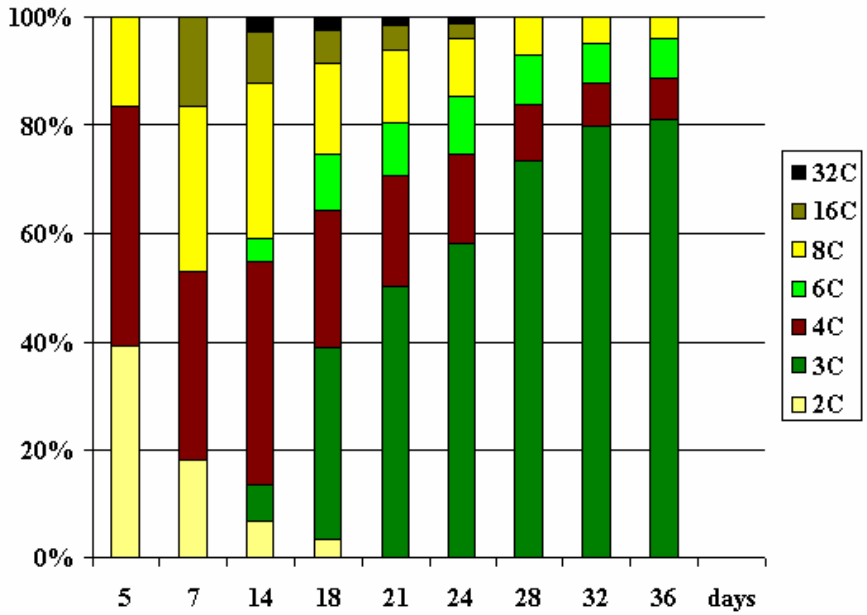

G_0/G_1
G_2
S
0
50
100
150
200
250
Relative DNA content

Flow cytometric analysis of seeds

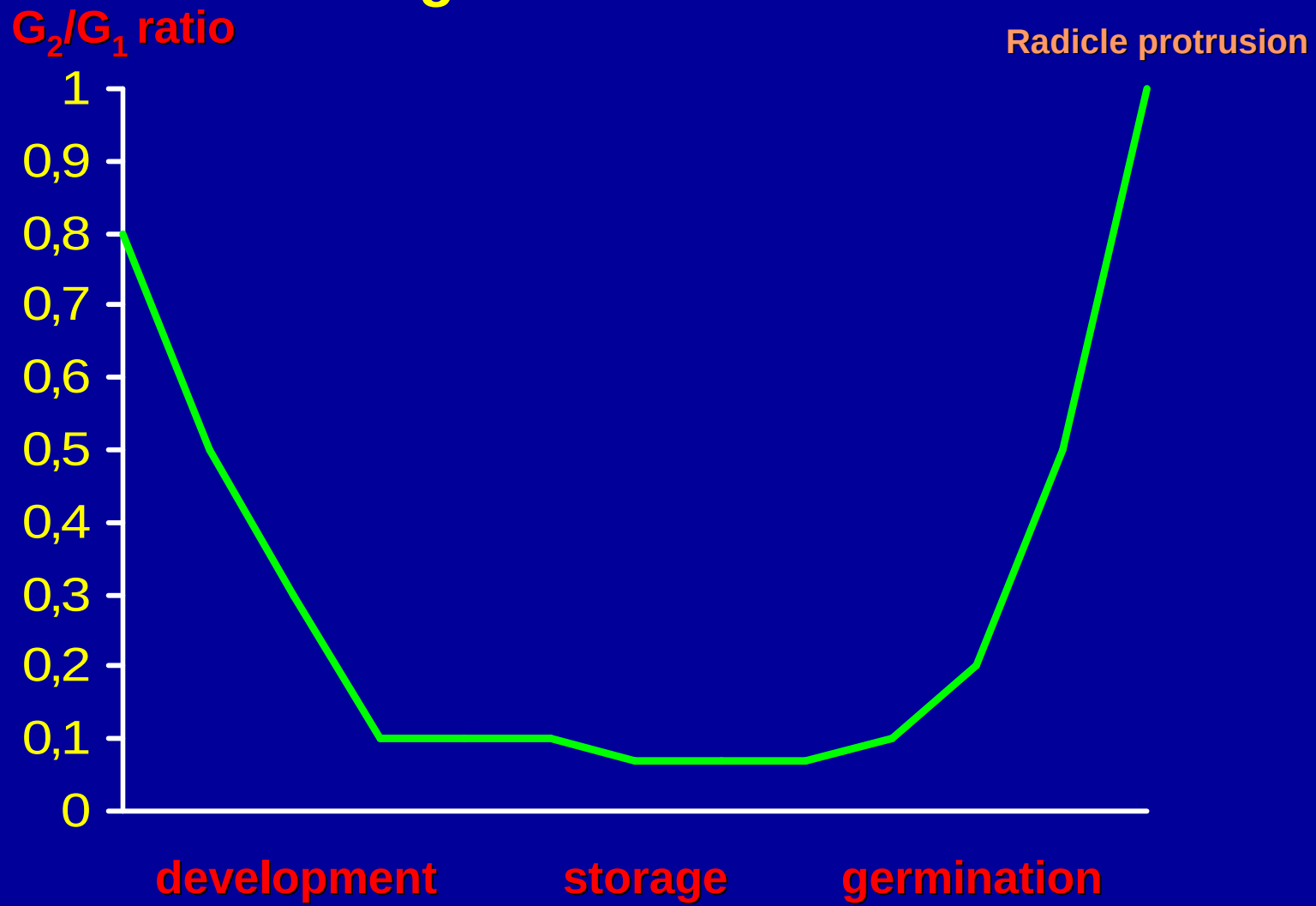
- Following seed development.
- Establishing seed maturity.
- Estimation of the progress of germination or seed treatment.
- Estimation of ploidy and genome size using seed tissue.
- Screening for reproductive pathways.

Changes in the cell cycle activity during development of triploid Sugar-beet seed

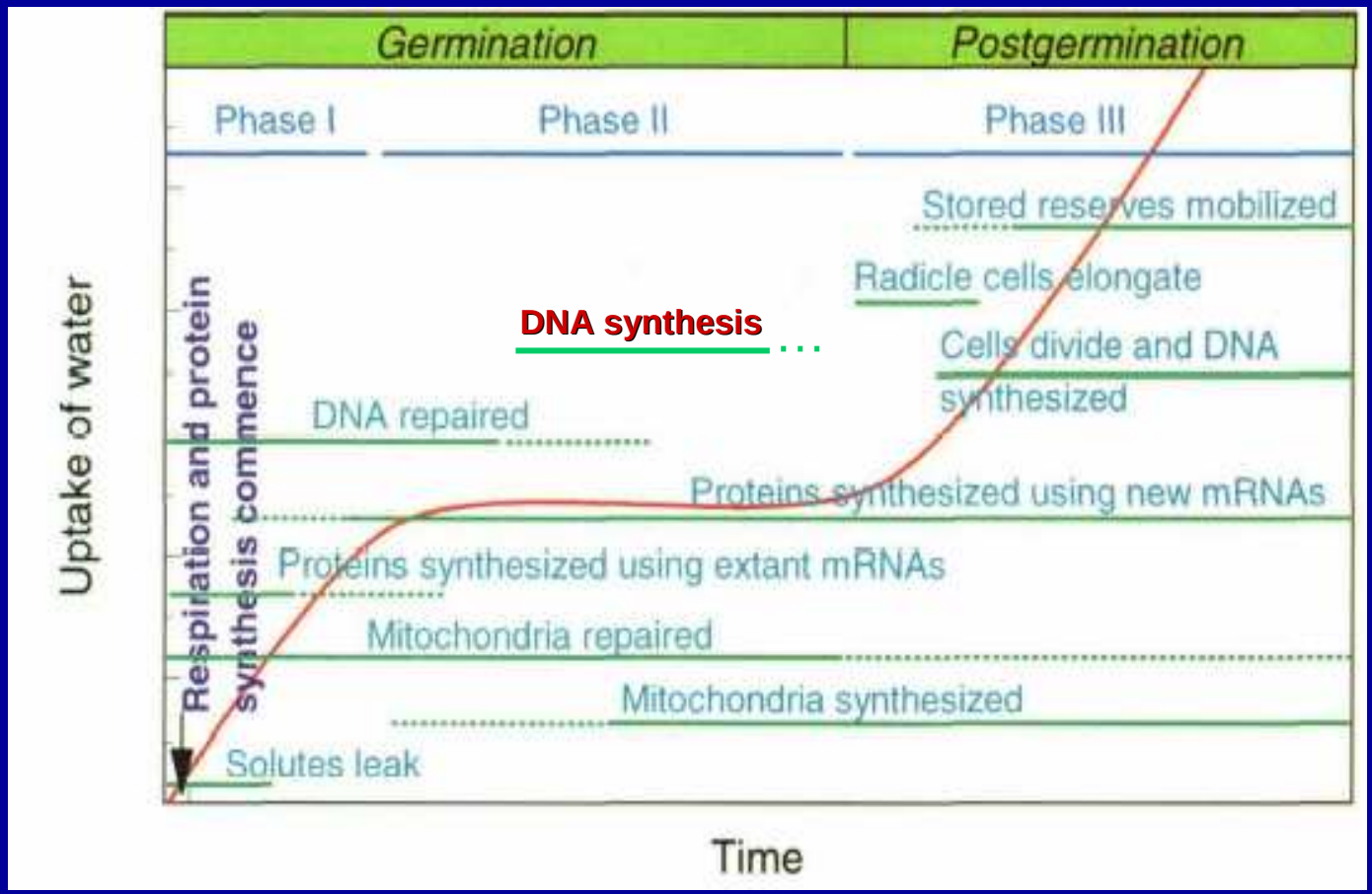




Changes of the G_2/G_1 ratio in the embryo during development, storage and germination of Sugar-beet seeds

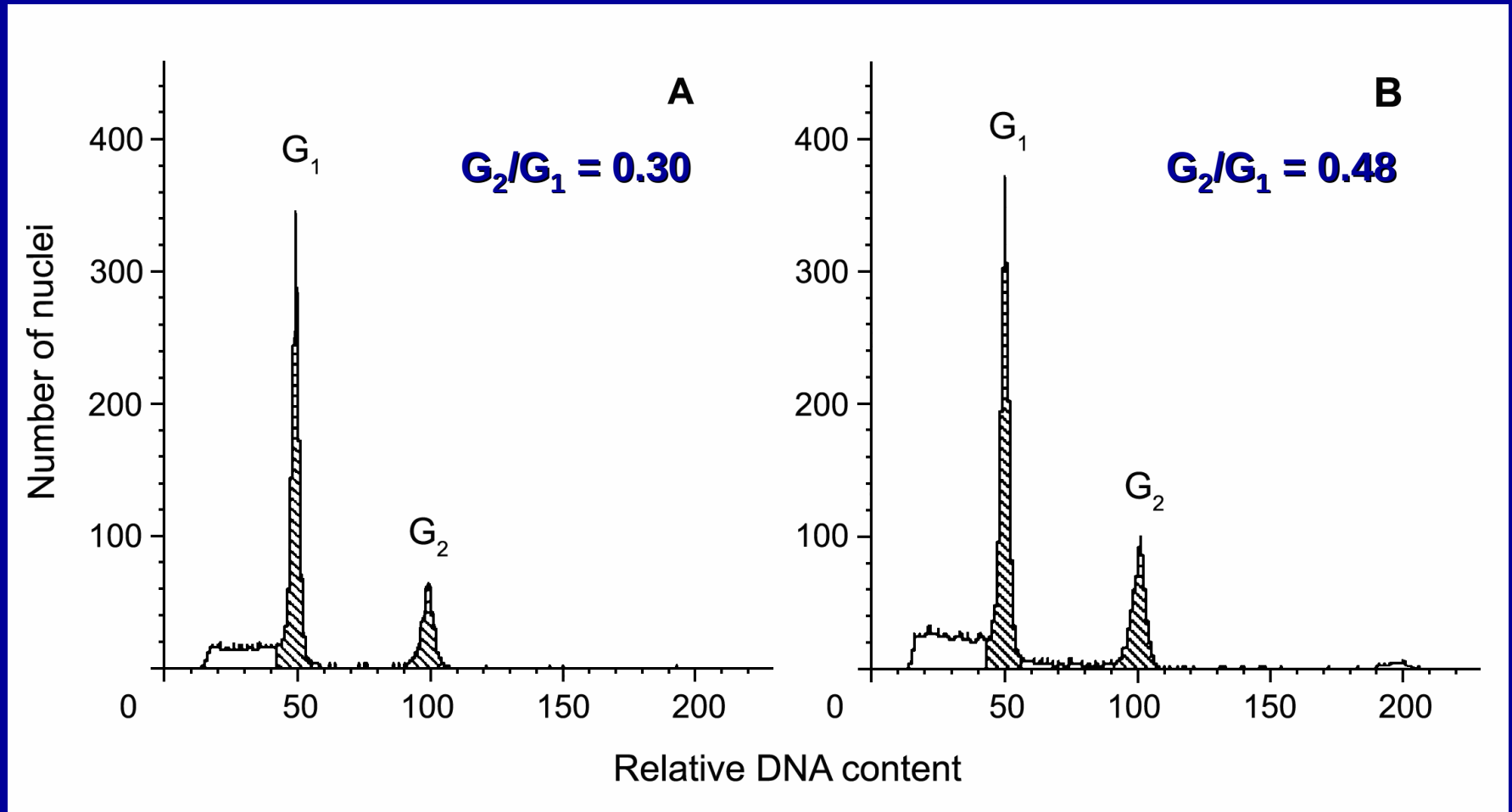


Seed priming – a technique of controlled hydration and subsequent drying that results in more rapid germination when the seeds are re-imbibed.



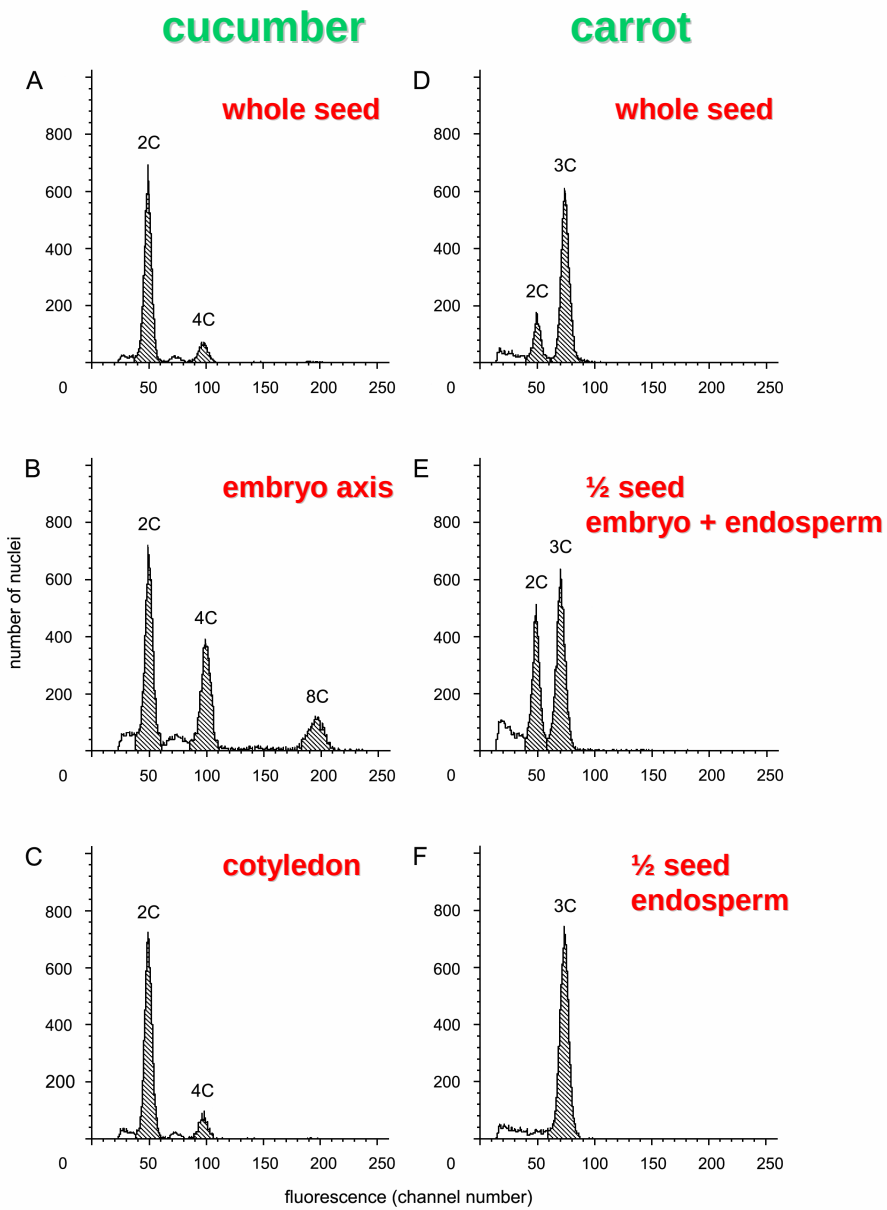
Untreated seed

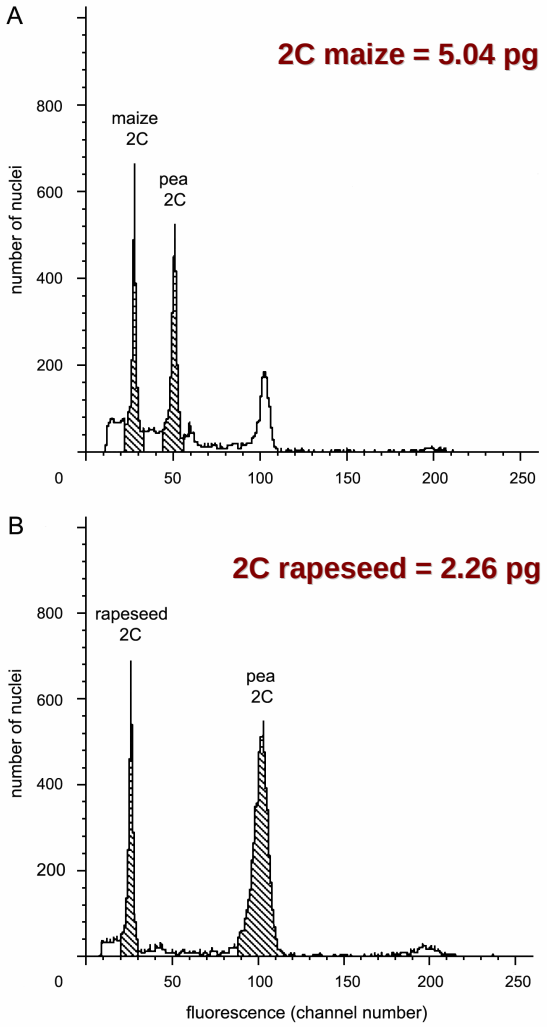
Treated seed



Matriconditioning of Lentil seeds

Estimation of genome size using seeds





Internal standard - radicle of *Pisum sativum*; 2C= 9.11 pg

Applications of flow cytometry to plant sciences

- Ploidy estimation.
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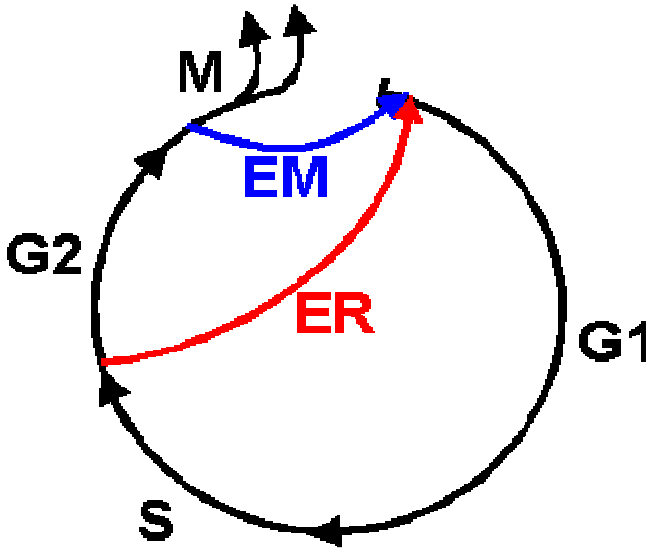
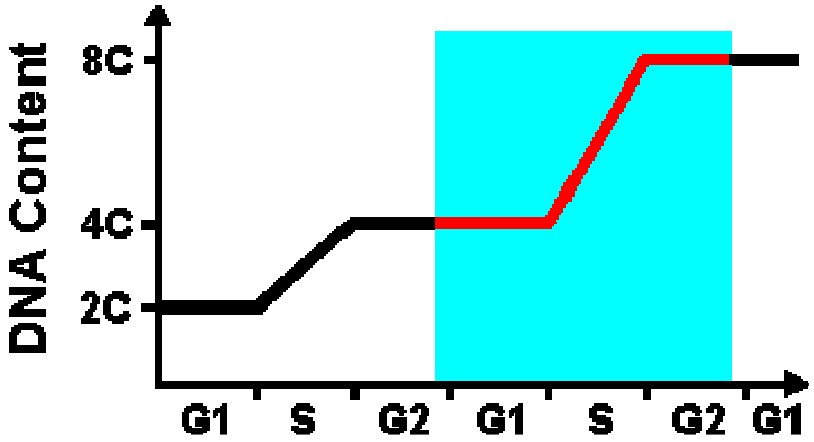
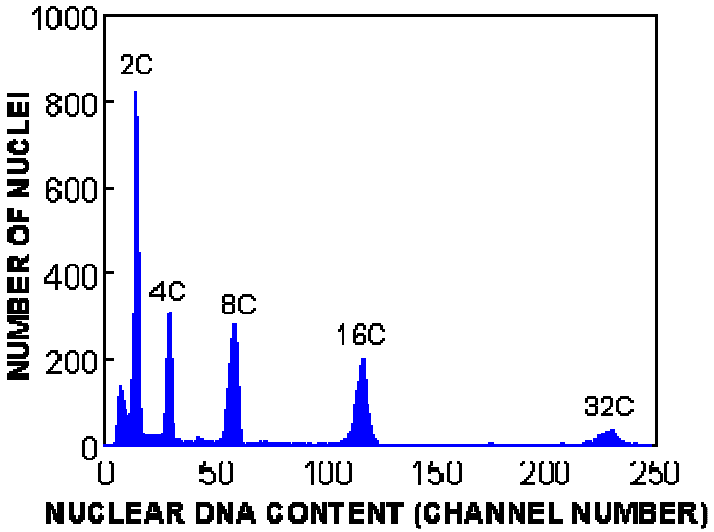
Endoreplication







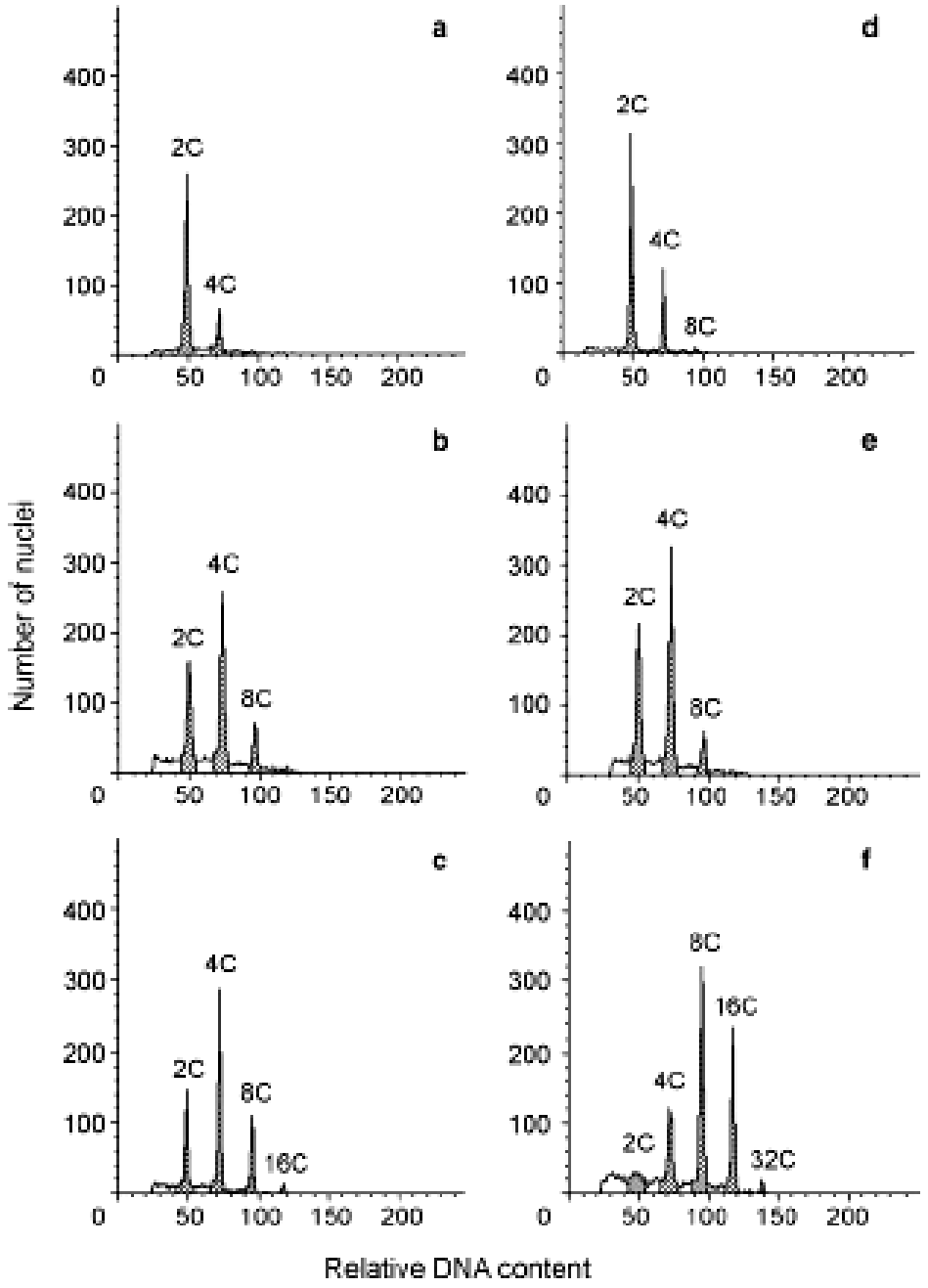
Occurrence and significance of endoreduplication

- Depends on systematic position of the species (polysomatic and non-polysomatic),
- Occurs during cell differentiation, mainly in highly specialized cell types, e.g. in storage (endosperm), and vascular tissues (tissue-specific),
- In some organs of the same plant more intensive than in others (organ-specific),
- Systemic endopolyploidy,
- Occurs in older organs rather than in younger ones (developmentally regulated),

Occurrence and significance of endoreduplication

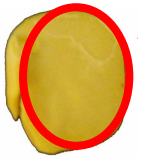
- Correlates with the size of cells,
- Functional significance (the need to coordinate gene expression required for the interaction of nuclear and organellar genomes),
- Characteristic for species with small genomes (evolutionary strategy which substitutes for a lack of phylogenetic increase in nuclear DNA content),
- ?

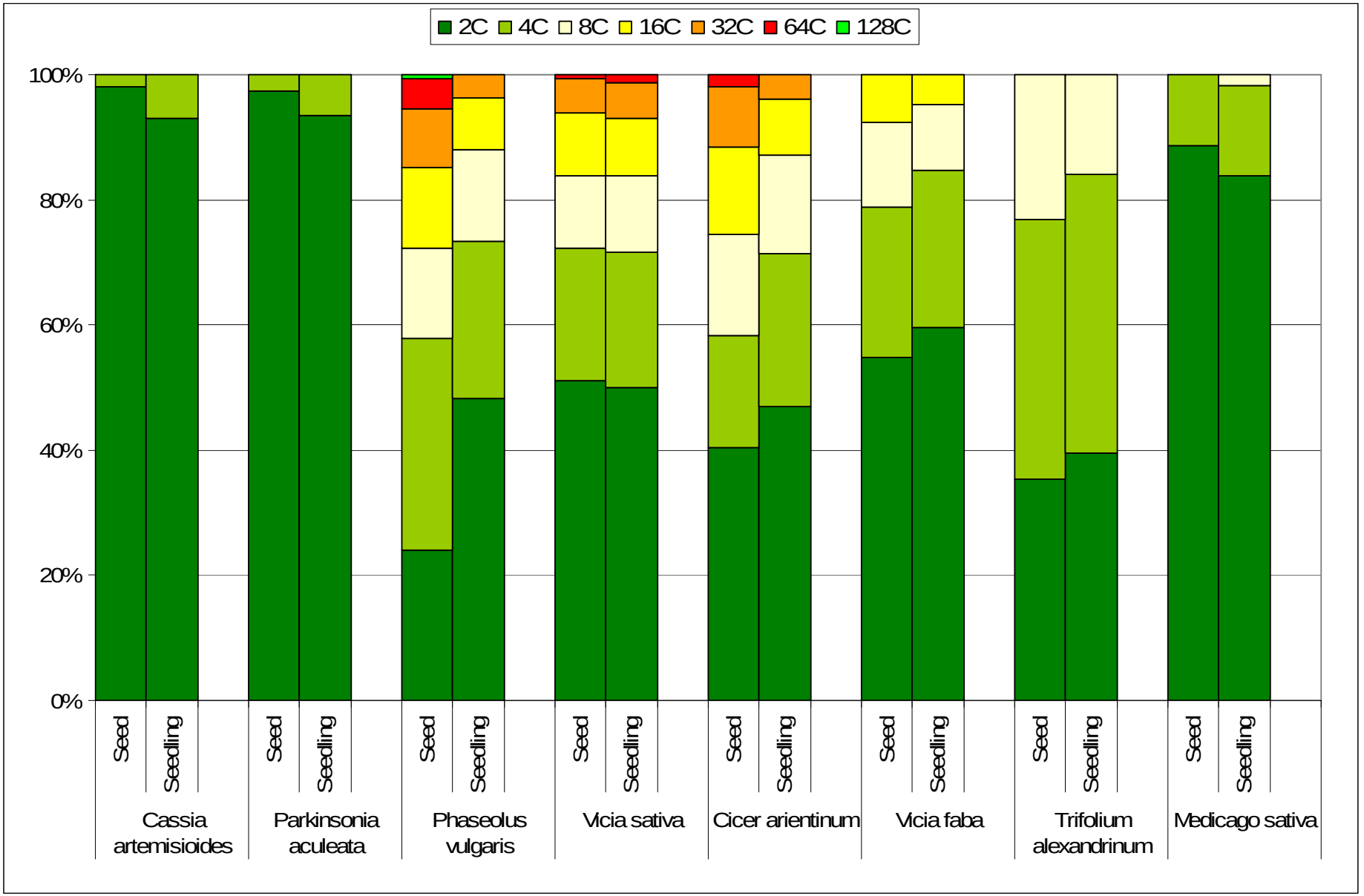
DNA histograms of nuclear preparations from diploid Sugar beet at the vegetative (a-c) and the reproductive (d-f) stage. (a) lamina of the youngest leaf; (b) petiole of the youngest leaf; (c,f) root storage parenchyma; (d) inflorescence bract; (e) lamina of the oldest leaf of a rosette.



Lukaszewska & Sliwinska 2007,
Sex. Plant. Reprod. 20, 99-107

Procentage of the nuclei with different DNA content in cotyledons of seeds and young seedlings of species from the Fabaceae family





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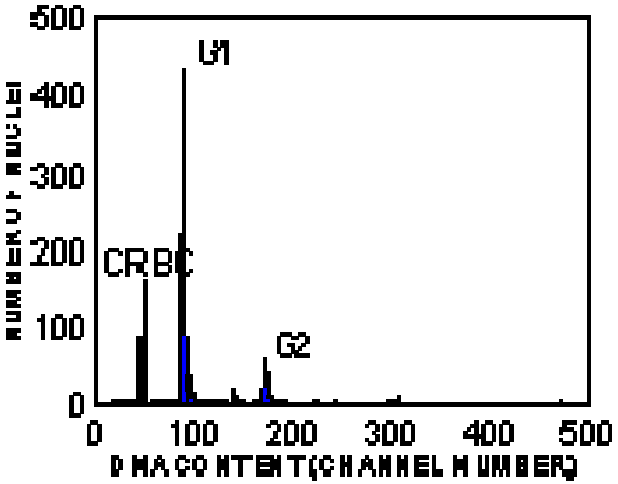
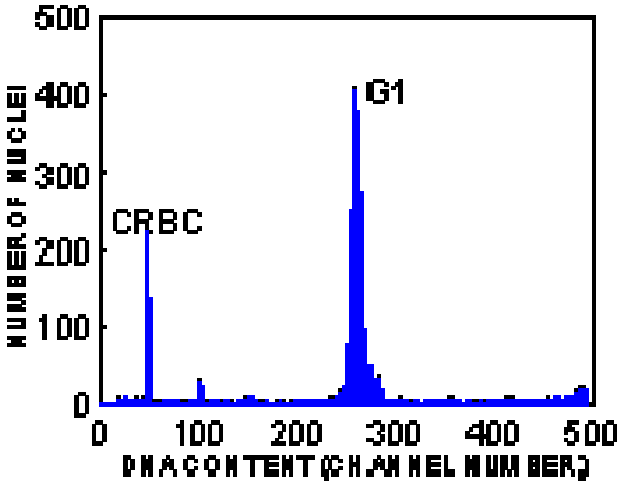
Interspecific hybridization

Lolium multiflorum (2n=14)

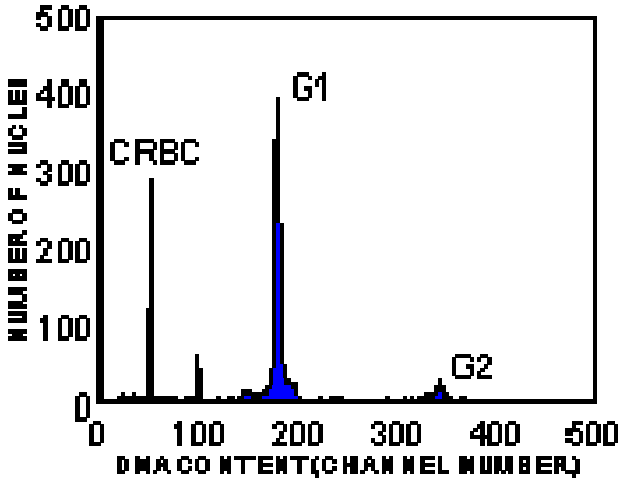


X

Festuca arundinacea (2n=42)



F1 HYBRID



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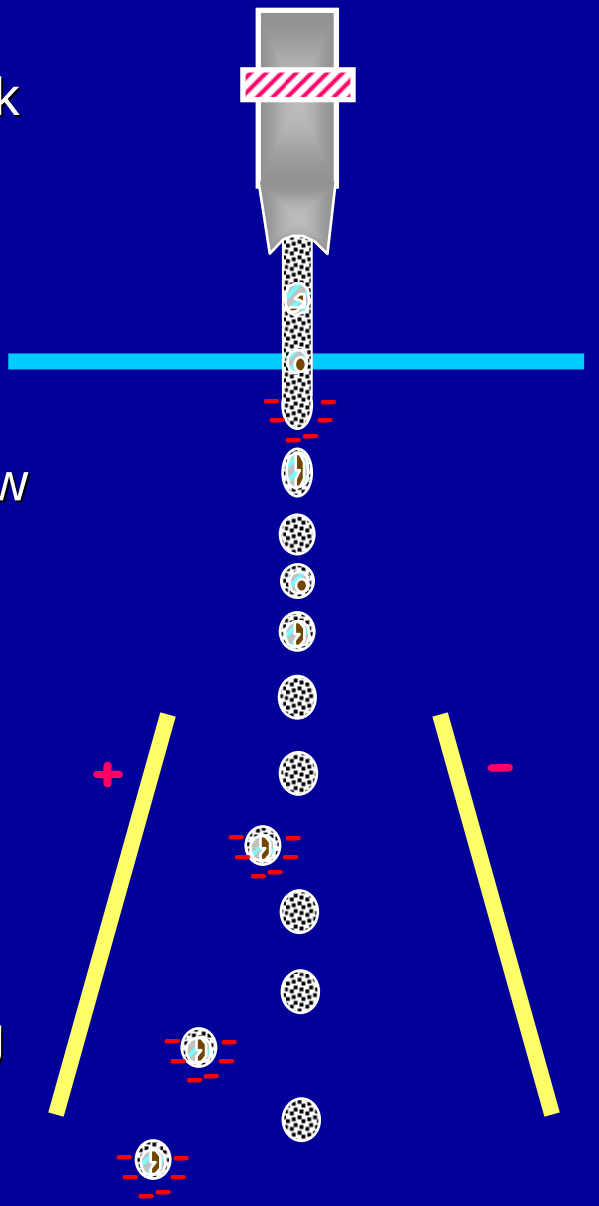
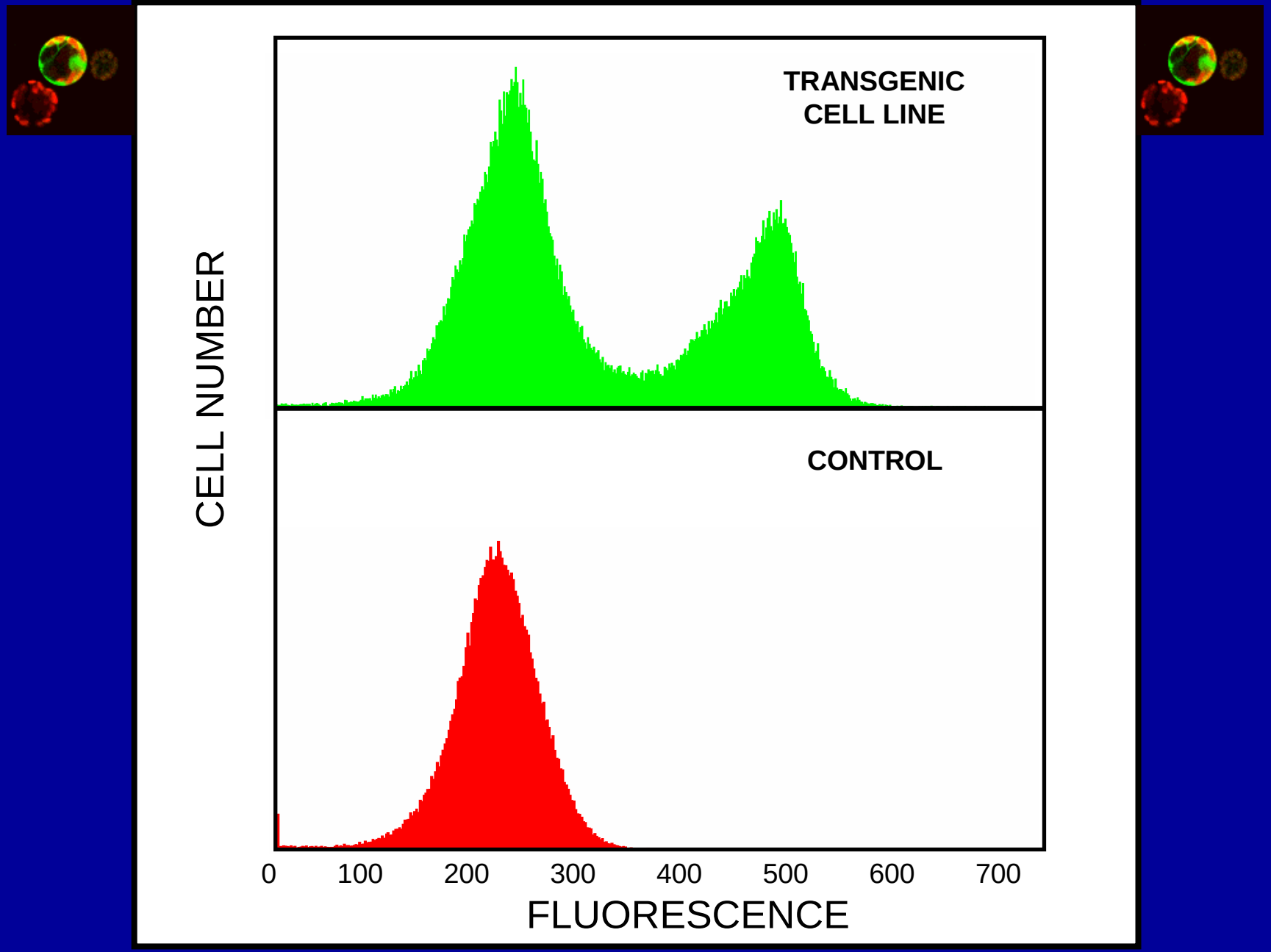
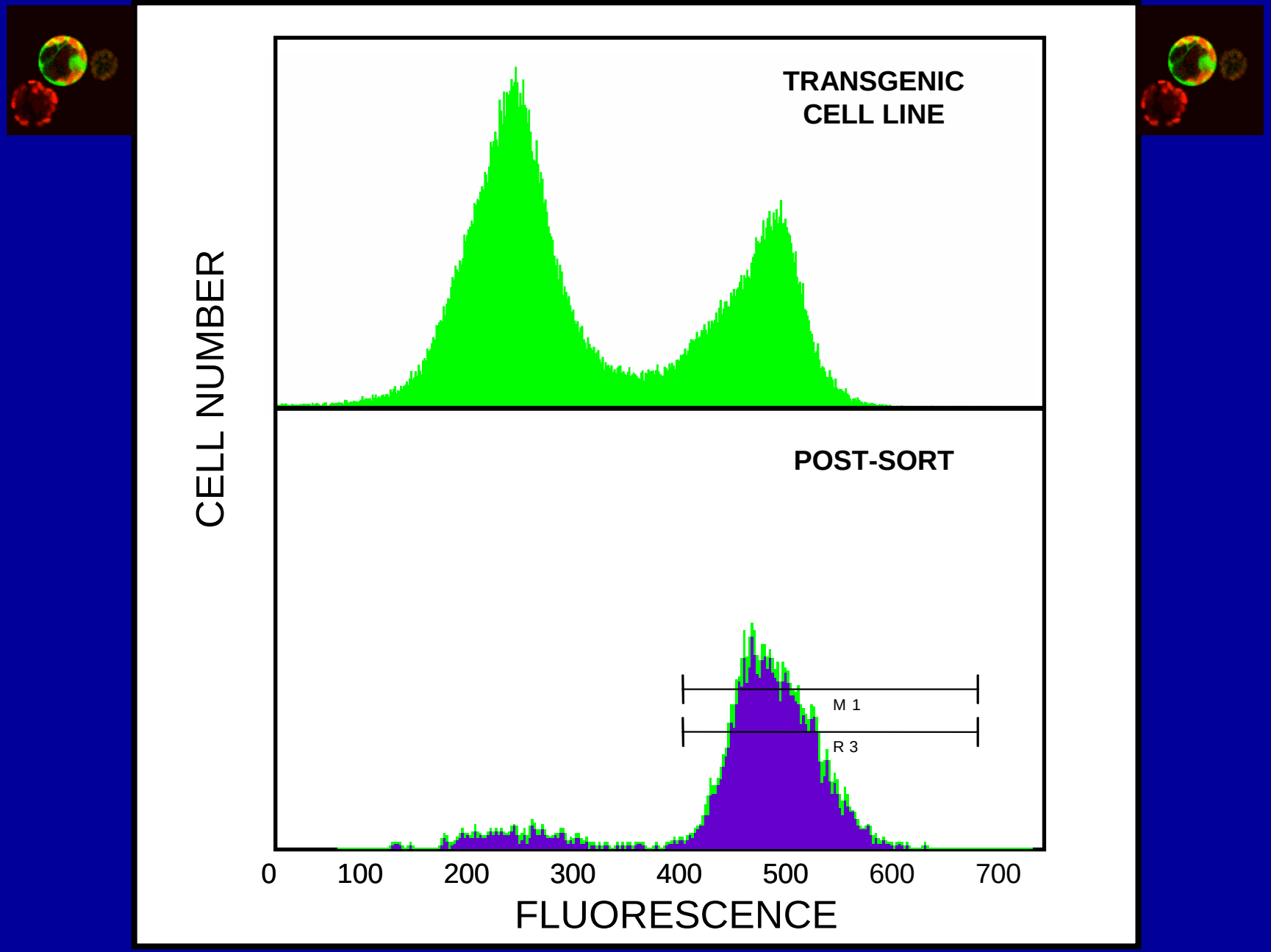
Sorting

Liquid jets in air are inherently unstable and break up into droplets.

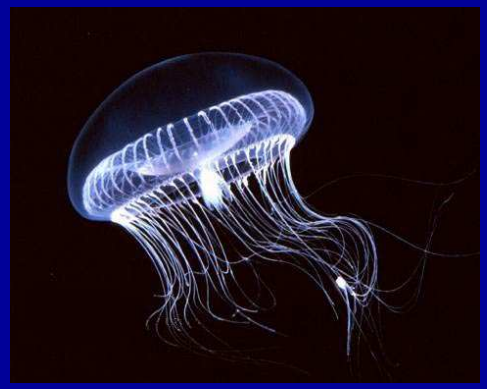
- If we mechanically vibrate the flow tip, the production of droplets is precisely synchronized.
- Droplets are produced at a precise distance below the flow tip, hence below the laser intersection point.
- If we put a charge on the flow stream at the point that the desired particle is entering the “last-attached” droplet, this droplet remains charged when it breaks off.
- We can subsequently deflect the cells by passing the droplets through an electrostatic field.

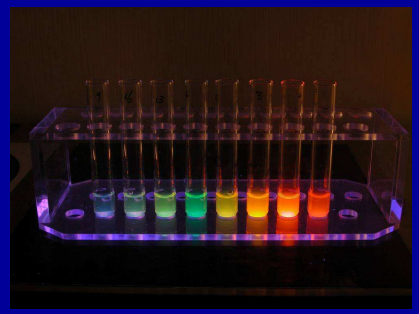



TRANSGENIC
CELL LINE
CONTROL
CELL NUMBER
0
100
200
300
400
500
600
700
FLUORESCENCE


TRANSGENIC
CELL LINE
POST-SORT
M 1
R 3
CELL NUMBER
0
100
200
300
400
500
600
700
FLUORESCENCE

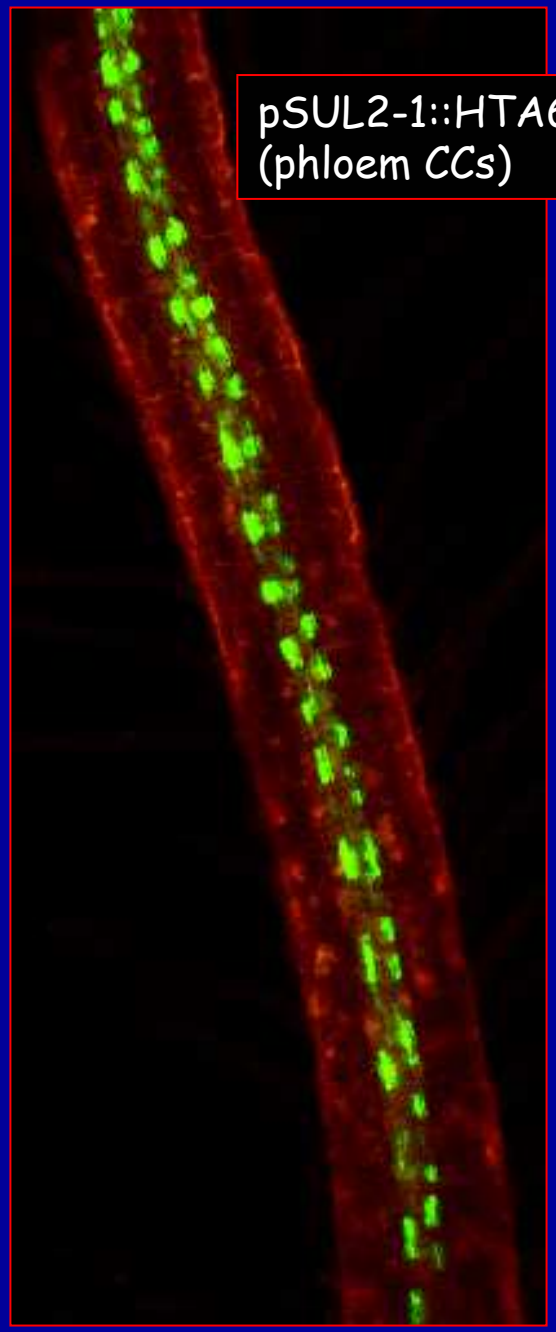
The **green fluorescent protein (GFP)** is a protein that exhibits bright green fluorescence when exposed to blue light. GFP was first isolated from the **jellyfish *Aequorea victoria***.

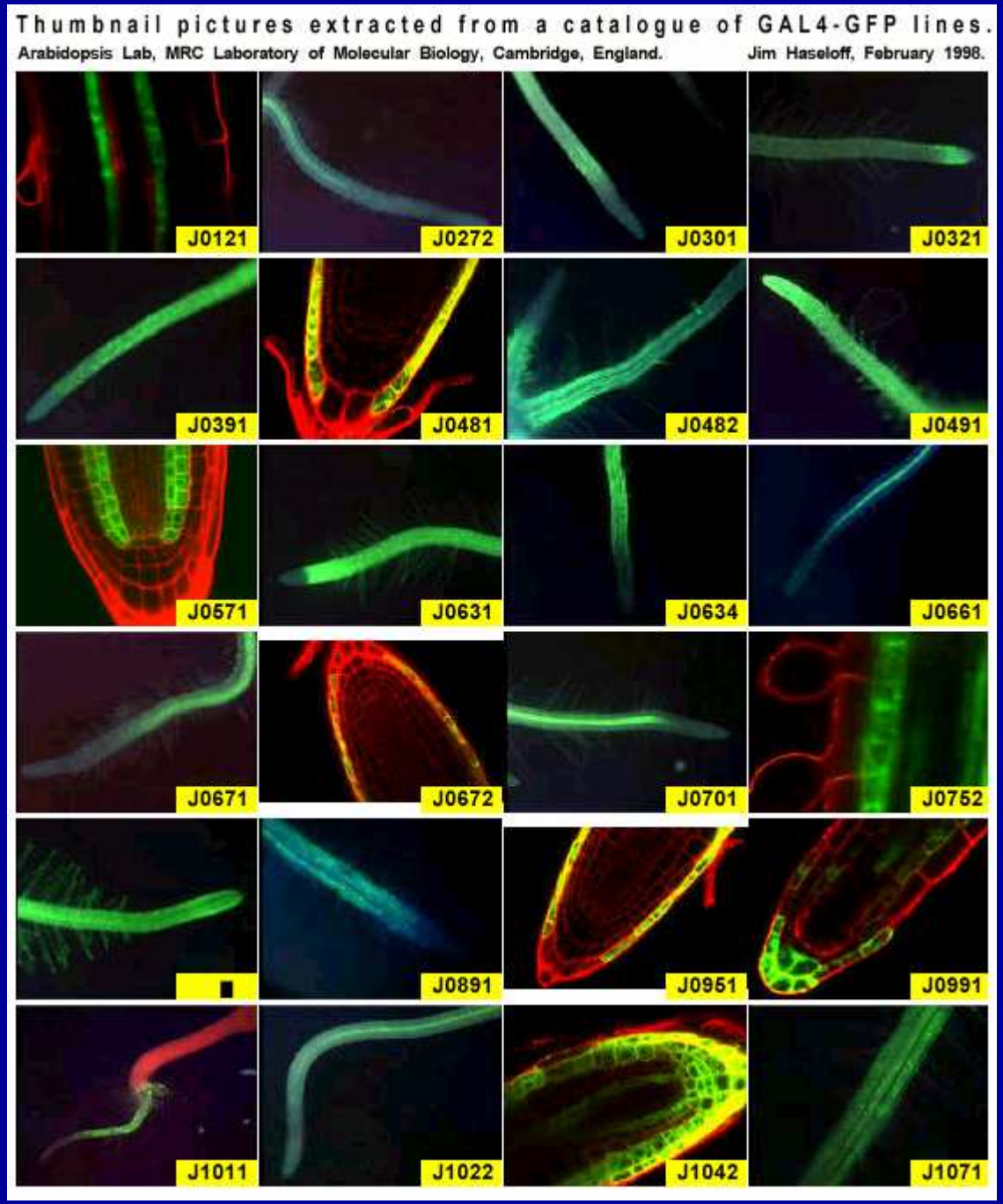




Julian Voss-Andreae's GFP-based sculpture *Steel Jellyfish* (2006). The image shows the stainless-steel sculpture on display at Friday Harbor Laboratories on San Juan Island (Wash., USA), the place of GFP's discovery (Nobel Price for M. Chalfie, O. Shimomura and R. Y. Tsien in 2008).

Cell type-specific labeling

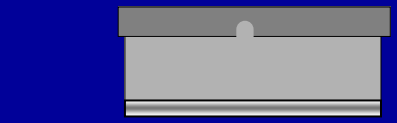


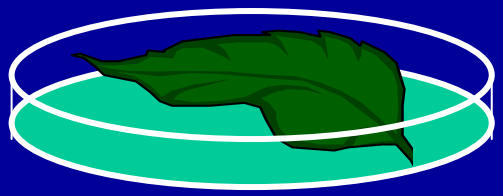


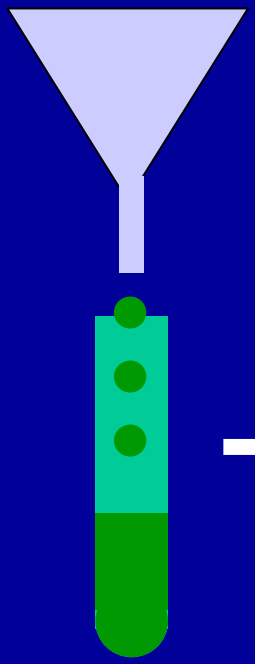
Cell-Specific GFP Expression

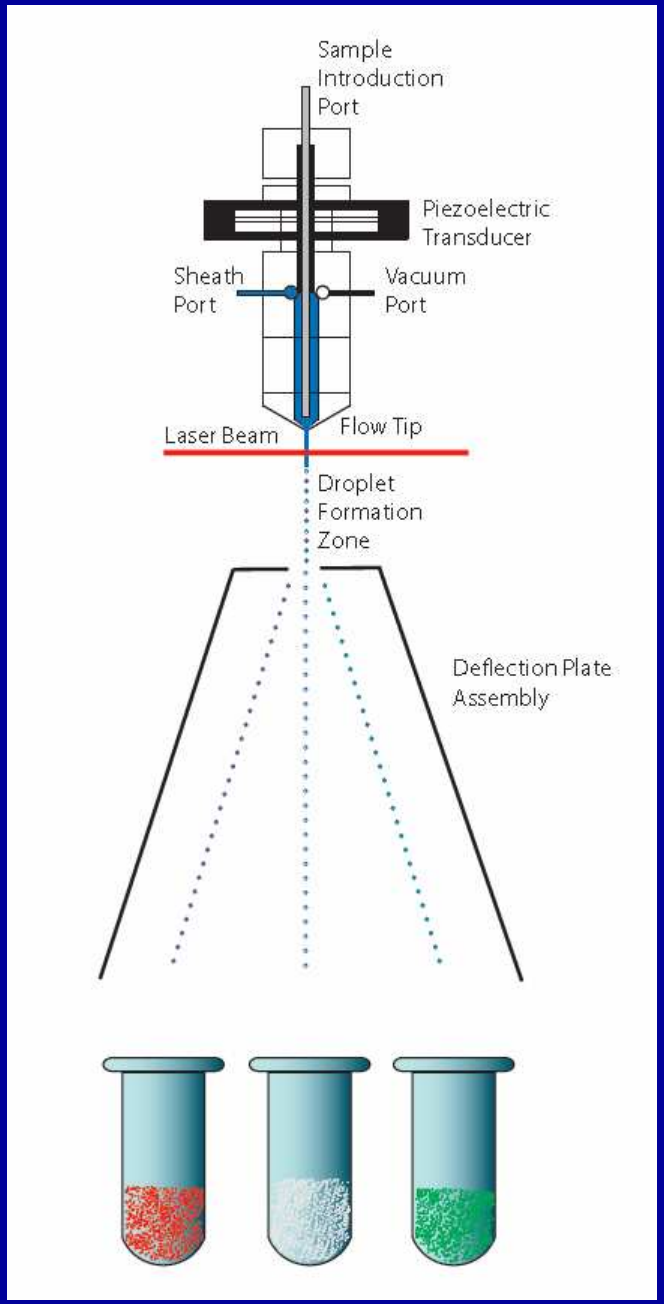
- Catalog of available transgenic *Arabidopsis* lines.
- Lines are available from the stock centers.
- However, the molecular basis for the observed phenotype is usually uncharacterized.

Image from Jim Haseloff









RNA isolation

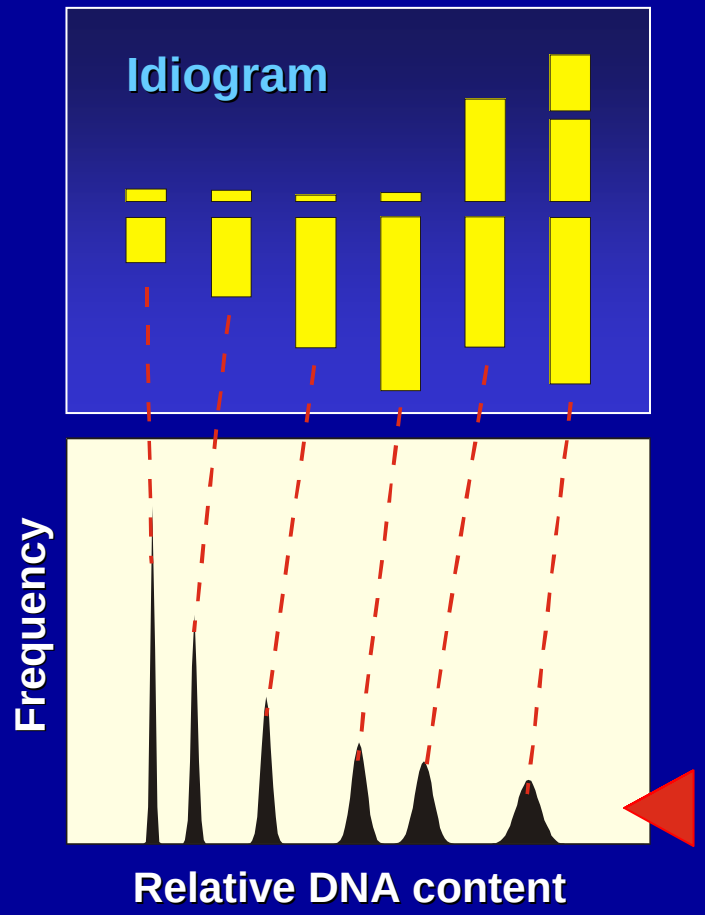
microarrays

CHROMOSOME SORTING

Applications:

- Chromosome-specific DNA libraries
- Targeted isolation of molecular markers
- Physical gene mapping
- Chromosome-specific cDNAs
- Chromosome painting probes
- FISH on extended chromatin fibres
- Chromosome-specific proteins
- Artificial plant chromosomes
- Chromosome-mediated gene transfer

Chromosome analysis by flow cytometry: flow karyotyping



Theoretical flow karyotype

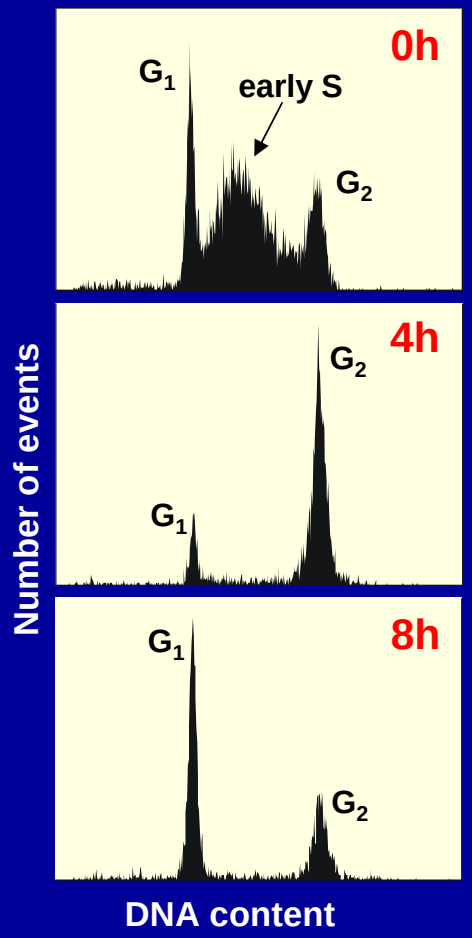
Flow karyotyping provides data quantifying both the frequency of occurrence and relative DNA content of chromosome types in a cell population

Application:

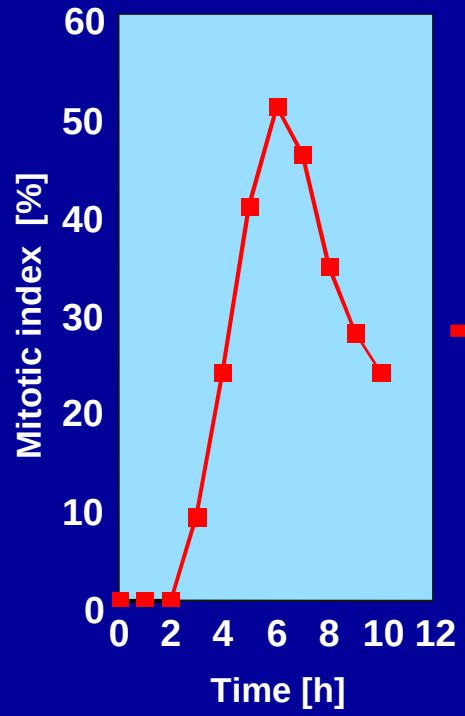
Quantitative detection of structural and numerical chromosome aberrations

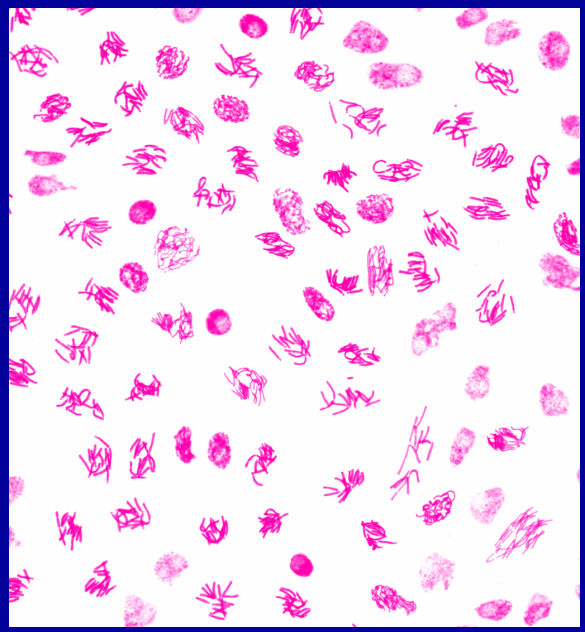
Doležel (1998)

Cell cycle synchronization



A combined treatment of root-tip meristems with hydroxyurea (1.25-2.5 mM / 18h) and amiprophos-methyl (2.5-10 μM / 2h) results in a high degree of synchrony and metaphase indices exceeding 50%.





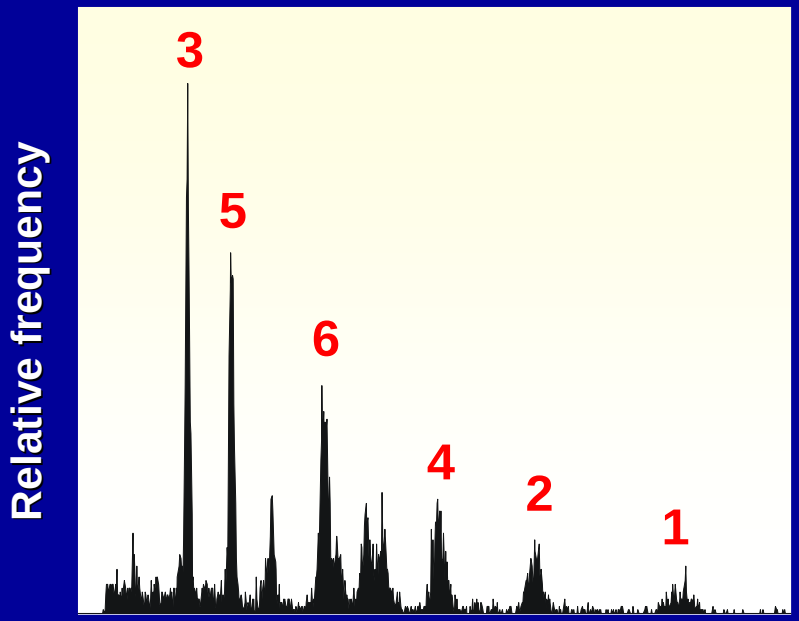
Metaphase accumulation

Cell cycle synchronization in root-tip meristems of *Vicia faba*

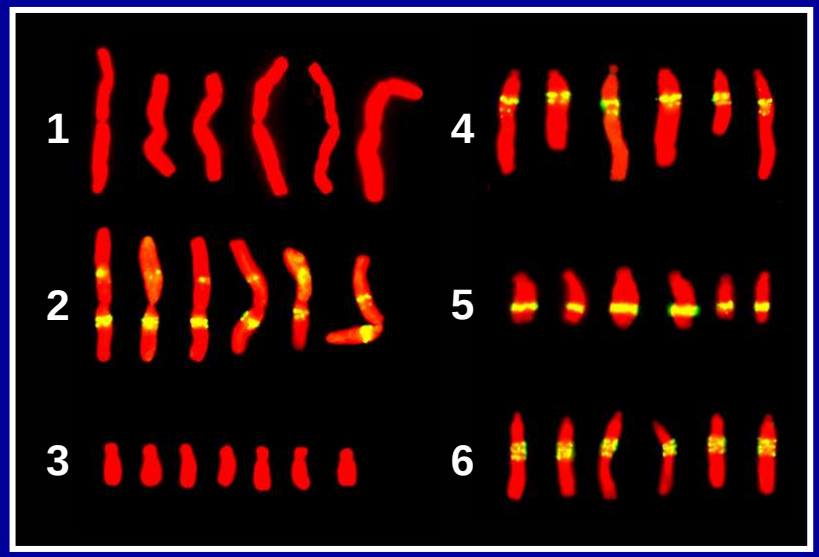
Doležel et al. (1992)

Sorting of chromosomes of *Vicia faba* (translocation line "EF")

Karyotype



Sorted chromosome fractions

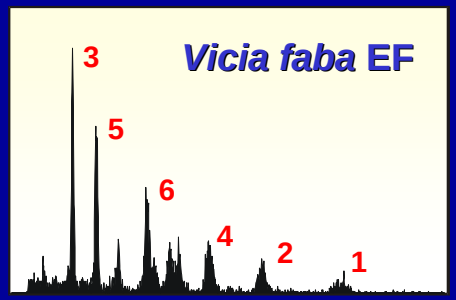


Chromosomes has been identified basing on *Fok*I repetitions

Doležel (1999)

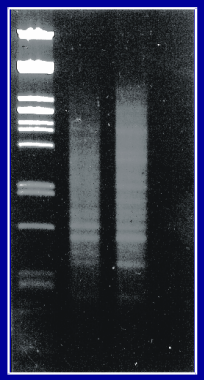
Construction of chromosome library

The chromosome library covering the whole *Vicia faba* genome has been constructed.

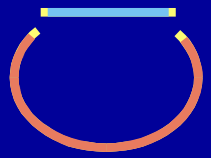


Analysis and sorting of the chromosomes

DOP-PCR

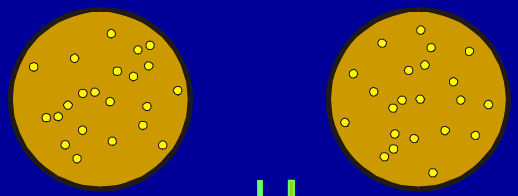


Construction of plasmid vector



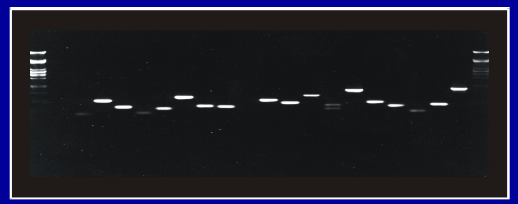
E. coli transformation

Colonies



Storage

Insert size 310 - 487 bp



Macas et al. (1996)

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http://wr.utp.edu.pl/genetyka/

Thank you!