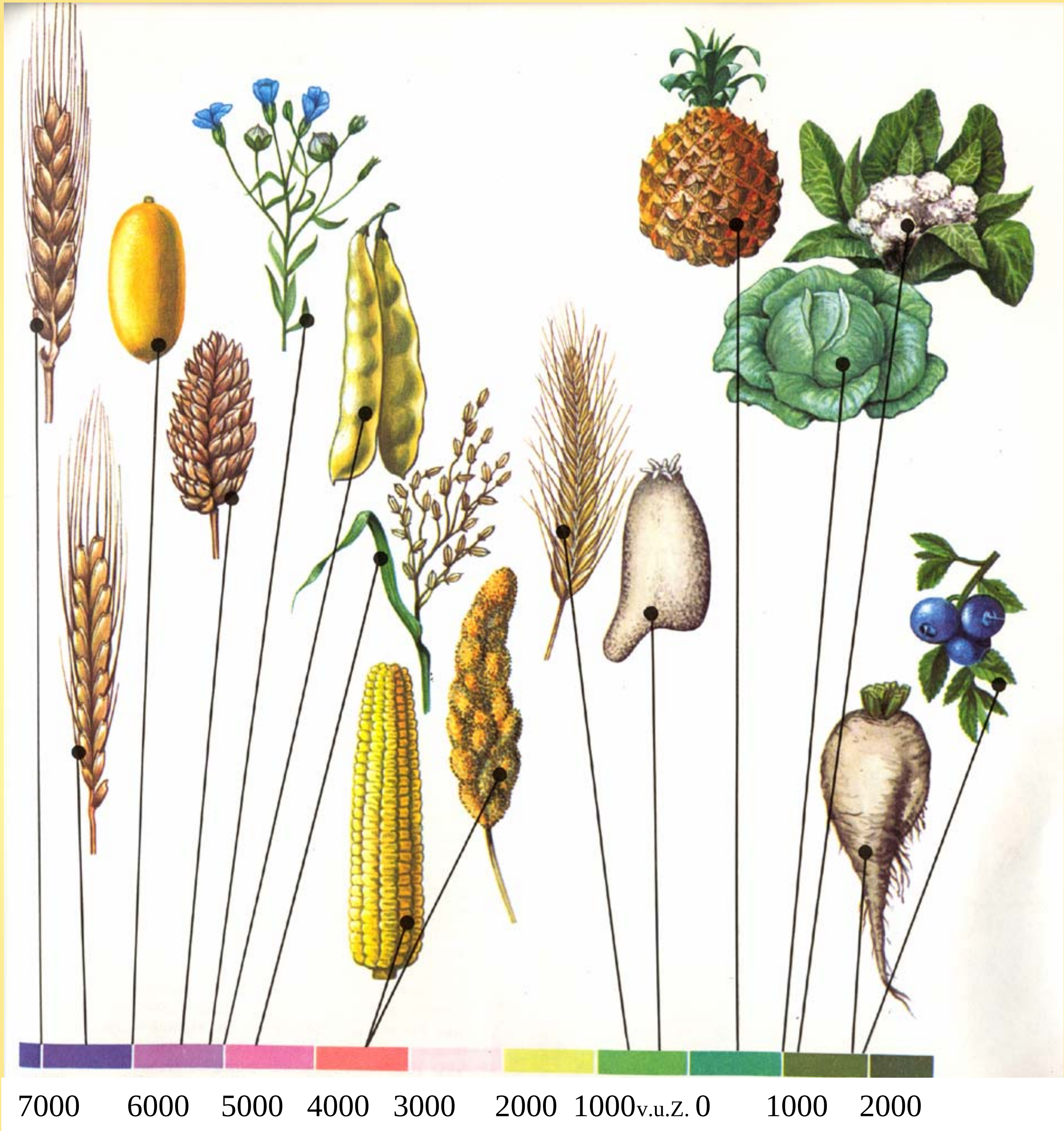
‘Green’ biotechnology

- Introduction
- DNA, Chromosomes, Genomes
- Plant Transformation
- **Modern Plant Breeding**
- Plant tissue culture
- Molecular Marker

Plant breeding:

the science of improving the heredity of plants for the benefit of humankind



The transition form wild plant to a cultivated plant started early in the history of mankind

Wild form

Cultivated form

Where Crops were Domesticated

- Five areas where crops were domesticated independently are:
- Southwest Asia (Middle East):
  - wheat, pea, olive

Where Crops were Domesticated

- China: Rice, Millet

Where Crops were Domesticated

- Mesoamerica: corn, beans, squash

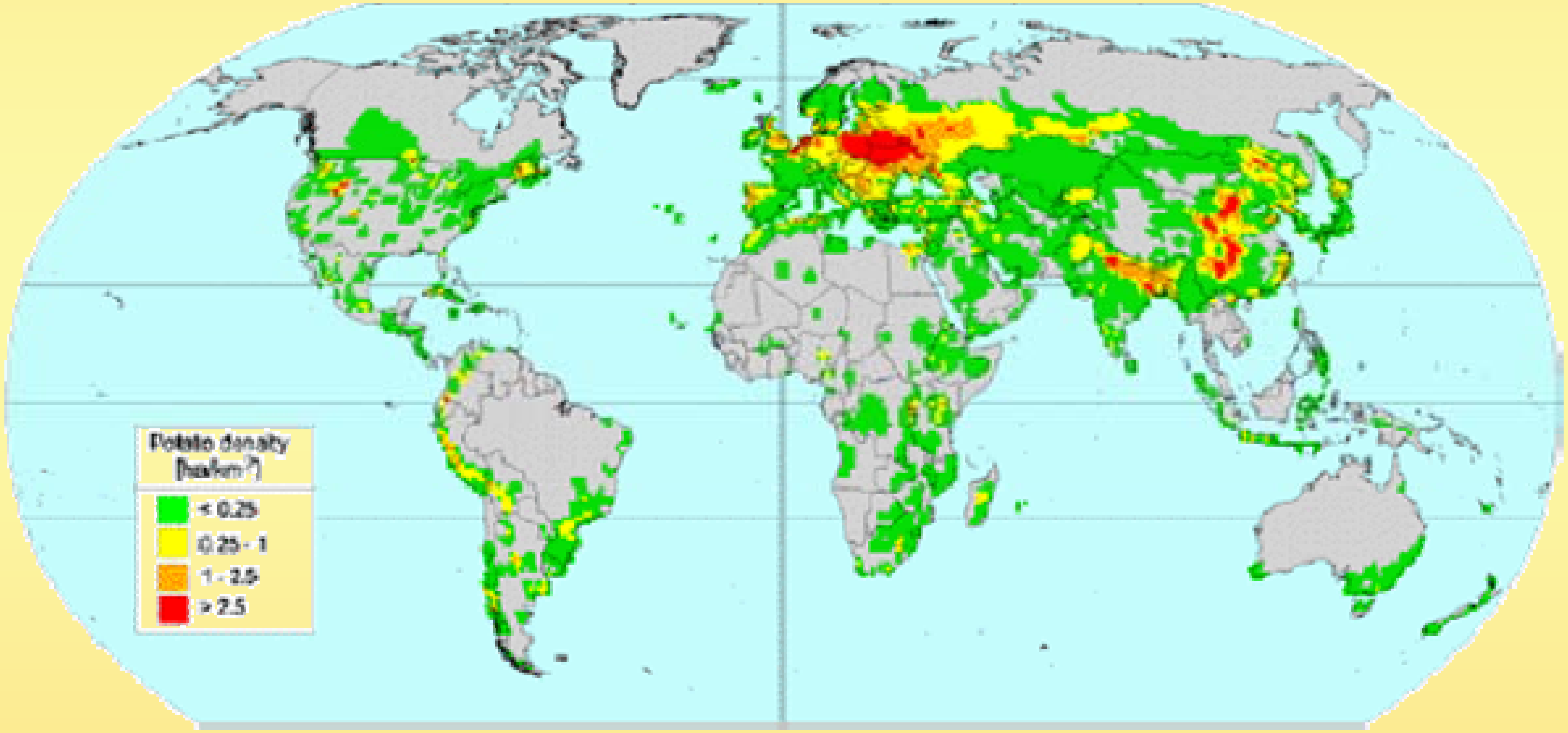
Where Crops were Domesticated

- Andes: potato

Where Crops were Domesticated

- America: sunflower

Often areas of most intense production are not where domestic crops originated.



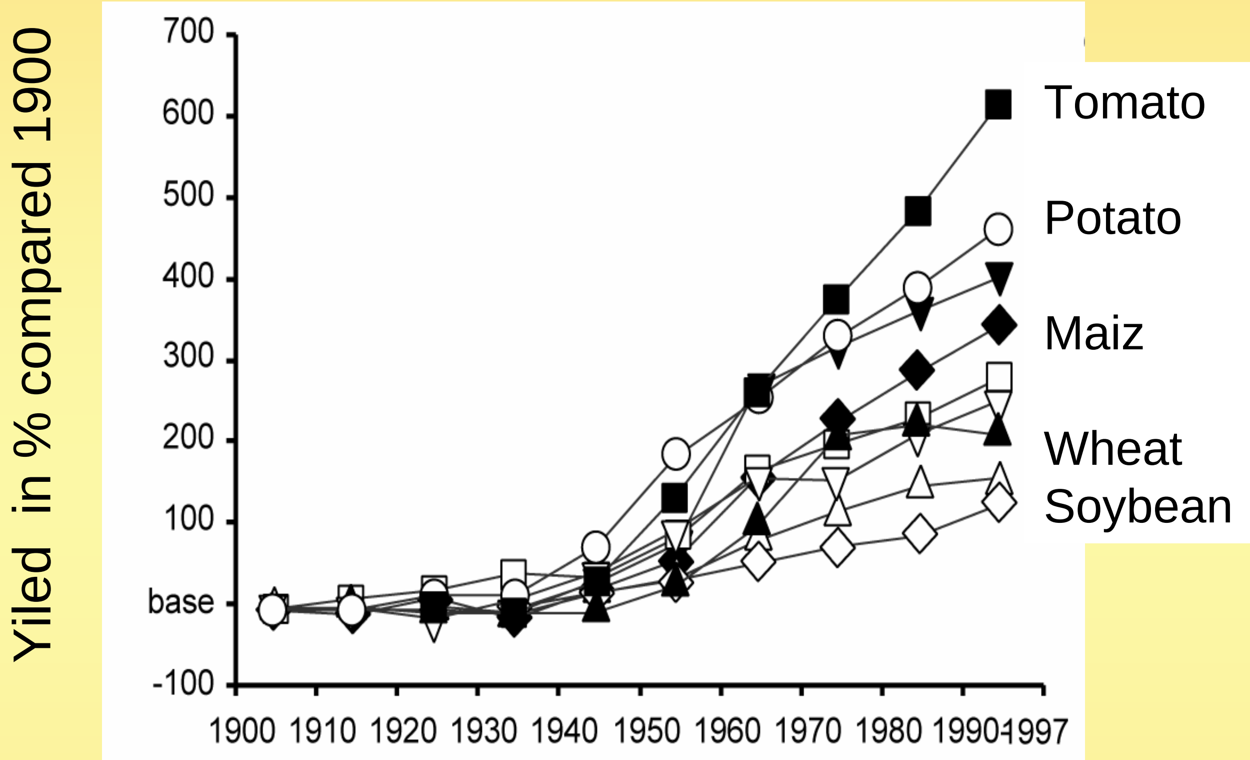
Global Potato Production

Wild plant *versus* cultivated plant

T. monococcum *T. aestivum*

Difference in:
- yield
- content
- germination synchronity
- maturaion
- resistance

Development of yield (example USA, 1900-1997)



Due to:

more <u>favorabel cultural conditions</u> (soil nutrients, water supply, pest control) and <u>improved genetic potential</u> of cultivars (plant breeding 20 – 30%)

Breeding objectives

- Yield
- Resistance to lodging (bending or breaking over of plants before harvest) and shattering (fall out of seeds before harvest)
- Winter hardiness (to survive winter stress)
- Heat and drough resistance
- Soil stress
- Resistance to plant pathogens
- Resistance to insect pests
- Product quality

Highligths of plant breeding

- 1865 Mendelian genetic principles
- 1923 first hybrid corn
- 1960 ‚Green Revolution‘
  - breeding, plant management (fertization, pest control..)
- 1983 first transgenetic plants

Plant Breeding: the process of selectively mating plants

- A basic type of plant breeding is Selection.
- Selection is when plants with desirable traits are chosen from a population and then reproduced.

Selection breeding

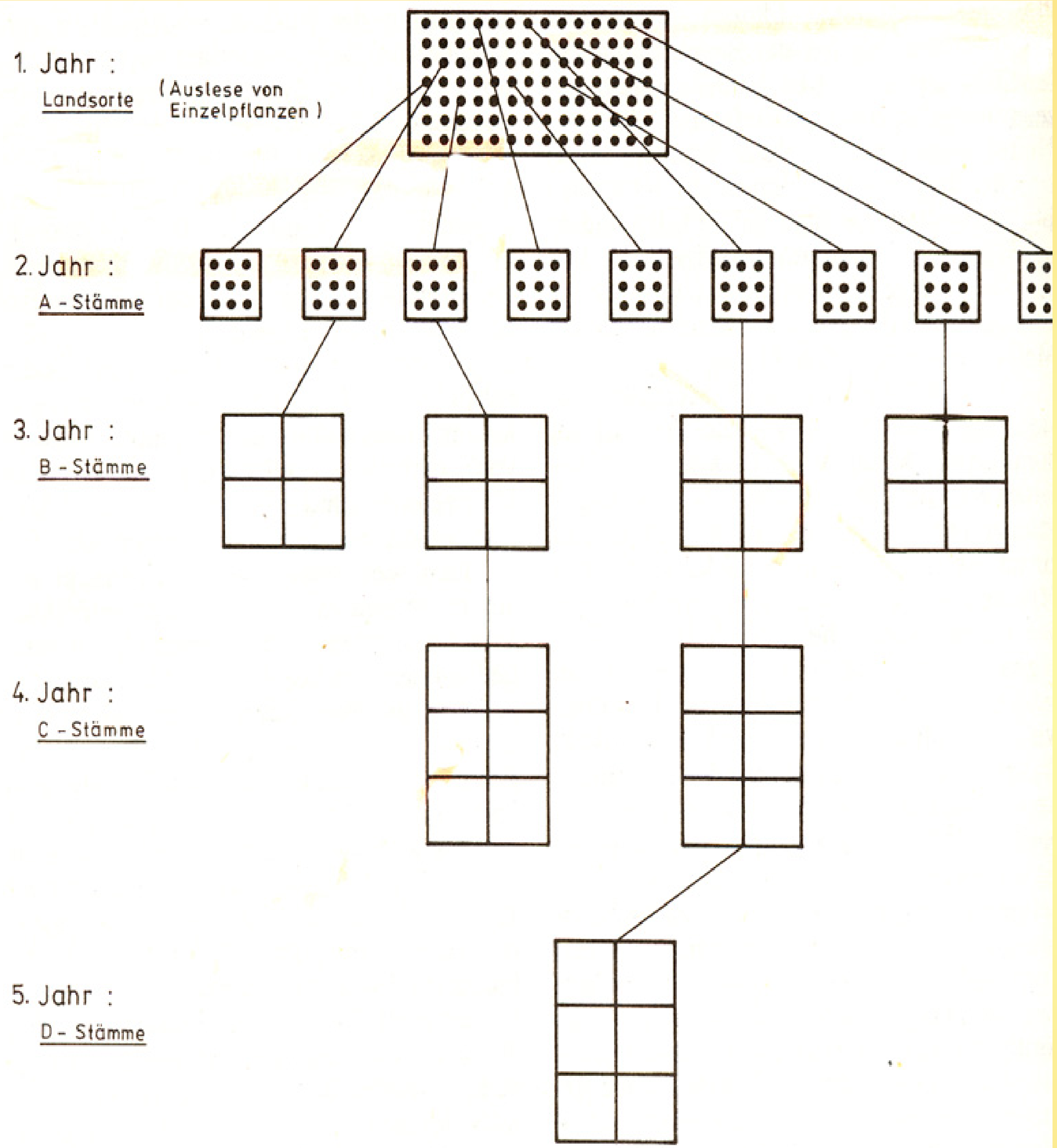
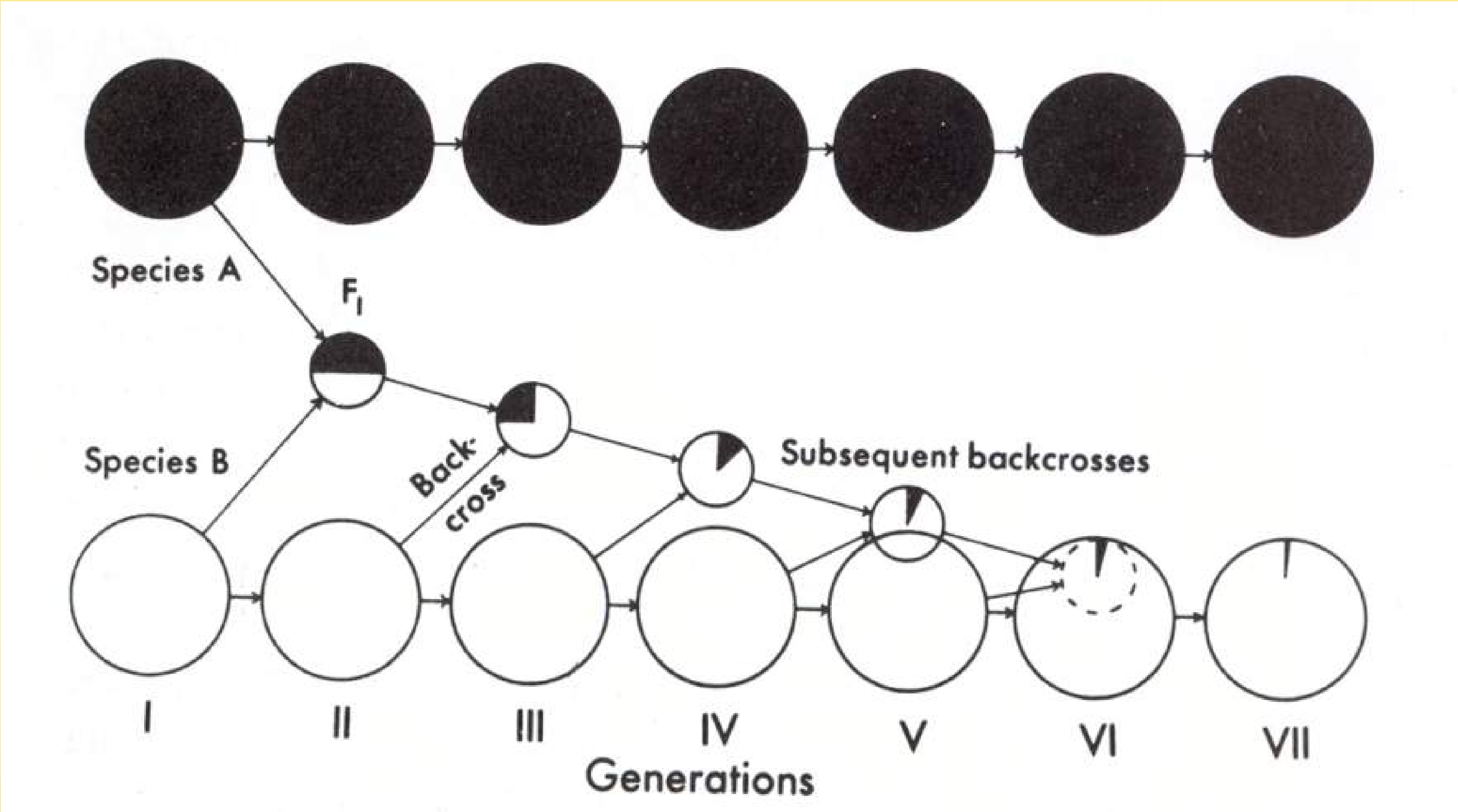


Abb. 4/1
Individualauslese bei einer Selbstbefruchter-Kulturpflanzenart
Punktierte Parzellen: Einzelpflanzenanbau

Introgression breeding

- Important for transferring genetic material from one species to the other
- Some might be adaptively important
- Some might be neutral genes

Introgression breeding



Briggs & Walters, Plant variation and evolution. 1997.

Modern breeding methods

examples:

- Mutation breeding

- Breeding of polyploids

- Generation of haploids

Mutation breeding

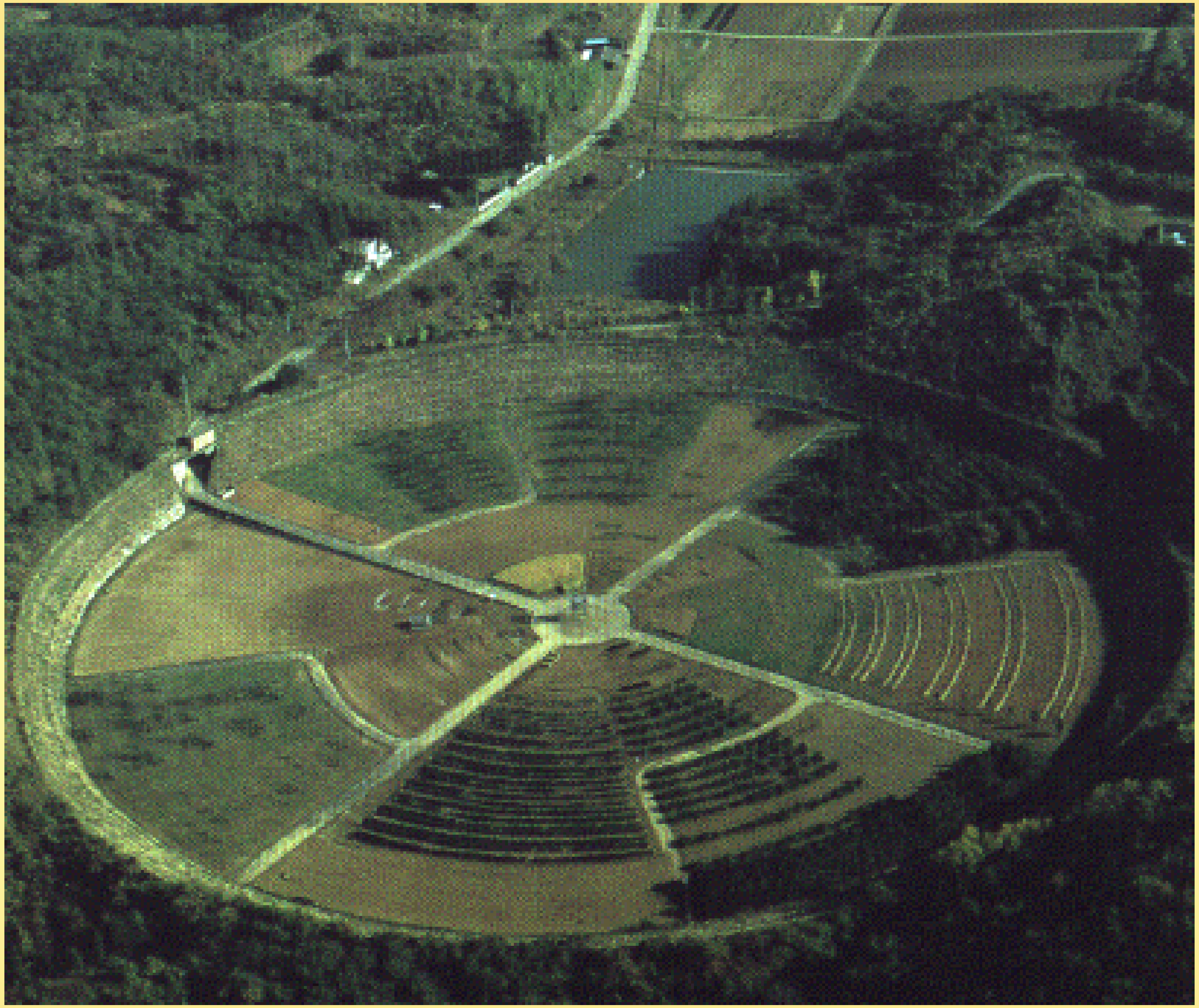
- 1927: Muller produced mutations in fruit flies using x-rays
- 1928: Stadler produced mutations in barley
- Mutation breeding became a bandwagon for about 10 years

(first claim to "replace breeders")

Inducing Mutations

- Physical Mutagens (irradiation)
  - Neutrons, Alpha rays
    - Densely ionizing (“Cannon balls”), mostly chromosome aberrations
  - Gamma, Beta, X-rays
    - Sparsely ionizing (“Bullets”), chromosome aberrations & point mutations
  - UV radiation
    - Non-ionizing, cause point mutations (if any), low penetrating
- Chemical Mutagens (carcinogens)
  - Many different chemicals
    - Most are highly toxic, usually result in point mutations
- Callus Growth in Tissue Culture
  - Somaclonal variation
  - Can screen large number of individual cells
    - Chromosomal aberrations, point mutations
    - Also: Uncover genetic variation in source plant

Mutation Breeding



The gamma field is a circular field of 100m radius with 88.8TBq Co-60 source at the center.

Gamma Greenhouse for chronic irradiation of sub-tropical plants. Cs137 source (4.81TBq, 130 Ci) is used in the octagonal greenhouse with 7 m radius at the Institute of Radiation Breeding, NIAR, Ibaragi, Japan.

Asian pear improved by radiation breeding

“Nijus-seiki” suscep-tible to black spot disease

“Gold Nijusseiki” resistant to black spot disease.

Institute of Radiation Breeding
Ibaraki-ken, JAPAN
http://www.irb.affrc.go.jp/

Mutation breeding

- Today there are three groups of breeders:
  - Mutation breeding is useless, we can accomplish the same thing with conventional methods,
  - Mutation breeding will produce a breakthrough given enough effort,
  - Mutation breeding is a tool, useful to meet specific objectives

Breeding of Polyploids

* Produced by:-
  - a) doubling of chromosomes in 1 species
    (autopolyploidy)
  - b) doubling after hybridization of 2 species
    (allopolyploidy)

* Common in plants (80% of species) - many commercially important examples.
* Rarer in animals (some fishes, flatworms, shrimp, amphibians). Triploid oysters (sterile)

Polyploids

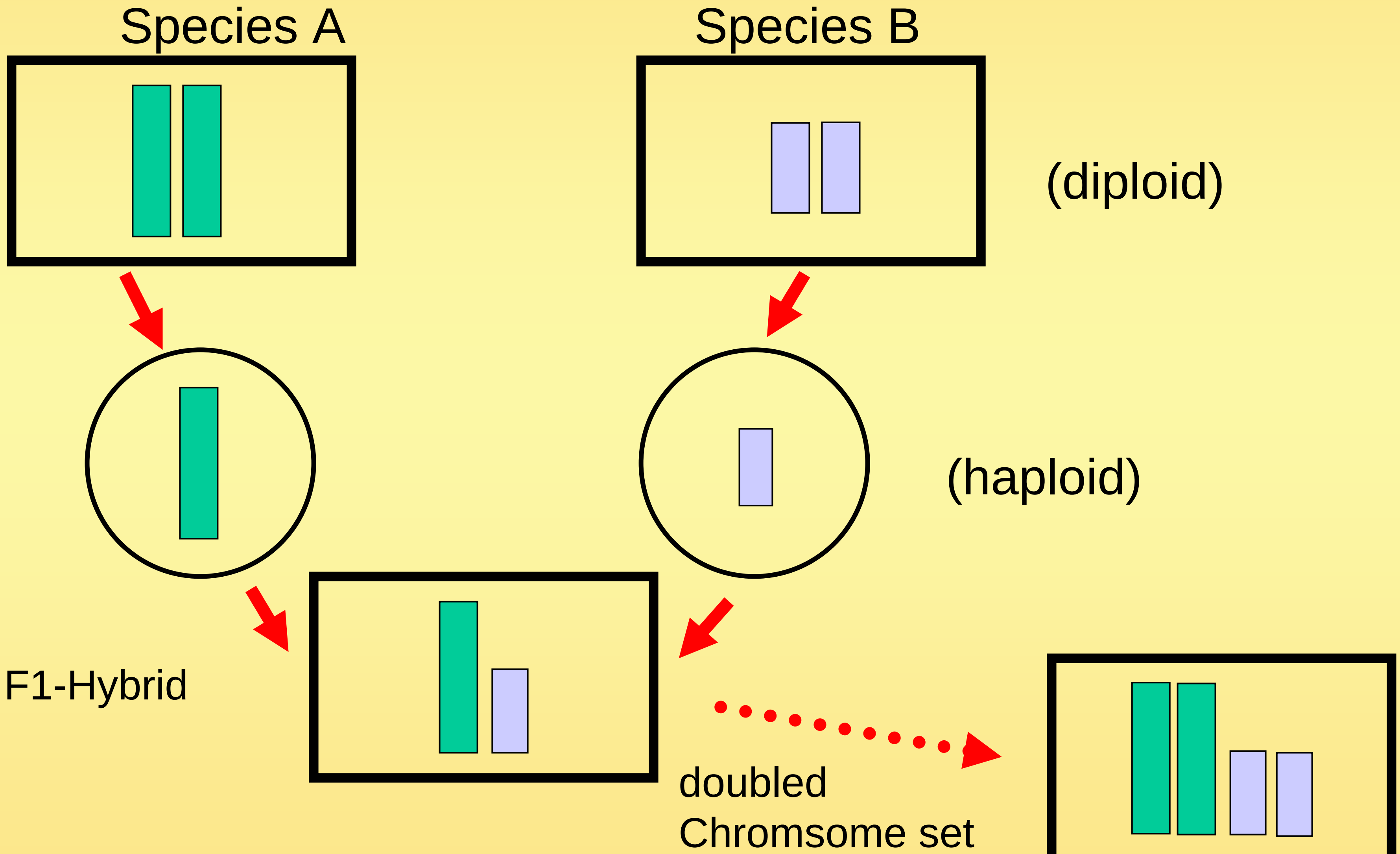
Autopolyploids

- between genetically very similar plants
- parents the same species
- chromosomes homologous
- at meiosis quadrivalents

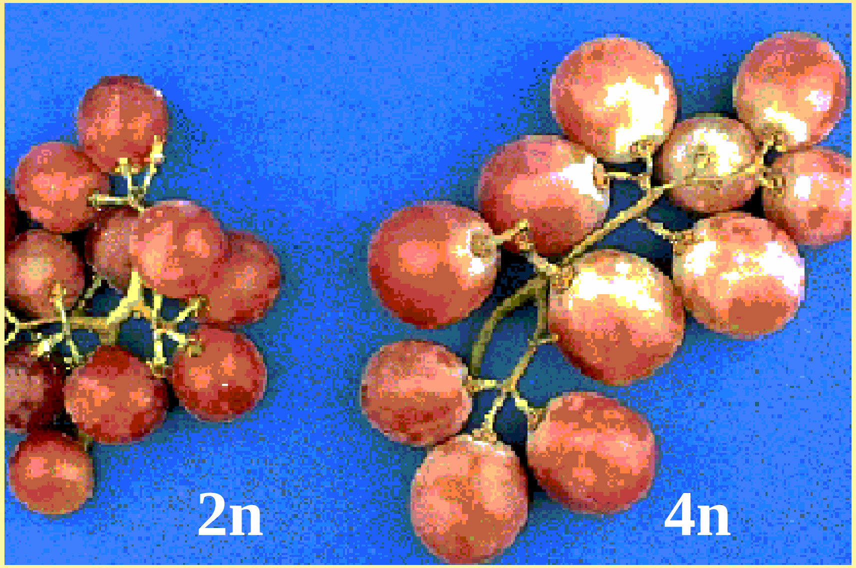
Allopolyploids

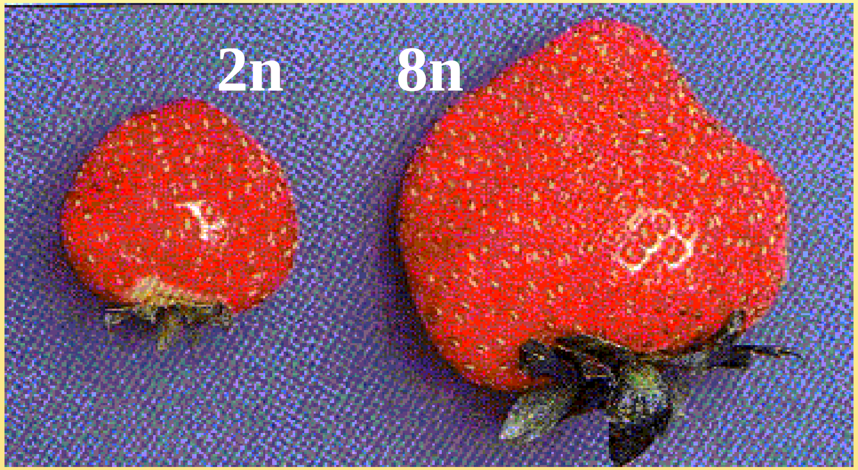
- between genetically dissimilar plants
- parents different species
- chromosomes not homologous
- at meiosis bivalents

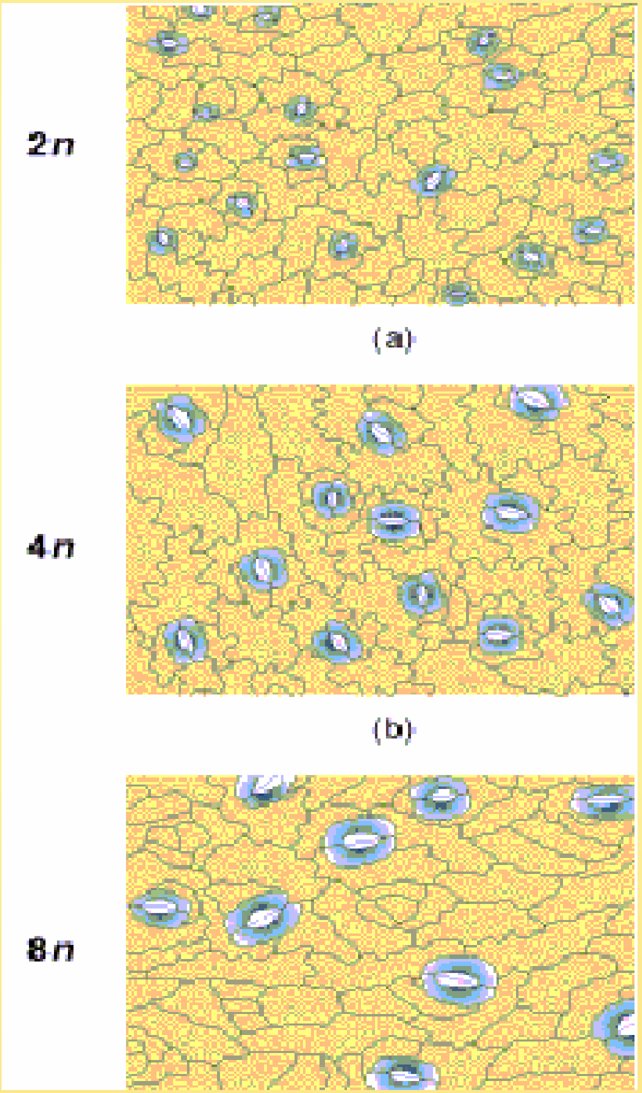
Allopolyploidy - doubling after hybridization of 2 species



Polyploidy typically increases cell size which results in larger tissues.

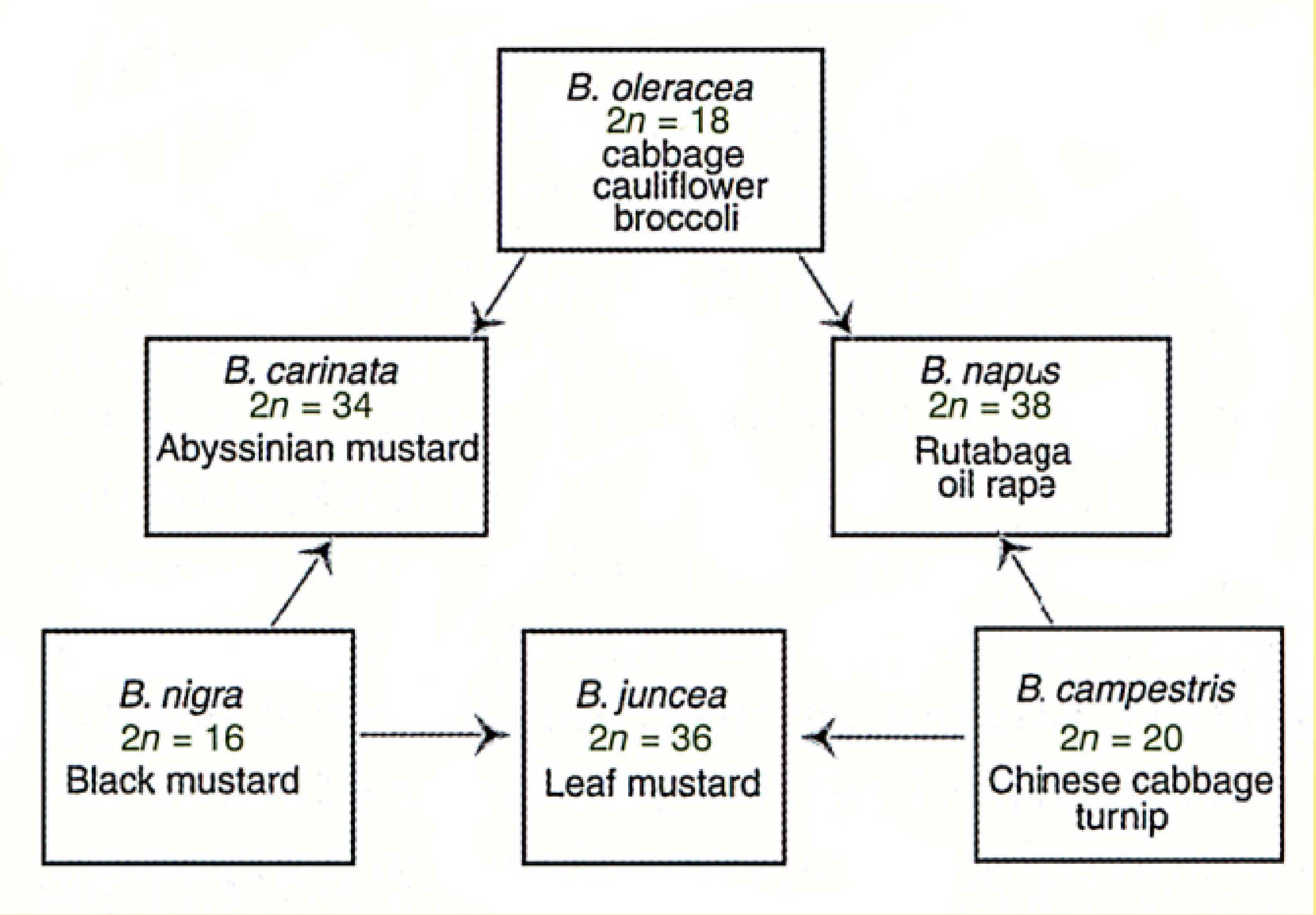






Stomatal cells in tobacco

Allopolyploidy in *Brassica*



Somatic Allopolyploidization

Diploid Protoplast of Species A.

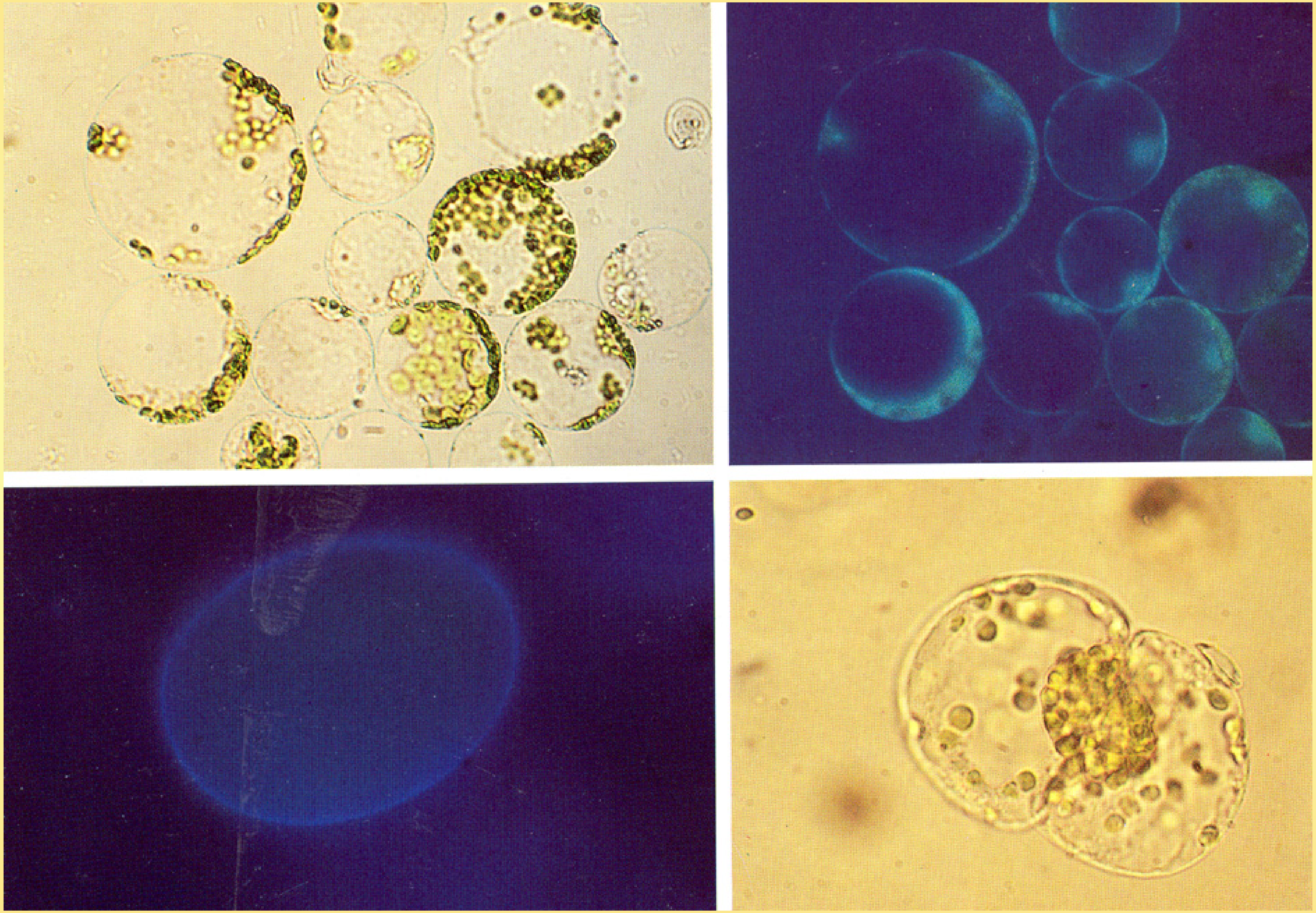
Diploid Protoplast of Species B.

Use polyethylene glycol to mediate fusion

Hybrid Tetraploid Cell

Grow up to Tetraploid plant.

Single protoplasts



Protoplasts after fusion

Possible changes taking place between the component genomes following allopolyploidyzation

- gain and loss of coding or uncoding DNA

- structural rearrangements at the chromosome level

- changes in gene expression - gene silencing or activation

Haploid plants

Haploid - gametic number of chromosomes

A. Reduce time for variety development, e.g. 10 to 6 years or less

B. Homozygous recombinant line can be developed in one generation instead of after numerous backcross generations

C. Selection for recessive traits in recombinant lines is more efficient since these are not masked by the effects of dominant alleles

Agricultural applications for haploids - Rapid generation of homozygous genotypes after chromosome doubling

Processes Leading to Production of Haploid Plants

Androgenesis – haploid plant derived from male gamete, most common method *in vitro*

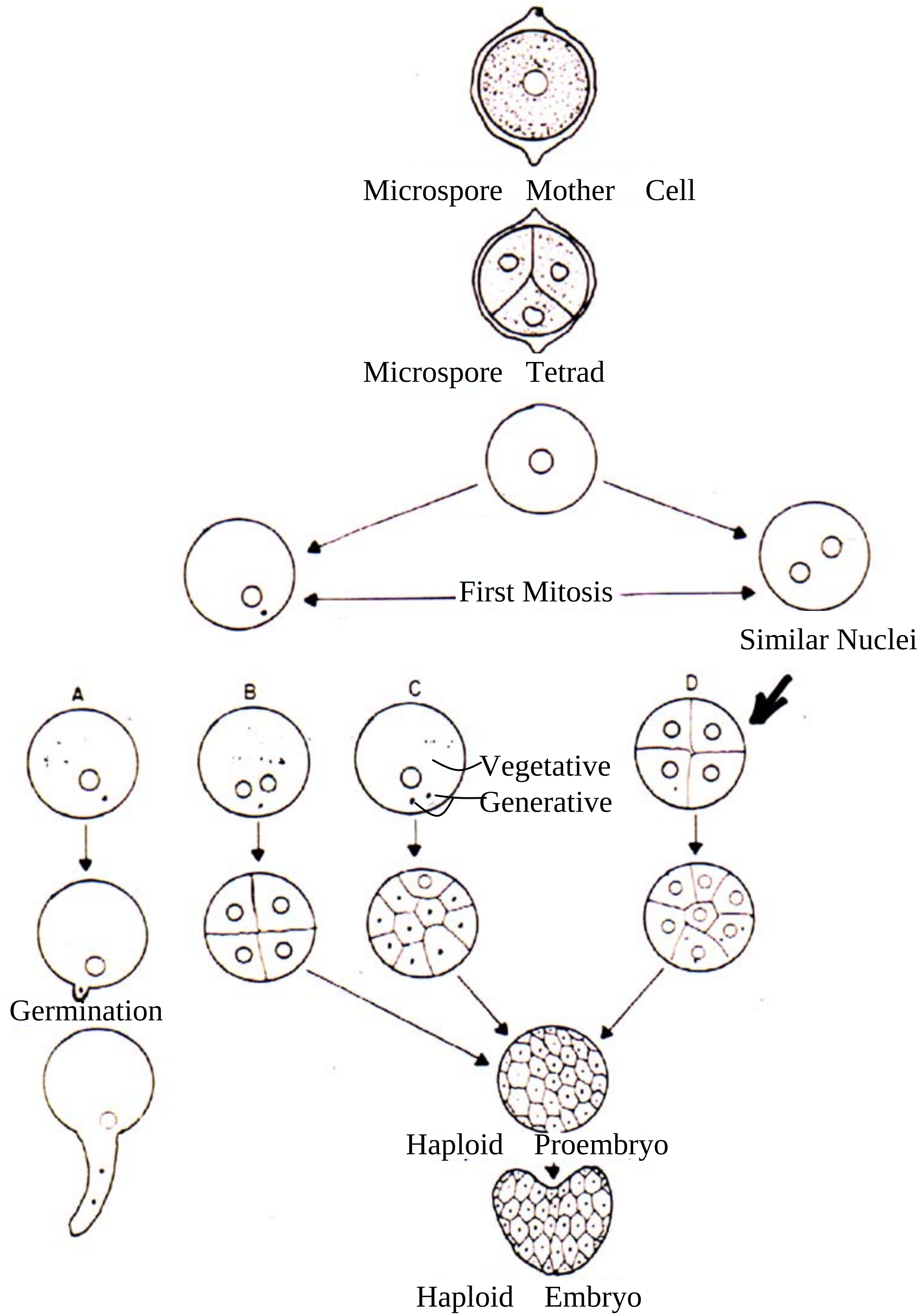
Parthenogenesis - from unfertilized egg

Chromosome elimination - chromosome elimination in somatic cells, most common method used with plant breeding

Production of Haploids through Anther and Microspore *In Vitro* Culture

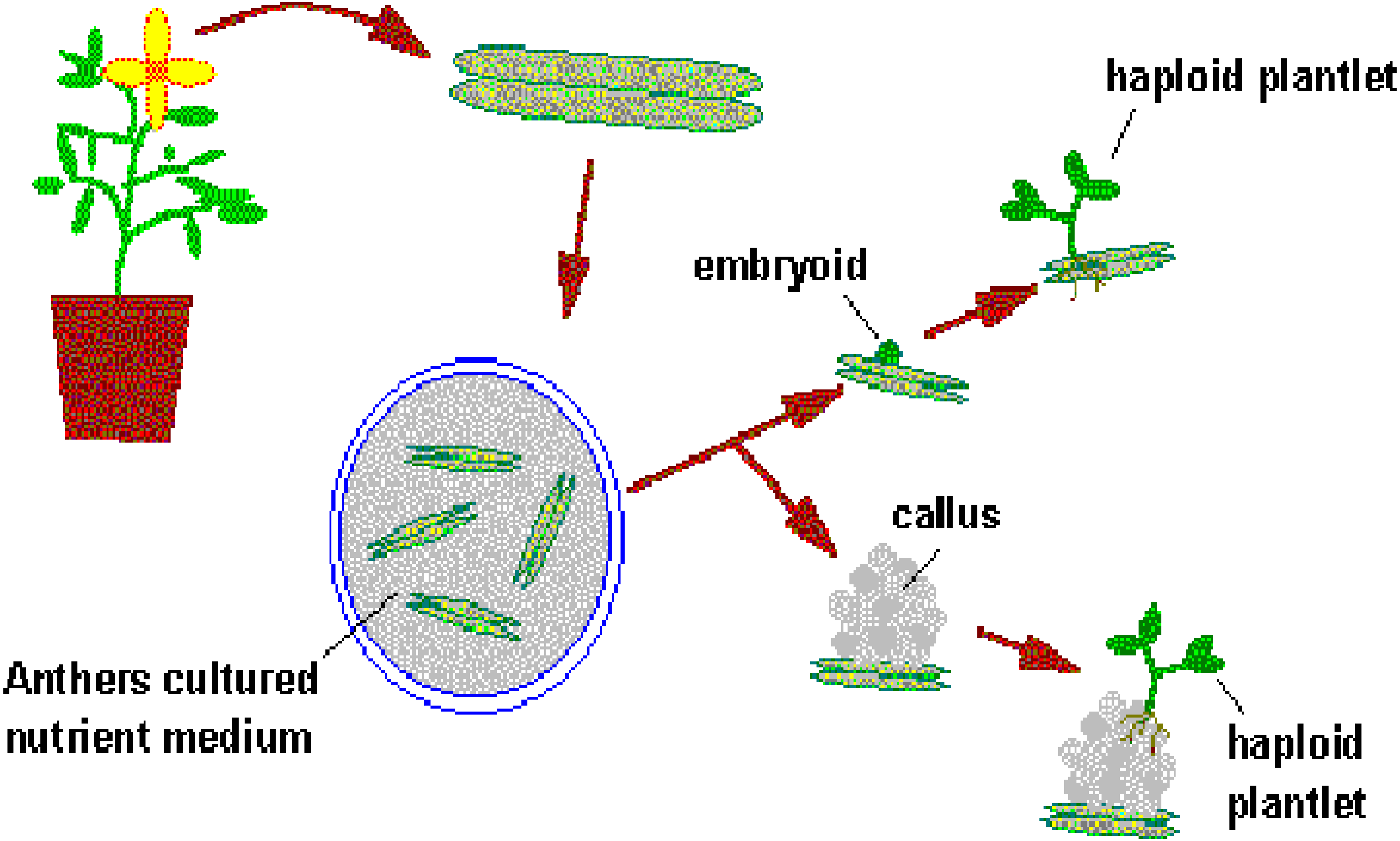
Anther and microspore (pollen) culture - haploid plants are derived from microspores (pollen) cultured individually or in anthers

Background – micro-sporogenesis and micro-gametogenesis leading to pollen development



Microspore Mother Cell
Microspore Tetrad
First Mitosis
Similar Nuclei
A
B
C
D
Vegetative
Generative
Germination
Haploid Proembryo
Haploid Embryo

Features of Anther/Microspore Culture



Anther/Microspore Culture Factors

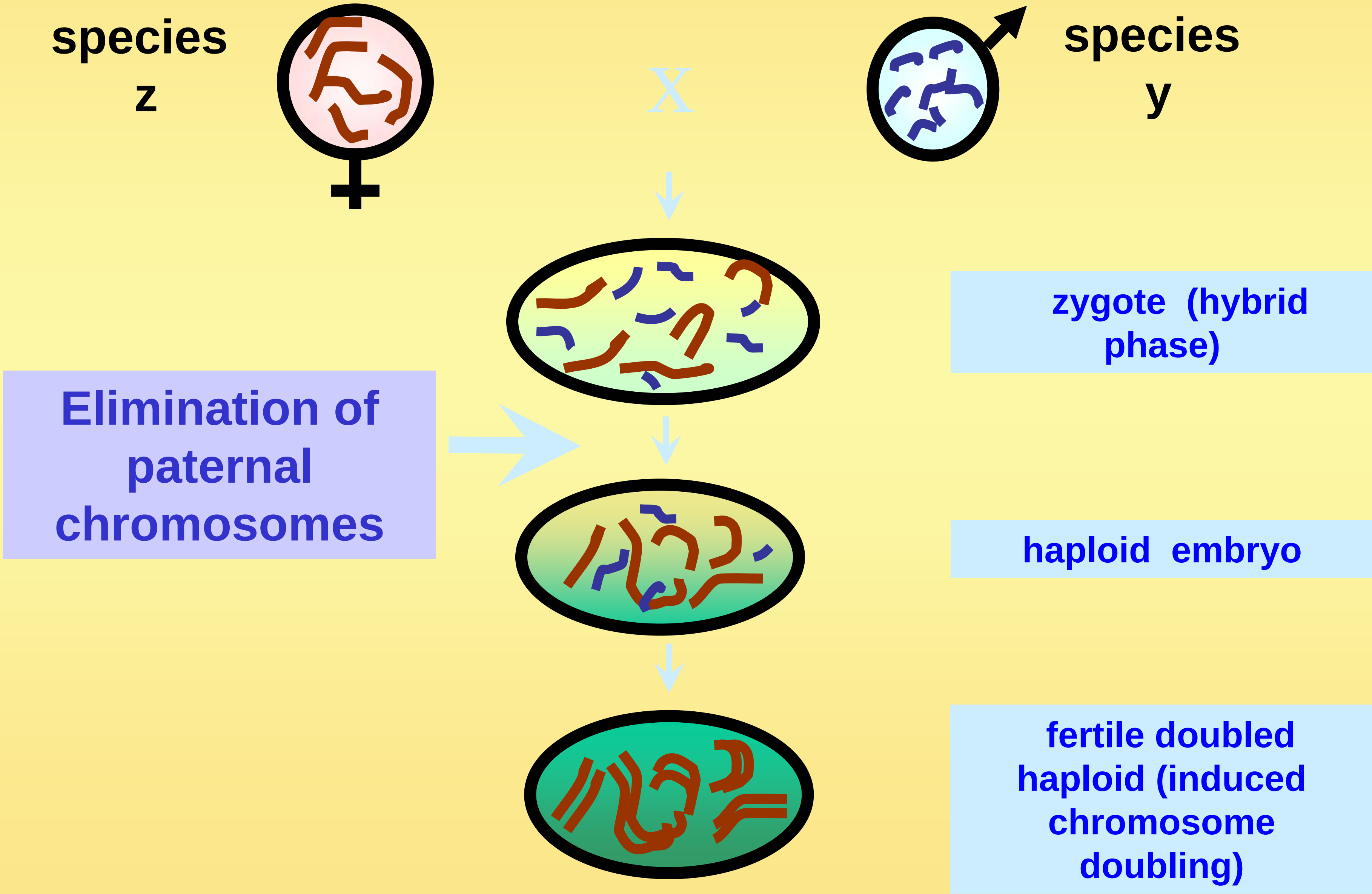
- Genotype
  - As with all tissue culture techniques
- Growth of mother plant
  - Usually requires optimum growing conditions
- Correct stage of pollen development
  - Need to be able to switch pollen development from gametogenesis to embryogenesis
- Pretreatment of anthers
  - Cold or heat have both been effective

Production Haploids through Chromosome Elimination and Embryo Rescue

Production of haploids by chromosome elimination - There are numerous examples, primarily achieved by wide crosses and embryo culture

eg.: oat, wheat, barley, potato

Generation of haploids by wide crosses



Monoploid Production of Barley (*H. vulgare*)

Day 0 - emasculation

Day 2 - pollination with *H. bulbosum* pollen

Day 3 (to 5) - 40% of the embryonic cells are haploid, endosperm abortion occurs, GA_3 treatment enhances retention of florets

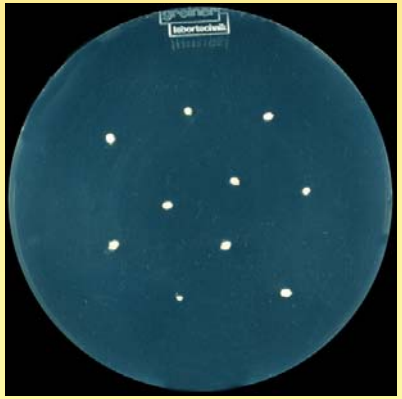
Day 11 - 94% of the embryonic cells are haploid

Day 14 (to 16) - embryos are dissected and cultured in the dark at 18 to 22 C, embryos develop *in vitro*

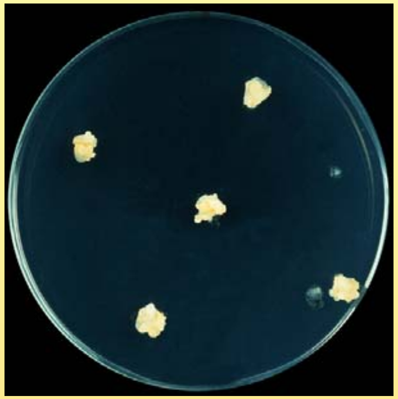
Day 22 (to 28) - embryos are transferred to light for seedling development

Embryo rescue

15 days



30 days



50 days

80 days

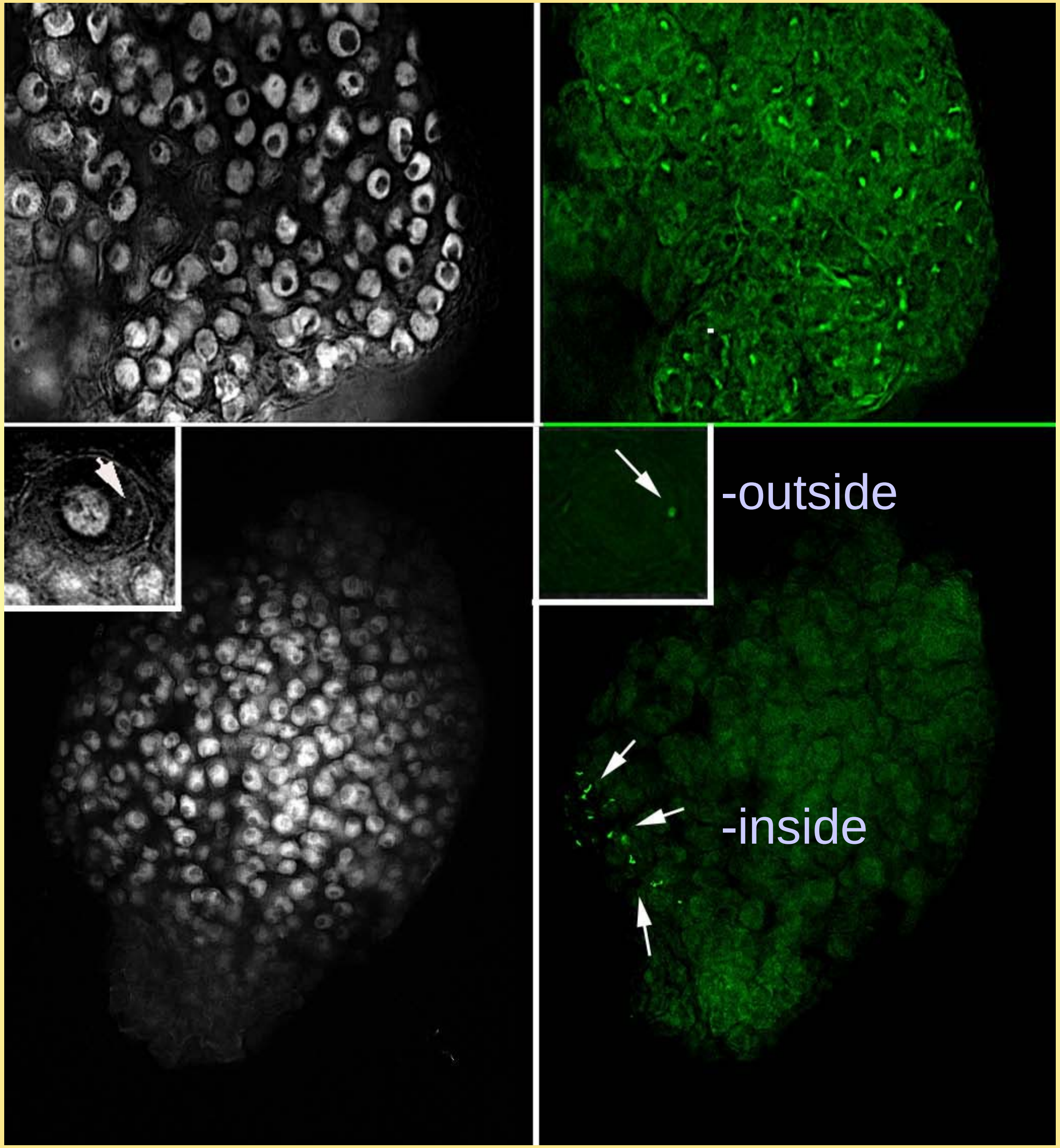
How does the elimination of chromosomes occur?

Mitosis-dependent elimination

- parent-specific centromere defects
- asynchrony of mitotic cycle times
- results in formation of micronuclei

Crosses

female		male
wheat (*Triticum aestivum*)	**x**	**pearl millet** (*Pennisetum glaucum*)
barley (*Hordeum vulgare*)	**x**	**wild barley** (*H. bulbosum*)



DAPI pearl millet-signals

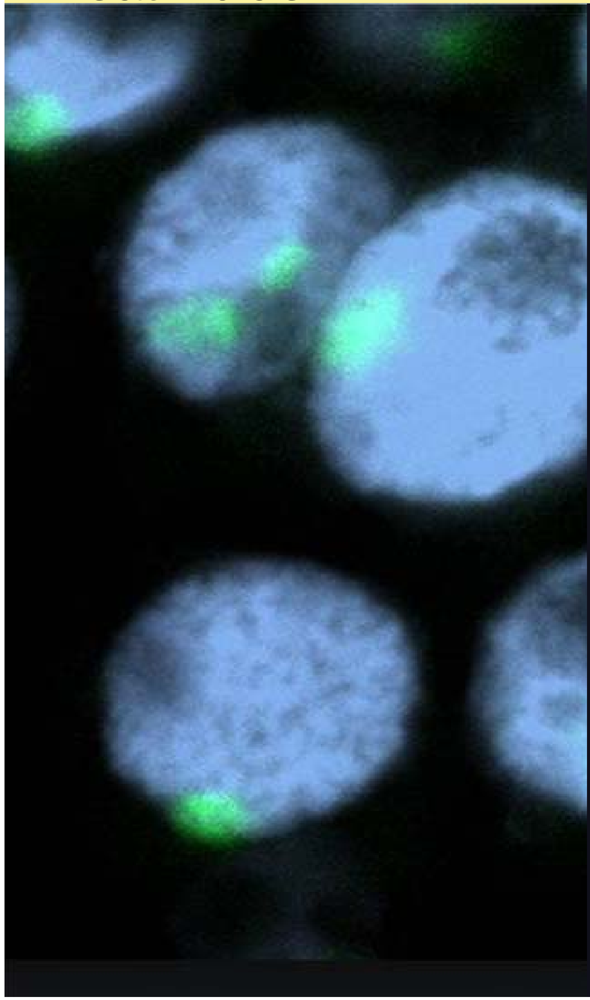
whole mount genomic *in situ* hybridization (GISH) on 6 day old embryos

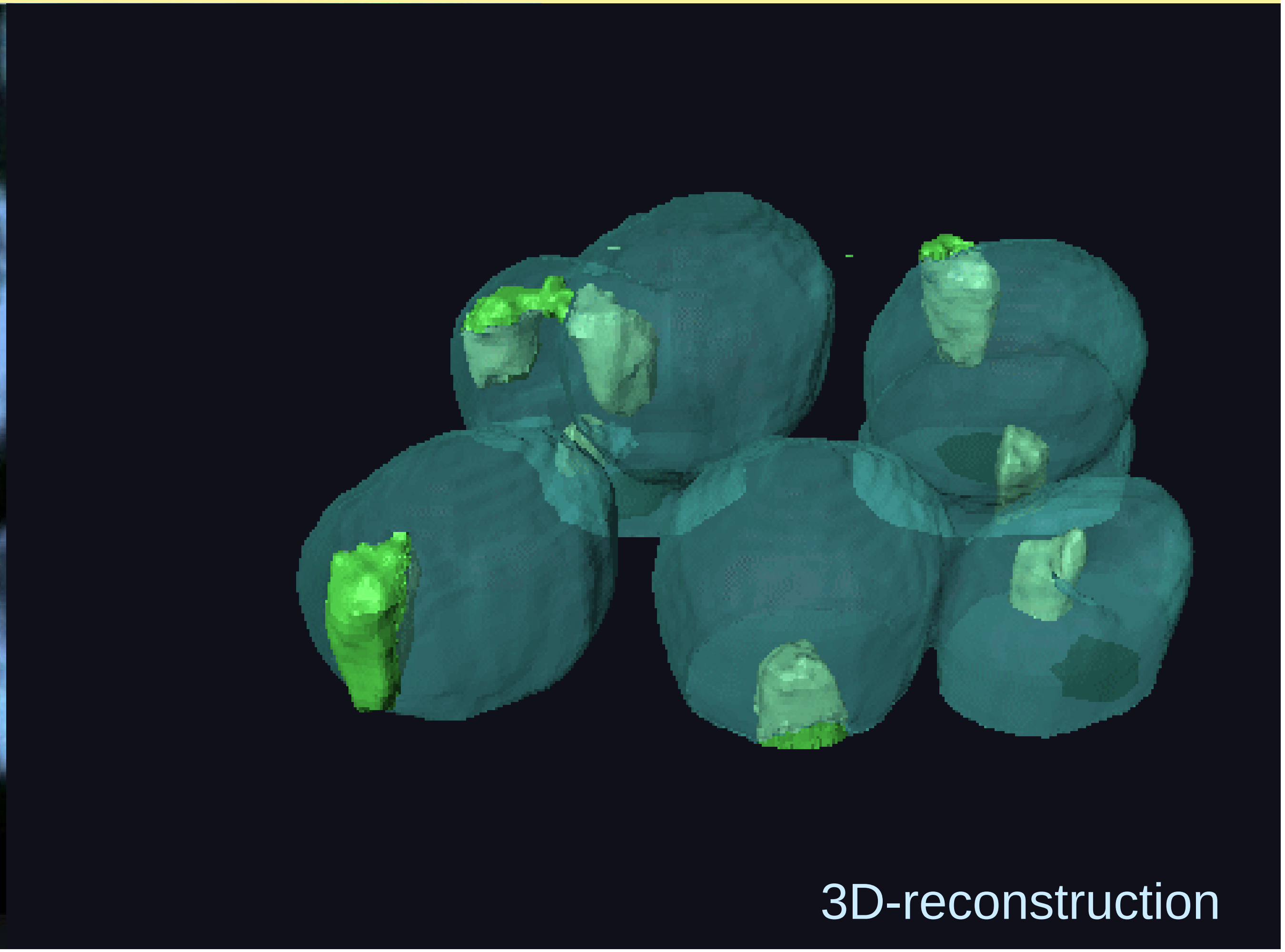
Number and location of pearl millet-positive cells varied between embryos at the same stage

Pearl millet chromatin occupies a distinct interphase territory

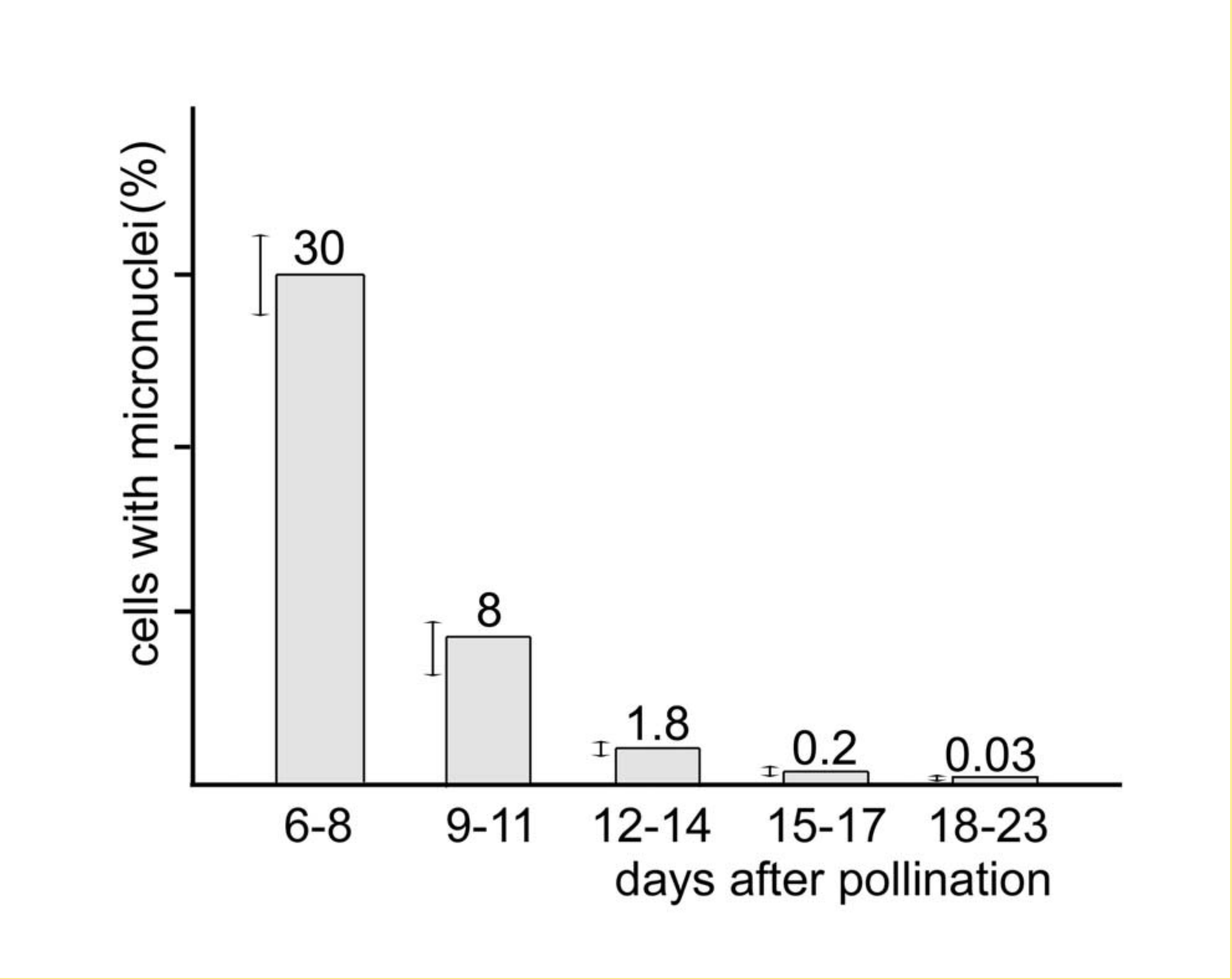
GISH
pearl millet - green
wheat - blue

- mainly at the periphery



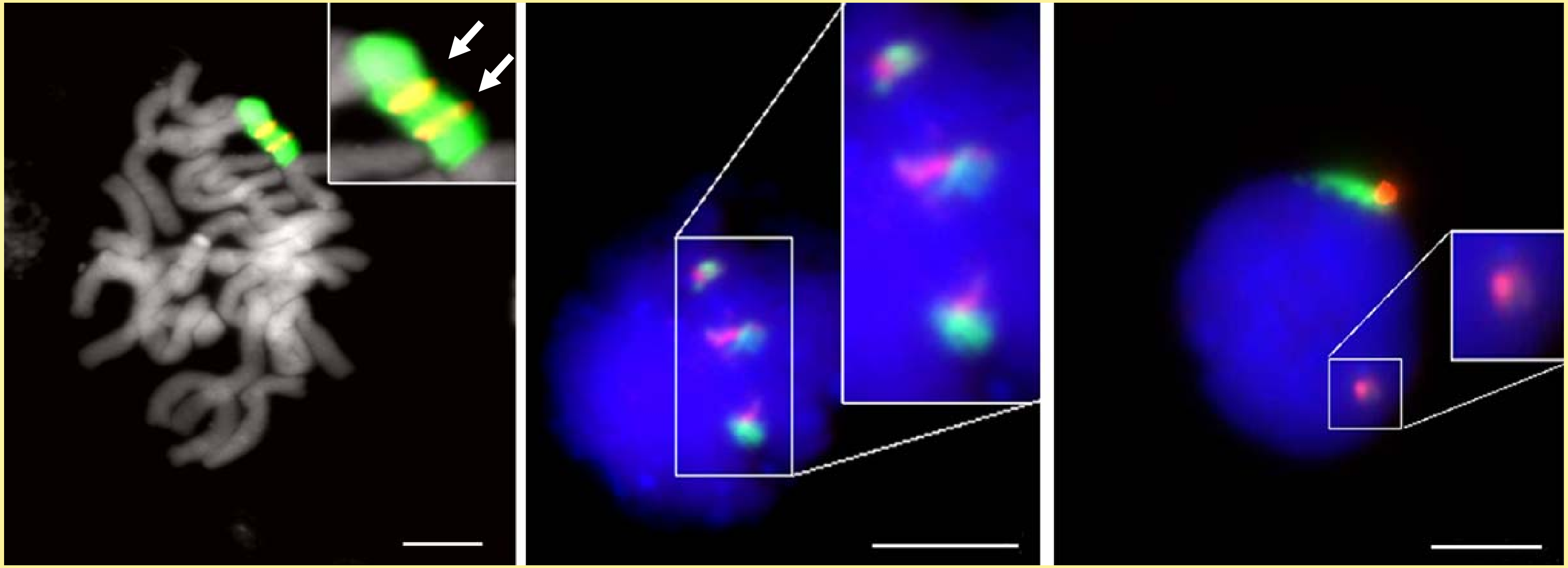


Elimination of pearl millet chromatin is sequential



Cells containing micronuclei

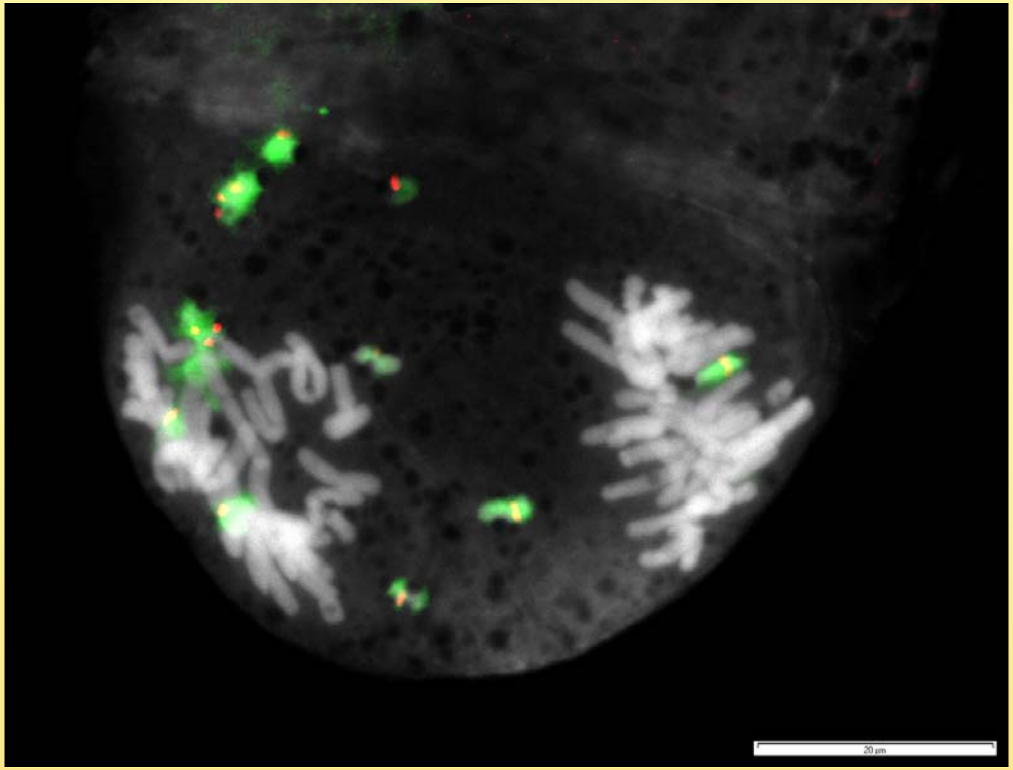
Pearl millet chromosomes – structurally rearranged and size reduced



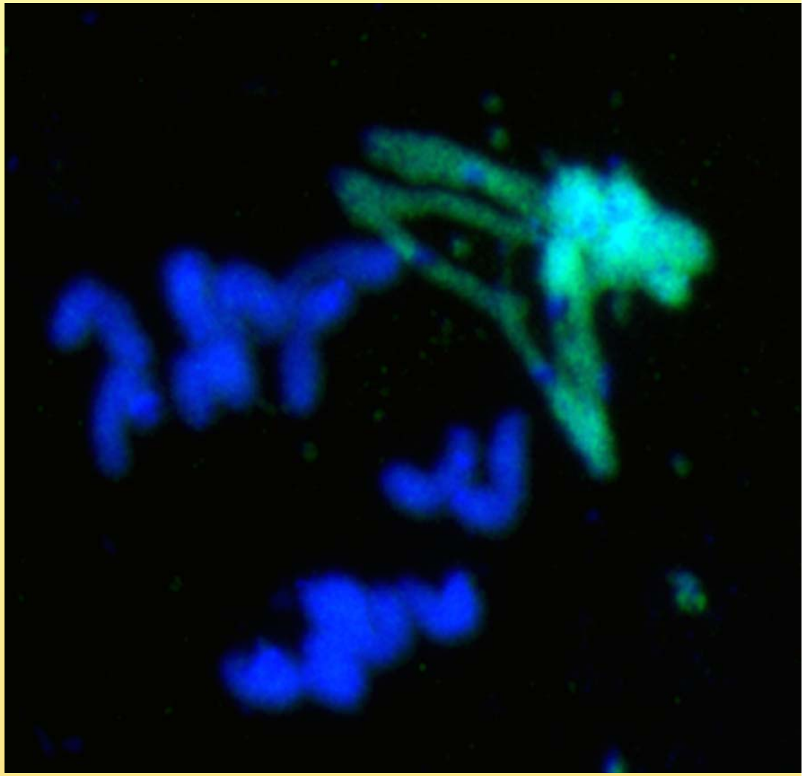
red - pearl millet centromere
green - pearl millet genomic DNA

Loss of paternal chromosomes during mitosis

- parent-specific centromere defects
- asynchrony of mitotic cycle times

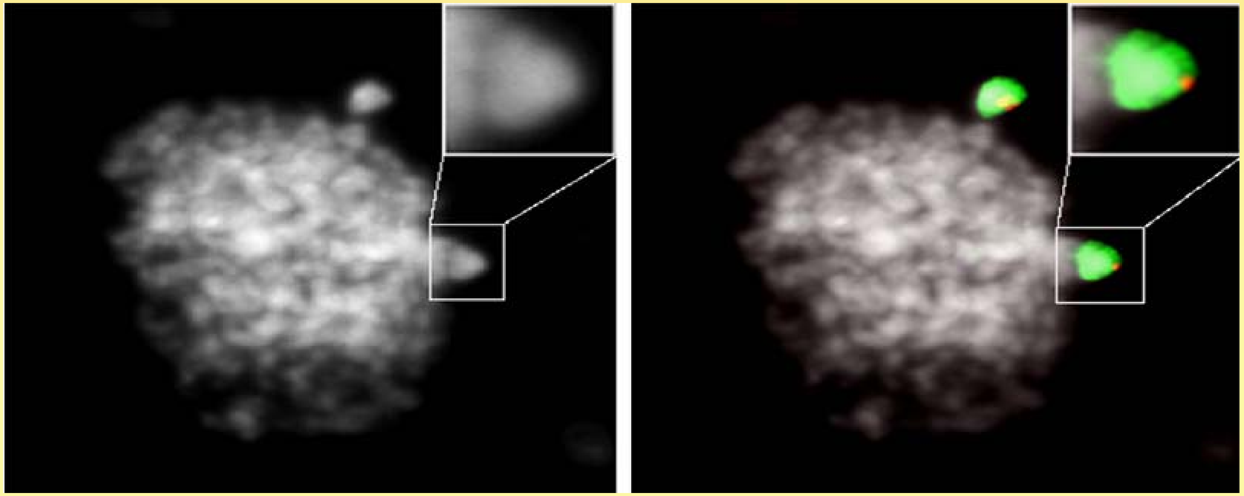


wheat x pearl millet

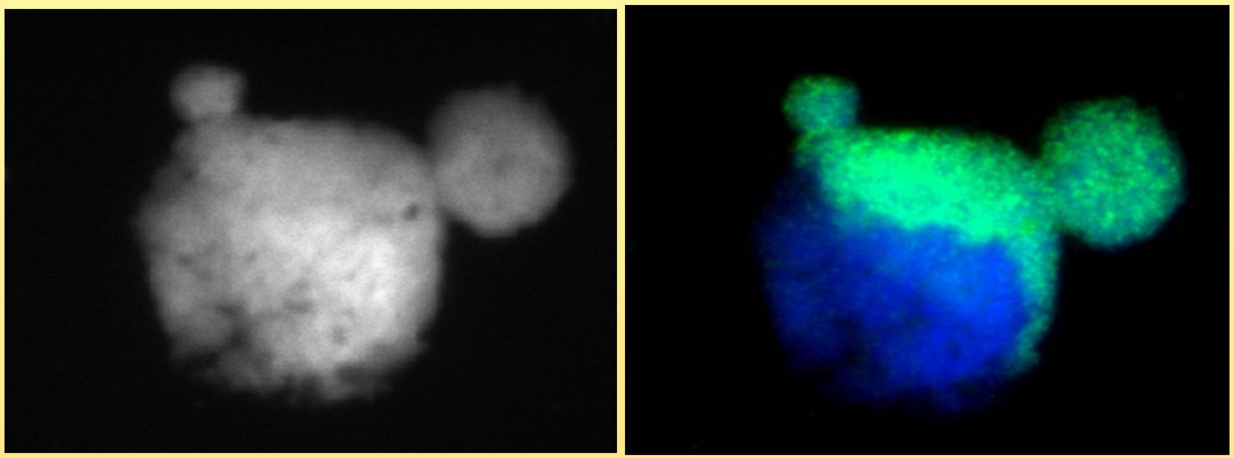


barley x *H. bulbosum*

‘Budding’ – mitosis-independent formation of micronuclei

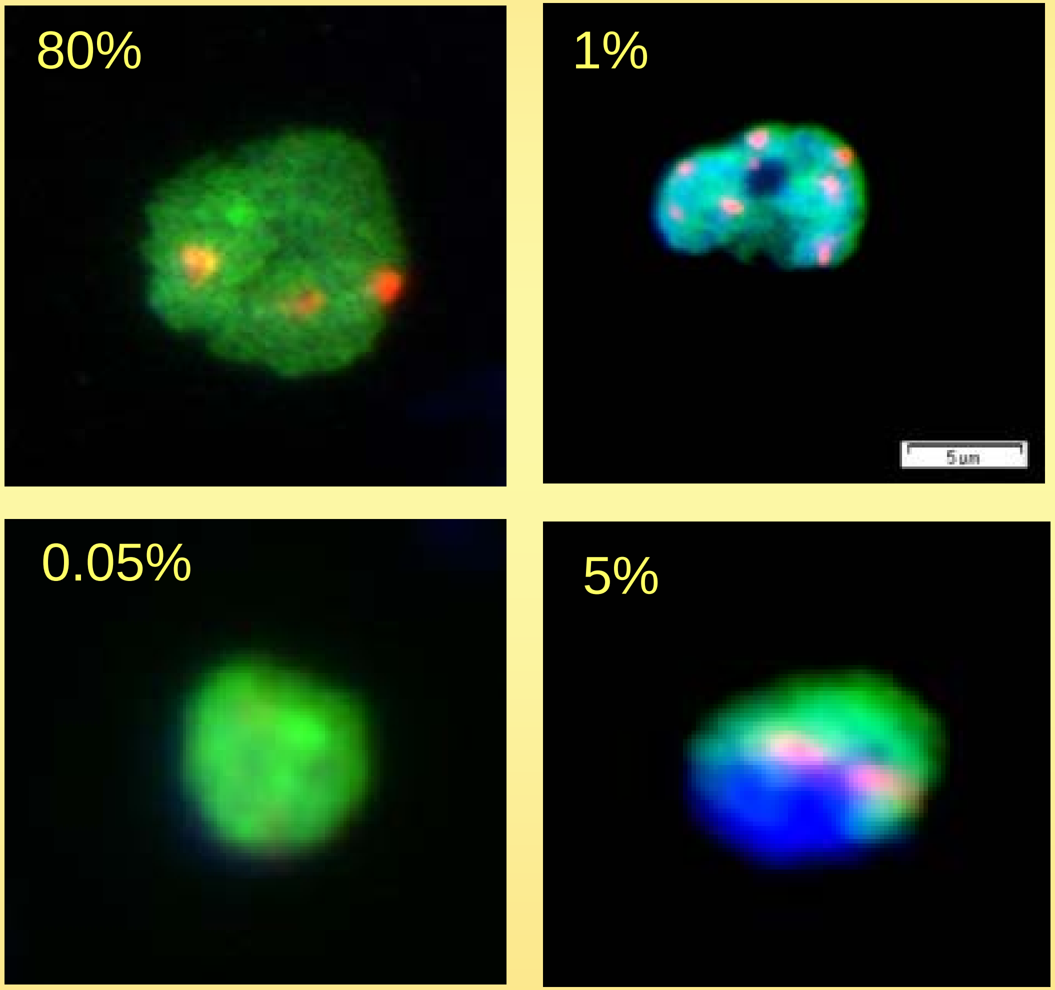


wheat x pearl millet



barley x *H. bulbosum*

Composition of micronuclei varies

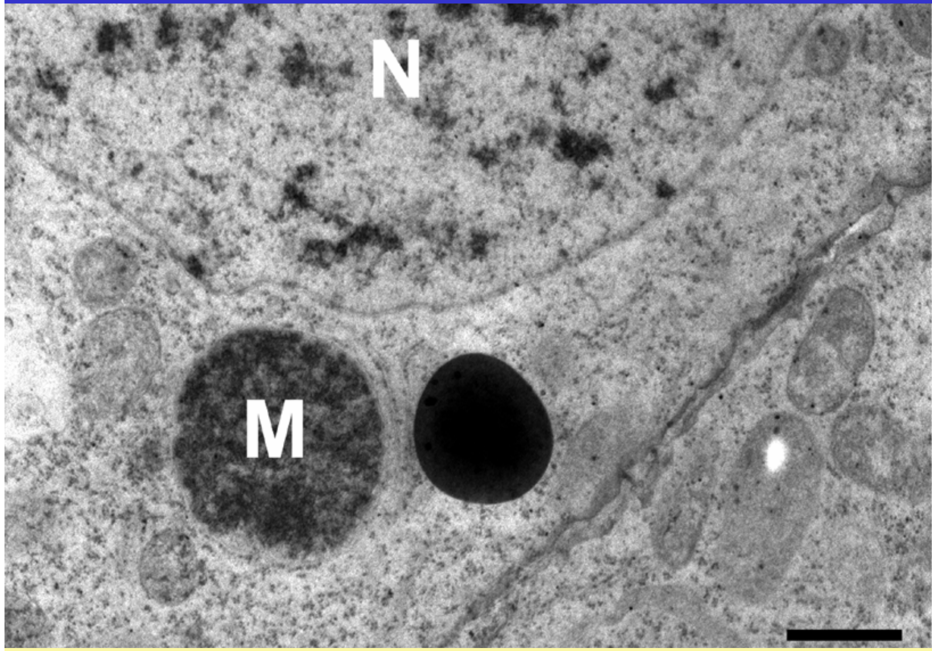


blue - wheat DNA

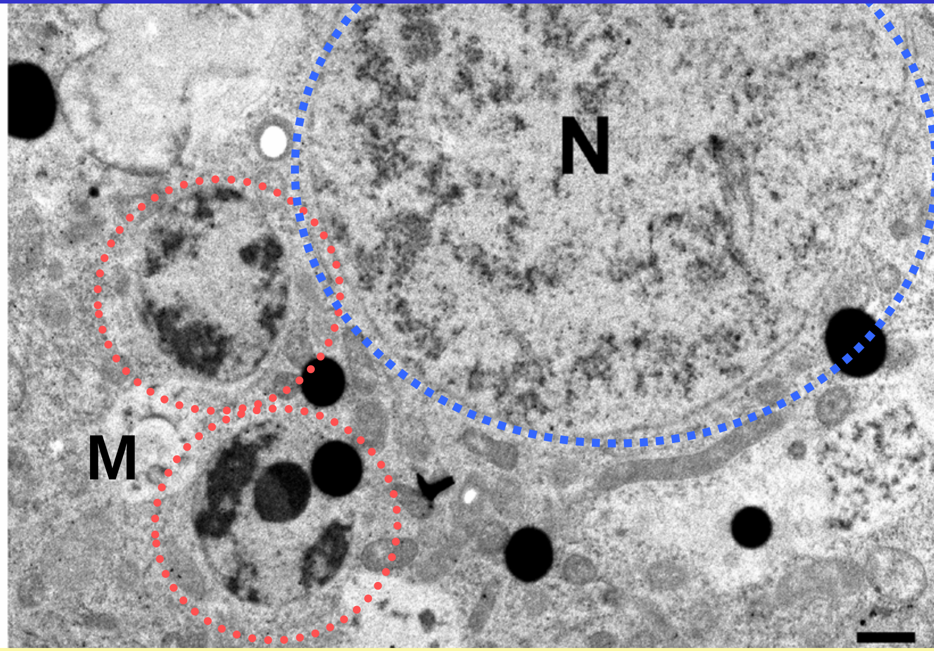
green - pearl millet DNA

red - pearl millet centromere

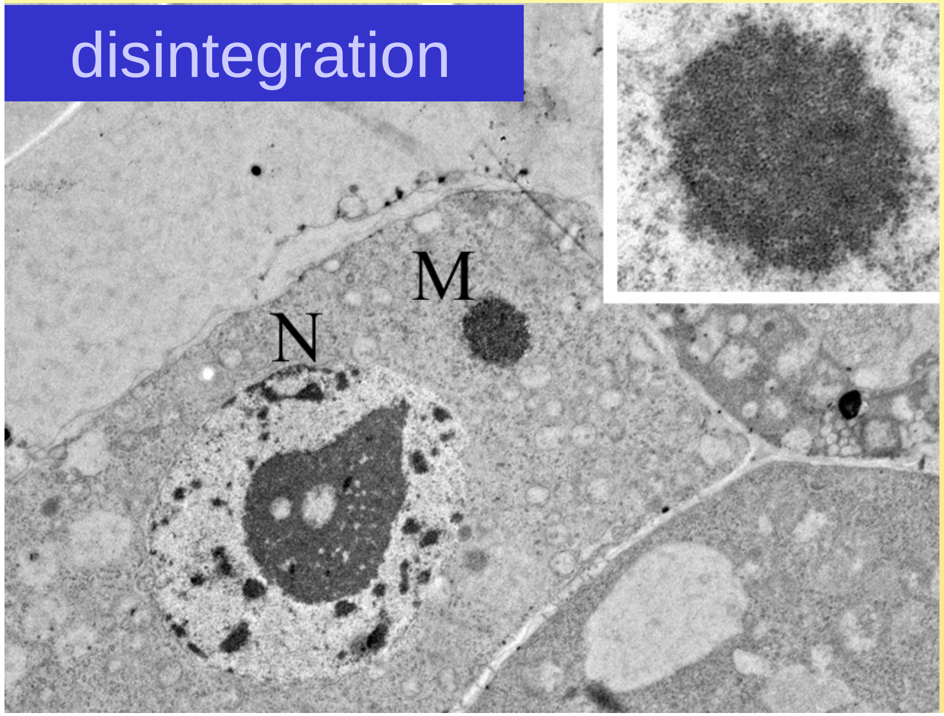
complete



partial heterochromatinization

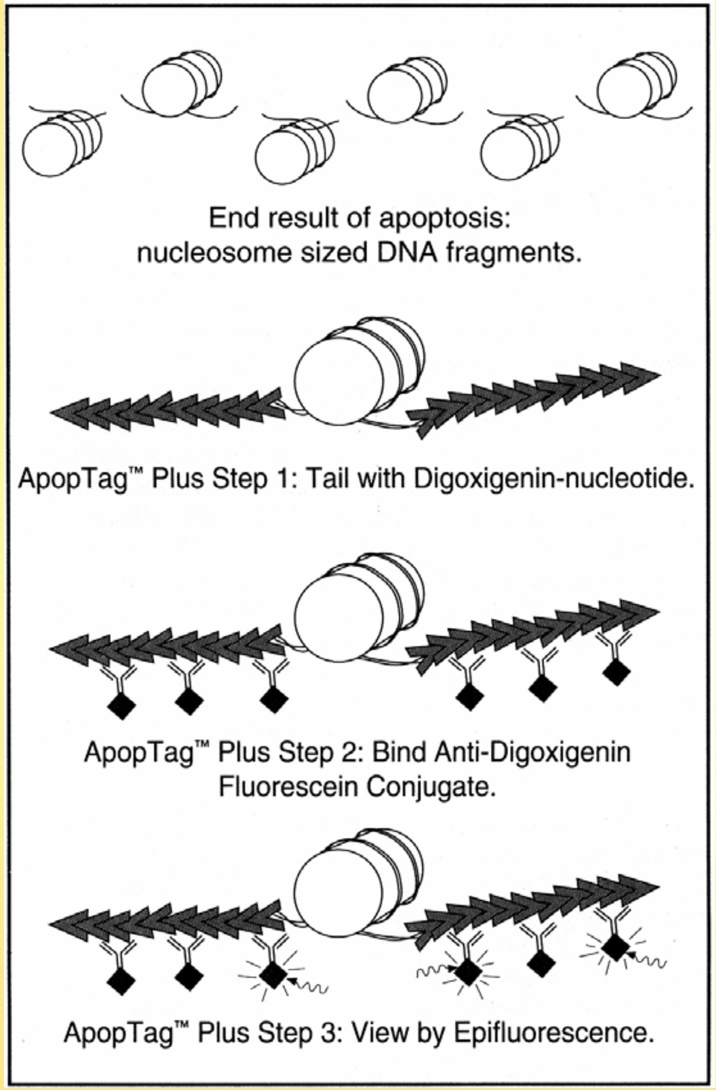


disintegration



Chromatin structure differs between both types nuclei

TUNEL assay - *in situ* detection of DNA fragmentation

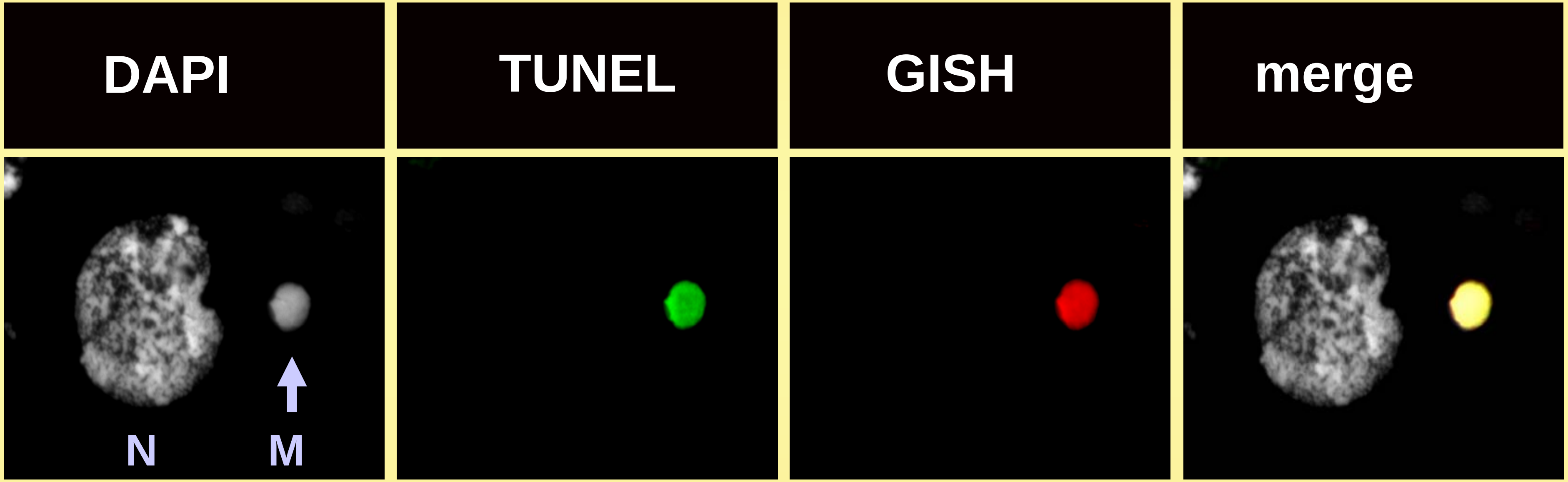


TdT adds nucleotides (digoxigenin-labeled) to the free 3' OH ends of DNA fragments

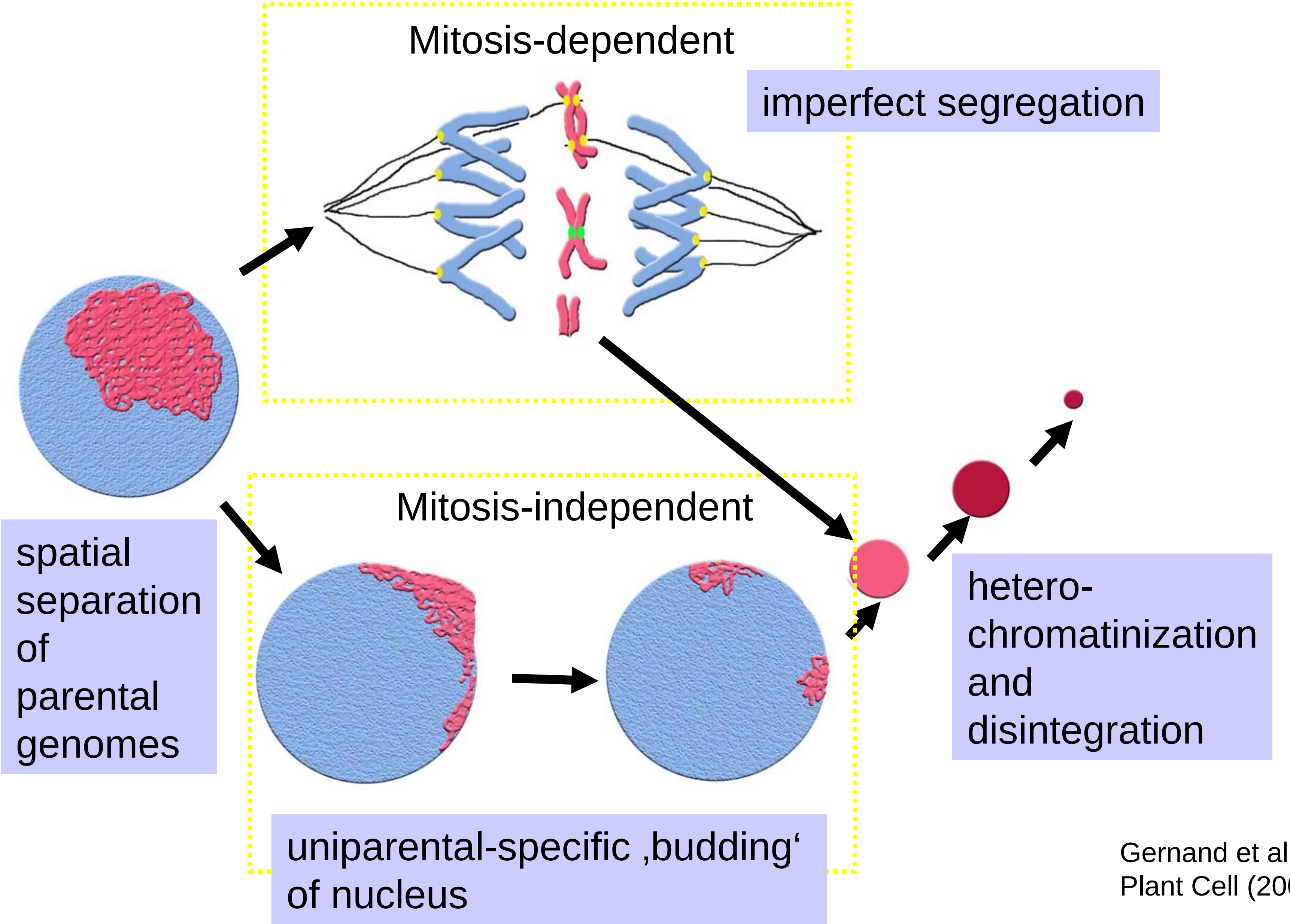
Labeled nucleotides - detected by anti-DIG antibodies

detection by microscopy

Fragmentation of micronucleated DNA – the final step of uniparental genome elimination



Proposed mechanism of uniparental genome elimination



Gernand et al.
Plant Cell (2005)

Modern breeding

Mutation breeding

Breeding of polyploids

Generation of haploids