Modern methods in plant genomics and breeding

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Outline

I. Introduction

- History/ background,
- Genesis of the process in plant genomics and breeding,
- DNA, Chromosomes,

11. Methods in plant breeding and their uses

Plant Tissue Culture: Micro-propagation, Somatic Embryos, Haploids, Protoplast, Somaclonal Variation, Embryo Culture, etc

Mutation induction with mutagens: Physical, Chemical, and Biological

111. Methods in plant genomics and their uses

Plant Genetic Engineering: Plant Transformation methods,

- Transgenesis & GMO
- Molecular markers,
- T- DNA mutagenesis
- How to become a rich man with novel methods/technology

1V. Conclusion

What is Plant Breeding?

- **The genetic adjustment of plants to the service of humankind**

  ---Sir Otto Frankel

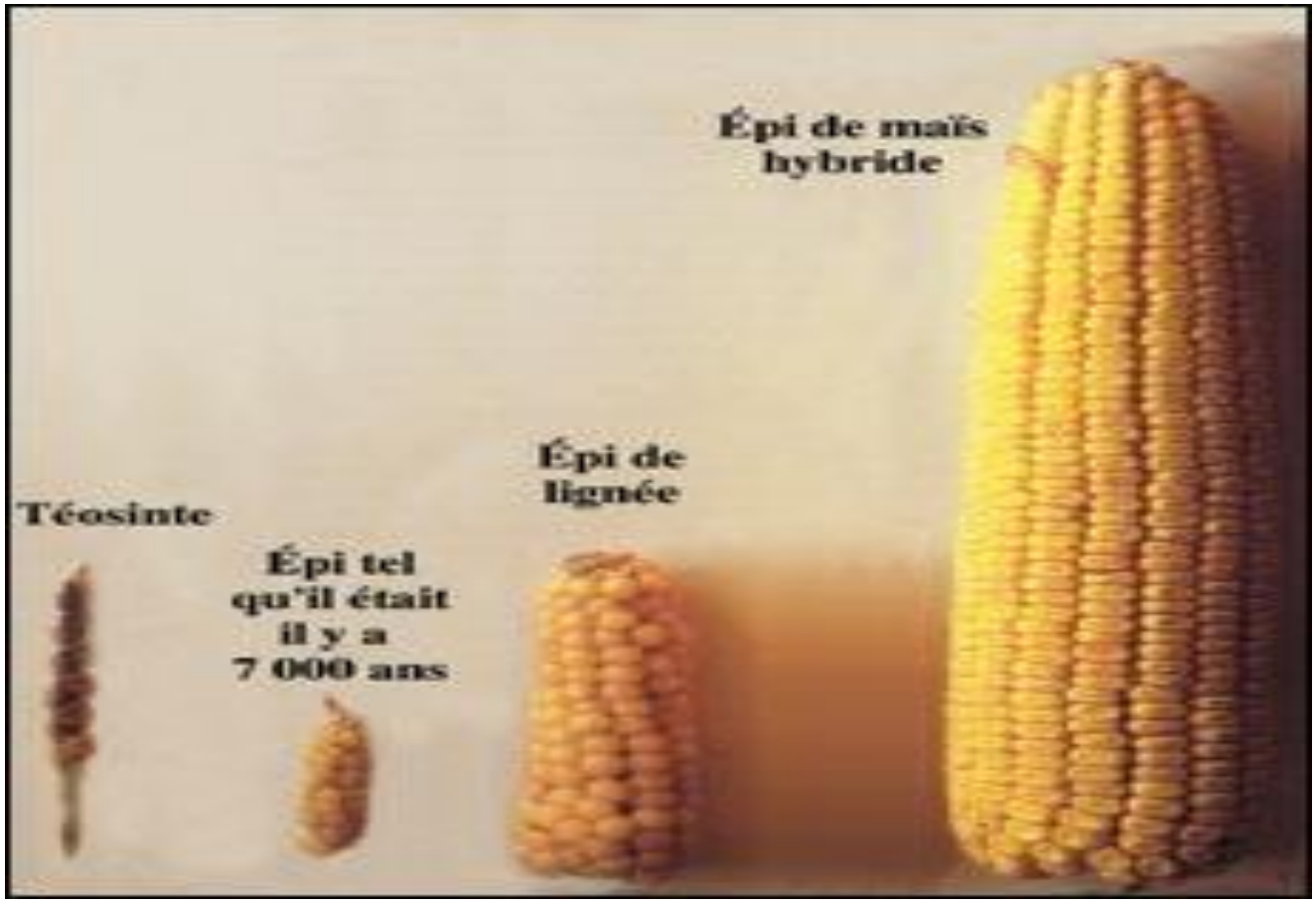
- **The science of improving the heredity of plants for the benefit of humankind**
- Genetic improvement through crossing plants with desired traits and selecting progeny with improved performance and/or improved combinations of traits.
- Systematic procedures used to improve trait phenotypes by crossing and selection, directed manipulation of the genotype at the DNA sequence level, and introduction of new genes.
- **Plant breeding** is the purposeful manipulation of plant species in order to create desired genotypes and phenotypes for specific purposes.

Plant breeding: A long impirical walk from Teosinte to modern corn - **Great productivity gains.**

Teosinte

Modern corn

From teosinte to modern corn



Long impirical Walk in Plant breeding: Domestication of corn. Modern breeding has greatly increased the size and number of seed per ear.

Plant breeding has been practiced for thousands of years - since near the beginning of human civilization.

Lesson 1: Selective breeding leds to higher-yielding varieties.

Crop improvement has been one of the most imp. Success story in the history: Great productivity gains,

Plant Breeding: History

Phase 1: <1960 **Phase 2: 1960-1980** **Phase 3: After 1980**

- Prehistoric: Impirical Plant Breeding
- Historic: Mendelian Laws
- Current: Modern Plant Breeding
- Way Forward: Targeted Plant breeding
- Plant breeding has played a great role in Agricultural development

- **10, 000 Years: Hunter to Cultivars - Agriculture/ farming**
- **Mendel's study: Inheritance 1900; Early 20th Century, slow progress**
- **2nd World war, 50s, onward**
- **leading to modern Agriculture; great progress, green revolution;**
- **80, 90s,00s onward: leading to Modern Genetics; Gene technology; Biotec/ GM crops**
- **Great productivity gains,**

Gregor Johann Mendel

Gregory Mendel working with peas made two innovations to the science of genetics:

1) **developed pure lines**
2) **counted his results and kept statistical notes**

Gregor Johann Mendel (July 20[1], 1822 – January 6, 1884) was an Augustinian abbot who is often called the "father of genetics" for his study of the inheritance of traits in pea plants. Mendel showed that the inheritance of traits follows particular laws, which were later named after him. The significance of Mendel's work was not recognized until the turn of the 20th century. Its rediscovery prompted the foundation of genetics.

Rediscovery of Mendel's work

It was not until the early 20th century that the importance of his ideas was realized.

In 1900, his work was rediscovered by Hugo de Vries and Carl Correns

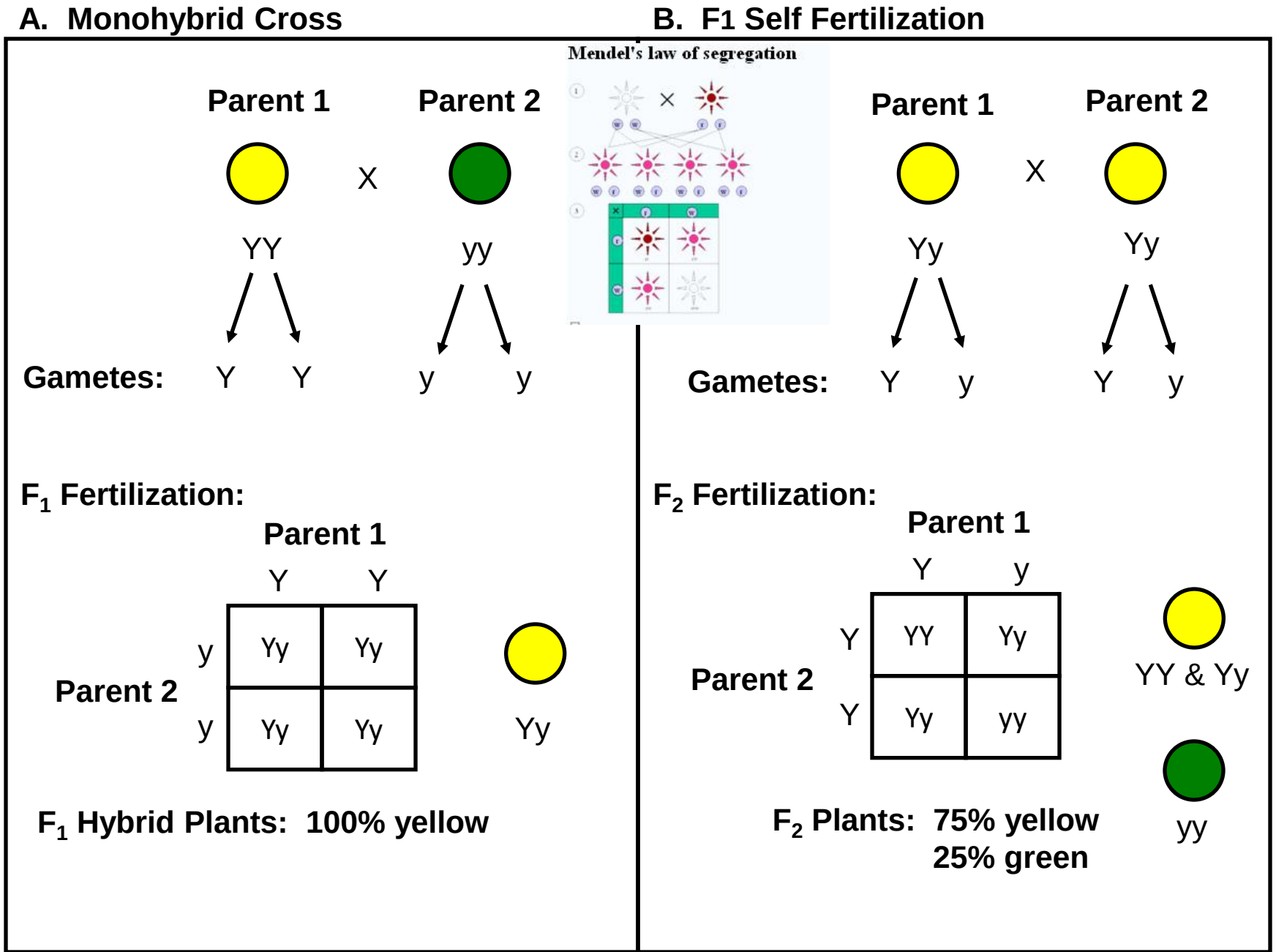
Mendel's Laws

Mendel's First Law - the law of segregation; during gamete formation each member of the allelic pair separates from the other member to form the genetic constitution of the gamete

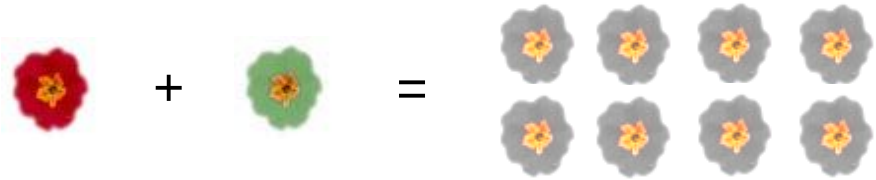
e.g. **A a** yields gametes with **A** **a**

Mendel's Second Law -- the law of independent assortment; during gamete formation the segregation of the alleles of one allelic pair is independent of the segregation of the alleles of another allelic pair

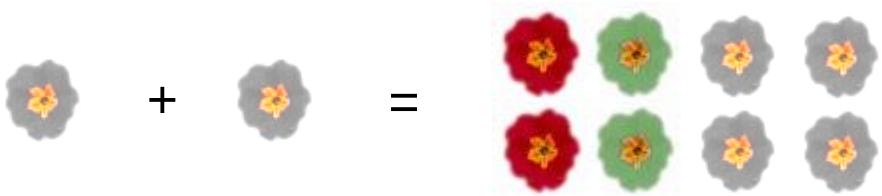
Figure 2.5. **Mendel's First Law** - the law of segregation; during gamete formation each member of the allelic pair separates from the other member to form the genetic constitution of the gamete



Law of Uniformity



Law of Segregation



Highlights of plant breeding:

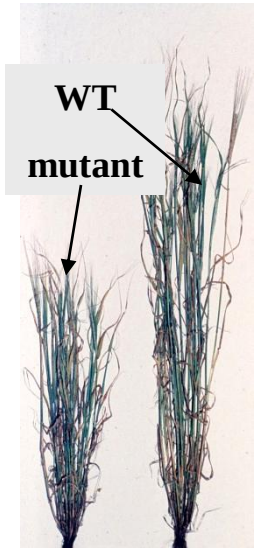
- 1865 Mendelian genetic principles
- 1923 first hybrid corn
- **1960 Green Revolution‘**

– breeding, plant management (fertization, pest control..)

- 1983 first transgenetic plants: GMO

Dr. Norman Borlaug
Nobel Peace Prize 1970
Father of the Green Revolution
1914-2009

Dwarf gene: High yielding varieties



Green revolution: Wheat in 60s & 70s, now is the time for **gene revolution**

Allelic Differences for Mendel's Genes
Plant Height Gene

Gene: gibberellin 3-β-hydroxylase
Function: adds hydoxyl group to GA_{20} to make GA_1
Role of GA_1: regulates cell division and elongation
Mutation in short allele: a single nucleotide converts an alanine to threonine in final protein
Effect of mutation: mutant protein is 1/20 as active

What is Biotechnology?

General Definition

The application of technology to improve a biological organism

A precise process to improve the plants

Detailed Definition

The application of the technology to modify the biological function of an organism by adding genes from another organism

Multidisciplinary

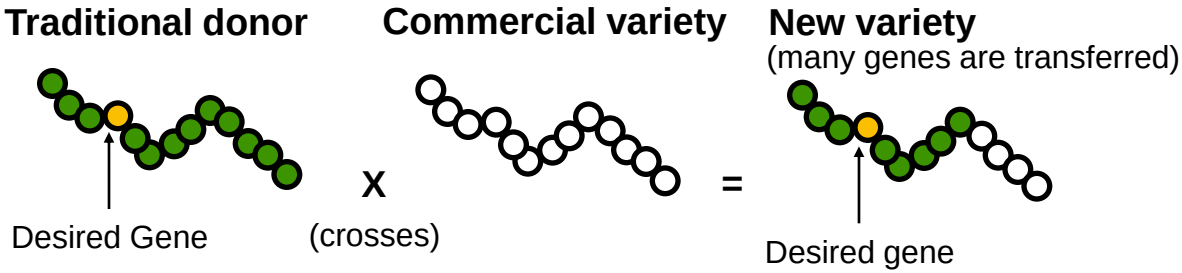
- Involves many disciplines or branches of learning, includes all areas of Life Sciences

Biotech examples

- Medicine
- Agriculture
- Environment
- Forestry
- Food and beverage processing

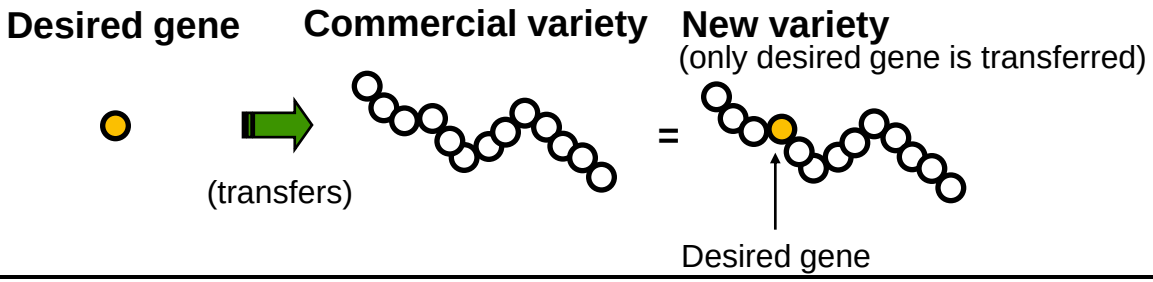
Traditional plant breeding

DNA is a strand of genes, much like a strand of pearls. Traditional plant breeding combines many genes at once.



Plant biotechnology

Using plant biotechnology, a single gene may be added to the strand.



Conventional Breeding

- limited to exchanges between the same or very closely related species
- little or no guarantee of obtaining any particular gene combination from the millions of crosses generated
- undesirable genes can be transferred along with desirable genes
- take a long time to achieve desired results

Genetic Engineering

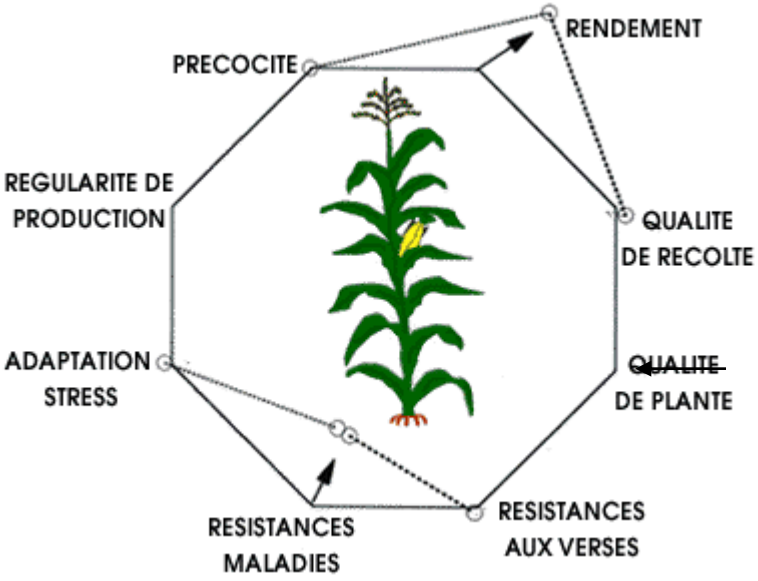
- allows the direct transfer of one or just a few genes, between either closely or distantly related organisms
- crop improvement can be achieved in a shorter time compared to conventional breeding

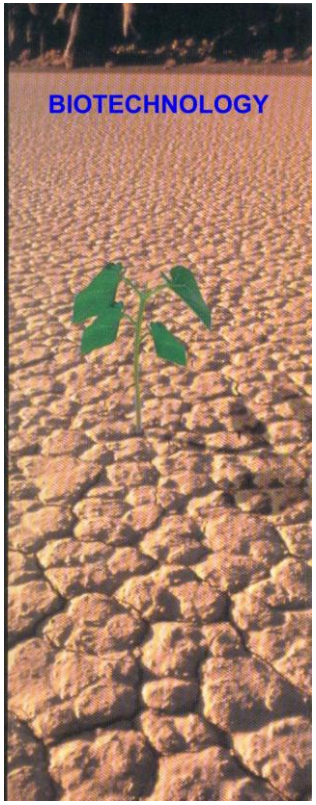
Uses /Aims of Modern Plant Biotechnology:

- develop plant varieties with specific properties for survival in their local regions
- environmentally sustainable, higher yielding and less expensive varieties
- varieties endowed with more nutritious constituents than the wild type species
- varieties that help to limit post-harvest crop losses
- novel plant varieties to boost biodiversity

Plant Biotechnology: Test-tube to the fields

GMO2007: Oslo, Norway





Use recombinant DNA technology

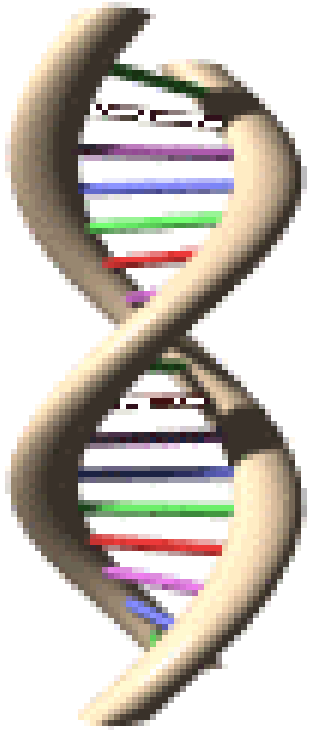
CONCEPTS ON BIOTECHNOLOGY

Genetic engineering

Technique that transfers gene(s) of interest to develop and improve plants, animals and other organisms

Biotechnology Timeline

- **1953 James Watson and Francis Crick, using crucial evidence gathered by Rosalind Franklin, discover the double-helix structure of DNA.**
-
- **1973 California biochemists Stanley Cohen and Herbert Boyer create the first recombinant DNA organism, work that forms the basis for modern biotechnology.**
- **1978 Harvard University professor Walter Gilbert, MIT professor; Phillip Sharp, and others found the biotechnology company Biogen**
- **.**

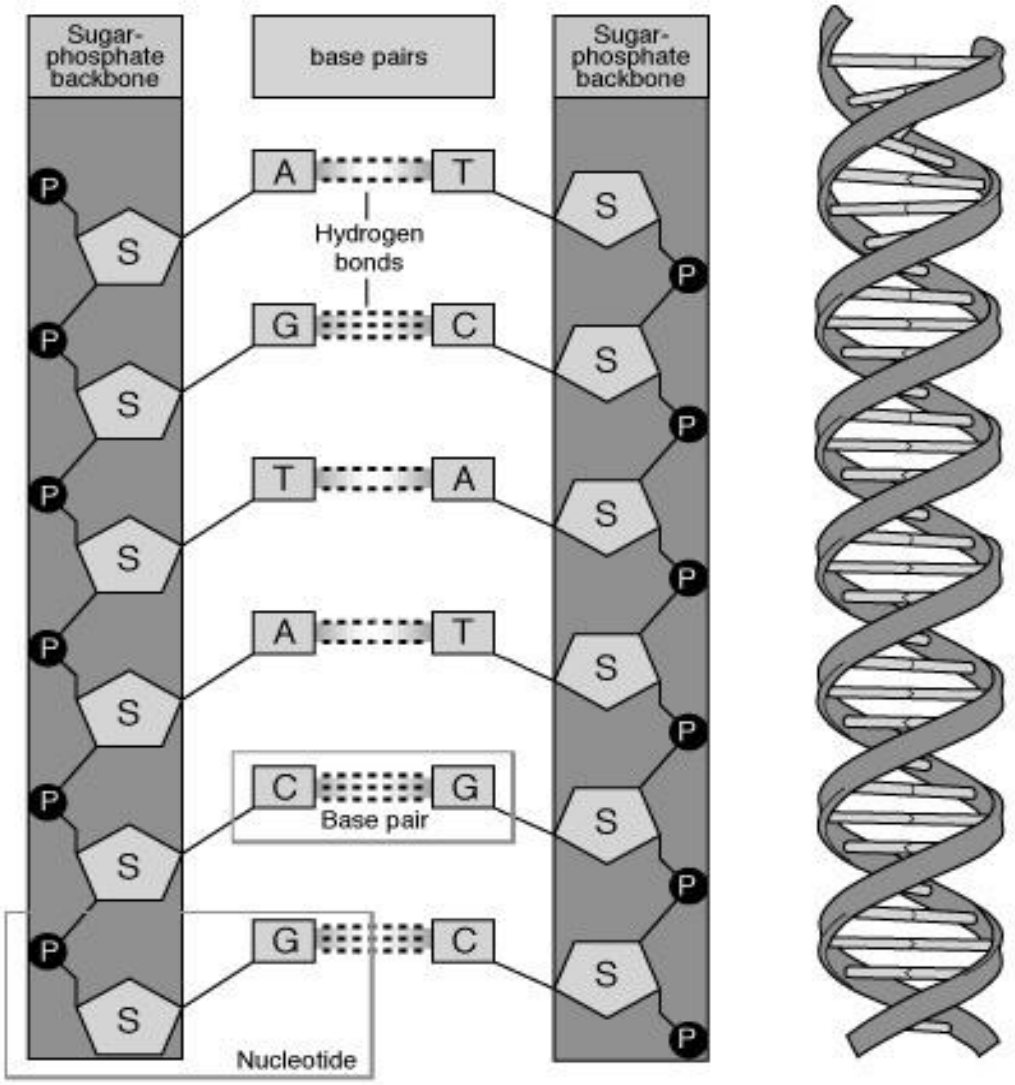


DNA

James D. Watson

In 1953, James D. Watson and Francis Crick demonstrated the molecular structure of DNA. Together, these discoveries established the central dogma of moleculJamar biology, which states that proteins are translated from RNA which is transcribed from DNA. This dogma has since been shown to have exceptions, such as reverse transcription in retroviruses.

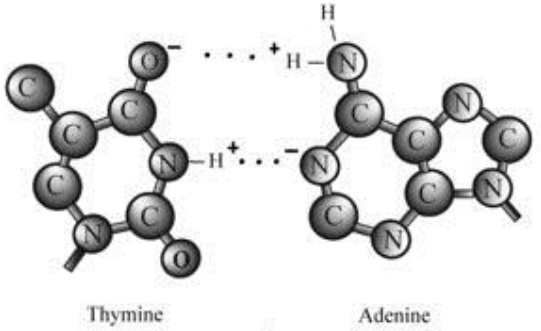
DNA Double Helical Structure

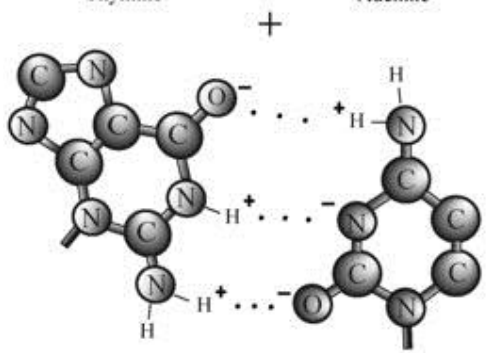


Nucleotide base pairing

A's pair with T's

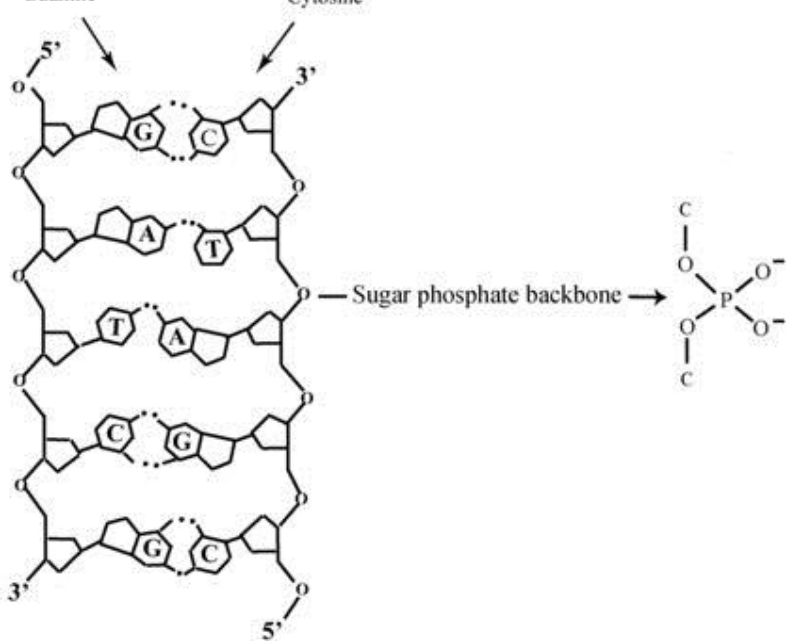
G's pair with C's



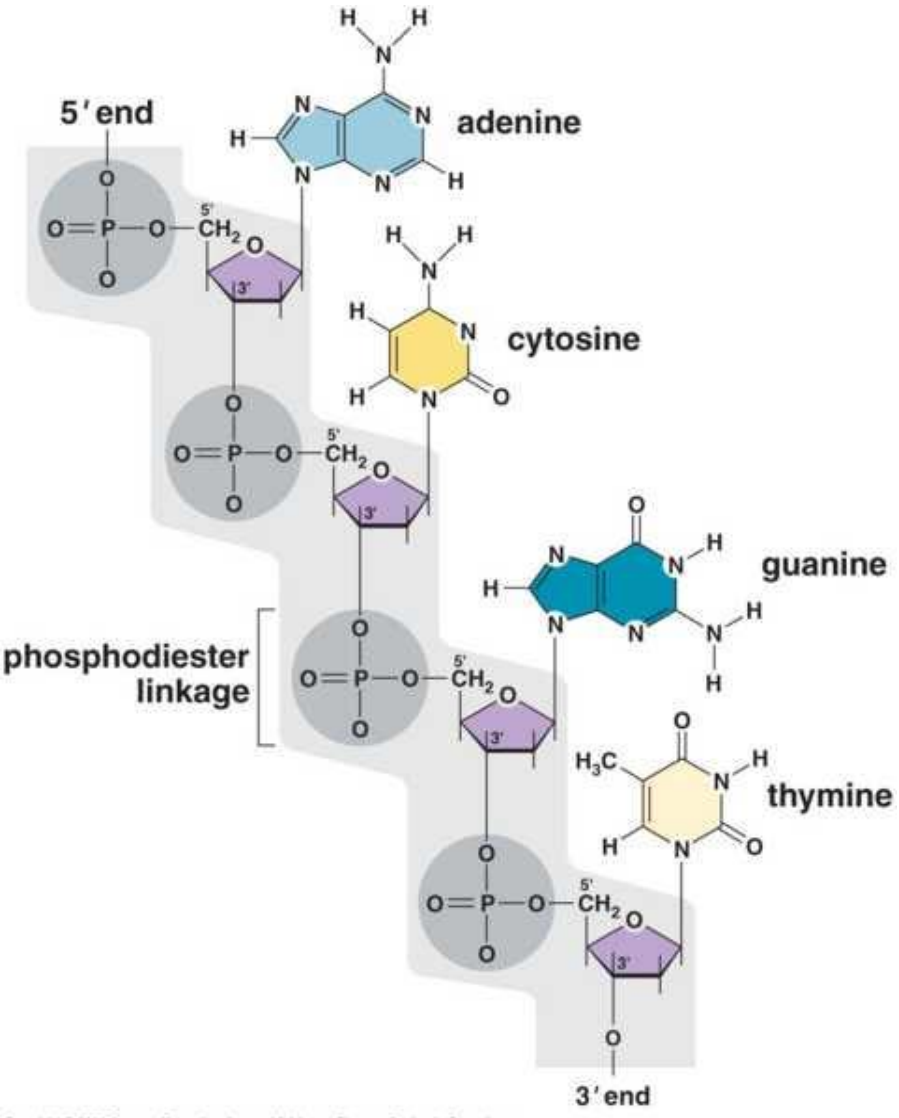


Nucleotide base pairing occurs through "hydrogen bonding"

Strands have directionality from 5' to 3' and when paired strands are in "antiparallel" orientation



DNA and RNA are structurally similar



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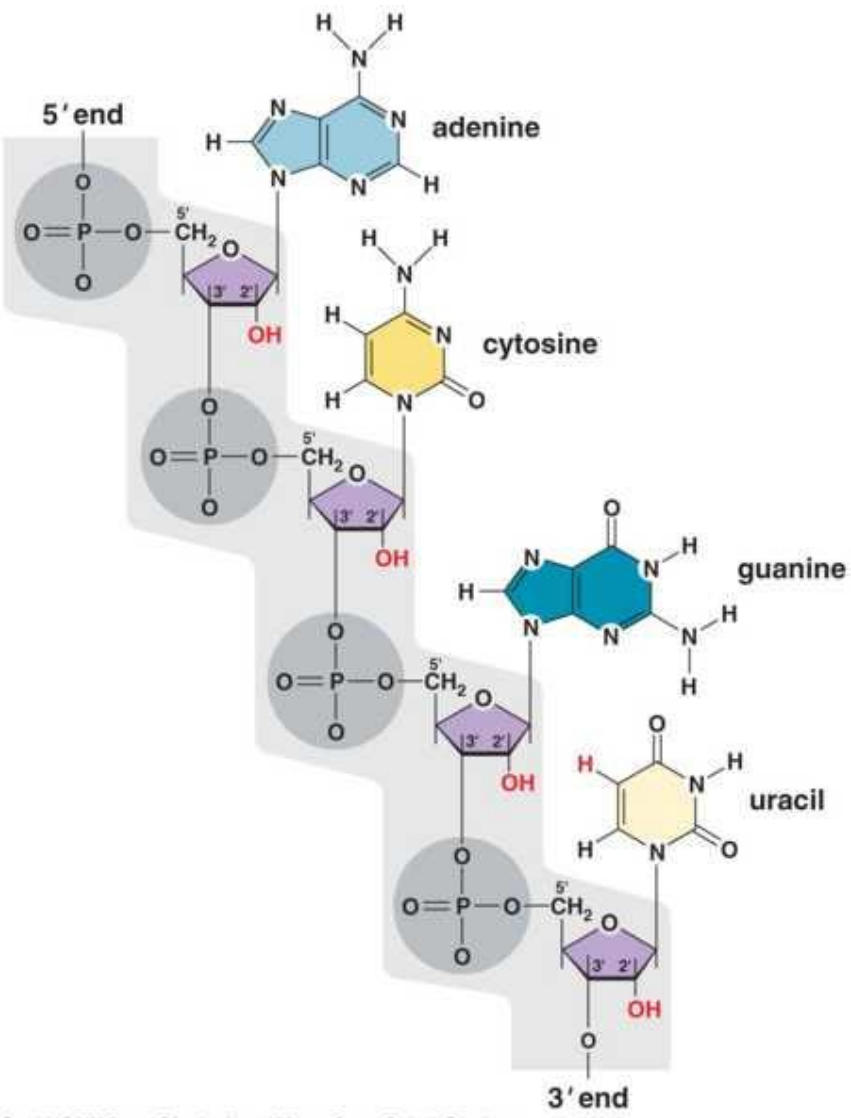

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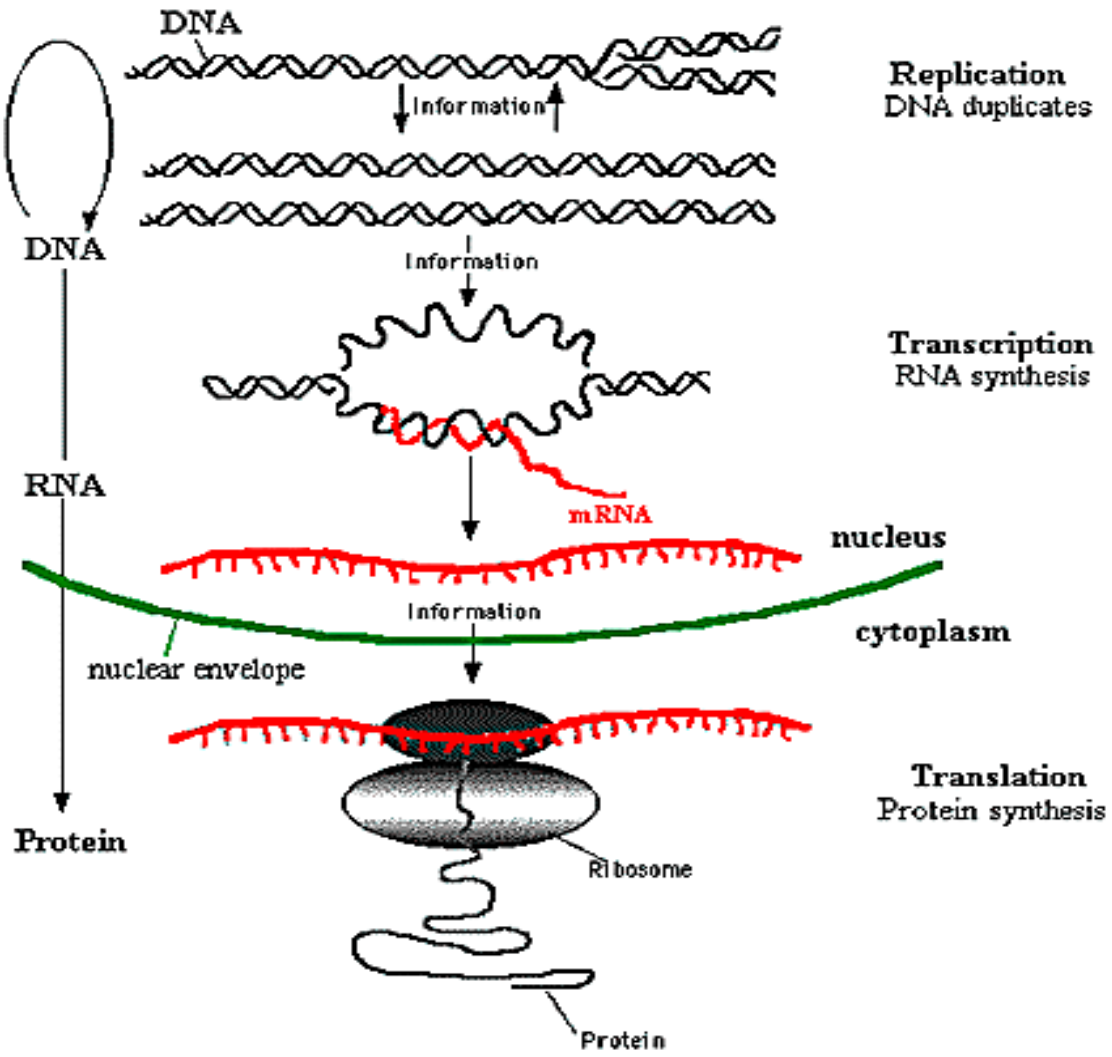
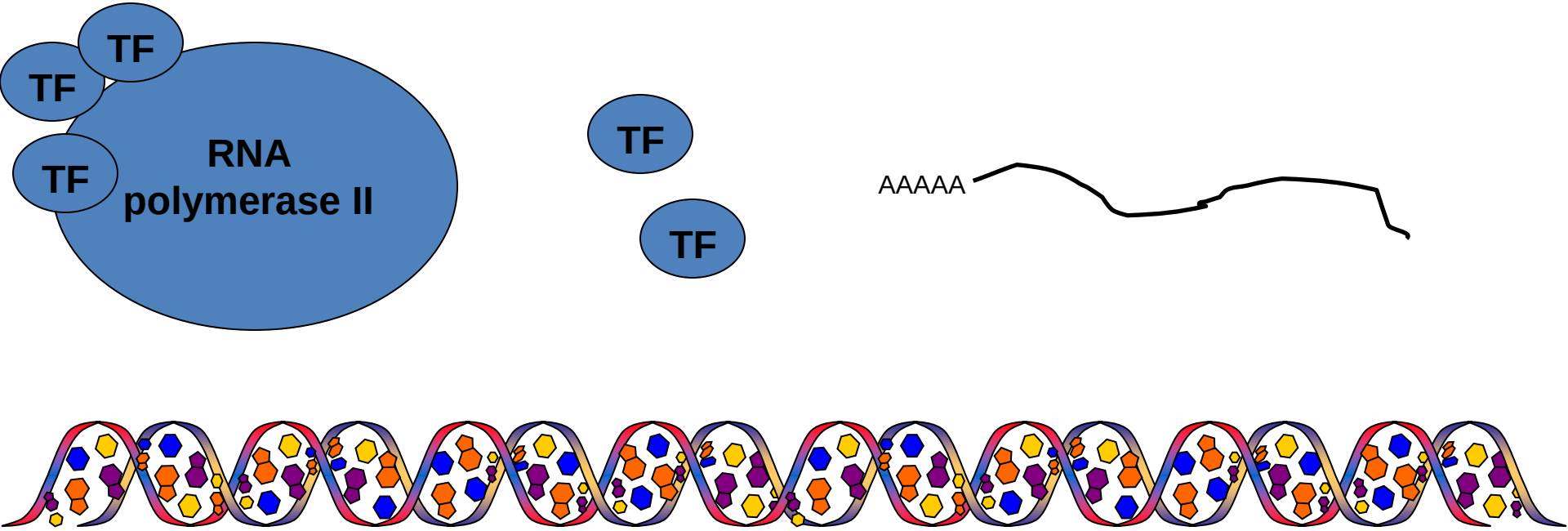
The Central Dogma of Life



The Central Dogma of Molecular Biology

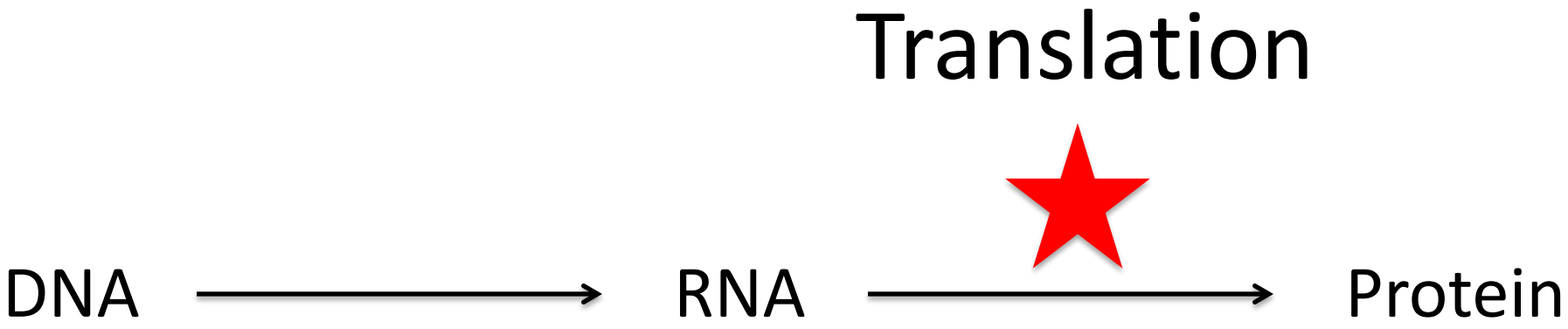
The Central Dogma of Molecular Biology

Transcription of DNA into mRNA



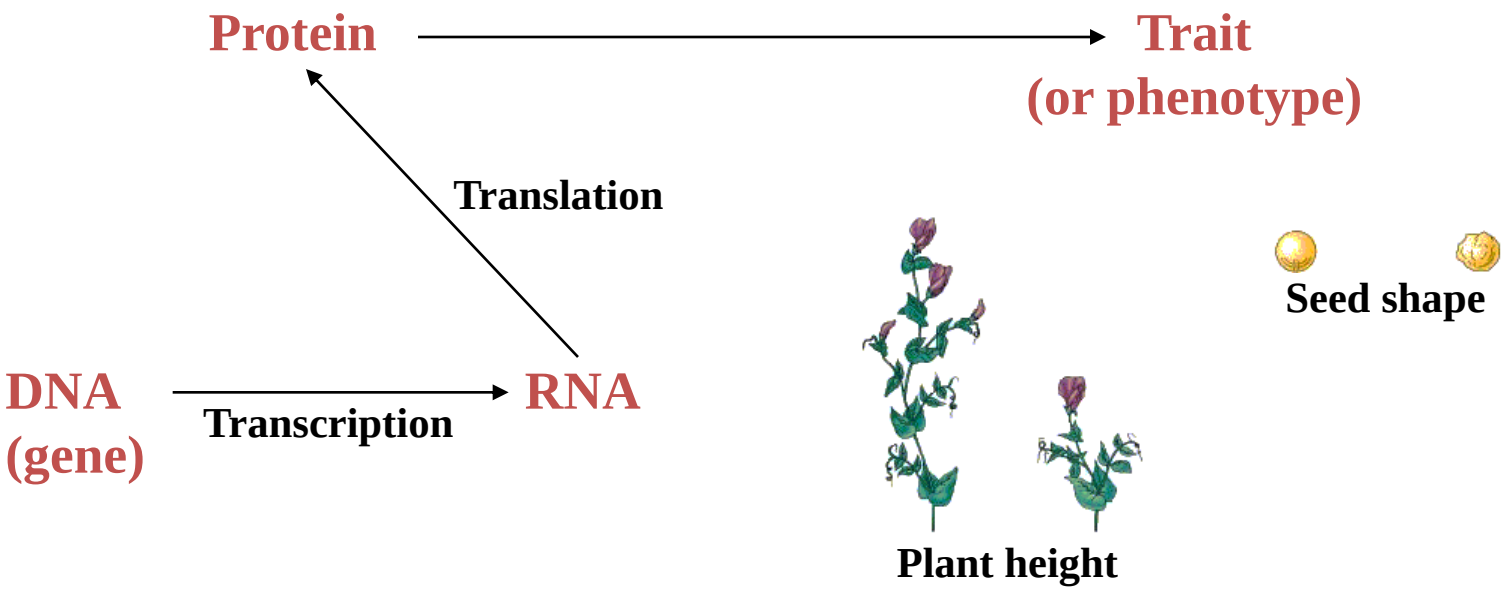
mRNA = messenger RNA

The Central Dogma of Molecular Biology



Central Dogma of Molecular Genetics

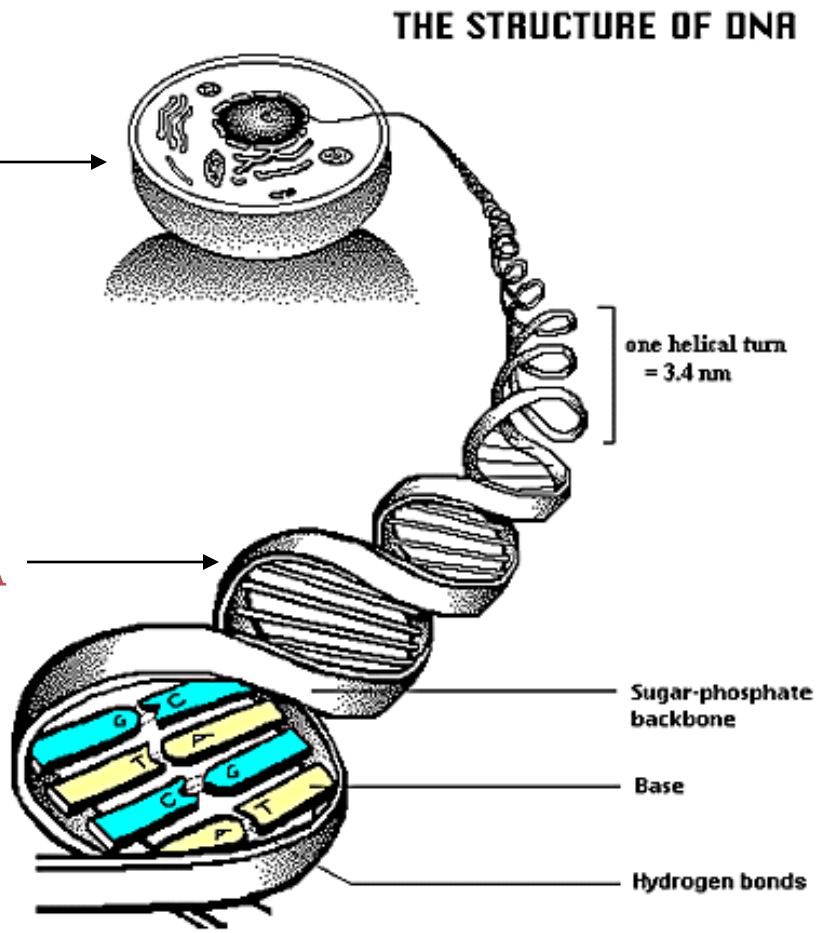
(The guiding principle that controls trait expression)



Gene Manipulation Starts At the DNA Level

The nucleus

contains DNA



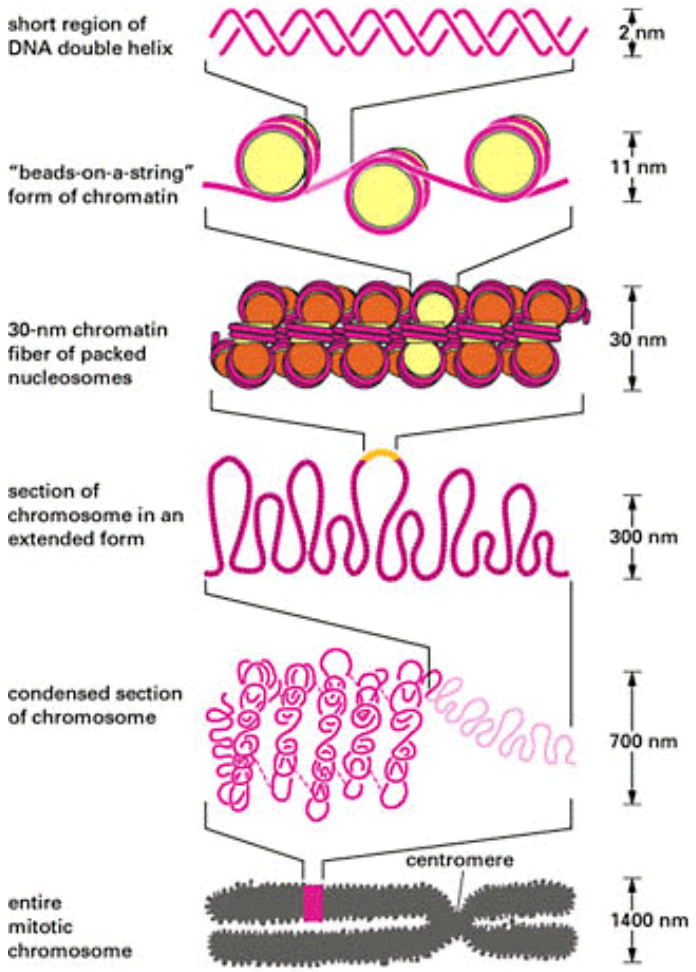
Source: Access Excellence

DNA Is Packaged

Double-stranded DNA →

is condensed into

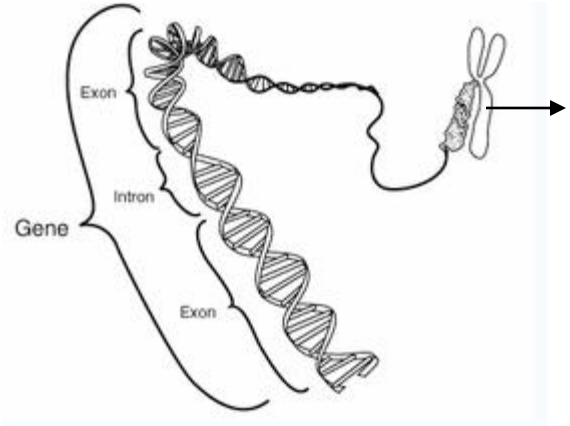
Chromosomes →



NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 50,000x SHORTER THAN ITS EXTENDED LENGTH

Source: Access Excellence

Chromosomes Contain Genes



Chromosome

Gene - a piece of DNA that controls the expression of a trait

Genes are the units of heredity in living organisms. They are encoded in the organism's genetic material (usually DNA or RNA), and control the physical development and behavior of the organism. During reproduction, the genetic material is passed on from the parent(s) to the offspring..

Genes Are Cloned Based On:

Similarity to known genes

Homology cloning **(mouse clone used to obtain human gene)**

Protein sequence

Complementary genetics **(predicting gene sequence from protein)**

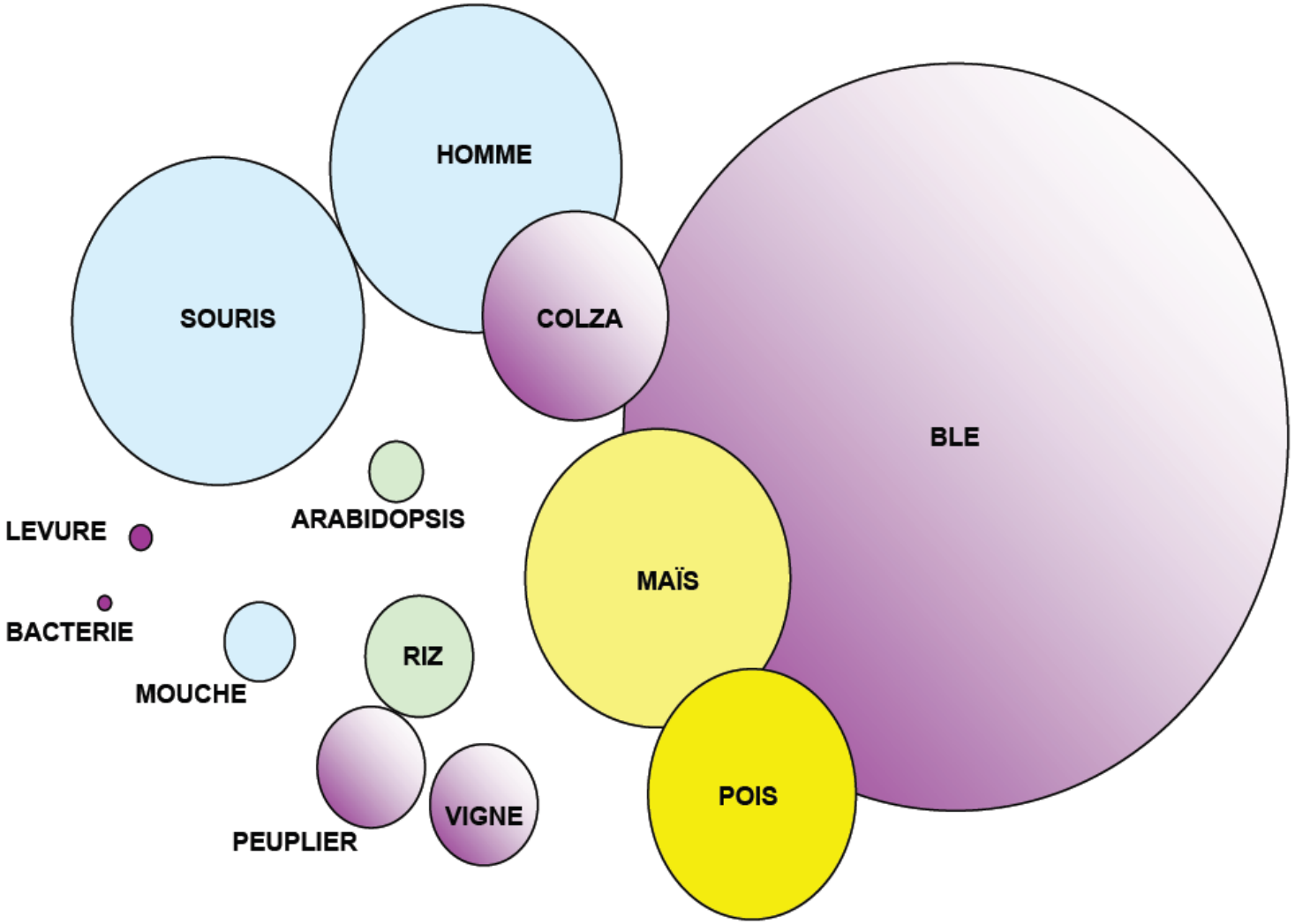
Chromosomal location

Map-based cloning **(using genetic approach)**

Typical numbers of genes in an organism

organism	genes	base pairs
Plant	<50,000	$<10^{11}$
Human, mouse or rat	25,000	3×10^{9}
Fruit Fly	13,767	1.3×10^{8}
Honey bee	15,000	3×10^{8}
Worm	19,000	9.7×10^{7}
Fungus	6,000	1.3×10^{7}
Bacterium	500–6,000	5×10^{5}–10^{7}
Mycoplasma genitalium	500	580,000
DNA virus	10–900	5,000–800,000
RNA virus	1–25	1,000–23,000
Viroid	0–1	~500

Taille comparée des génomes de différentes espèces



Recombinant DNA history

1966 The genetic code is deciphered when biochemical analysis reveals which codons determine which amino acids.

1970 Hamilton Smith, at Johns Hopkins Medical School, isolates the first restriction enzyme, an enzyme that cuts DNA at a very specific nucleotide sequence. Over the next few years, several more restriction enzymes will be isolated.

1972 Stanley Cohen and Herbert Boyer combine their efforts to create recombinant DNA. This technology will be the beginning of the biotechnology industry.

1983 Kary Mullis does PCR. 1985 Kary Mullis publishes method. Patents follow.

http://www.accessexcellence.org/RC/AB/WYW/wkbooks/SFTS/sidebarmilestone.html

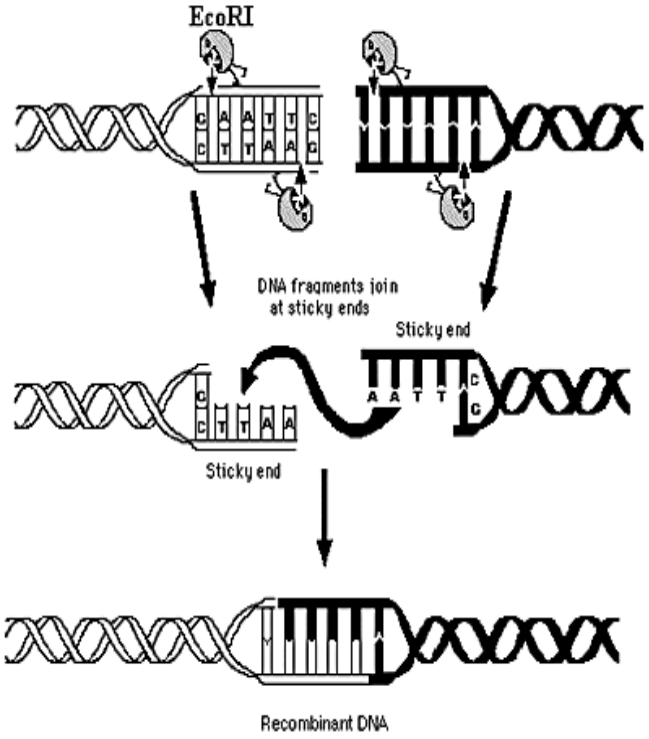
Cloning platforms

- Restriction enzymes/ligase
- PCR-based methods

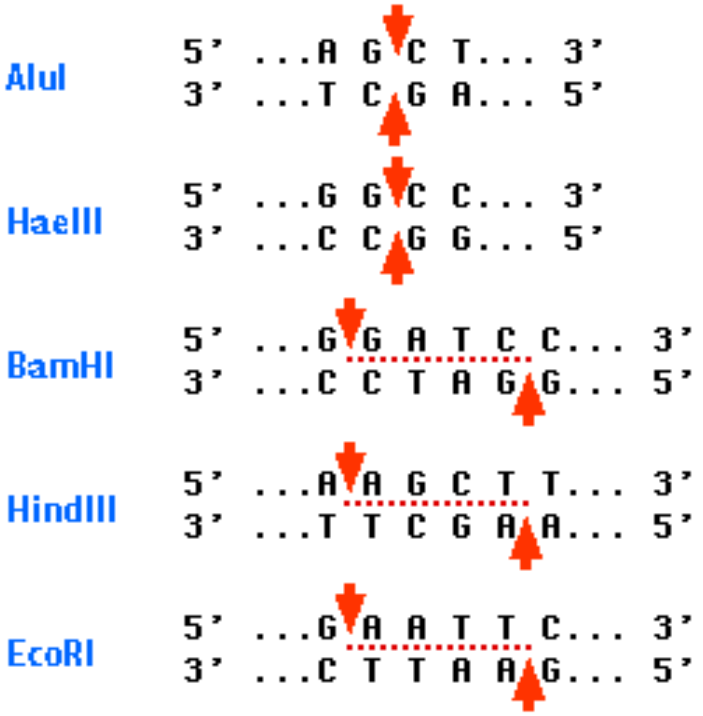
Key enzymes

- Restriction endonuclease
- DNA ligase
- Taq DNA polymerase

Restriction Enzymes

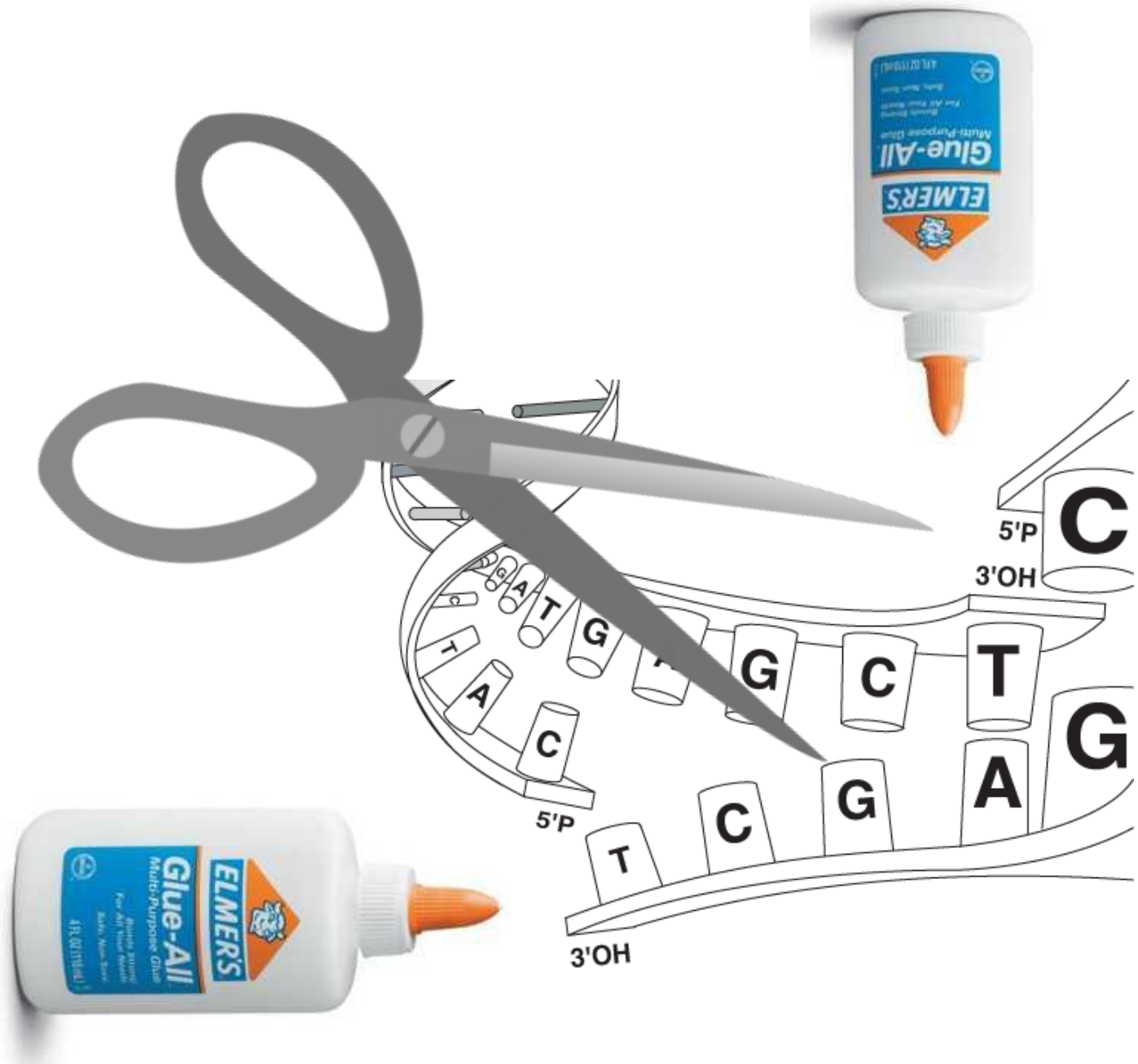


Restriction Enzyme Action of EcoRI



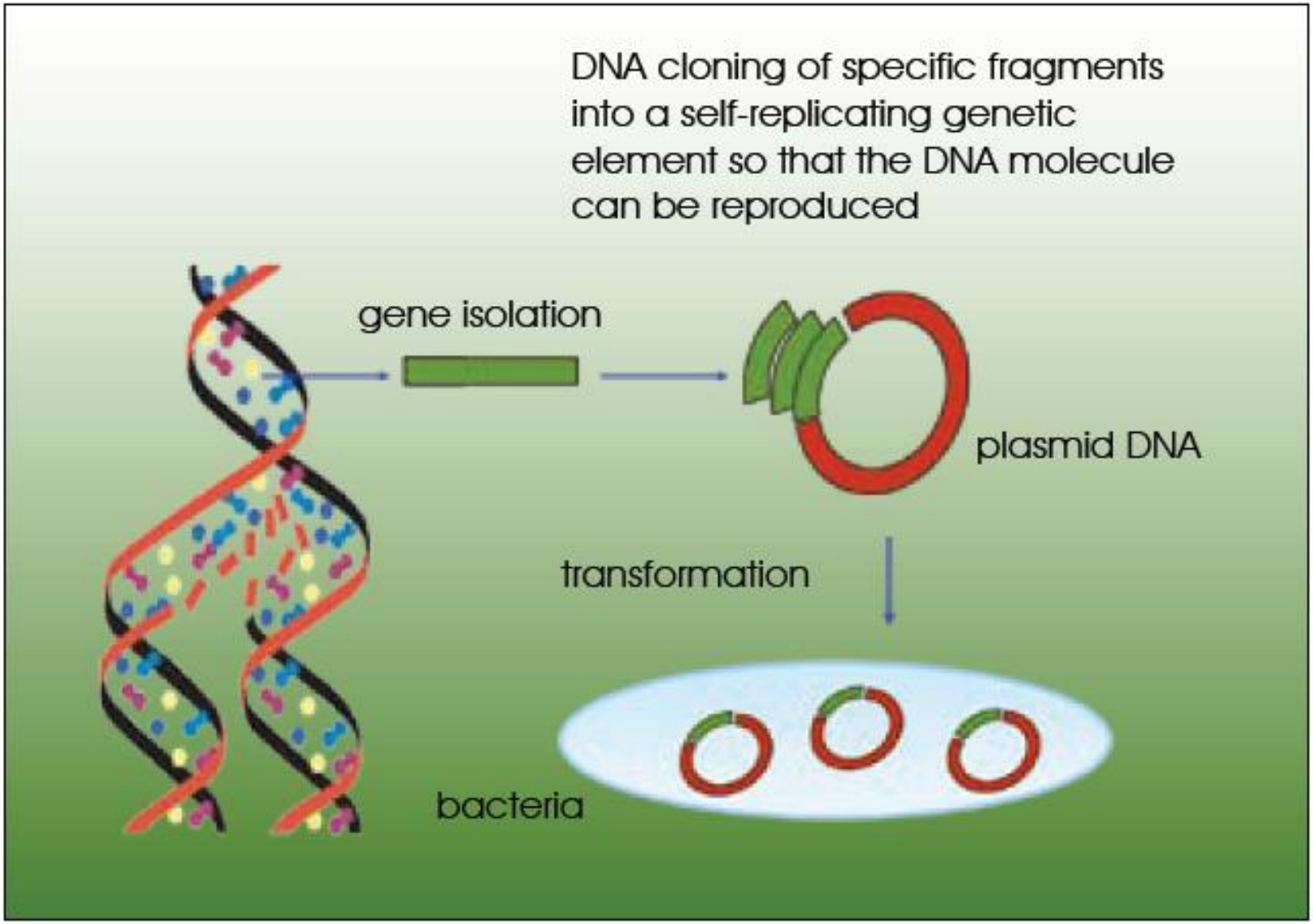
AluI and **HaeIII** produce blunt ends

BamHI **HindIII** and **EcoRI** produce "sticky" ends



http://upload.wikimedia.org/wikipedia/commons/thumb/6/66/Scissors.svg/540px-Scissors.svg.png

Figure 8. Gene cloning



Introducing PCR

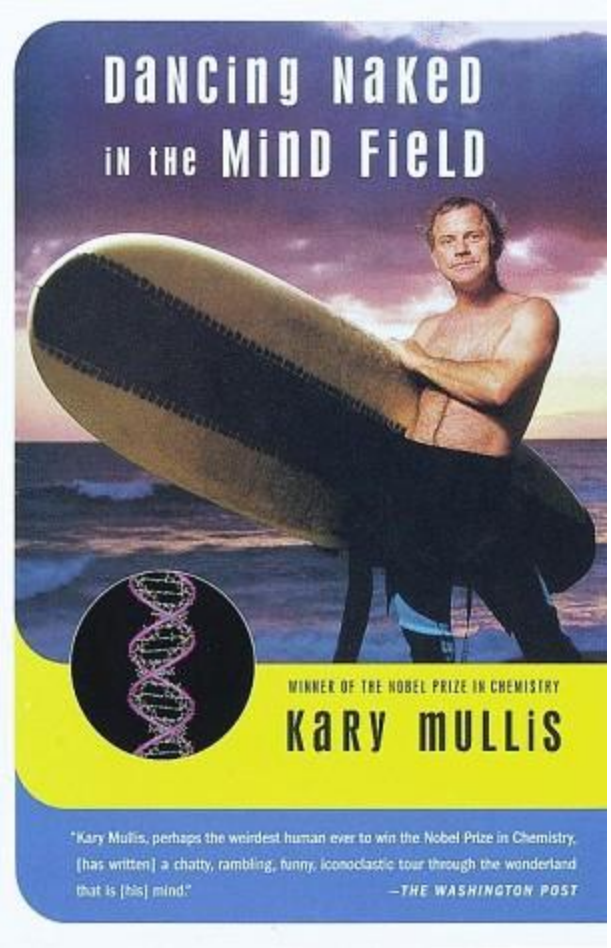
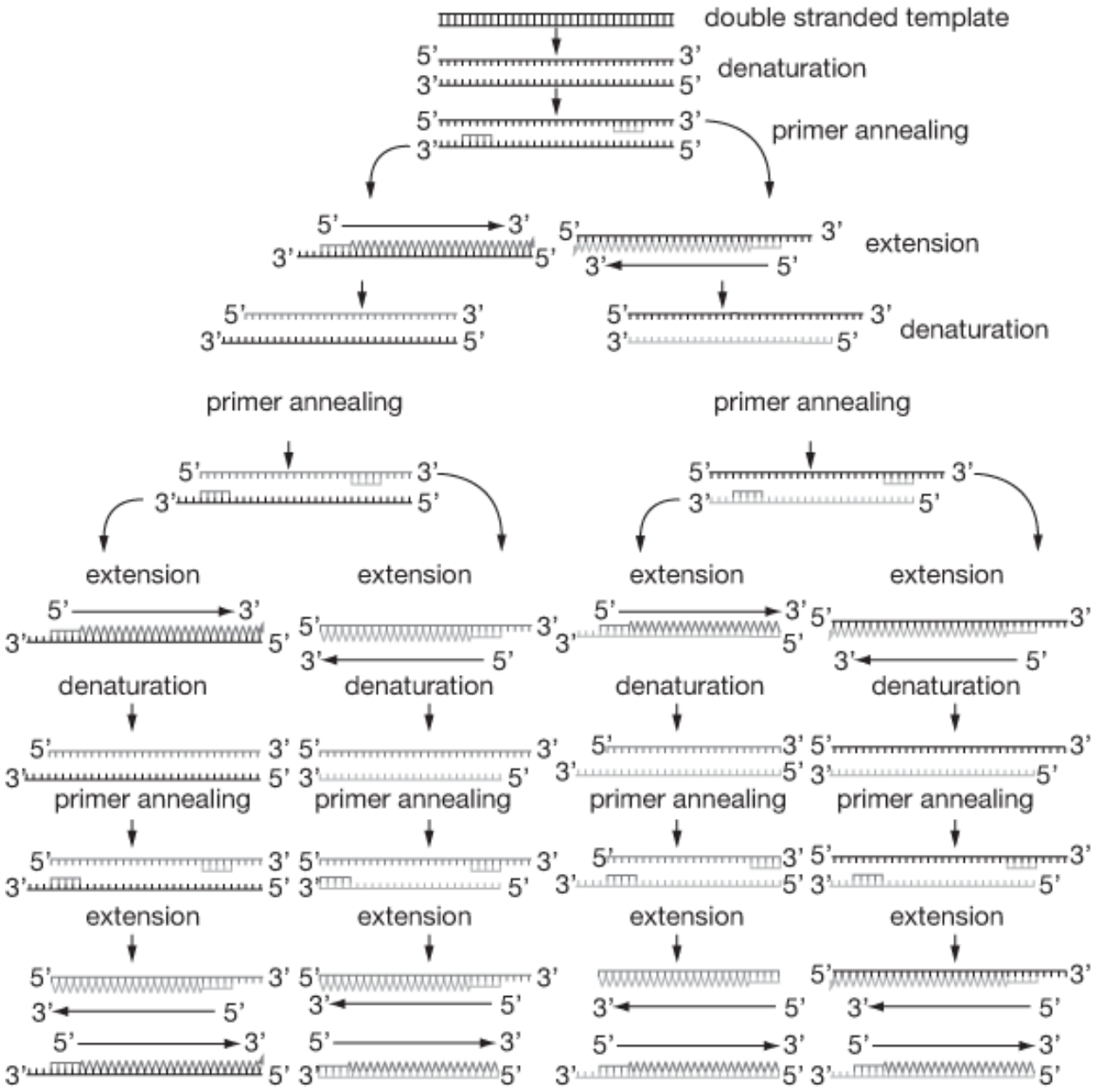


Figure 7.10


double stranded template
denaturation
primer annealing
extension
denaturation
primer annealing
primer annealing
extension
extension
extension
extension
denaturation
denaturation
denaturation
denaturation
primer annealing
primer annealing
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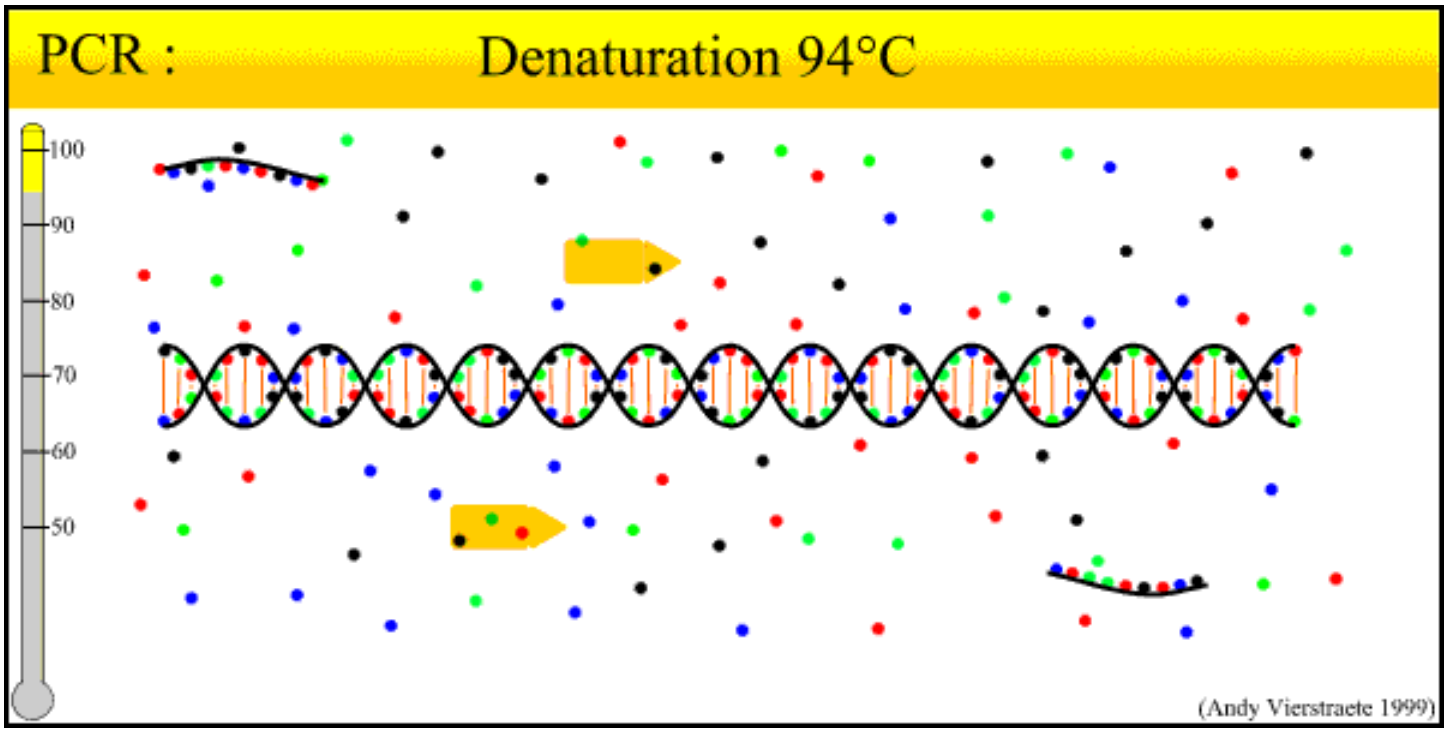
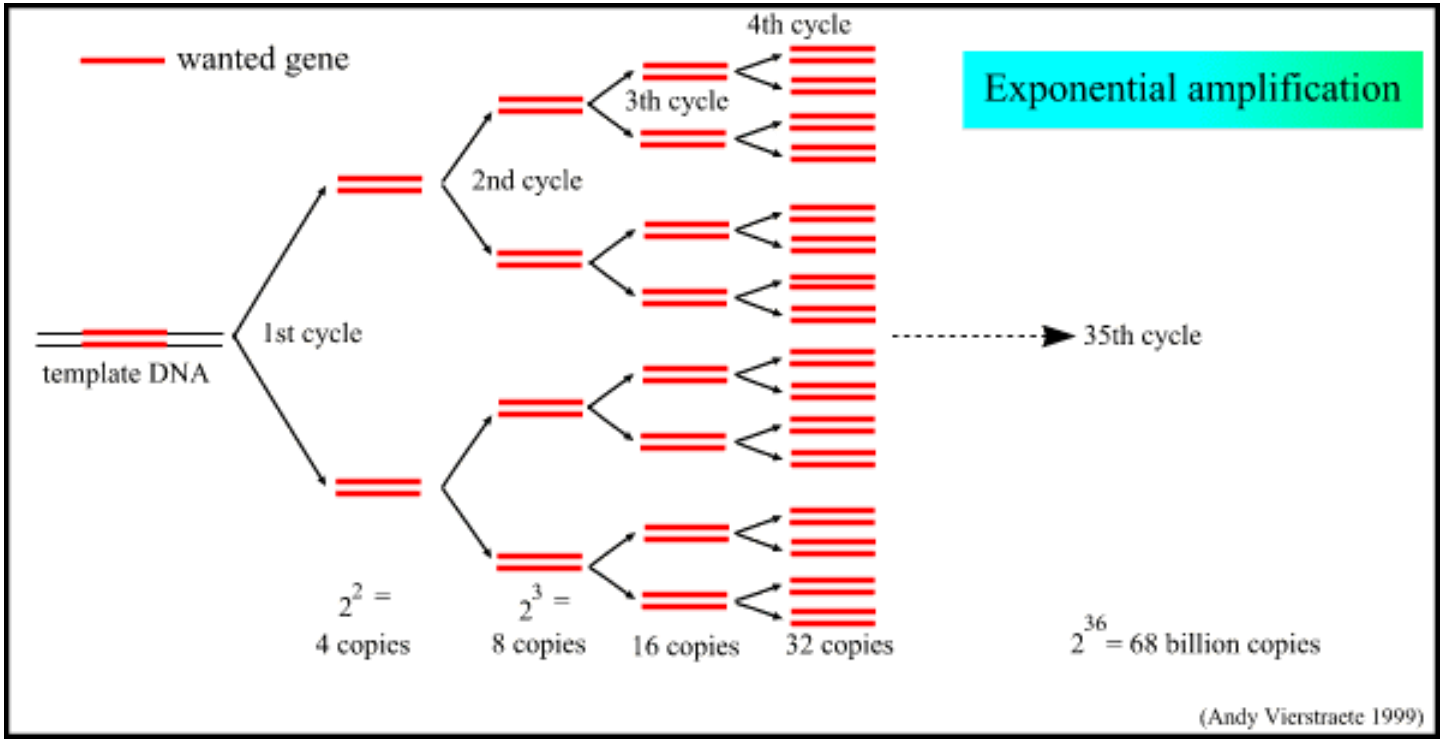
extension
extension
extension
extension

PCR Animation



Denaturation: DNA melts
Annealing: Primers bind
Extension: DNA is replicated

PCR Again


wanted gene
4th cycle
Exponential amplification
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
2^2 =
4 copies
2^3 =
8 copies
16 copies
32 copies
2^{36} = 68 billion copies
(Andy Vierstraete 1999)

In General, Plant Biotechnology Techniques Fall Into Two Classes

Gene Manipulation

- **Identify a gene from *another species* which controls a trait of interest**
- **Or modify an existing gene (create a new allele)**

Gene Introduction

- **Introduces that gene into an organism**
- **Technique called *transformation***
- **Forms *transgenic organisms***

Introducing the Gene or
Developing Transgenics

Steps

1. Create transformation cassette

2. Introduce and select for transformants

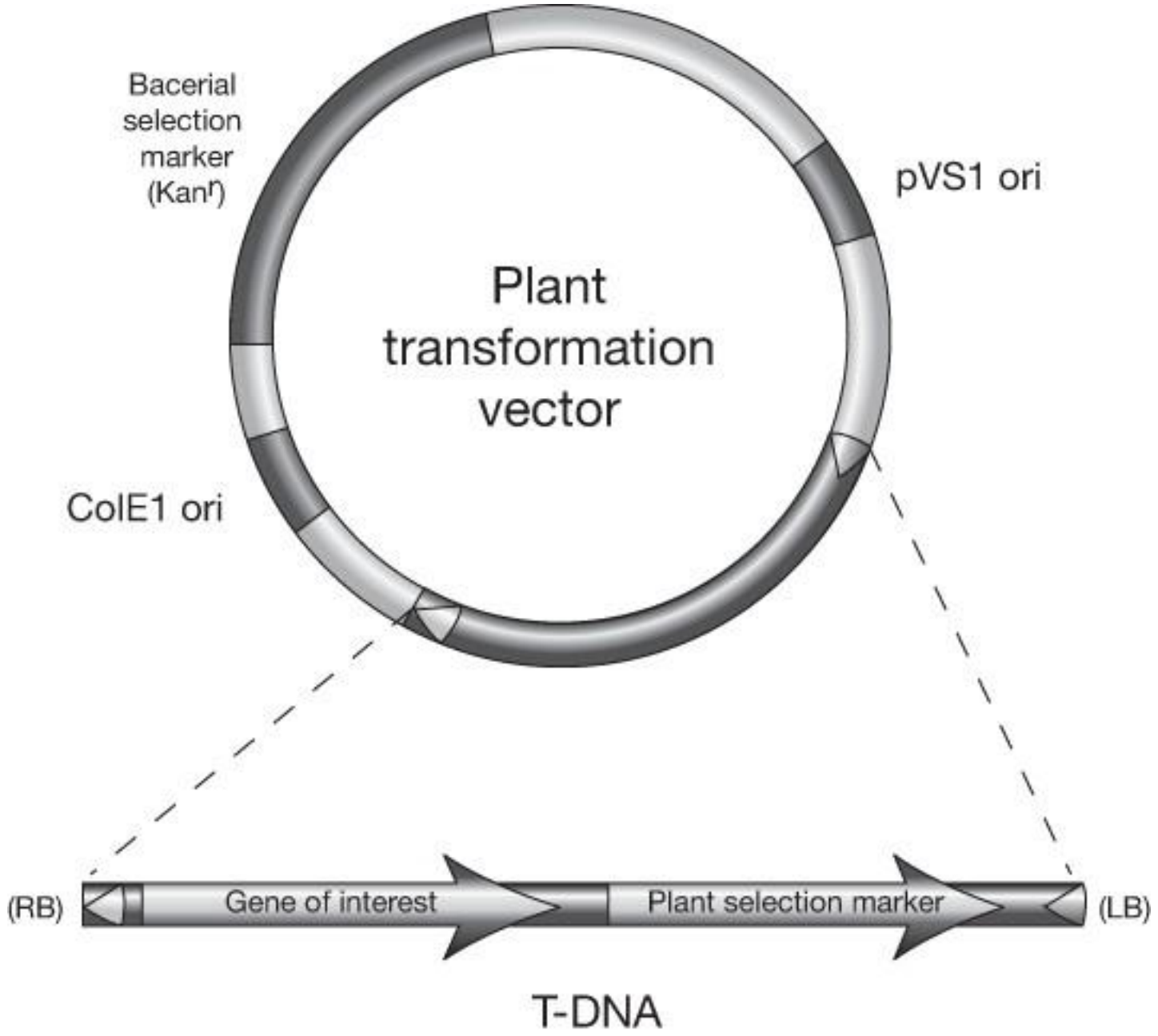
Transformation Cassettes

Contains

1. **Gene of interest**
    - **The coding region and its controlling elements**

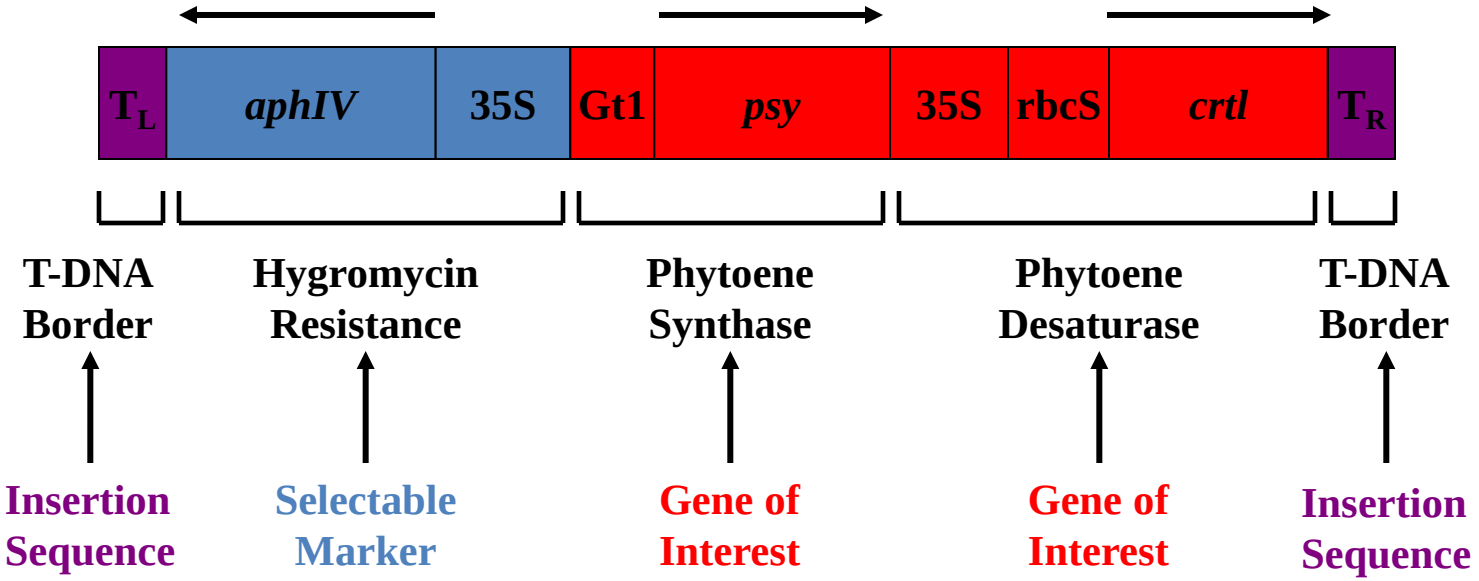
2. **Selectable marker**
    - **Distinguishes transformed/untransformed plants**

3. **Insertion sequences**
    - **Aids *Agrobacterium* insertion**


Bacerial selection marker (Kan^r)
pVS1 ori
Plant transformation vector
ColE1 ori
(RB)
Gene of interest
Plant selection marker
(LB)
T-DNA

Let's Build A Complex Cassette

pB19hpc (Golden Rice Cassette)



Crop Biotechnology: lab to field

Outline

1. In vitro Plant Tissue Culture methods

Micro-propagation, Somatic Embryos, Haploid production, Embryo culture

II. Mutational methods

Physical, Chemical, Biological

III. Plant Genetic Engineering and molecular marker

- **Plant Transformation**
- **Transgenic Plant-GMO**
- **Molecular marker**
- **T-DNA methods**
- **How to become a rich man with novel methods/technology development**

1V. Conclusions

Different *in vitro* techniques

- In vitro multiplication/cloning
- organ culture, cell suspension, callus, embryo rescue, embryo culture, protoplast, somatic hybridization

- **Haploid production, anther & pollen culture**, ovule culture
- Gene transfer, etc

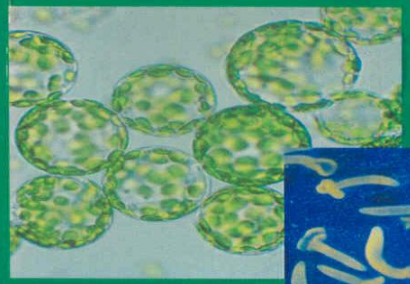
The greatest propagation potential

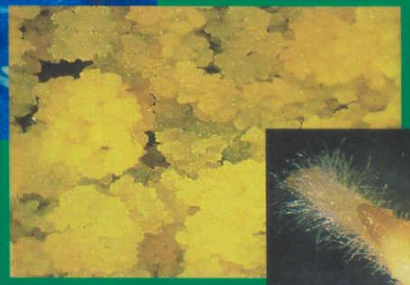
Process & Case studies:

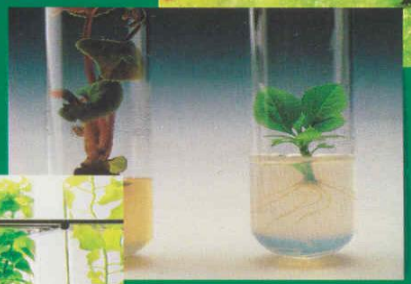
Brief overview of in vitro plant regeneration since1930s

- Gautheret 1939, carrot root: callus formation
- White 1943,
- Skoog, F. and co-workers 1957, 1958: Discovered cytokinins,
- Auxin /cytokinin balance for shoot and root formation
- Steward et al.1958: Somatic embryogenesis
- Reinert 1958: Somatic embryogenesis
- Cocking 1960 Protoplast culture
- Guha and Maheshawari 1964, 1966: Discovered haploid production from anther culture
- **Nitsch et al 1965, 1969: haploid production Sangwan et al. 1975**
- Schell & Van Montagu, Nester, Schilperoort, Chilton: Recombinant DNA technology

Great progress in Plant regeneration in >1000 sp. –
mainly using the impirical system



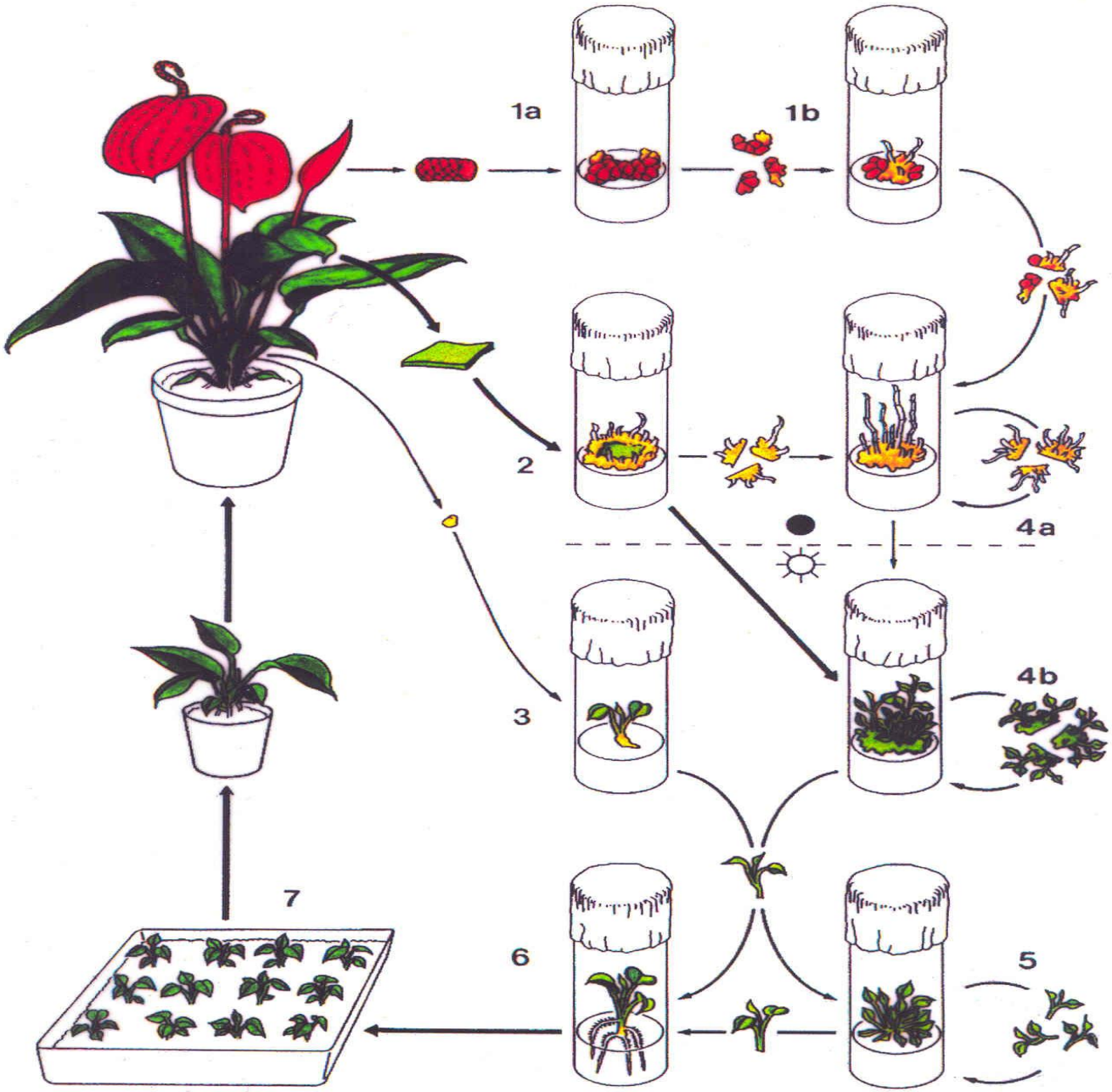




Use of tissue culture ;

In breeding (genetic engineering, haploids, embryo rescue, cryopreservation)

- In propagation (micropropagation)
- In freeing plants from diseases
- Case study: Cassava

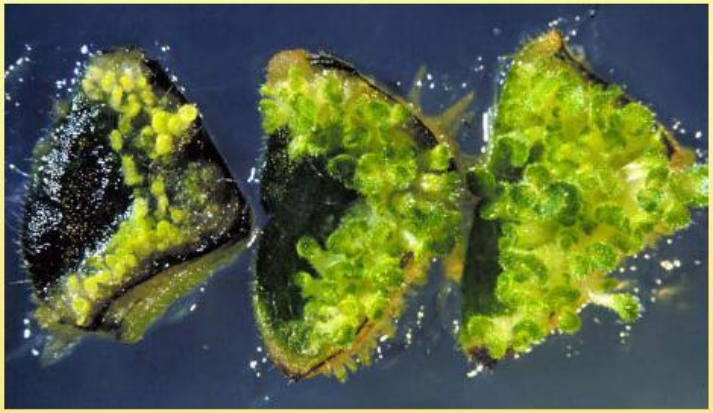

1a
1b
2
3
4a
4b
5
6
7

Plant Regeneration

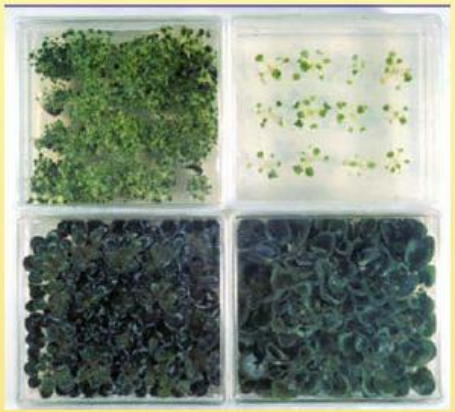
Under the right conditions, plant tissues regenerate into whole plants via two distinct processes; “somatic embryogenesis” and “shoot morphogenesis”.

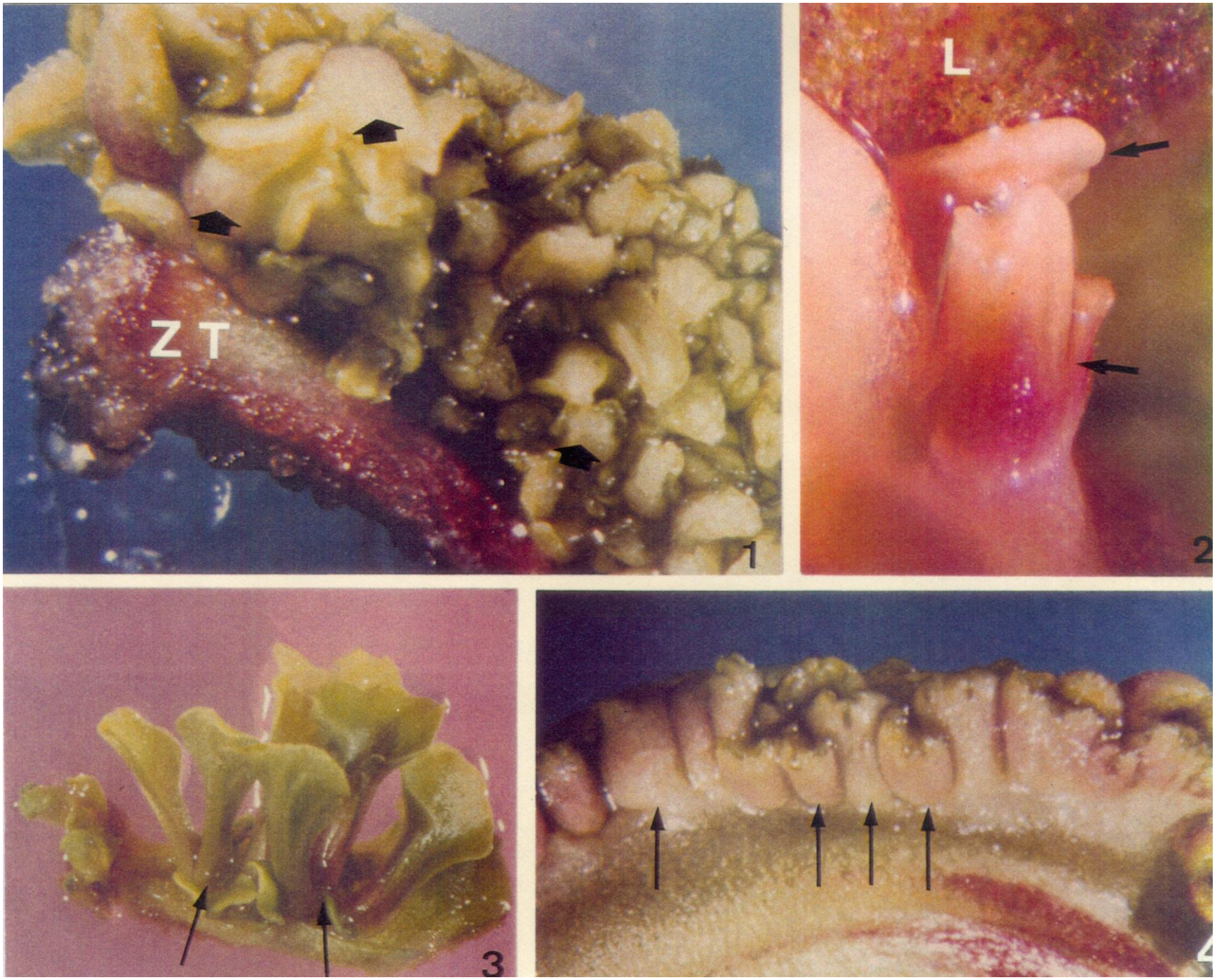
Micropropagation

- allows rapid propagation of new varieties
- economical in time and space
- disease free
- elite propagules



example: violets




ZT
1
L
2
3
4

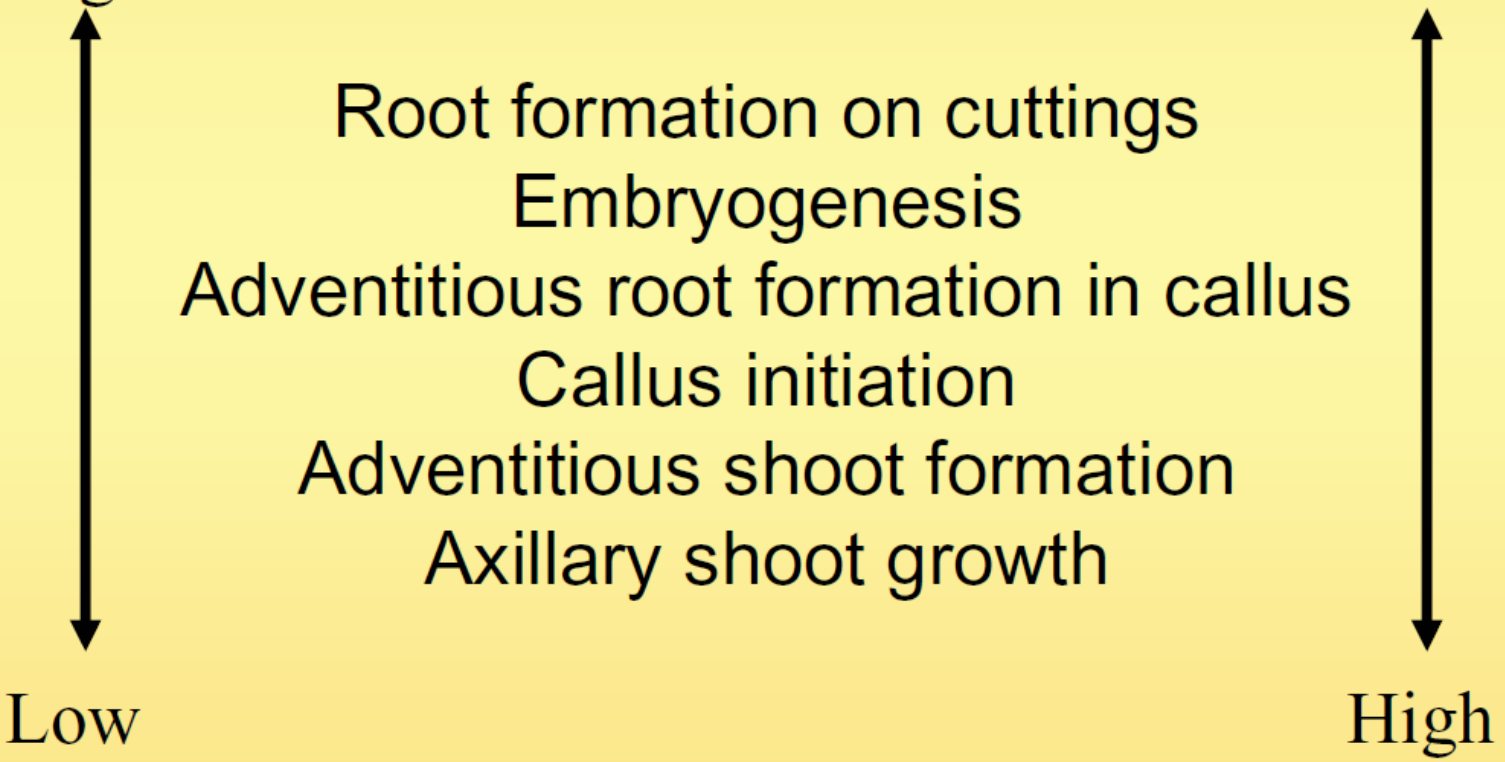
Essential Nutrients

Macronutrients (required content in the plant - 0.1% or % per dry weight) - **C, H, O, P, K, N, S, Ca, Mg**

Micronutrients (requirement - ppm/dry weight) - **Fe, Mn, Zn, Cu, B, Cl, Mo**

Na, Se and Si are essential for some plants

Hormone Balance

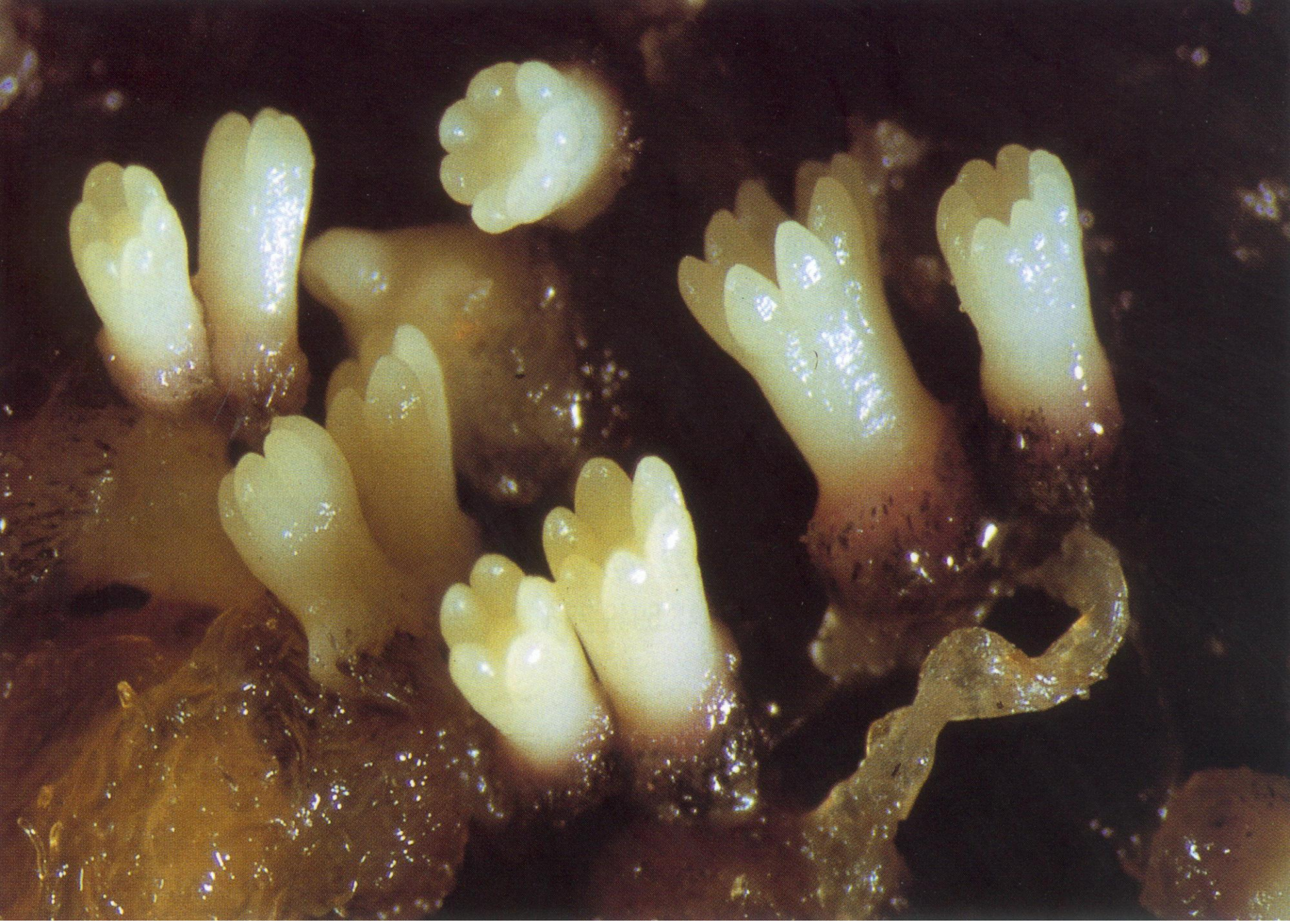


In a larger scale in a
commercial greenhouse

The mother plant vi startet
With gave this result….
What went wrong?

Somatic Embryogenesis

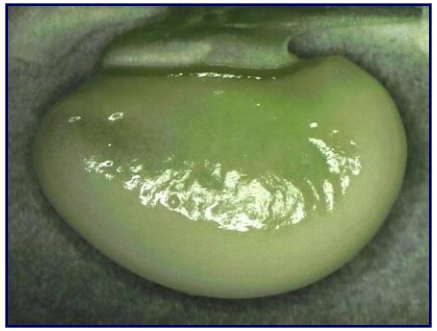
For somatic embryogenesis, embryos form, which can develop and then germinate into a whole plant.



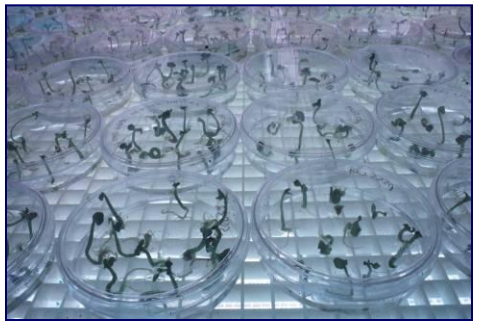
Soybean Embryogenesis

Plant Recovery

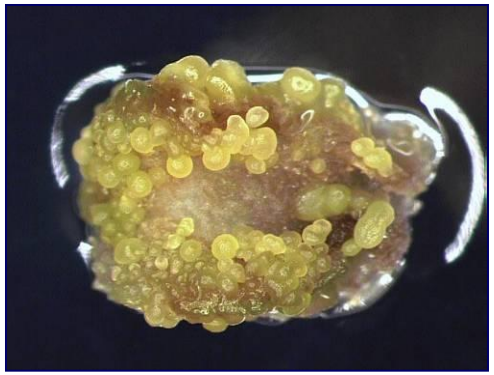
Starting Material
Immature seeds



Germination

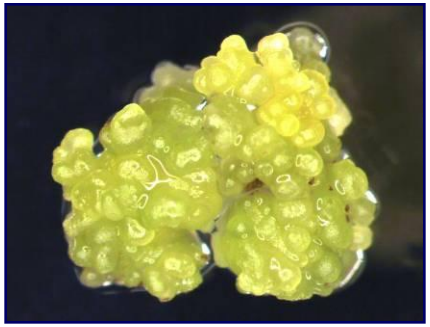


Induction



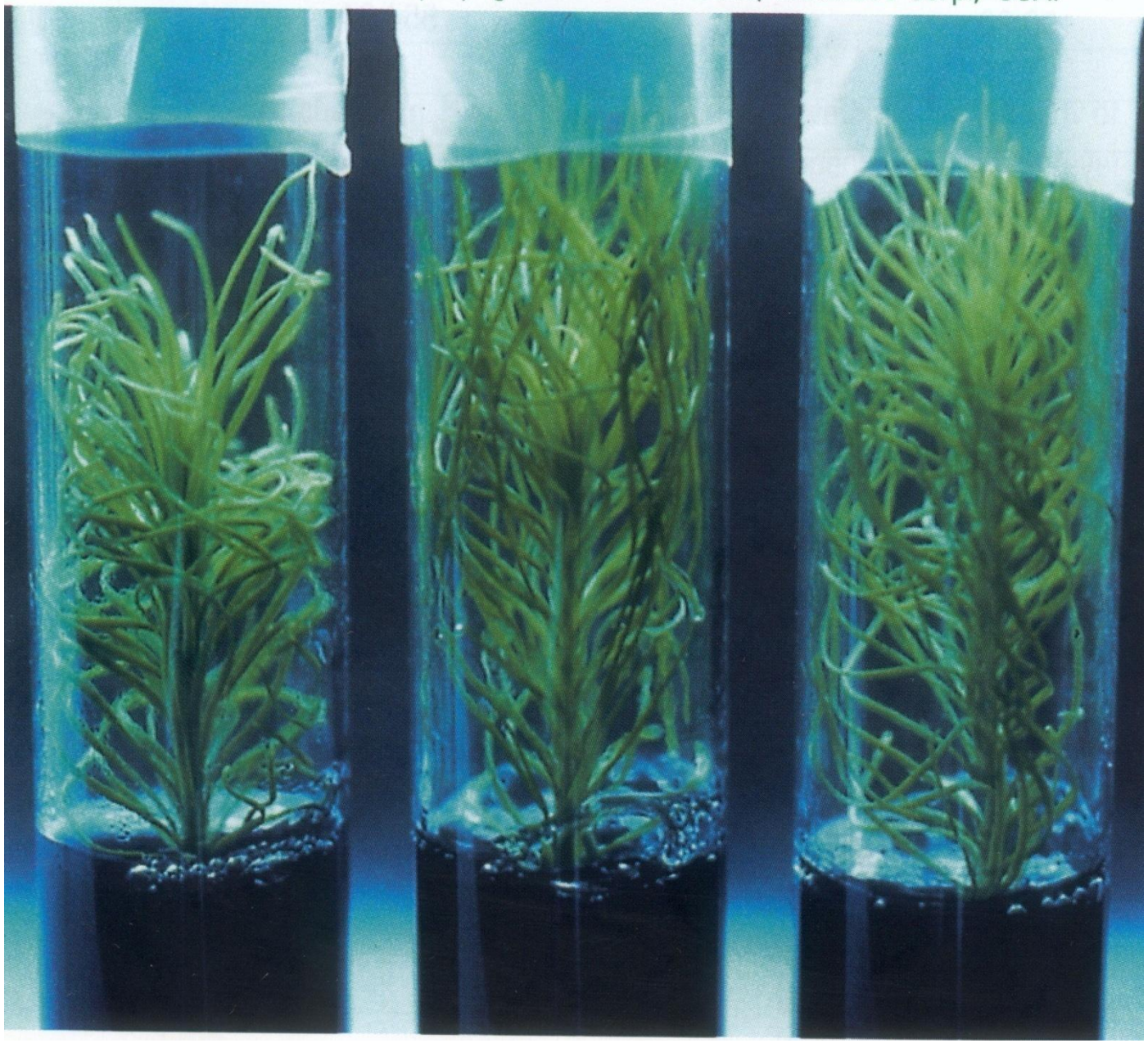
Development

Proliferation

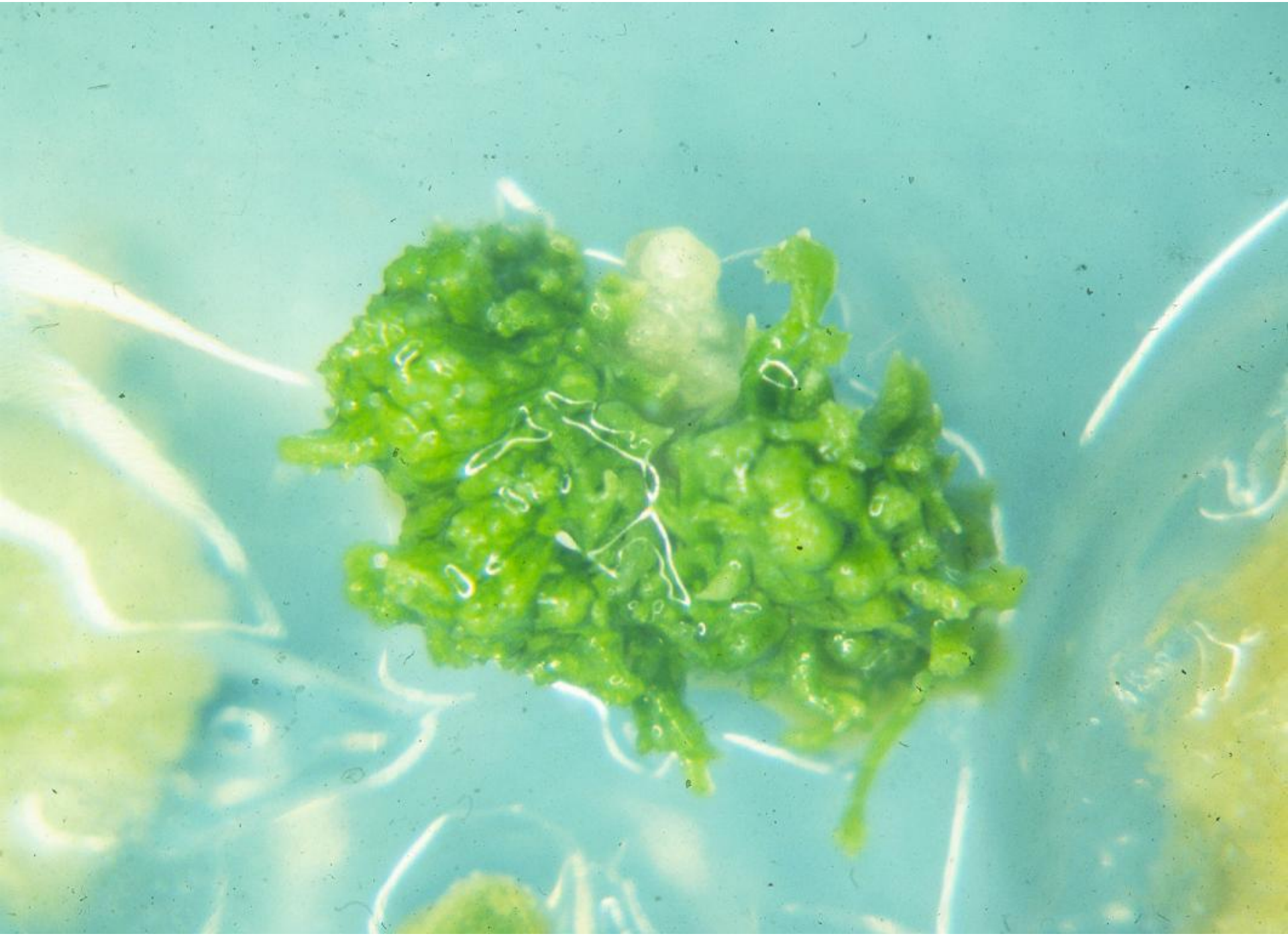


Bioreactor

▼ *Pinus Taeda*; Micropropagated shoots J. Coke, Westvaco Corp., USA.



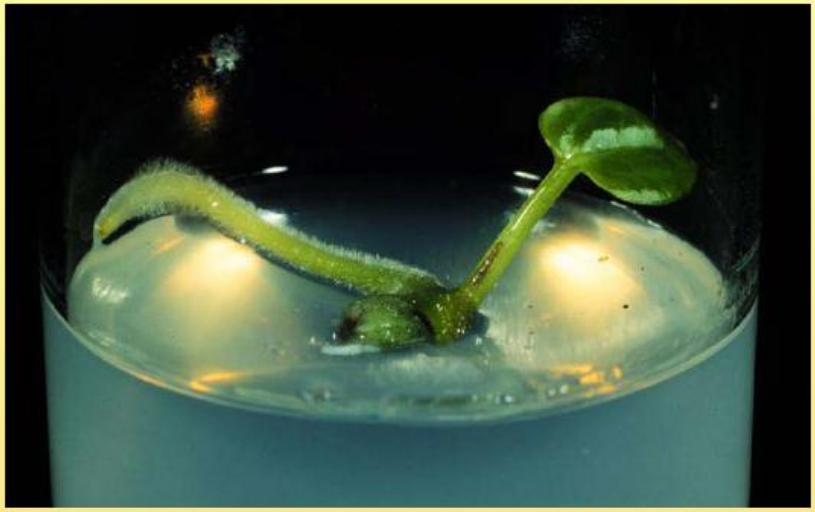

N.K. Konan



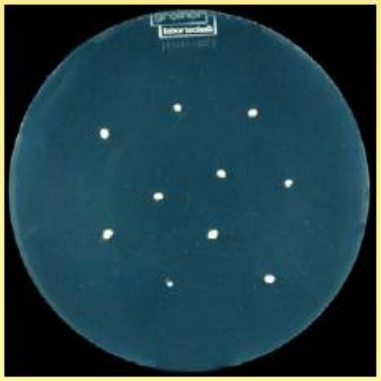
Embryo Culture Uses

Rescue F1 hybrid from a wide cross

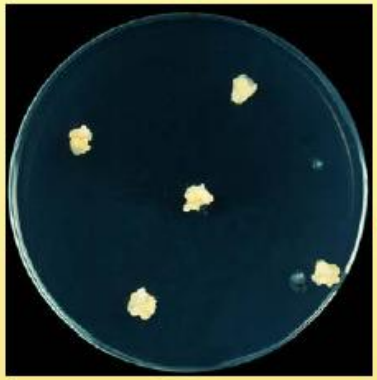
Example: Anthurium



Embryo rescue process



15 days



30 days

50 days

80 days

Potential uses for tissue culture in plant breeding

- Eliminate virus from infected plant selection

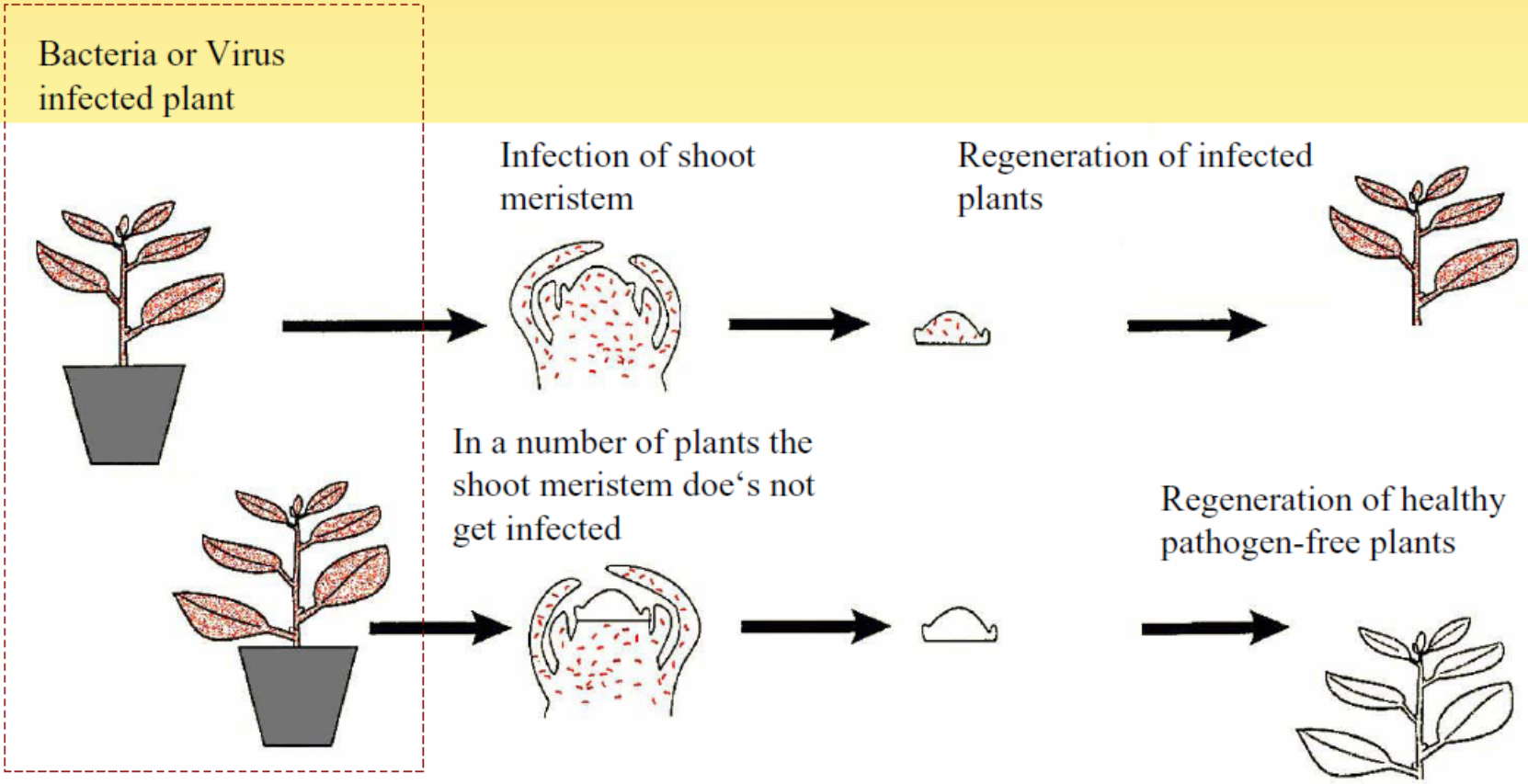
  – Either via meristem culture or sometimes via heat treatment of cultured tissue (or combination)

Eliminate virus from infected plant selection.

often used for potato, strawberry, banana, citrus

Isolation of the shoot meristem

Phytosanitation



Somaclonal Variation

- There are two general types of Somaclonal Variation:
  - Heritable, genetic changes (alter the DNA)
  - Stable, but non-heritable changes (alter gene expression, epigenetic)

  - used in mutation breeding

Somaclonal variability

Kohleria „Orange Glow“, eine durch mutage
Behandlung von Gewebekulturen erhaltene
(Oben) im Vergleich zur Ausgangsform (lin

Somaclonal/Mutation Breeding

- Advantages
  - Screen very high populations (cell based)
  - Can apply selection to single cells
- Disadvantages
  - Many mutations are non-heritable
  - Requires dominant mutation (or double recessive mutation); most mutations are recessive

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

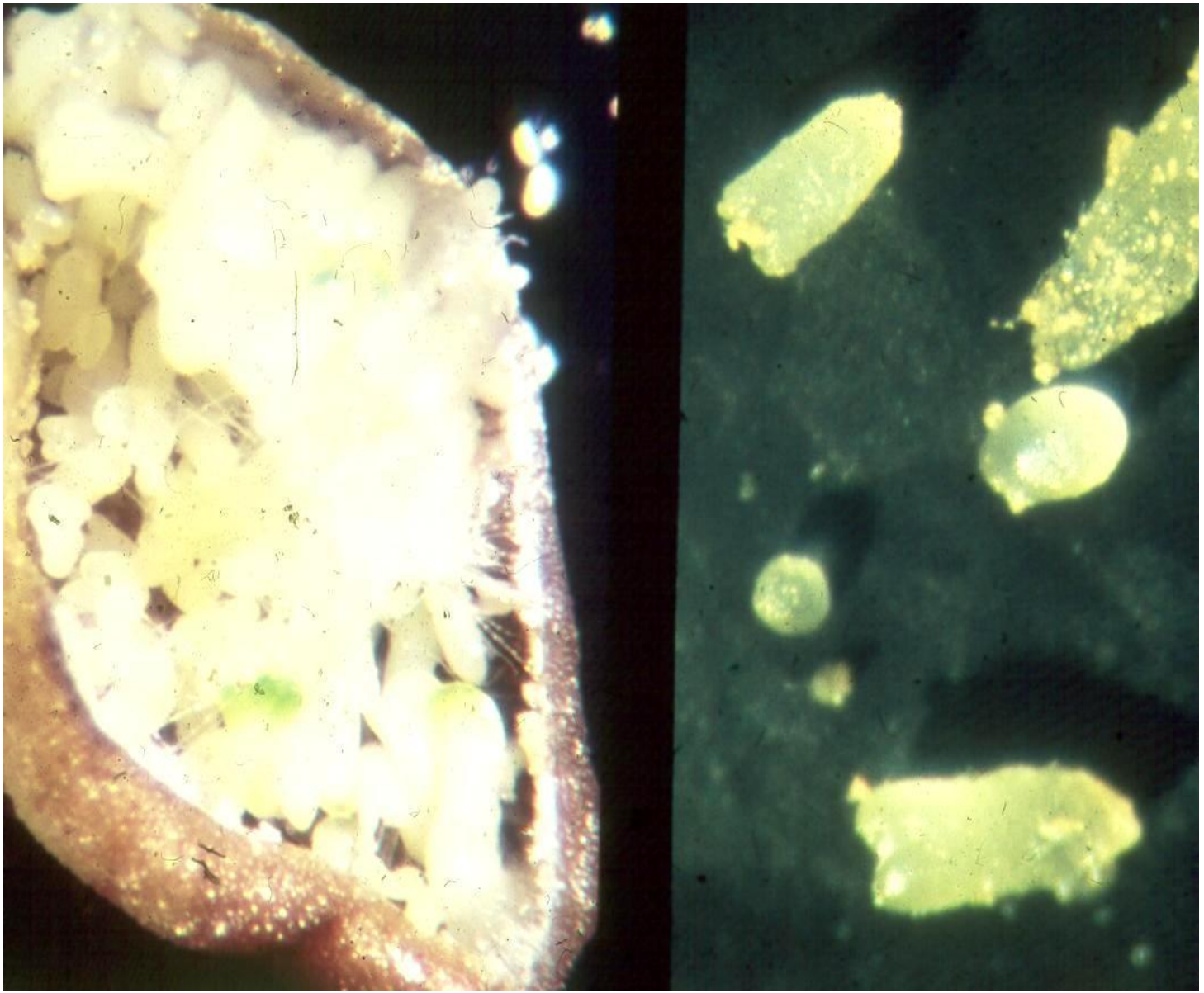
Agricultural applications for haploids - Rapid generation of homozygous genotypes after chromosome doubling

Processes Leading to Production of Haploid Plants

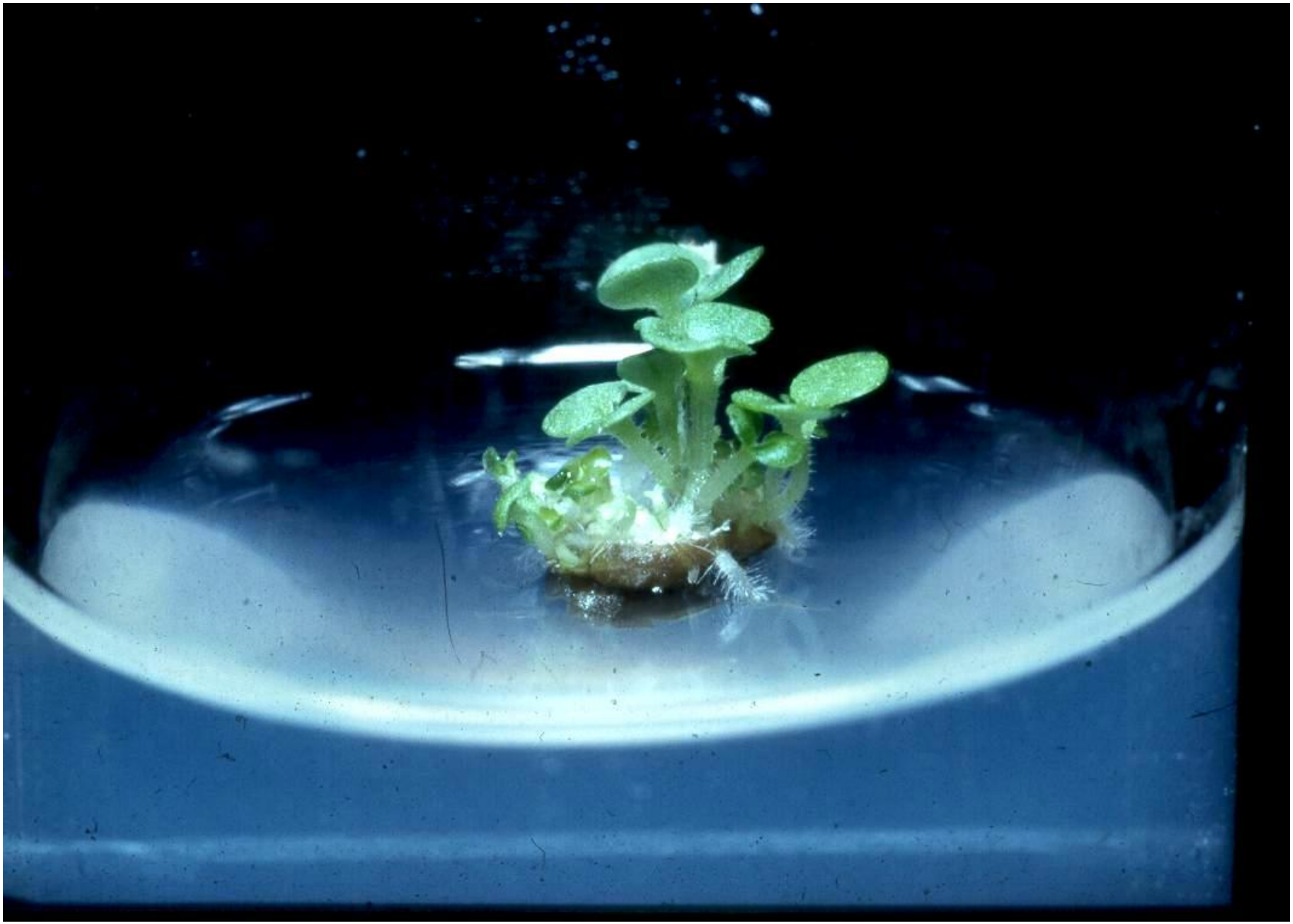
Androgenesis – haploid plant derived from male gamete, most common method *in vitro*

Parthenogenesis - from unfertilized egg

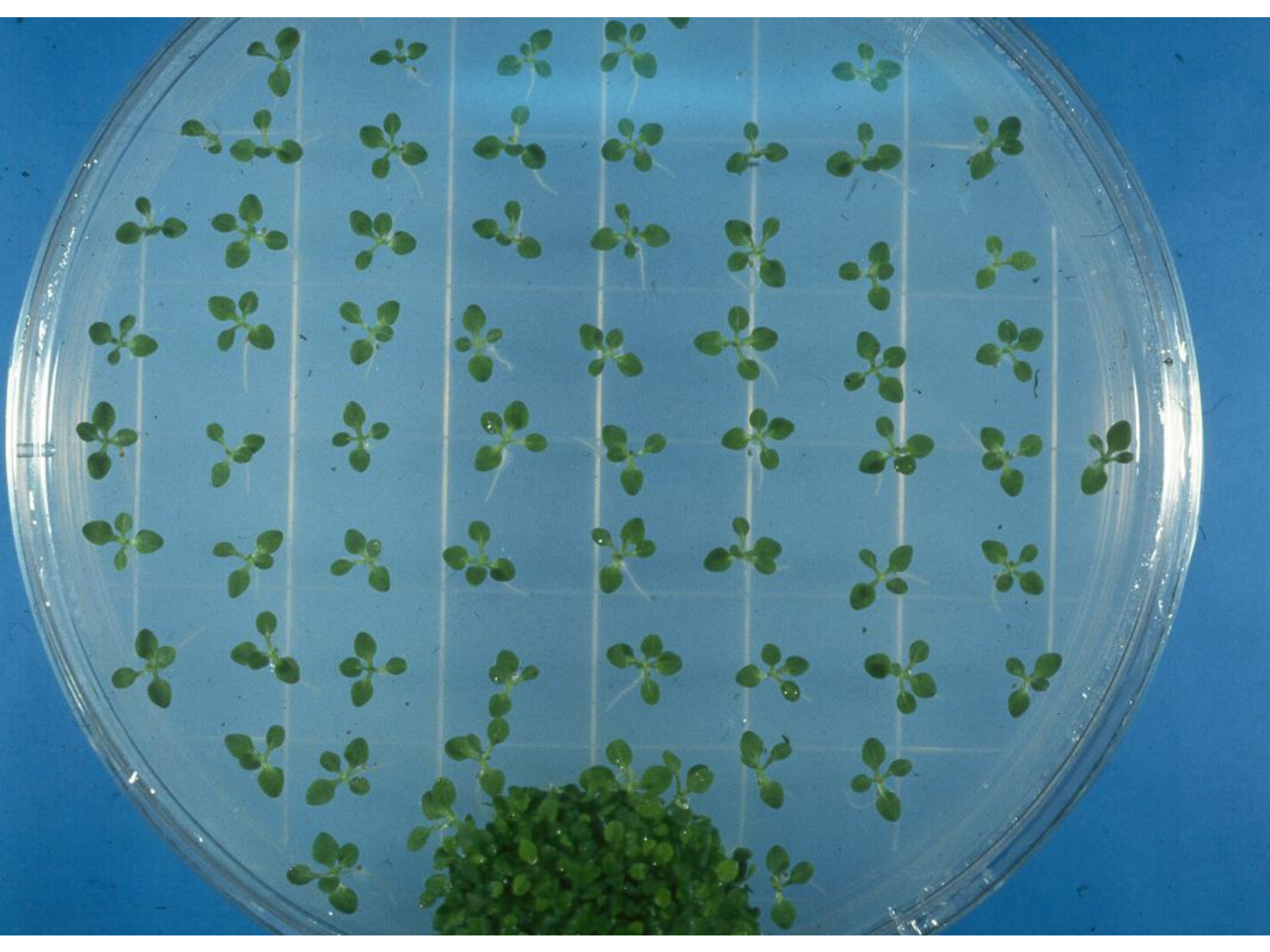
Chromosome elimination - chromosome elimination in somatic cells, most common method used with plant breeding



Sangwan, R.S. and B. Norreel (1975) Induction of plants from Pollen grains. NATURE, 257, 222-224



Sangwan, R.S. and B. Norreel (1975) Induction of plants from Pollen grains. NATURE, 257, 222-224



Microspore culture of rice

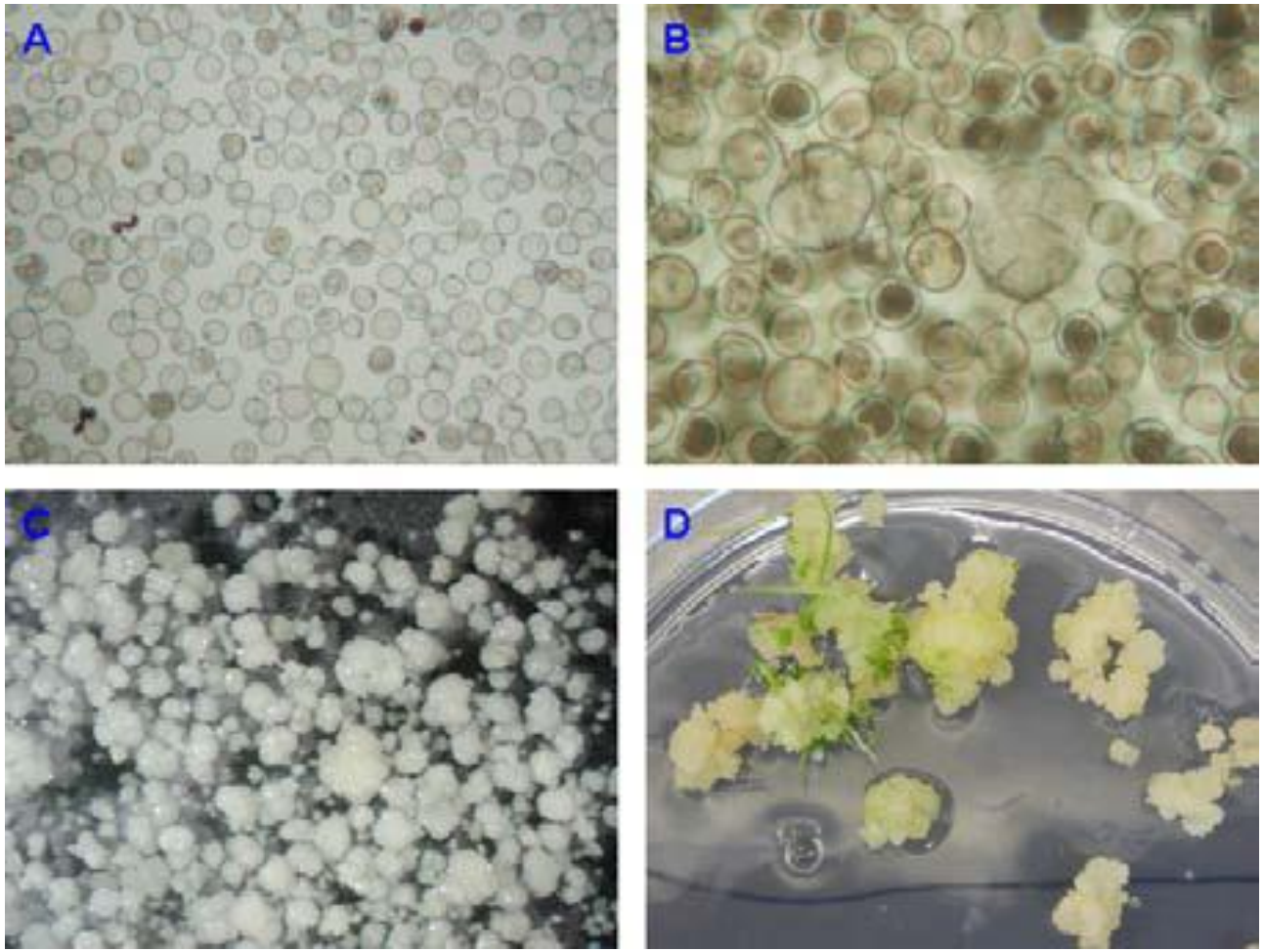
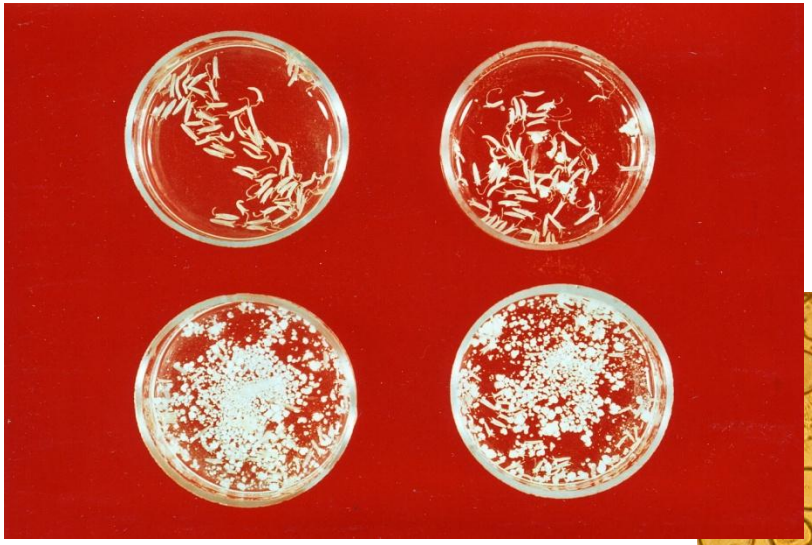
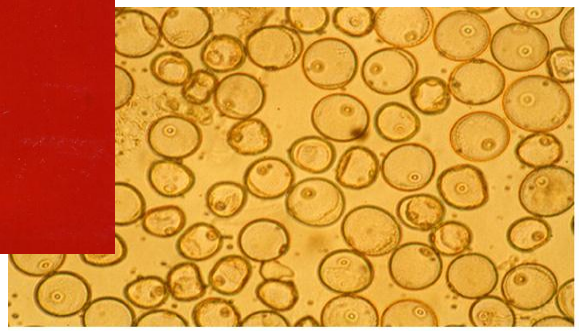


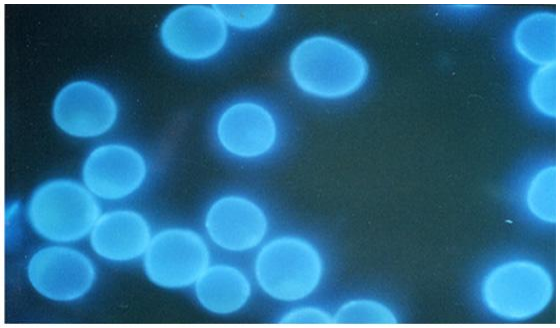
Figure 1. Isolated microspore culture

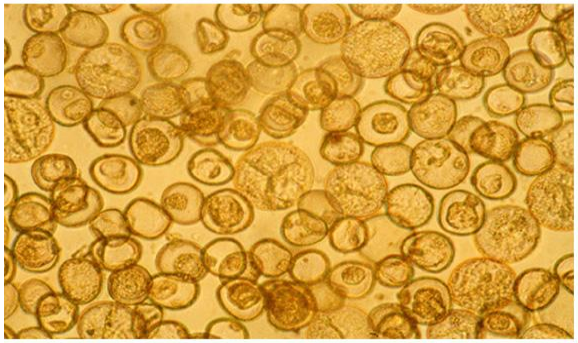
A: Fresh isolated microspores; B: Microspore cell division and multicellular structure; C: Calli; D: Haploid plantlets

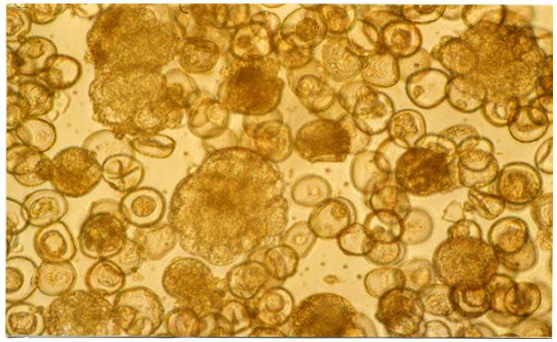


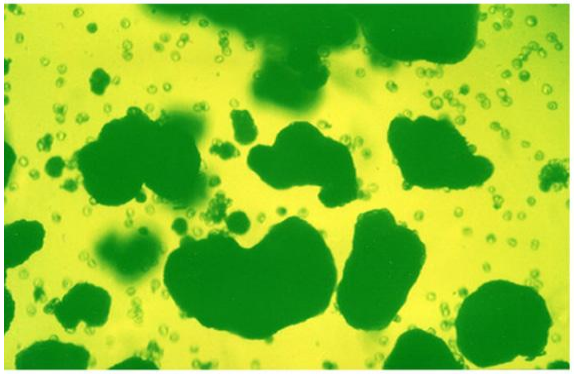
FDA (fluorescein diacetate) stain to determine if the cultured microspores are survived











Shed

microspore

culture of barley

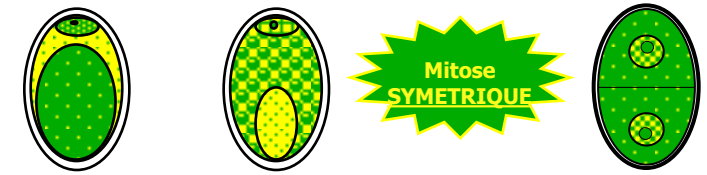
The protocol is similar to

that of wheat

Pollen Embryogenesis in Barley : How

Different stages of Androgenesis

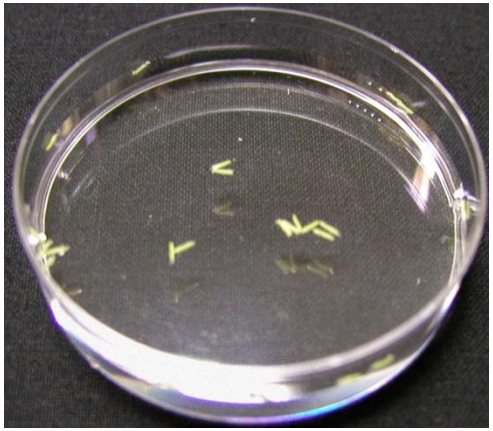
Pretreatment

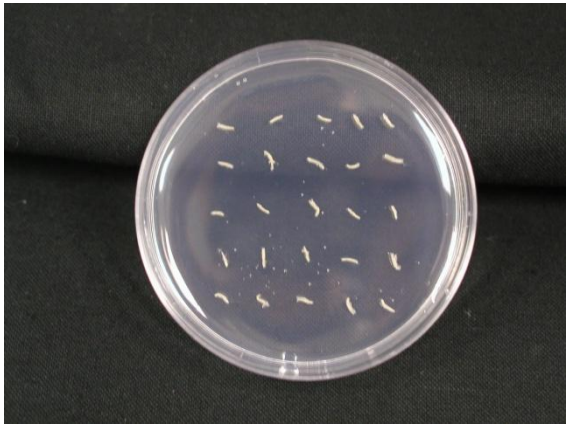


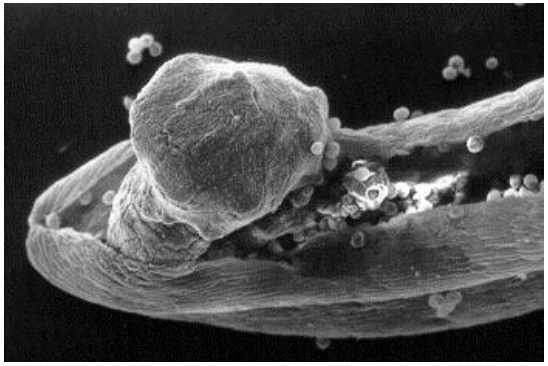
Uninucleate

Binucleate pollen

2equal nuclei



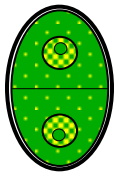




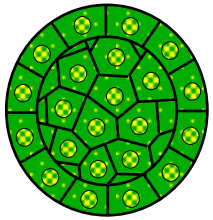
Pollen embryogenesis:

How?

Culture



2equal nuclei



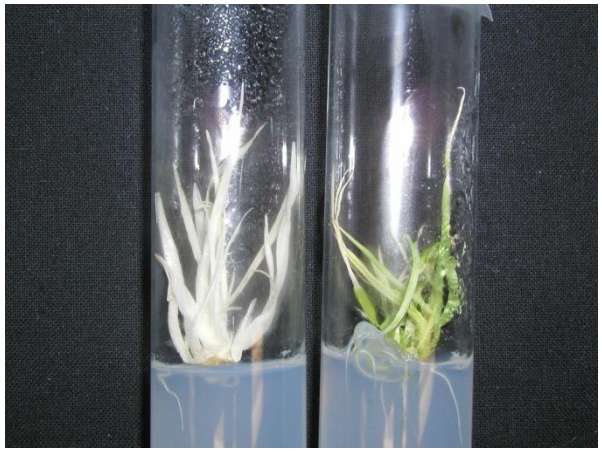
Embryo

Regeneration

Haploid plants

Diploïdisation

Dihaploid plants



Field evaluation

2. **Methodologies/protocols:** Anther culture, Pollen culture

Culture Media: MS, N&N, N6, White, Heller, B5, Lin and Staba, etc etc.

Phytohormones: IAA, GA3, 2,4-D, Kinetin, BAP, Zeatin,

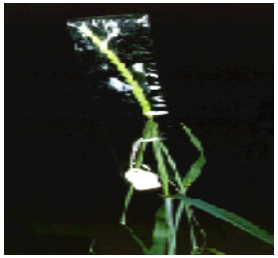
Other substances: coconut milk, plum juice, potato extract etc

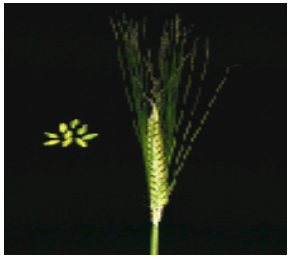
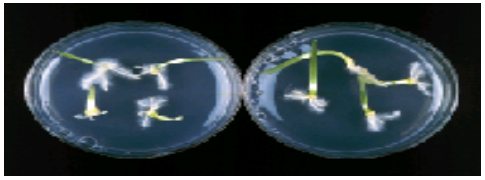
Pre-treatments: Cold shock, high temperature, centrifugation, reduced atmospheric pressure

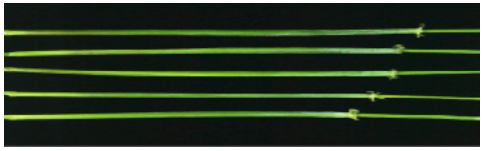
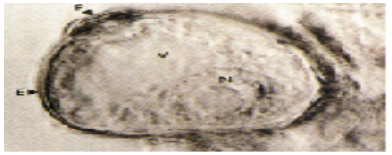
Anther culture versus pollen culture

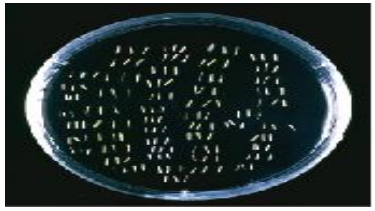
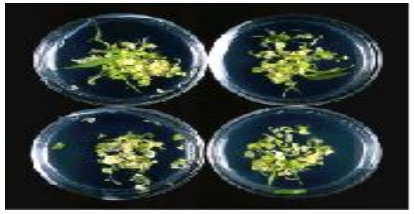
Doubled haploid production in barley via anther culture

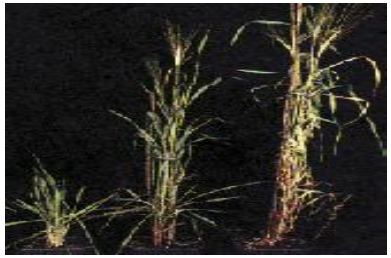


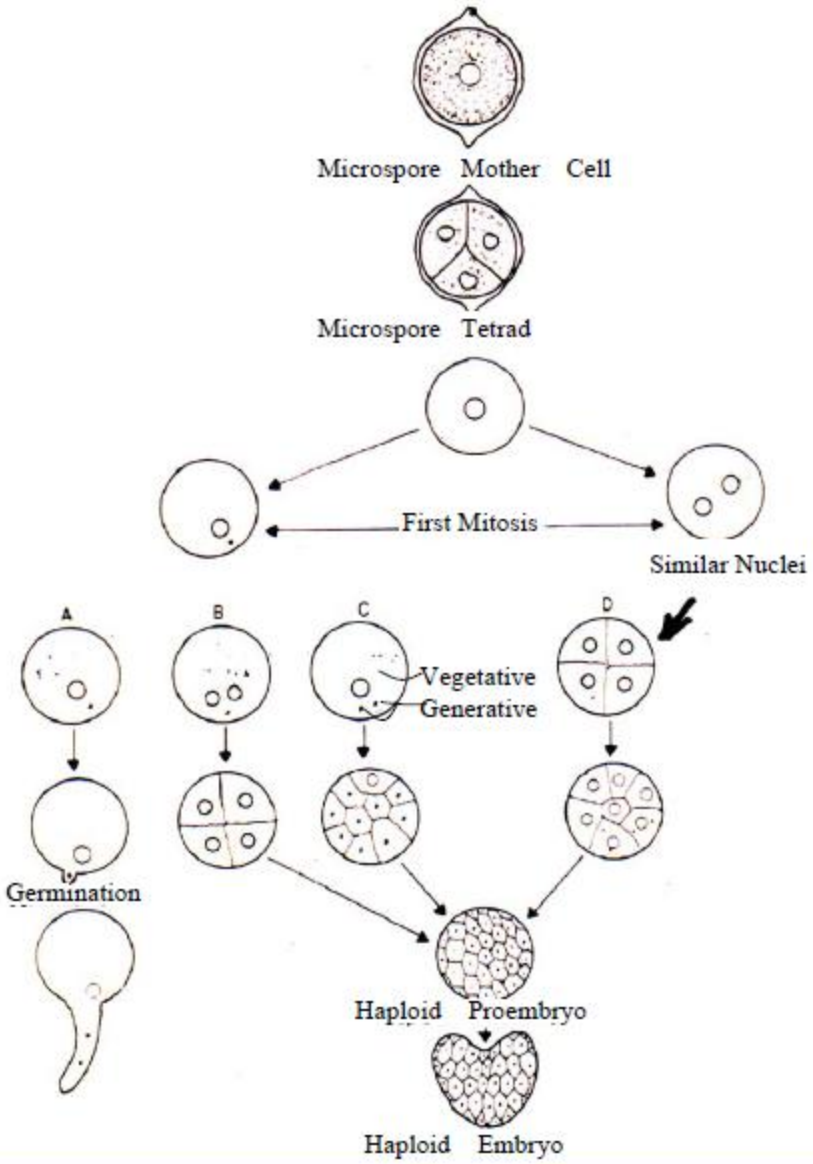




Microspore Mother Cell
Microspore Tetrad
First Mitosis
Similar Nuclei
A
B
C
D
Vegetative
Generative
Germination
Haploid Proembryo
Haploid Embryo

Anther/Microspore Culture Factors

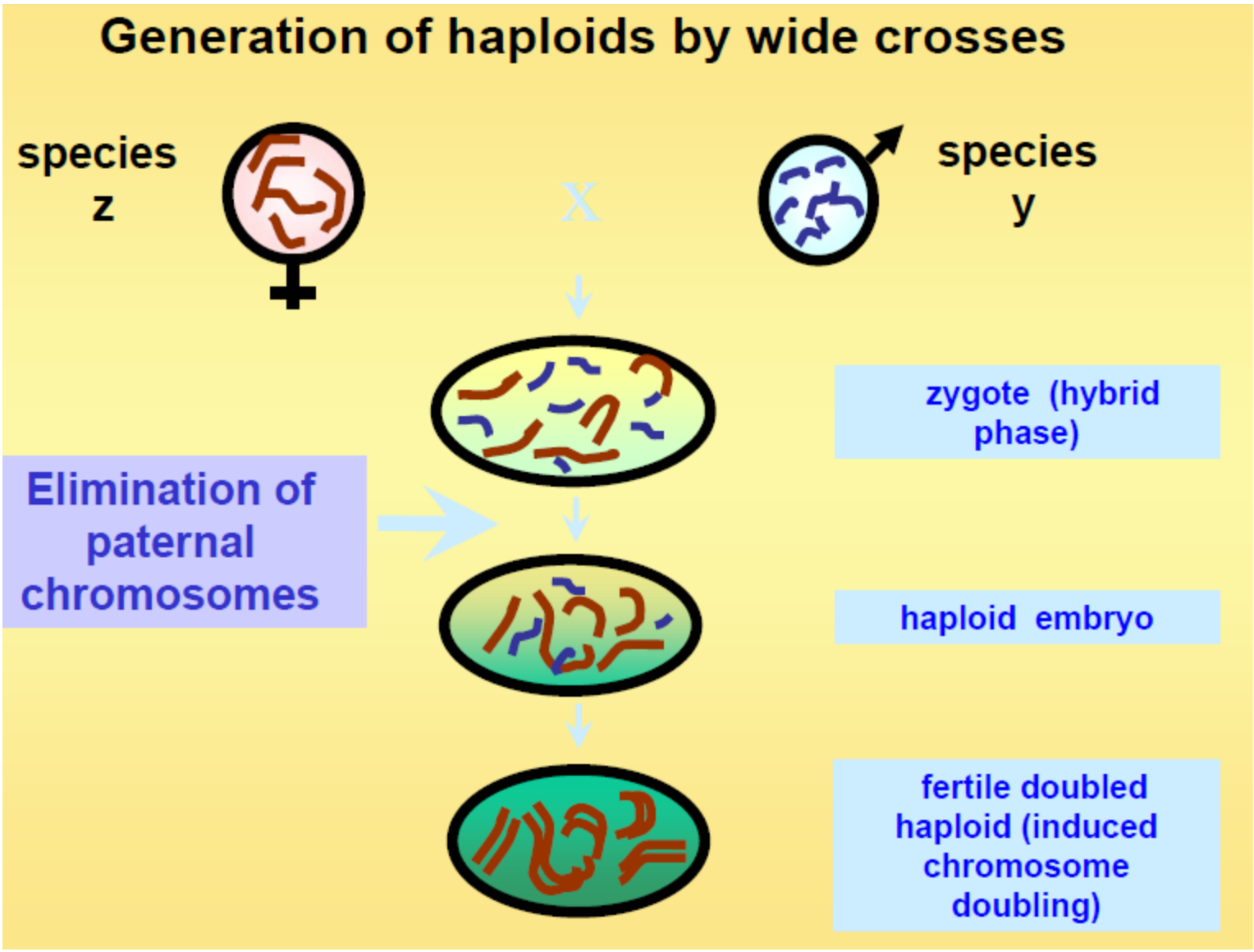
- Genotype
  - As with all tissue culture techniques
- Growth of mother plant
  - Usually requires optimum growing conditions
- Correct stage of pollen development
  - Need to be able to switch pollen development from gametogenesis to embryogenesis
- Pretreatment of anthers
  - Cold or heat have both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

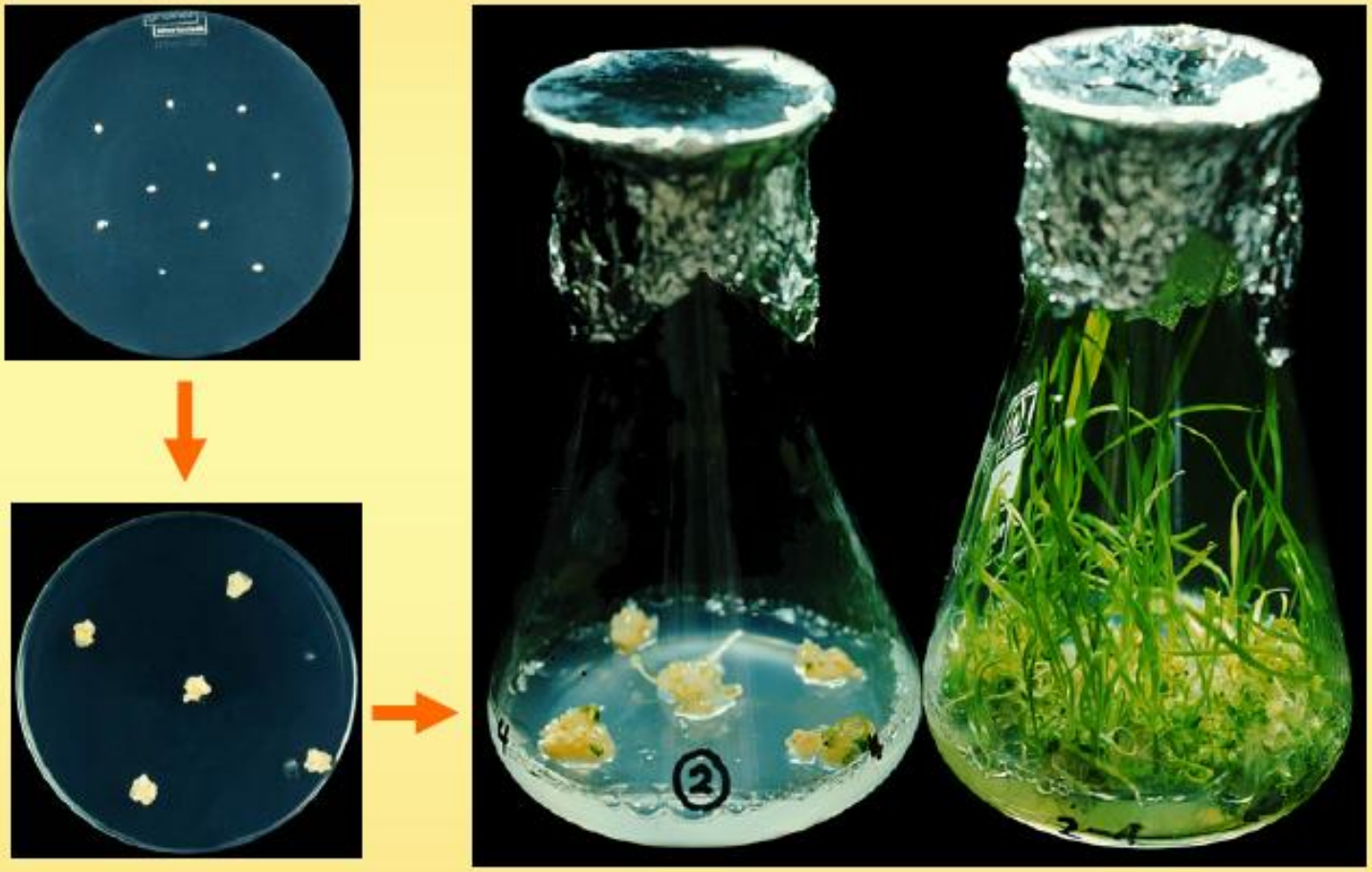
Day 3 (to 5) - 40% of the embryonic cells are haploid, endosperm abortion occurs, GA_3 treatment enhances retention of florets

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



Haploids from eggs

Parthenogenesis

No fertilisation

- **in seeds**
- **ovary culture**

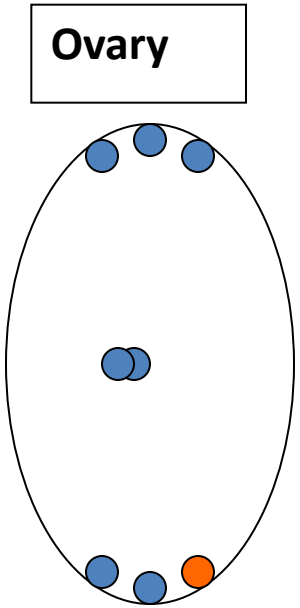
Chromosome elimination (interspecific pollination)

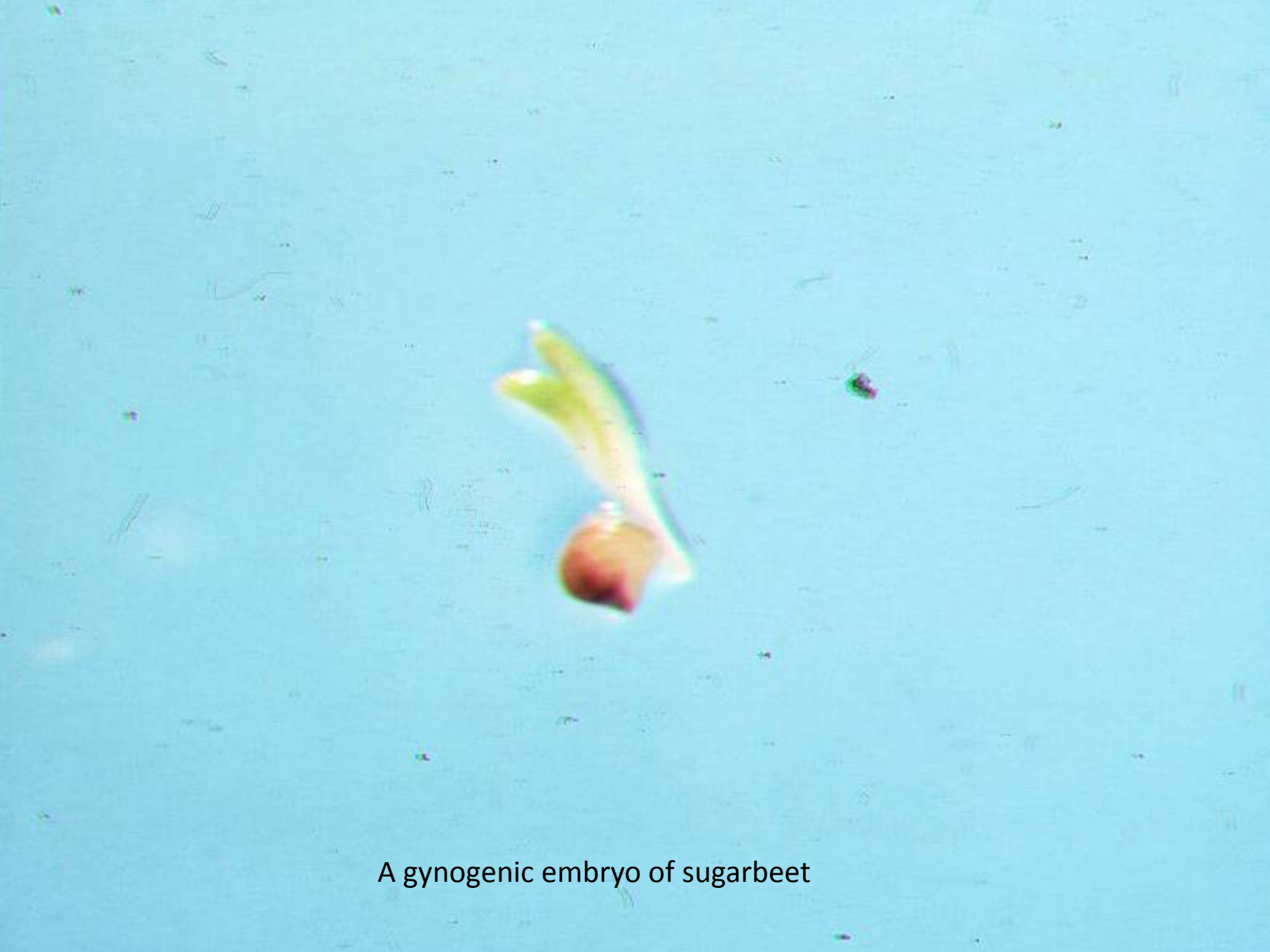
Fertilisation plus chromosome loss

Antipodals 1n

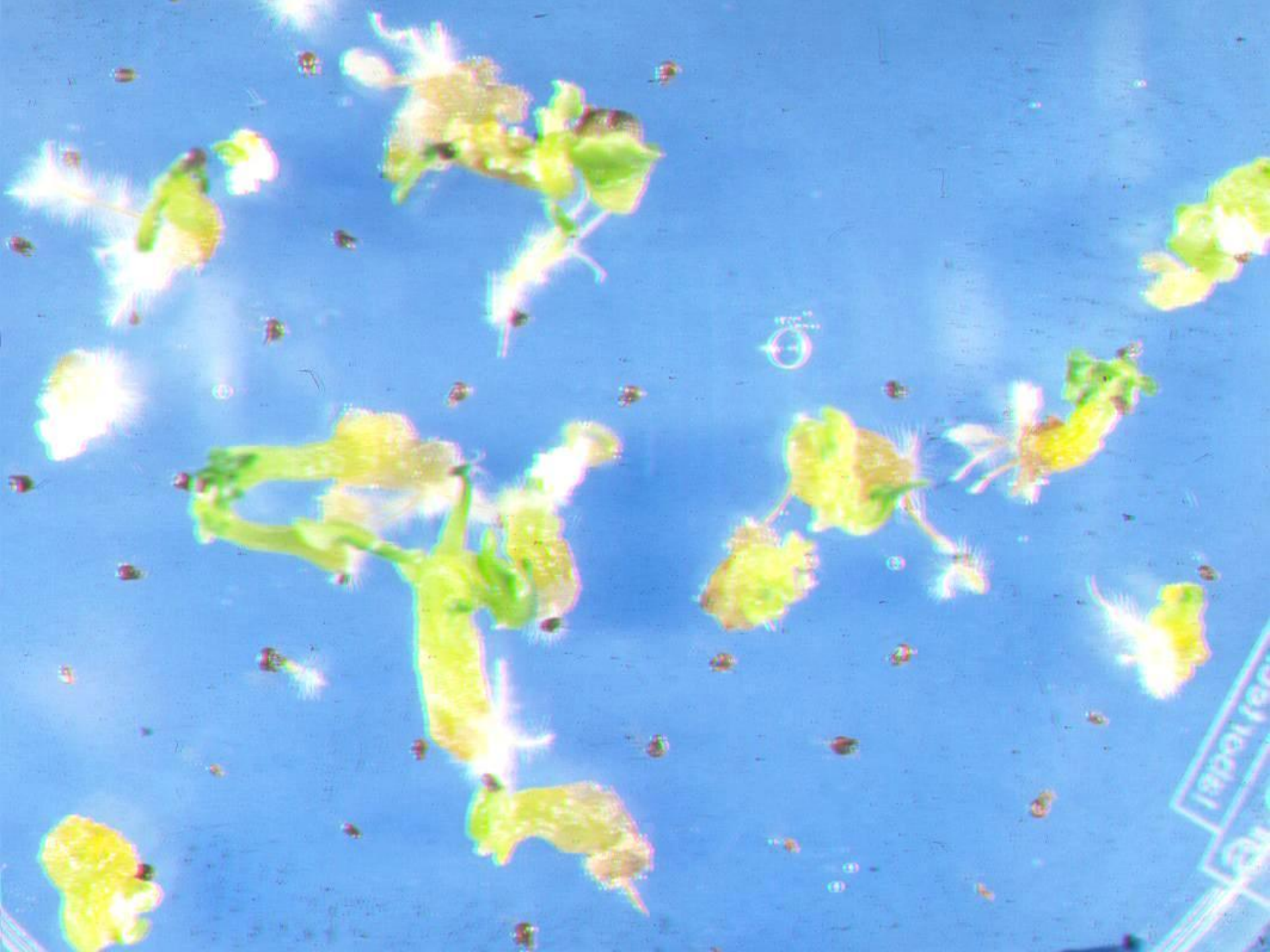
Central nucleus 2n

Synergids and egg 1n





A gynogenic embryo of sugarbeet



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

- In vitro tissue culture

– Somaclonal variation; Callus Growth in Tissue Culture

– Can screen large number of individual cells
- Chromosomal aberrations, point mutations
- Also: Uncover genetic variation in source plant

Asian pear improved by radiation breeding

"Nijus-seiki" suscep-tible to black spot disease

"Gold Nijusseiki" resistant to black spot disease.

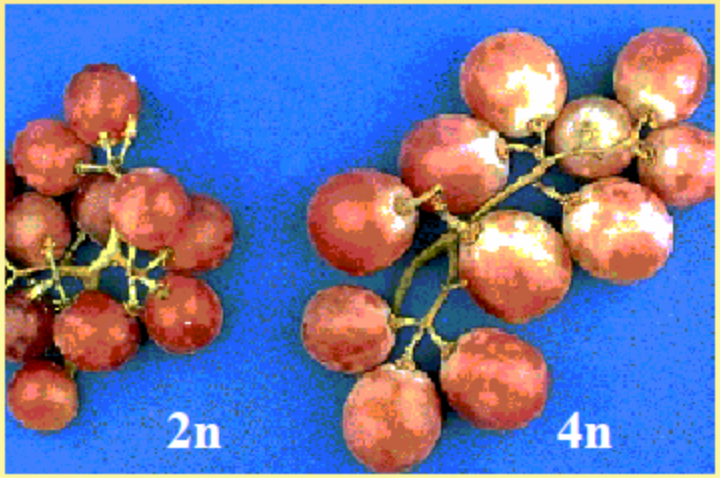
Institute of Radiation Breeding
Ibaraki-ken, JAPAN
http://www.irb.affrc.go.jp/

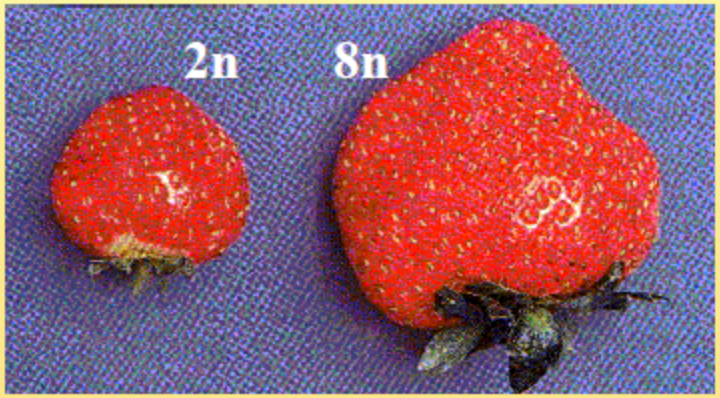
Breeding of Polyploids

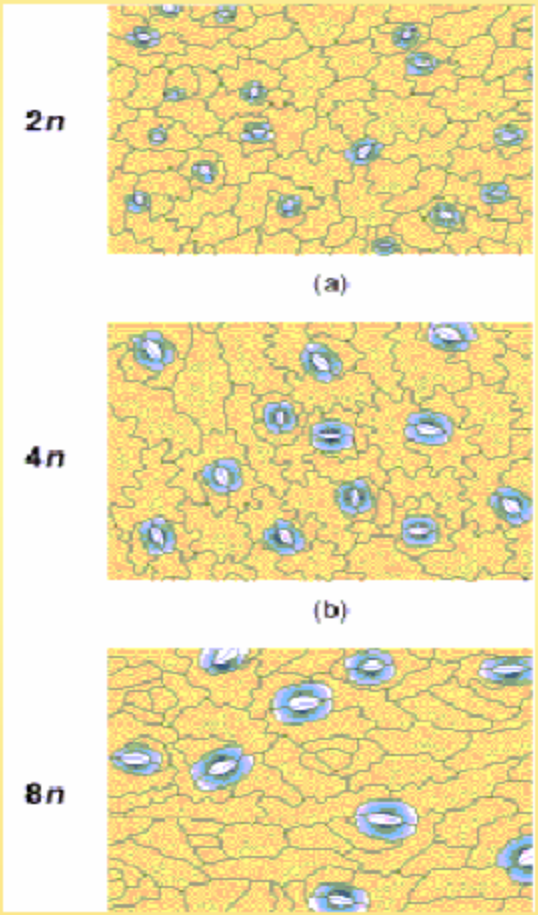
* Produced by:-
  a) doubling of chromosomes in 1 species (autopolyploidy)
  b) doubling after hybridization of 2 species (allopolyploidy)

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

Polyploidy typically increases cell size which results in larger tissues.

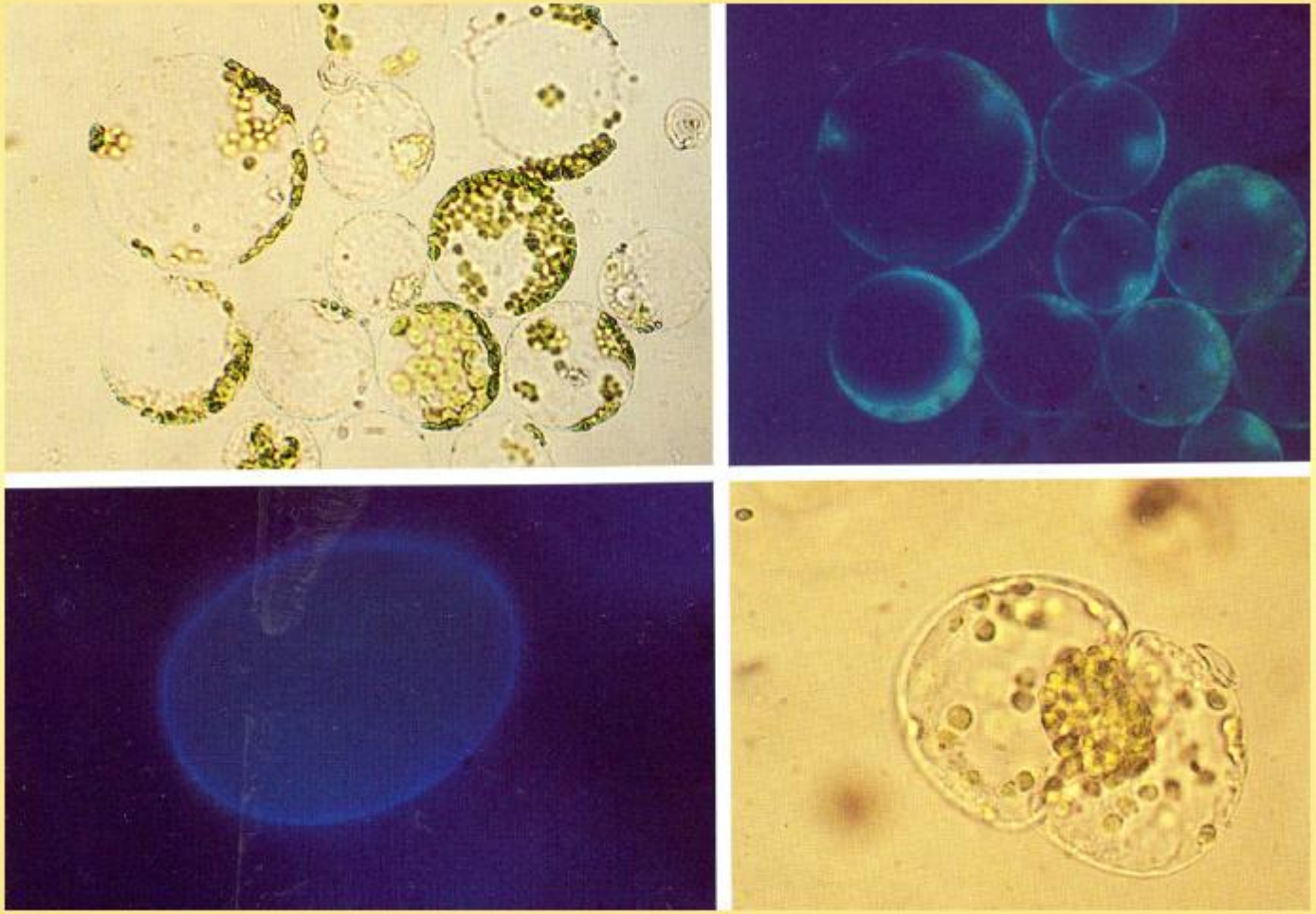






Stomatal cells in tobacco

Single protoplasts



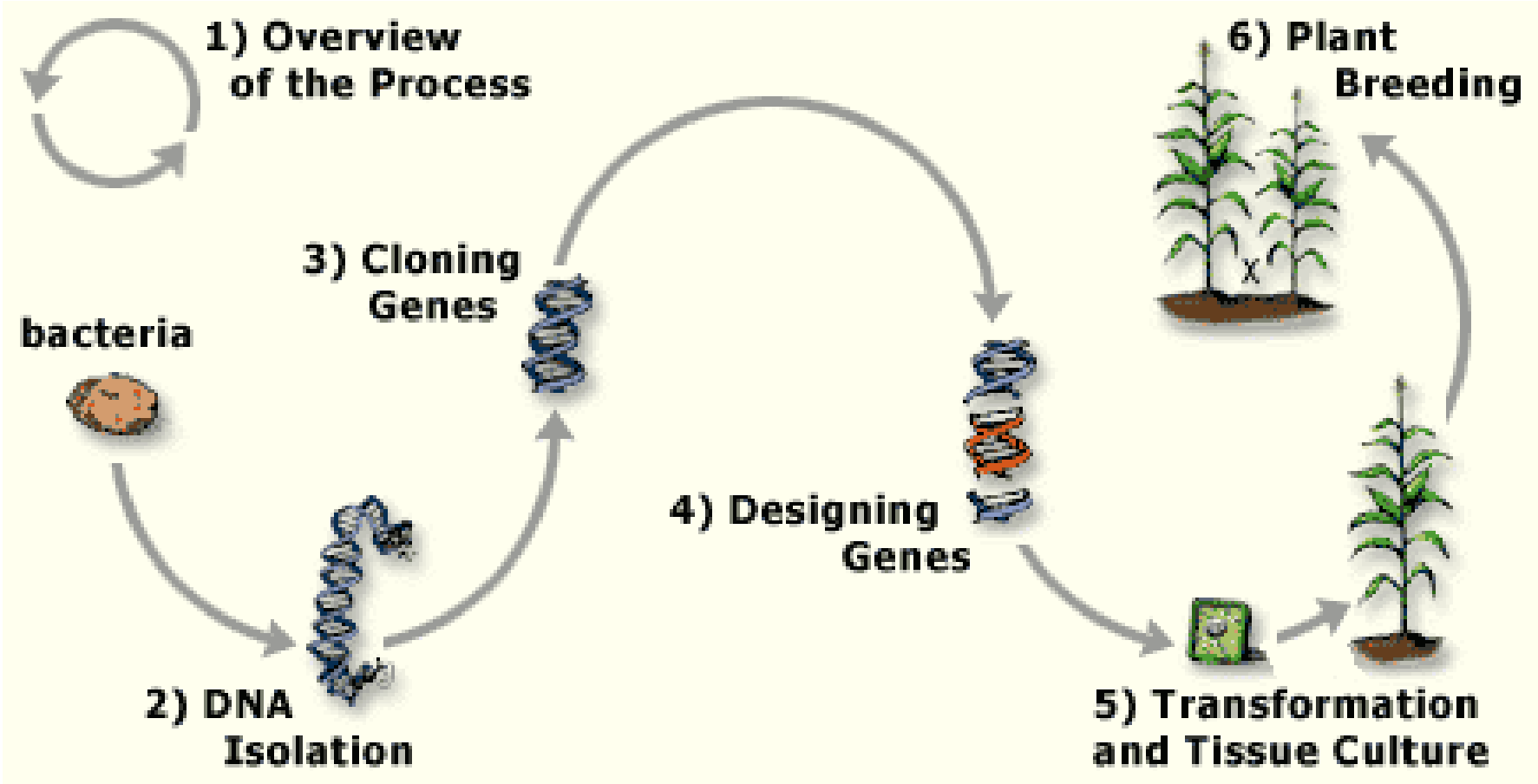
Protoplasts after fusion

Plant Genetic Engineering

For successful production of transgenic plants,

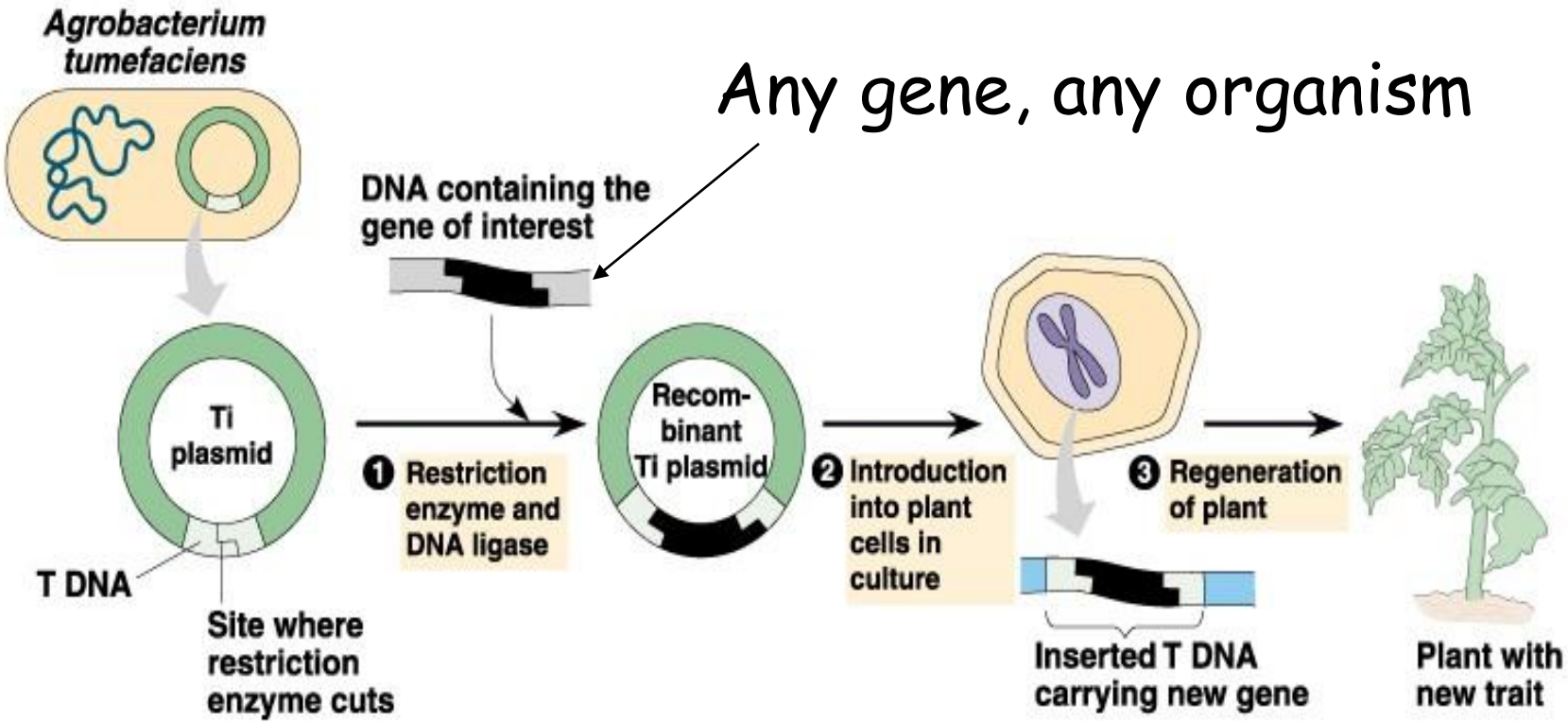
1) DNA delivery
2) Regeneration from single cells

GMO: Process
PTC + MB: GMO



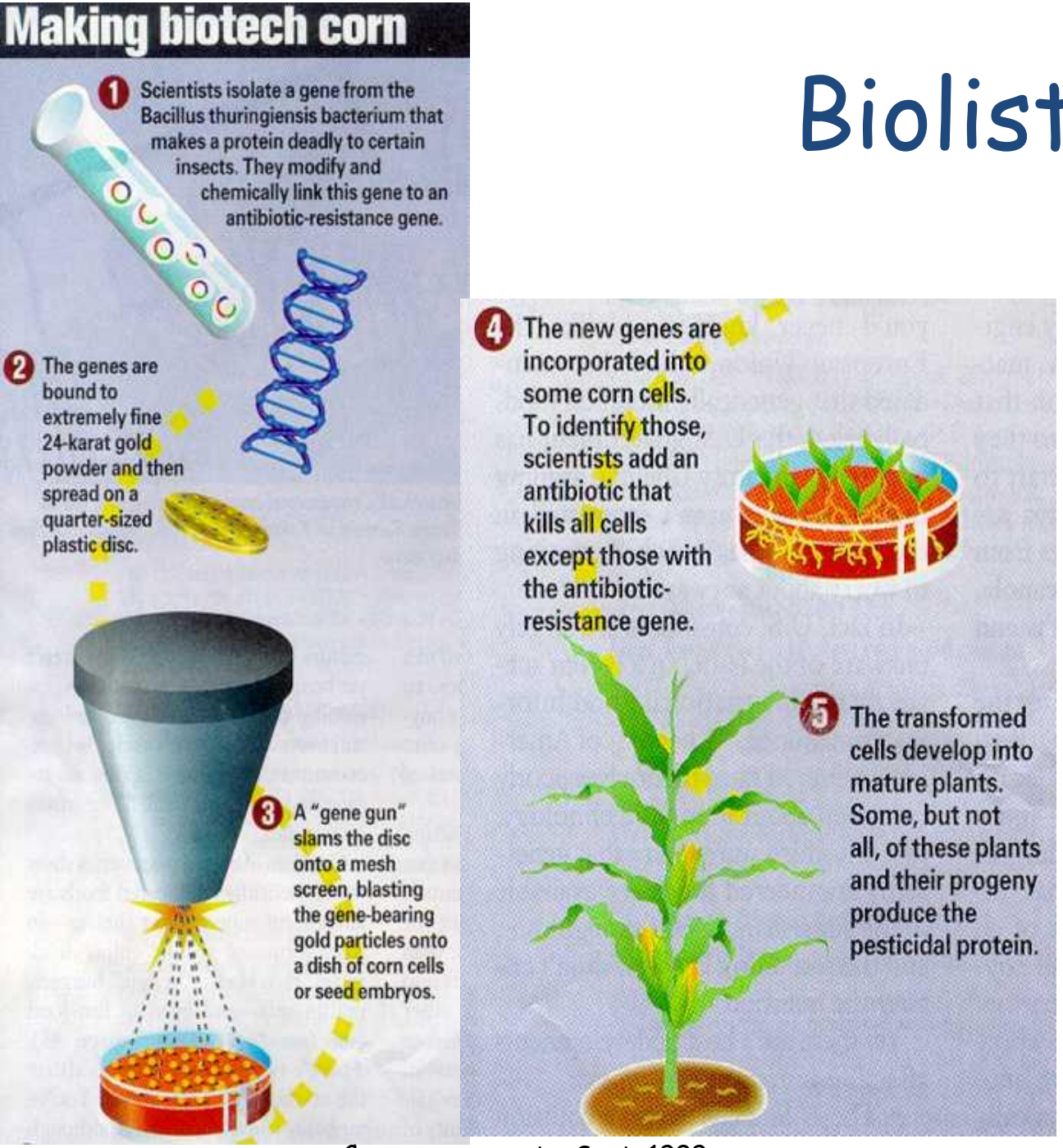
Much of the progress with improvement of crop plants via Biotechnology relies on the ability to introduce DNA into single cells and then, generate whole fertile plants from those single modified cells.

Transgenic plants- *Agrobacterium*



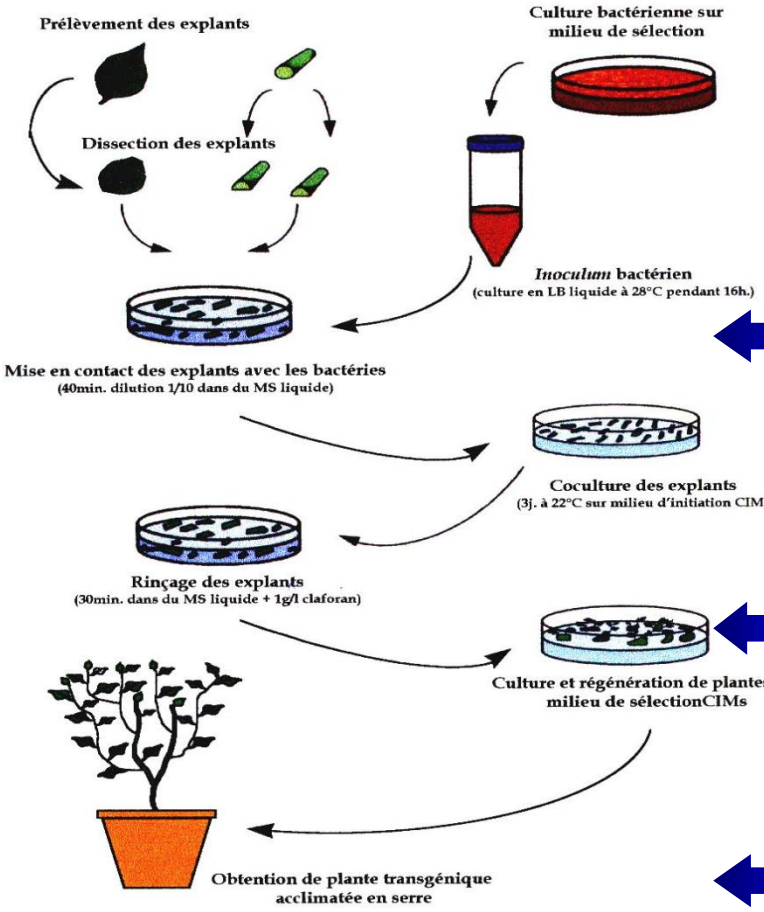
The new plant will pass the transgene to its progeny through seed.

Biolistics



Consumer reports, Sept. 1999

Gene Transfer Technology: Agrobacterium tumefaciens-mediated genetic transformation system in higher plants



Early events

ORGANOGENESIS (bud formation)

Transgenic plants

Timeline

- 1962 Murashige and Skoog invent tissue culture media that is very effective for tobacco and other plants
- **1982 First stably transgenic plant—marker gene in tobacco**
- 1987 Gene gun invented.
- 1994 Flavr Savr tomato commercialized
- 1996 First wide-scale planting of soybean and corn
- 2006 Billionth acre of transgenic crop planted somewhere in the world

Introducing the Gene or

Developing Transgenics

Steps

1. Create transformation cassette

2. Introduce and select for transformants

Transformation Cassettes

Contains

1. **Gene of interest**
    - **The coding region and its controlling elements**

2. **Selectable marker**
    - **Distinguishes transformed/untransformed plants**

3. **Insertion sequences**
    - **Aids *Agrobacterium* insertion**

Gene of Interest

Promoter Region

- *Controls when, where and how much the gene is expressed*

ex.: CaMV35S (constitutive; on always)

Glutelin 1 (only in rice endosperm during seed development)

Transit Peptide

- *Targets protein to correct organelle*

ex.: RbCS (RUBISCO small subunit; choloroplast target

Coding Region

- *Encodes protein product*

ex.: EPSP

β-carotene genes

Selectable Marker

Promoter Region

- *Normally constitutive*

ex.: CaMV35s (Cauliflower Mosaic Virus 35S RNA promoter

Coding Region

- *Gene that breaks down a toxic compound; non-transgenic plants die*

ex.: *nptII* [kanamycin (bacterial antibiotic) resistance]
***aphIV* [hygromycin (bacterial antibiotic) resistance]**
***Bar* [glufosinate (herbicide) resistance]**

Effect of Selectable Marker

Non-transgenic = *Lacks Kan or Bar Gene*

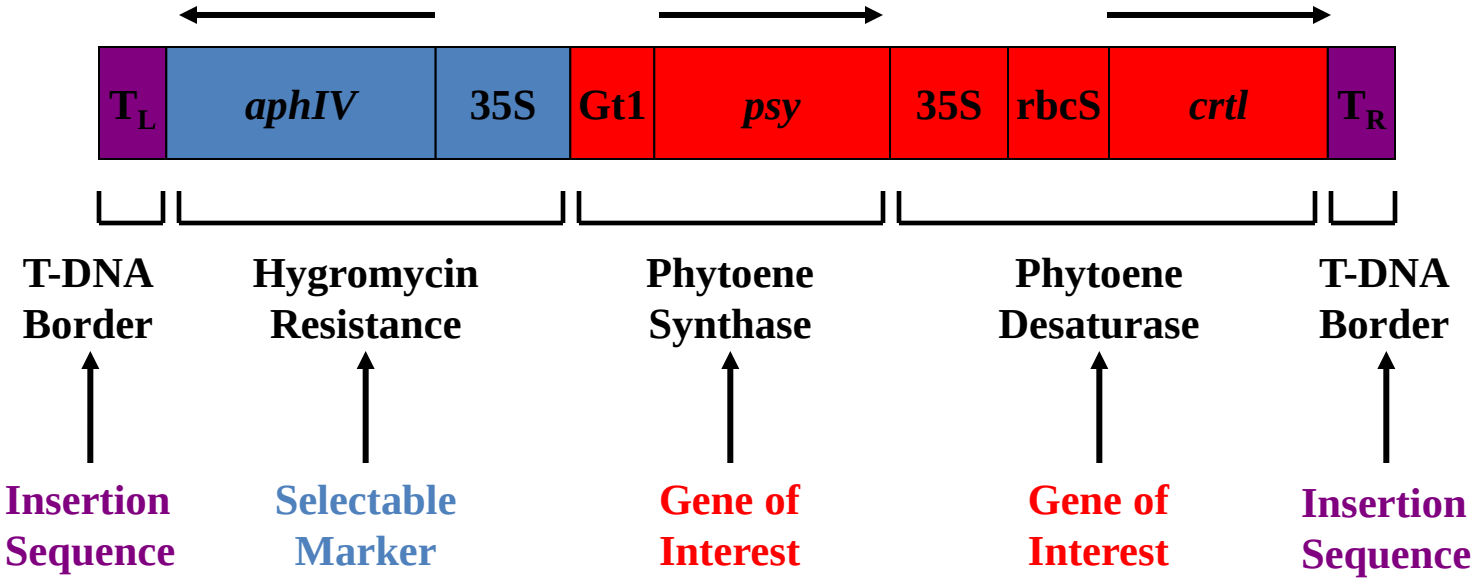
Plant dies in presence of selective compound

Transgenic = *Has Kan or Bar Gene*

Plant grows in presence of selective compound

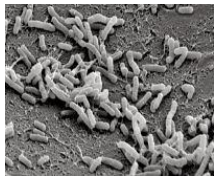
Let's Build A Complex Cassette

pB19hpc (Golden Rice Cassette)



Delivering the Gene to the Plant

- Transformation cassettes are developed in the lab
- They are then introduced into a plant
- Two major delivery methods
  - *Agrobacterium*

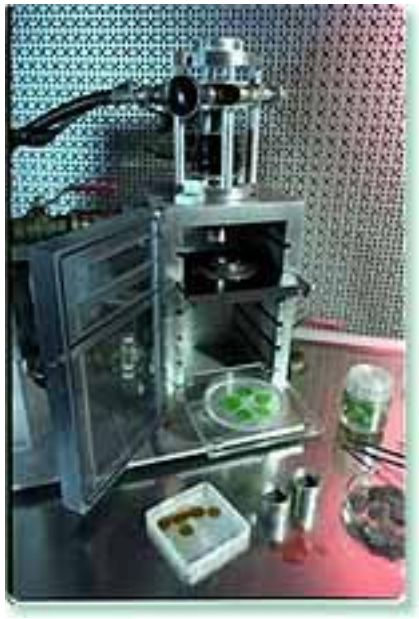


  - Gene Gun

Tissue culture required to generate transgenic plants

The Gene Gun

- **DNA vector is coated onto gold or tungsten particles**
- **Particles are accelerated at high speeds by the gun**
- **Particles enter plant tissue**
- **DNA enters the nucleus and incorporates into chromosome**
- **Integration process unknown**



Transformation Steps

Prepare tissue for transformation

- **Tissue must be capable of developing into normal plants**
- **Leaf, germinating seed, immature embryos**

Introduce DNA

- ***Agrobacterium* or gene gun**

Culture plant tissue

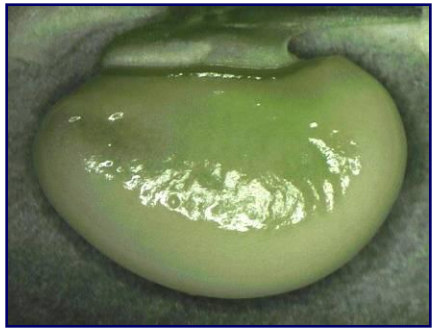
- **Develop shoots**
- **Root the shoots**

Field test the plants

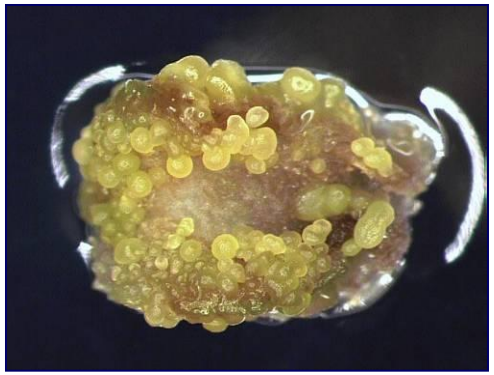
- **Multiple sites, multiple years**

Soybean Embryogenesis

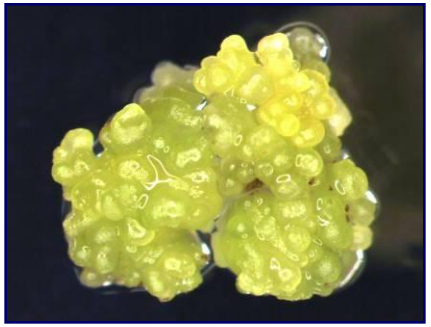
Starting Material
Immature seeds



Induction

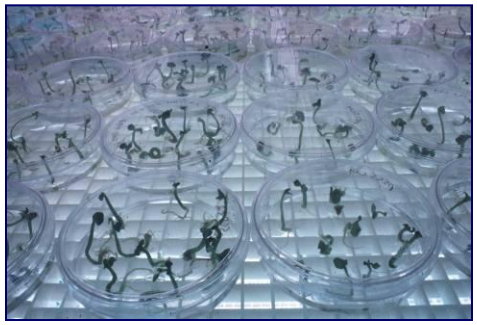


Proliferation



Development

Germination



Plant Recovery

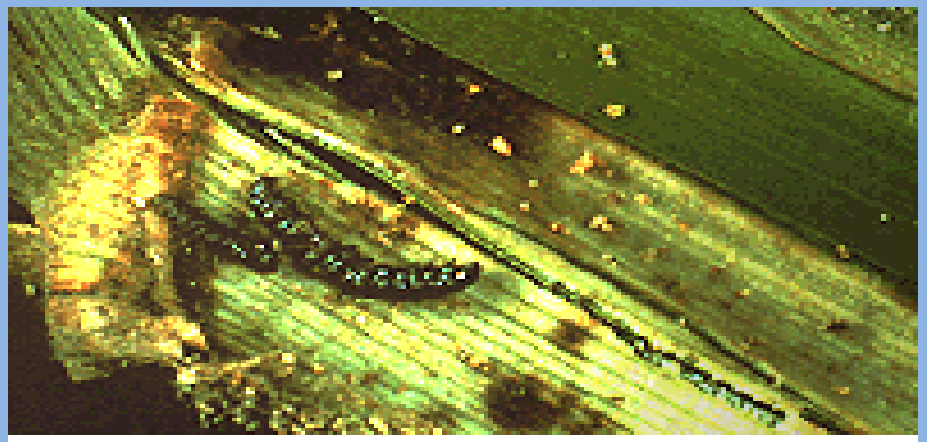
Important Globally Approved Genetically Modified Plants

Product	Genetically Altered Traits
Tomato	Delayed ripening: Gene sequence for polygalaturonase production in tomato rearranged and reversed to minimise its expression by Antisense technology.
Cotton	Bt gene incorporated plants (ballworm & budworm resistant): CRY 1A c gene from Bt Kurstaki.
Soybean	Reisitant to glyphosate for control of weeds: Enolpyruvylashikimate-3-phosphate synthase gene from Agrobacterium sp.CP4
Potato	Bt gene incorporated (Colorado potato beetle resistant) : Cry III (A) gene from Bt. Tenebrionis.
Maize/Corn	Bt gene incorporated (resistant to comborer) : Cry 1A b gene from Bt. Kurstaki
Rapeseed / Canola	Altered oil composition (high lauric acid content): 12:0 acyl carrier protein thioesterase gene from Umbellularia californica. Resistant to glufosinate for Male sterility properties
Squash	Resistant to viruses: Coat protein genes of watermelon mosaic virus 2 and Zucchini yellow mosaic virus.
Papaya	Resistant to Papaya ring spot virus: Coat protein gene of p type of PRSV HA-5-1 from Hawai.
Chicory	Male starility resistant to glufosinate and fertility restores genes from bacteria.

Herbicide tolerant soybean

Insect Resistance

- *Bacillus thuringiensis* ('*Bt*')
  - Insecticidal bacterium marketed worldwide for control of many important plant pests
  - *Bt* products represent about 1% of the total 'agrochemical' market (fungicides, herbicides and insecticides) across the world



European corn borer larvae infected with *Bacillus thuringiensis*.
Courtesy Nova Nordisk Entotech, Inc.

Insect Resistance GM Crops

- **Bt corn**
  - 72% reduction in insecticide use
  - 10% yield increase, net benefit $44/ha
- **Bt cotton**
  - 5.3 million less insecticide applicat. in 1998

Bt corn

Bt cotton

Golden Rice Facts

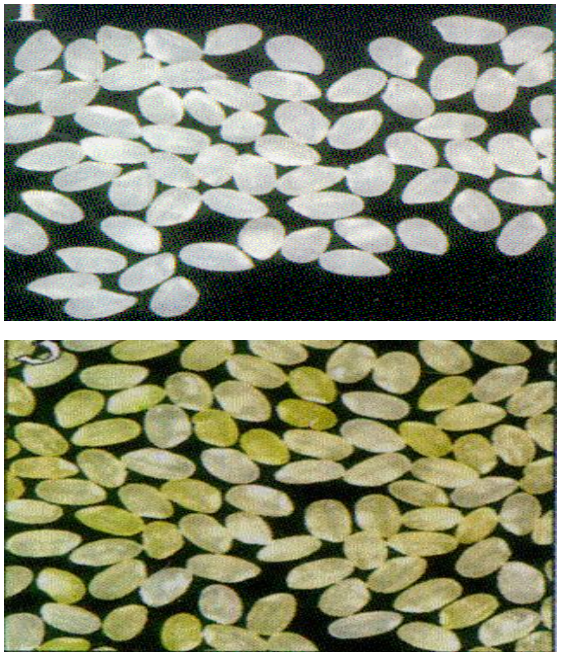
- **"Golden Rice"** grains produce pro-vitamin A (β-carotene) → mammals make vitamin A from β-carotene

- **"Golden Rice"** → it glows with the golden color of β -carotene, carrot's yellow-orange compound - world's most common source of vitamin A

Golden Rice

Transgenic technology produced a type of rice that accumulates beta-carotene in rice grains. Once inside the body, beta-carotene is converted to vitamin A.

More than 120 million children in the world suffers from vitamin A deficiency. Golden Rice has the potential to help prevent the 1 to 2 million deaths each year caused by a deficiency in this vitamin.



"Golden" rice

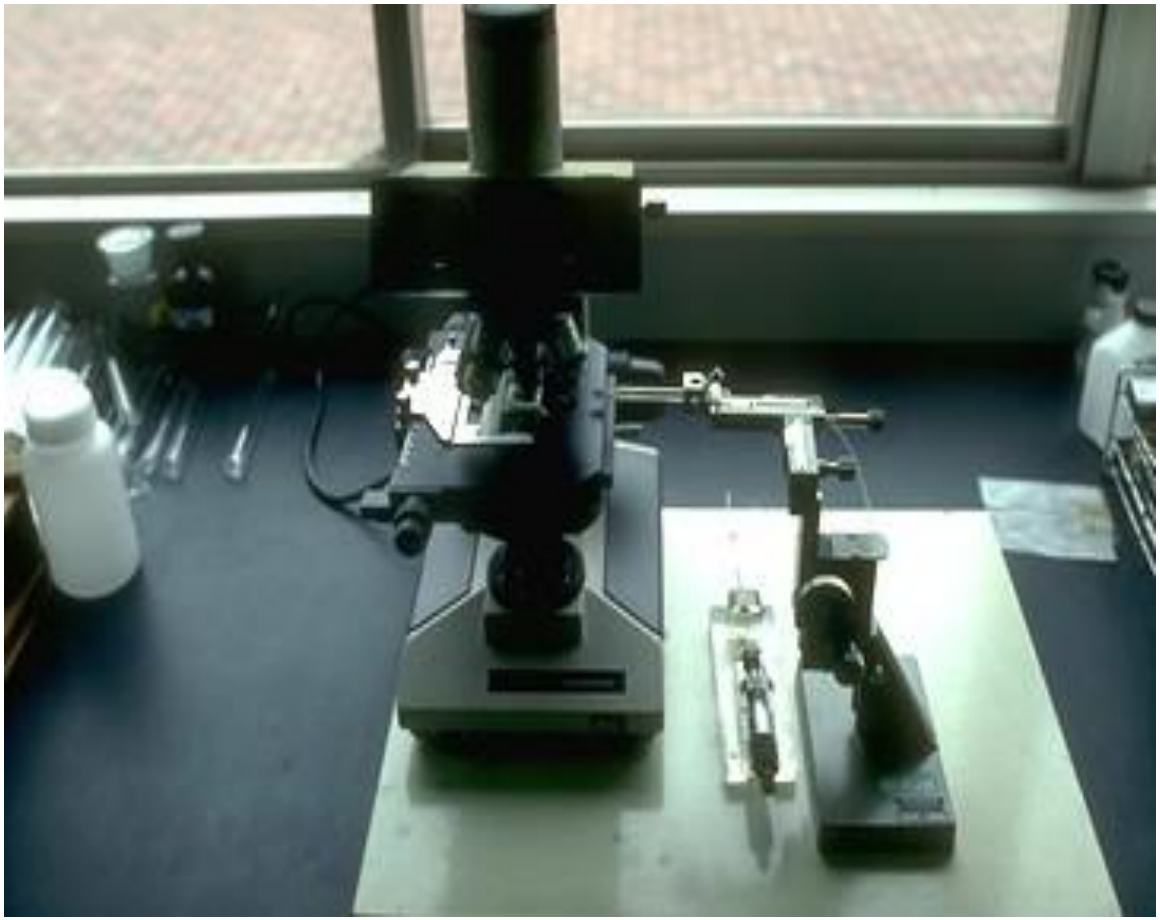
Microinjection

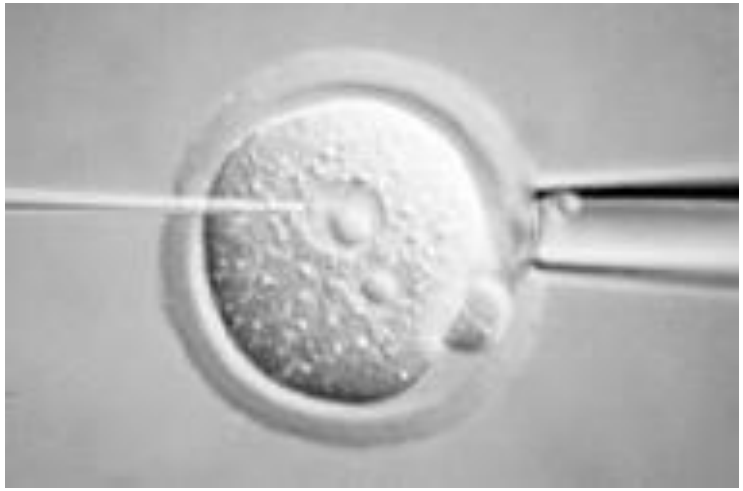
- The process of directly injecting foreign DNA into cells
- This technique is effective with plant protoplasts and tissues

Microinjection

Problems:

- Cell damage, low success rate
- Labor intensive
- Expensive equipment



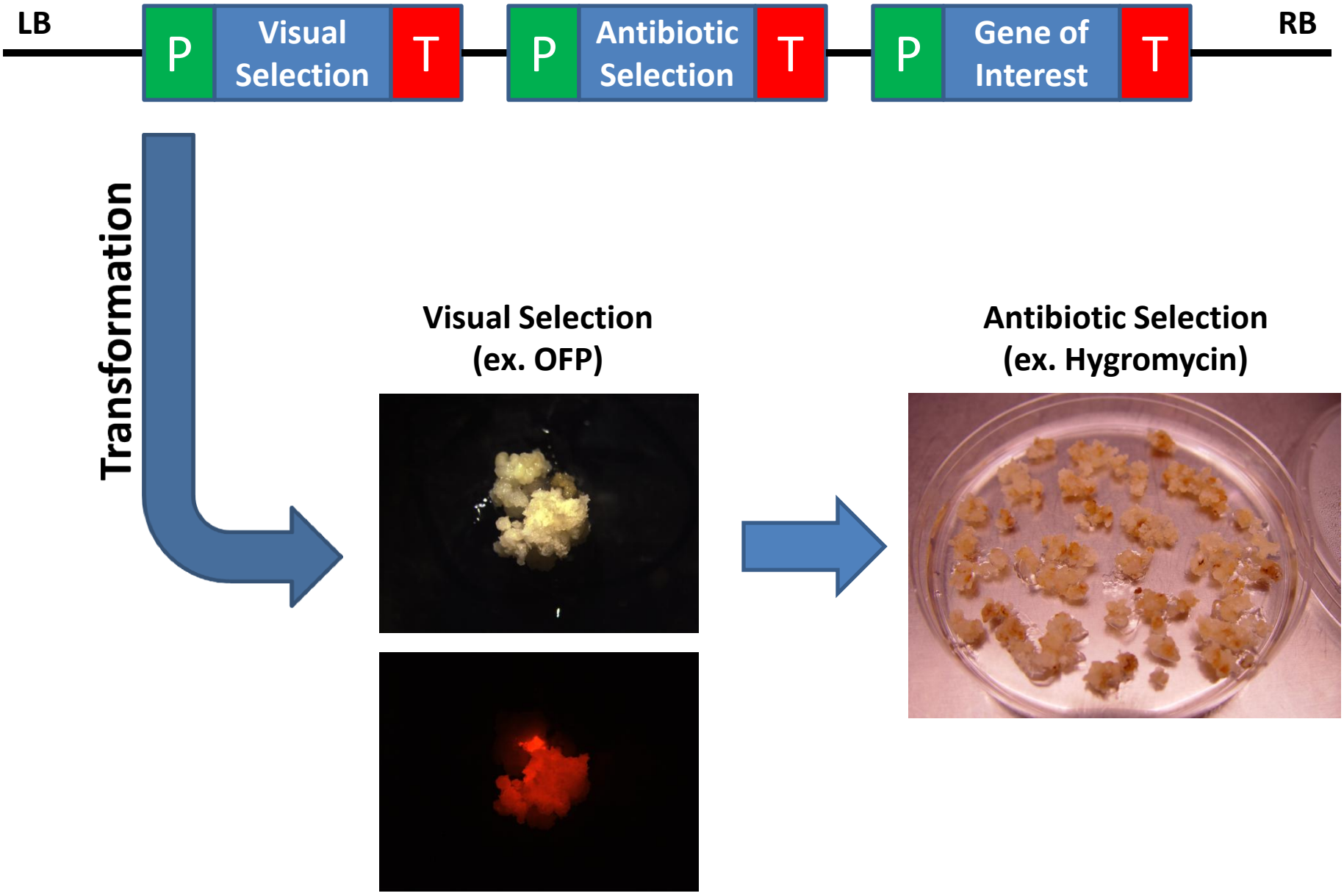


Electroporation

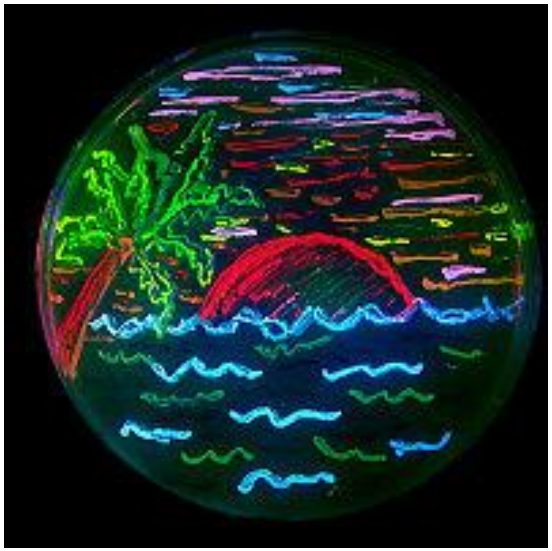
- Use of electrical impulses to increase cell wall and membrane permeability to DNA that is contained in the surrounding solution
- Electroporation is successful in both monocots and dicots, but is used in a relatively small proportion compared to other techniques

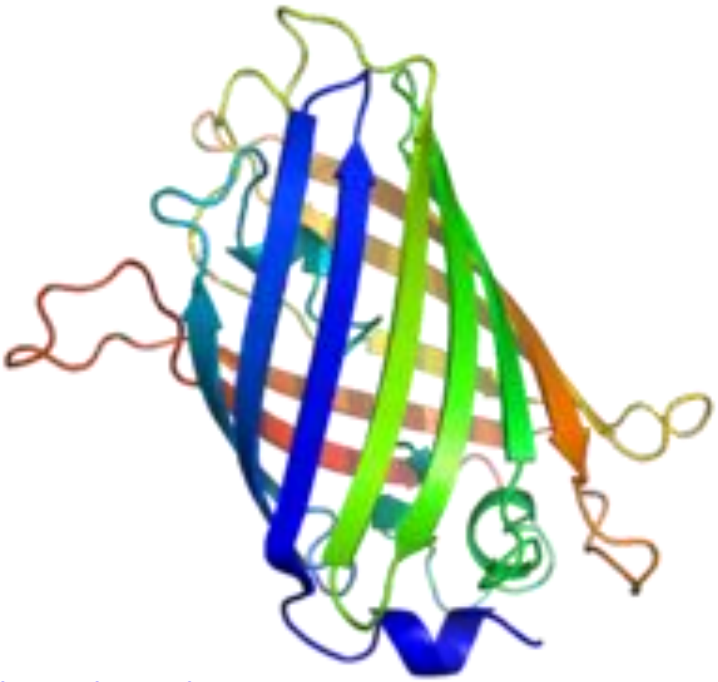
Molecular characterization of transgenic plants

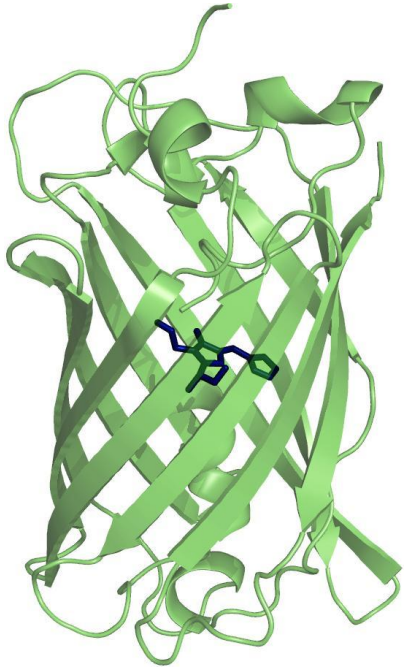
- **PCR**- Simplest and fastest method. Prone to false positives.
- **Southern Blot**- Confirms insertion of the tDNA into the genomic DNA of the target organism, as well as provides insertion copy number.
- **Northern Blot**- Confirms the presence of RNA transcript accumulation from the transgene of interest.
- **Western Blot**- Confirms presence of the PROTEIN produced from the inserted transgene of interest.
- **qRT-PCR**- Provides a relative expression level for the gene of interest—transcript—like Northern blot.


LB
P
Visual Selection
T
P
Antibiotic Selection
T
P
Gene of Interest
T
RB
Transformation
Visual Selection
(ex. OFP)
Antibiotic Selection
(ex. Hygromycin)

Fluorescent Proteins

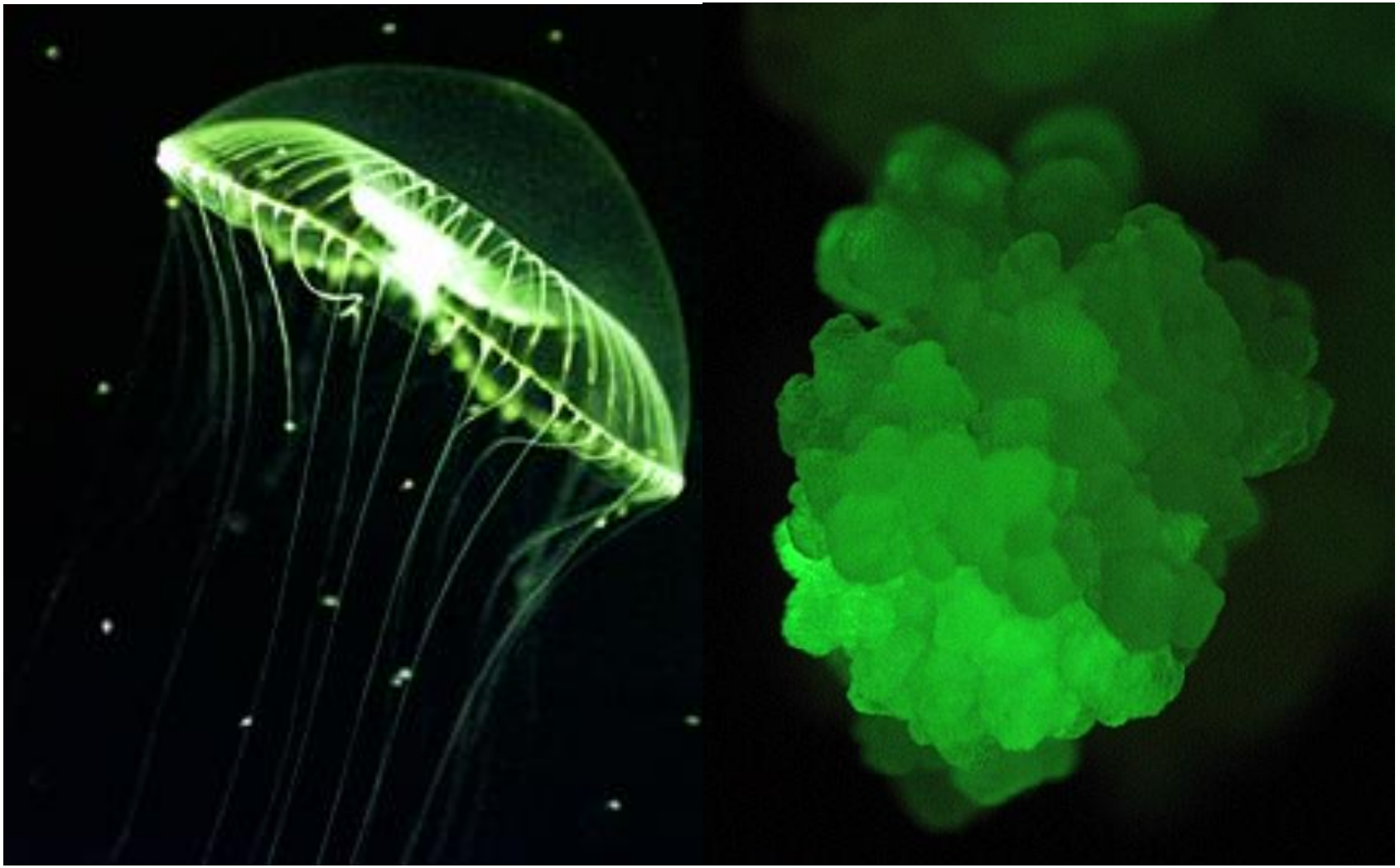






http://en.wikipedia.org/wiki/File:FPbeachTsien.jpg

GFP - Jellyfish Green Fluorescent Protein

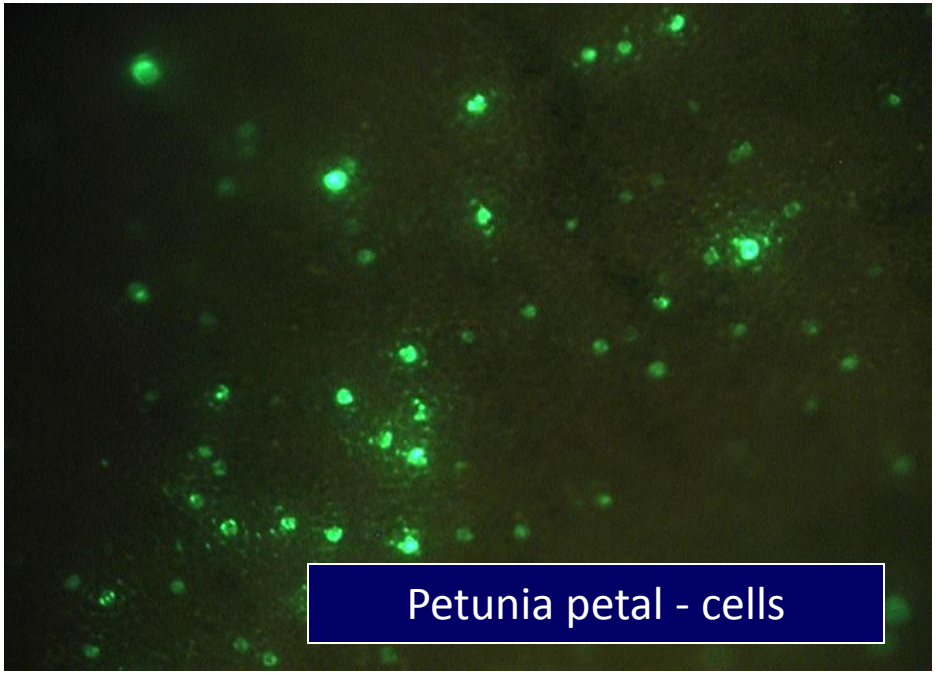


GFP - Jellyfish Green Fluorescent Protein

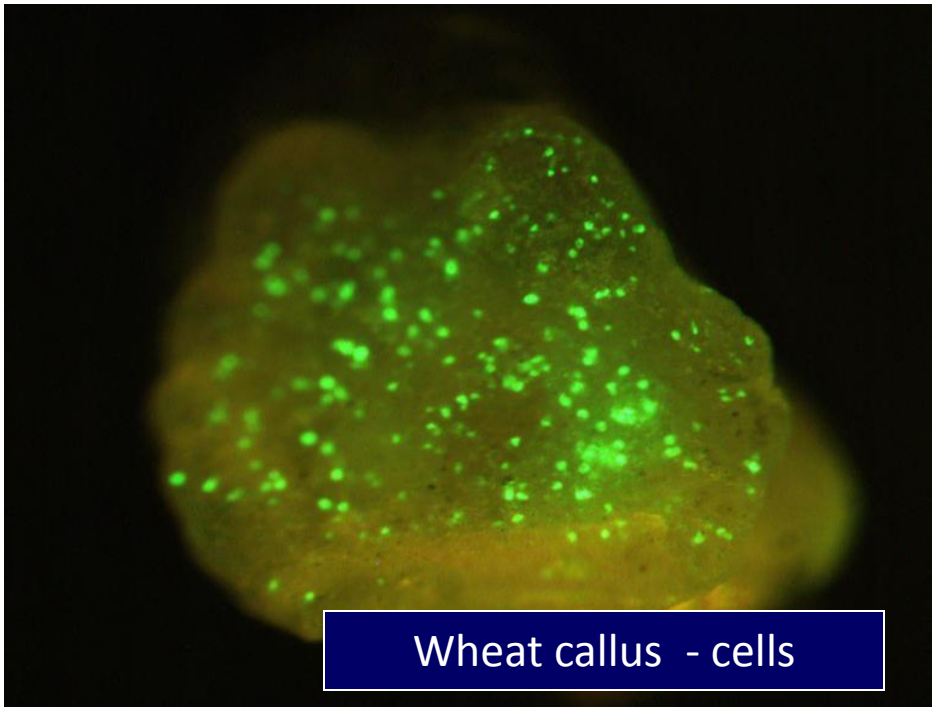
GFP is a marker gene used in DNA transfer studies. The jellyfish green fluorescent protein gene has been modified for optimum expression in plants. The protein from the gene will fluoresce green when illuminated with high intensity blue light.

Introduction of the *gfp* gene into different target tissues

Soybean seed – whole seedling (on right)



Petunia petal - cells



Wheat callus - cells

GFP expression in wheat seeds (left seed, on left) and roots (below)

PCR and DNA Gel Electrophoresis

PCR- Polymerase chain reaction, uses DNA primers to amplify a target sequence of DNA, producing billions of copies of identical DNA.

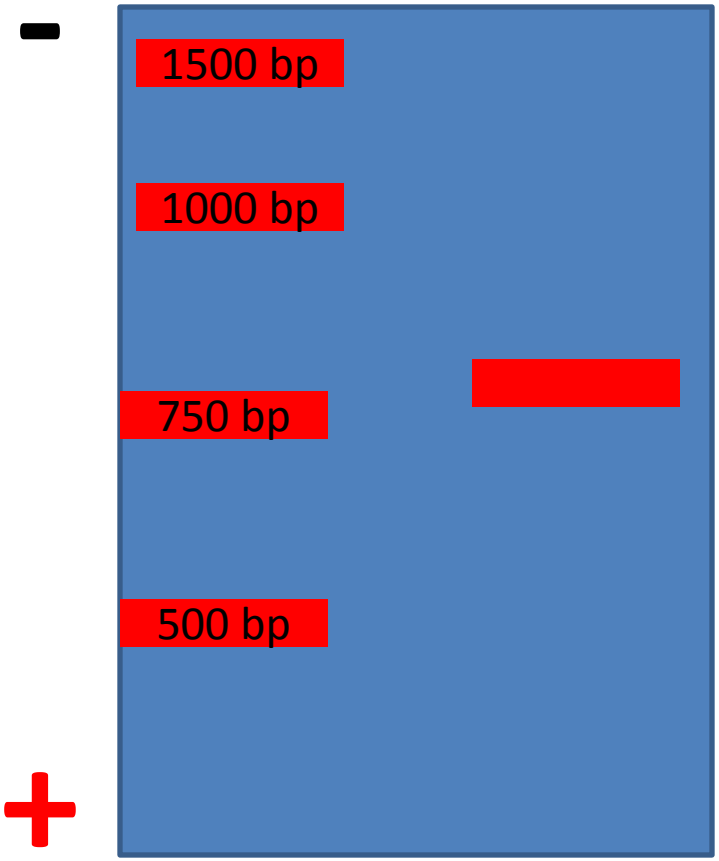
Gene cloning

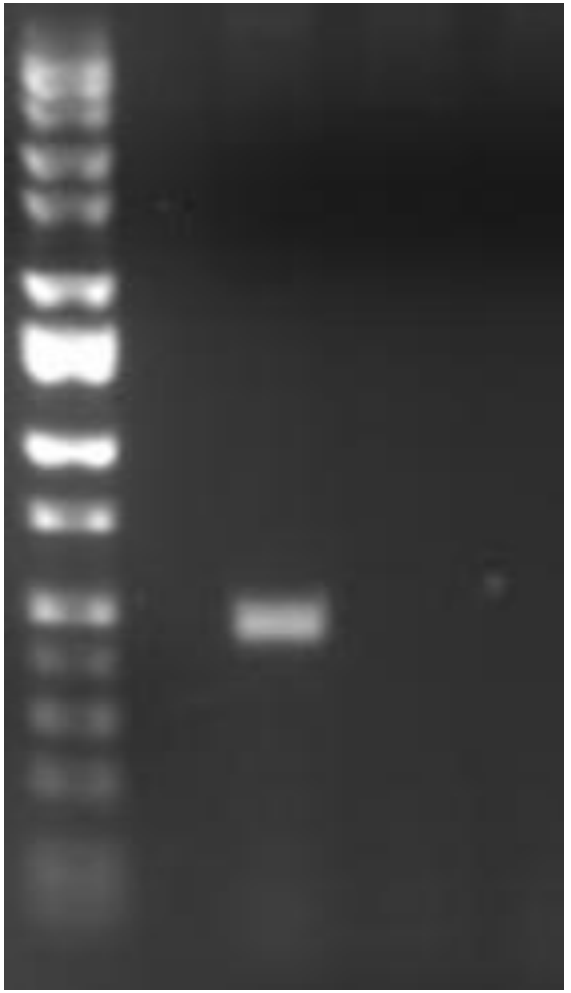
Molecular analysis

(Confirmation of the presence of a particular fragment of DNA in a pool of DNA)

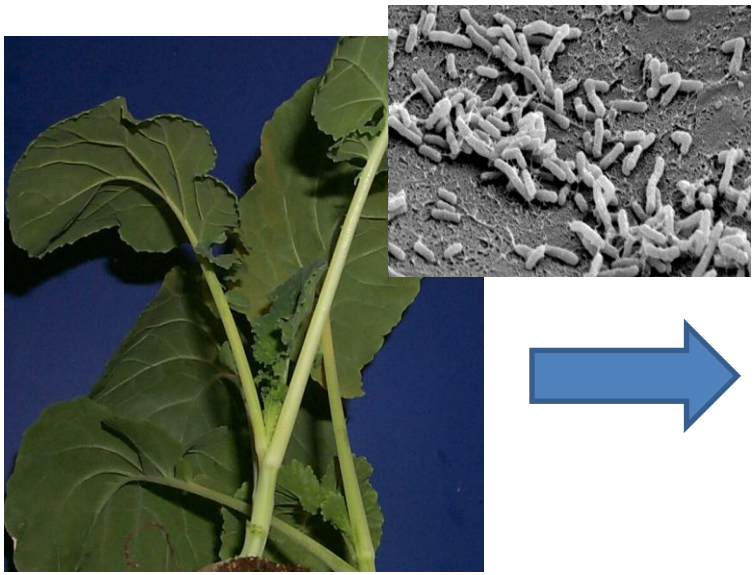
PCR analysis by gel electrophoresis

Ladder Sample



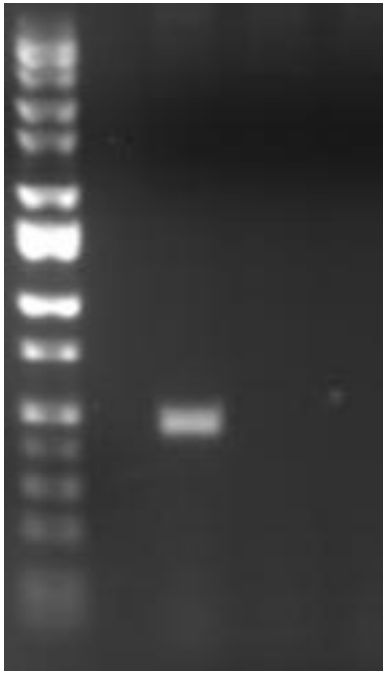


PCR and False Positives



Transgenic plant produced from *Agrobacterium*-mediated transformation

Genomic DNA



- In T_0 plants, *Agrobacterium* left over from the initial transformation is still present in all tissues.
- Contamination of the genomic DNA with the initial transformation vector that is still present in the *agrobacterium* can produce a PCR band.

Southern Blot

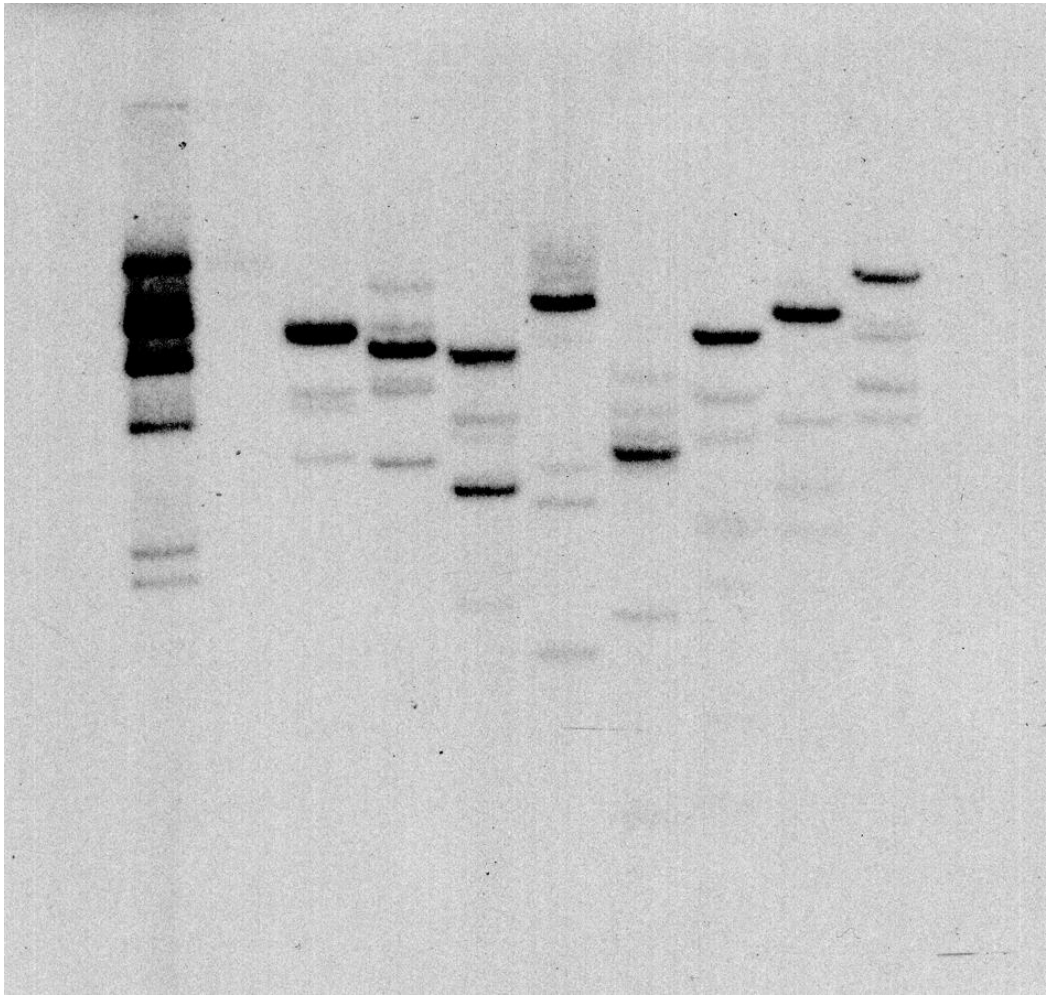
- Southern blotting confirms the presence of the gene of interest in the genomic DNA of the target plant and avoids the pitfalls of potential false positives.
- Steps
  - Genomic DNA isolation
  - Restriction enzyme digestion of genomic DNA
  - Running digested DNA on agarose gel to separate fragmented DNA by size.
  - Transfer of separated DNA to nylon membrane
  - Hybridization with radioactive DNA probe

Southern Blotting

Lane 1- Ladder
Lane 2- Negative Control
Lanes 3-8- Experimental Events

- Bands at different places from event to event indicate insertion at different places in the genome.
- The number of bands in each well indicates how many insertions there were in each event.

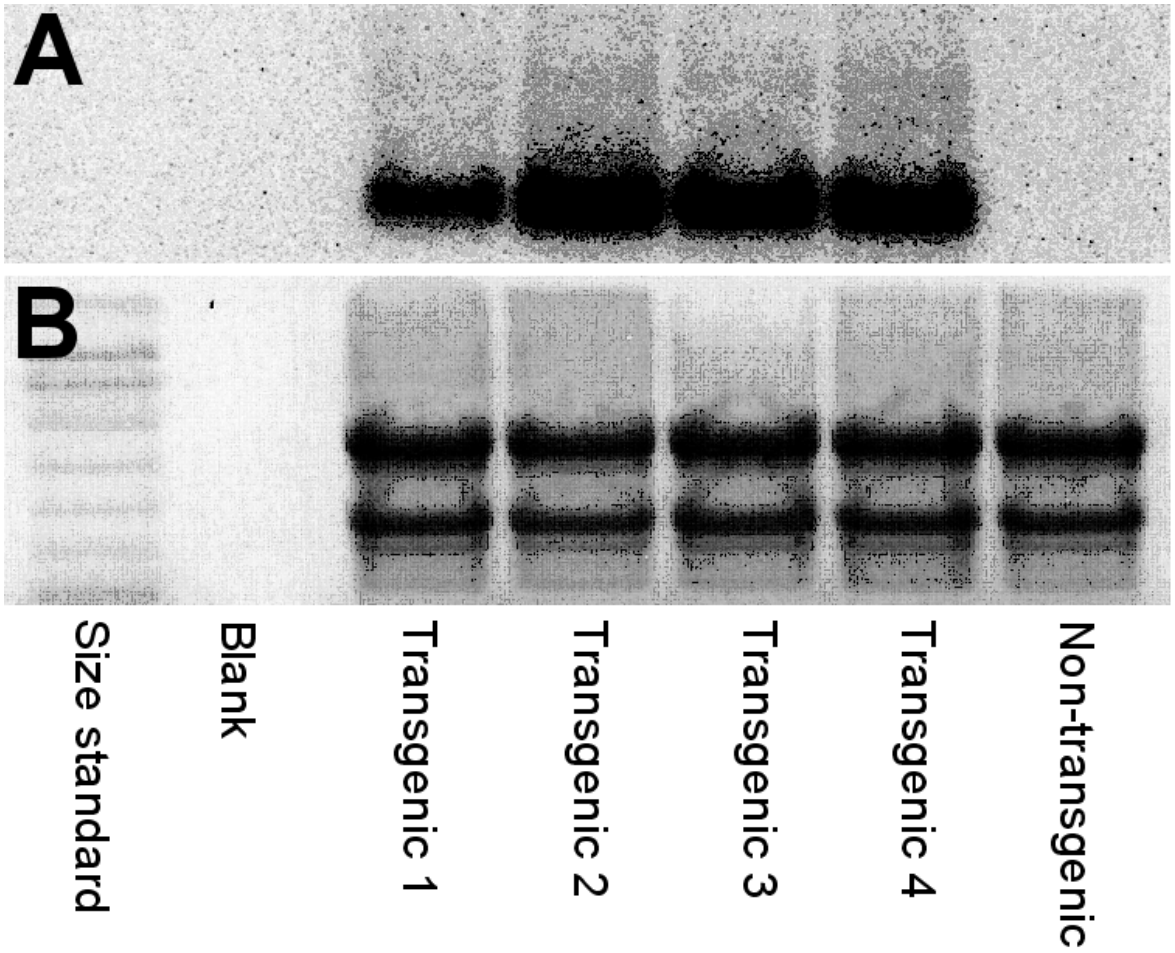
The Final product



Northern Blot

No digestion necessary… why is this?

RNA loading controls are necessary to ensure an equal amount of RNA is loaded in each well.



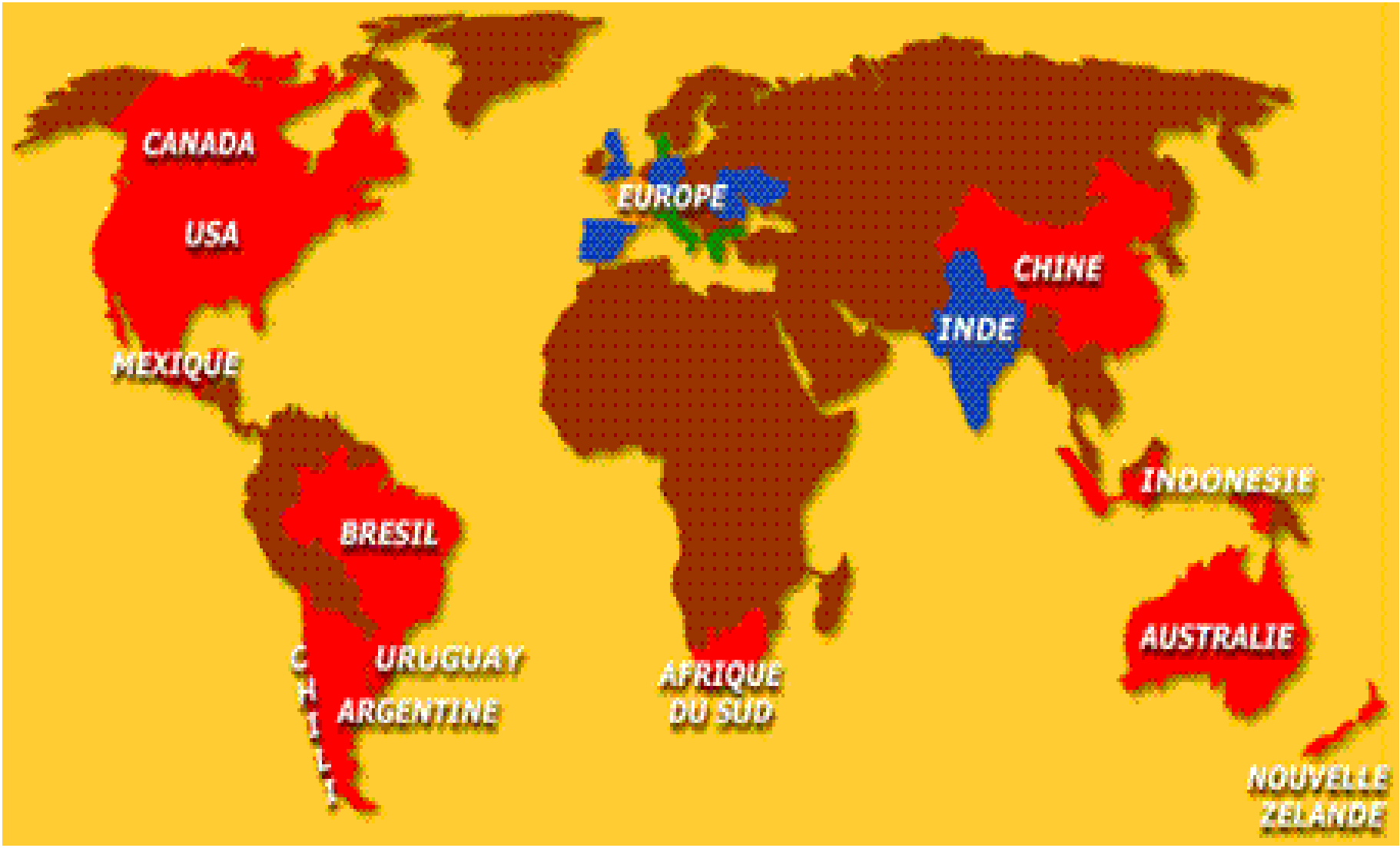
Antibiotic Selection

- When a mixture of transformed and untransformed callus is placed on antibiotic selection media, only the transformed callus carrying the antibiotic selection cassette is able to survive and grow.
- In most cases, the untransformed callus dies, making it “easy” to select for callus carrying the T-DNA.

Trait/Gene Examples

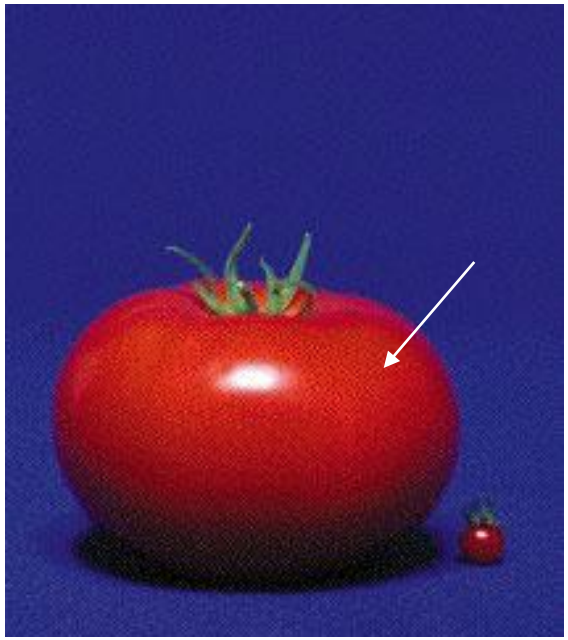
Trait	Gene
RoundUp Ready	Bacterial EPSP
Golden Rice	Complete Pathway
Plant Virus Resistance	Viral Coat Protein
Male Sterility	Barnase
Plant Bacterial Resistance	p35
Salt tolerance	AtNHX1

Map - GMO


CANADA
USA
MEXIQUE
EUROPE
CHINE
INDE
INDONESIE
BRESIL
URUGUAY
ARGENTINE
CHILI
AFRIQUE DU SUD
AUSTRALIE
NOUVELLE ZELANDE

GM Food

GM Tomato



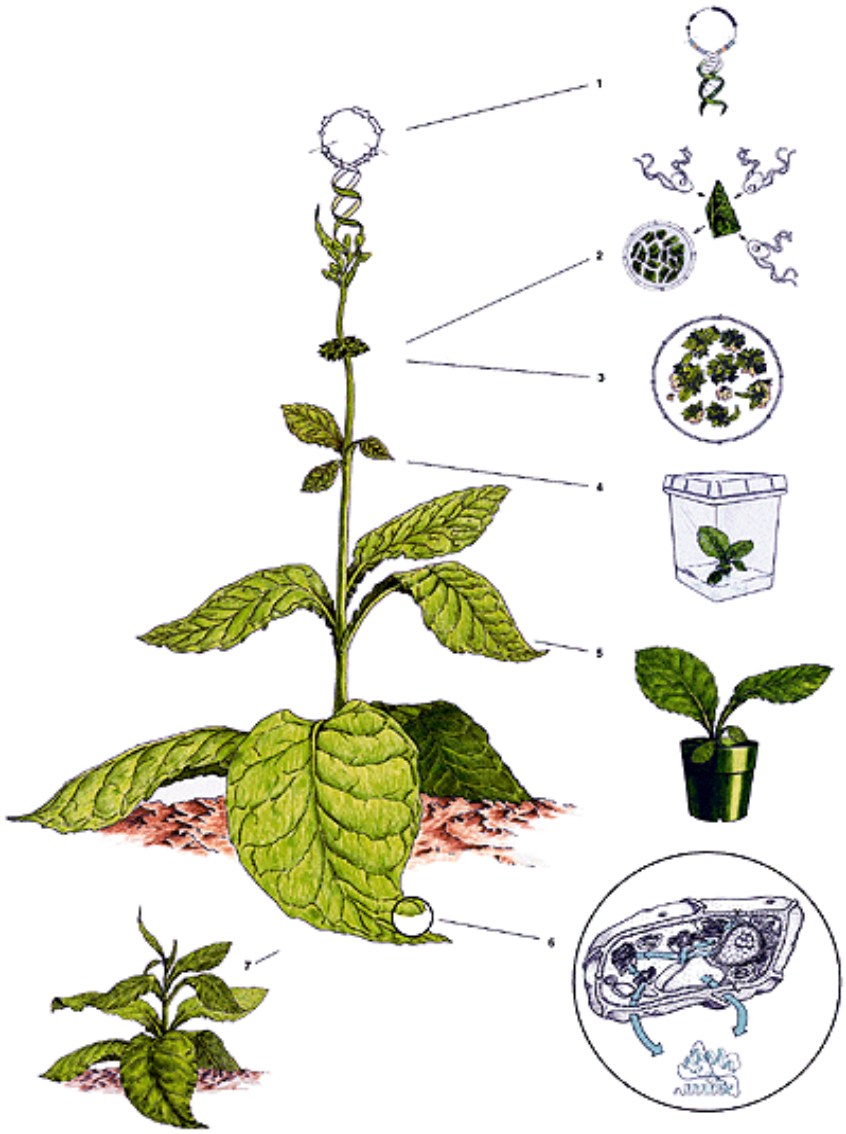
GM Rice

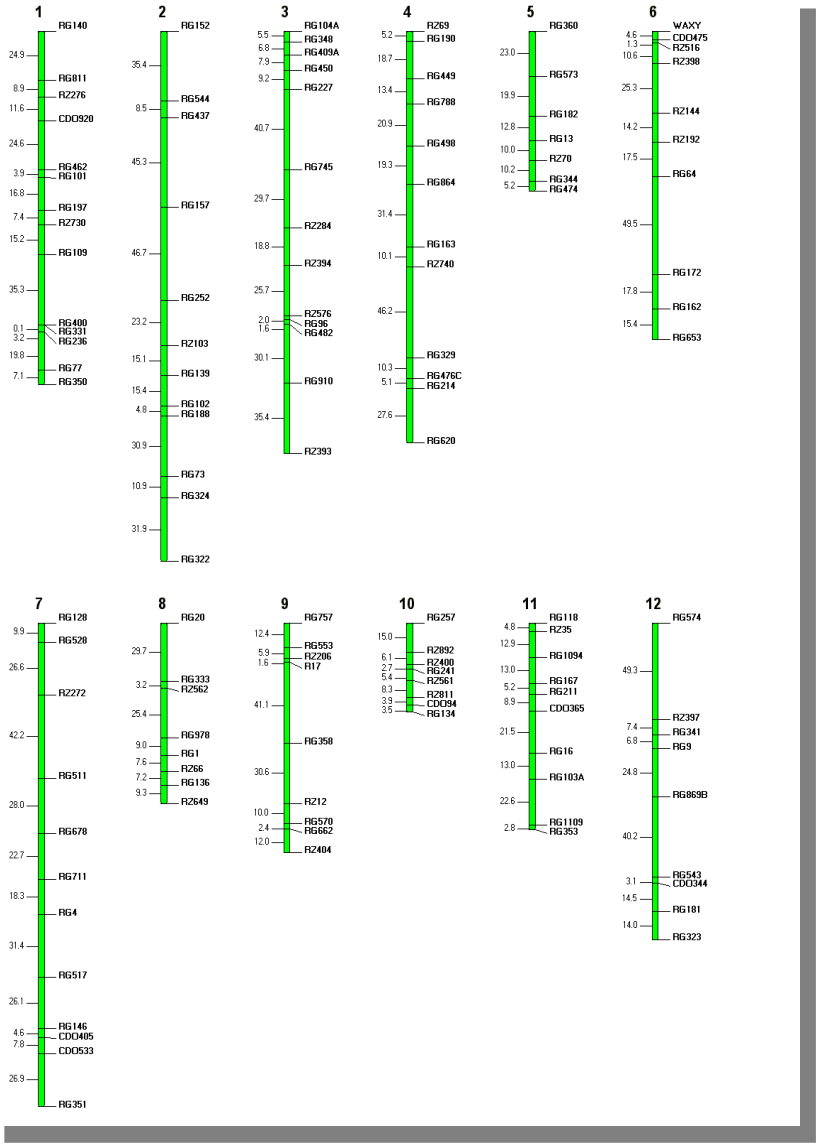
Controversies Surrounding the Risks and Benefits of Genetically Modified Food

Debate about GM Food

The appearance of genetically modified foods in the marketplace has resulted in a firestorm of **public debate, scientific discussion, and media coverage**. A variety of ecologogical and human health concerns come with the new advances made possible by genetic modification.

PLANT BIOTECHNOLOGY





Molecular markers are classified into different groups according their major features, such as composition, structure, function, etc.

1.2 Classification of molecular markers

There exist two major systems for molecular marker classification:

1.2.1 Classified according to their structure and composition

In this system the molecular markers developed up to now are divided into three generations as follows:

- First generation: Classical, Southern hybridization based markers
- Second generation: PCR-based markers
- Third generation: Genome sequence based markers

First generation:

Classical, Southern hybridization based markers

RFLP: Restriction Fragment Length Polymorphism

SSCP-RFLP: Single Strand Conformation Polymorphism-RFLP

DGGE-RFLP: Denaturing gradient gel electrophoresis-RFLP

表1. 续

Second generation:

PCR-based markers

RAPD: Randomly Amplified Polymorphic DNA
AFLP: Amplified Fragment Length Polymorphism
SSR: Simple Sequence Repeat
STS: Sequence Tagged Site
EST: Expressed Sequence Tag
SCAR: Sequence Characterized Amplified Region

RT—PCR: Revert Transcription PCR
DDRT-PCR: Differential Display Reverse Transcription-PCR
SSH: Subtractive suppression hybridization

Third generation:

Newly developed genome sequence based markers

SNP: Single Nucleotide Polymorphisim

InDel: Insertion and Deletion

cSSR: cDNA simple sequence repeat (cDNA SSR, developed from cording sequence)

1.2.2 Classified according to their practical applications

- **Simple marker:**

  Variety specific markers:

  Specific markers of hybrid seeds —

  Complementary amplification

- **Multiple markers, DNA fingerprinting**

  (DNA analysis, DNA profiling）

Part 2 : Application of molecular markers

2.1 Molecular marker linkage map

2.2 Forensic and eugenic sciences

2.3 Classification and evolution studies

2.4 Crop breeding

2.5 Germplasm identification (both reality and purity)

2.1 Molecular marker linkage map

Molecular marker has been used almost in all fields of biology, especially in genome research, construction of genetic linkage map, forensic and eugenic sciences, gene tagging and mapping, crop breeding and germplasm identification. Now I would like to mention some good examples.

2.4.1.3 Wild rice high-yielding genes

During the long history of rice cultivation, rice yield increased about 6-8 times, meanwhile, almost all of the high-yielding genes of rice cultivars have been exploited.

In order to exploit new gene resources, recently people pay attention to the exploitation and application of high-yielding genes from wild rice and weeds.

Professor Yan Longping co-liberated with Tanksley, Cornell University, USA, detected two high-yielding genes (major QTLs) from wild rice, *Oryza rufipogon*. These 2 high-yielding genes have the potential to increase the yield by 17% and 18% respectively.

The original result published in "Nature", in which the high-yielding genes were mapped on chromosome 1 and 2 respectively. But the linkage markers were too far to be used in MAS. Later, four new SSR markers, more closely linked with *yld1.1* and *yld2.1* respectively, were developed based on the rice the molecular linkage map and genome sequencing information data.

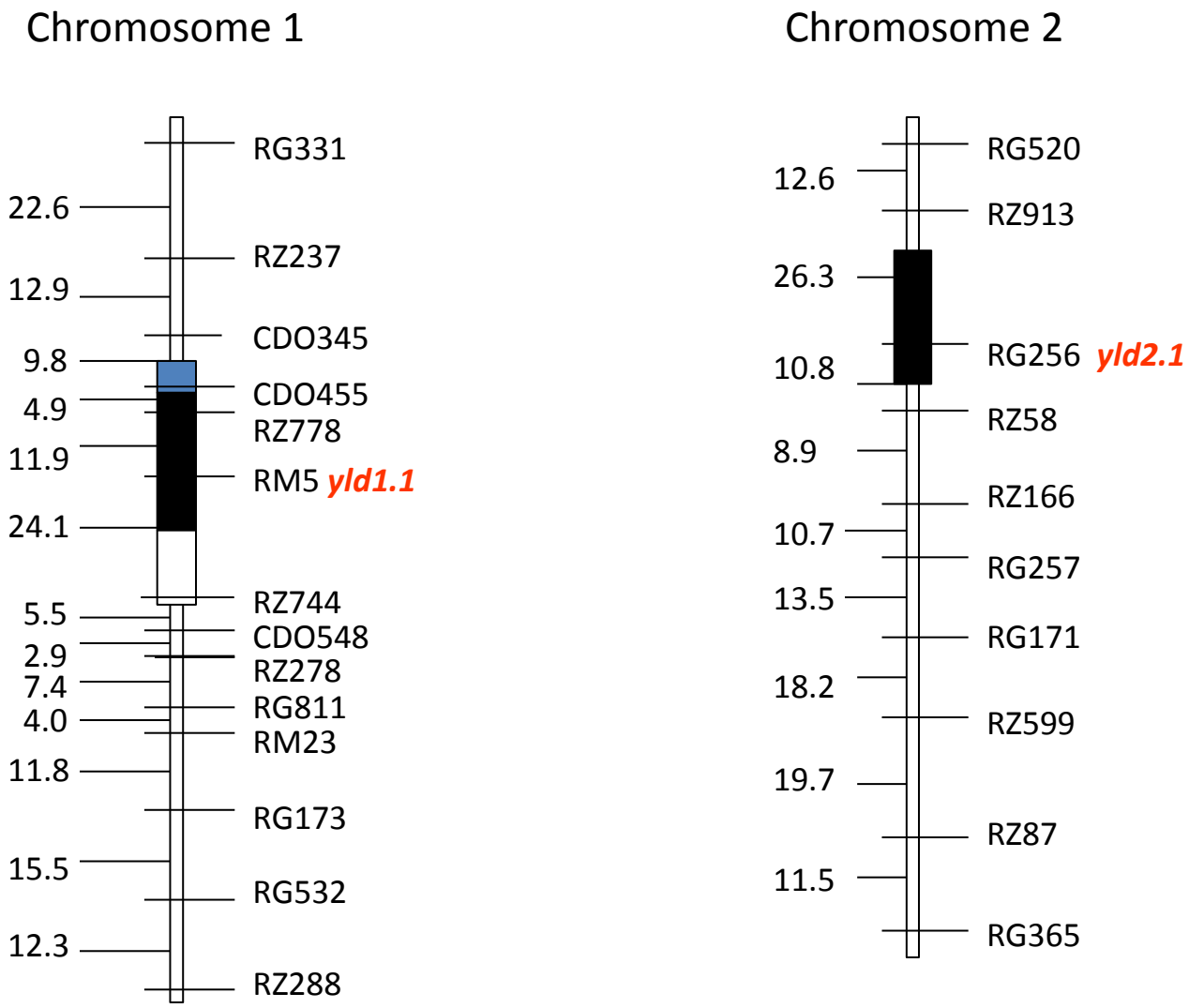


Fig.1 Chromosome location of the two high-yielding genes from wild rice

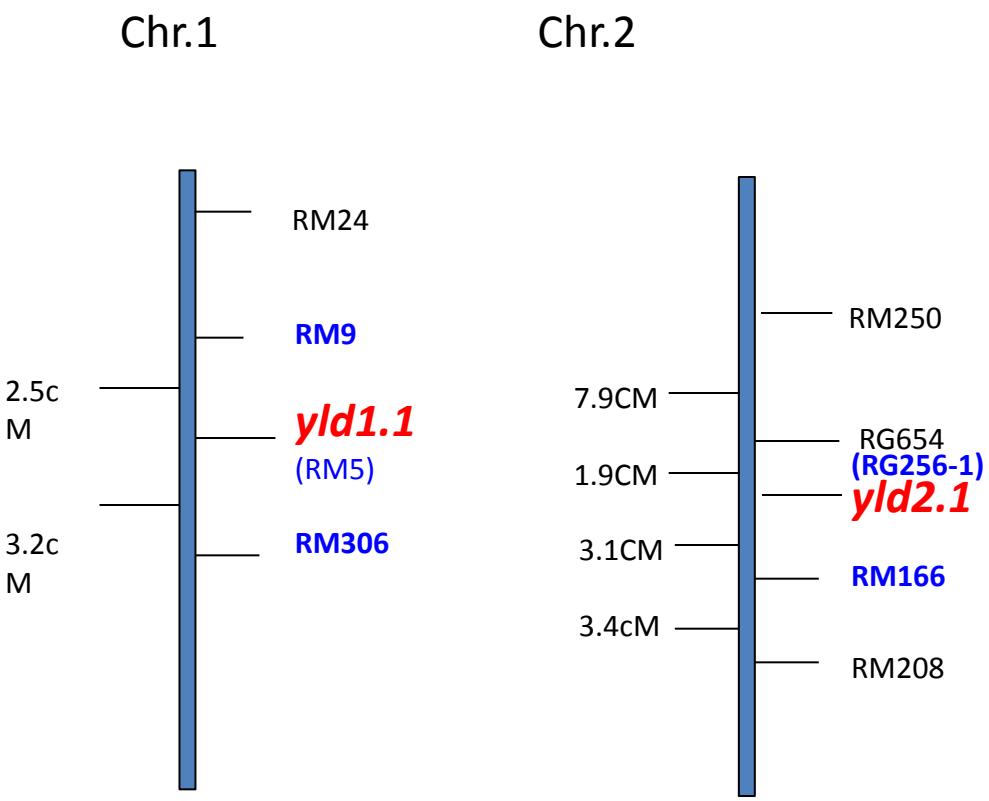


Fig. 3 The linkage markers selected for MAS of *yld1.1 and yld2.1*

Then 4 pairs of linkage markers were designed and synthesized as and used in rice variety improvement by MAS.

For *yld1.1*

RM9： 2.5 cM from ***yld1.1***

F 5’ GGTGCCATTGTCGTCCTC 3’

R 5’ ACGGCCCTCATCACCTTC 3’

RM306: 3.2 cM from ***yld1.1***

F 5’ CAAGGTCAAGAATGCAATGC 3’

R 5’ GCCACTTTAATCATTGCATC 3’

For *yld2.1*

GG256-1: co-segregated with ***yld2.1***

F 5’ TCATGACAGAGCCTTGTCTGATG 3’

R 5’ TGAGTACGATGATTGTGTGGAC 3’

RM166: 3.1 cM from ***yld2.1***

F 5’ GGTCCTGGGTCAATAATTGGGTTACC 3’

R 5’ TTGCTGCATGATCCTAAACCGG 3’

F: Forward markers; R: Reverse markers.

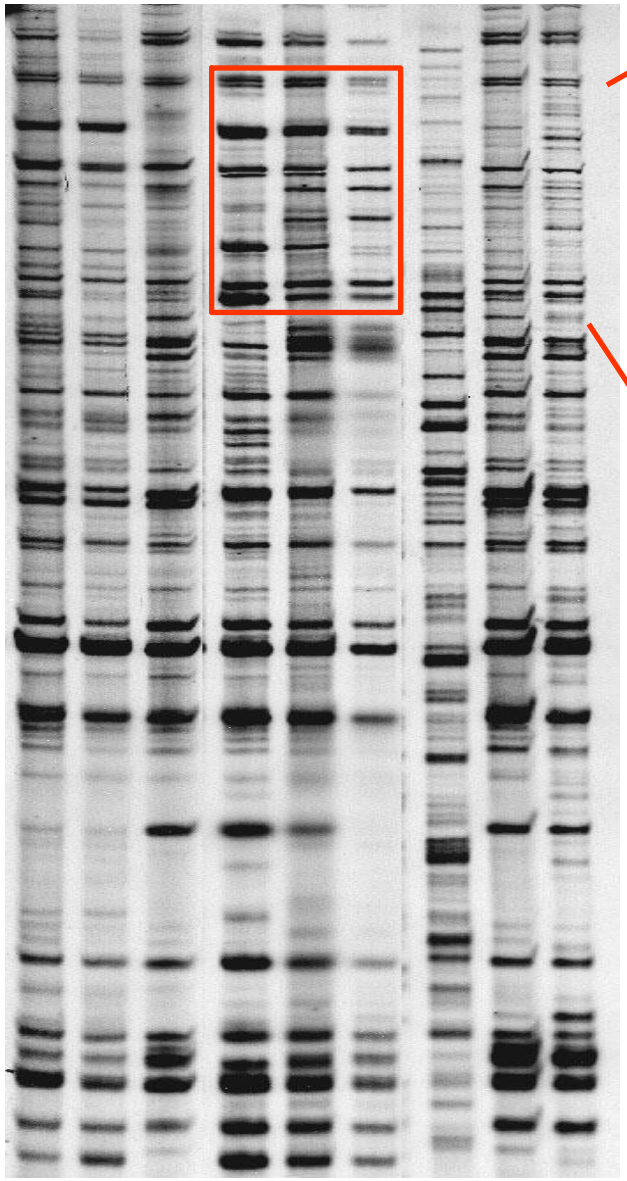
High-yielding genes, *yld1.1* and *yld2.1,* were transferred into rice cultivars Ce64, Minghui63 and 9311 respectively via traditional hybridization and MAS. The yields of the corresponding improved cultivar was increased about 30%.

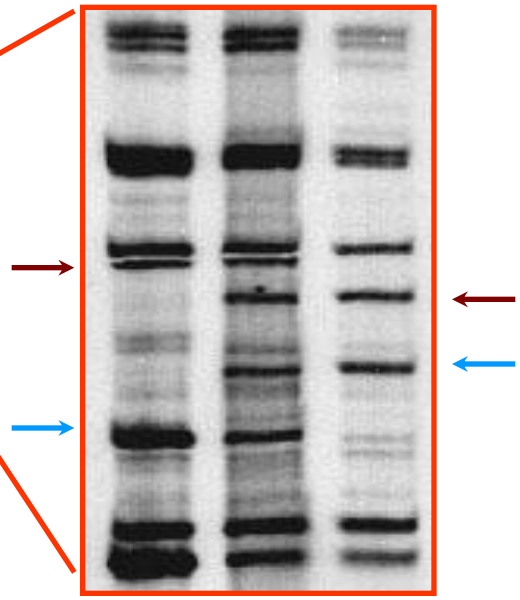
V77(CK) J23A/Q611

Individual ear of J23/Q611 and V77

2.5.1.2 Hybrid variety identification

(Rice, maize and *Eucalyptus* tree)





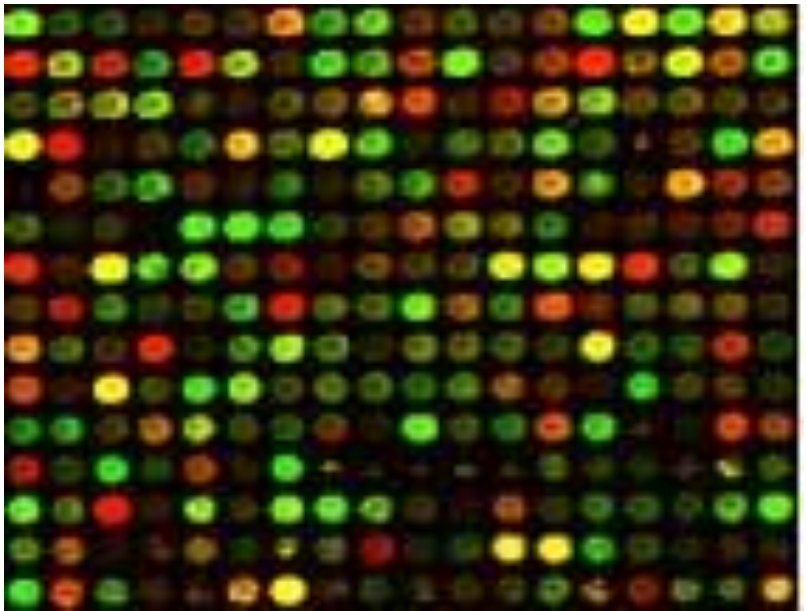
Complementary pattern amplified with AFLP primer pair *EcoR*I-AAG/*Mse*I-CAA.

1. Female parent Zhenxian 97A;

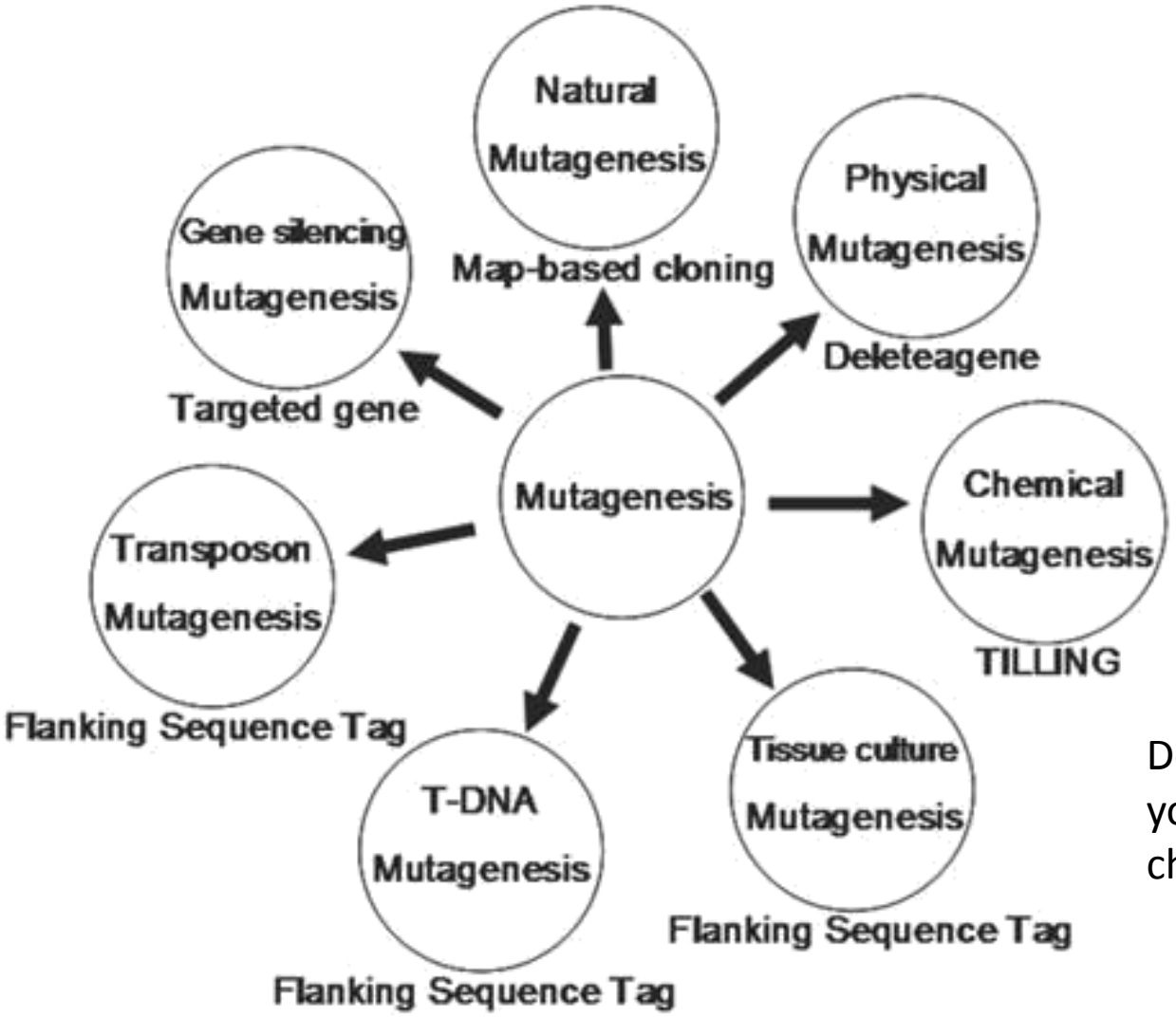
2. Shanyou 63(Hybrid);

3. Male parent Minghui63.

There is a broader context to the methodology e.g., quantitative methods used in plant breeding are currently being applied to the analysis of “DNA chip” experiments

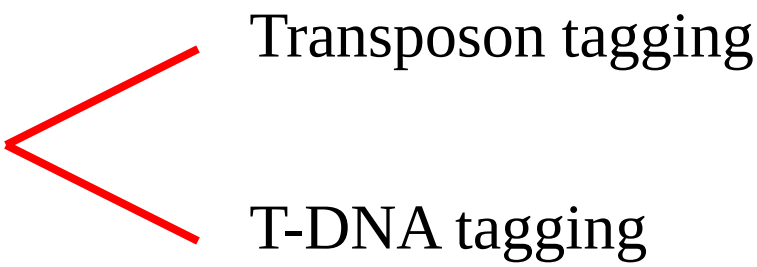


Summary: Mutate a gene in general..... Lot of methods

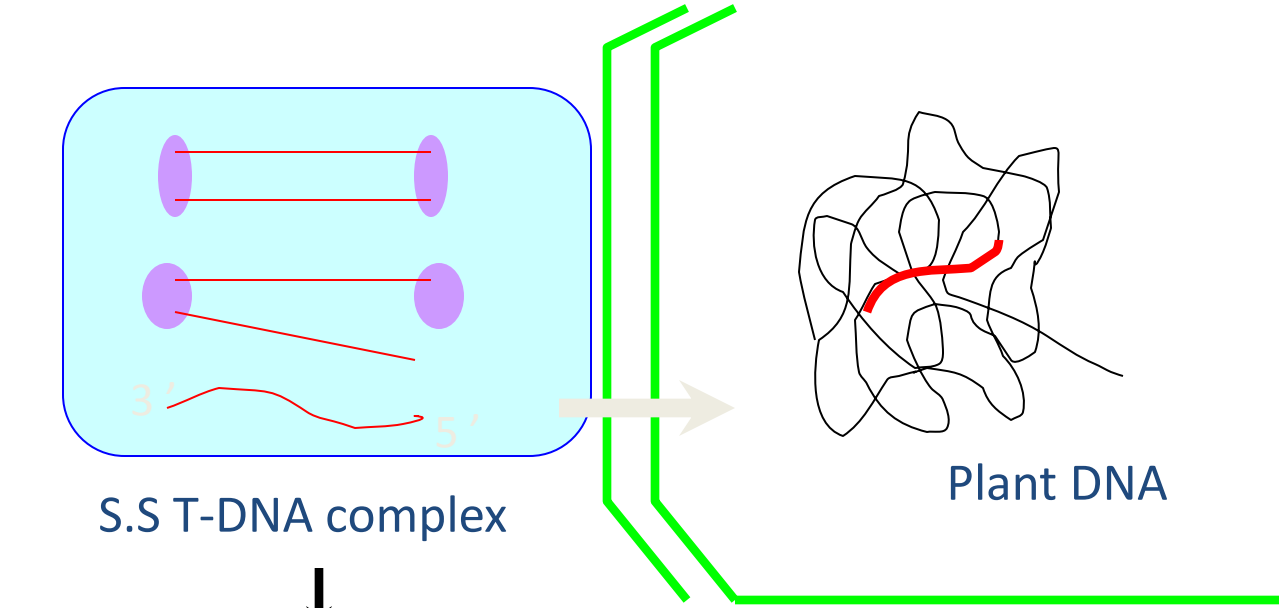


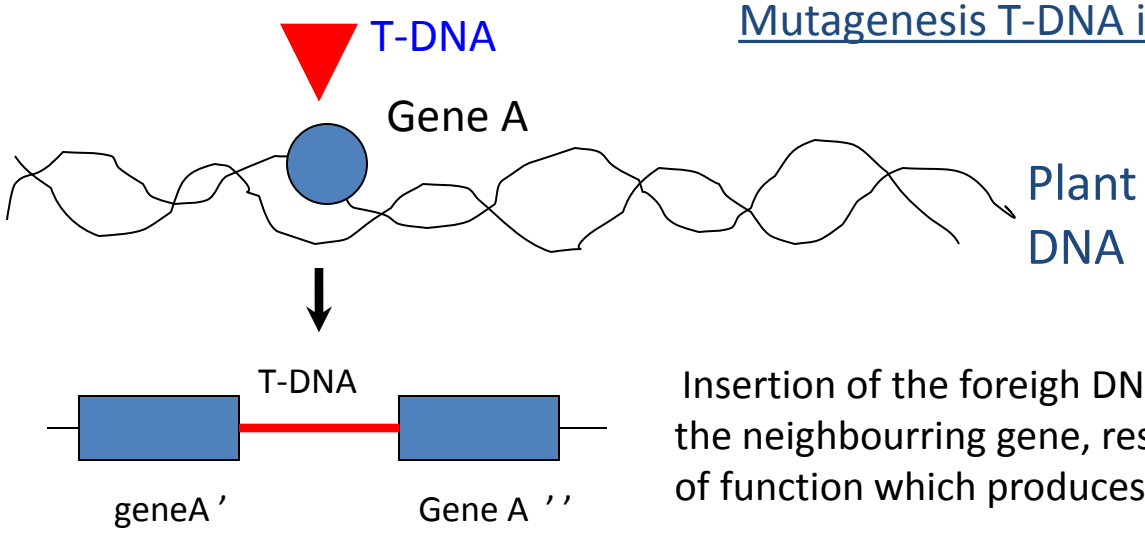
Depending on what you want to do, you can choose either of these methods.

You know the fonction but not the gene - to identify the gene you have to tag it

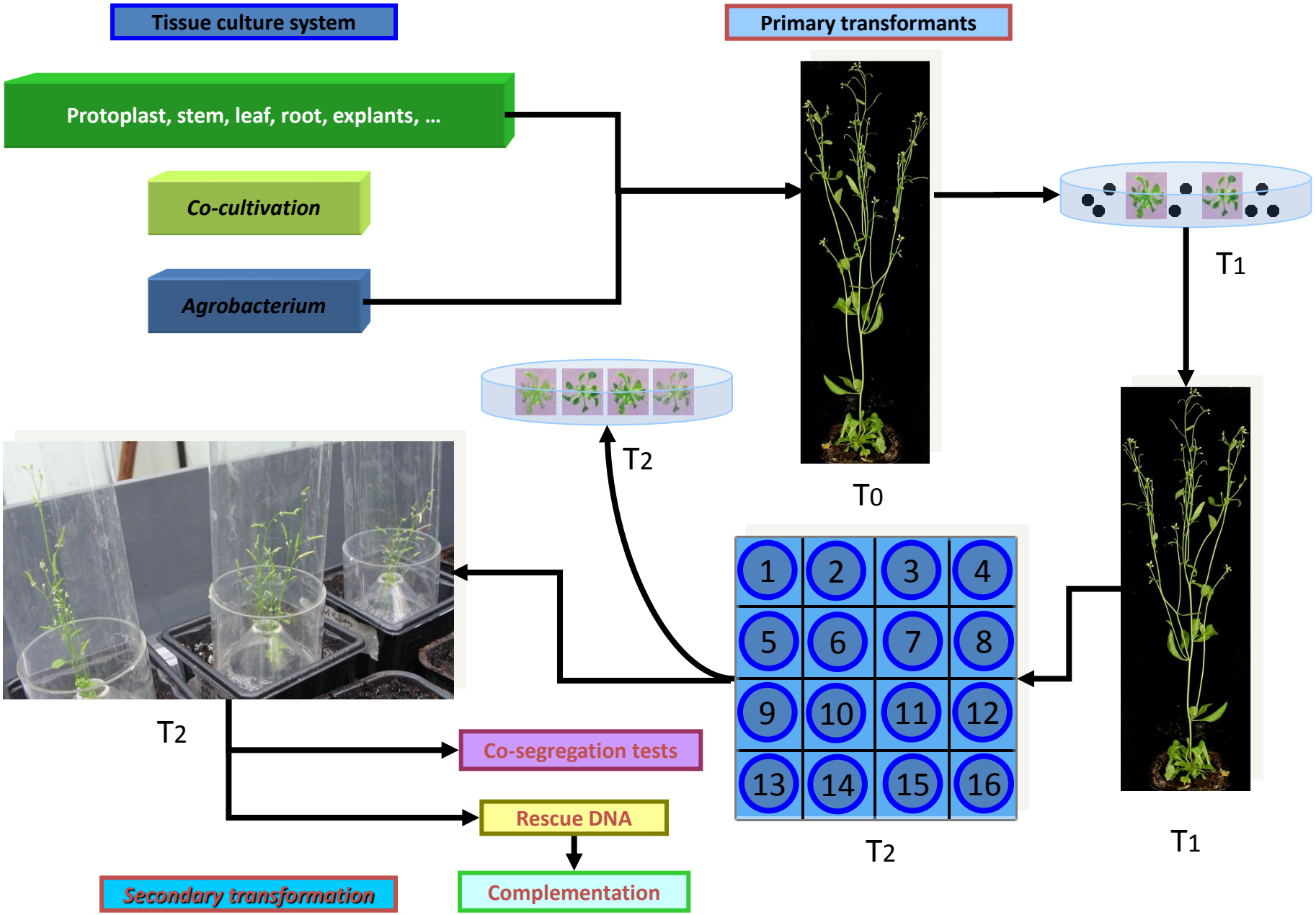


T-DNA TAGGING SYSTEM





Insertion of the foreigh DNA can alter the expression of the neighbourring gene, resulting either a gain or a loss of function which produces a screenable phenotype.


Tissue culture system
Primary transformants
Protoplast, stem, leaf, root, explants, …
Co-cultivation
Agrobacterium
T1
T2
T0
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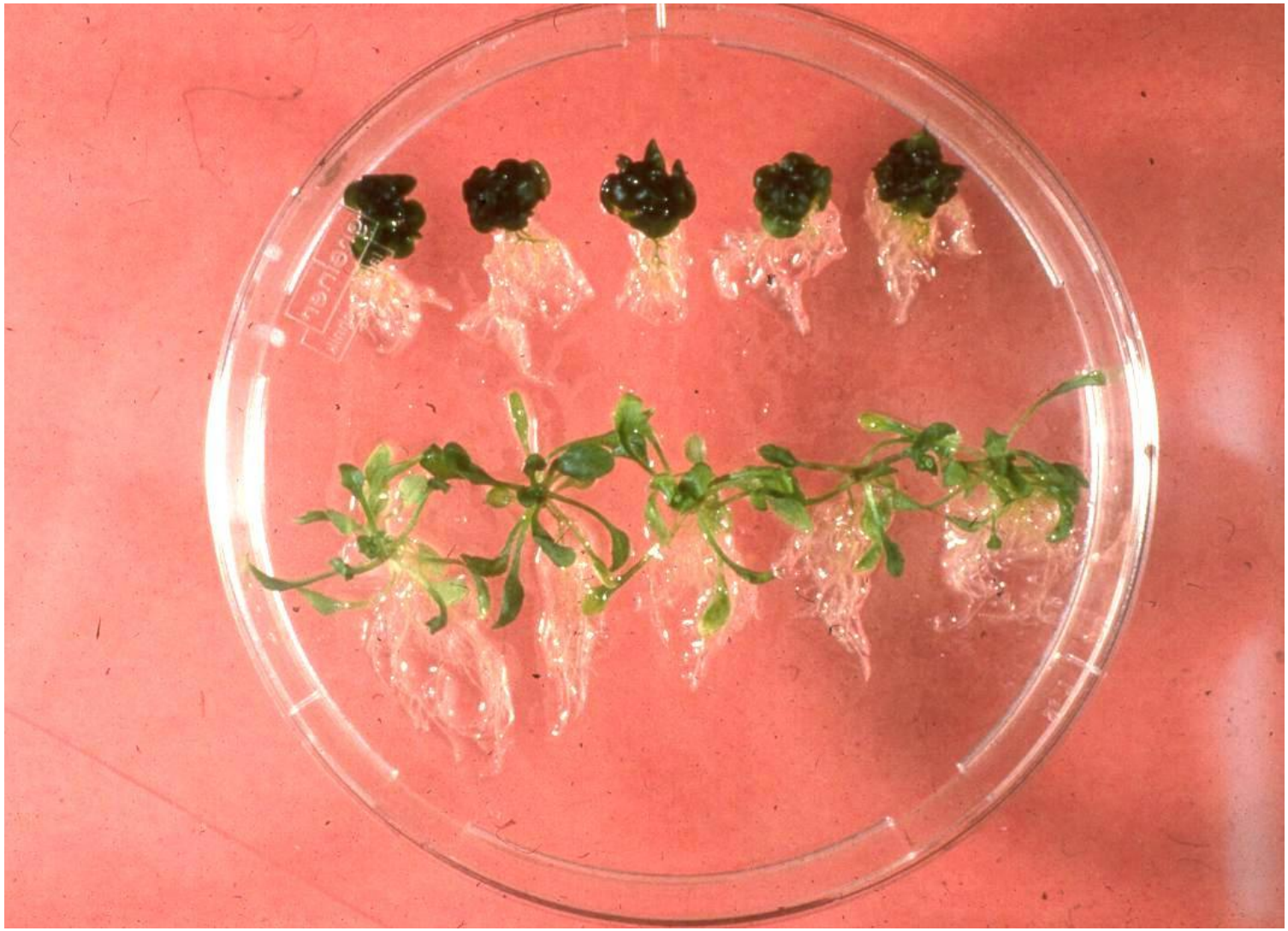
T2
Co-segregation tests
Rescue DNA
Complementation
Secondary transformation



Arabidopsis sweetie mutant

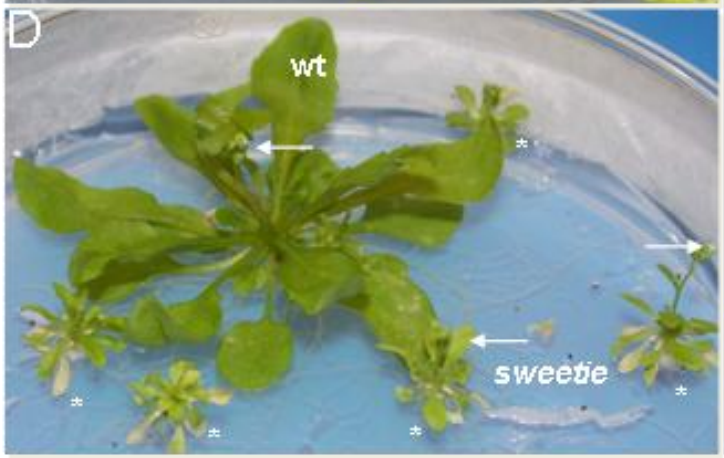
Taille réduite

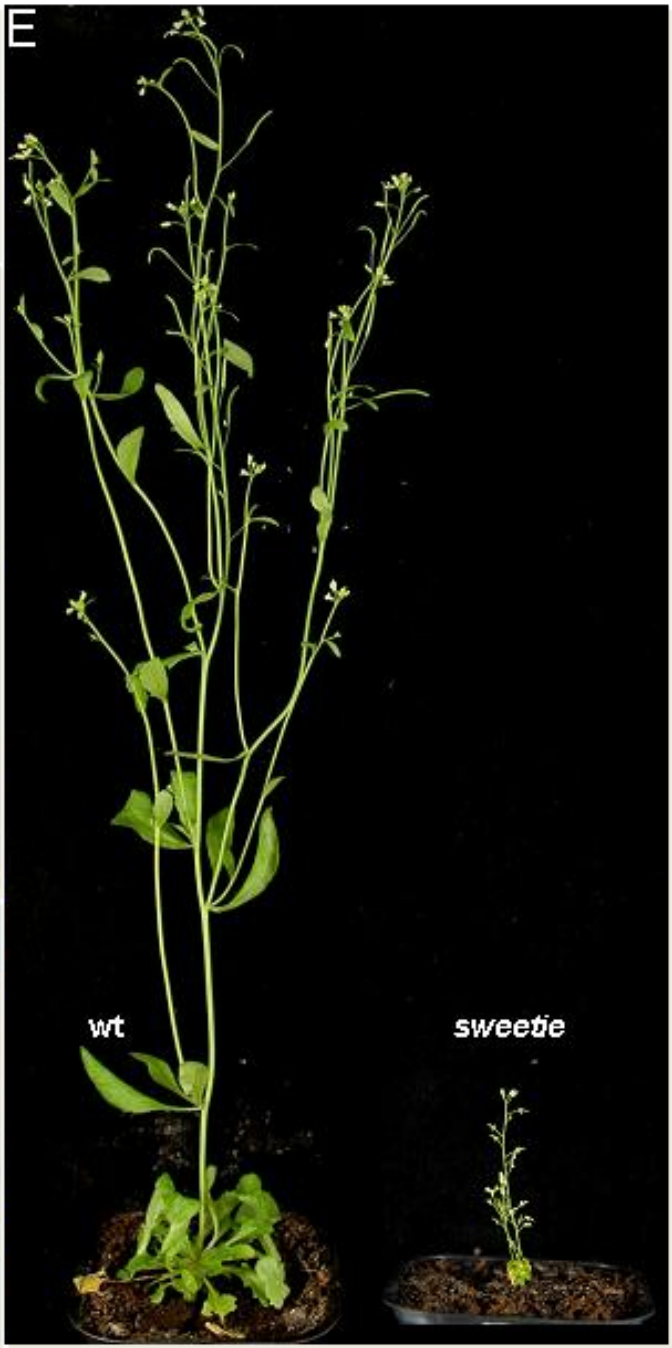
Feuilles lancéolées

Sénescence précoce

Stérilité

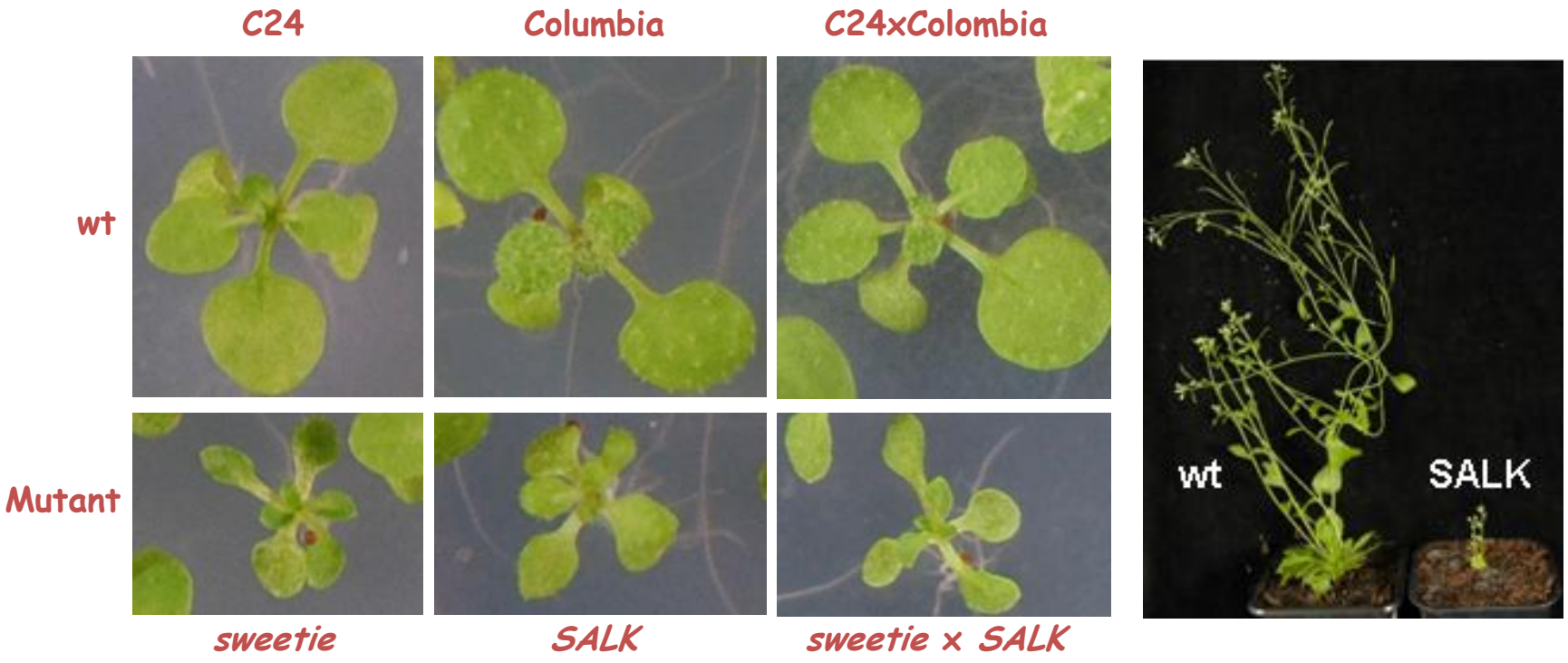
Surproduction de Tréhalose





Test d'allélisme

- ✓ Le mutant SALK a le phénotype de *sweetie*.
- ✓ Croisement des hétérozygotes et analyse de la F1.



Arabidopsis nan1 mutant

Taille réduite

Feuilles incurvées

Pigmentation

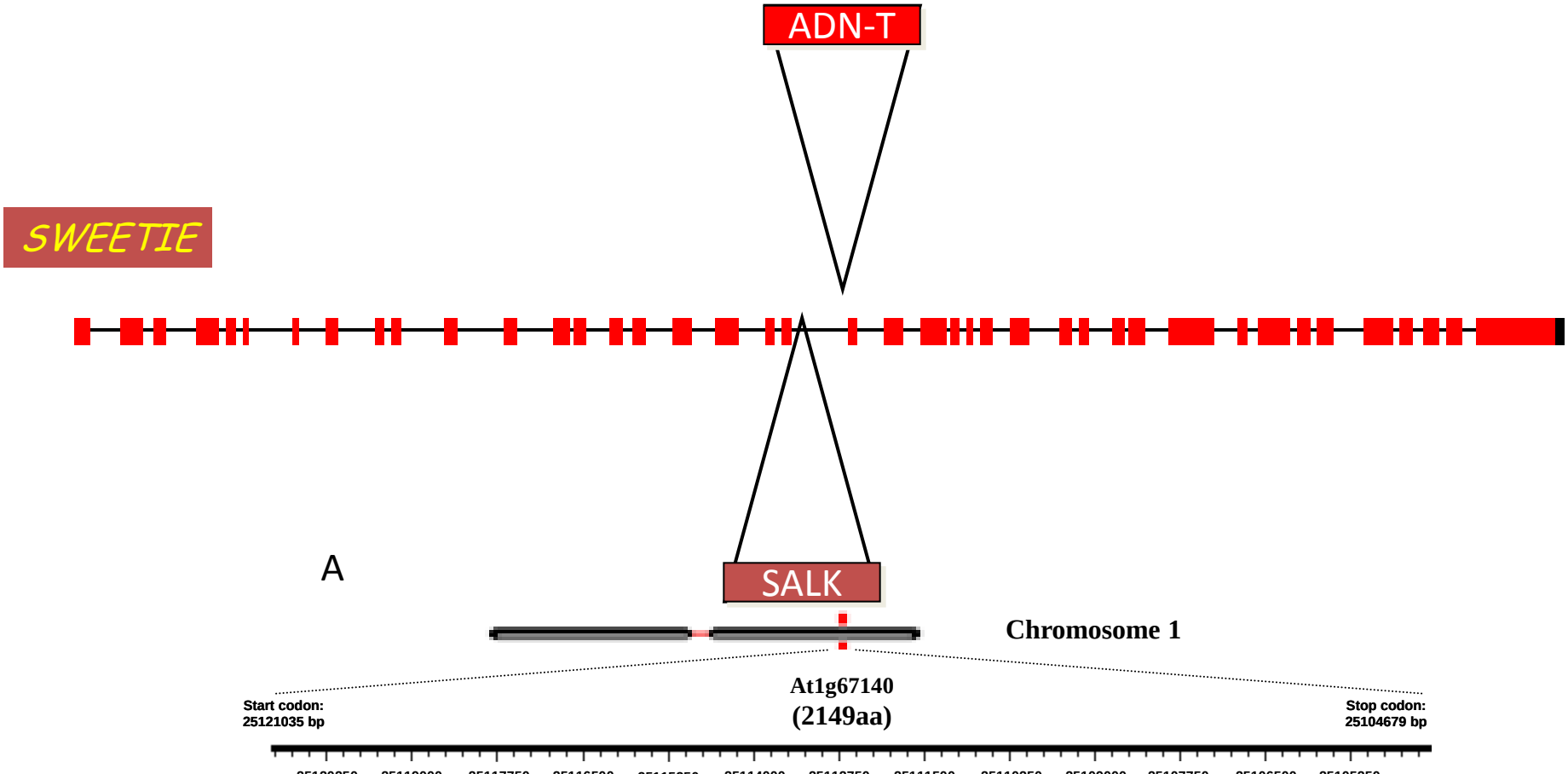
Stérilité

Trehalose

Le gène *sweetie*

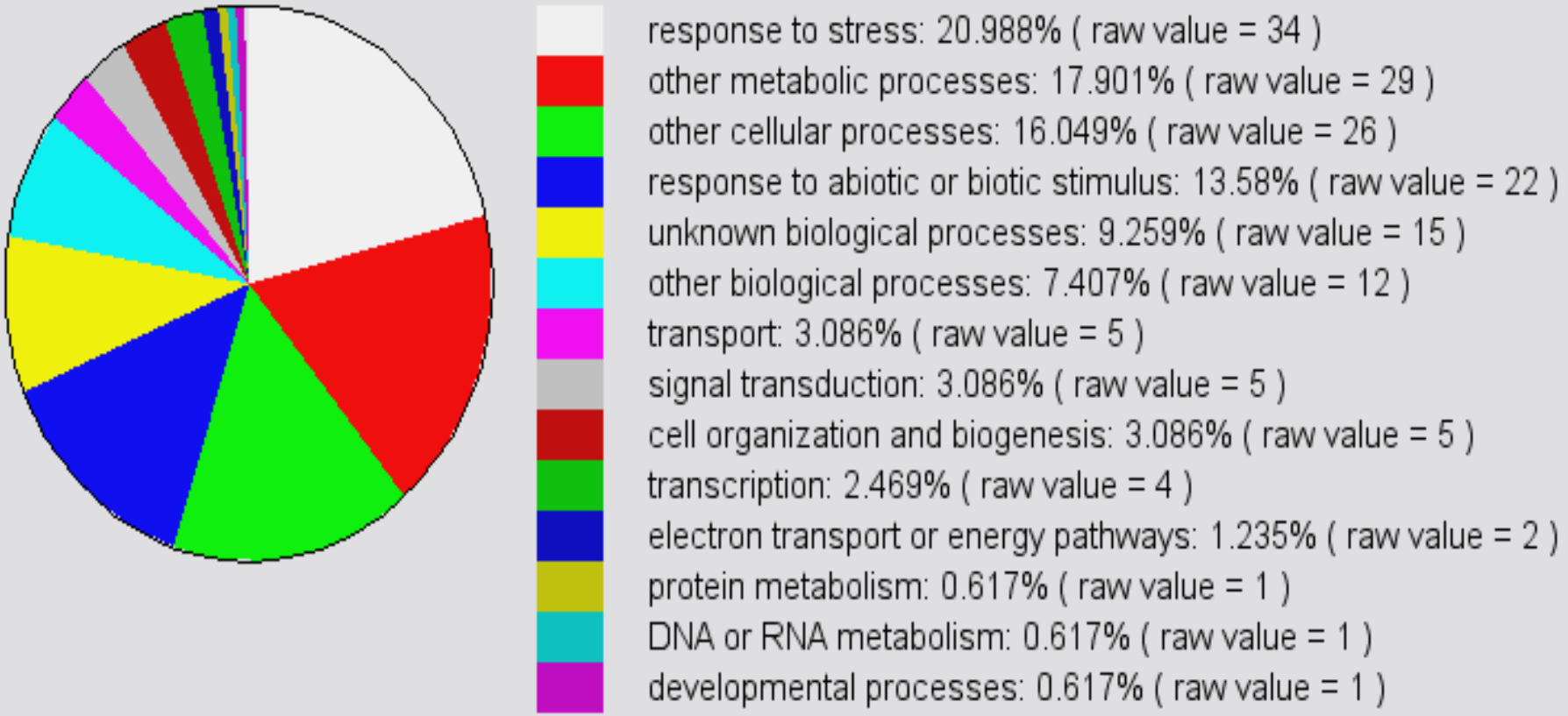
- ✓ Insertion localisée dans un gène de 16,3 kb, 41 exons, protéine: 2149 AA.
- ✓ protéine unique, fonction inconnue.
- ✓ Mutant SALK.

Veyres, N et al. 2008 - The *Arabidopsis sweetie* mutant… .**Plant Journal**, 55, 665-686



Functional Categorization by annotation for : GO Biological Process

Microarray analysis

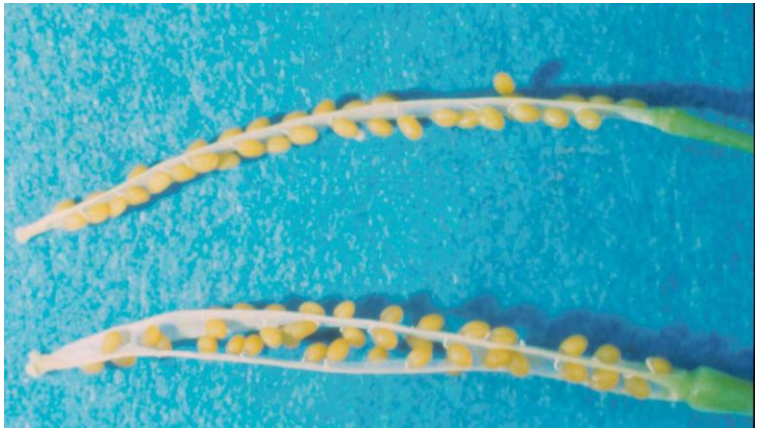


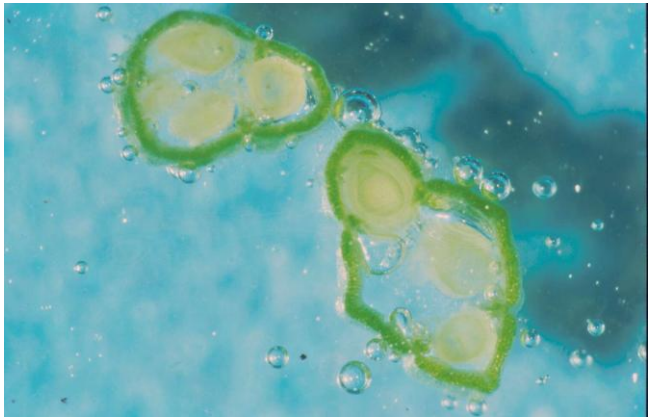
From the pie charts, over-representation of genes involved in "response to stress" and "response to abiotic or biotic stimulus" is evident.

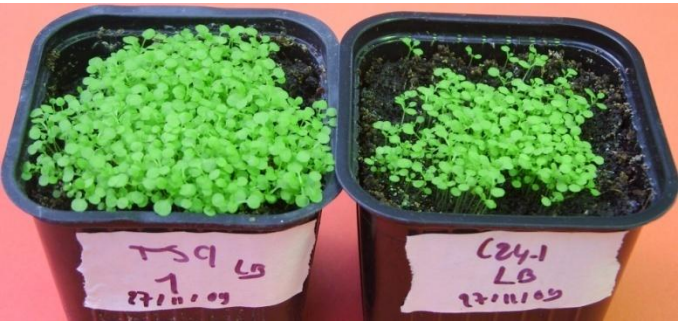
By Dr. M. Aono

Silique mutant and wild type with removed carpels

Silique mutant with extra carpel and septum in silique






TS9 LB
1
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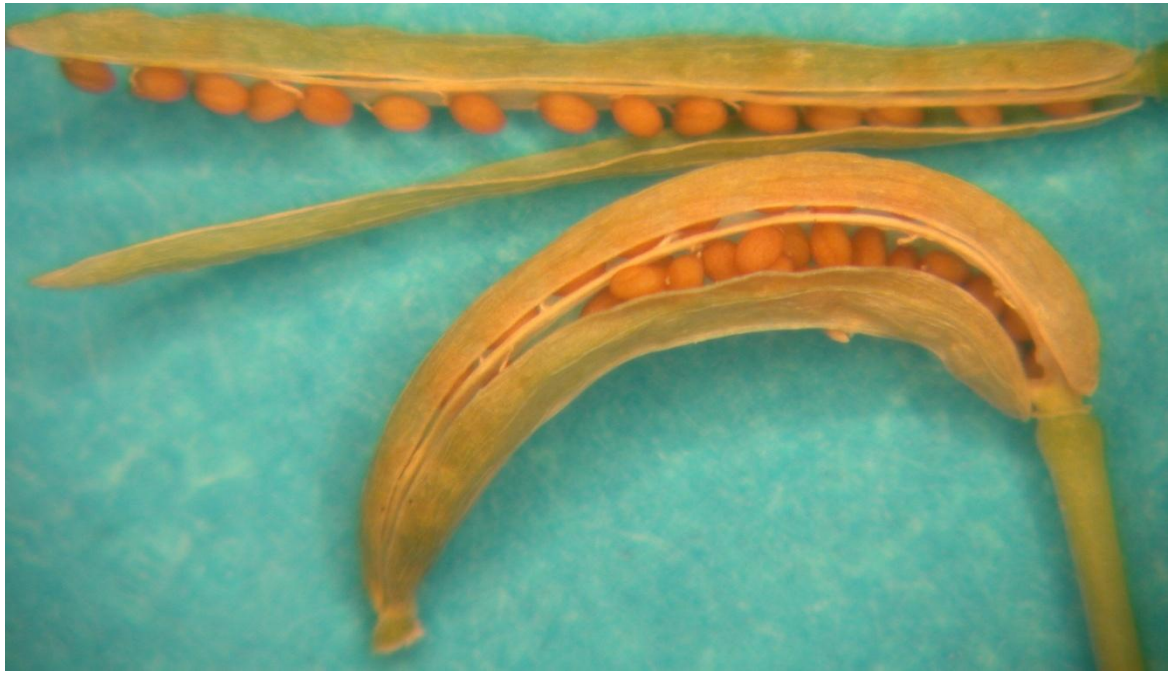
C24-1
LB
27.11.09



PLANTS: AT THE CELLULAR LEVEL

1- Cell division - essentially similar to animal cells

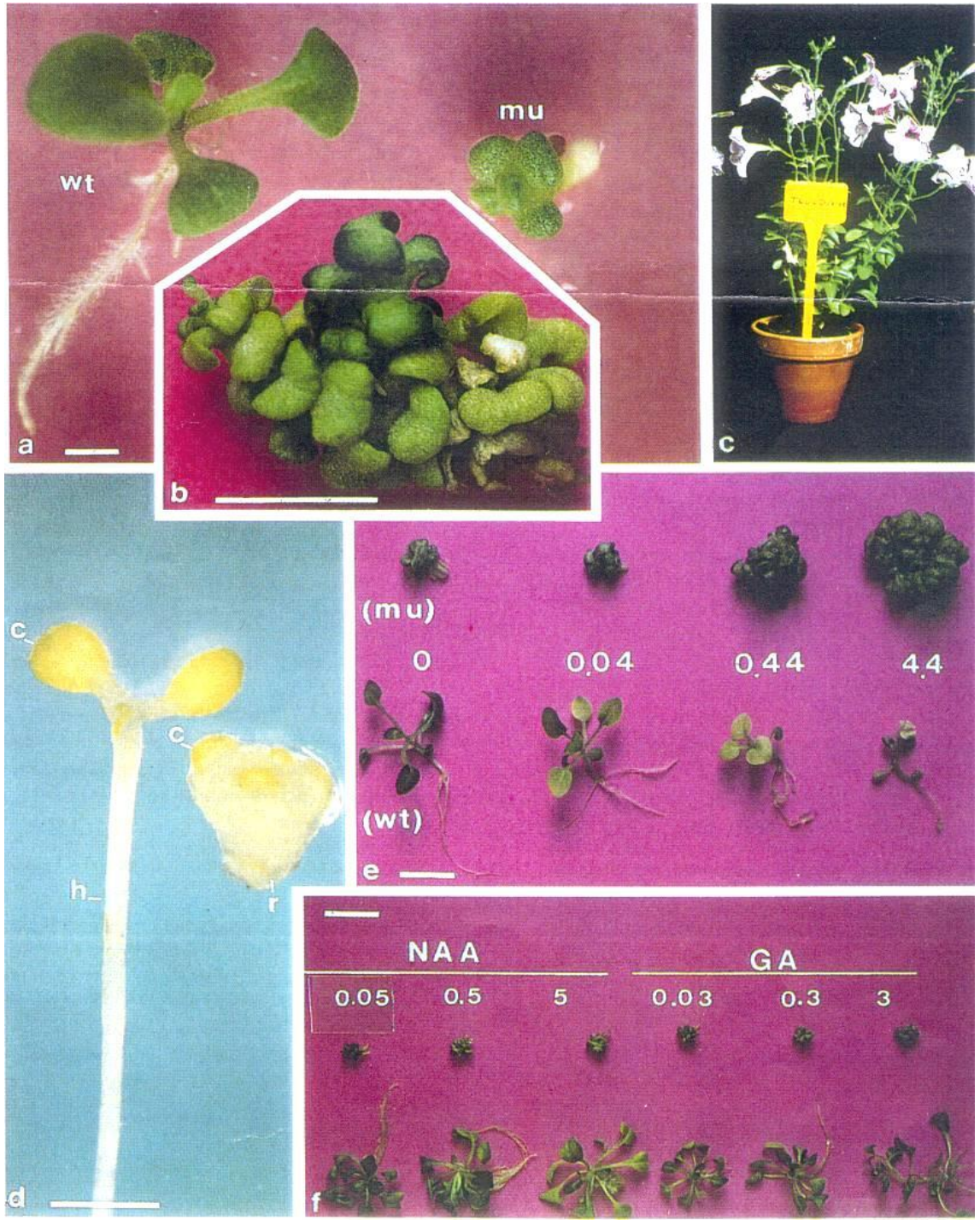
2- Cell enlargement - plant growth and final shape are due to cell elongation

3- Cell diferentiation - third phase along with enlargement

- The molecular control of cell elongation, one of the basic process of plant morphogenesis , is still largely unknown.

- After increase of osmotic pressure, elongation occurs in the direction of least resitance from the Cell Wall.

- The study of mutants provides a unique tool.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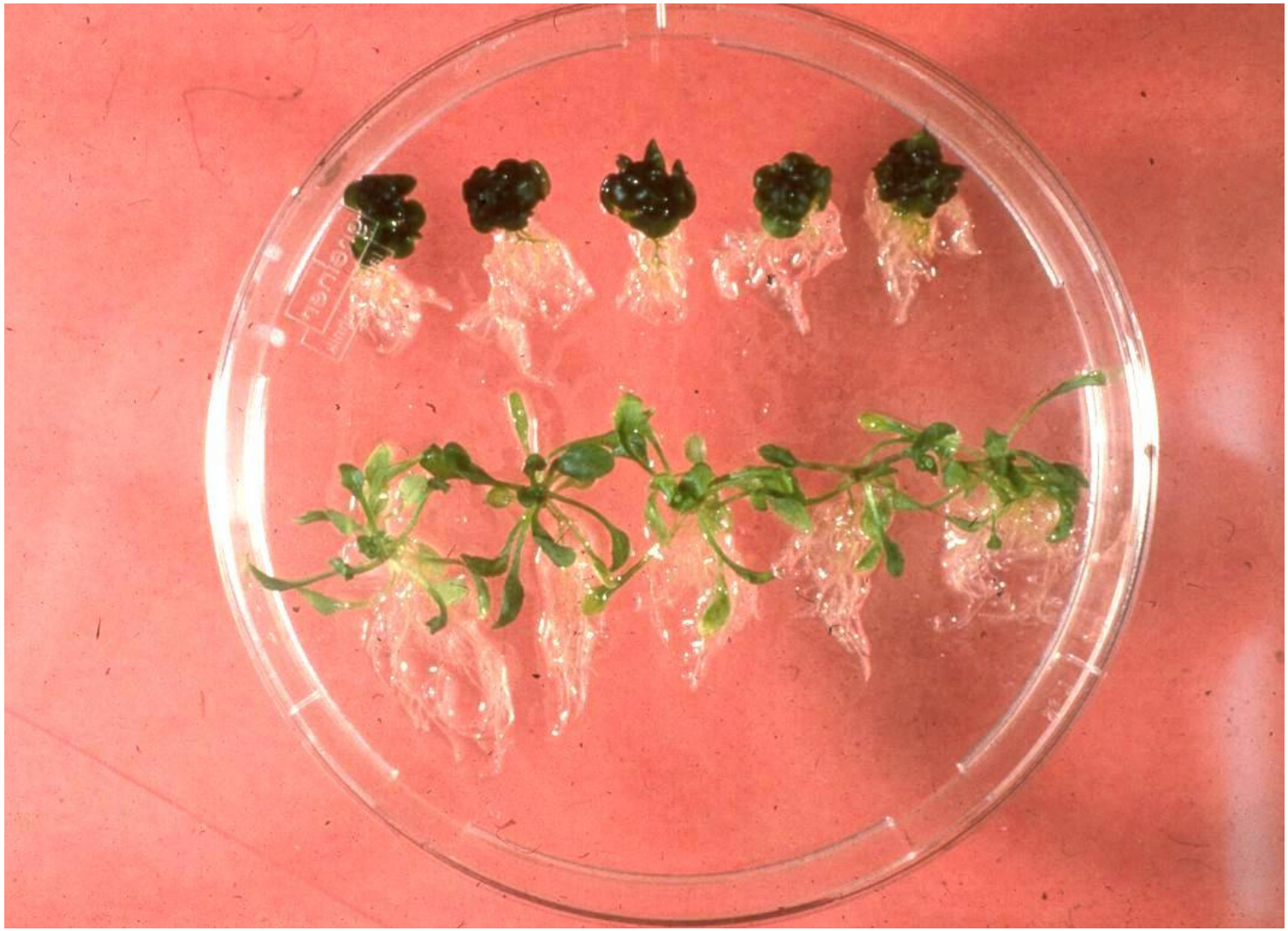

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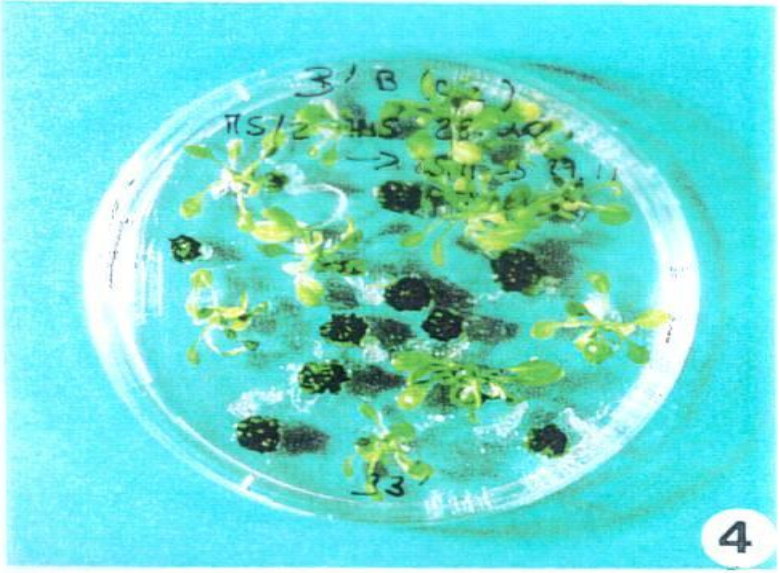

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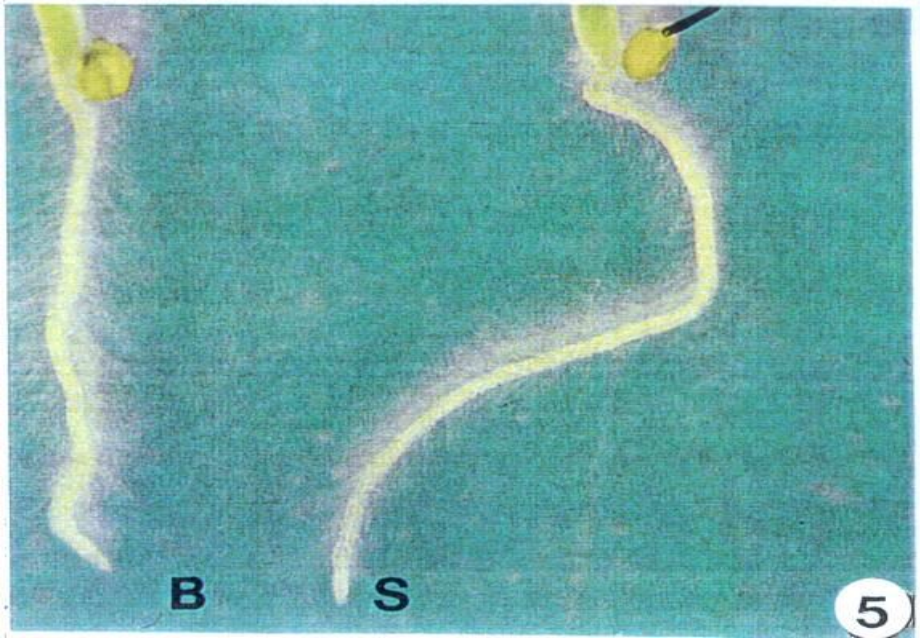

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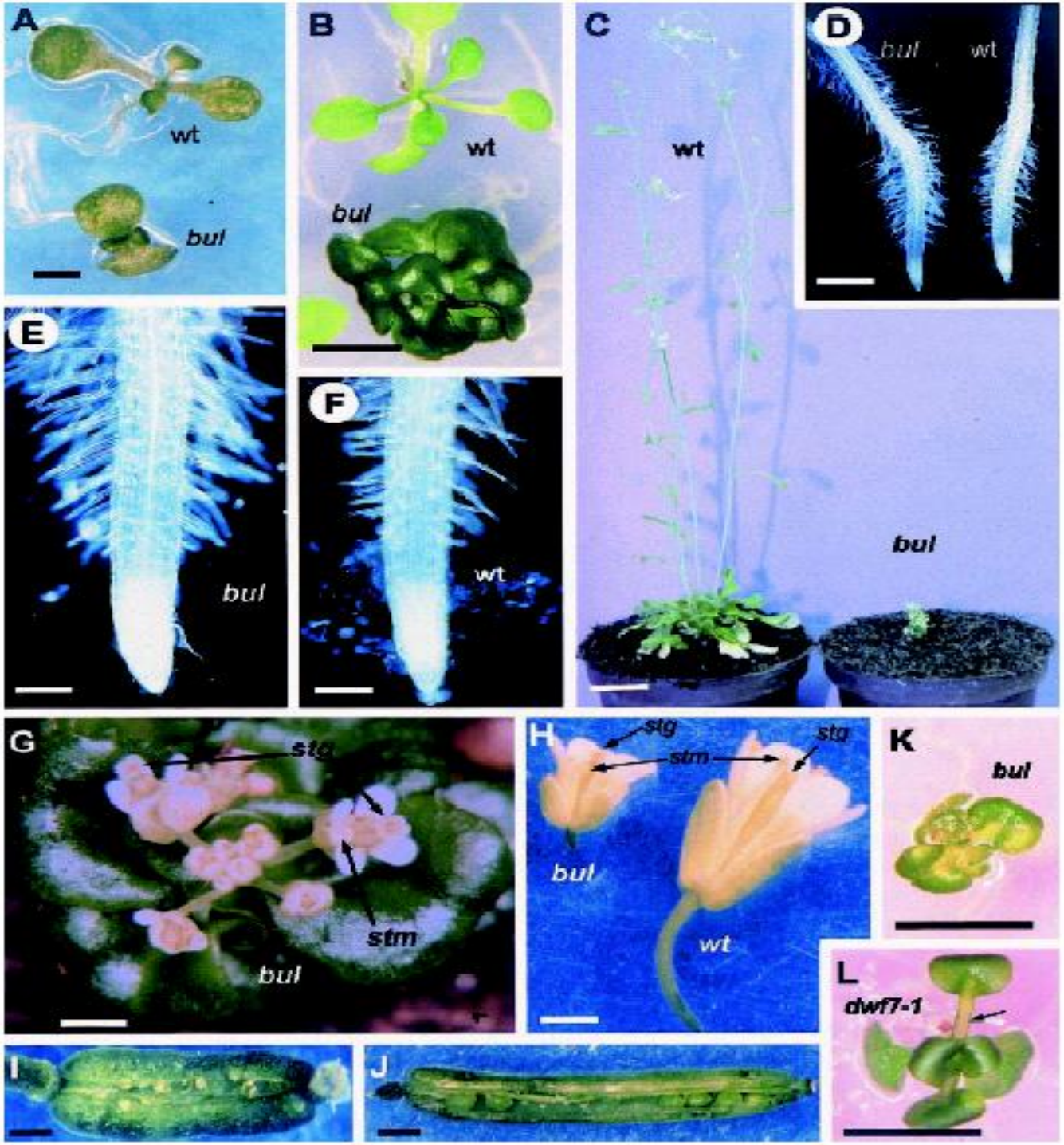
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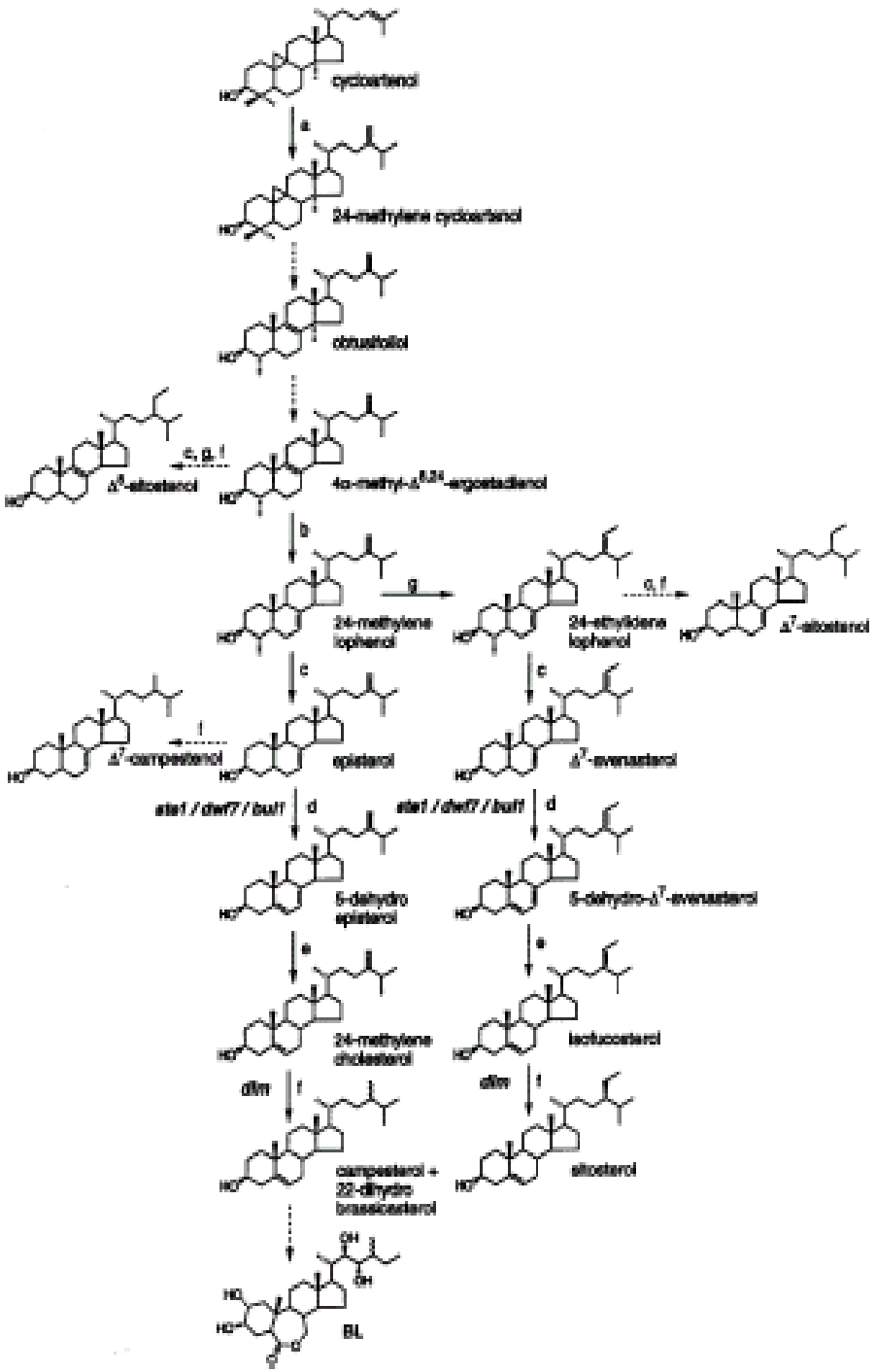


Fig. 3. Simplified biosynthetic pathway of sterols in wild-type and *bul1 Arabidopsis*. The *dashed arrows* indicate more than one biosynthetic step (not shown here). *a*, C-24 methylation; *b*, Δ^8-Δ^7-sterol isomerisation; *c*, C4-demethylation; *d*, C5(6)-desaturation; *e*, Δ^7-reduction; *f*, C24-isomerisation/reduction; *g*, C24^1-methylation

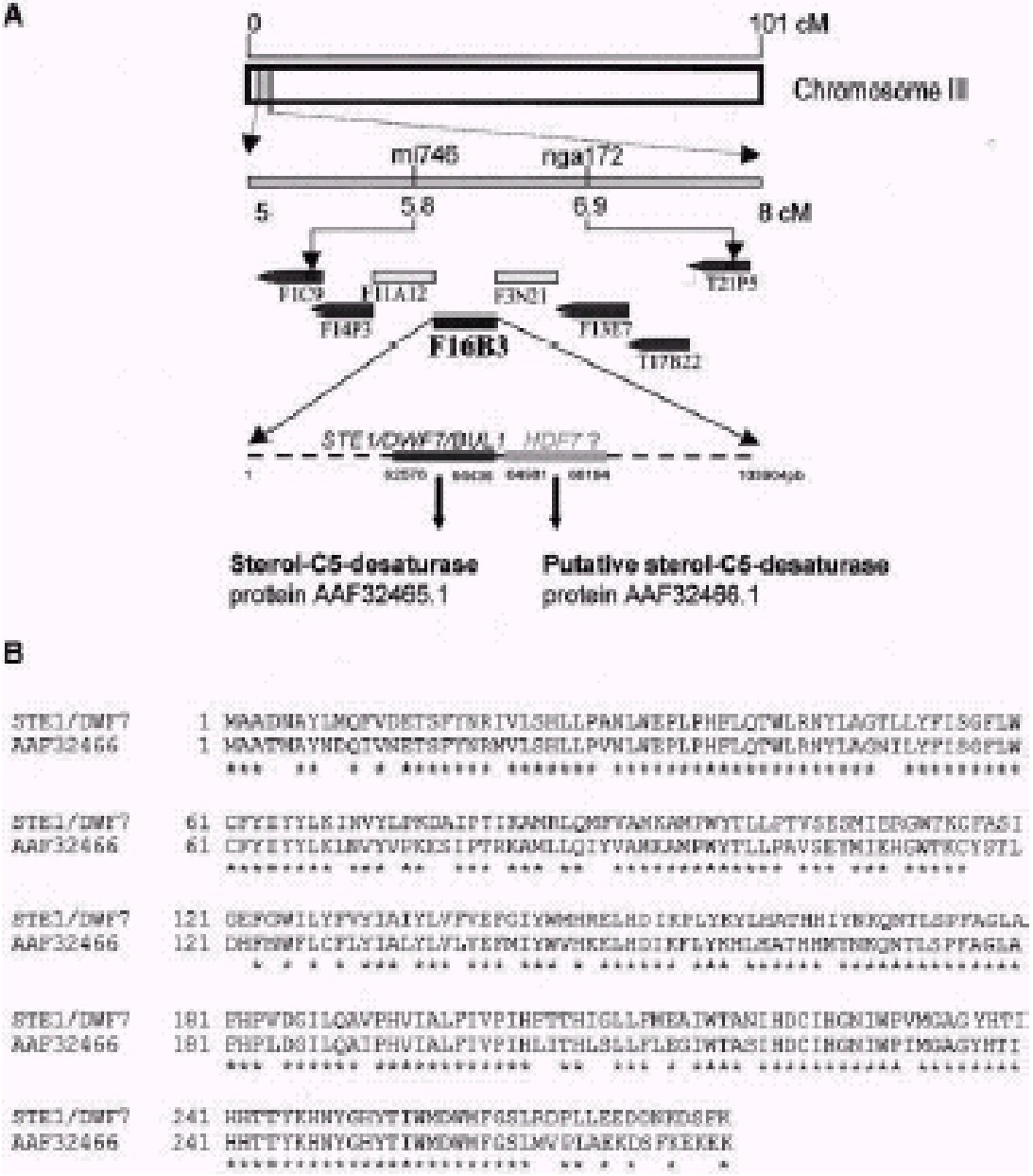


Fig. 7A,B. Schematic view of the duplicate genes of *Arabidopsis*, coding the Δ^7 sterol C-5 desaturase, and amino acid sequence alignment. **A** Both genes are mapped on the F16B3 BAC of chromosome III. The first gene, *STE1/DWF7*, was isolated by Gachotte et al. (1996) and Choe et al. (1999a). Sequencing of the *Arabidopsis* chromosome III revealed another gene with the same function, located just behind the *STE1/DWF7* gene. **B** Sequence alignment of STE1/DWF7 with this putative Δ^7 sterol C-5 desaturase (AAF32466) indicates that this new protein is 80% identical (90% similarity) in amino acid sequence to the STE1/DWF7. To explain the weaker phenotype of their *dwf7-1* mutant, Choe et al. (1999a) reported the existence of a second Δ^7 sterol C-5 desaturase gene, named *HDF7* (*HOMOLOG OF DWF7*). This *HDF7* gene may be this new gene identified in GenBank. Sterol analyses and an allelism test demonstrate the allelic nature of *bul1*, *ste1* and *dwf7*. The severity of the *bul1-1* mutant phenotype therefore suggests a complete null mutation in the *STE1/DWF7* gene function. To explain the extreme dwarf phenotype of *bul1-1*, we suppose that this mutation is affecting both duplicate genes. GenBank accession numbers for the STE1/DWF7 sequence are AAF324465, AAD12944 or AAD38120. *Asterisks* below amino acids indicate identity in both proteins. Sequence alignment was done using binary alignment from Expasy Proteomics Tools

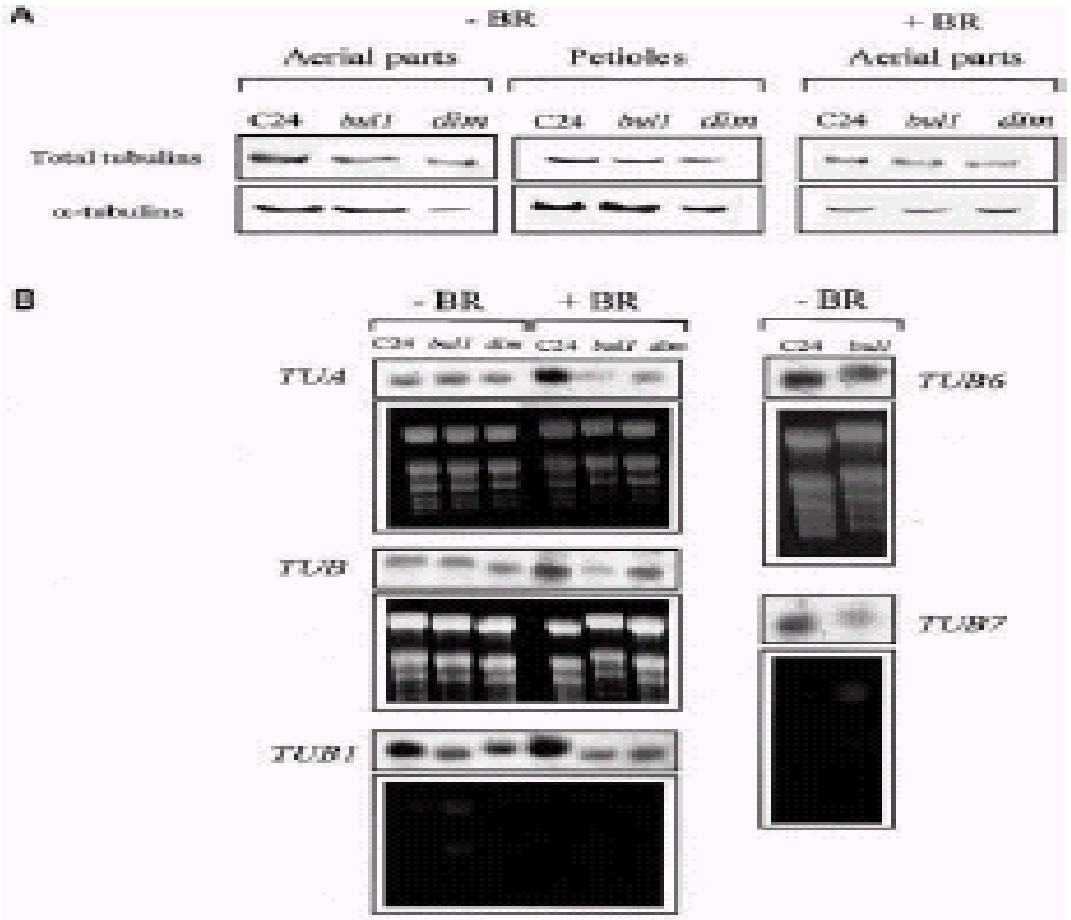
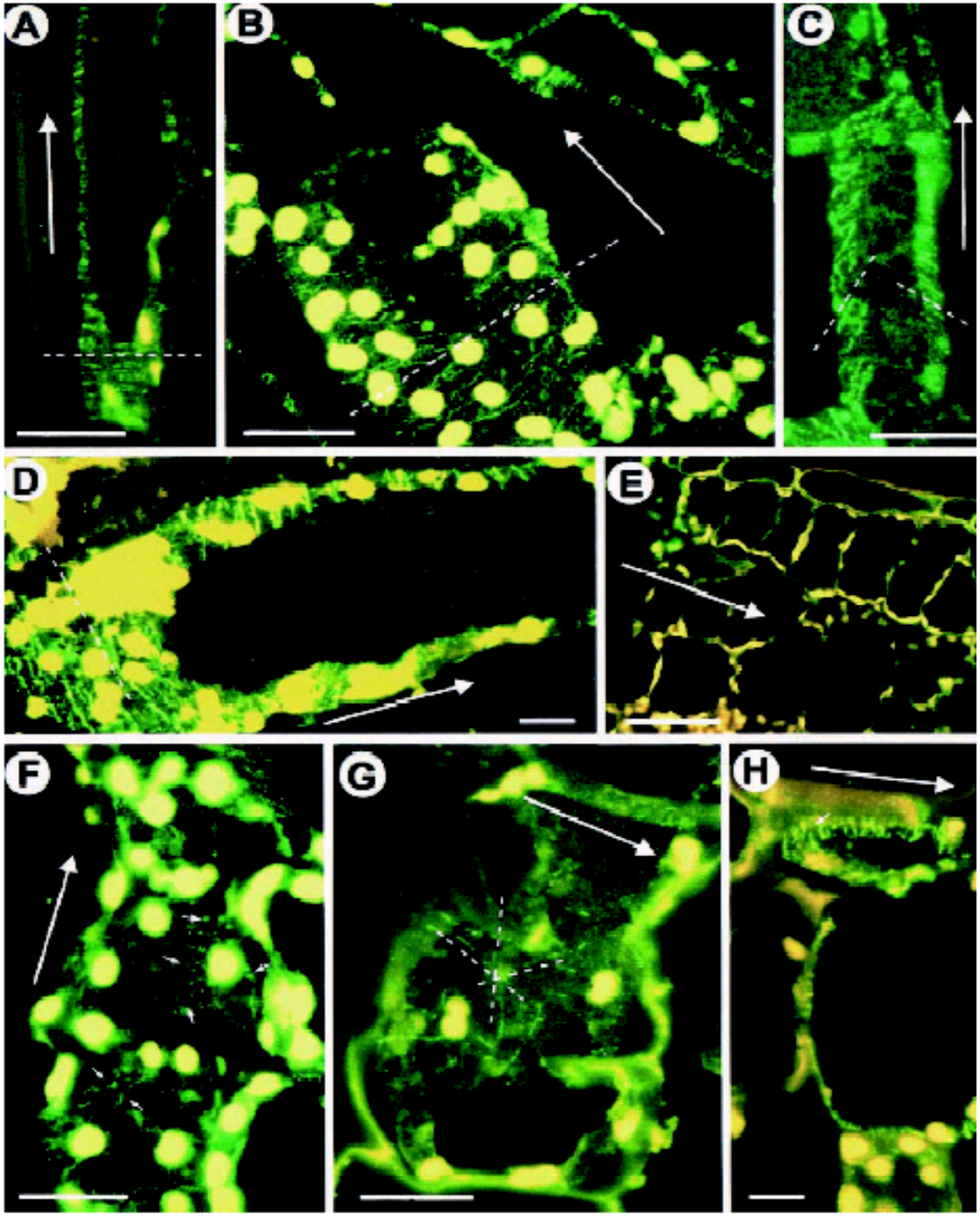


Fig. 3A,B. Western and Northern blot analyses of tubulin genes of *Arabidopsis thaliana* wild type, and *bul1-1* and *dim* mutants, and effect of brassinosteroids on tubulins and expression of tubulin genes. **A** Western blot analysis of total tubulins and α-tubulins. Total proteins were extracted from aerial parts or petioles of 15-d-old light-grown plants. The plants were grown for 3 d on basal medium without BR and then transplanted for 12 d on MS/2 medium supplemented (+BR) or not supplemented (−BR) with 1 μM of homoBR. Fifty micrograms of total proteins was loaded in each lane. **B** Northern blot analysis of tubulin genes. RNA was isolated from aerial parts of 15-d-old light-grown seedlings. As for Western blotting, the plants were grown for 3 d on basal medium without BR and then transplanted for 12 d onto MS/2 medium supplemented (+BR) or not supplemented (−BR) with 1 μM of homoBR. Hybridization was performed with gene-specific probes for tubulin genes (*TUA*: α-tubulin; *TUB*: β-tubulin; *TUB1*: β1-tubulin; *TUB6*: β6-tubulin; *TUB7*: β7-tubulin). The ethidium bromide-stained gel is shown to demonstrate that 15 μg of total RNA was loaded in each lane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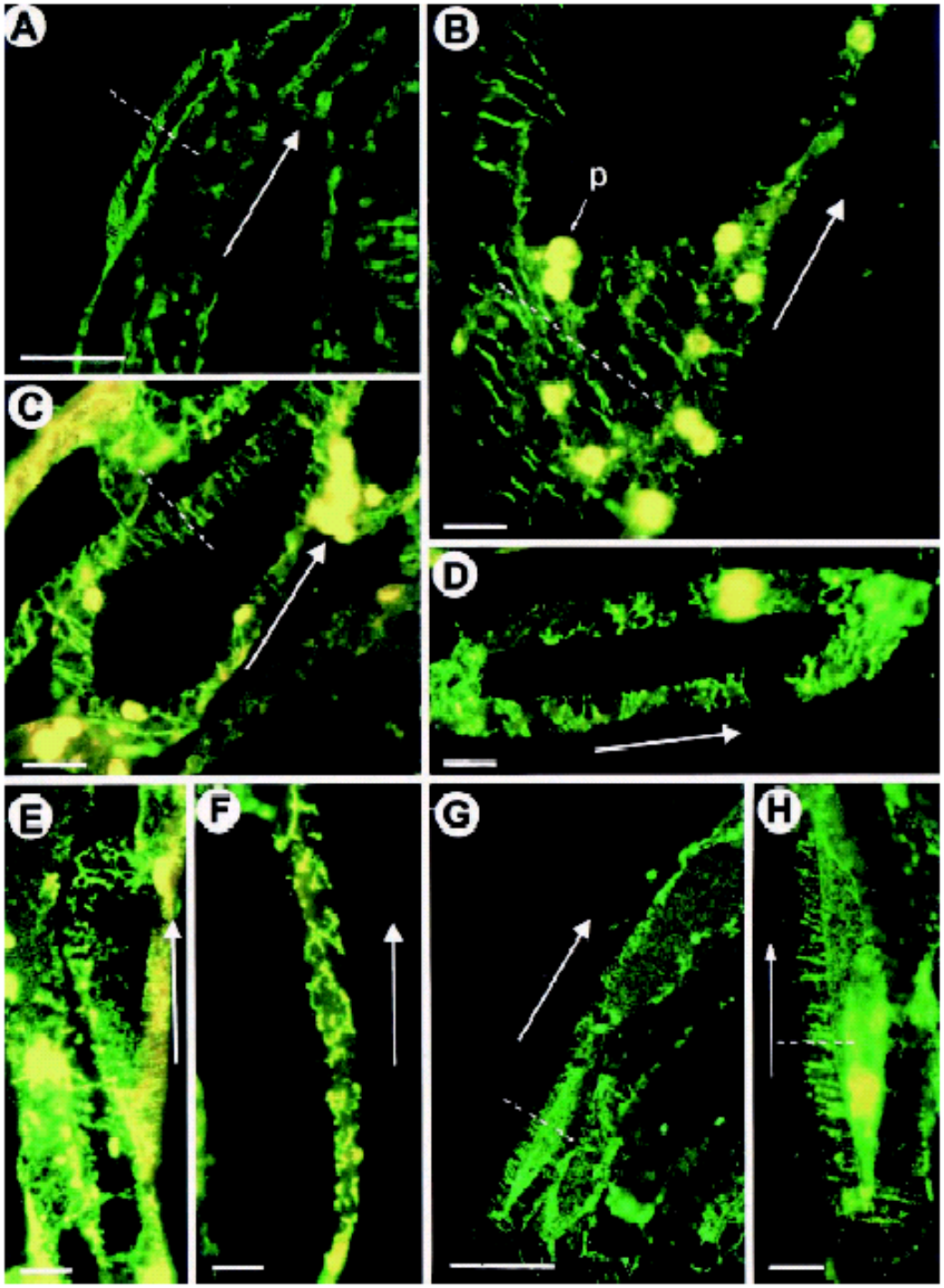
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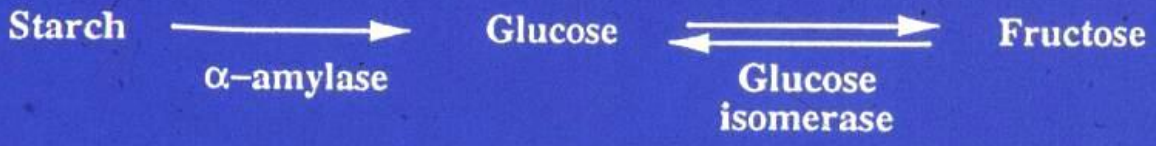
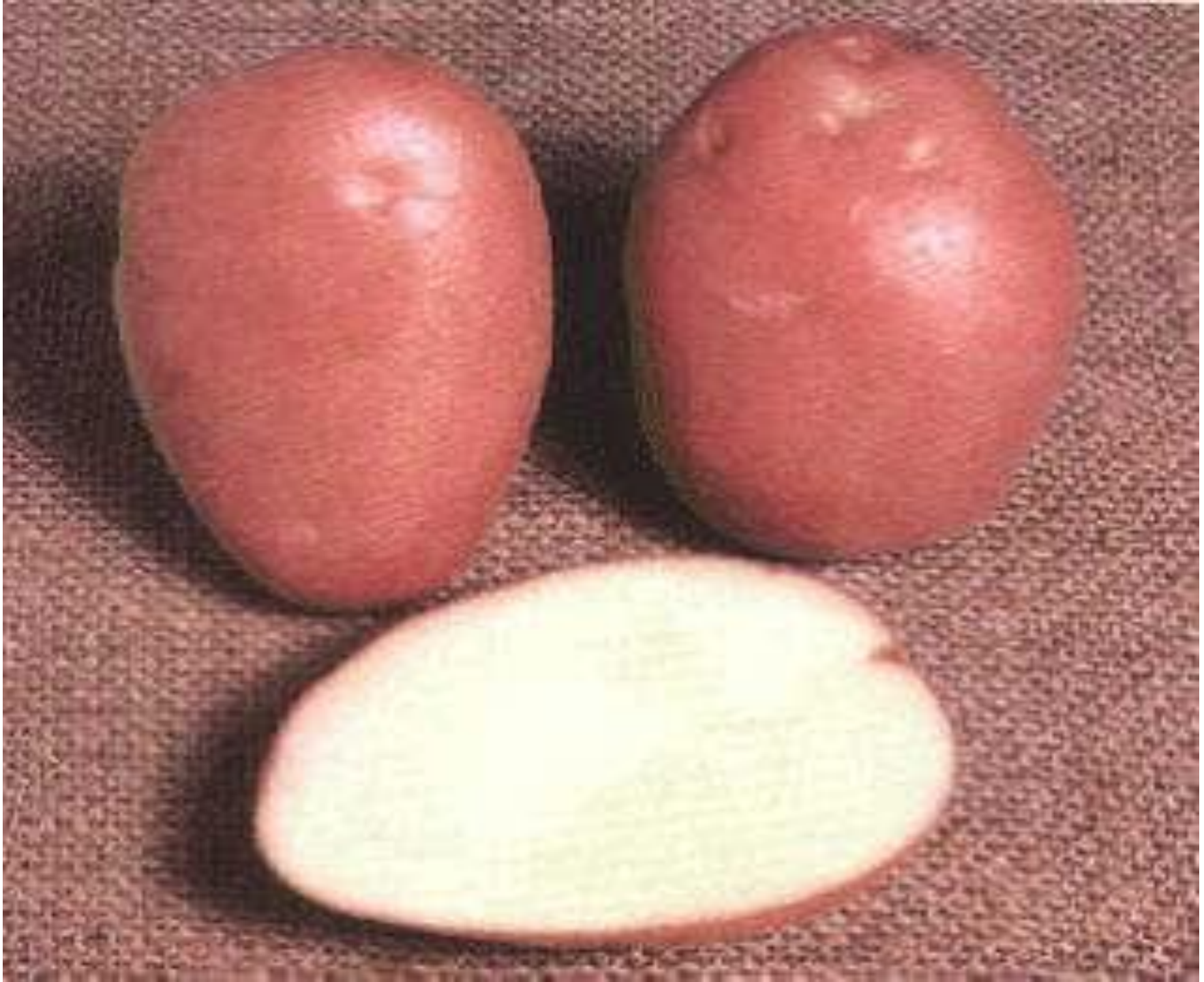
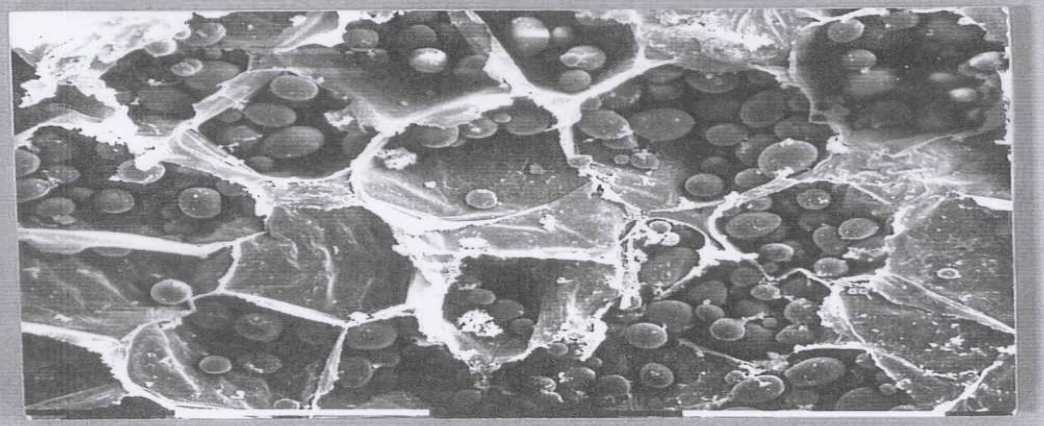
GENE FUSION TECHNOLOGY



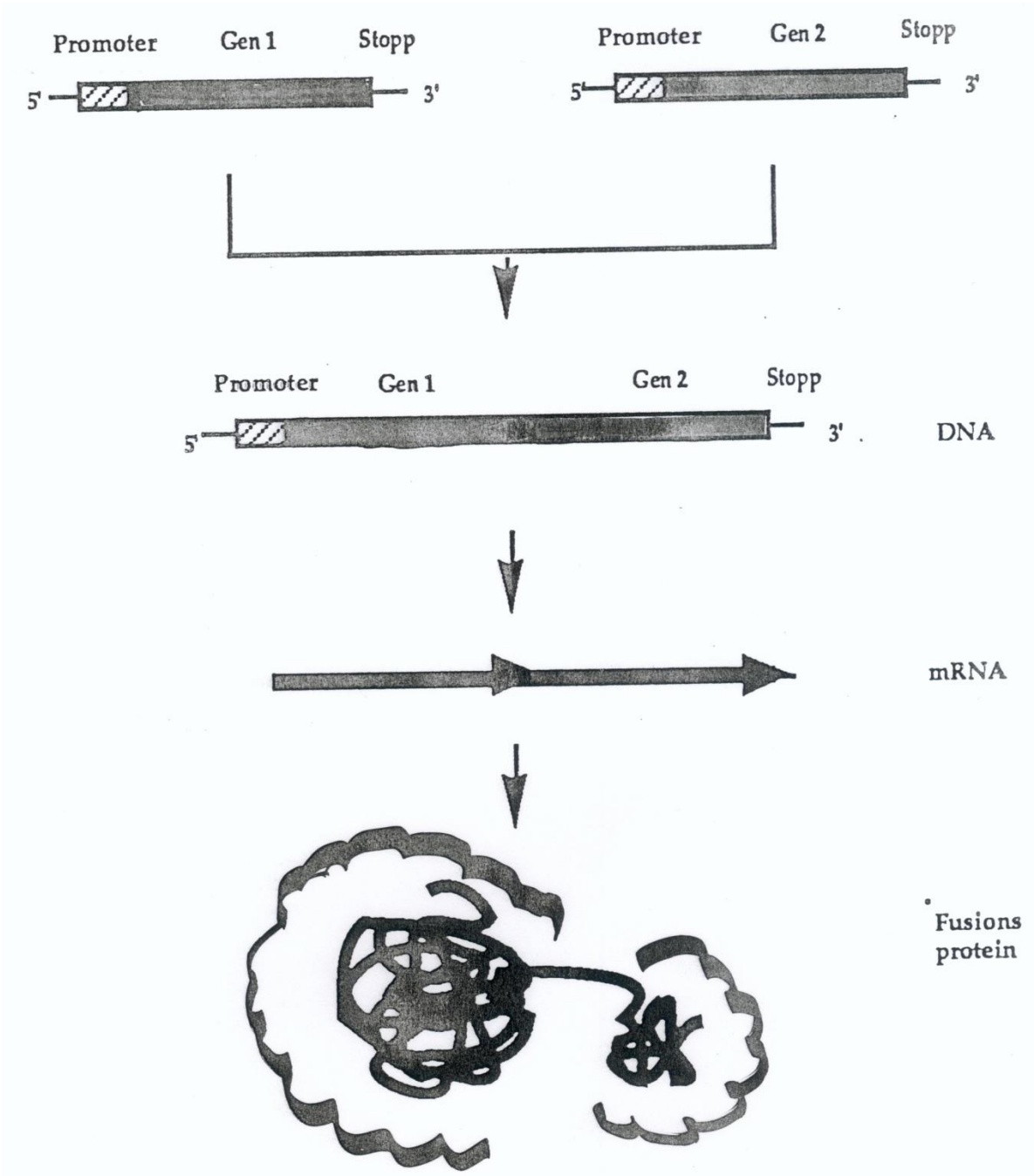


De larges lenticelles (L) sont observées en surface des microtubercules cultivés en présence de dose élevée de saccharose. Site d'attachement (sa). Grossissement 20.4



Cellules centrales de microtubercule obtenu avec 80 g/l de saccharose. Grossissement 300

A bifunctional enzyme can be prepared by joining the structural genes of two enzymes; the translational stop signal at the 3’- end of the first gene is removed and ligated in –frame to the ATG start codon of the second gene. Upon fusion, the novel chimaeric gene encodes a single polypeptide chain carrying both active sites.


Promoter
Gen 1
Stopp
5'
3'
Promoter
Gen 2
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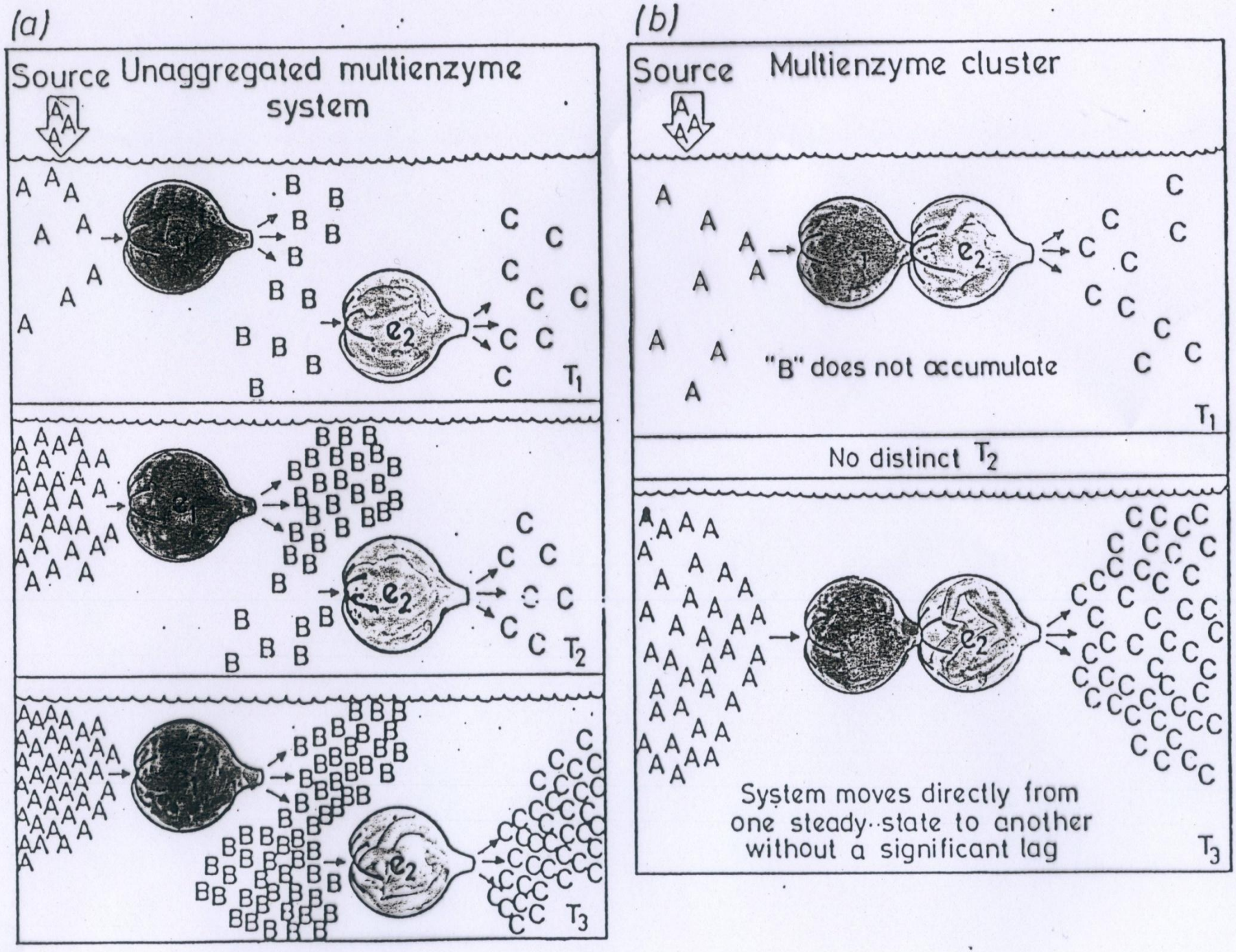
5'
3'
Promoter
Gen 1
Gen 2
Stopp
5'
3'
DNA
mRNA
Fusions
protein

WHY GENE FUSION ?

1. Proximity effects (transient time, steady-state rate).
2. Purification is simplified.
3. Homogeneous conjugates (1:1 ratio).
4. Protein stabilization.


(a)
Source
Unaggregated multienzyme system
A
B
C
e_1
e_2
T_1
T_2
T_3
(b)
Source
Multienzyme cluster
"B" does not accumulate
T_1
No distinct T_2
System moves directly from one steady-state to another without a significant lag
T_3

Gene Fusion Encoding the Thermostable Enzymes :

α - Amylase from *Bacillus stearothermophilus*

Glucose Isomerase from *Thermus thermophilu*

– This Chimeric Gene under GBSS : Granule – Bou
Starch Synthase promoter

-Only active at high temp. > 65, 80℃

-Fructose production > 20 times

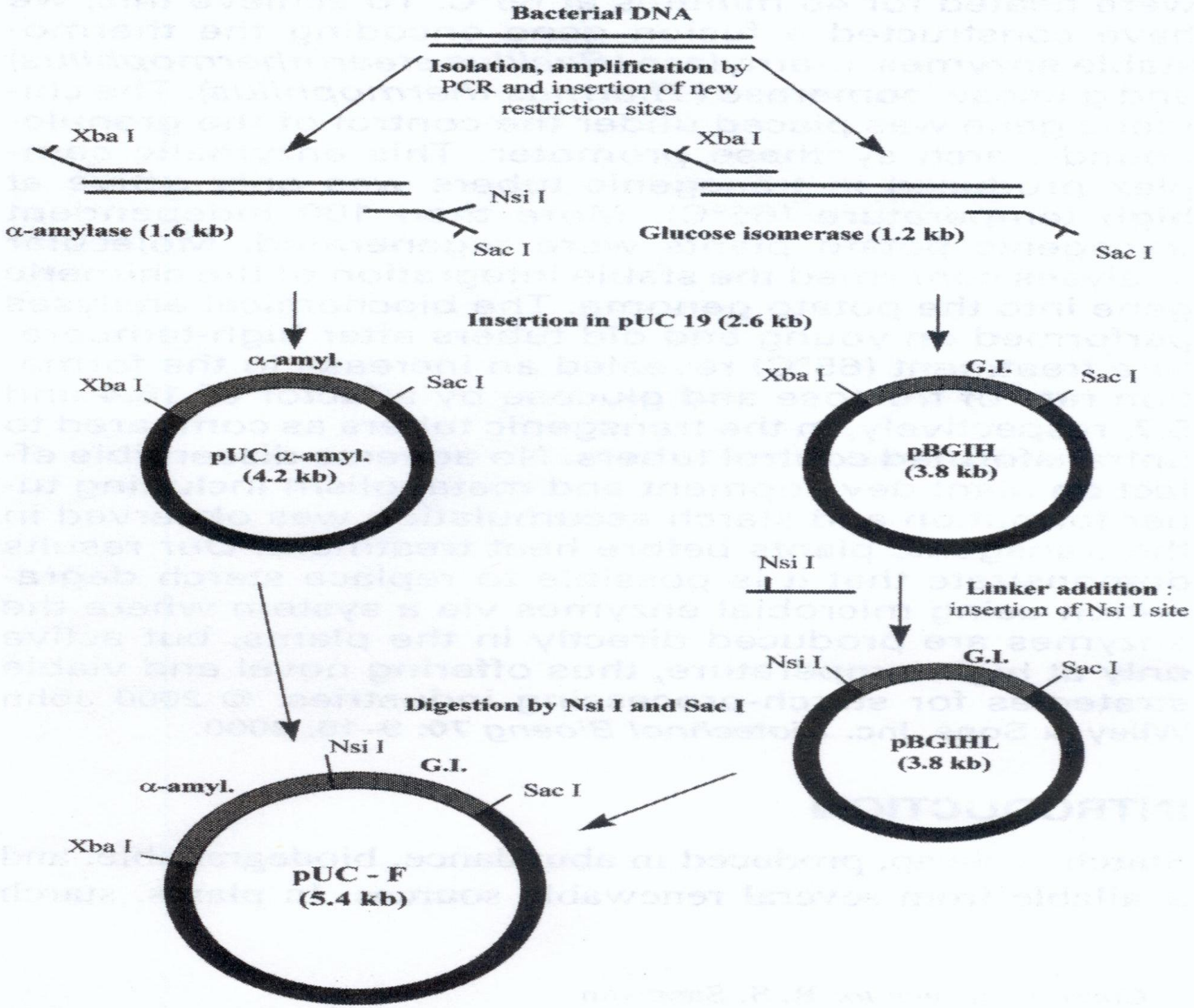
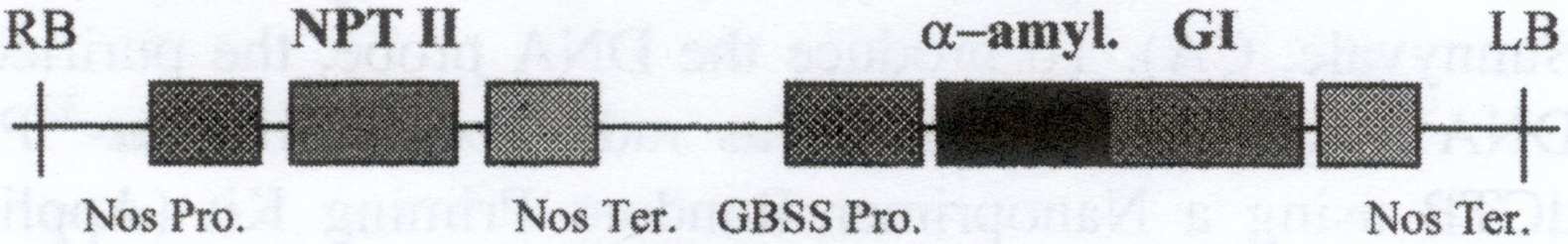


Figure 1. Construction of the plasmid pUC-F containing α-amyl/GI chimeric gene.



(a)

V R W M H P R M G H H H H H H V D M Y E

(b)

Figure 2. Construction of the fused α-amyl/GI gene complex. (A) Schematic representation of the T-DNA of pHO3-F. RB: right border; LB: left border; NPT II: neomycin phosphotransferase gene; α-amyl.: α-amylase gene; GI: glucose isomerase gene; Pro.: promoter. NPT II is under the control of the nopaline synthase promoter (Nos). α-amyl/GI fused gene is under the control of the GBSS (granule-bound-starch synthase) promoter as detailed in the Material and Methods sections. (B) Amino acid sequence of the linker region between the two genes. The amino acid sequence between the two genes (α-amyl and GI genes) is underlined.

Over the 5 years period:

- Generated >250 independant transgenic potato plants/lines.
- Morphometric analysis on 250 plants.
- Molecular analysis (Copy number, gene dosage effect etc) were done on 150 plants.
- 50 plants were selected for gene expression and fructose production analysis.

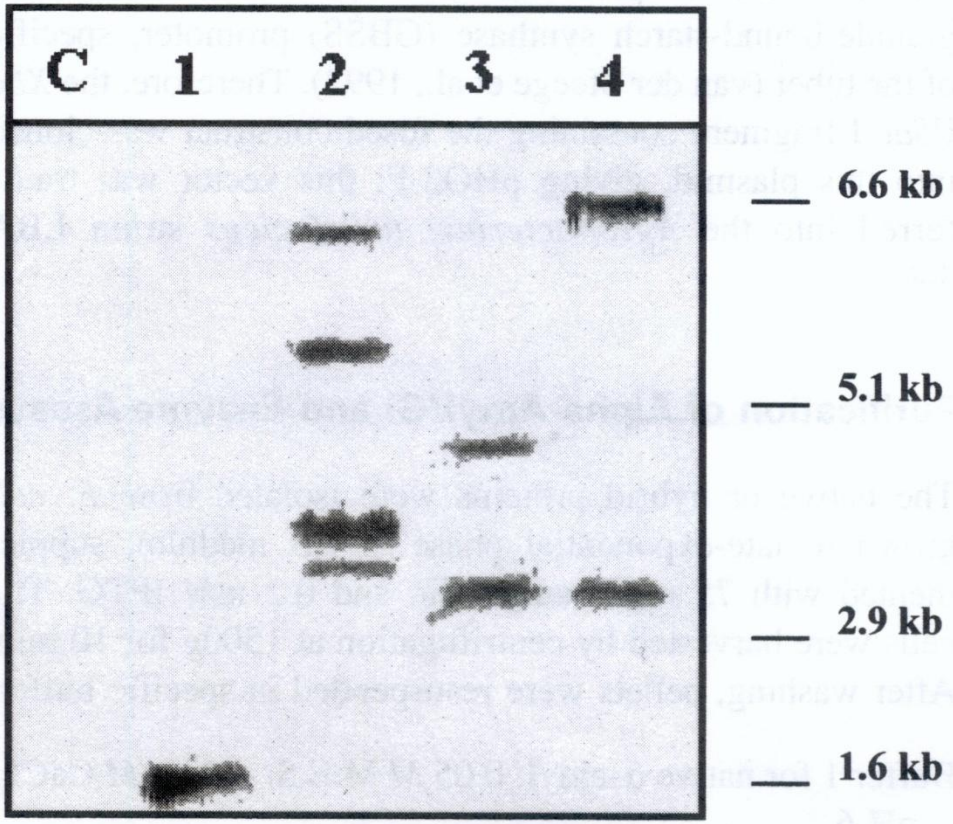


Figure 3. Southern blot analysis of total genomic DNA isolated from leaves of transformed plants (b7, b30, b36, b48) and an untransformed control plant (c). Genomic DNA was digested with *Hind* III and electrophoresed on 0.8% agarose gel as described in Material and Methods. The GI gene was used as probe. Lane C: DNA from untransformed control potato plant. Lane 1–4: Total DNA isolated from four independent transgenic plant lines (b7, b30, b36, and b48). All transformants contain band(s) hybridizing with the GI probe. b30, b36, and b48 present more than one band indicating that these plants have integrated more than one copy of the transgene (4, 2, and 2 copies, respectively).

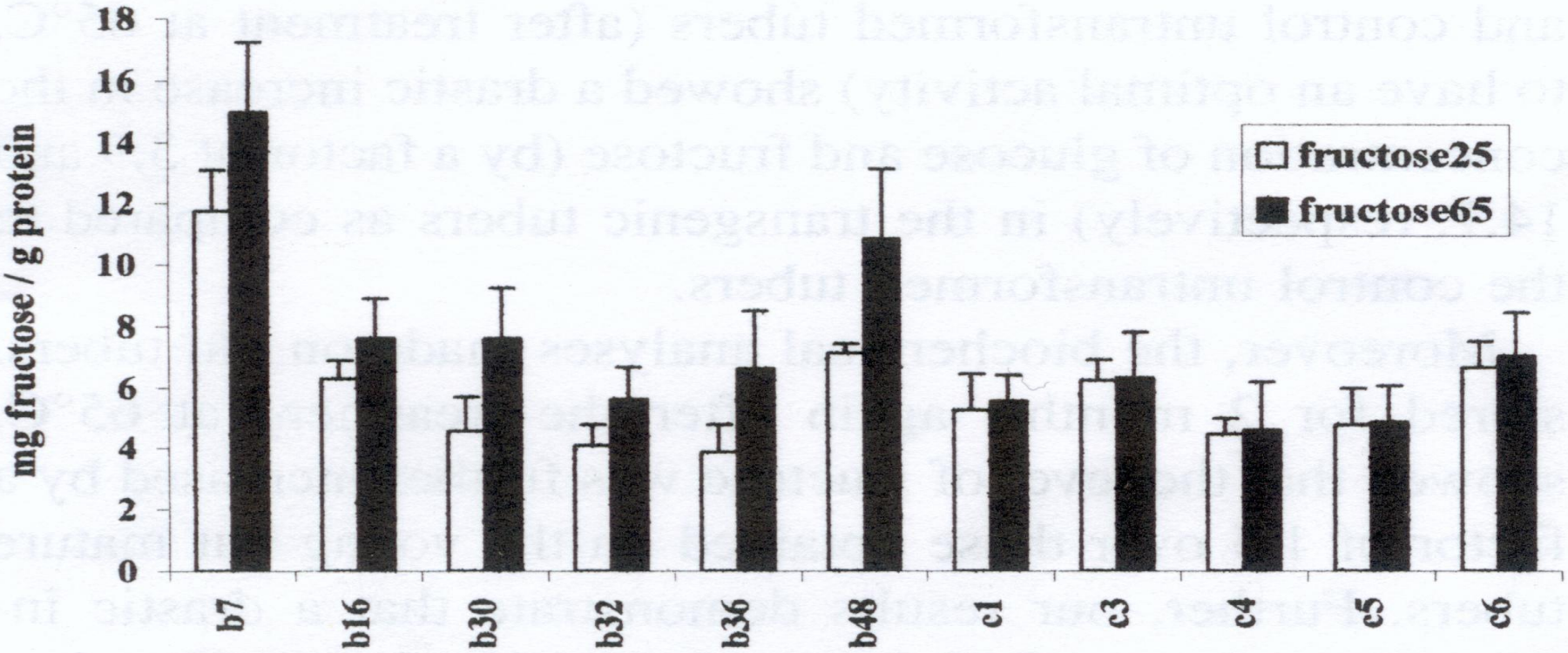


Figure 5. Measurement of fructose content in transgenic and control tuber samples before and after heat treatment at 65°C. Fructose content at 25°C and after heat treatment at 65°C for 45 min was determined in young tubers from transformed (b) and untransformed control (c) potato tuber extracts as described in Material and Methods. Each number refers to an independent transgenic plant line. The data are presented in mg fructose/g protein.

Engineering Direct Fructose Production in Processed Potato Tubers by Expressing a Bifunctional Alpha-Amylase/Glucose Isomerase Gene Complex

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biotechnology : Sweet potato

DAVID ADAM

GM researchers in France have built a potato that produces massive amounts of fructose -- the sweetest of all natural carbohydrates -- when mashed and heated. The sugary spuds could one day be harvested to produce the sweeteners used in everything from diabetic chocolate to soft drinks.

Genes coding enzymes that convert starch to sugars can be spliced into the potato plant, Rajbir Sangwan of the Université de Picardie Jules Verne in Amiens, France, and his colleagues now report. All potatoes produce and store starch; the enzymes in the new GM variety only convert it to fructose when the potato is heated. So normal potato metabolism and development is not affected.

Fructose and glucose look very similar but chemically they behave differently -- fructose is often used as a diabetic sweetener, for instance, because it does not seem to significantly increase the demand on insulin. Some diet products also use it because less of it (so fewer calories) is needed to sweeten food compared with table sugar.

Millions of tons of high-fructose syrup are produced each year in large-scale chemical plants. Starch extracted from maize is combined with water to produce glucose, which is then converted to fructose. In recent years, enzymes produced by bacteria have speeded up the process.

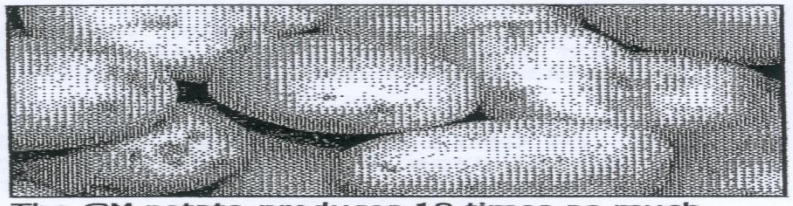
But Sangwan's team goes one better -- bundling the entire conversion, enzymes and all, inside a spud. They go from chemical plant to potato plant. And scaling up the process is no problem, Sangwan points out. Just grow more potatoes.

His team modify the potato by inserting genes coding the enzymes 'alpha amylase' and 'glucose isomerase'. The first breaks down starch to glucose, the second changes glucose to fructose.

"The possibility of modifying the carbohydrate composition should be particularly valuable to the food industry," Sangwan says. Potatoes heated to different temperatures have different glucose/fructose concentrations because of varying glucose isomerase

Monday, 9 October, 2000, 13:56 GMT 14:56 UK

GM 'mashed potato' revealed



The GM potato produces 19 times as much fructose as an unmodified potato

French scientists have developed a GM potato that releases sugars when it is cooked.

Two genes encoding enzymes capable of converting the starch stored in potatoes into fructose have been added to the plant. When the potato is heated and mashed, fructose is released, turning the humble spud into a miniature chemical factory.

The researchers believe the GM potato could revolutionise the food industry, enabling fructose to be produced more cheaply.

A variety of products, including some sweeteners, diabetic foods and soft drinks, contain fructose.

But the researchers warn that anti-GM feeling in Europe may delay future applications of the technology.

Food products

Current methods for producing the huge quantities of fructose syrup needed each year rely on large-scale industrial processes using starch from maize.

The starch is converted into fructose in a chemical plant using bacterial enzymes that can function at high temperatures.

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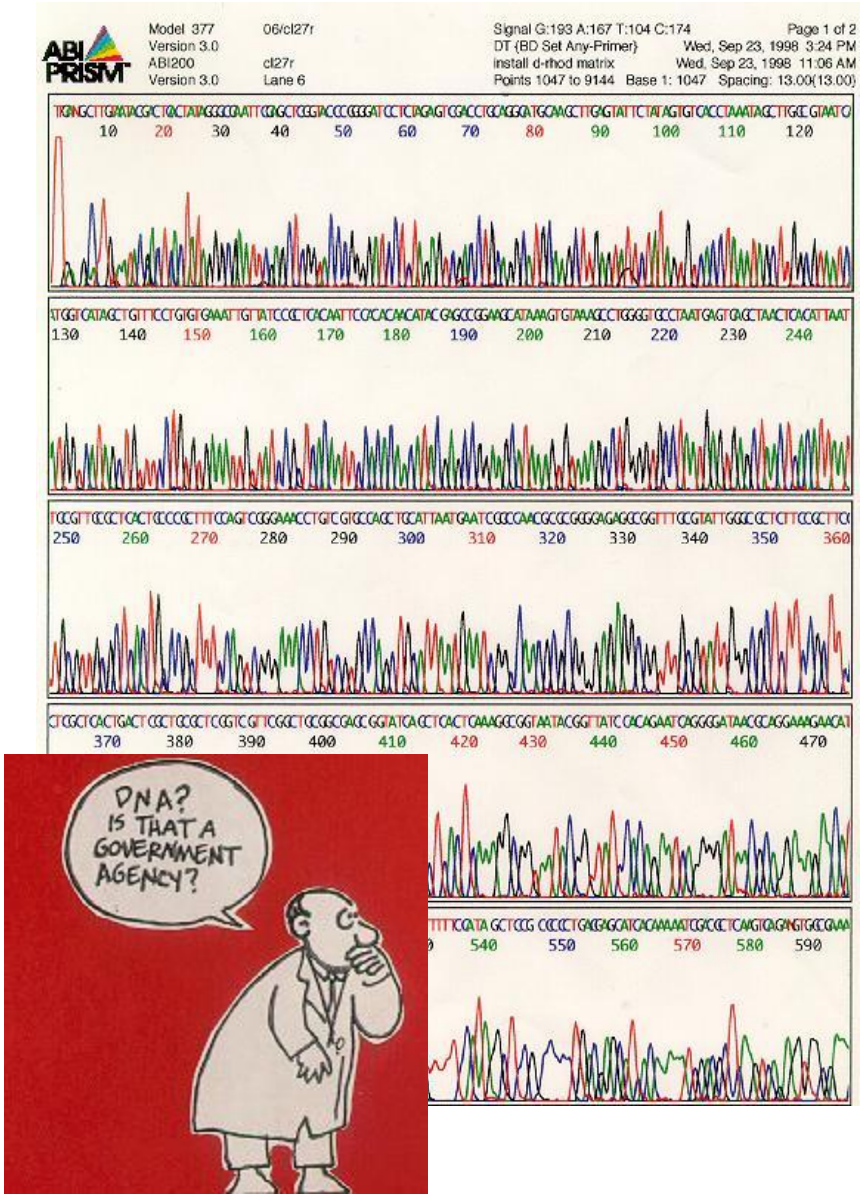
'They're the controls –
potato-free potatoes !'
CONTROL
HIGH STARCH
AMYLOSE FREE
GMG

Complementarity of Biotechnology & Breeding.

- Many of the potential plant-related benefits from modern biotechnologies rest on principle that they enable unique and/or faster genetic improvements.

Conclusions

How can we use new information ?



What is the most appropriate breeding technology?

- How can new technologies enhance the all ready proven techniques of traditional breeding without diverting resources?
- How important are trans-genes to the future of crop improvement?

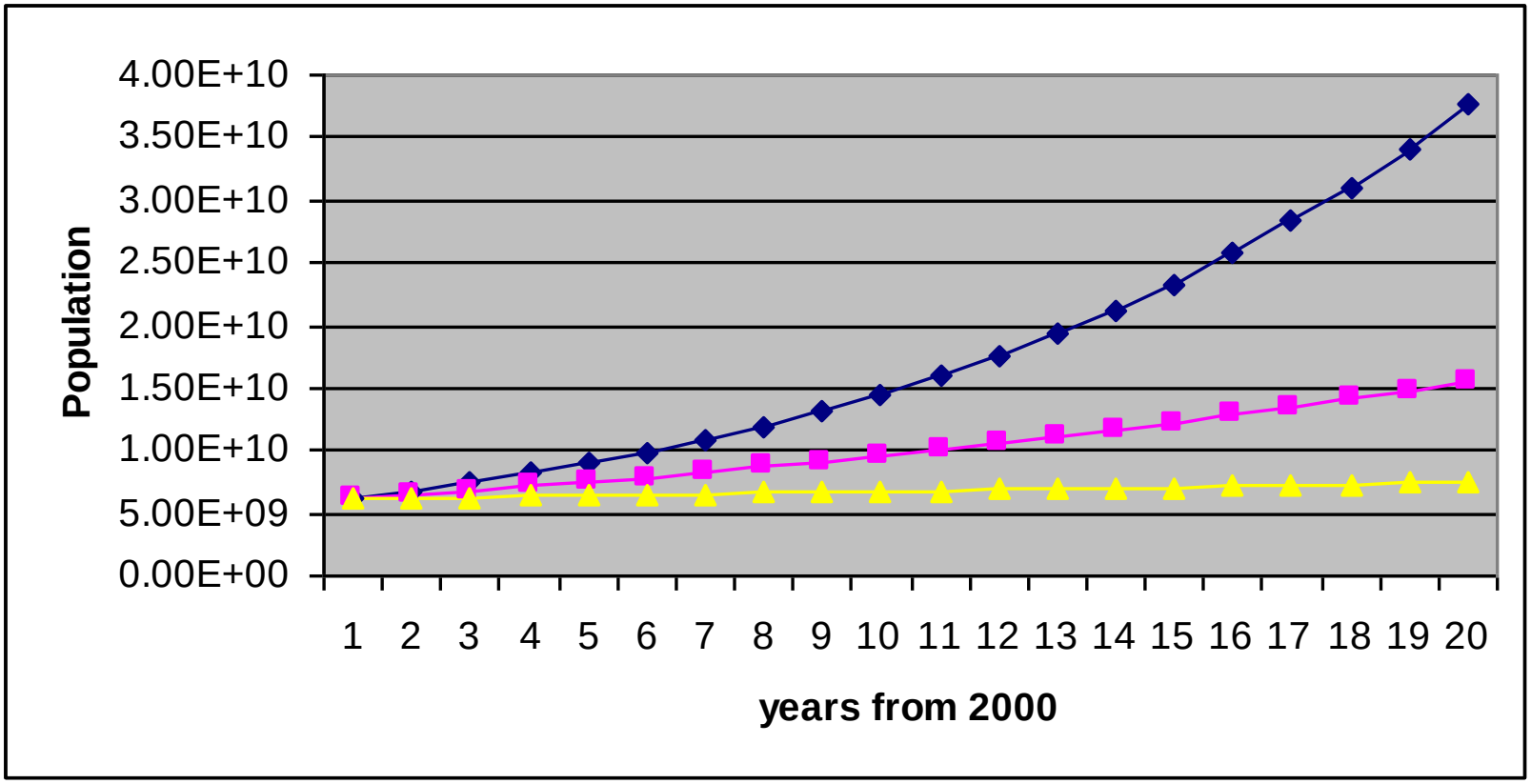
More research is needed for "knowledge based approaches"

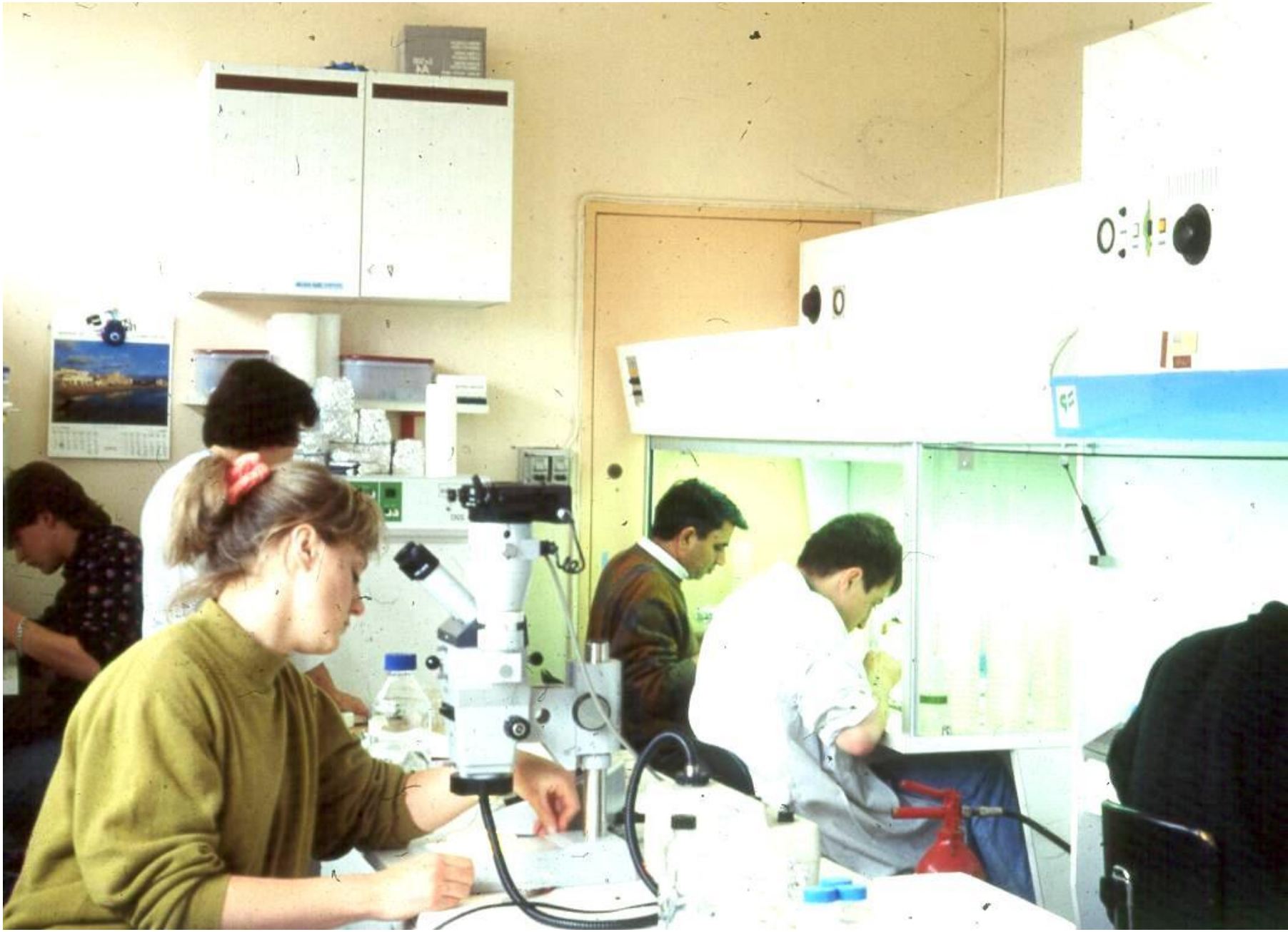
- (i) genetic architecture of the trait combinations we seek to manipulate
- (ii) the nature of the genetic changes that were brought about by phenotypic selection
- (iii) the power that can be attained in conventional breeding strategies
- (iv) the power that can be obtained by molecular breeding strategies

- **“Boost the power of conventional breeding by marrying it to genomic and other molecular-genetic techniques”**

- Adaptation of high-throughput approaches
  - Breeding can benefit form genomics approaches
  - Breeding can also offer genomics approaches e.g. experimental designs for gene expression studies.

Projected population increase under different assumptions of reproduction and mortality





Thank You

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