Master 1
BP04 - Introduction to Biotechnology
December, 2013

Plant Biotechnology: Principles and Techniques

Instructors:
Eric BONCOMPAGNI
Nice-Sophia Antipolis University - France

Plant Biotechnology - Schedule

Lecture Eric Boncompagni	**Monday 23 December** 9 – 12h **201, 1H Building, USTH** • First contact with the students and discussion related to course syllabus and particular topics. • Introduction in Plant Biotechnology, history and general information. • What is Plant Biotechnology/Molecular Biology? • Film: Plant health institute - Inra – France

Lecture Eric Boncompagni	**Tuesday 24 December** 9 – 12h **201, 1H Building, USTH** • Techniques of plant *in vitro* plant tissue/cell cultures • Sterile techniques, media preparation • Plant hormones and growth regulators. **=> See laboratory practical 1**

Lecture Eric Boncompagni	**Tuesday 24 December** 13h30 – 16h30 **201, 1H Building, USTH** • Diversity of organisms. • Traits (tolerance, metabolism, oil production, vaccines) and plants (important crops and model organisms) to improve. • *Arabidopsis thaliana* • Contribution of biotechnology to improvement of quality and quantity of crop production.
	Thursday 25 December 9-12 h **304, A18 Building, USTH** • Methods of improvement: selection and transgenesis • Techniques enabling introduction of foreign genes into the plant genome. • *Agrobacterium tumefaciens* mediated transformation • Floral dip *vs.* other target tissues • Selection and regeneration of transgenic plants.

Plant Biotechnology – Schedule (continued)

Exercises Eric Boncompagni	**Thursday 26 December** 9-11 h **USTH** • Exercises

Exercises Eric Boncompagni	**Thursday 26 December** 13h30– 15h30 **USTH** • Exercises

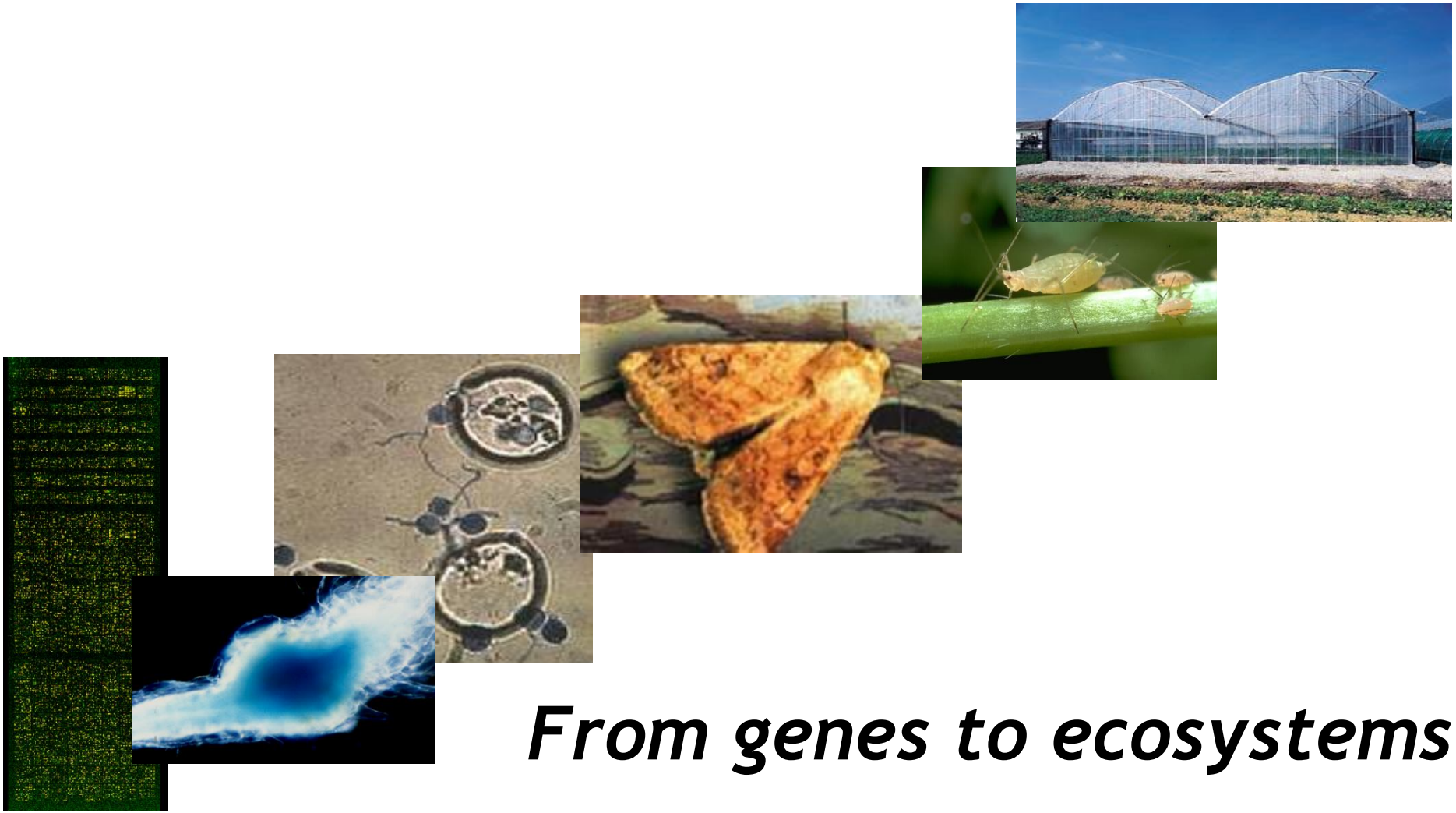
Laboratory Practical => **Agricultural Genetics Institute**

Laboratory Practical 1 Eric Boncompagni	**Friday 27 December** 8-12h • Culture *in vitro* • **Group 1**	**Friday 27 December** 13-17h • Culture *in vitro* • **Group 2**

Laboratory Practical 2 Eric Boncompagni	**Monday 30 December** 8-12h • Protoplast's fusion • **Group 1**	**Tuesday 31 December** 8-12h • Protoplast's fusion • **Group 2**

Institut Sophia Agrobiotech

Institut Sophia Agrobiotech



From genes to ecosystems

Research Area: Biotic Interactions and Plant Health

Research for Sustainable Agriculture and the Environment

- **Molecular bases of plant-pathogen interactions**

  Reduction of pesticide use:

  - Plant Breeding for resistance
  - Stimulate defence mechanisms
  - Inhibit pathogen development

- **Molecular bases of plant- symbiotic bacteria interactions**

  Reduction of fertilizer use:

  - Optimize the symbiosis
  - Reduce the negative impact of stress

http://www.paca.inra.fr/

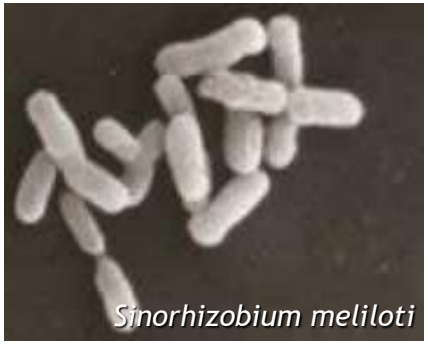
Rhizobium – Medicago truncatula symbiosis (P. Frendo & R. Brouquisse)

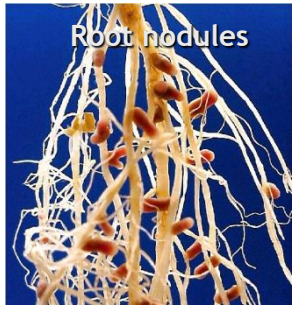
Legumes

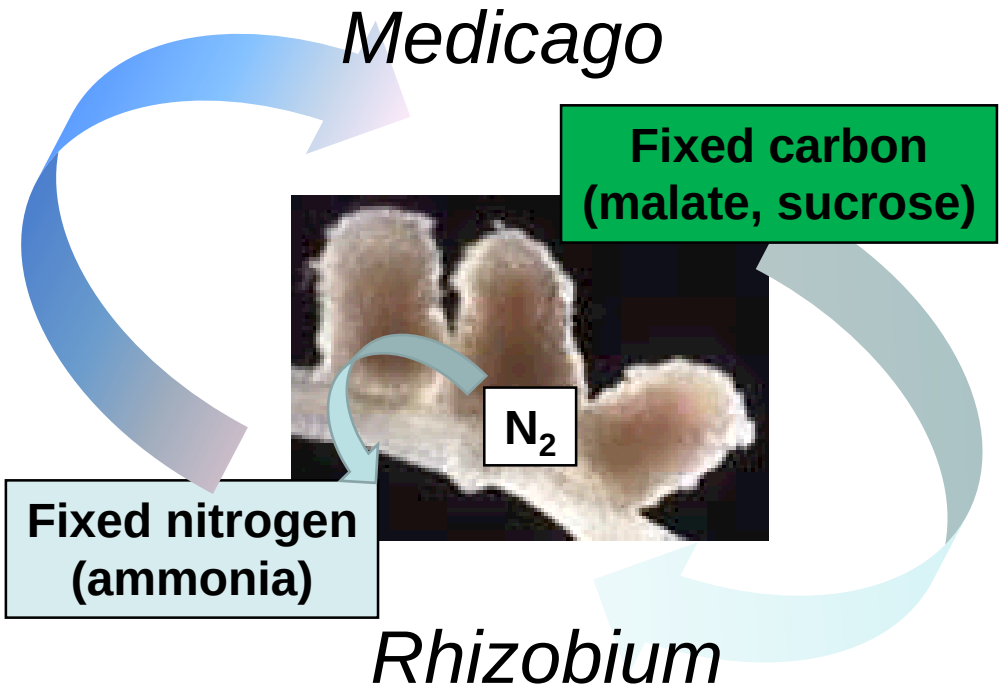
Legumes represent a crop with nutritional properties particularly **valuable for food and feed (**20 to 40% proteins in seeds, production of health-promoting secondary compounds, blood cholesterol-reducing effect …).

Rhizobium – Medicago truncatula symbiosis

Sinorhizobium meliloti / *Medicago sp.* Interactions

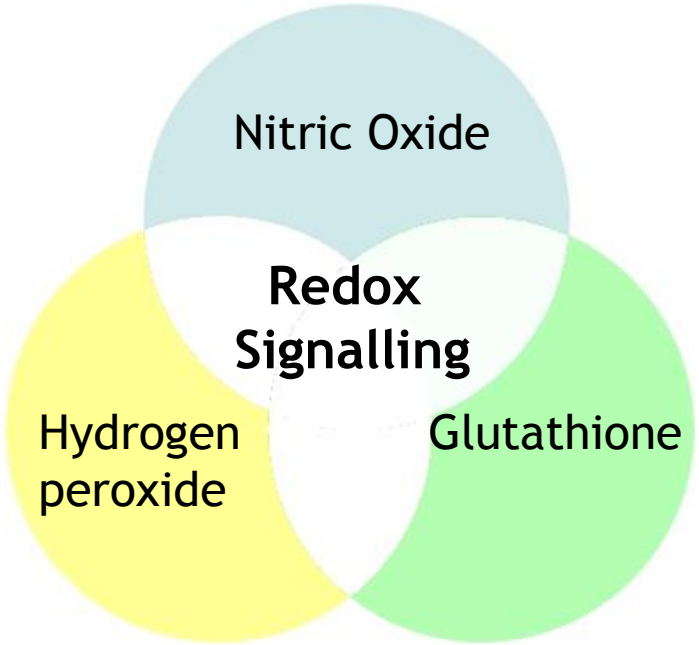






Rhizobium – Medicago truncatula symbiosis

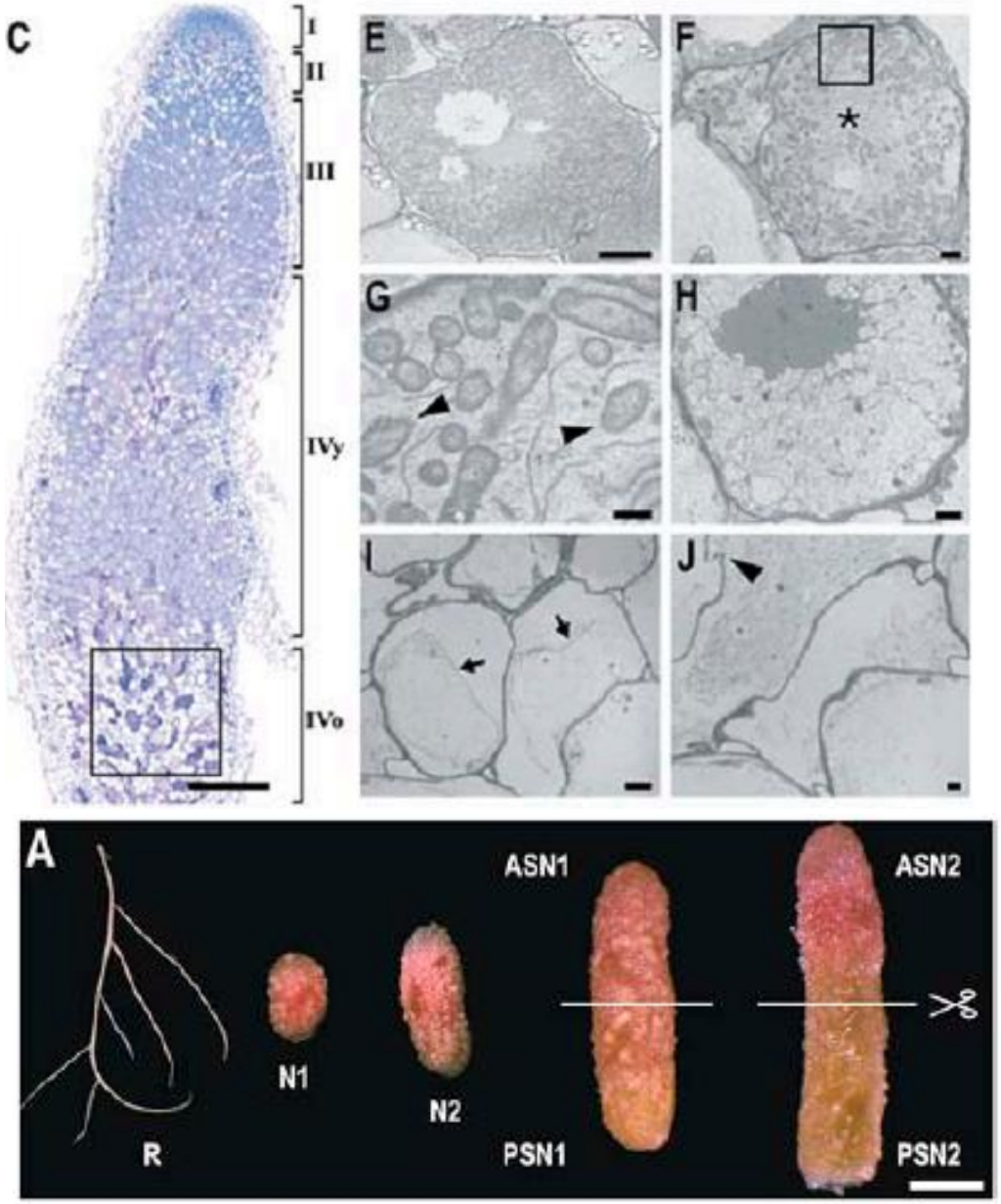
1. Redox signalling: From symbiose establishment to nodule functionning



- Spatiotemporal dynamics
- Cross-talks
- Production systems
- molecular targets

2. Understanding senescence mechanism in the root nodule

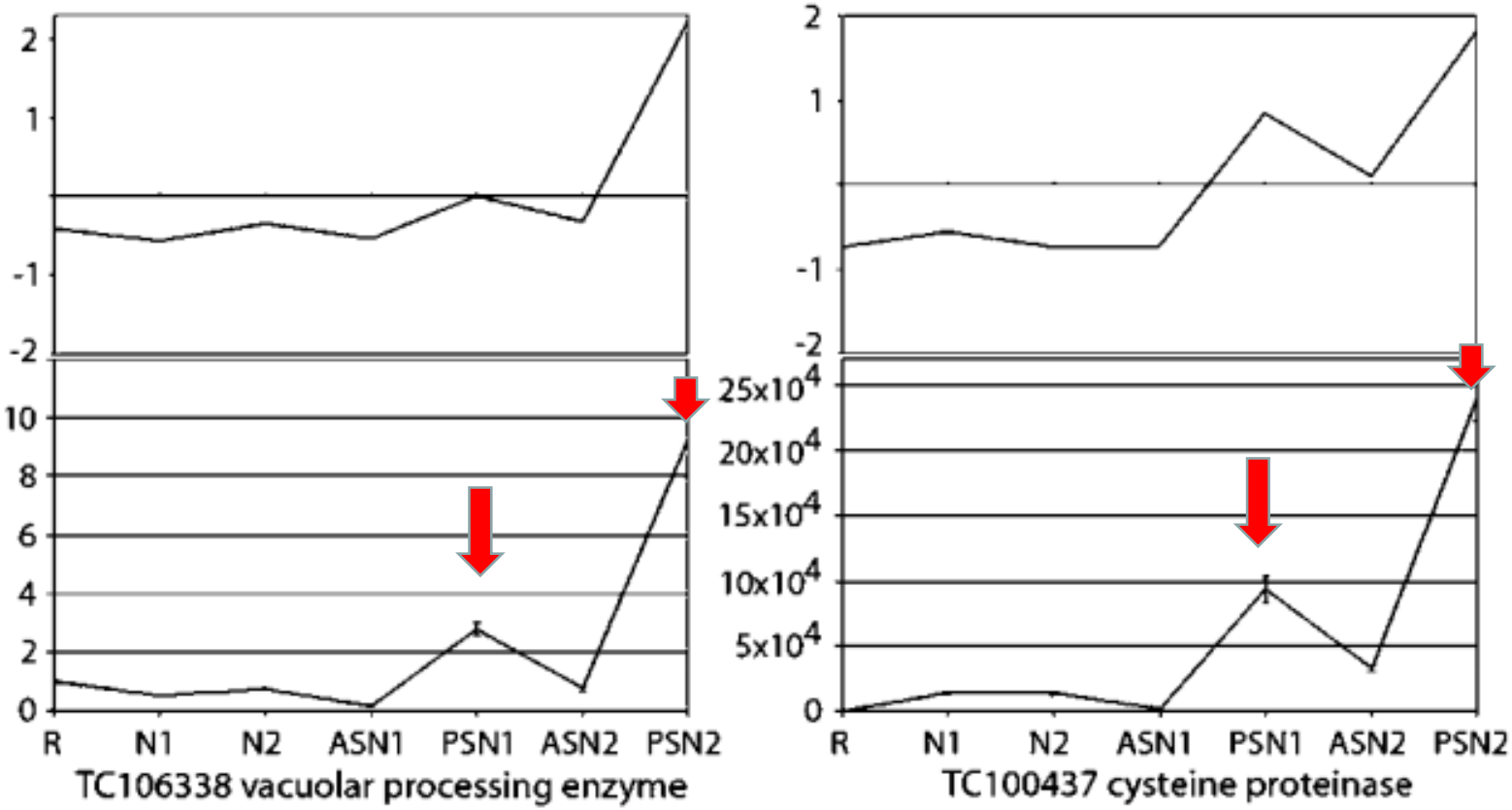
Nodule senescence



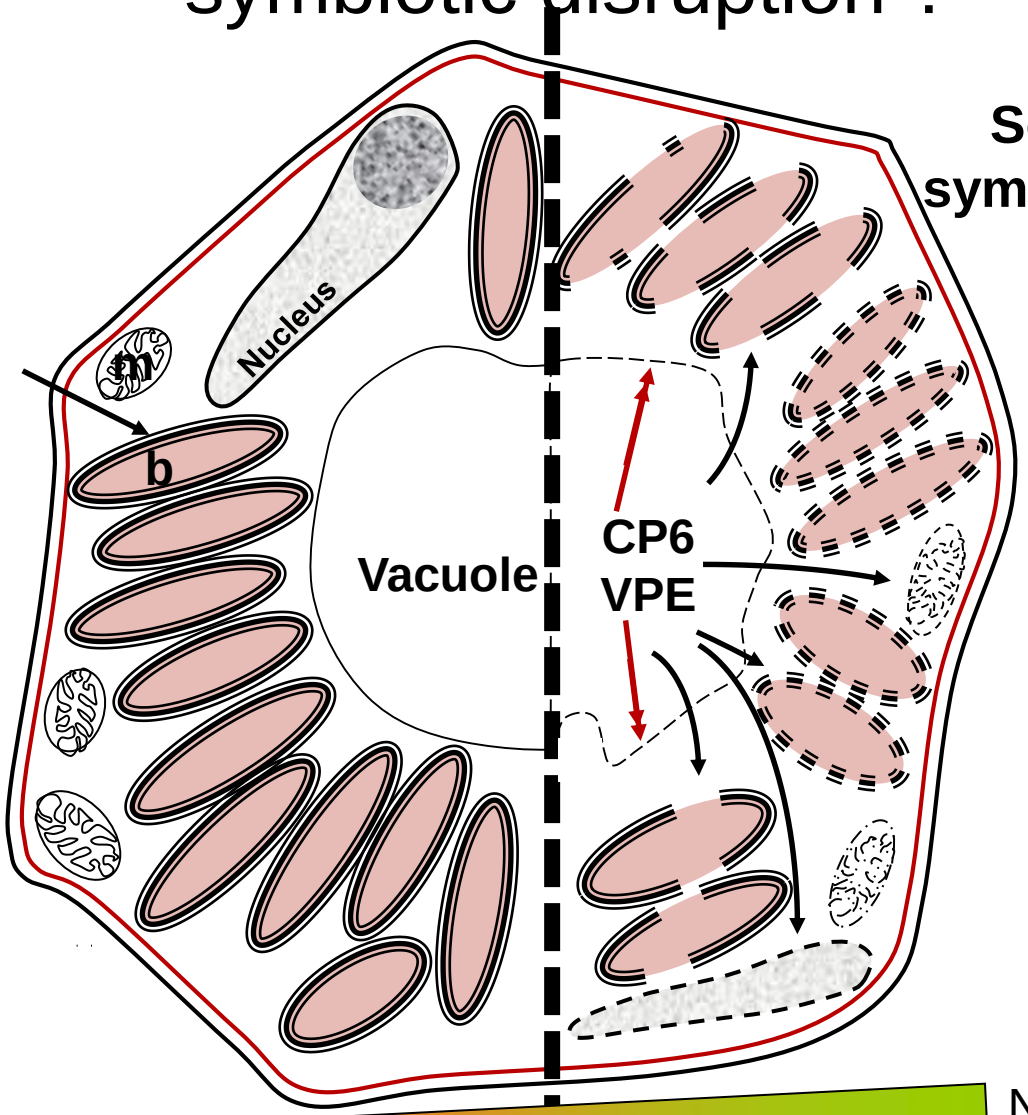
Van de Velde *et al.* (2006) Plant Physiol.

Confirmation by qRT-PCR of cDNA-AFLP profiles

Van de Velde *et al.* (2006) Plant Physiol.



Contribution of cysteine proteases in the symbiotic disruption ?



Peribacteroid membrane

Senecence of symbiotic host cell

(Zone IV)

Symbiotic disruption

b : bacteroid

m : mitochondria

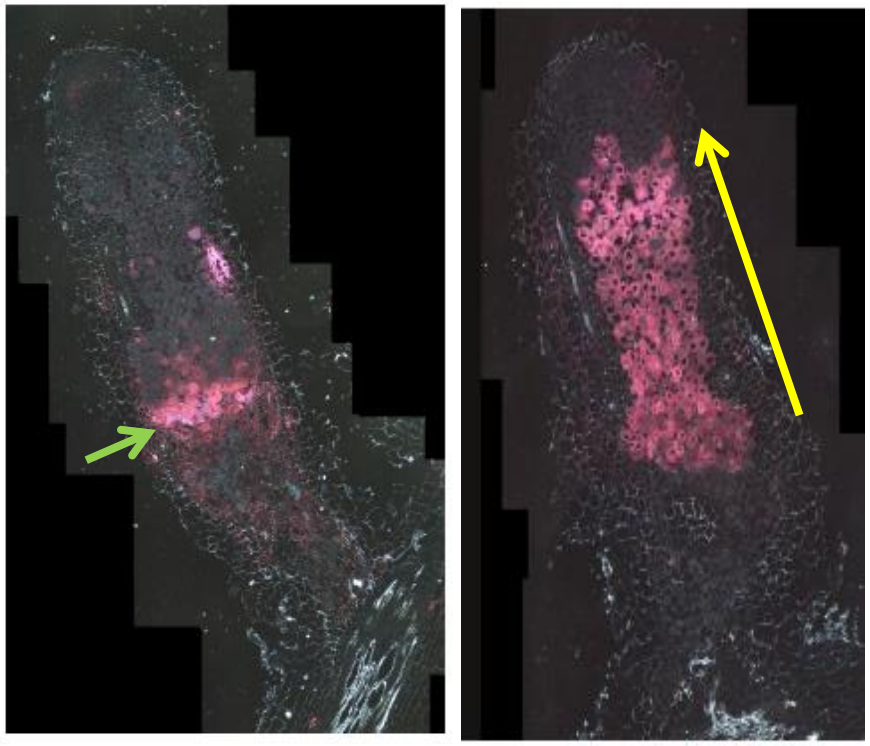
Symbiosis

Nodule senescence

Tissue localization of cysteine proteases

PCP6::GUS 6WPI

Ctrl 3DS



PVPE::GUS 6WPI

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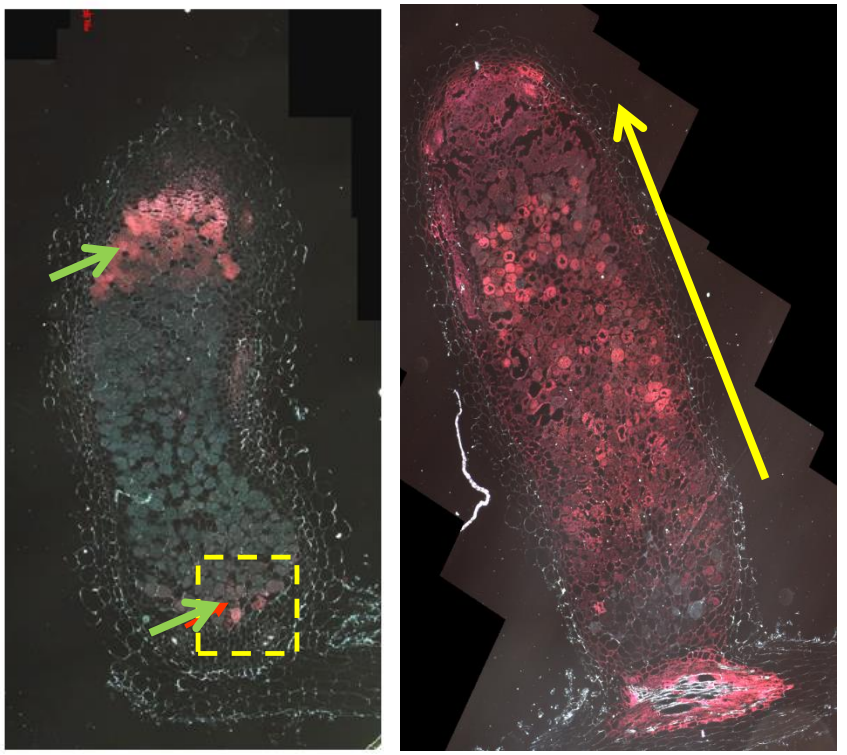
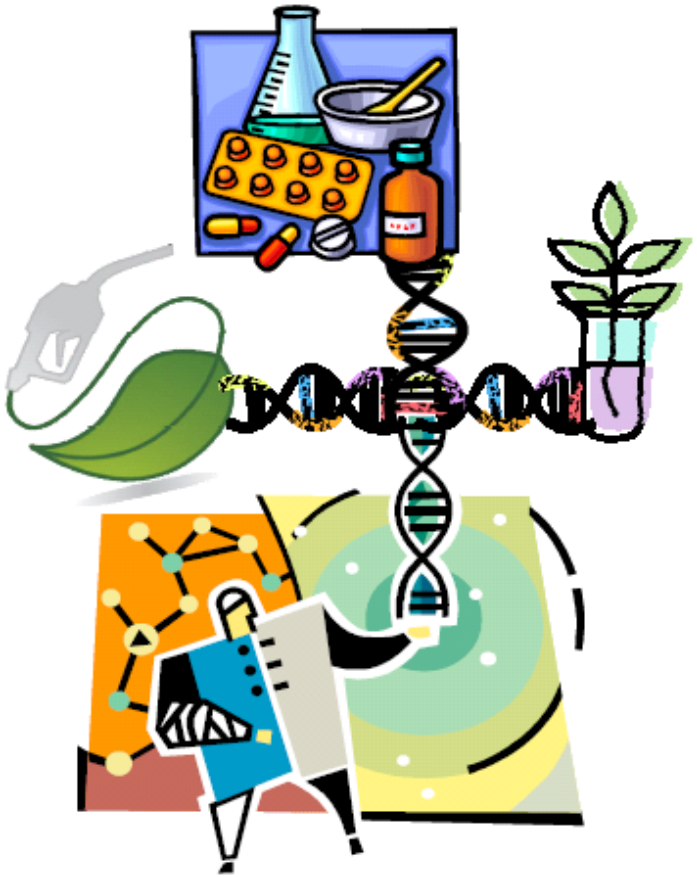


→ Extension of expression zones of both cysteine proteases under dark stress?

Introduction in Plant Biotechnology, history and general information

Introduction in Plant Biotechnology, history and general information



Biotechnology is the **use or manipulation of an organism** or the components of an organism.

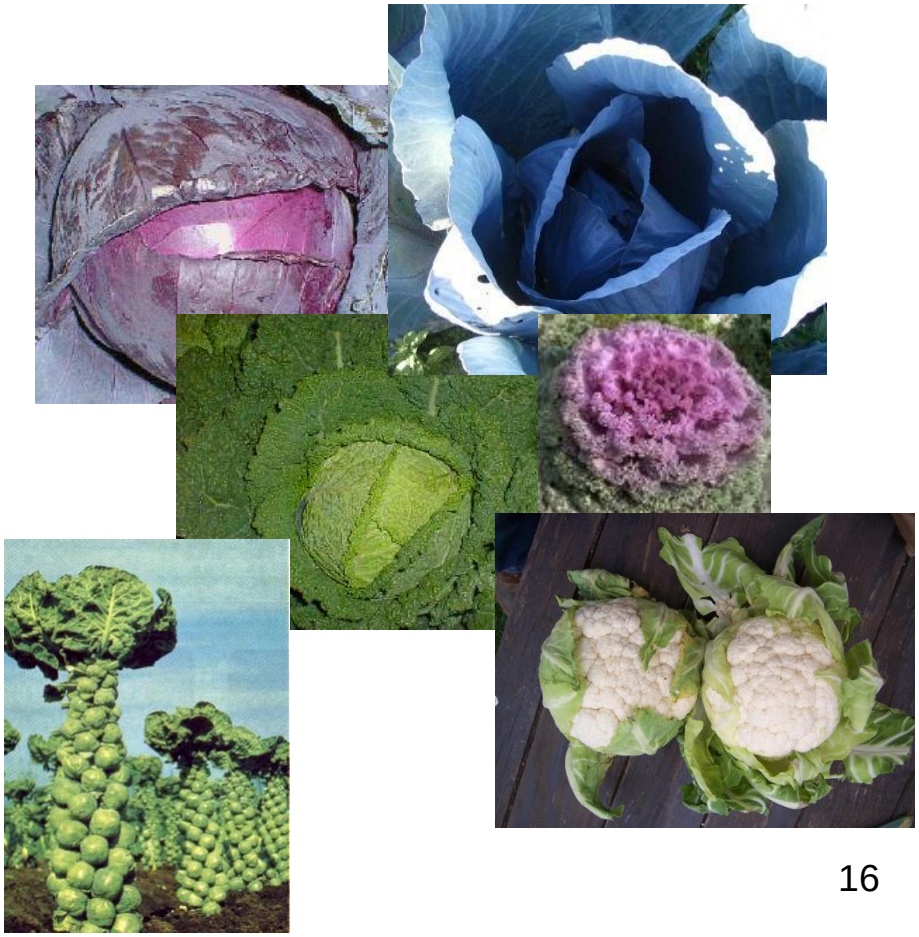
By this definition, the origins of biotechnology date back to when people first begin to domesticate cultivate food crops. While those early applications are certainly still employed today, modern biotechnology is primarily associated with **molecular biology, cloning and genetic engineering**.

Within the last 50 years, the biological sciences were revolutionized by several key discoveries that enabled the rapid evolution of the biosciences. These discoveries enabled scientists to **isolate and manipulate genes**, which has facilitated the growth of the biotechnology industry.

Improve plant species

First step toward selection: domesticate cultivate food crops

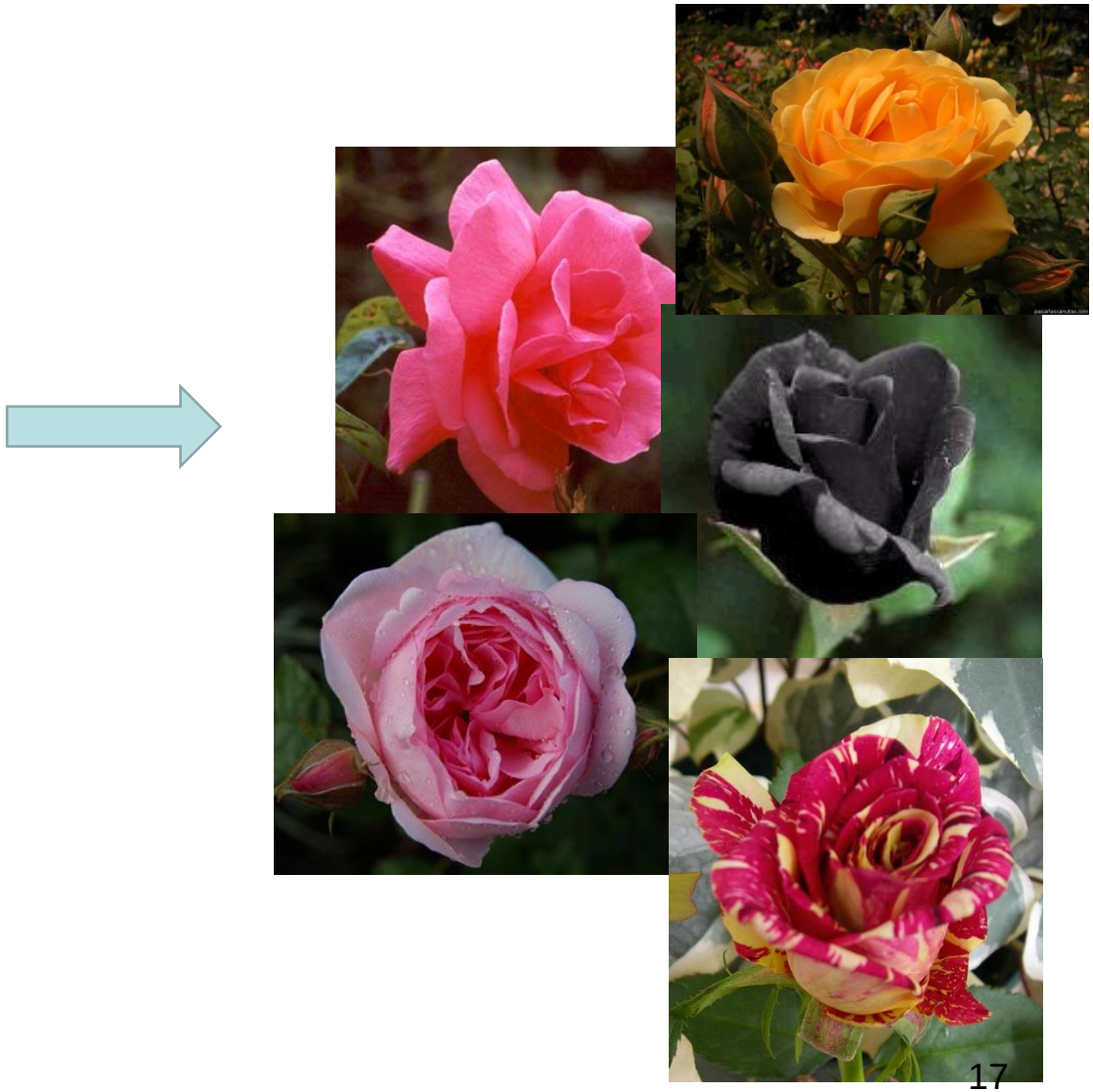
For instance: Cauliflower



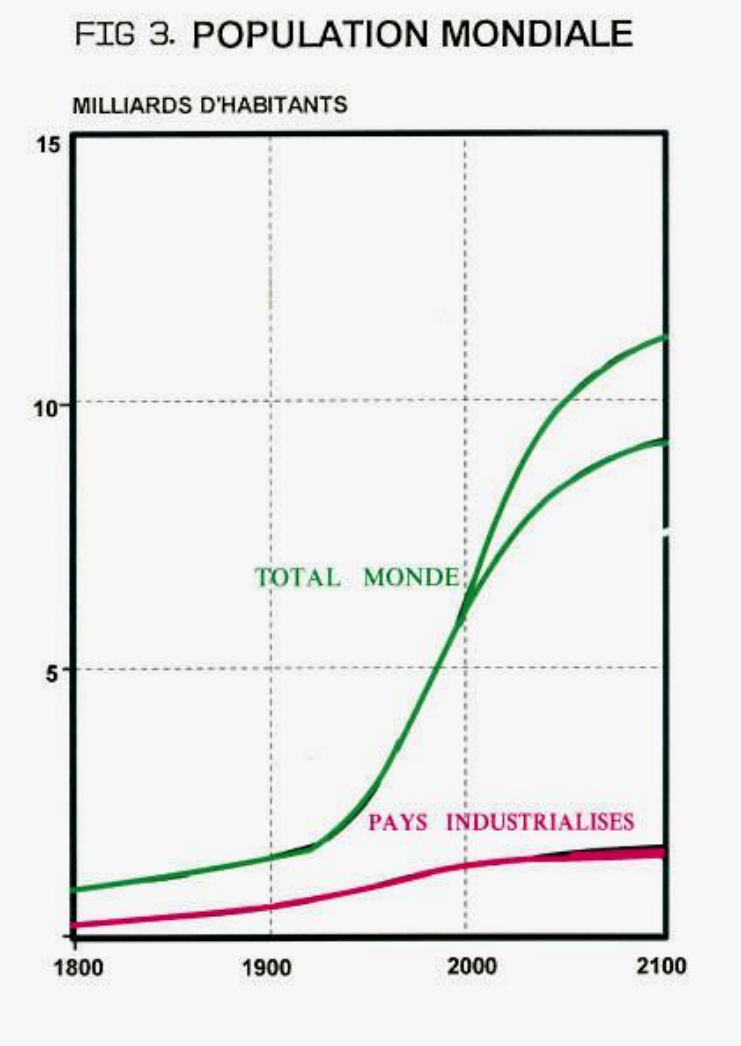
First step toward selection: domesticate crops for their flowers… with high money value!!!!

Example of roses

Églantier



Biggest problem: Feed all the people



- **Population on earth is increasing…**

  1950: 2.5 billions

  2008: 6.7 billions

  Projection 2050 : 9 Billions

- **Evolution of field productivity in the world:**

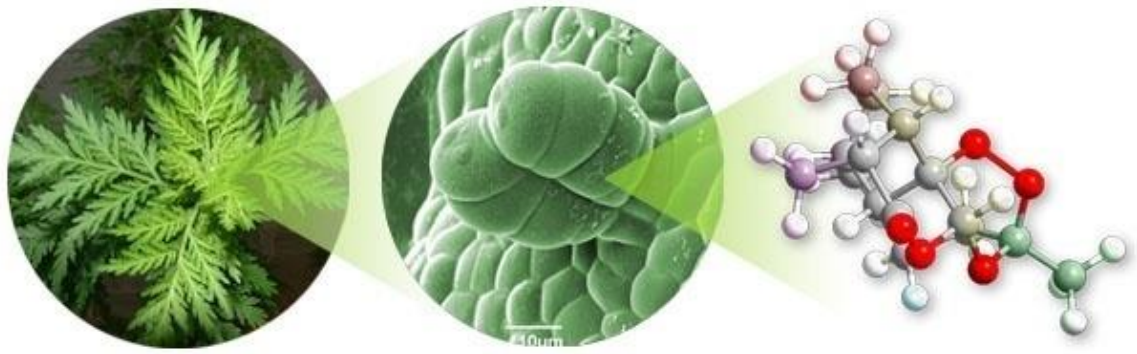
  1960 : 1 hectare feed 2.4 persons

  2005: 1 hectare feed 4.5 persons

  En 2050: 1 hectare will need to feed 6.1 à 6.4 persons… need new solution!!!!!!!!!!

Plants provide us with more than food!!!

- Plants are sources of novel therapeutic drugs
- Plants provide better fibers for paper or fabric
- Plants are sources of biorenewable products
- Plants provide renewable energy sources



Plants produce hundreds of compounds we use as medicines or drugs, for instance:

- **Willow** (*Salix*) bark as a source of aspirin => acetylsalicylic acid;
- **Foxglove** (*Digitalis purpurea*) as a source of digitalis => treatment for cardiac problems;
- **Pacific yew** (*Taxus brevifolia*) as a source of taxol => treatment for cancer;
- **Coffee** (*Coffea arabica*) and **Tea** (*Camellia sinensis*) as sources of caffeine => stimulant

BIOTECHNOLOGY INDUSTRY FACTS

• The biotechnology industry emerged in the 1970s, based largely on new **recombinant DNA** technology.

• Biotechnology has created more than 200 new therapies and vaccines, including products to treat cancer, diabetes, HIV/ AIDS and autoimmune disorders.

• Agricultural biotechnology benefits farmers, consumers and the environment—by **increasing yields** and farm income, **decreasing pesticide** applications and **improving soil and water quality**, and providing **healthful foods** for consumers.

• Environmental biotech products make it possible to clean up hazardous waste more efficiently by harnessing pollution => **Bioremediation**

• In the USA, the biotech industry is regulated by the U.S. Food and Drug Administration (FDA), the Environmental Protection Agency (EPA) and the Department of Agriculture (USDA).

=> **Biotechnology is the use of organisms, cells and biological molecules to solve problems or make useful products.**

Implications of genomics on biotechnology

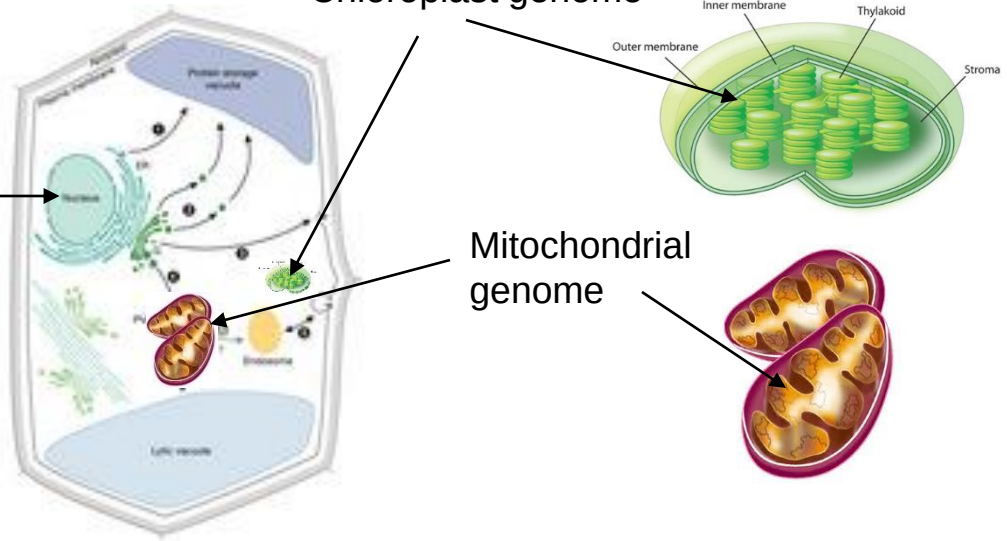
Genomics is the scientific study of the **genome** and the role **genes** play in determining cell structure, directing growth, and controlling biological functions.

Knowing the complete or partial **DNA sequences** of individual genes or markers provides useful information, even if the precise details of gene function remain unknown.

Plant cell:

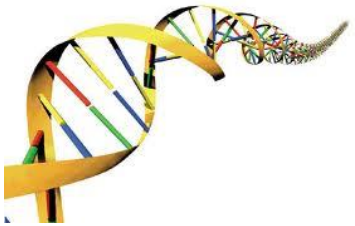
Nuclear genome

Chloroplast genome

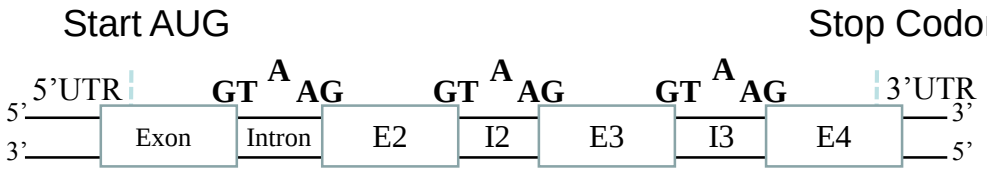


Mitochondrial genome

DNA:



Eukaryotic gene:



Nuclear genome sizes…

Organism	Haploid Genome Size (Mb)	Number of Genes	Genes per Mb
Bacteria			
Haemophilus influenzae	1.8	1,700	940
Escherichia coli	4.6	4,400	950
Archaea			
Archaeoglobus fulgidus	2.2	2,500	1,130
Methanosarcina barkeri	4.8	3,600	750
Eukaryotes			
Saccharomyces cerevisiae (yeast)	13	6,200	480
Caenorhabditis elegans (nematode)	100	20,000	200
Arabidopsis thaliana (plant)	118	25,500	215
Drosophila melanogaster (fruit fly)	180	13,700	76
Oryza sativa (rice)	390	40,000	140
Danio rerio (zebrafish)	1,700	23,000	13
Mus musculus (house mouse)	2,600	22,000	11
Homo sapiens (human)	3,200	20,500	7
Fritillaria assyriaca (plant)	120,000	ND	ND

*Some values given here are likely to be revised as genome analysis continues. Mb = million base pairs. ND = not determined.

C-value

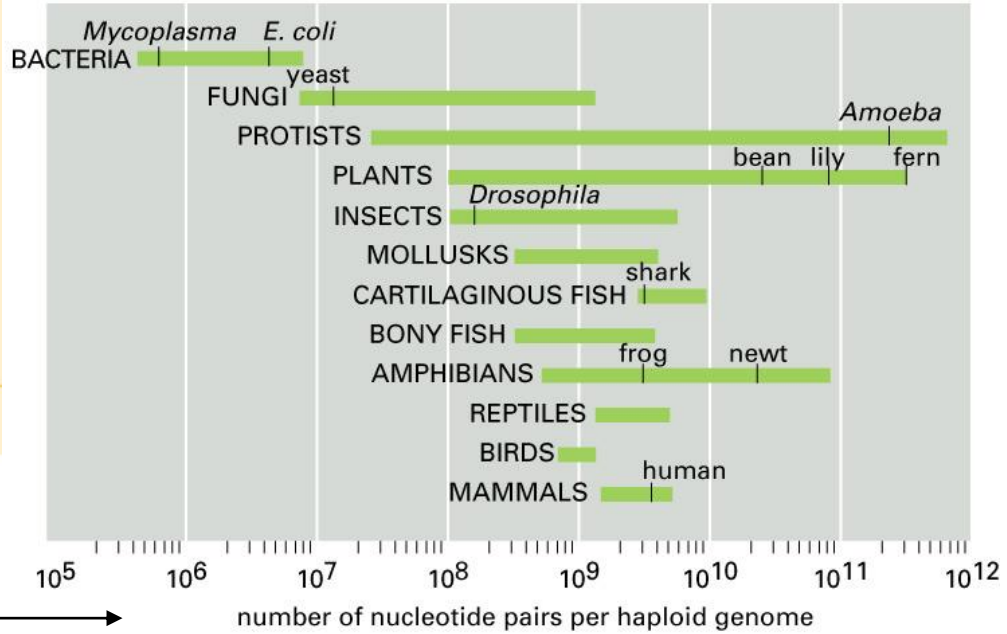
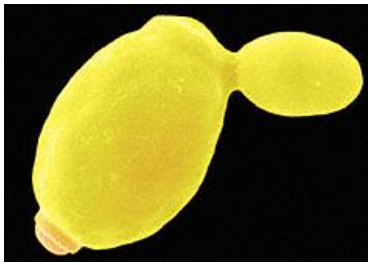


Figure 1–38. Molecular Biology of the Cell, 4th Edition.

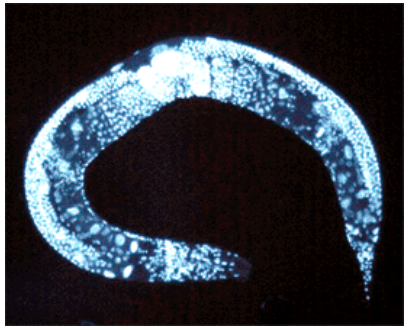
… and organism complexity!!!

Yeast



1 cell
~5,000 genes

Worm (*C. elegans*)



♀♂: 959 cells
♂ : 1031 cells

19,000 genes

Fly (*D. melanogaster*)

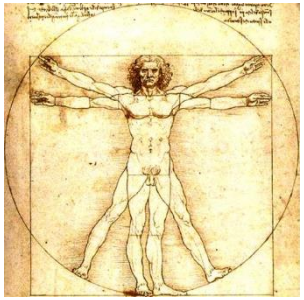
~10^8 cells

13,600 genes

Rice

30-50,000 genes

Human



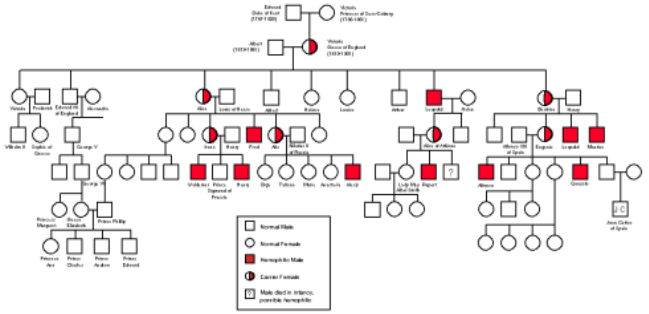
~10^{14} cells

25,000 genes

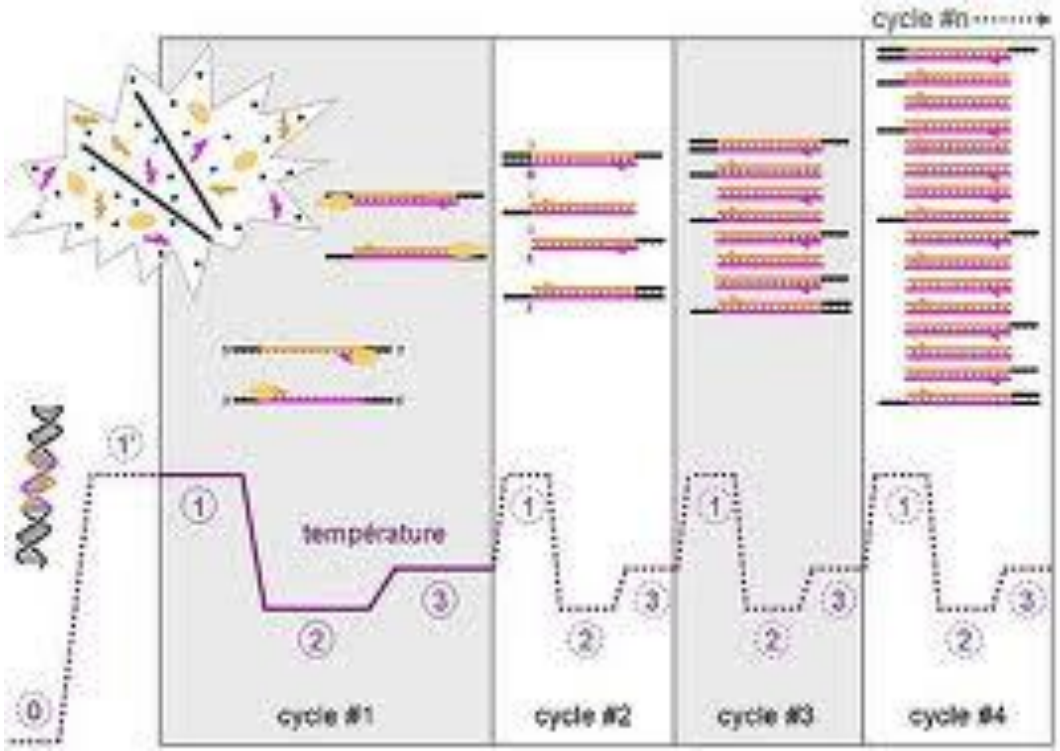
C-value paradox!!!

ADN quantity is not related to organism complexity (or evolution) nor its cell number neither its gene number.

In 1865, Gregor **Mendel** studies garden peas and discovers that genetic traits are passed from parents to offspring in a predictable way—the **laws of heredity**.



In 1983, Kary Mullis invents the **polymerase chain reaction** (PCR) **technique**. PCR, which uses heat and enzymes to make unlimited copies of genes.



For example, genomics data can:

- Use genetic information to develop individual drugs and therapies
- Understanding how genes affect one another in different species
- Identify the genes involved in disease processes
- Improve crop yield and pest resistance…

Stages in Product Development

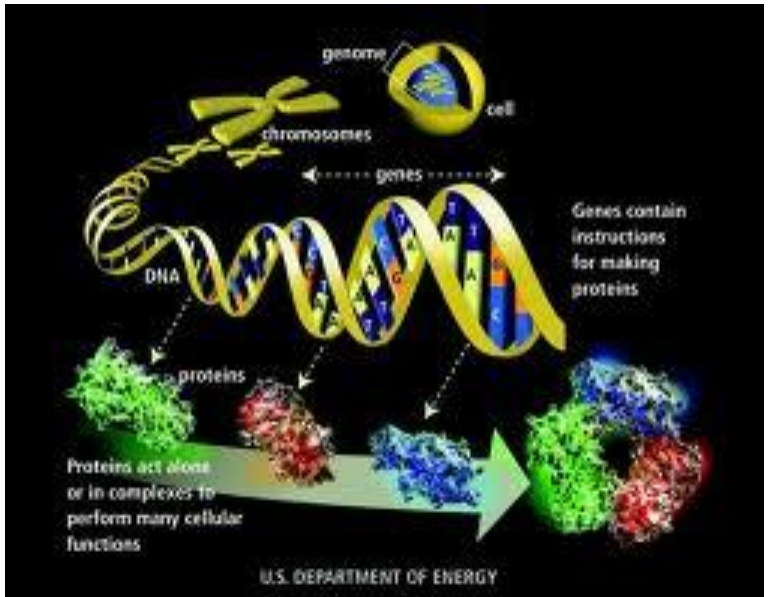
Product Identification => **Research & Development** => Small-scale Manufacturing => Testing for Safety and Efficacy => Manufacturing => Sales and Marketing

Implications of proteomics on biotechnology

Genes exert their effects through **proteins**, so gene expression results in protein production.

The collection of proteins in a cell is known as its **proteome**, and proteomics is the study of the structure, function, location and interaction of proteins within and between cells. The collection of proteins in an entire organism is also referred as its proteome (e.g., the Rice proteome).

The sequence of **amino acids** and modification after translation affects the shape and, therefore, the **function of a protein**. Any changes to a protein affect a protein's form and function, which might explain how the ~50,000 rice genes in the genome can make the hundreds of thousands of proteins that comprise the plant proteome.



Proteomics research tools may be used to address many questions such as:

- which proteins are produced by certain cells
- how do age, environmental conditions and diseases affects the protein production
- how can biotechnology alter protein production in plant organisms
- discovering the functions of all proteins.
- understanding how proteins changes occur in disease development
- discerning how a protein interacts with other proteins.

Studying plants **increases our knowledge** about life in general and helps us to work with them to keep us fed, healthy, sheltered, clothed… => Understand plant biology to modify the plant and to solve problems and make useful products => **Biotechnology**





Film: Plant health institute - Inra - France

The aim of this institute is to mobilize multidisciplinary know-how in order to propose new solutions for **controlling plant pests** based on an understanding of their interactions with crops, changes in their populations, their relationships to the rest of the cultivated ecosystem and on the knowledge of how these crop systems function.

The challenges facing all sectors of French and European agriculture are particularly significant for specialized agriculture and especially the fruit, vegetable and greenhouse horticulture sectors. This results in close partnerships at the regional level between plant health poles and integrated horticultural production.

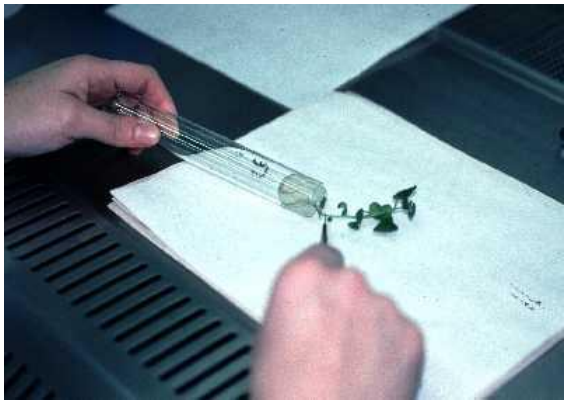
Institut SOPHIA AGROBIOTECH

http://www.international.inra.fr/

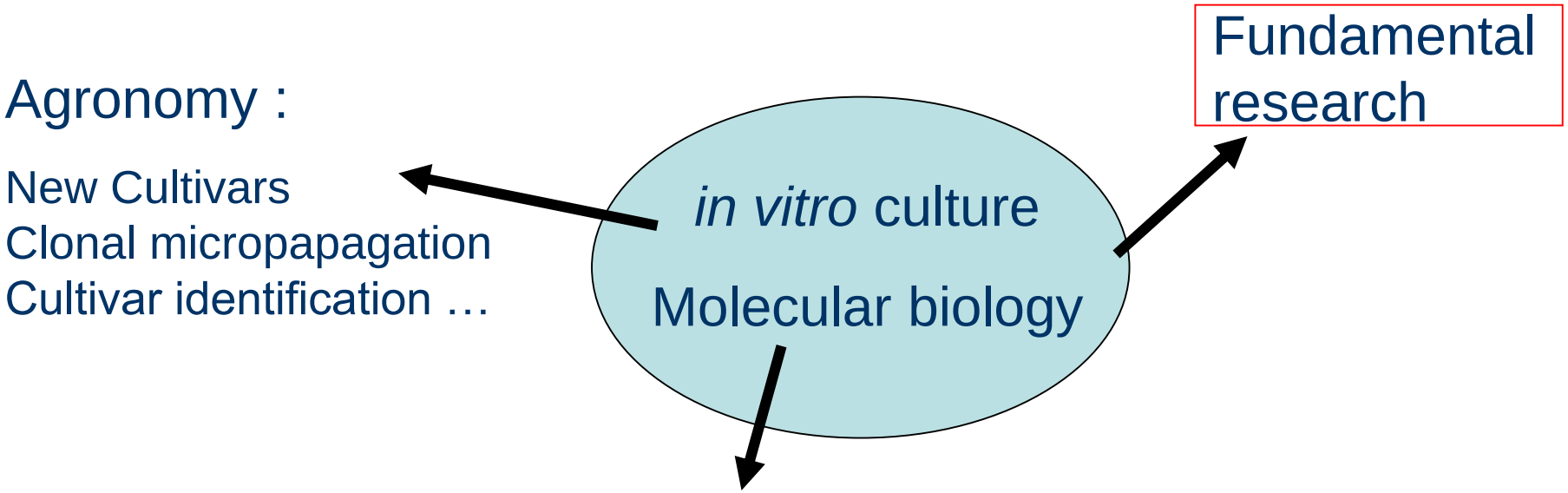
INRA
Institut National de la Recherche Agronomique

PLANT BIOTECHNOLOGY

- Haplodiploïdisation
- Culture of meristems
- Micropropagation
- Save of embryo
- Protoplast fusion
- Induction of variability
- …



- Plant biotechnologies:



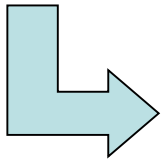
Plant totipotency

Overview

- Definition
- Birth and development of *in vitro* culture
- Application et limitation of totipotency
- Mecanisms underlying totipotency
- Biological significance of plant cell totipotency

Some definitions

- In plants, the totipotency can be defined as the property of some cells that may regenerate a plant when they are placed under appropriate conditions (possibly via a stage of dedifferentiation)

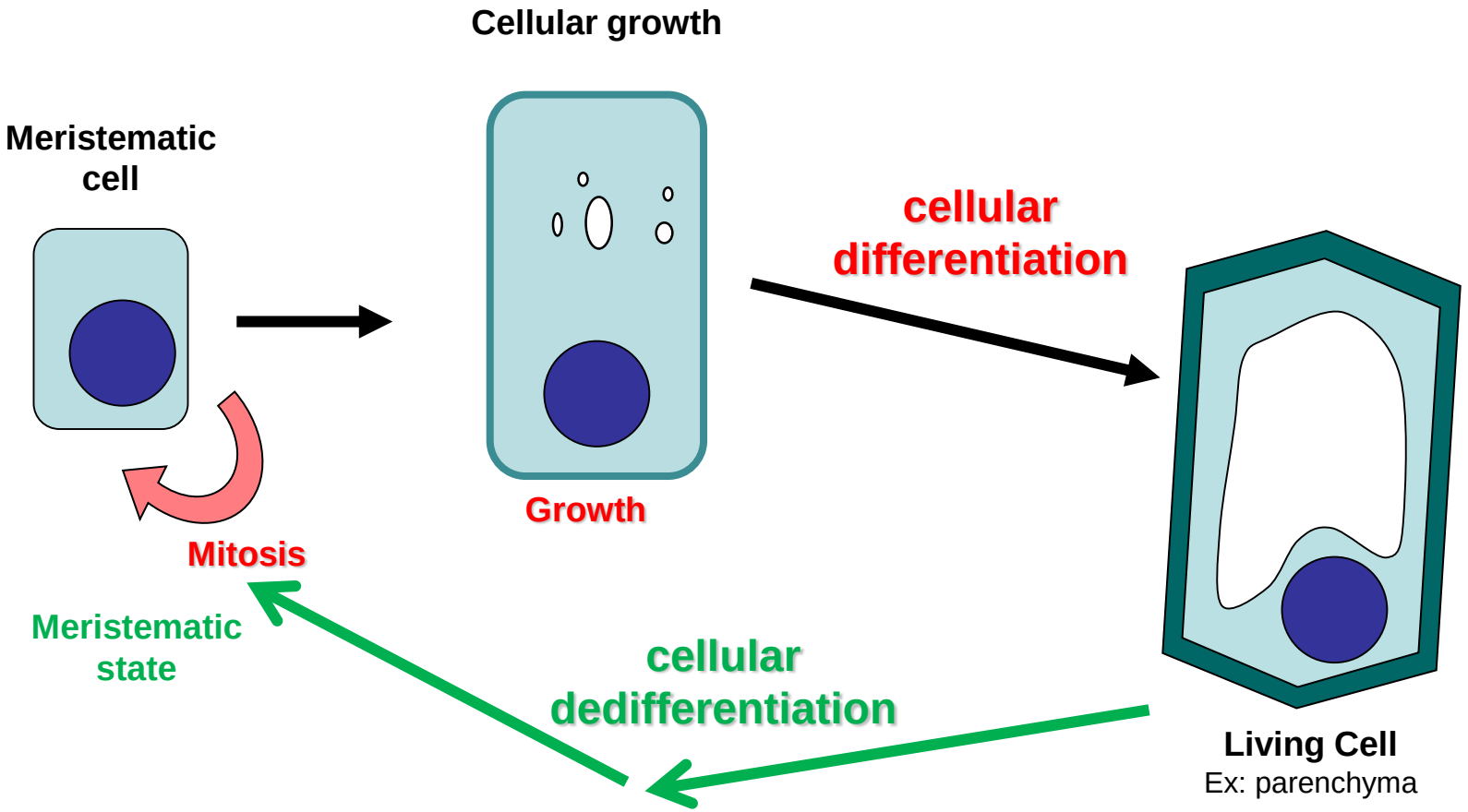


Specific to plants

Exemple : micropropagation of *Saint paulia*

2 months later

Plant totipotency : Cellular dedifferenciation

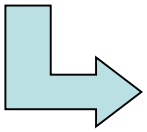


Historical aspects

Totipotency as the theme of plant biotechnology birth

Historical of tissue culture and plant organs

- Scientific context in the beginning of XXth century
  - Cellular theory (Schleiden et Schwann, 1838)



How to study the behavior of single cells?

  - Microbiology and biochemistry

Growth in axenic cultures

Characterisation of growth substances

G. Haberlandt : the concept of totipotency of the plant cell (1902)

Two main important ideas:

- culture of isolated cells constitute a potential research model
- keep alive isolated cells
  - No cellular multiplication

- We can potentially regenerate a whole plant from a single cell … → totipotency
  - Failure (bad choice of explants, ignorance of growth substances)

Emergence of culture techniques

– Haberlandt (1902) : concept of totipotency
– White (1934) : *in vitro* culture of tomato root
– Gautheret (1935) : use of auxin to grow willow cambium
– 1939 : First callus culture of carrot

Tissue culture is possible using growth substances and / or meristematic tissues

Emergence of culture techniques

– Braun (1941) : work on crown gall

→ *In vitro* tumor growth without hormone supply!!

– Miller (1955) : cytokinins

– Murashige and Skoog (1962): development of effective culture media containing cytokinins and auxins

Validation of Haberlandt hypothesis

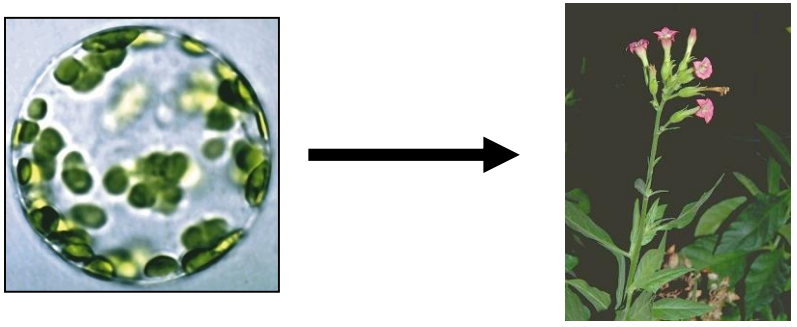
- 1956 (Muir) cell suspension cultures

- 1958 (Reinart et Stewart) Carrot somatic embryogenesis

Validation of Haberlandt hypothesis

- 1965 (Vasil et Hilderbrandt) Regeneration of a tobacco plant from a single cell

- 1971 (Nagata et Takabe) Regenerating a whole plant from a protoplast

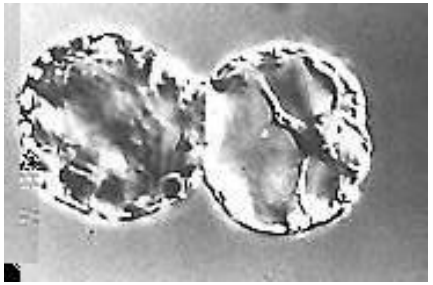


Development: agronomic tools

- 1965 (Morel) *in vitro* propagation of orchids

- 1972 (Sharp) : tomato plants from haploid pollen

- 1973 : hybrid from a protoplast fusion



Development: production of secondary metabolites

- 1977 : culture of tobacco cells in a reactor of 20,000 liters
- 1983 (Mitsui Petrochemical) : industrial production of secondary metabolites

Development : GMO

- Van Montagu (1983) : kanamycin resistant tobacco plants
- 1994 : Flavr Savr (Calgene)
- 1996 : GM maize in the United States

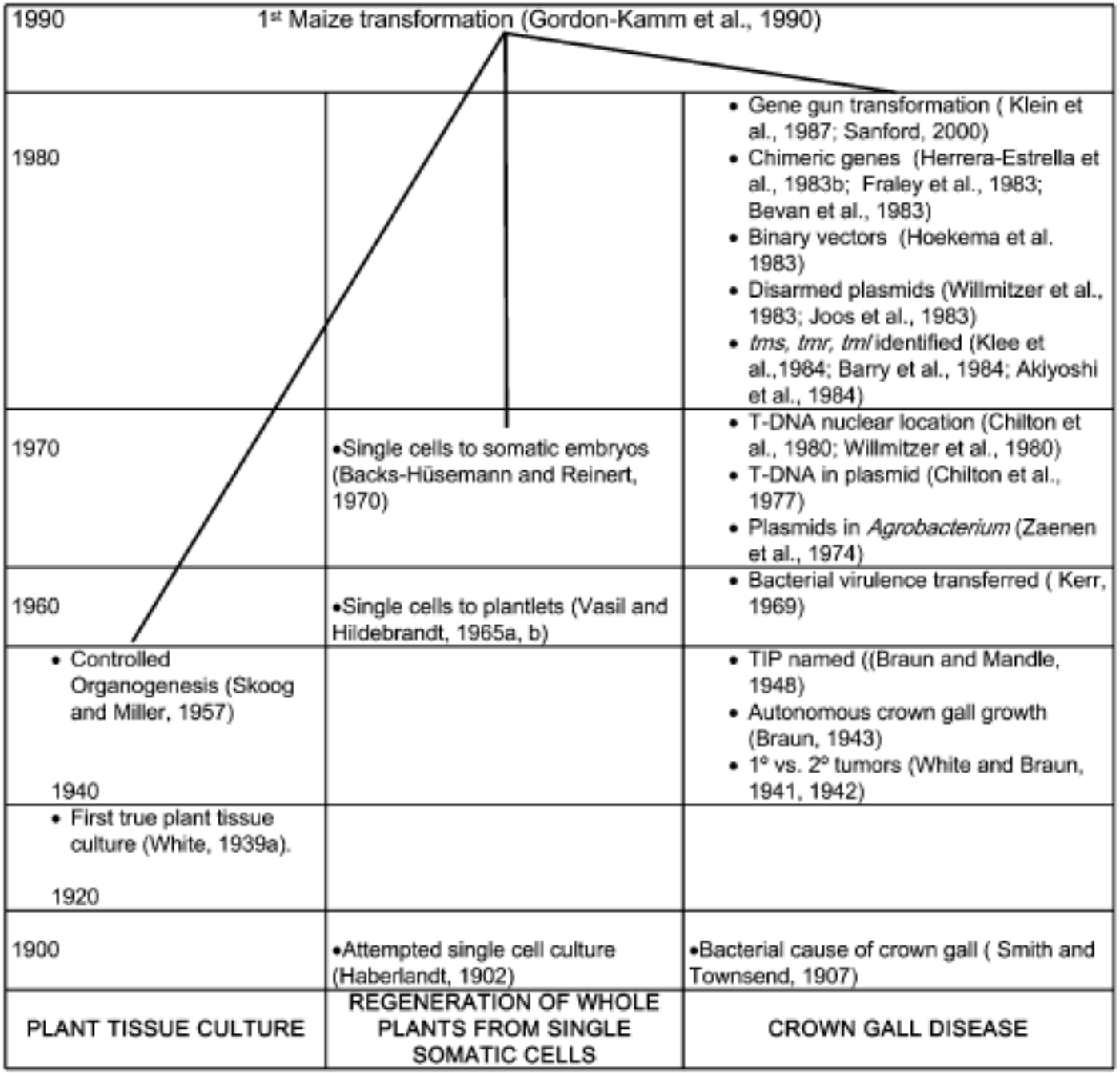


Figure 1. Chronology of Research Leading to Modern Plant Biotechnology.

Sussex, Plant Cell 2008

Conclusions

- Initial problem :
  - Search for a isolated cell model
  - Validation of plant cell totipotency