Talla Asirían (870 B.C.)
Polinización artificial de las higueras

¿Que cultivos fueron desarrollados desde estas plantas?

maiz

zanahoria

lechuga

Mejorando los Cultivares

Desarrollo de Nuevas Variedades de Cultivares

- **Hibridación**
  - **Cruce con Variedades Silvestres**
  - **Híbridos**
- **Mutaciones**
  - **Irradiación**
  - **Mutágenos Químicos**
- **Cultivos Celulares**
  - **Rescate de Embriones**
  - **Variación Somatoclonal**

History of Plant Biotechnology (Monsanto)

Tens of thousands of years ago...

People wandered the earth, collecting and eating only what they found growing in nature. By about 8,000 BC, however, the first farmers decided to stay in one place and grow certain plants as crops — creating agriculture and civilization, in that order.

Thousands of years ago

People first learn to use bacteria to make new and different foods, and to employ yeast and fermentation processes to make wine, beer and leavened bread.

1700s

Naturalists begin to identify many kinds of hybrid plants — the offspring of breeding between two varieties of plants.

History of Plant Biotechnology (Monsanto) Continued…..

1856 Gregor Mendel begins a meticulous study of specific characteristics he found in various plants which were passed to future plant generations.

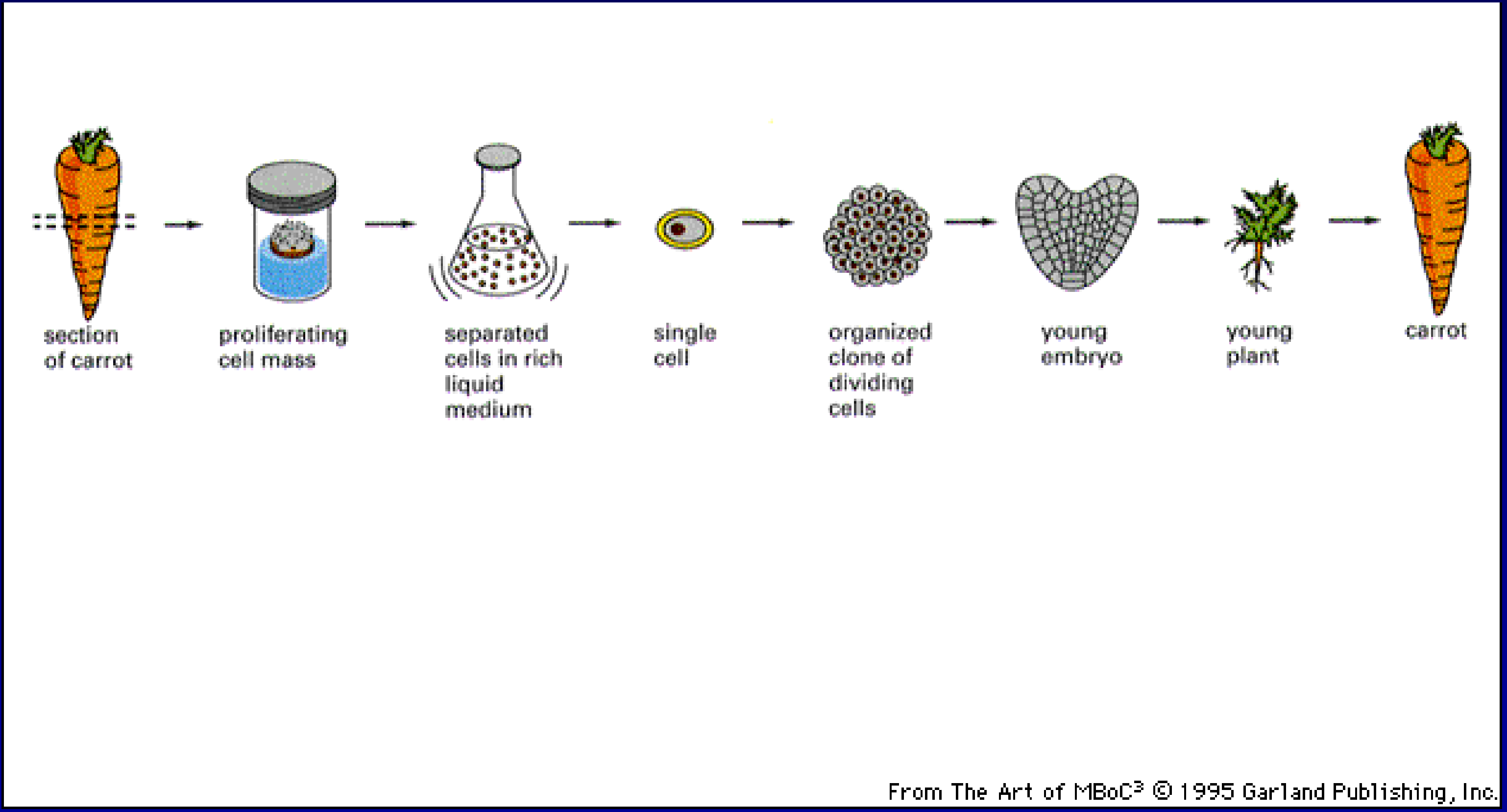
1861 Louis Pasteur defines the role of micro-organisms and establishes the science of microbiology.

1900 European botanists use Mendel's Law to improve plant species. This is the beginning of classic selection.

1950 First regeneration of entire plants from an in vitro culture.

1950

First regeneration of entire plants from an in vitro culture.



History of Plant Biotechnology (Monsanto) Continued 2…..

1953 James Watson and Francis Crick discover the double helix structure of deoxyribonucleic acid, commonly known as DNA. Proteins are made up of strings of amino acids. The number, order and kind of amino acids determine the property of that protein. DNA holds the information necessary to order the amino acids correctly. The DNA transmits this hereditary information from one generation to the next. But it wasn't until three decades later that even larger strides occurred in the field. Watson and Crick would later receive the Nobel Prize for their work.

History of Plant Biotechnology (Monsanto) Continued 3…….

1970s The Green Revolution introduces hybrid seeds into food-short Third World countries.

1973 Researchers develop the ability to isolate genes. Specific genes code for specific proteins.

1980s Scientists discover how to transfer pieces of genetic information from one organism to another, allowing the expression of desirable traits in the recipient organism. This is called genetic engineering, one process
used in biotechnology. Using the technique of "gene splicing" or "recombinant DNA technology" (rDNA), scientists can add new genetic
information to form a new protein which creates traits that protect plants
from diseases and pests.

History of Plant Biotechnology (Monsanto) Continued 4…….

1982The first commercial application of this technology is used to develop human insulin for diabetes treatment.

1983 The first transgenic plant: a tobacco plant resistant to an antibiotic.

1985 Genetically engineered plants resistant to insects, viruses, and bacteria are field tested for the first time.

1990 The first successful field trial of genetically engineered cotton plants (bt cotton) is conducted. DEKALB receives the first patent for transformed corn.

History of Plant Biotechnology (Monsanto) Continued 5…….

1994 The Flavr-Savr tomato, designed to resist rotting, is approved by the FDA for sale in the United States.

1995-1996 Monsanto's Roundup Ready soybeans, which are resistant to herbicides, and YieldGard Corn, which is protected from the corn borer, are approved for sale in the United States. Bollgard cotton first commercialized in the US.

1996 Posilac bovine somatotropin, designed to increase milk efficiency in dairy cattle, is approved for use in the United States.

1997 Roundup Ready cotton first commercialized in the US.

History of Plant Biotechnology (Monsanto) Continued 6…….

1998 DEKALB markets the first Roundup Ready corn. YieldGard® Corn is approved for import into European Union.

2000 Scientists achieve major breakthrough in rice; data to be shared with worldwide research community.

On Nov. 27, President and CEO Hendrik Verfaillie announced the
New Monsanto Pledge, the company's commitments to achieving sustainable agriculture.

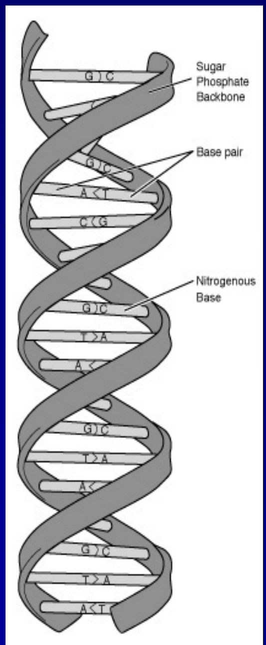
Genética: estudio de la herencia

Gregor Mendel: desarrollo un set de experimentos que nos indicaron la existencia de elementos biológicos llamados genes

Cruzamiento: un cruzamiento es un apareamiento controlado entre dos organismos específicos. El propósito de hacer cruzas en genética es obtener progenie de un genotipo específico o usar las proporciones de diferentes fenotipos de la progenie para deducir los genotipos de los padres.

Algunas plantas pueden ser autopolinizadas (autofecundados). La mayoría de los animales no pueden ser autofecundados.

A gene is a DNA segment that encodes a specific protein that contributes to expression of a trait.



→ **mRNA** → **protein** ⋯→ **trait**

transcription

translation

expression

Producing transgenic plants

- Isolate and clone gene of interest
- Add DNA segments to initiate or enhance gene expression
- Add selectable markers
- Introduce gene construct into plant cells (transformation)
- Select transformed cells or tissues
- Regenerate whole plants

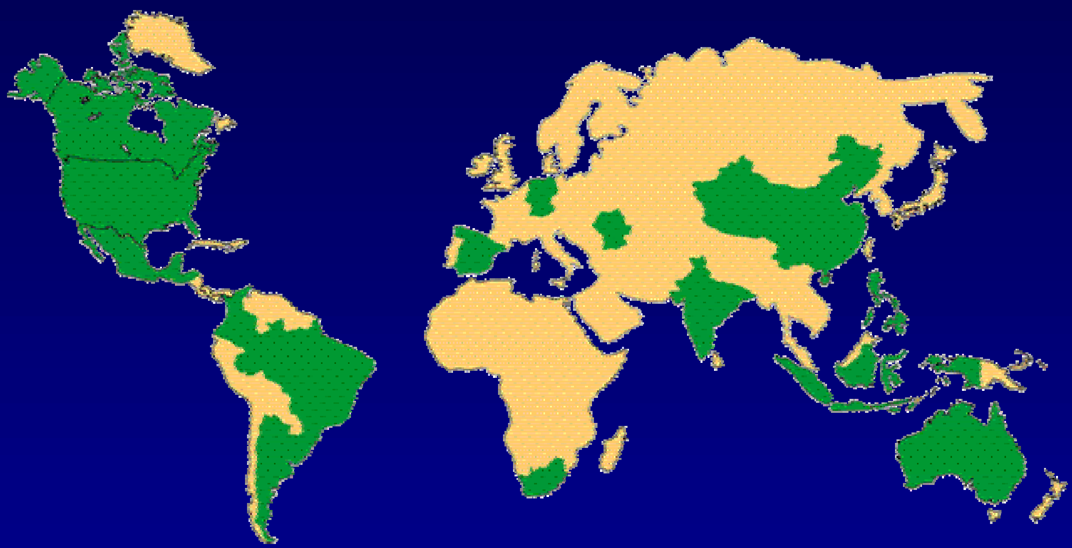
Identify and clone the gene of interest

- The most limiting step in the transgenic process.
- Public and private labs are directing huge efforts to locate, identify, characterize, and clone genes of agricultural importance.

PUBLIC – PRIVATE PARTNERSHIPS
In AGRICULTURAL BIOTECHNOLOGY
SOME EXAMPLES IN ASIAN AGRICULTURE

Randy A. Hautea
Global Coordinator, ISAAA
and Director, ISAAA SEAsia Center

Global Area (million hectares) of Transgenic Crops, 1996 to 2003, by Country and for the Top Six Countries



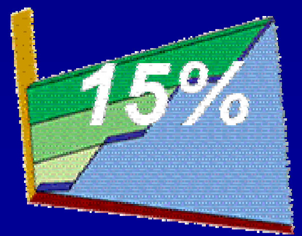
50,000 hectares, or more

USA	42.8 million
Argentina	13.9 million
Canada	4.4 million
Brazil	3.0 million
China	2.8 million
South Africa	0.4 million
Australia	0.10 million
India	0.10 million
Romania	>0.05 million
Uruguay	>0.05 million

Less than 50,000 hectares

Spain	Bulgaria
Mexico	Honduras
Philippines	Germany
Colombia	Indonesia

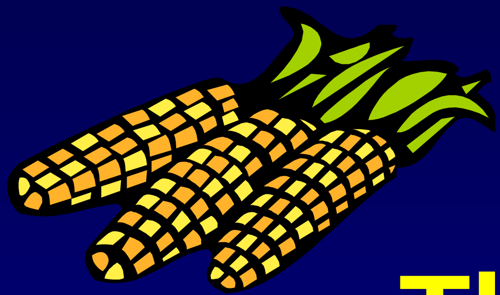
Increase over 2002

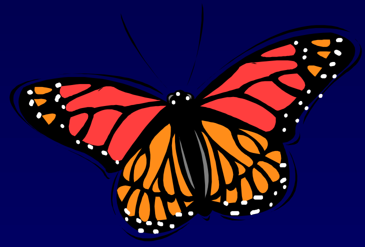


■ 18 countries which have adopted biotech crops

In 2003, global area of biotech crops was 67.7 million hectares, representing an increase of 15% over 2002.

Source: Clive James, 2003





The Basics of Transgenic Technology

What are transgenic plants?

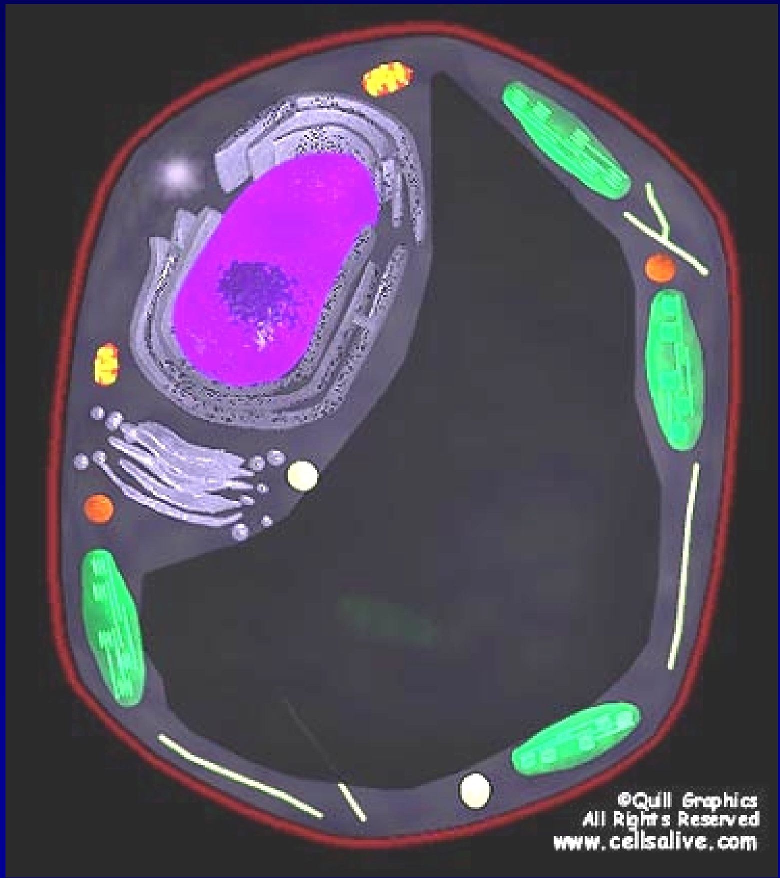
- **Transgenic** indicates gene transfer using recombinant DNA technology. The transferred gene is usually, but not necessarily, from outside the normal range of sexual compatibility.
- **Synonyms:**

Genetically modified organism (GMO) Genetically engineered organism (GEO)

Plant
Transformation Technology

- Nuclear Transformation
- Plastid Transformation

Plant Cells contain 3 genomes



- Nuclear
- Plastid
- Mitochondria

Clonamiento de un gen especifico

Elección de un vector de clonamiento

a) Plasmidios

b) Fago λ

c) Cosmidos

d) YACs

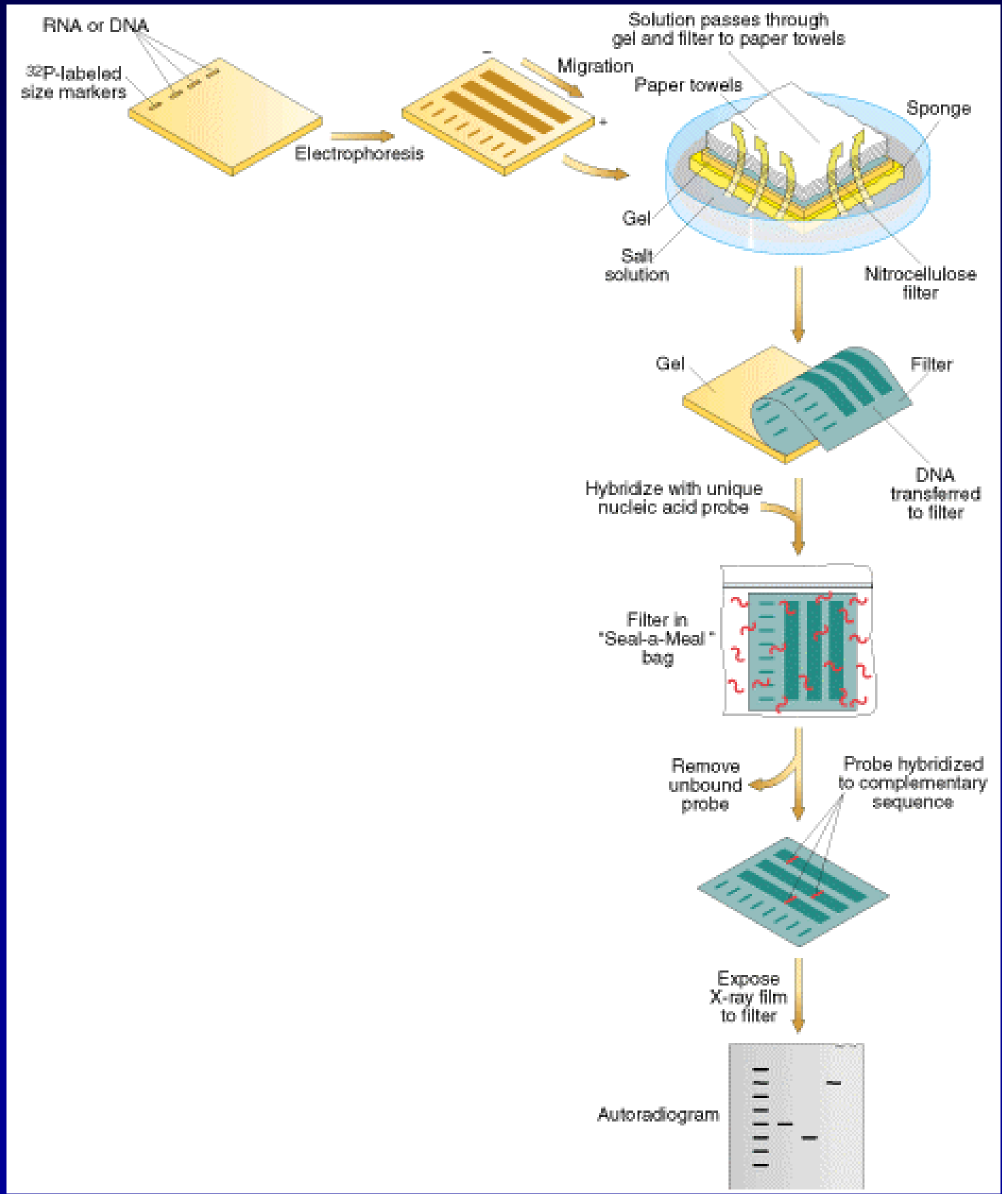
e) BACs

Construcción de una genoteca

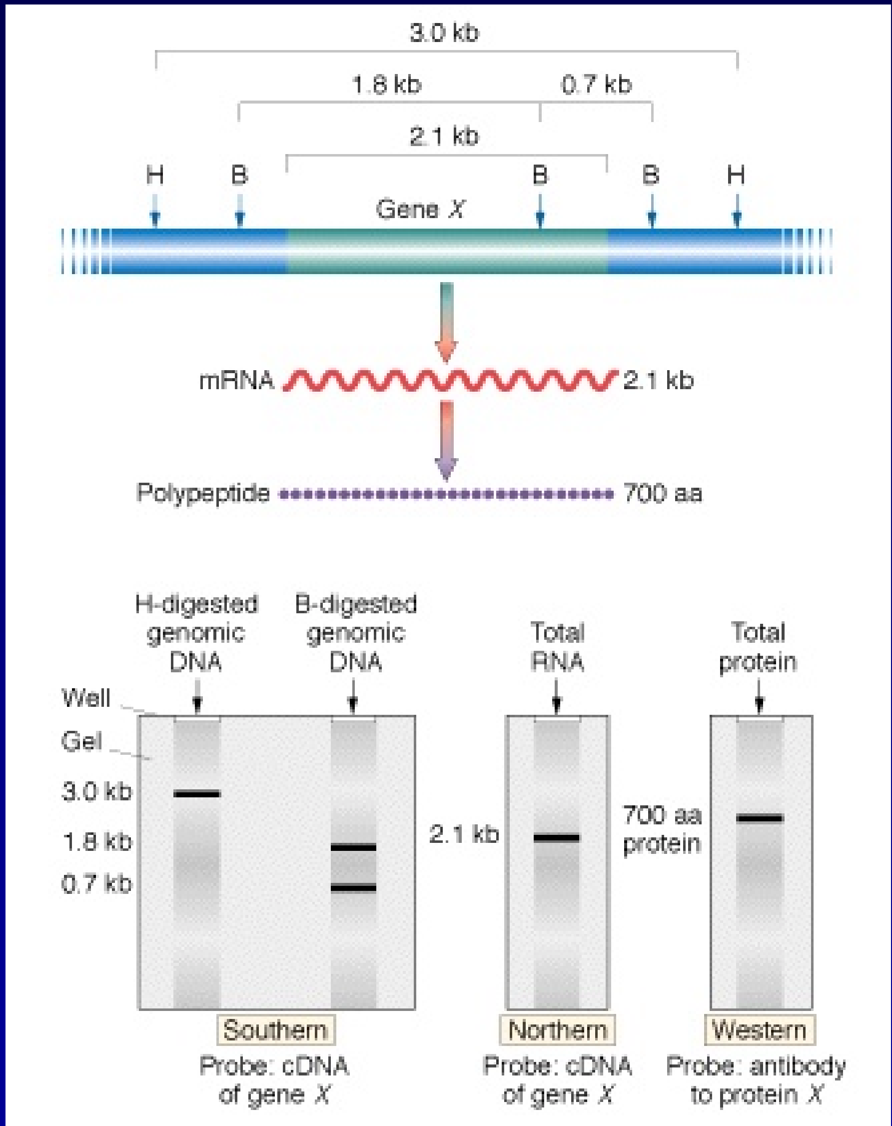
a) Genoteca genómica

b) Genoteca de cDNA

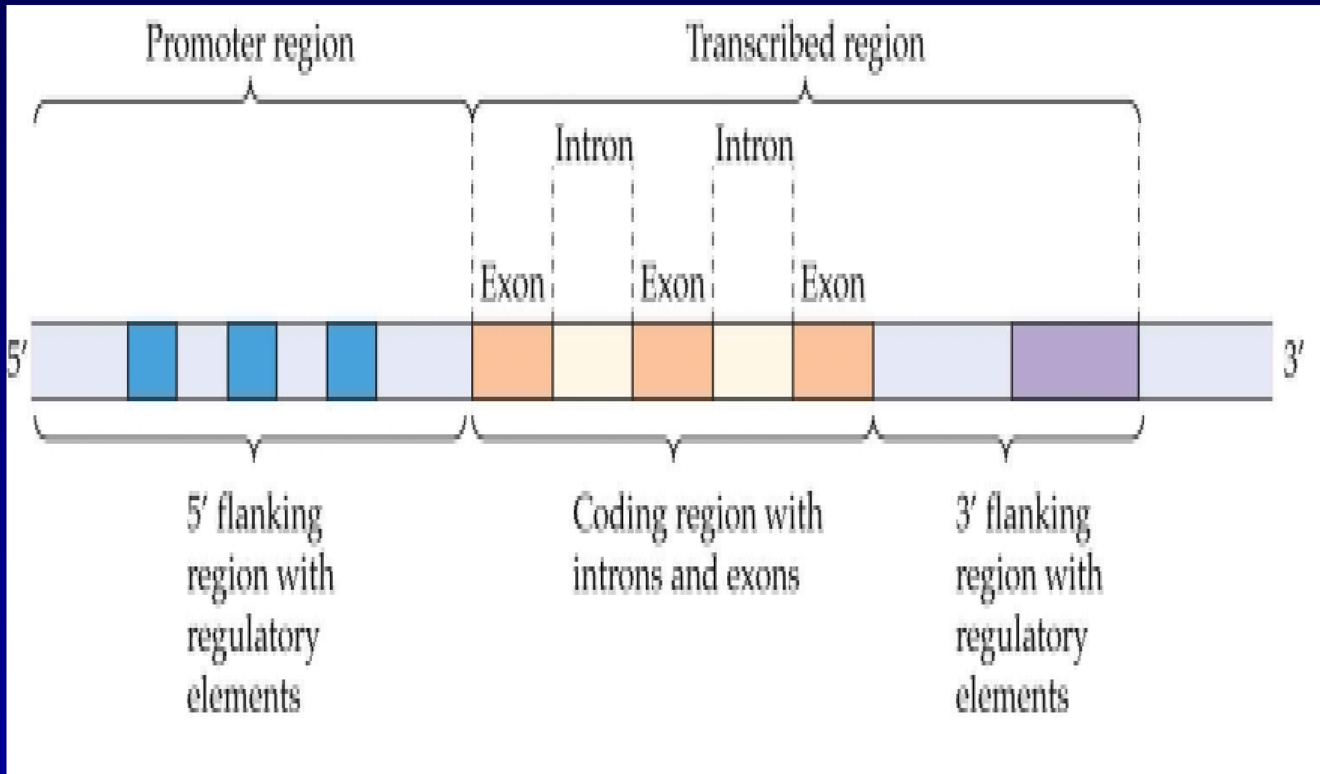
Gel electrophoresis and blotting in identifying specific cloned genes



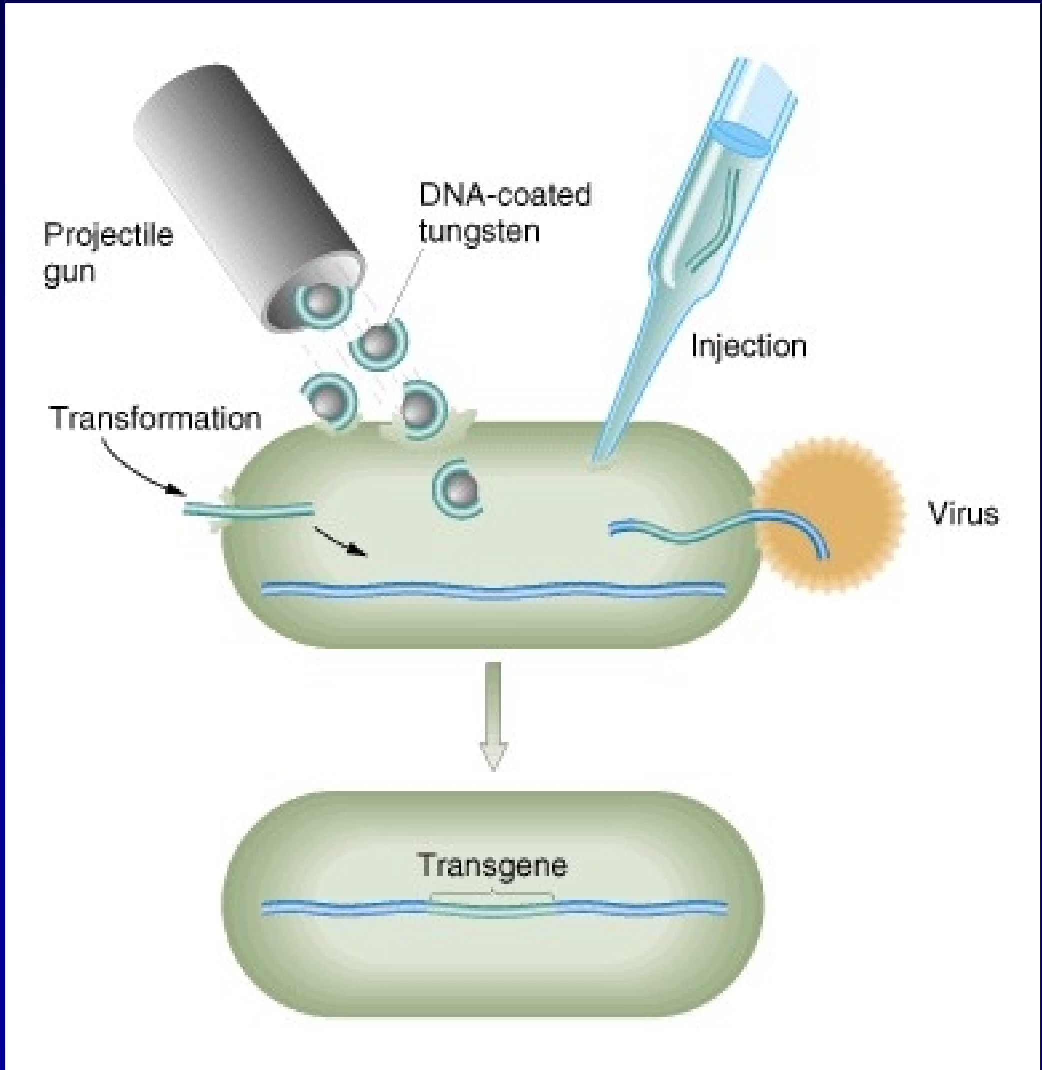
Comparison of Southern, Northern, and Western analyses of gene X



The structure of a gene

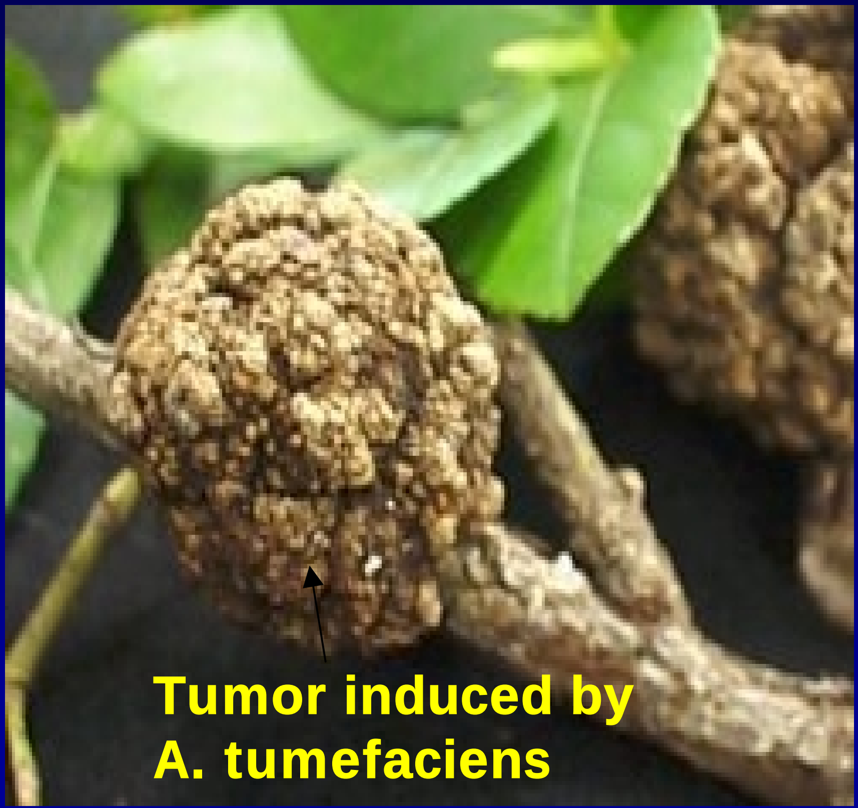


Some of the different ways of introducing foreign DNA into a cell



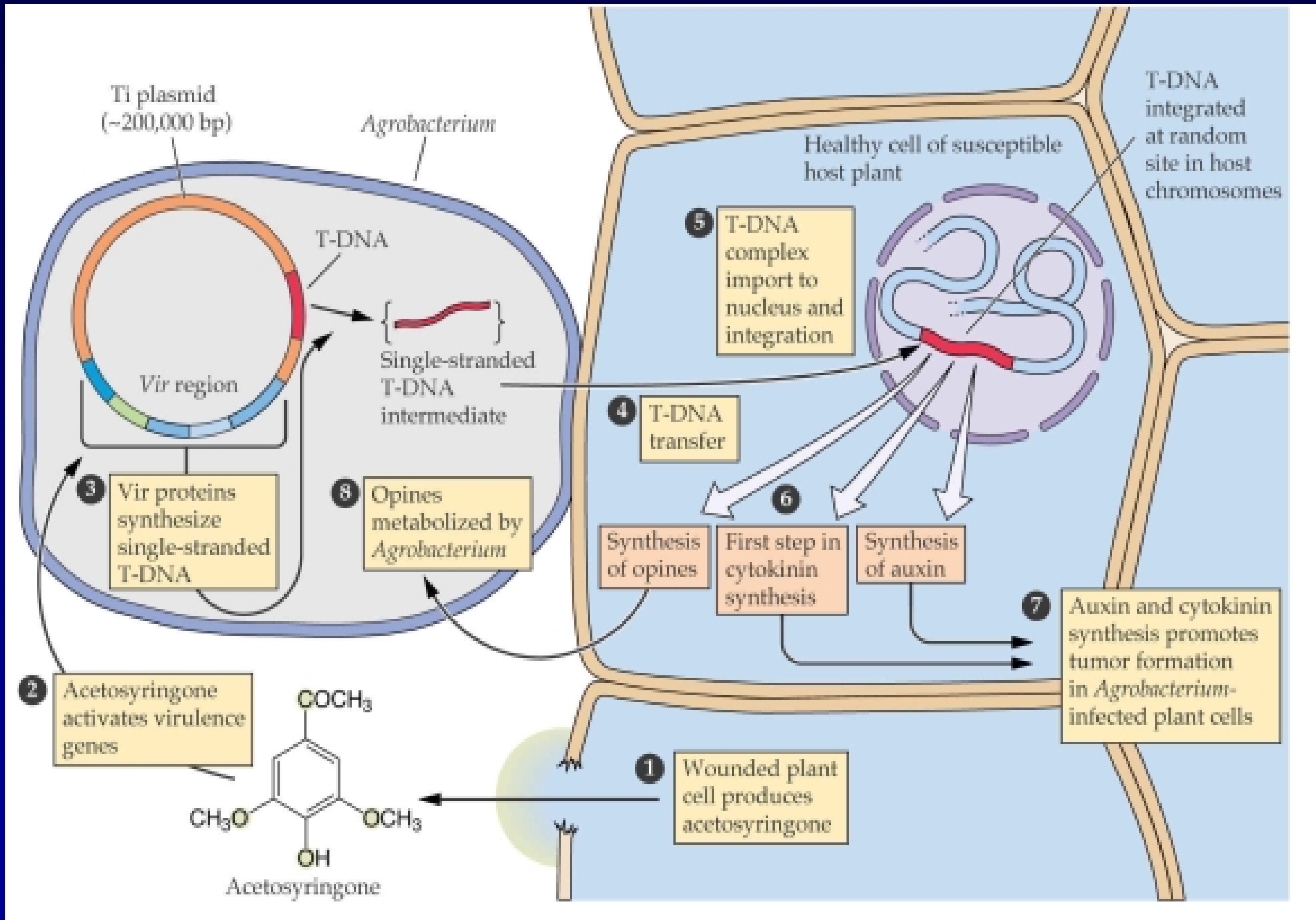
Genetic engineering of plants with *Agrobacterium tumefaciens*

- *A. tumefaciens:* used extensively for genetic engineering of plants.
- engineering selected genes into the T-DNA of the bacterial plasmid
- integrated into the plant chromosomes when the T-DNA is transferred.

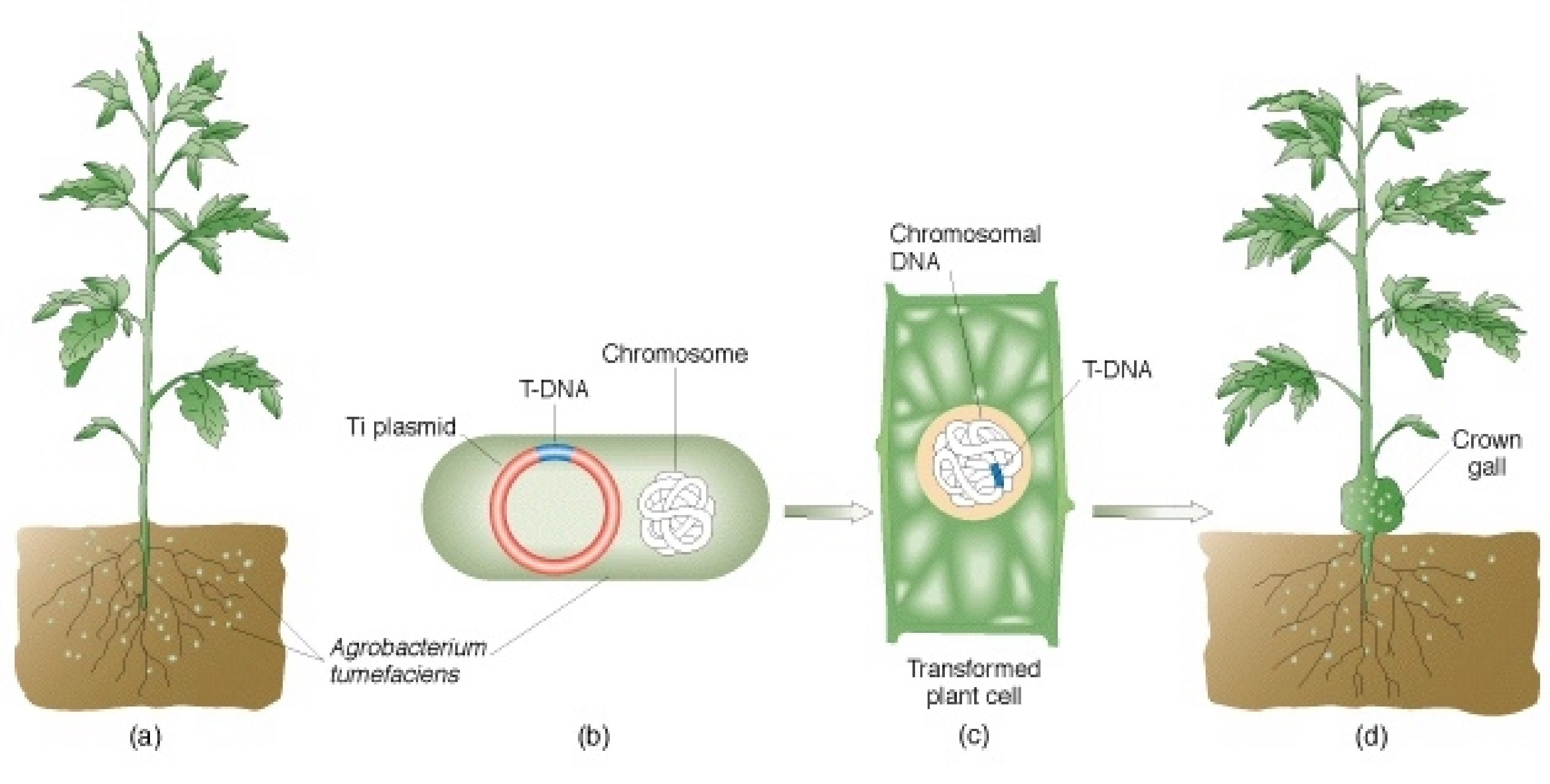


http://courses.washington.edu/z490/gmo/natural.html

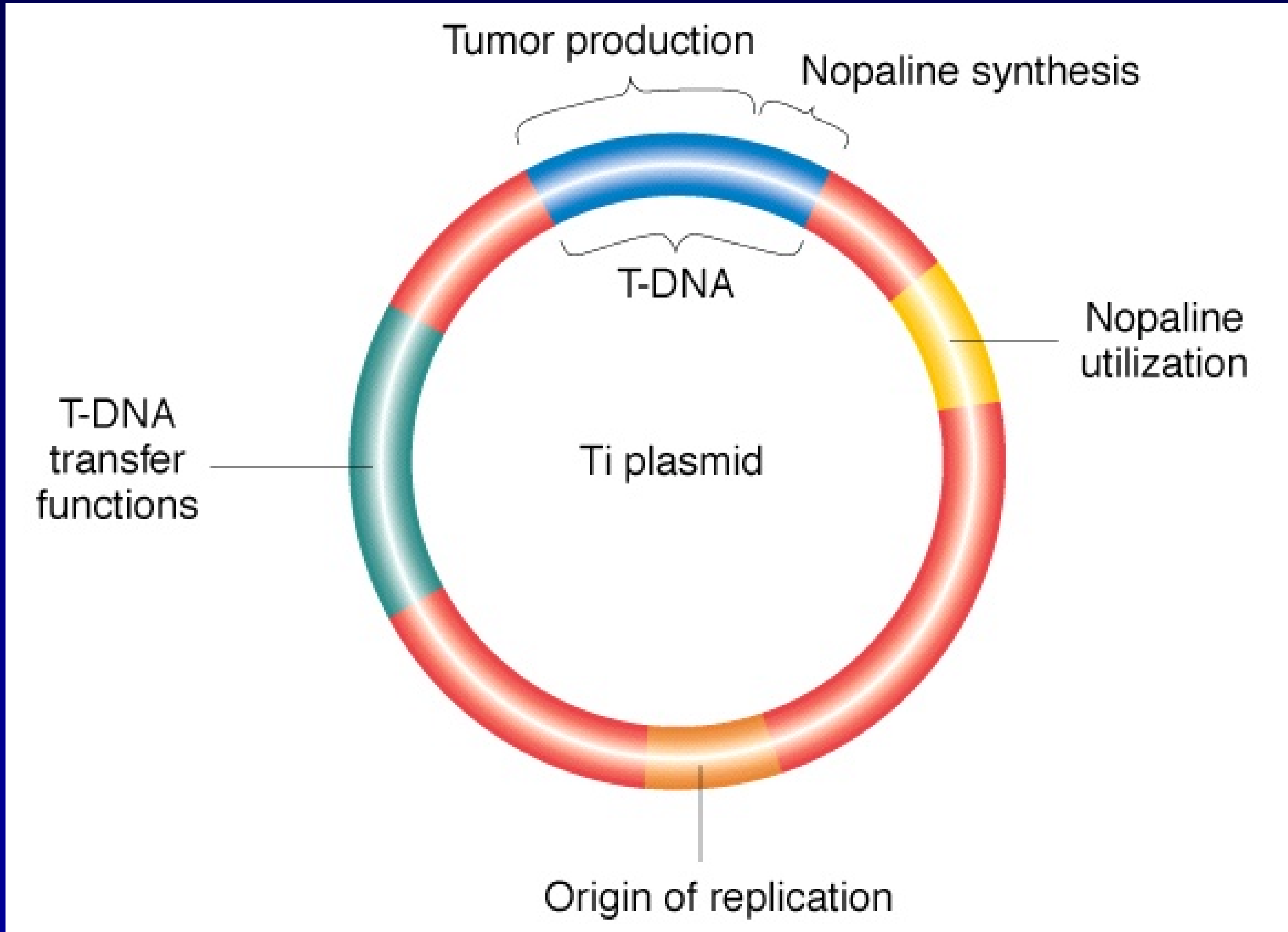
Agrobacterium-mediated T-DNA transfer



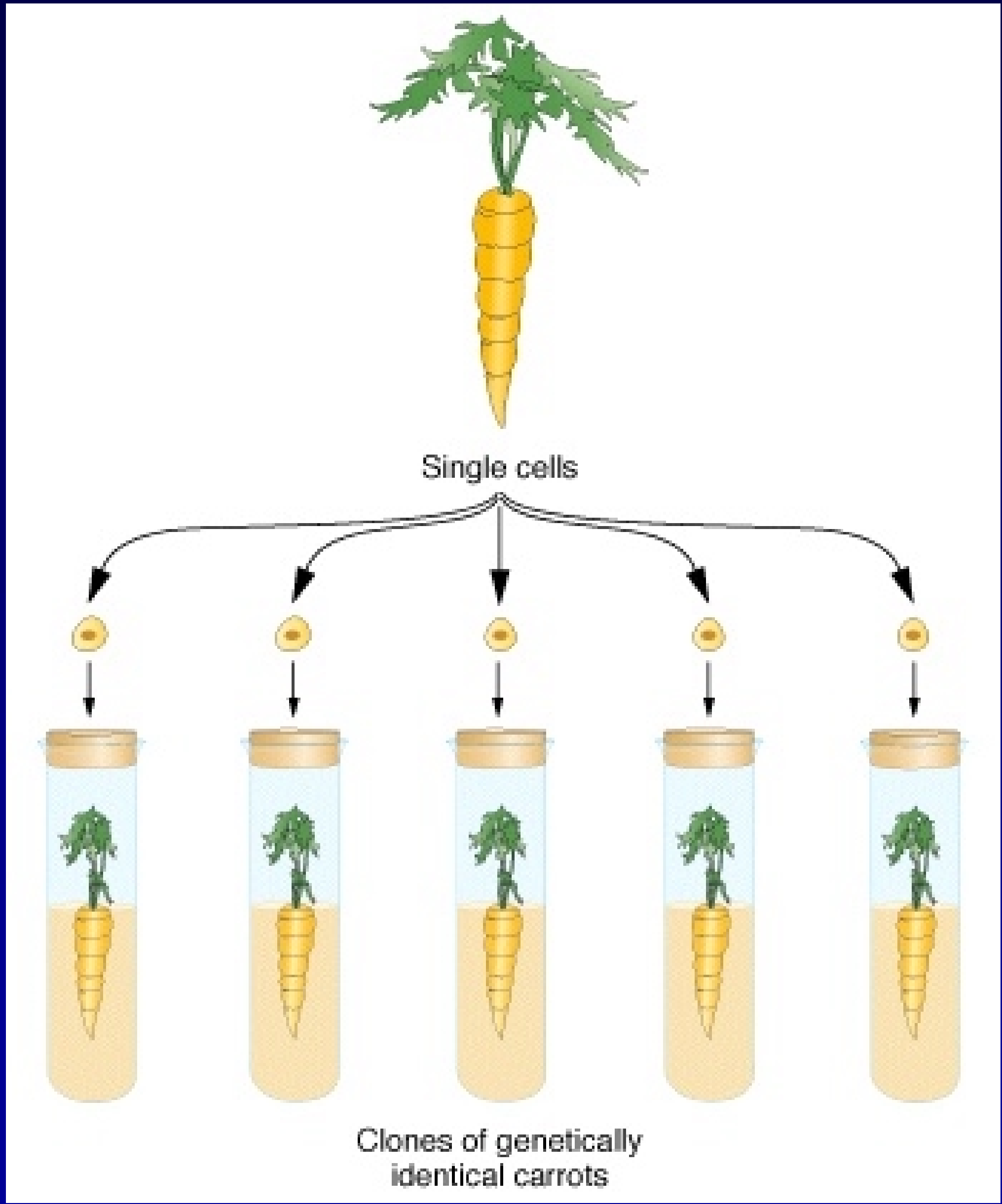
Crown gall disease by the bacterium *Agrobacterium tumefaciens*



Simplified representation of the major regions of the Ti plasmid of *A. tumefaciens*



Genetically identical plants



Future plant genome objectives

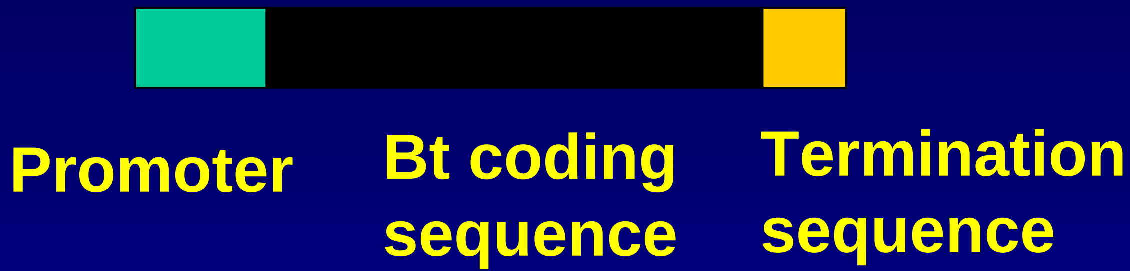
- Determine function of all Arabidopsis genes by 2010.
- Sequence the rice genome (smallest genome of grain crops), both public and private sectors.
- Sequence *Medicago truncatula* as a model system for legume biology.
- Sequence selected gene-rich regions of crops with large genomes, e.g., corn, wheat.

Bt genes

- **Spores of the soil bacterium *Bacillus thuringiensis* (Bt) contain a crystalline (Cry) protein. In the insect gut, the crystal breaks down and releases a toxin that binds to and creates pores in the intestinal lining.**

- **A truncated Cry gene is used in Bt crops.**

Add DNA segments to control gene expression



- **Promoter** initiates transcription; affects when, where, and how much gene product is produced.
- **Termination sequence** marks end of gene.

Transgene promoters

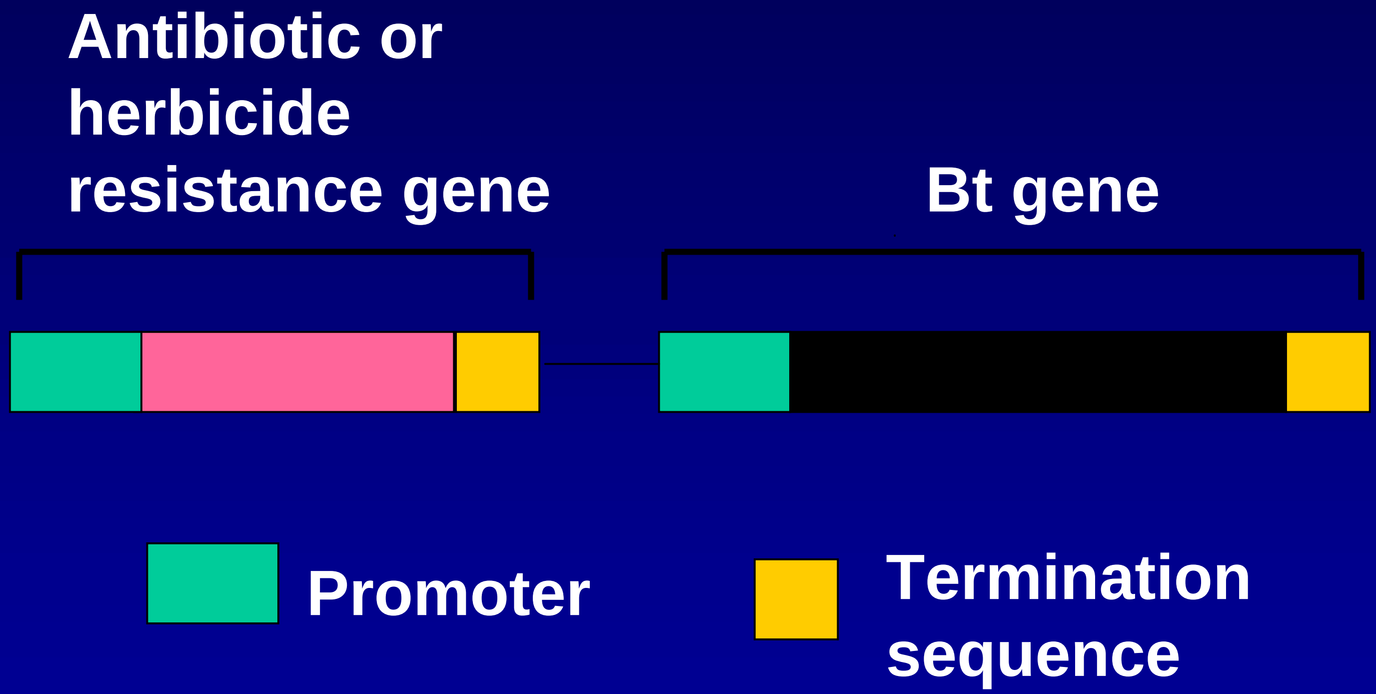
- **Most commonly used is the CaMV 35S promoter of cauliflower mosaic virus. It is a <u>constitutive</u> promoter (turned on all the time in all tissues), and gives high levels of expression in plants.**
- **More specific promoters are under development: tissue-, time-, and condition-specific.**
- **Most commonly used is the nopaline synthase (*nos*) transcription terminator sequence from *Agrobacterium tumefaciens*.**

Add selectable markers

- **Because gene transfer is an inefficient process (1 to 5% success rate), a system is needed to identify cells with the new genes.**

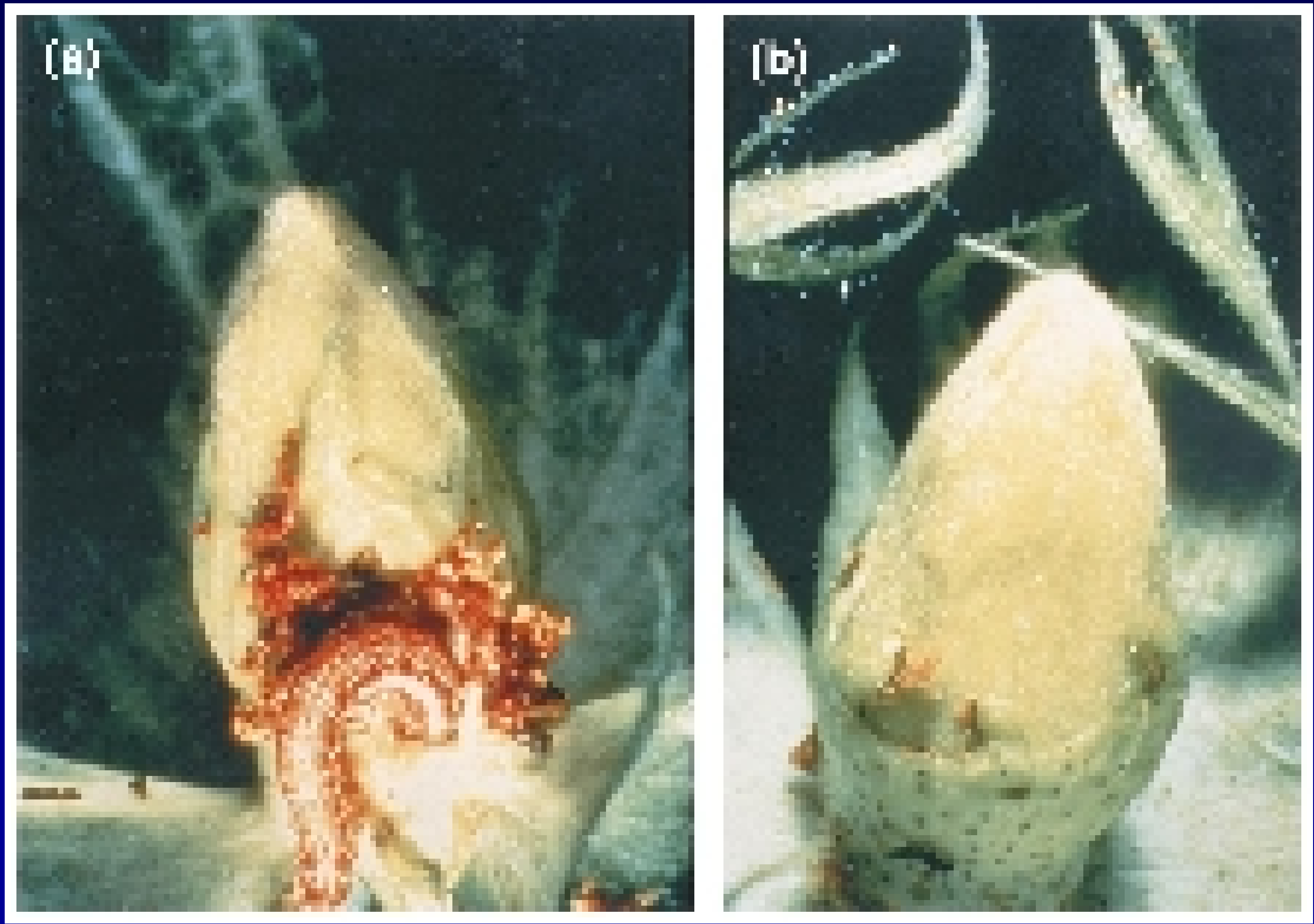
- **Typically, antibiotic or herbicide resistance genes are used as markers.**

Bt gene construct

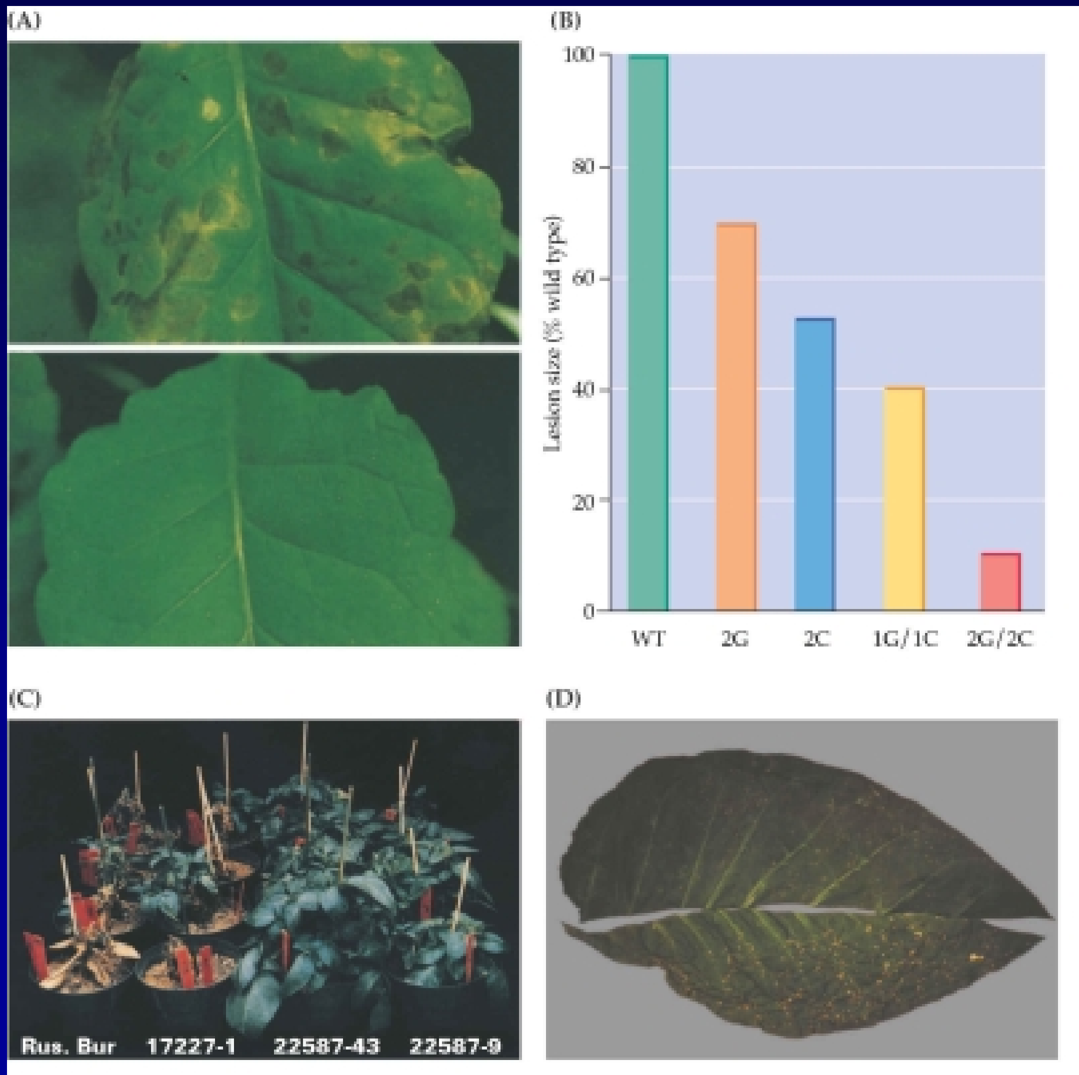


Transgenic tobacco plant expressing the luciferase gene from a firefly

The bolls of cotton plants engineered to resist the cotton boll worm, *Heliothis zea*



Disease control using transgenic plants



Floral Dipping

Update on Plant Transformation

Arabidopsis in Planta Transformation. Uses, Mechanisms, and Prospects for Transformation of Other Species[1]

Andrew F. Bent*

Plant Physiology, December 2000, Vol. 124, pp. 1540–1547,

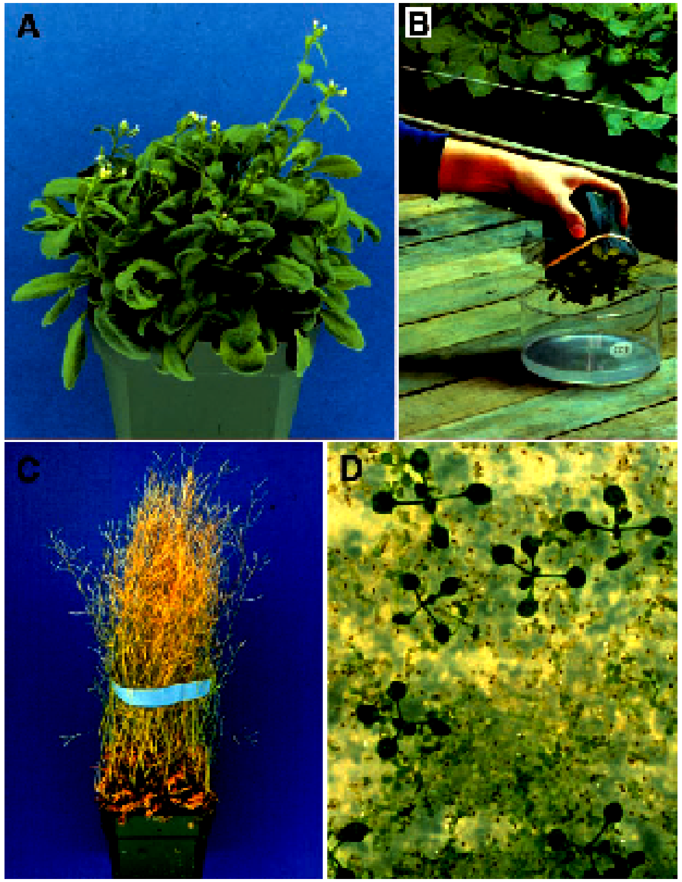
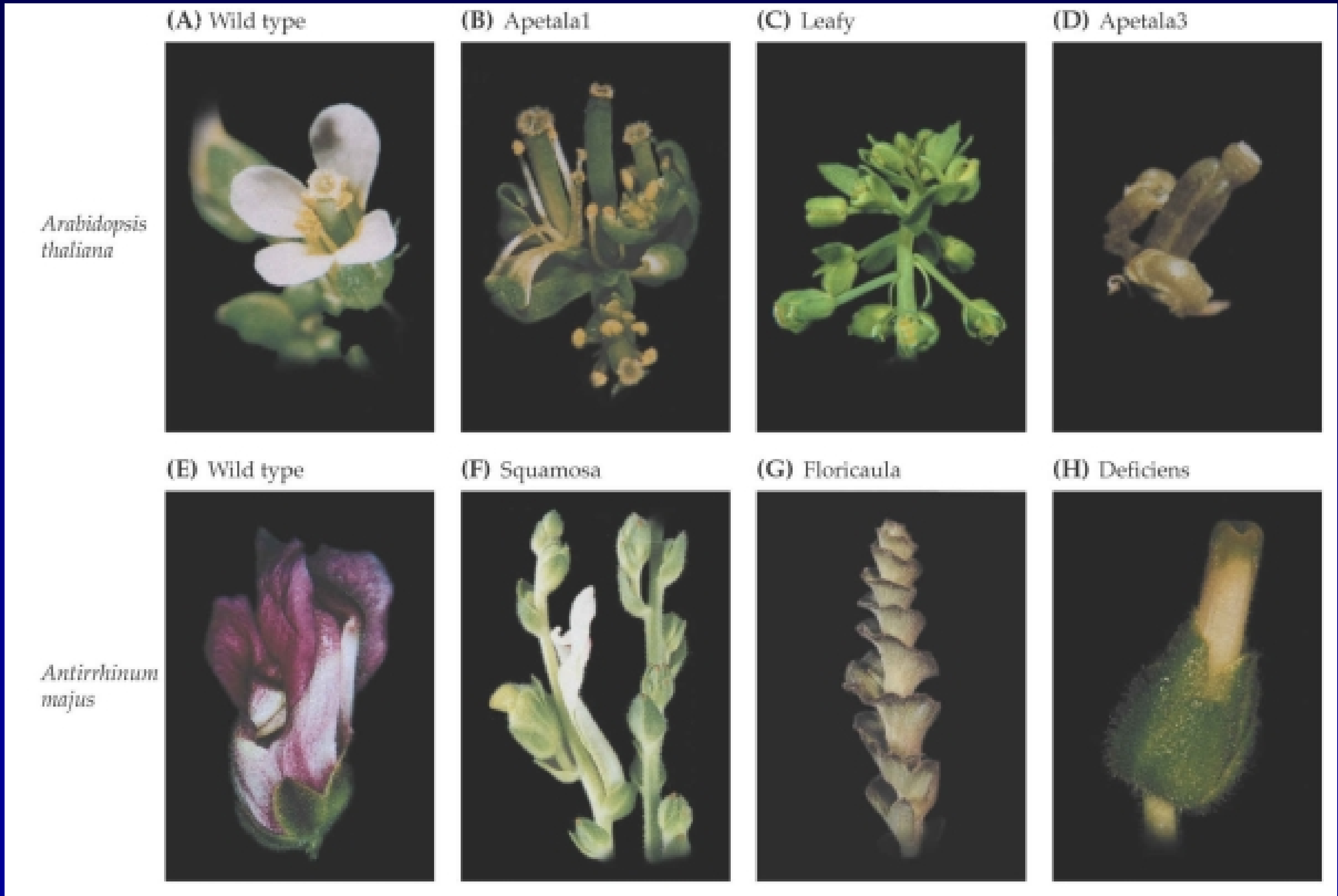


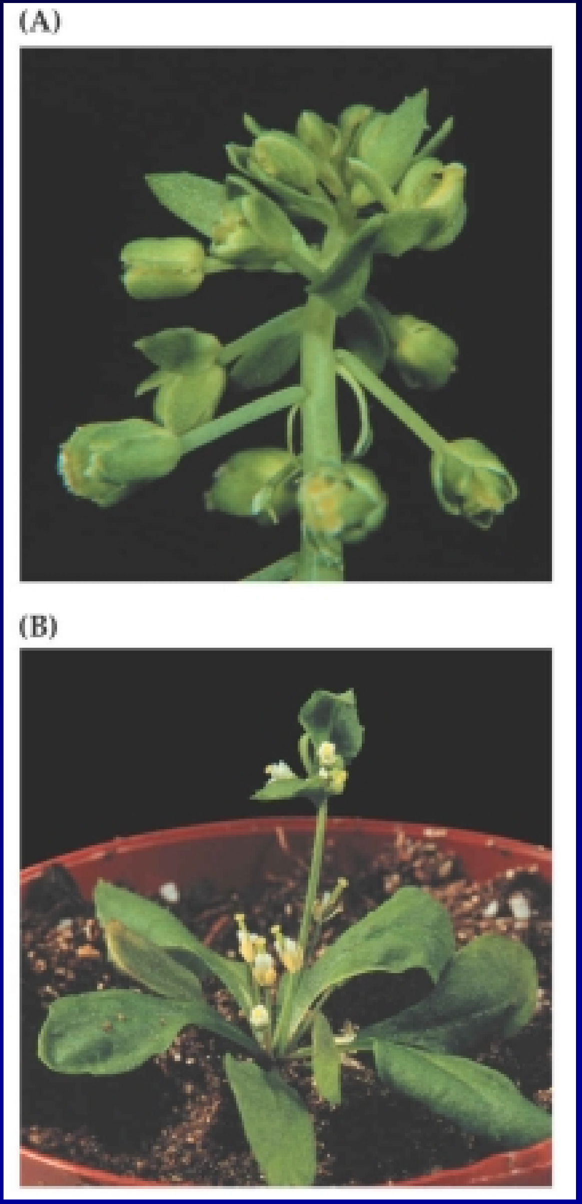
Figure 1. It's really that simple. For floral dip transformation of Arabidopsis, plants are grown to a stage when they have just started to flower (A), plants are dipped briefly in a suspension of *Agrobacterium*, Suc, and surfactant (B), plants are maintained for a few more weeks until mature and then progeny seeds are harvested (C), and seeds are germinated on selective medium (e.g. containing kanamycin) to identify successfully transformed progeny (D).

Similar genes participate in the formation of very different flowers



LEAFY (LFY), a gene for floral meristem identity, is necessary and sufficient to convert indeterminate shoots into flowers in *Arabidopsis*

a) *lfy* mutant
b) 35S::*LFY* plant



Overexpression of *LEAFY* in citrus transgenic plants

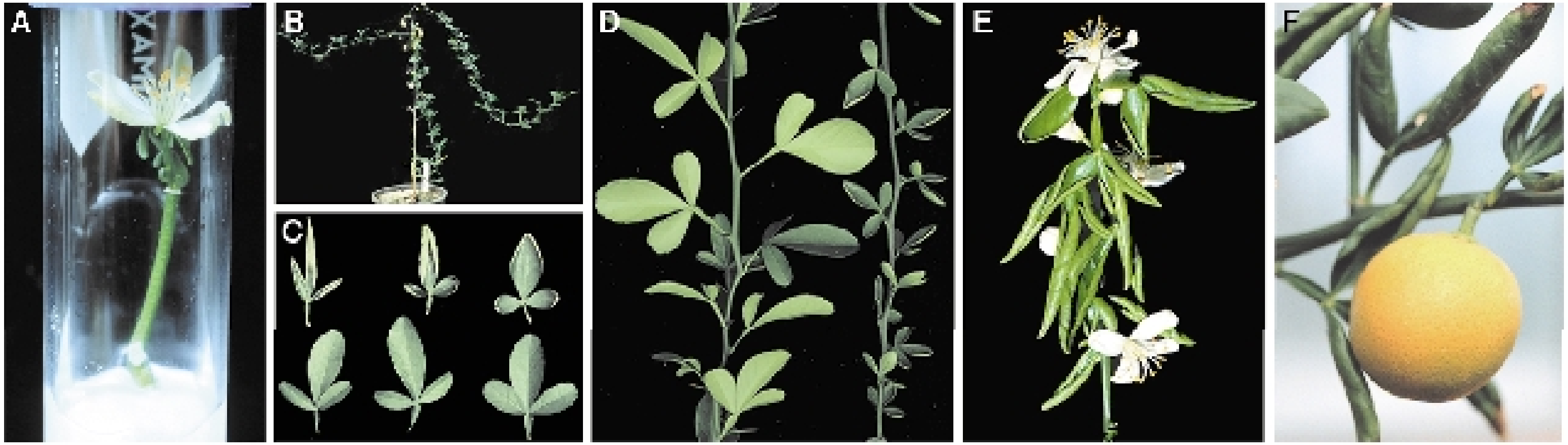


Figure 1. Overexpression of *LEAFY* in citrus transgenic plants. (A) Transgenic shoot grafted *in vitro* on a nontransgenic rootstock showing a precocious terminal flower five weeks after regeneration. (B) Transgenic plant showing a weeping growth habit. (C) Leaves from transgenic plants showing various degrees of curling (top) compared to leaves from nontransformed control plants (bottom). (D) Vegetative shoot from a transgenic plant showing the reduction of thorns and small curled leaves (right) compared to a vegetative shoot from a nontransformed control plant of the same age (left). (E) Transgenic plant flowering 16 months after its transfer to the greenhouse. (F) Ripened fruit from a transgenic plant grown in the greenhouse.

Effect of antisense ACC-oxidase genes on the ripening of tomatoes

Evaluate transformed plants

- Presence and activity of introduced gene
- Other effects on plant growth
- Environmental effects
- Food or feed safety

Presence and activity of introduced gene

- Southern blot -- is the introduced DNA present in the plant's genome?
- Northern blot -- is mRNA produced?
- Western blot -- is the protein produced?
- Is the expected phenotypic trait observed?

Backcross transformed plant into an improved variety

- For most plant species, only a few lines or varieties will give high rates of transformation. Often they are lines with poor agronomic or quality characteristics.
- Therefore, an improved variety must be backcrossed for several generations to the transformed plant.

Plant Biotechnology

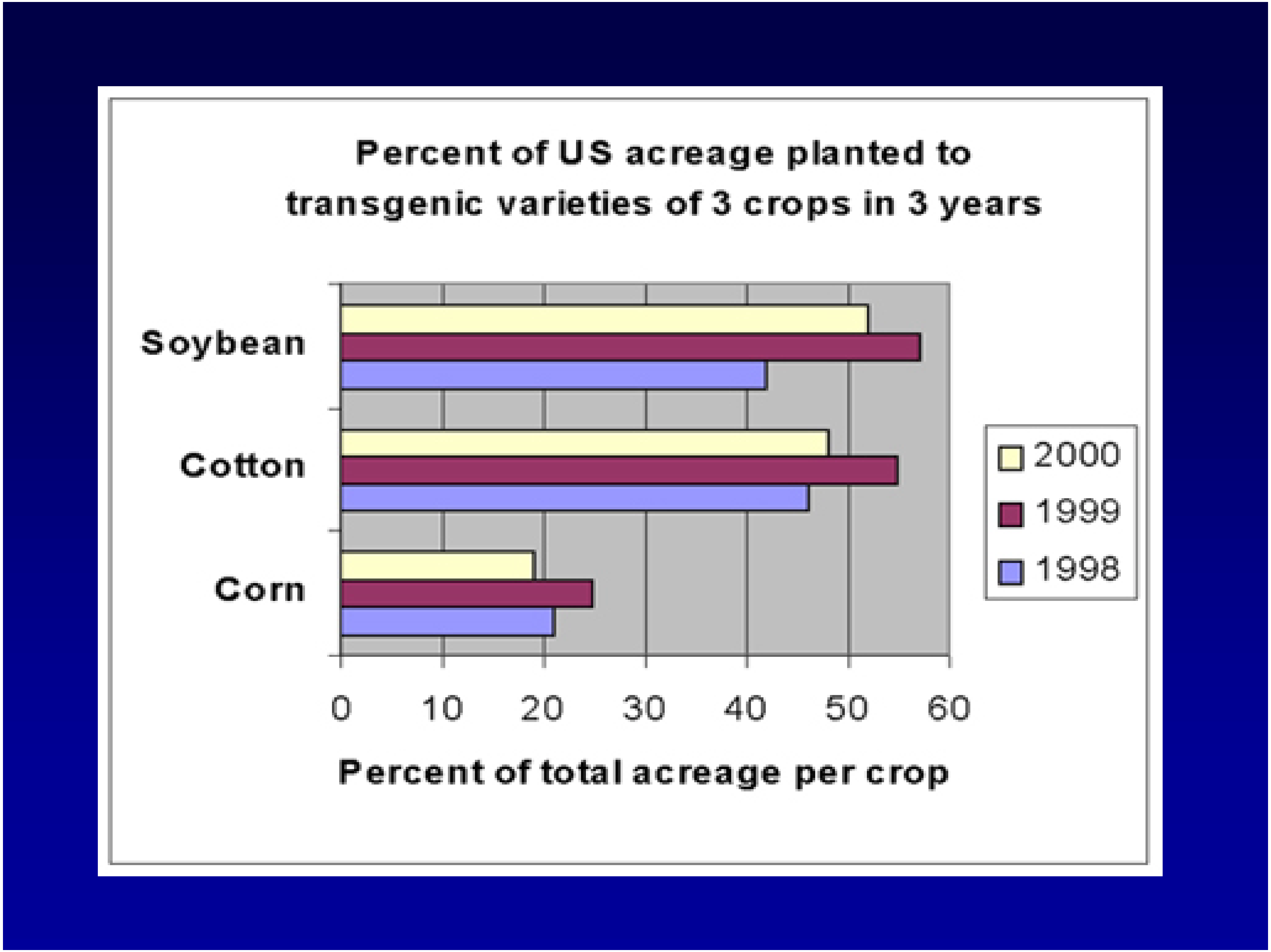
- 1º Generation
  - Insect Resistance (Bt)
  - Herbicide Resistance (Roundup)

- 2º Generation
  - improved nutritional value
  - improved uptake of nitrogen
  - factory for pharmaceuticals
  - factory for plastic production
  - phytoremediation

Applications Of Transgenic Plants

Most Abundant Transgenic Crops

1. Soybeans 53 million acres
2. Corn 27.4 million acres
3. Cotton 9.1 million acres
4. Canola 8.4 million acres
5. Potato <0.3 million acres
6. Squash <0.3 million acres
7. Papaya <0.3 million acres

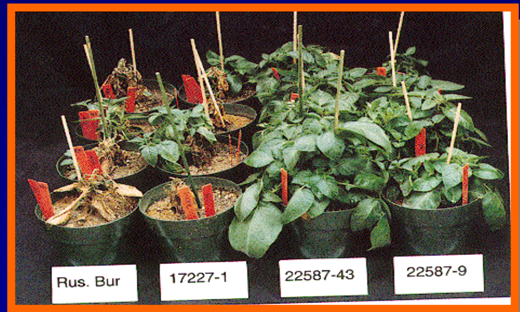

Percent of US acreage planted to transgenic varieties of 3 crops in 3 years
Soybean
Cotton
Corn
0
10
20
30
40
50
60
Percent of total acreage per crop
2000
1999
1998

What Transgenic Traits are Used

1. Herbicide Tolerance — 69.4 %
2. Bt Insect Tolerance — 22.0 %
3. Bt & Herbicide Tolerance — 7.2 %
4. Virus Resistance — <0.3 %

Biotechnology Can Add Value to Global Agriculture!

- Environmental Impact - Decreased Use of Pesticides
- Reduce Losses from Pests and Diseases
- Improve Nutrient Efficiency
- Improve Productivity

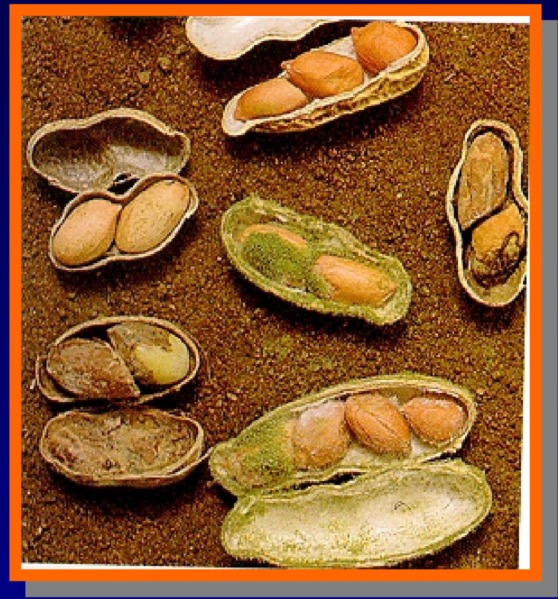


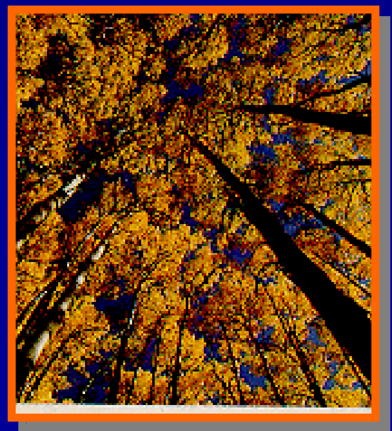
Benefits of Biotechnology…..

- **Post Harvest Quality - Prolong Shelf Life of Fruits, Vegetables and Flowers**
- **Extend Crop Area and Season**
- **Stress Tolerance - Drought, Acidity, Salinity, Heat. Flooding**

Enhancing Food and Agriculture!

- More Nutritious Food
- Healthy Produce. Low Toxins
- Pharmaceutical Proteins
- Clean Up Environment
- Industrial Products
- Value-Added Products





Genes, greens, and vaccines

Julian K-C. Ma

NATURE BIOTECHNOLOGY VOL 18 NOVEMBER 2000 *http://biotech.nature.com*

Figure 1. Pharmaceutical production, present and future. (A) A traditional fermentation factory with fermentation cylinders. (B) A field of transgenic tobacco producing a subunit vaccine.

"Terminator" Genes

- Transgenes introduced into crop plants to make them produce sterile seeds.

The process introduces three transgenes into the plant:

- A gene encoding a toxin which is lethal to developing seeds but not to mature seeds or the plant. (This gene is usually silenced (inactive) because of a stretch of DNA inserted between it and its promoter).
- A gene encoding a recombinase - an enzyme that can remove the spacer in the toxin gene thus allowing to be expressed.
- A repressor gene whose protein product binds to the promoter of the recombinase making it inactive.

How Terminator genes work

- When the seeds are soaked (before sale to farmers) in a solution of tetracycline synthesis of the repressor is blocked
- The recombinase gene becomes active and the
- spacer segment is removed from the toxin gene and it becomes expressed.
- The toxin keeps the seeds sterile but has no effect on the plant

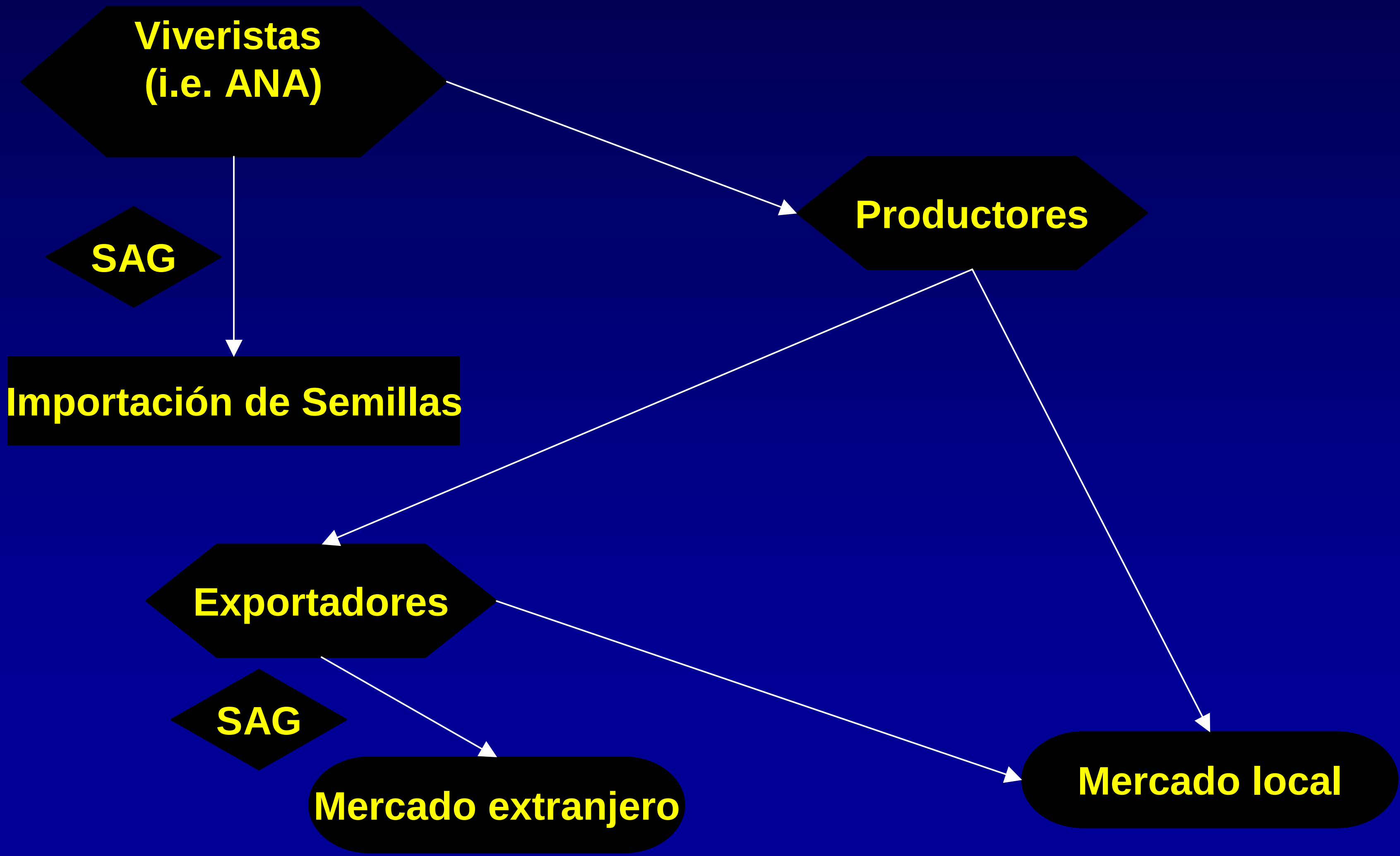
Controversy:

- Farmers want to be able to save some seed from their crop to plant the next season.
- Seed companies want to be able to be able to keep selling seed.

Issues

- Transgenic crop plants are opposed by some people and groups because of the potential risk of transgenes in commercial crops endangering native species.
- Examples:
- A gene for herbicide resistance in, e.g. corn, escaping into a weed species could make control of the weed far more difficult.
- The gene for B.t. toxin expressed in pollen might endanger pollinators like honeybees, and other non-target insects.

La Industria Agrícola en Chile



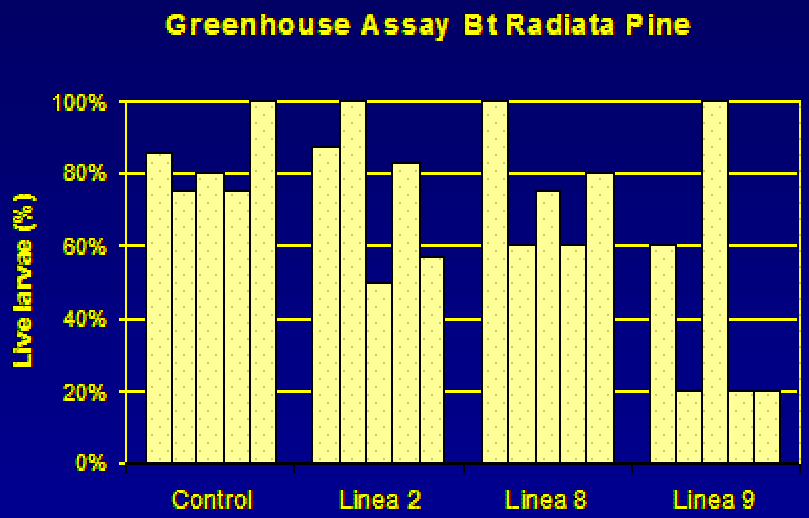
Important themes for the Chilean Agricultural Community

- **Sanitary Concerns**
- **Production**
- **Quality**
- **Coexistance**
- **Tracebility**
- **Royalies and Intelectual Property Rights**

Plant Genetic Engineering Projects

- ***Radiata Pine***
  1999 - Insect resistance
  2000 - Modification of lignin and increased cellulose content
  2001 - Resistance to fungal diseases
  2002 - Herbicide tolerance
- ***Fruits***
  2000 - Fungus resistance in grapes
  2002 - Genetic engineering of stone fruit quality
- ***Wheat***
  Phosphate Uptake
- ***Tomato***
  Resistance to pathogens
  Salt-stress resistant

Radiata pine somatic embryogenesis established in Valdivia lab



Insecticidal activity of Bt protein against shoot tip moth in transgenic radiata pine demonstrated in greenhouse assays

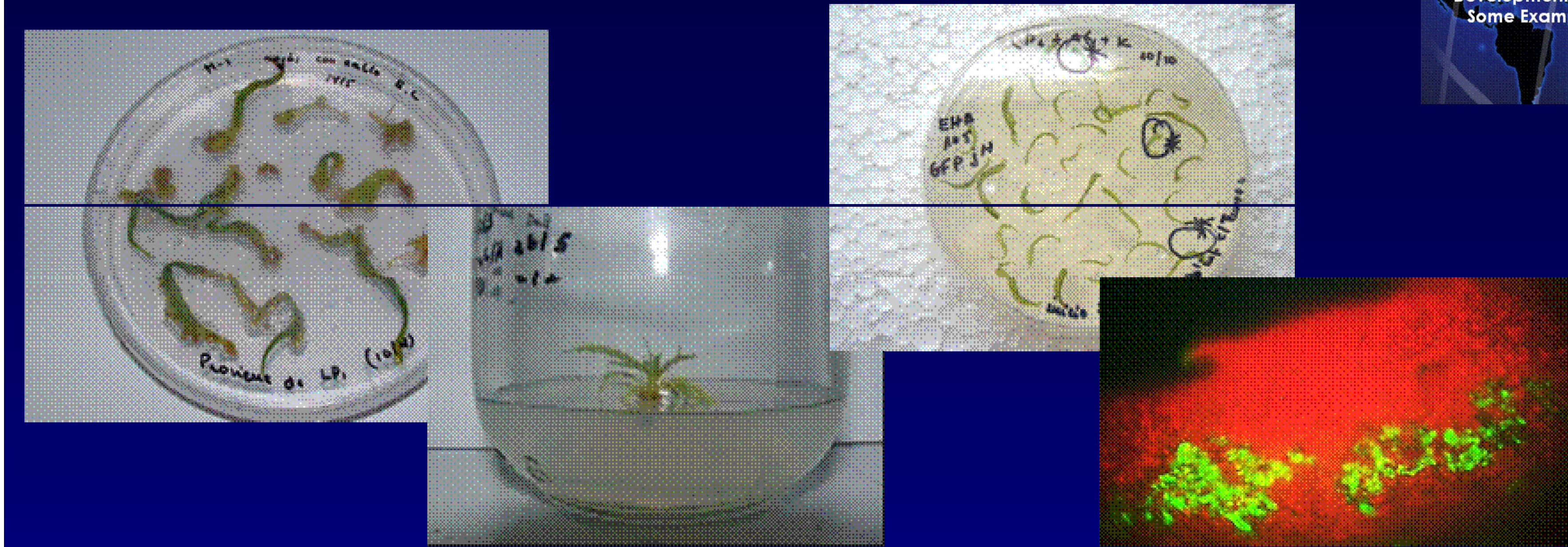
Fundación Chile

Collaborative Biotechnology Development and Transfer: Some Examples in Chile

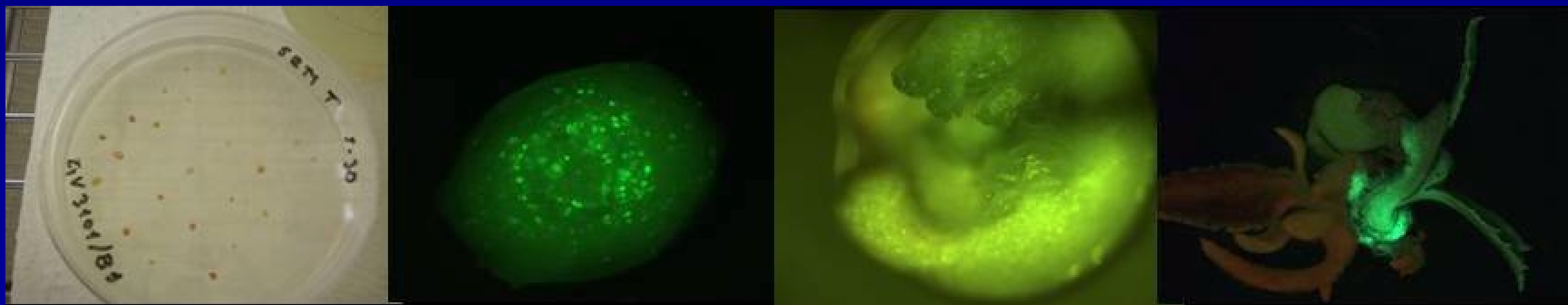
M. Moynihan
Santiago
February 2004

Grape somatic embryogenesis technology transfered to INIA

Transgenic grape plants established in greenhouse



Peach leaf explants regeneration and transformation



Plum hypocotyl transformation system (USDA protocol)

Transgenic apples under evaluation in Chile

Permits issued by SAG in Chile

1993 - 2002

Corn	Glyphosate RR, Gluphosinate, Bromoxynil, *Ostrinia nubilalis* Lepidopter resistance. High lisine contents.
Soybean	Glyphosate RR and Gluphosinate resistance
Tomato	Poligalacturonase, Lepidopter resistance, herbicide tolerance. Delayed ripening
Canola	Glyphosate RR and Gluphosinate resistance. High oil contents.
Squash	Virus resistance
Pine	Resistance to *Rhyacionia bouliana*
Eucalyptus	Glyphosate resistance.
Melon	Ethylene, virus resistance
Potato	Resistance to *Erwinia carotivora* spp *atroseptica*
Sugarbeet	Glyphosate RR and Gluphosinate resistance
Sunflower	Resistance to *Sclerotinia slerotiorum,* insect resistance
Wheat	Gluphosinate resistance
Tobacco	Resistance to PVY, *Botrytis cinerea*

Source: SAG

National Commission for Biotech Development

- Create proposal with strategic guidelines and concrete actions
- Health: regulate use of biotech
- Agriculture: commercial use of biotech crops
- BSP: review country position
- Regulate research and other areas
- Creates National Biotech Commission

Current situation - regulations

- Agricultural regulatory framework considers environmental risk assessment for the import of propagation material (Resolution 1523/01).
- Import of transgenic material for food processing and other uses is not regulated.
- CALT: Advisory Committee on Environmental Release of LMOs.

Biosafety Regulations

- SAG Resolution 1523/01 establishes requirements for the use of transgenic material:
  – produced in Chile or overseas
  – authorized by SAG's National Director on a case by case basis
  – submission of an application including technical data and information of experience in country of origin.
  – only materials to be multiplied/trialed
  – comply with biosafety requirements.

SAG
Application for the introduction of transgenic material

CALT

I Verify that required information is complete
Timing: 10 days

II Technical review of documents
Timing: 60 days

Analysis and evaluation based on submitted information

Complementary information is required

Biosafety measures cannot be established

SAG does not authorize

Biosafety measures are established

SAG authorizes (case by case resolution)

Biosafety quarantine

Permit Issued by SAG considers:

- Transformation event
- Port of entry
- Location and area of the production (quarantine)
- Variety
- Amount of seed to be imported
- Use of the seed
- Biosafety measures (isolation, handling of material and production procedures)

Biosafety regulations

- Resolution 1523/01 has taken into consideration:
  - Confidentiality
  - other uses of transgenic material
    - products/by products for animal feed
  - considers new and familiar events
  - events with/without quarantine
  - refuge plan (maize)
  - public consultation (new events)
  - Fees

Procedures for GM seed Production

Solicitud de ingreso de material transgénico: normativa 1.523

Evaluación del riesgo: CALT

Rechazo

Resolución aprobatoria: normativas de bioseguridad

S A G

Ingreso de semilla

Evaluación y ensayos: Estación Experimental

ANPROS – Sistema GPS

Multiplicación

Envasado y reexportación semillas

Procesamiento semilla en planta

Eliminación de desechos de acuerdo a normativas de bioseguridad

F I S C A L I Z A C I O N

E M P R E S A S

Sin cosecha no hay postcosecha...y hasta aquí todo bien..

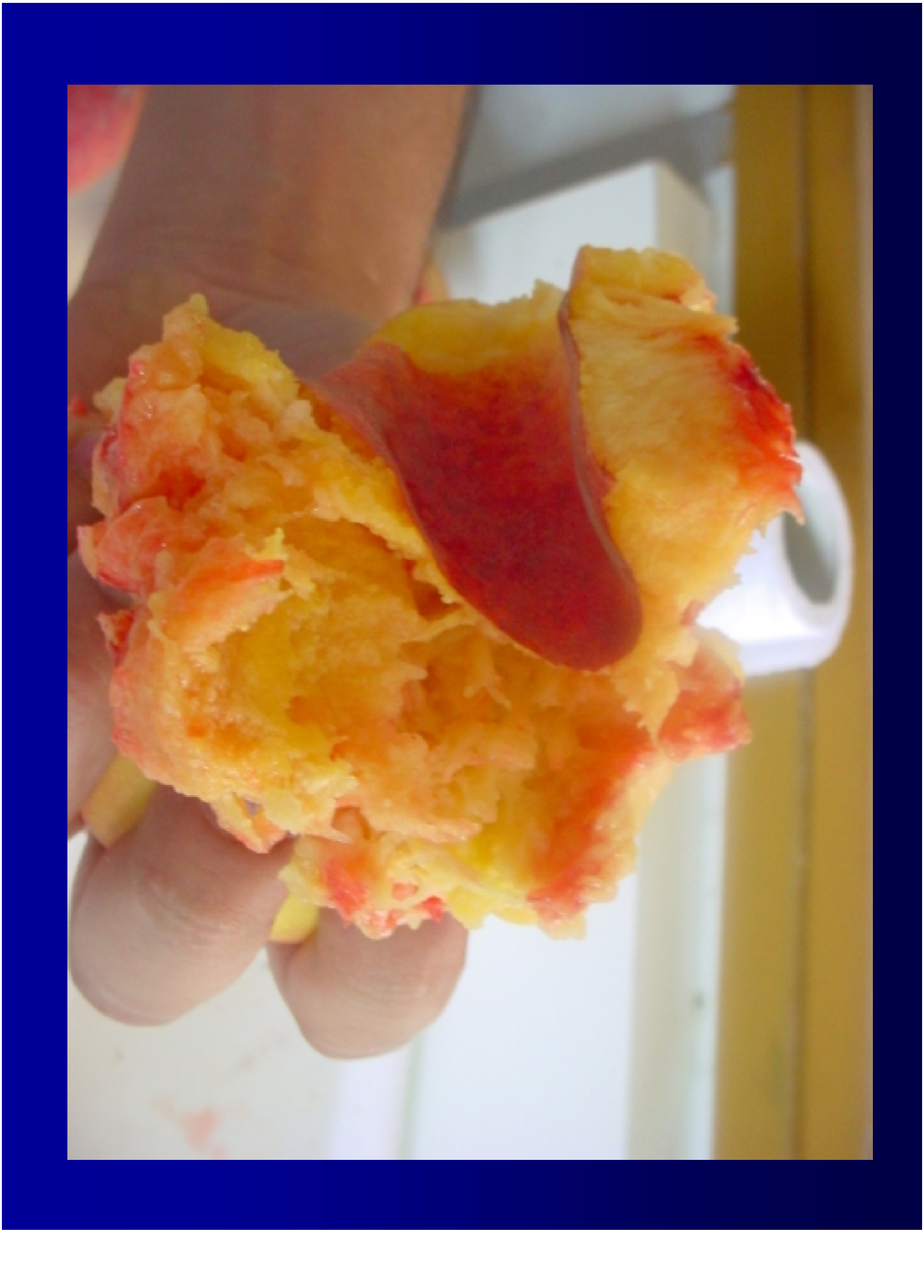
...y qué les pasó en la postcosecha?

Harinosidad

Pardeamiento

Desórdenes fisiológicos en fruta de carozo derivados de daños por frío (“chilling injury”)

Fig. 23.2. Chilling injury symptoms in stone fruits: (*a*) dry ("woolly") texture in nectarine, fruit lacking juiciness; (*b*) flesh browning in nectarine; (*c*) black pit cavity with flesh browning in peach; (*d*) black pit cavity with flesh translucency in nectarine; (*e*) flesh translucency in plum; (*f*) red flesh development ("bleeding") in nectarine; (*g*) red flesh development in plum; (*h*) external symptoms of severe flesh browning in peach.




4°C
10°C

O´Henry 15 días a 10º C
+ “ripening”

O´Henry 15 días a 4º C
+ “ripening”

TEMPERATURA!!!!!

TEMPERATURA!!!!