What is Biotechnology?

General Definition

The application of technology to improve a biological organism

Detailed Definition

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

What controls this natural variation?

Allelic **differences at** ***genes*** **control a specific trait**

Definitions are needed for this statement:

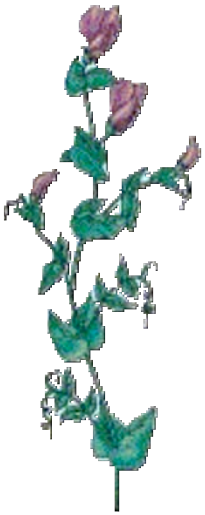
Gene - **a piece of DNA that controls the expression of a trait**

Allele - **the alternate forms of a gene**

What is the difference between genes and alleles for Mendel's Traits?

Mendel's Genes

Plant height



Tall ***Short***

Allele

Seed shape

Smooth ***Wrinkled***

Allele

Allelic Differences for Mendel's Genes
Plant Height Gene

Gene: gibberellin 3-□-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

Allelic Differences for Mendel's Seed Shape Gene

Gene: strach branching enzyme (SBE) isoform 1
Function: adds branch chains to starch
Mutation in short allele: transposon insertion
Effect of mutation: no SBE activity; less starch, more sucrose, more water; during maturation seed looses more water and wrinkles

Back crossing-manipulating genes via sexual reproduction

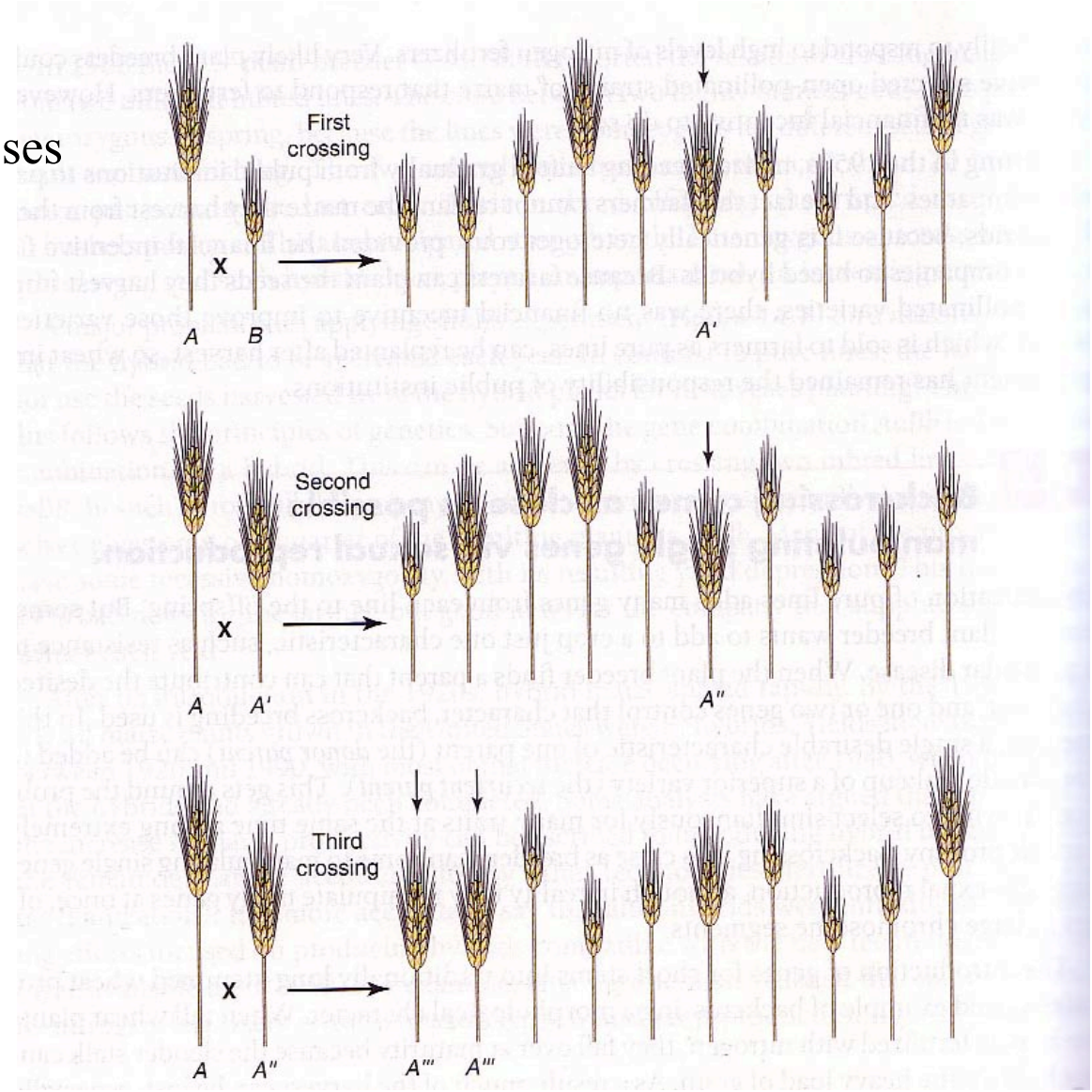
No.of Back crosses

2-12.5%

3-6.25%

4-3.12%

5-1.56%

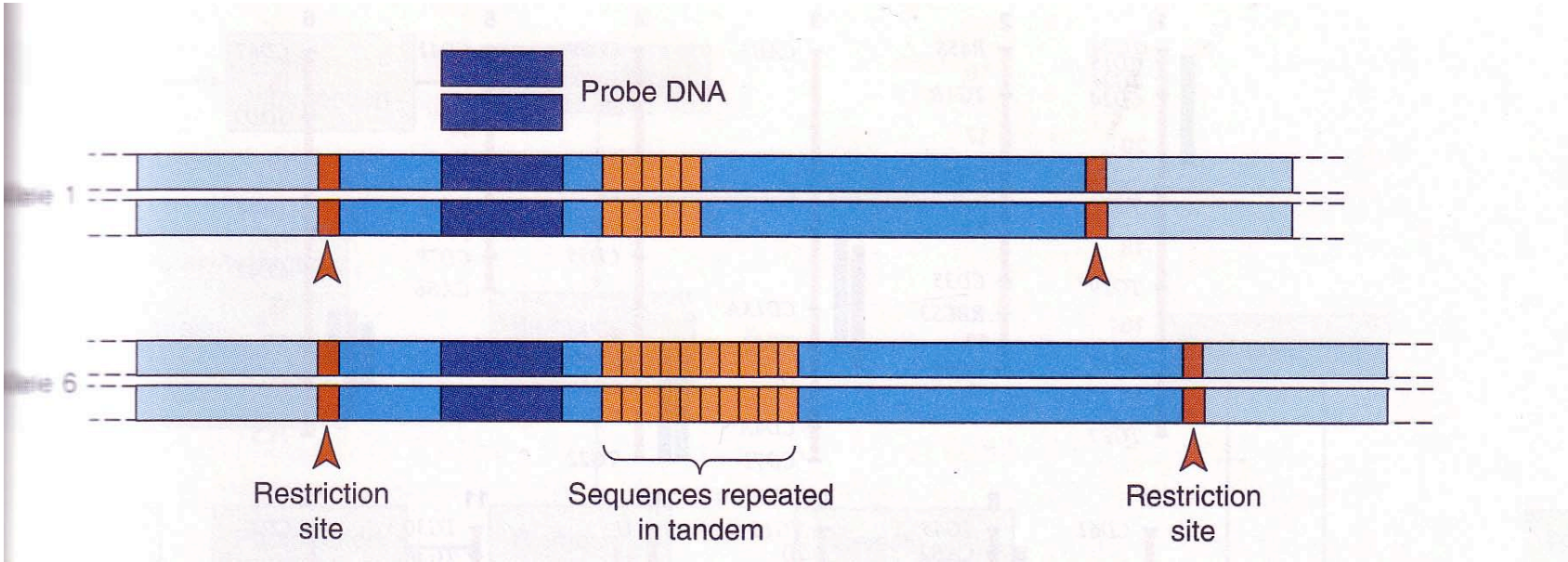


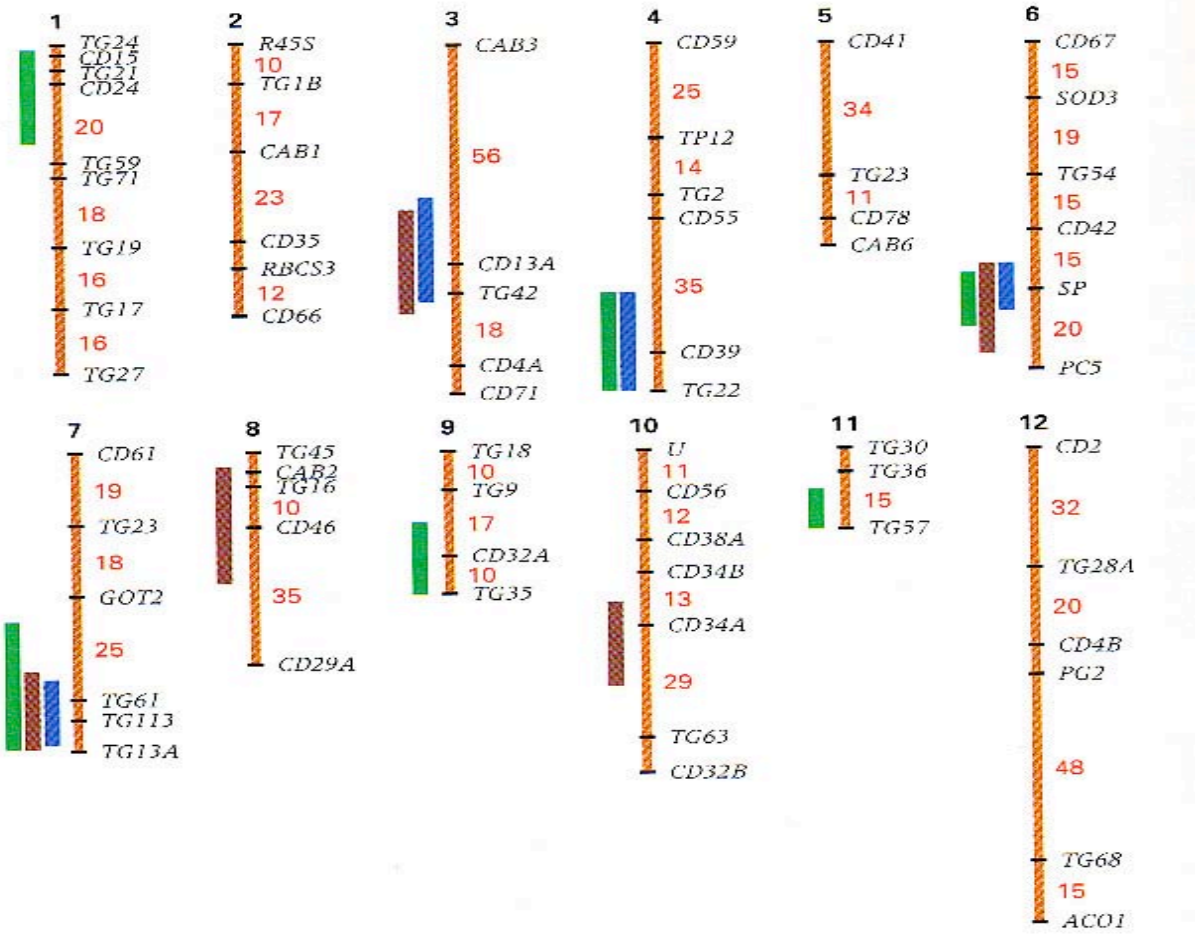
Conventional breeding program for a new cereal

Generations		Evaluation/Selection/Testing	
P	Selected parents		
F_1	800 crosses		
F_2	2 million plants	Disease resistance	
F_3	400,000 plants	Disease resistance	
F_4	12,000 lines	and Field characteristics	
F_5	1,200 lines		
F_6	300 lines	Disease resistance	
F_7	50 lines	Yield	
F_8	5 lines	Field characteristics	Industrial uses
F_9	3 lines	Uniformity	Quality testing
F_{10}	2 lines		
F_{11}	1 line!		

A conventional breeding program for a new cereal starts with selected parents and careful hand pollination to produce 800 crosses. This F_1 progeny is uniform. Then, 2 million plants are grown (2500 from each parent cross) and evaluation begins. After 10 generations the breeder ends up with one line.

Marker Assisted Breeding

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CD15
TG21
CD24
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TG59
TG71
18
TG19
16
TG17
16
TG27
2
R45S
10
TG1B
17
CAB1
23
CD35
RBCS3
12
CD66
3
CAB3
56
CD13A
TG42
18
CD4A
CD71
4
CD59
25
TP12
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TG2
CD55
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CD39
TG22
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CD41
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CD67
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SOD3
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CD56
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CD38A
CD34B
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CD34A
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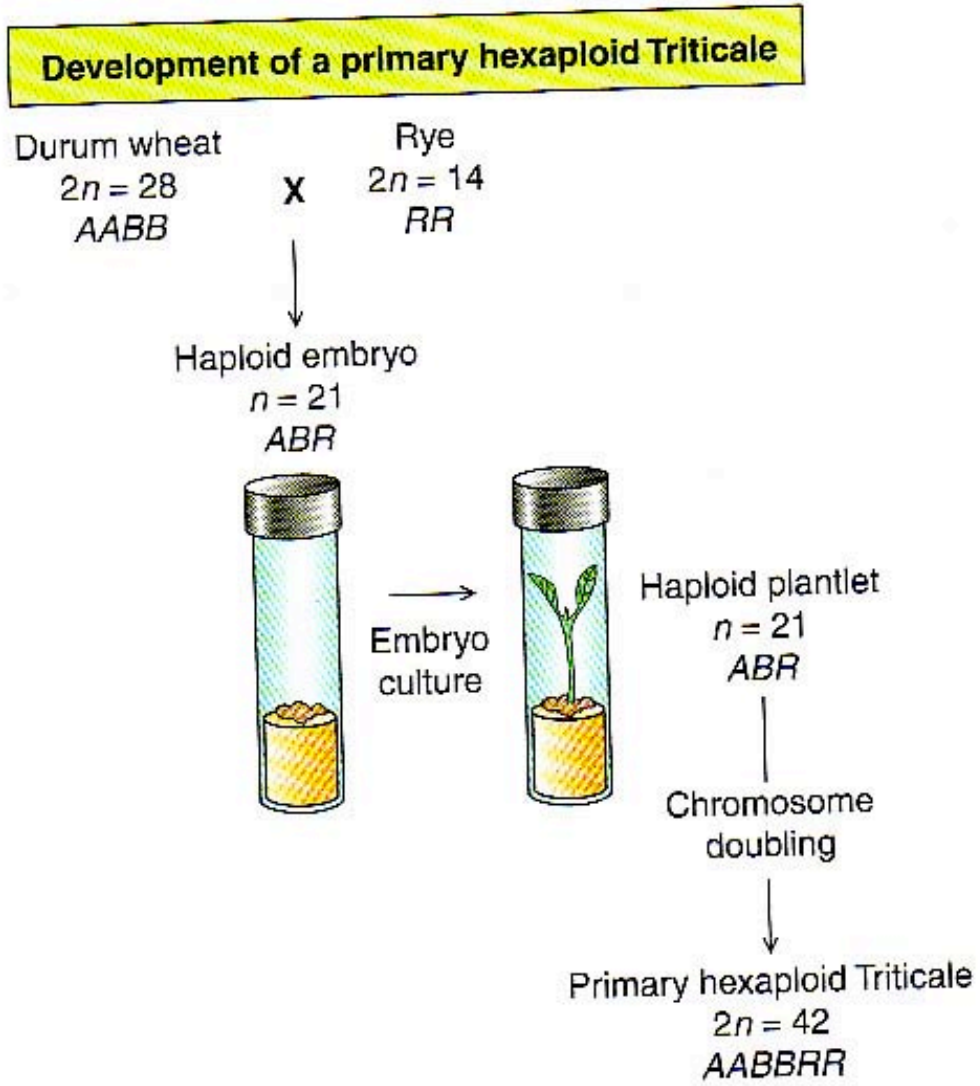
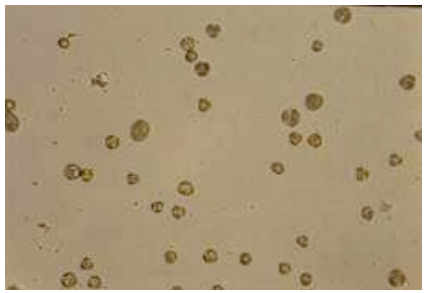
TG63
CD32B
11
TG30
TG36
15
TG57
12
CD2
32
TG28A
20
CD4B
PG2
48
TG68
15
ACO1
Fruit weight
Acidity
Soluble solids

Embryo Rescue

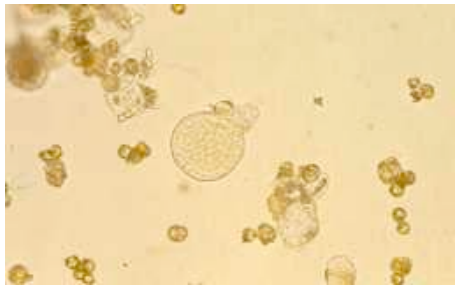


Anther Culture for Pure Line Breeding

Microspores



Developing Microspores



Suspensor of a
Developing microspore

Heart shaped embryos

Mature green embryos

In General, Plant Biotechnology Techniques Fall Into Two Classes

Gene Introduction

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

Gene Manipulation

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

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	TP	Coding Region

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

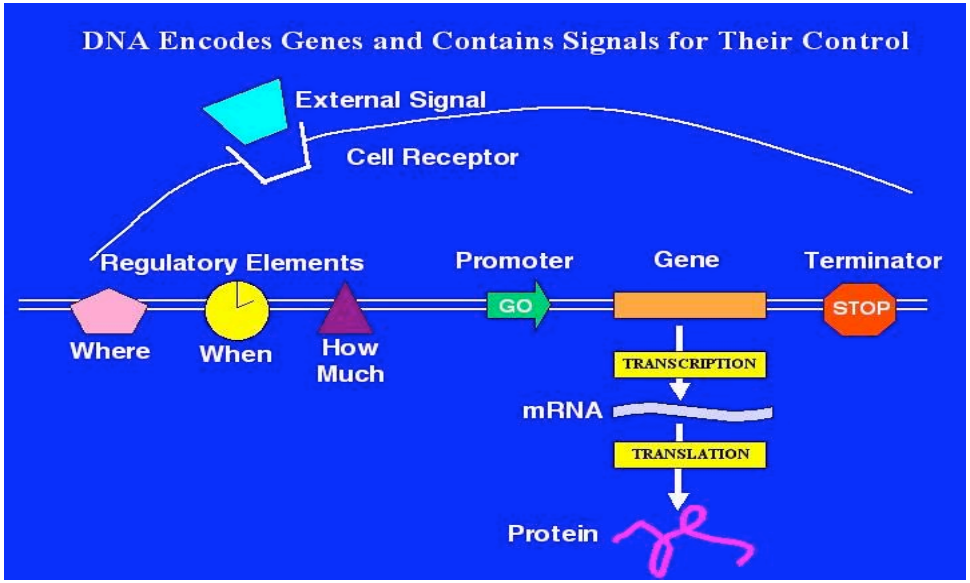
- *Targets protein to correct organelle*

ex.: RbCS (RUBISCO small subunit; choloroplast target

Coding Region

- *Encodes protein product*

ex.: EPSP



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]**

Delivering the Gene to the Plant

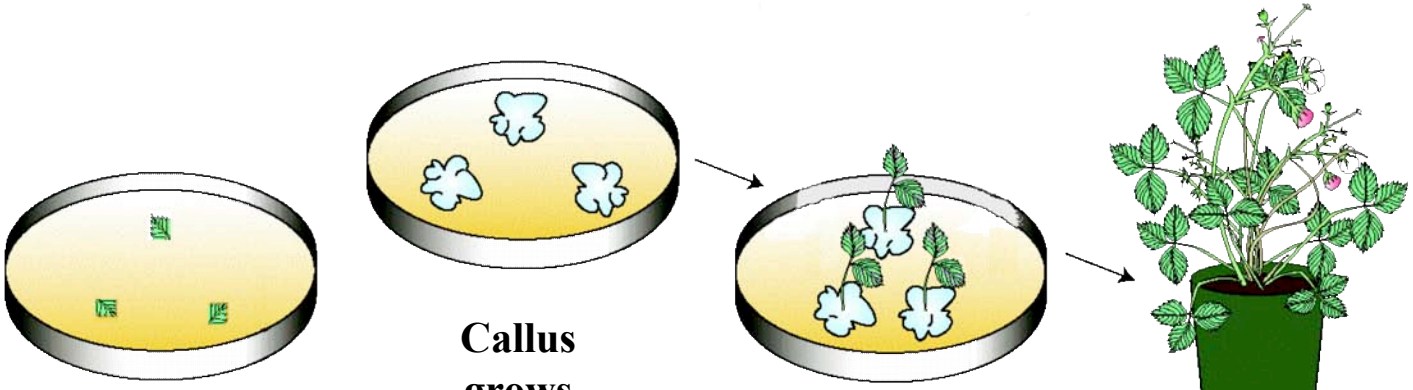
- Transformation cassettes are developed in the lab
- They are then introduced into a plant
- Two major delivery methods
  - *Agrobacterium*

  - Gene Gun

Tissue culture required to generate transgenic plants

Plant Tissue Culture

A Requirement for Transgenic Development

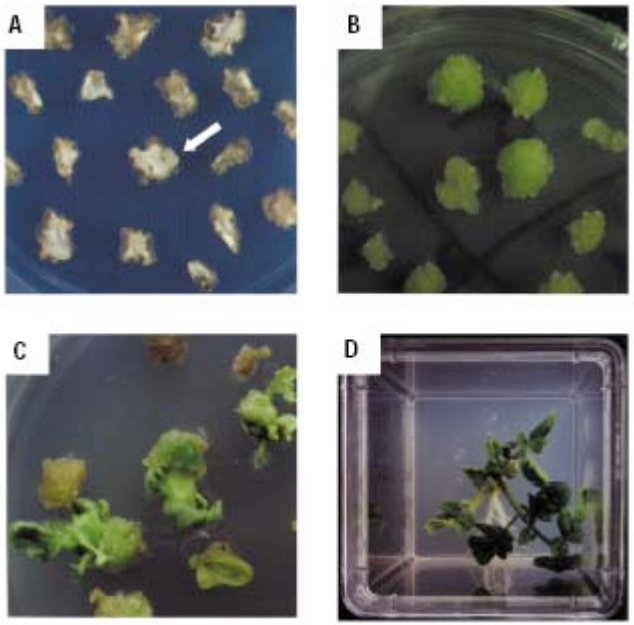


A plant part
Is cultured

Callus
grows

Shoots
develop

Shoots are rooted;
plant grows to maturity



Agrobacterium

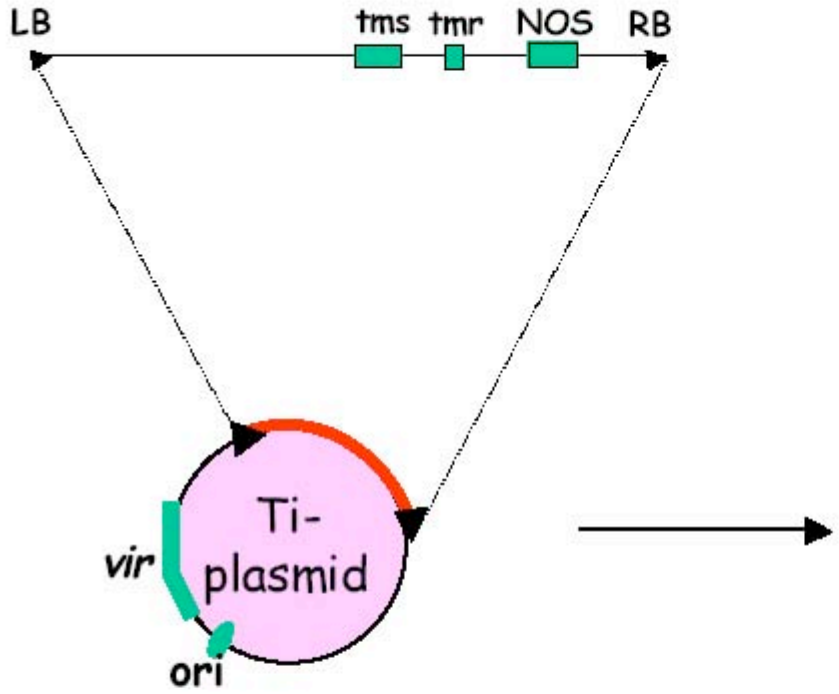
A natural DNA delivery system

- **A plant pathogen found in nature**
- **Infects many plant species**
- **Delivers DNA that encodes for plant hormones**
- **DNA *incorporates* into plant chromosome**
- **Hormone genes expressed and galls form at infection site**

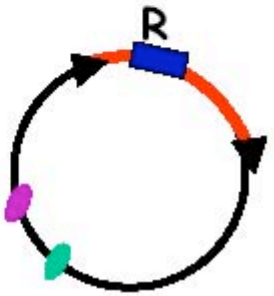
Gall on stem

Gall on leaf

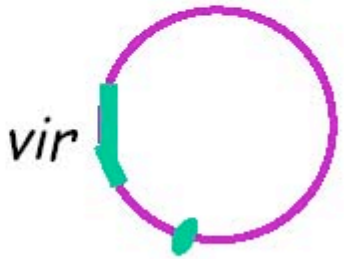
Binary gene vector for plant transformation



E. coli plasmid with T-DNA



Ti-plasmid without T-DNA



LB, RB: 25 bp repeat, left and right border
tms: tumor morphology shoot
tmr: tumor morphology root
ori: origin of replication
vir: virulence region

But Nature's *Agrobacterium* Has Problems

Infected tissues cannot be regenerated (via tissue culture) into new plants

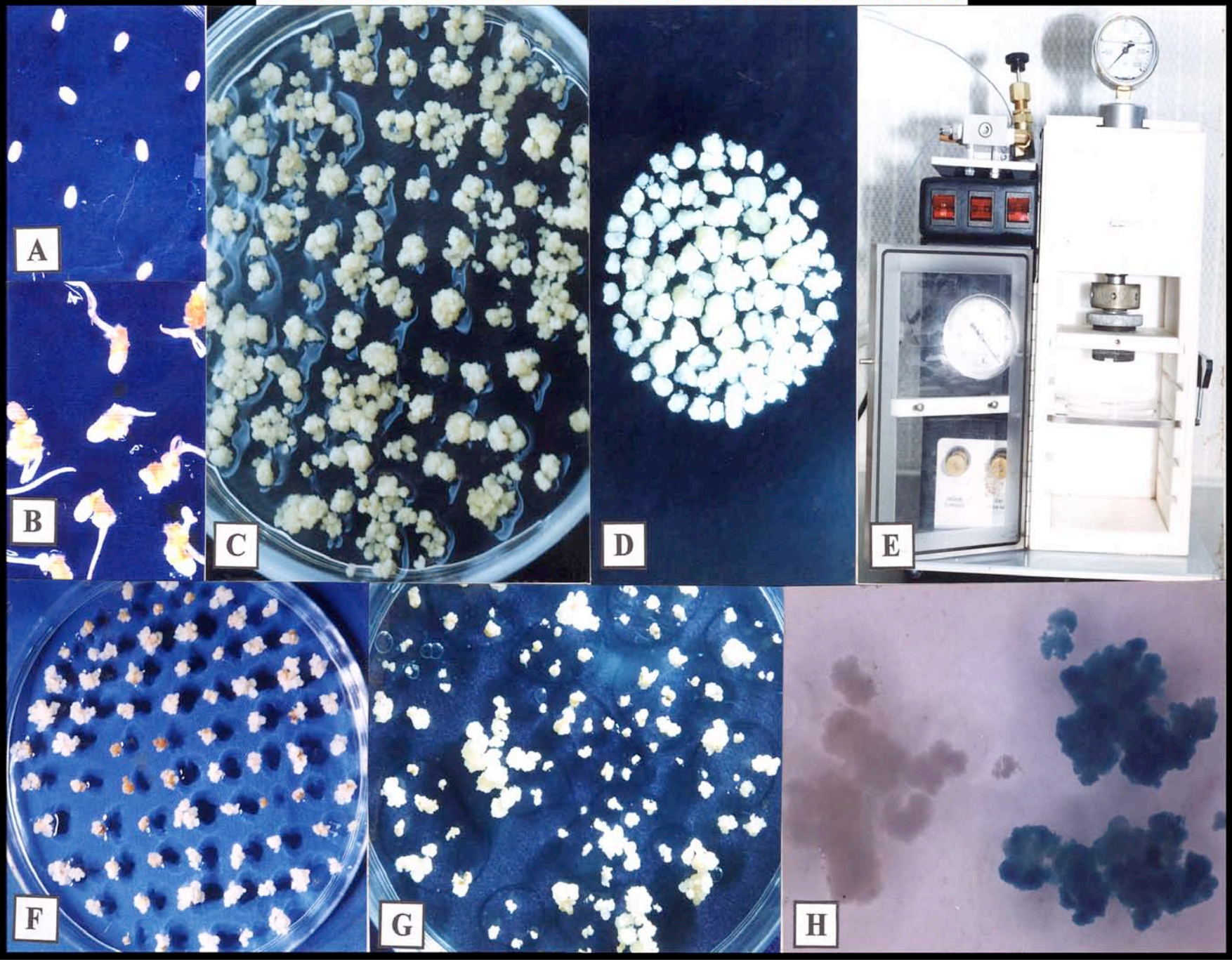
Why?

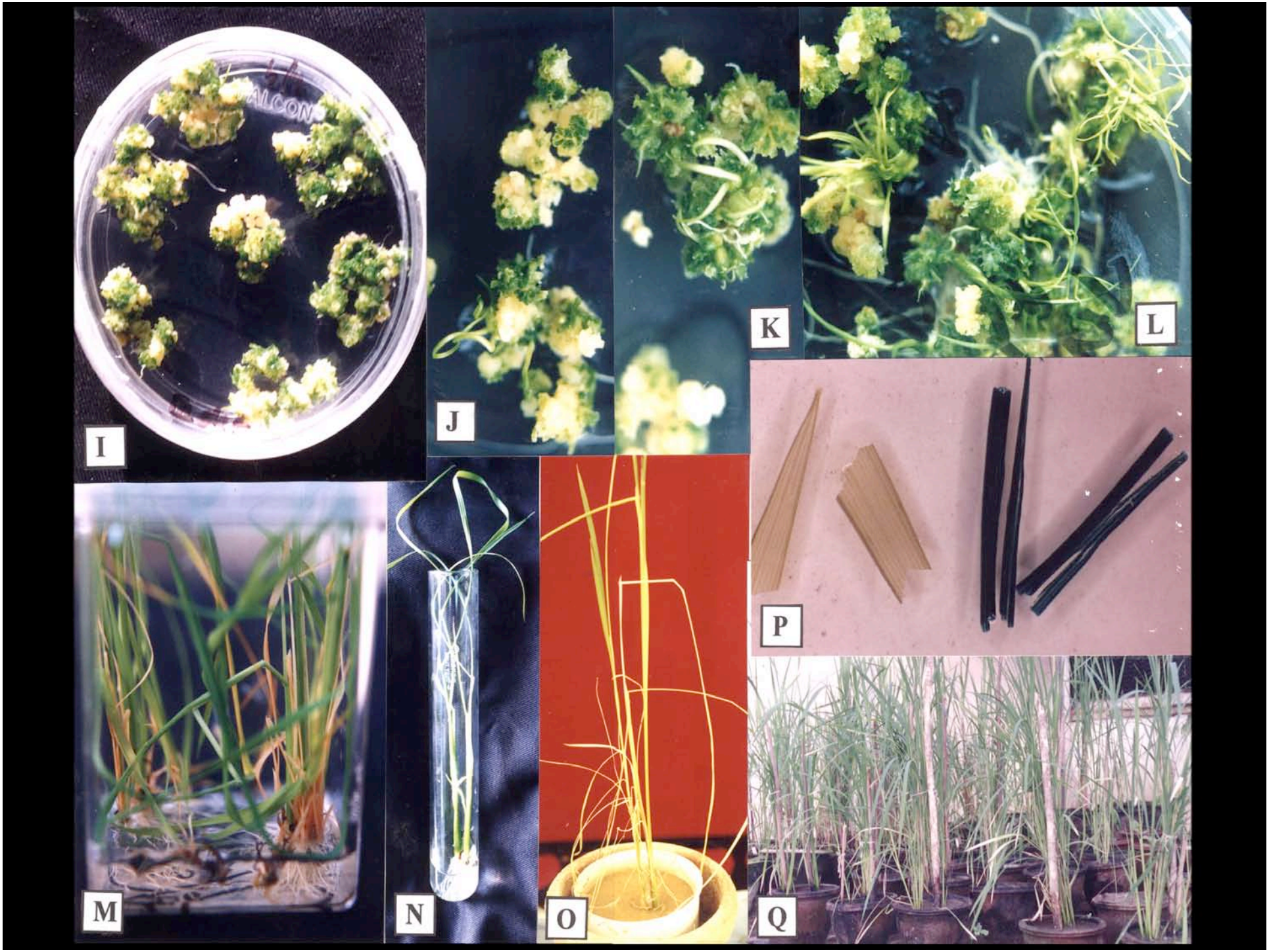
- **Phytohormone balance incorrect regeneration**

Solution? ***Transferred DNA (T-DNA) modified by***

- **Removing phytohormone genes**
- **Retaining essential transfer sequences**
- **Adding cloning site for gene of interest**

Protocol for Biolistic Rice Transformation

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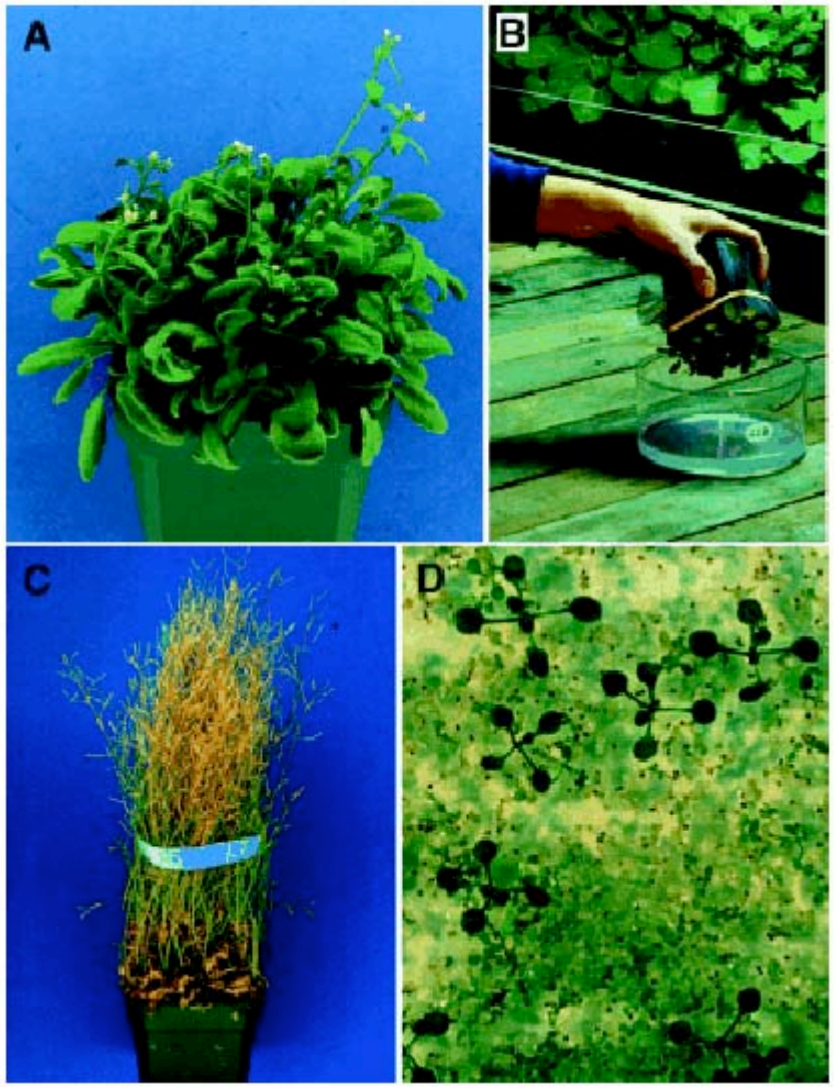
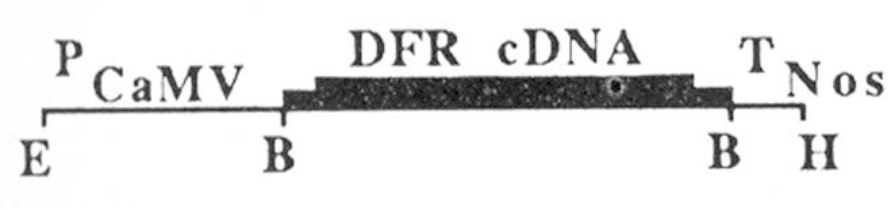


Floral Dip (Vacuum infiltration) for Arabidopsis

(A) Plants are grown to just flowering.
(B) Plants are dipped briefly in a suspension of *Agrobacterium*.
(C) Plants are grown until mature and then progeny seeds are harvested.
(D) Seeds are germinated on selective medium (e.g. containing kanamycin) to identify successfully transformed progeny.

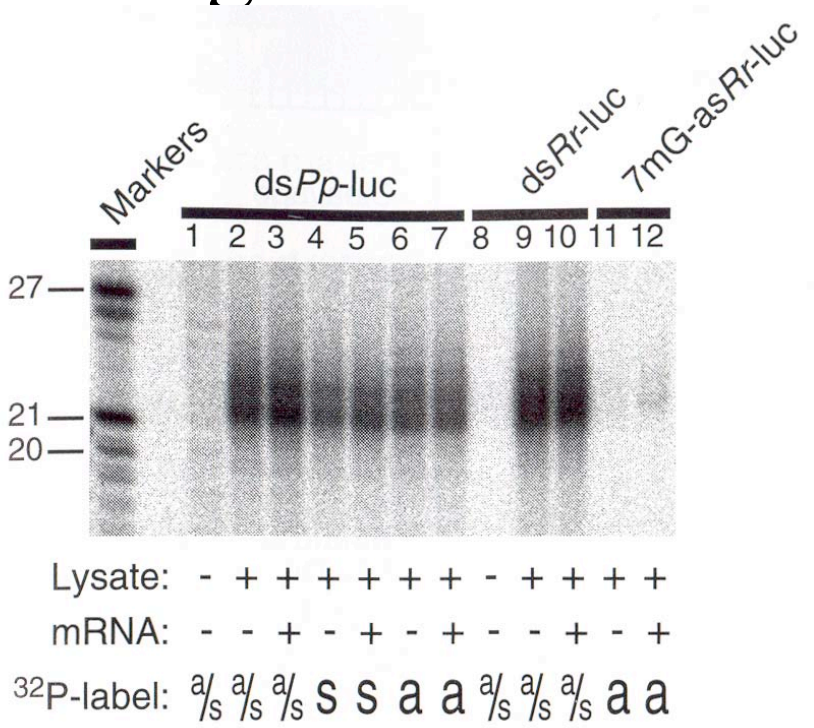
Gene Silencing in Transgenic Plants

DFR construct introduced into petunia
CaMV - 35S promoter from
Cauliflower Mosaic Virus
DFR cDNA – cDNA copy of the DFR
mRNA (intronless DFR gene)
T Nos - 3' processing signal from the
Nopaline synthase gene

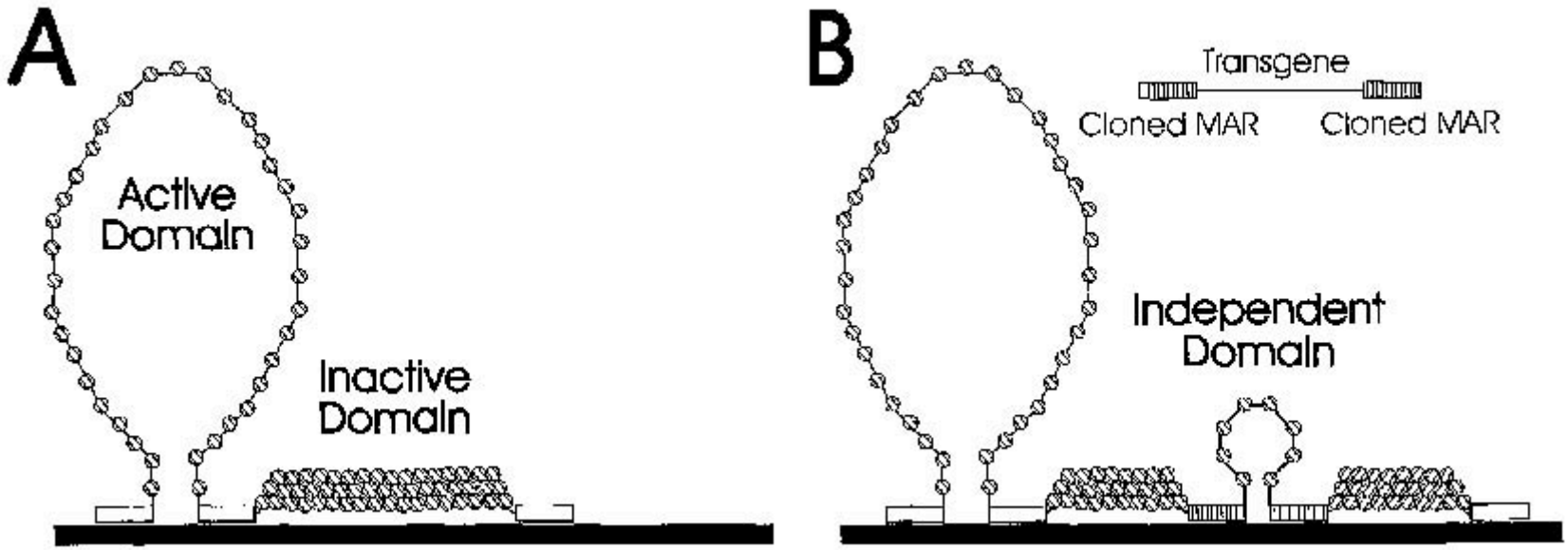


Causes of Gene Silencing

- DNA methylation.
  - DNA methylation via DNA-DNA pairing
  - Transgene recognition.
  - Insertion into hypermethilated genomic r
- Homology-dependent gene silencing.
  - Inactivation of homologous transgenes.
  - Paramutation.
  - Cosuppression.
- Suppression by antisense genes.
  - Antisense transcripts affect the target ger
  - Antisense RNA may block the processing of mRNAs by masking the sequence recognized by splicing and polyadenilation apparatus.
  - Many antisense RNAs complementary to ribosome binding site have been shown to inhibit translation initiation.
  - Antisense RNA prevents the accumulation of target mRNA.
- Position effect.
- Increased copy number.



Matrix Associated Region



Gene Manipulation

- **It is now routine to isolate genes**
- **But the target gene must be carefully chosen**
- **Target gene is chosen based on desired phenotype**

Function:

Glyphosate (RoundUp) resistance

EPSP synthase enzyme

Increased Vitamin A content

Vitamin A biosynthetic pathway enzymes

The RoundUp Ready Story

- **Glyphosate is a broad-spectrum herbicide**
  - **Active ingredient in RoundUp herbicide**
  - **Kills all plants it come in contact with**
  - **Inhibits a key enzyme (EPSP synthase) in an amino acid pathway**
- **Plants die because they lack the key amino acids**
- **A resistant EPSP synthase gene allows crops to survive spraying**

RoundUp Sensitive Plants

Shikimic acid + Phosphoenol pyruvate

+ Glyphosate

Plant EPSP synthase

3-Enolpyruvyl shikimic acid-5-phosphate (EPSP)

Aromatic amino acids

Without amino acids, plant dies

RoundUp Resistant Plants

Shikimic acid + Phosphoenol pyruvate

+ Glyphosate

Bacterial EPSP synthase

RoundUp has no effect; enzyme is resistant to herbicide

3-enolpyruvyl shikimic acid-5-phosphate (EPSP)

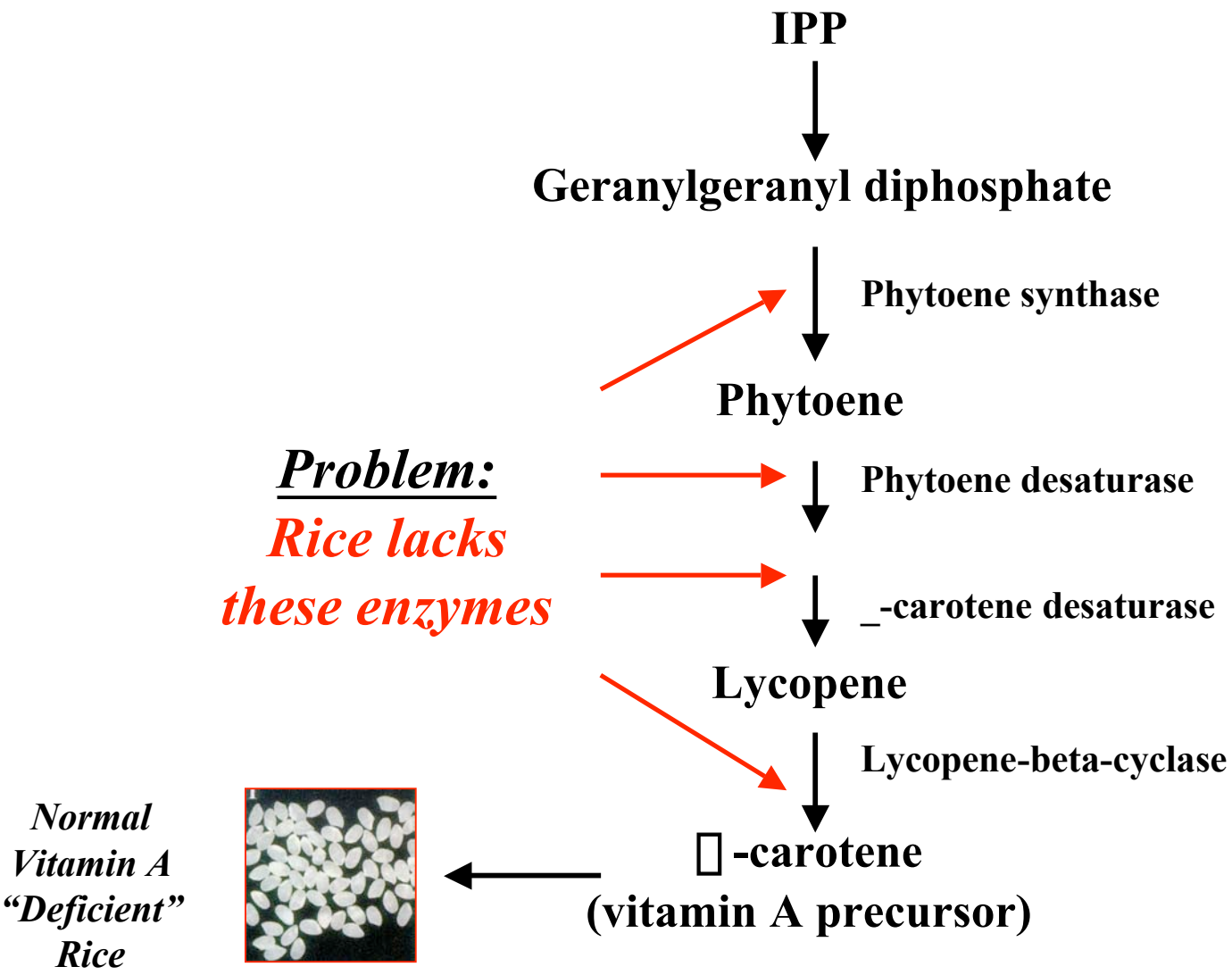
With amino acids, plant lives

Aromatic amino acids

The Golden Rice Story

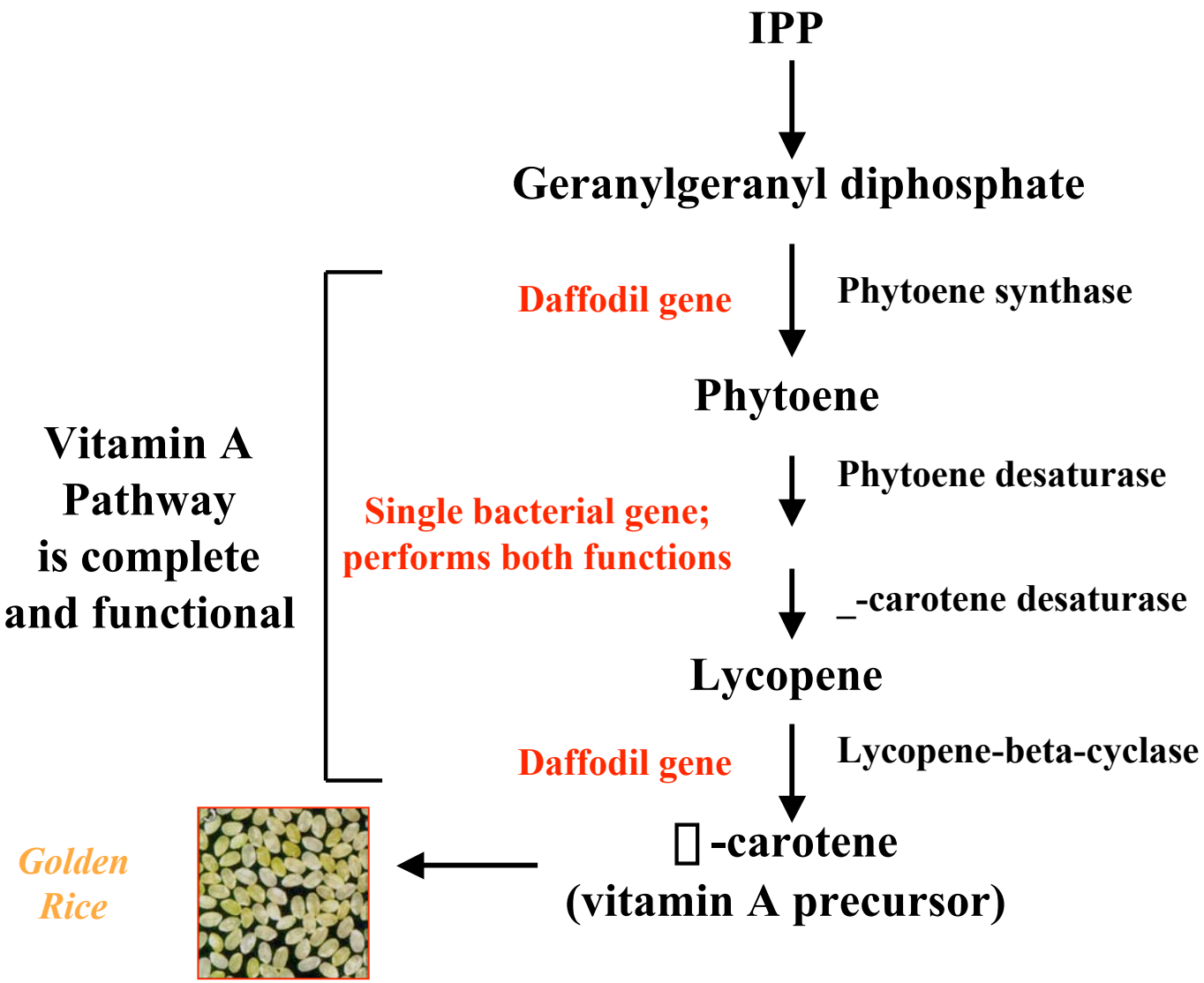
- **Vitamin A deficiency is a major health problem**
  - **Causes blindness**
  - **Influences severity of diarrhea, measles**
- **>100 million children suffer from the problem**
- **For many countries, the infrastructure doesn't exist to deliver vitamin pills**
- **Improved vitamin A content in widely consumed crops an attractive alternative**

-Carotene Pathway in Plants



The Golden Rice Solution

-Carotene Pathway Genes Added



Virus-resistant Papaya

Papaya, a tropical fruit high in vitamins C & A, is an important food crop worldwide.and the 2nd largest export crop in Hawaii.

A virus, papaya ringspot potyvirus (PRSV), was discovered in Hawaii in the 1940's and had wiped out papaya production on Oahu by the 1950's.

The papaya industry moved to the Puna district on the Big Island of Hawaii.

PRSV was discovered in Puna in 1992, by late 1994, PRSV had spread throughout Puna and many farmers were going out of business.

Virus-resistant Papaya

In anticipation of a new virus outbreak, scientists at Cornell, began a project to develop transgenic virus-resistant papaya in 1986.

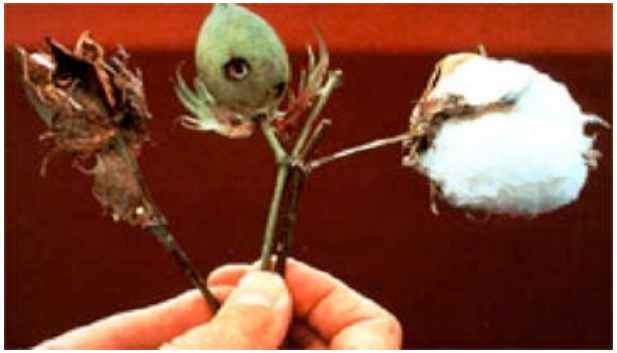
Transgenic Non-transgenic

Non-transgenic Transgenic

Papaya transformation was greatly facilitated by the recent invention of the "gene gun" at Cornell.

The coat protein of the virus was engineered into papaya to confer resistance, similar to a vaccine.

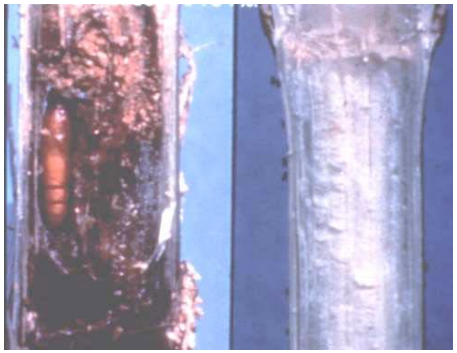
Insect Resistance



Source: USDA

Insect resistant cotton

- ✓ Bt toxin kills the cotton boll worm
- ✓ toxin gene from a ***bacteria***

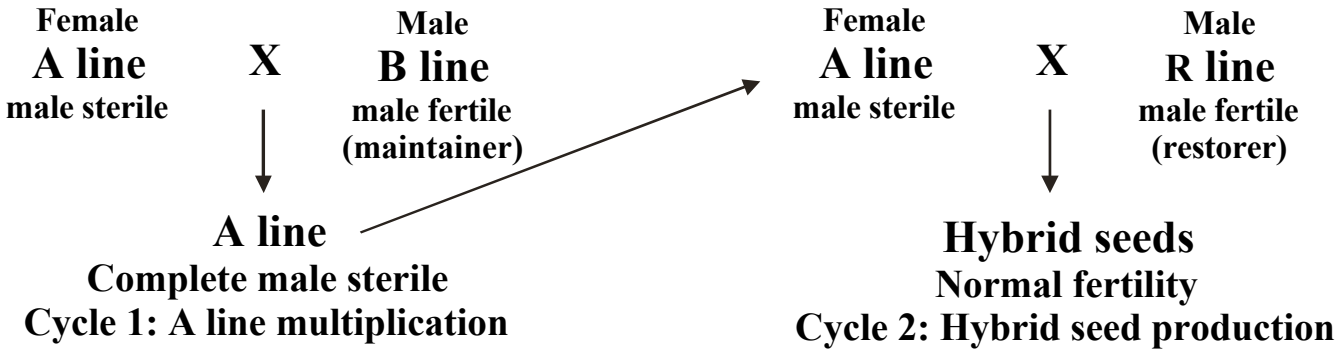


Normal **Transgenic**

Insect resistant corn

- ✓ Bt toxin kills the European corn borer
- ✓ toxin gene from a ***bacteria***
- ✓ Rootworm GM approved (2/26/03)

Cytoplasmic male sterility (CMS) three line system



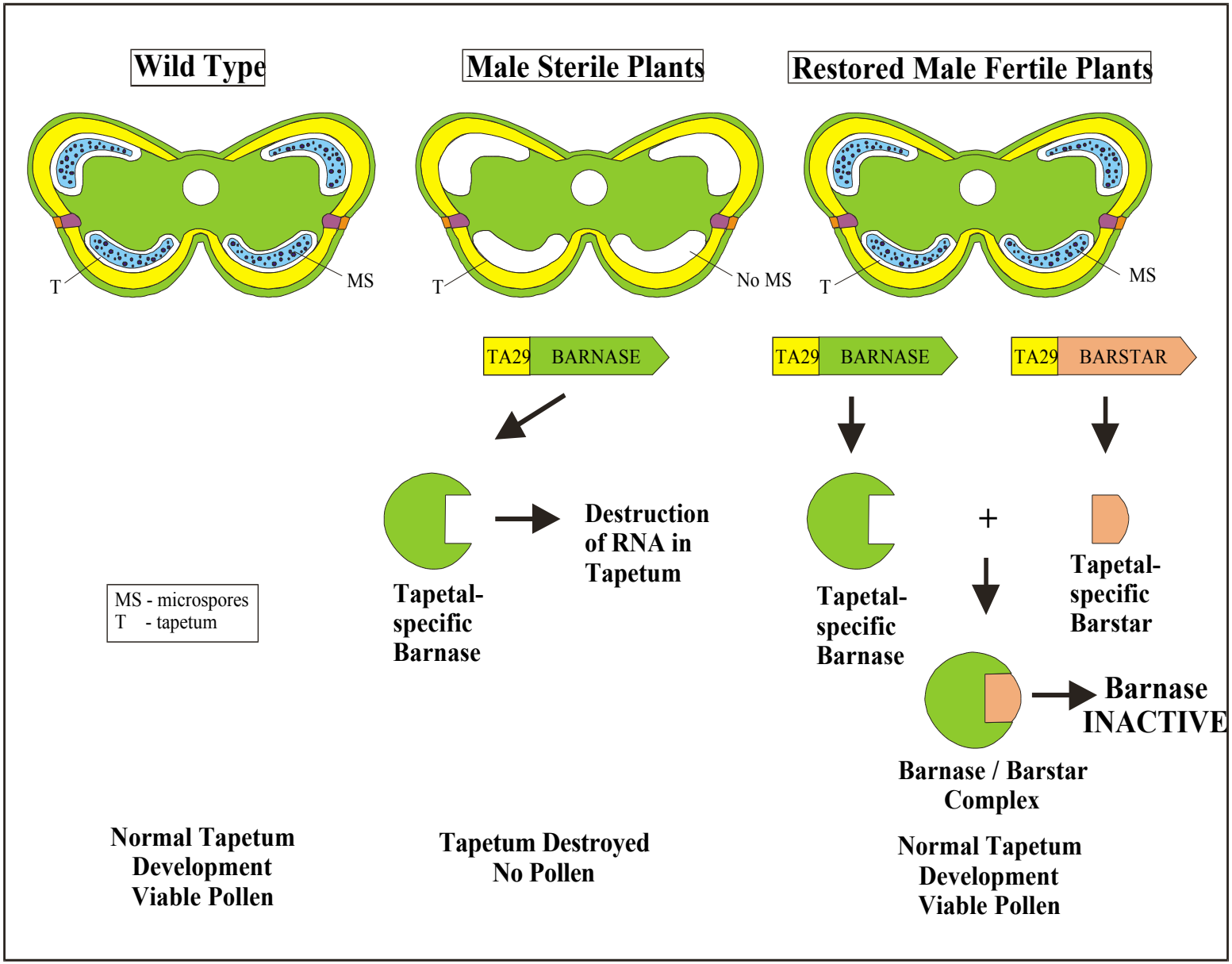
1. Strict genetic match among A, B and R lines is required for male fertility conversion, which limits parent selection and heterosis improvement.

2.The genetic match is hard to be made, which leads to genetic uniformity and vulnerability such as in corn before 1970's.

3. Labor-intensive and time-consuming procedure raises the cost of production. Contamination can happen in any steps of the cycles.

Studies made in rice, wheat, corn, sorghum, rapeseed, cotton etc., successful in corn before 1970's, rice currently (especially in China).

Engineered male sterility with barnase



Edible Vaccines – A Biopharming Dream

Biotech Plants Serving Human Health Needs

- A pathogen protein gene is cloned
- Gene is inserted into the DNA of plant (potato, banana, tomato)
- Humans eat the plant
- The body produces antibodies against pathogen protein
- Human are ***"immunized"*** against the pathogen
- Examples:
  - ✓Diarrhea
  - ✓Hepatitis B
  - ✓Measles

Future Health-related Biotech Products

Vaccines

- ✓ Herpes
- ✓ hepatitis C
- ✓ AIDS
- ✓ malaria

Tooth decay

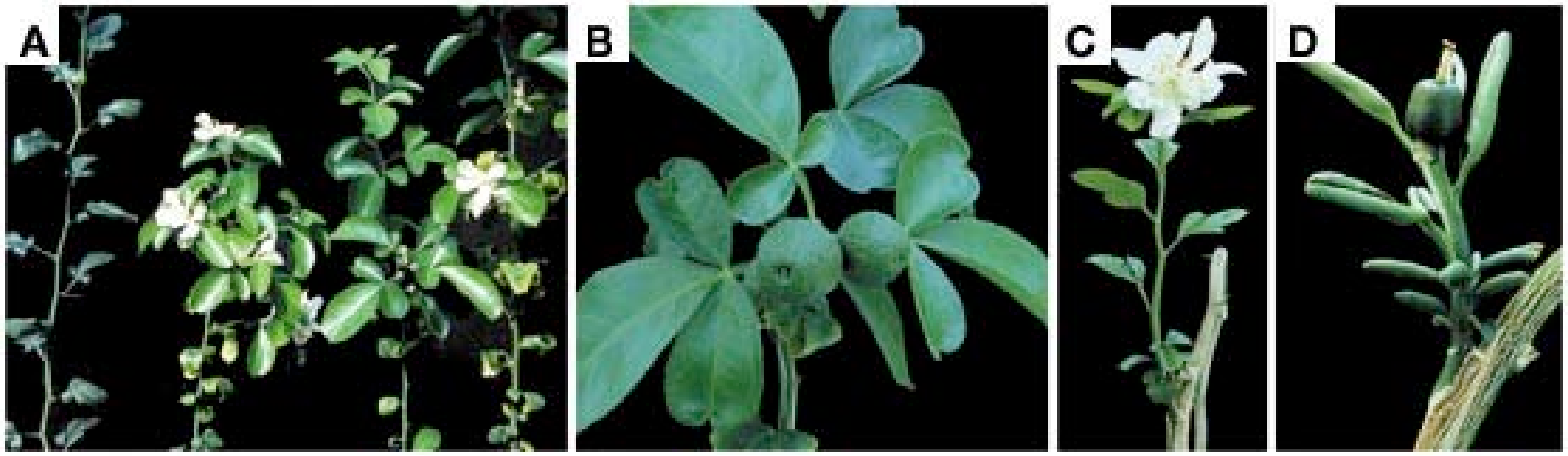
- ✓ *Streptococcus mutans*, the mouth bacteria
- ✓ releases lactic acid that destroys enamel
- ✓ engineered *Streptococcus mutans*

  does not release lactic acid

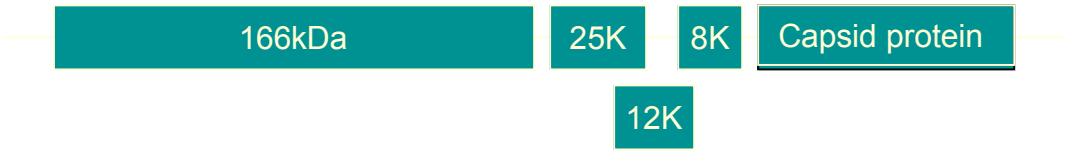
  destroys the tooth decay strain

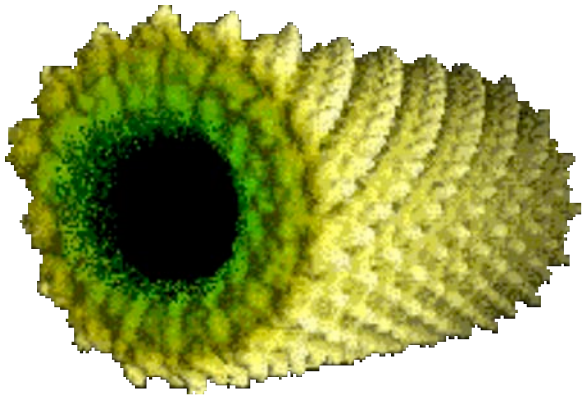
Constitutive Expression of *Arabidopsis* LEAFY and Apetala1 Genes in Citrus reduces their Generation time.

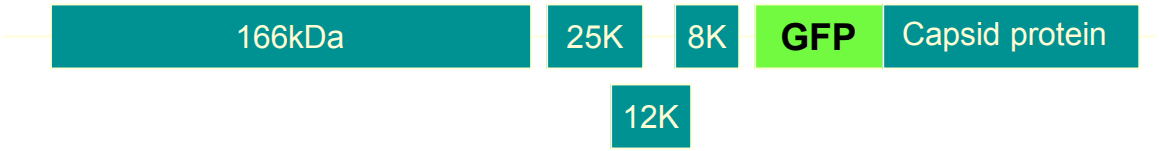
- Both LFY and AP1 genes cause reduction in the juvenile phase in citrange
- AP1 gene is more effective than LFY in the reduction of juvenile traits and the promotion of flowering without causing abnormal leaf development effects
- Short juvenile phase and early flowering are stable traits maintained in the offspring of transgenic plants

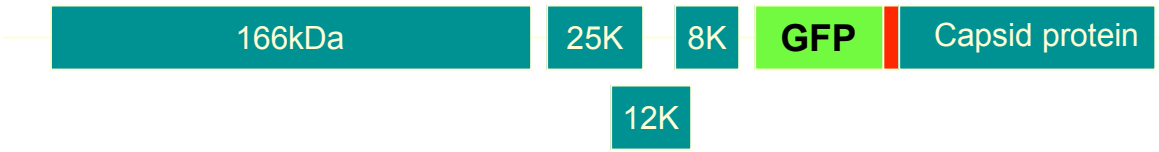


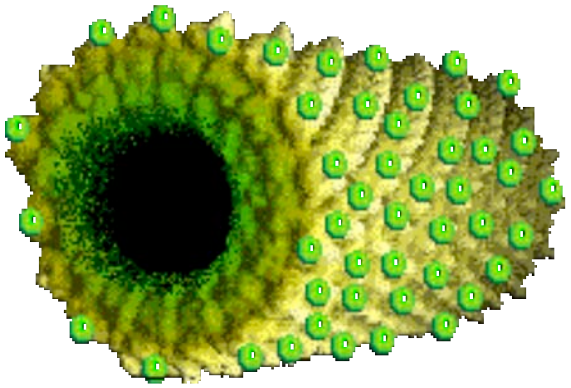
‘Overcoat’ Technology



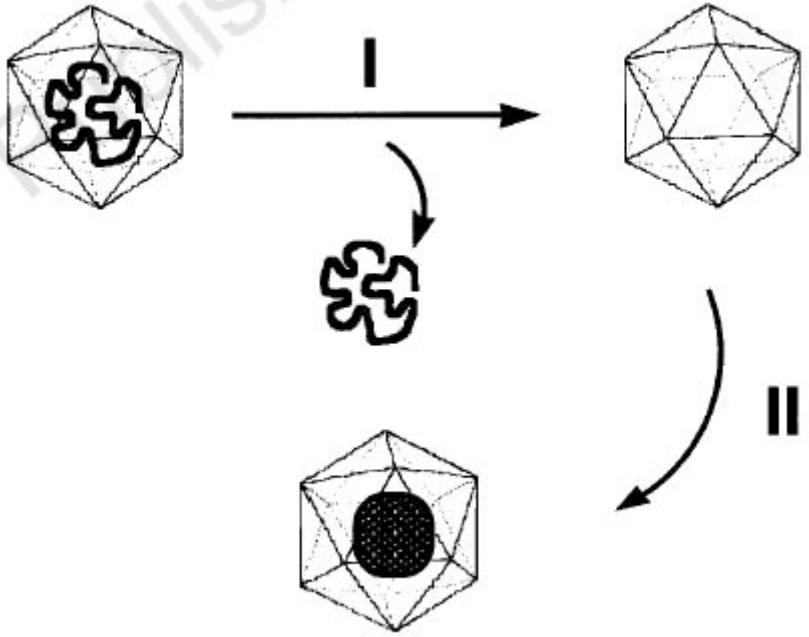


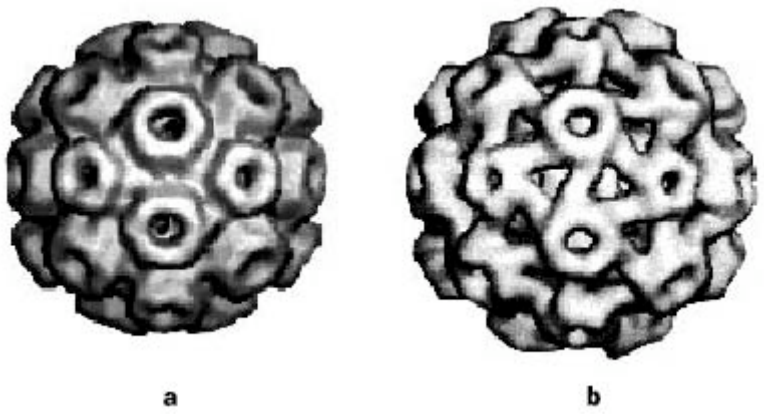






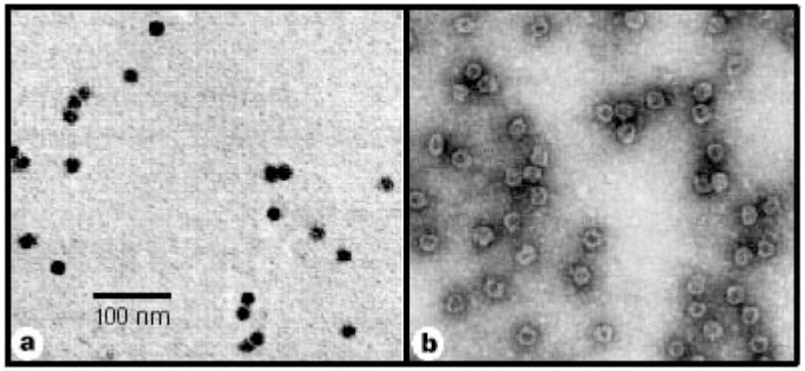
Nanofabrication using Plant Virus





I. High pH to remove viral RNA. Clean up particles by Ultracentrifugation and,

II. add reactants and drop pHto allow mineralization.



Concerns Regarding Bt Crops

Appearance of resistant insects

- US Bt planting rules to minimize chances of resistance:
  (1) must plant 20% non-BT corn (50% non-Bt cotton) in field
  (2) monitoring must take place
- Introduce multiple resistance genes
- Increase expression levels

Impact on non-target insects

- voluntary 'buffer zone' of conventional corn surrounding Bt corn

'Transgenic pollen harms monarch larvae". J.E. Losey *et al.,* Nature 20 May 1999 (correspondence). Subsequently very harshly criticised for being short-term non-field trial, using most susceptible larval stage, very preliminary science

- Later work suggests little, if any effect
- Correct comparison is with effect using conventional insecticide

Concerns About Virus Resistant Plants

Transcapsidation

If plant infected by another virus, could its genome be encapsidated by the coat protein encoded by the transgene? ‘Masked’ virus.

Is there sufficient coat protein being produced to produce masked virus?

If one was produced, would it have any new biological properties?

Synergy

If plant infected by another virus, would symptoms be more severe because of coat protein gene?

Recombination

Would frequency be increased? Would recombinants be ‘novel’?

What Are the Public Concerns?

Economics

Are we changing the economics on the farm?

Environmental

Are we irreversibly modifying the environment?

Globalization

Is technology becoming centralized in too few hands?

Social

Will we develop a class of genetic outcasts?

Religious

Are we playing God?

“Responsible biotechnology is not the enemy; starvation is. Without adequate food supplies at affordable prices, we cannot expect world health, or peace”

President Jimmy Carter

February 1999