GENETIC TRANSFORMATION – EXAMPLES AND ACHIEVMENTS

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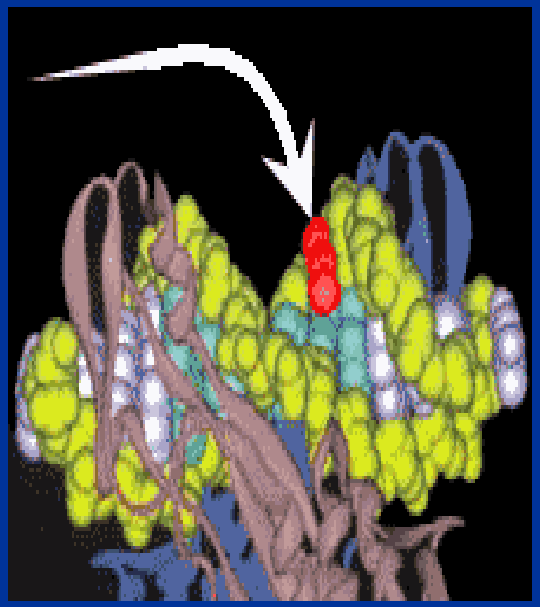


Transgenics are a Biotechnology Product

How about some definitions

Biotechnology - General Definition

The application of technology to improve a biological organism

Biotechnology - Detailed Definition

The application of the technology to improve the biological function of an organism by ***adding genes from another organisms***

What About the Term Genetic Engineering?

Genetic engineering is the basic tool set of biotechnology

Genetic engineering involves:

- ✓ Isolating genes
- ✓ Modifying genes so they function better
- ✓ Preparing genes to be inserted into a new species
- ✓ Developing transgenes

Plant transformation

Introduction of exogenous DNA into a plant cell

- Transient – no incorporation of exogenous DNA into the genome
- Stable – incorporation into genome

Transformation of multicellular organisms:

- Cannot directly transform every cell - Transformation involves one cell which then regenerates an entire organism

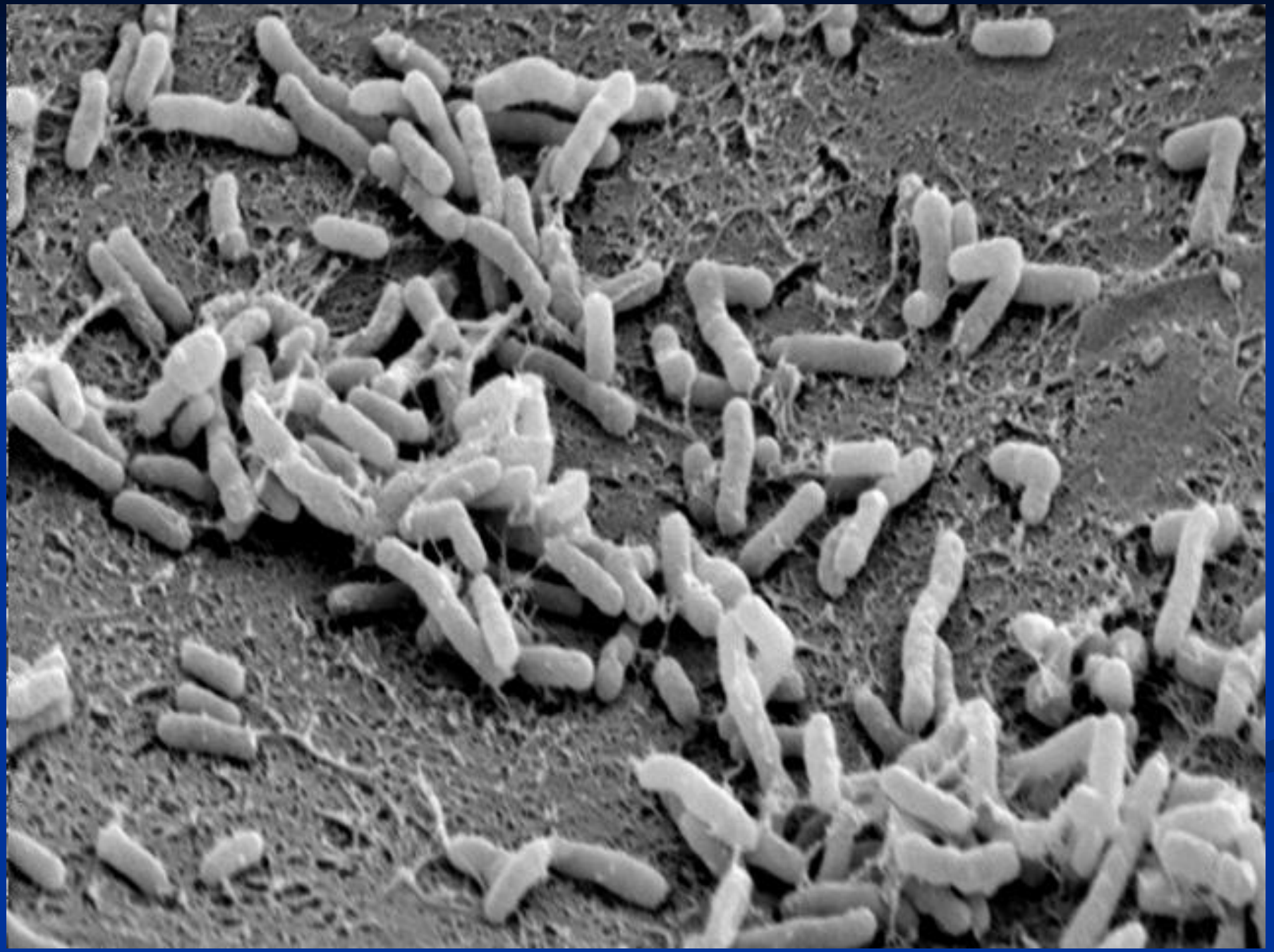
METHODS

- INDIRECT
- DIRECT

Agrobacterium tumefaciens: a natural tool for plant transformation

Soil gram positive bacterium

Agrobacterium tumefaciens attached to a plant cell



Naturally,

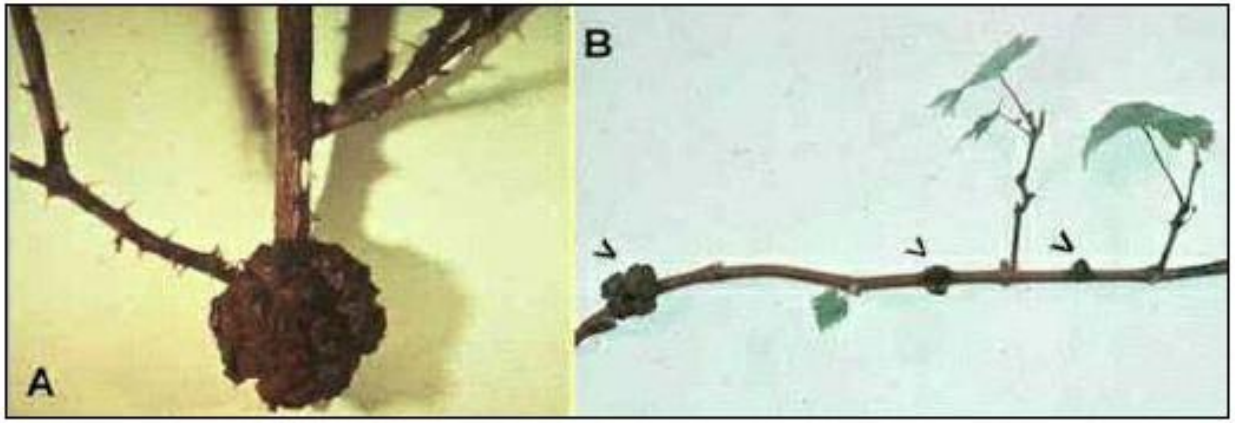
Agrobacterium tumefaciens causes crown gall disease of a wide range of dicotyledonous (broad-leaved) plants, especially members of the rose family such as apple, pear, peach, cherry, almond, raspberry and roses.

Agrobacterium tumefaciens

Gram negative bacterium
Soil borne
Naturally attacks certain plants at wound site causing crown gall
Infection results in formation of galls on lower trunk, soil level, roots
Galls produce opines - specialized nitrogenous compounds
Opines used by Agrobacterium as source of nutrients

Agrobacterium tumefaciens: a natural tool for plant transformation

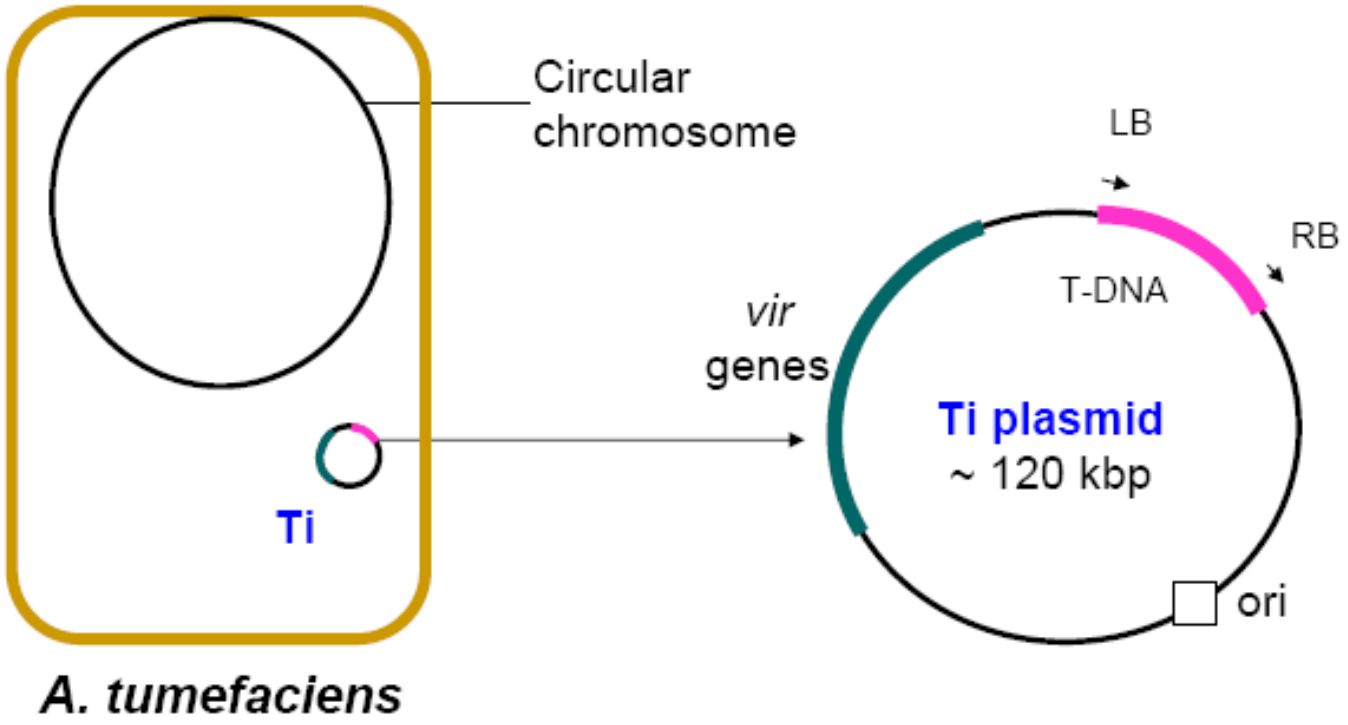
- Causes Crown Gall disease - tumors (galls) form at base of stem in many dicots



Photographs supplied by Sharon von Broembsen, Oklahoma State University

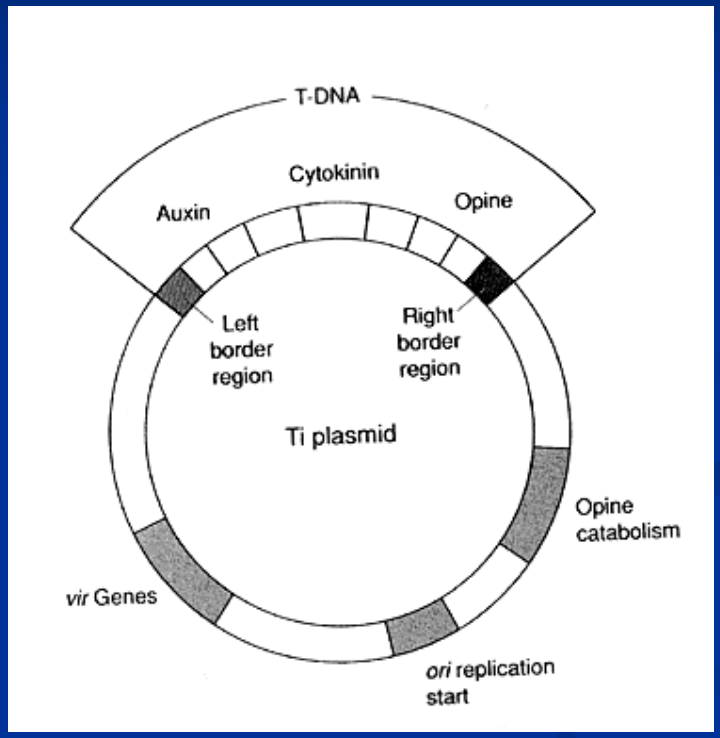
- production of tumors is caused by the transfer of bacterial DNA to the plant, which integrates into the plant genome

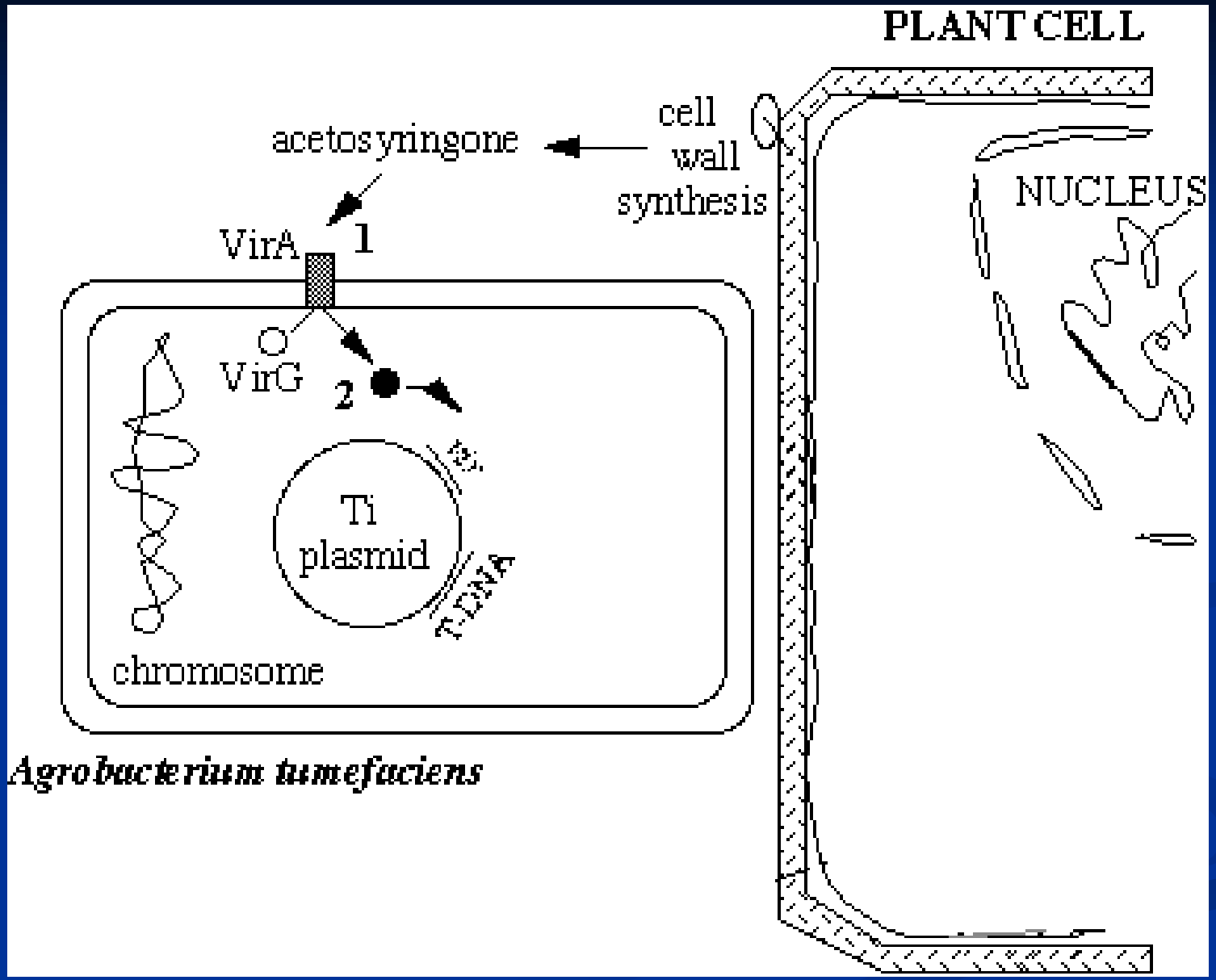
- Genes involved in crown gall disease are not present on the chromosome of *A. tumefaciens* but on a large **plasmid**, called the **Ti (tumor-inducing)** plasmid.

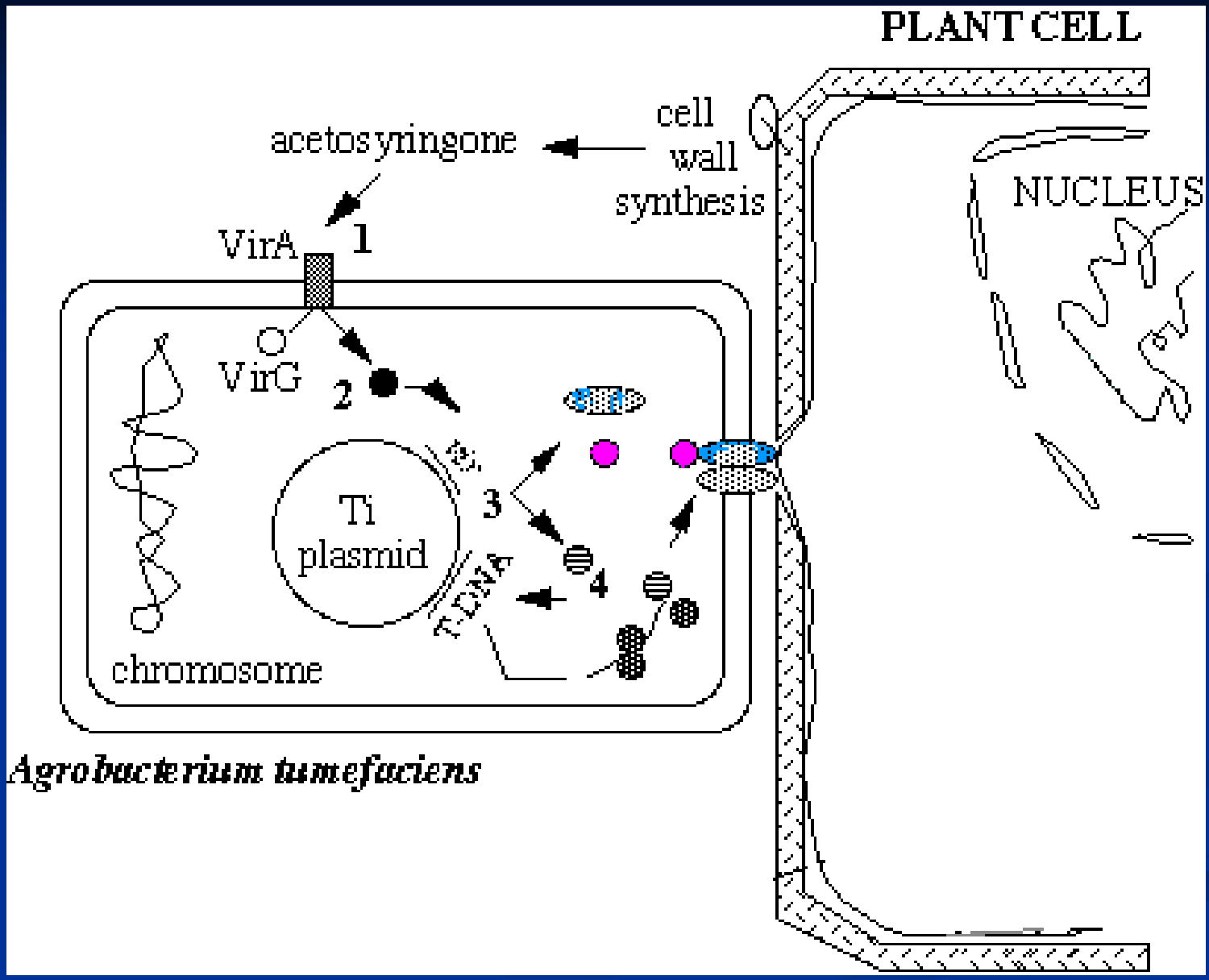


Ti Plasmid

- 200 kb in size
- vir genes - responsible for packaging excised T-DNA and transferring it into host cells
- 7-8 vir genes
- Opine catabolism genes




PLANT CELL
cell
wall
synthesis
acetosyringone
NUCLEUS
VirA
1
VirG
2
Ti
plasmid
T-DNA
chromosome
Agrobacterium tumefaciens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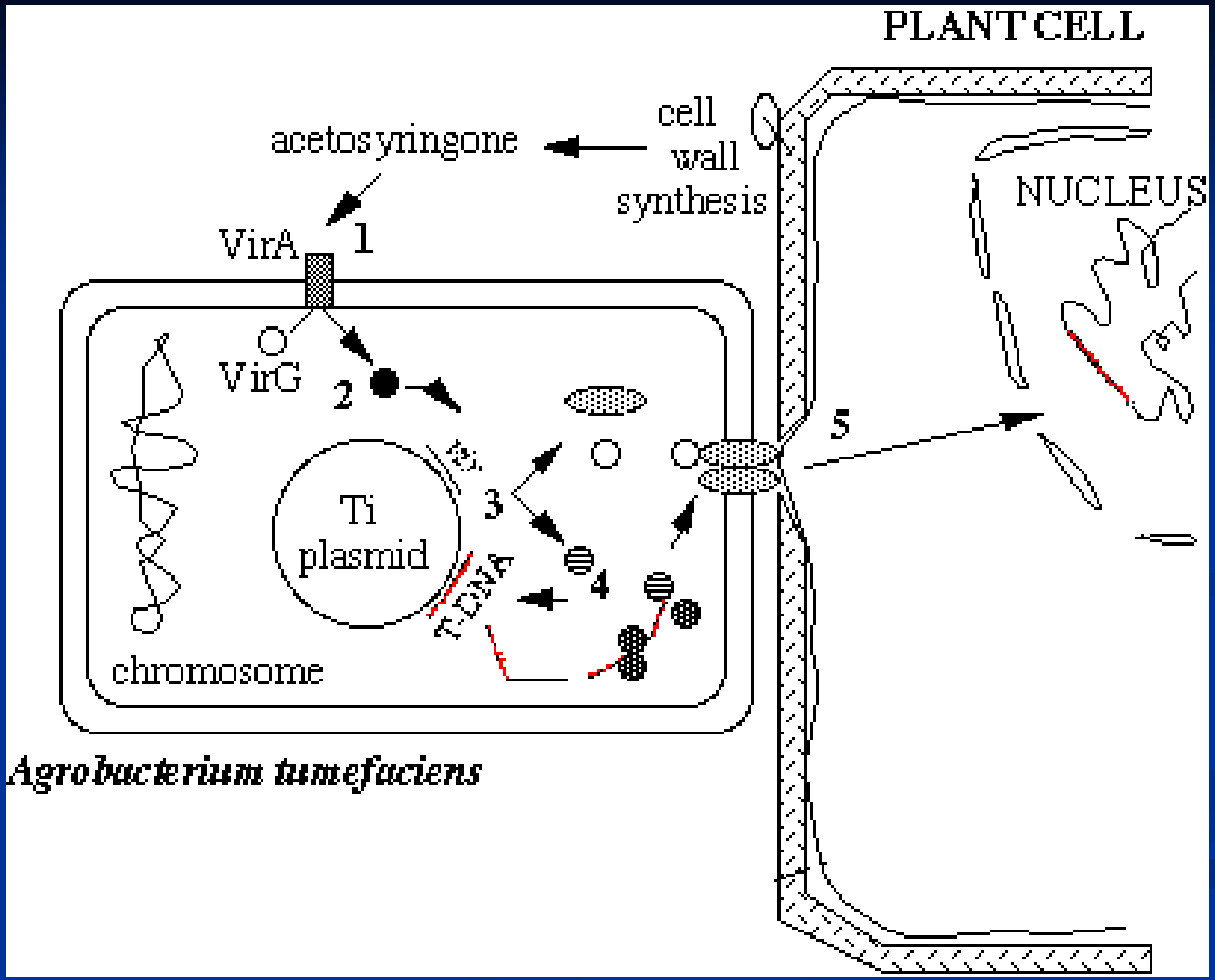
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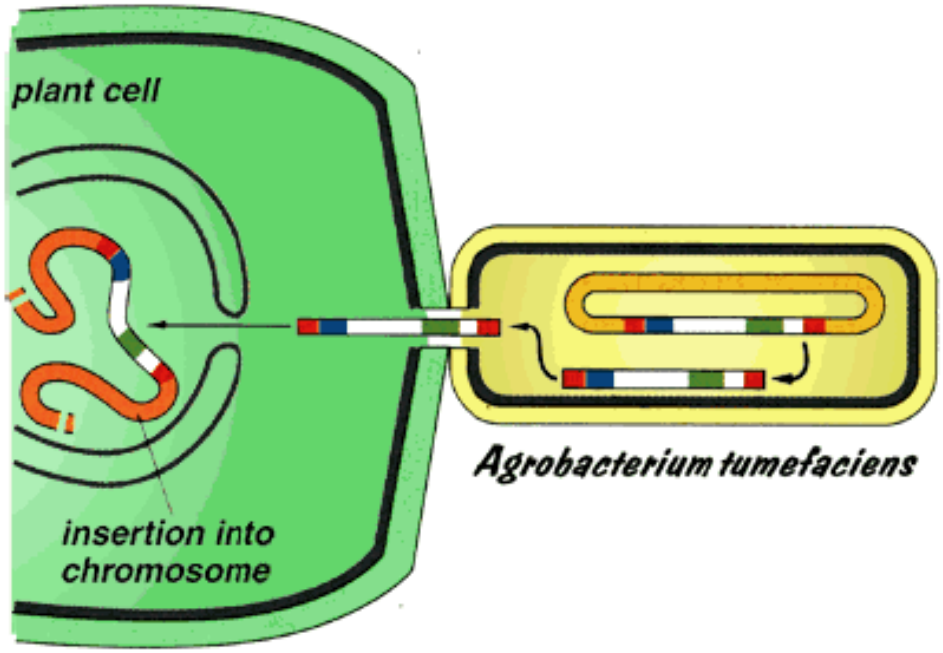
5
NUCLEUS
Agrobacterium tumefaciens

T-DNA transfer into plants



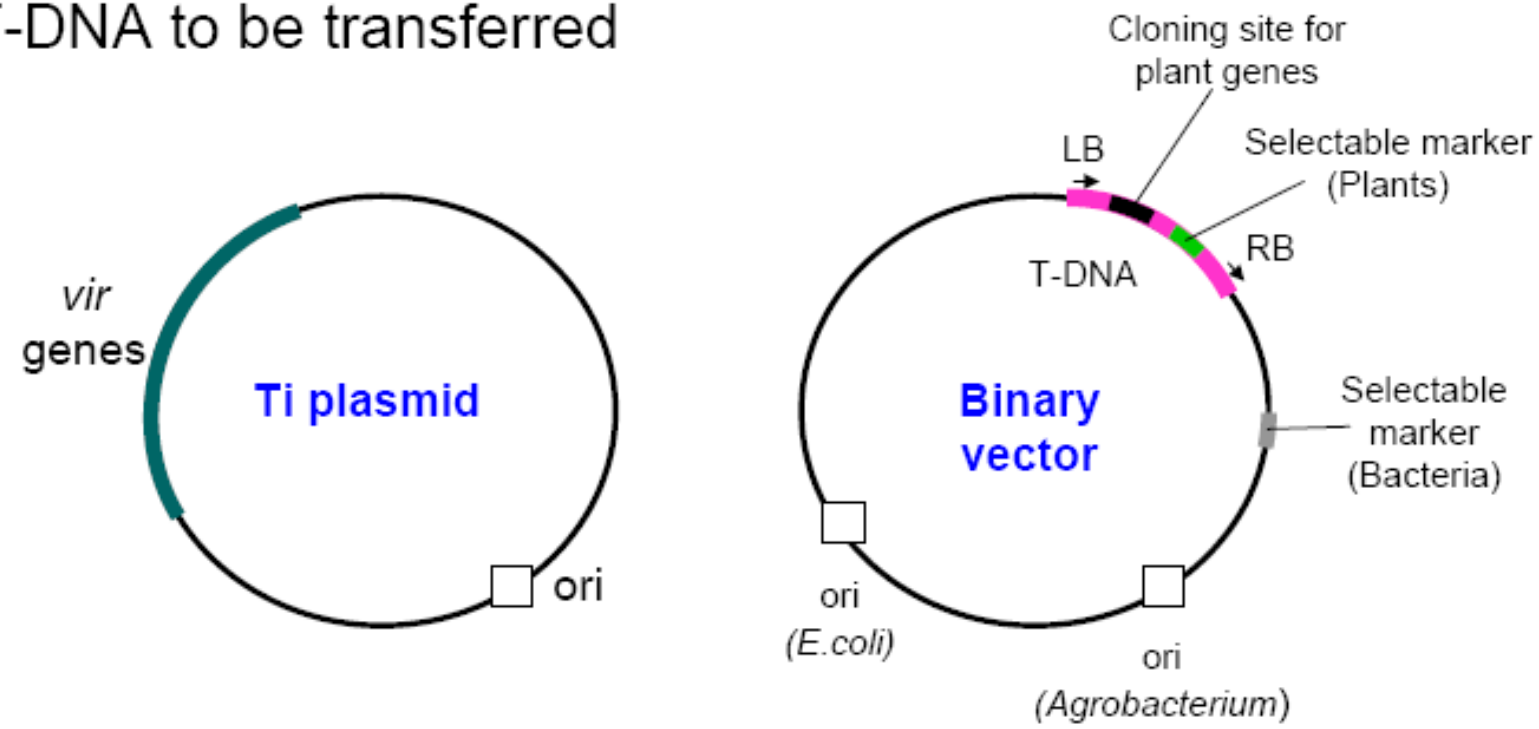
- T-DNA transfer process is activated when *Agrobacterium* gets in contact with damaged plant tissue

- T-DNA is nicked at the RB, single stranded DNA gets replicated to the LB and moved into the plant cell - this catalyzed by products of vir genes

http://www.plantsci.cam.ac.uk/Haseloff/SITEGRAPHICS/Agrotrans.GIF

Agrobacterium tumefaciens as a tool for genetic engineering

- *vir* genes and T-DNA can be on separate plasmids
- only left and right borders (LB & RB) are required for T-DNA to be transferred



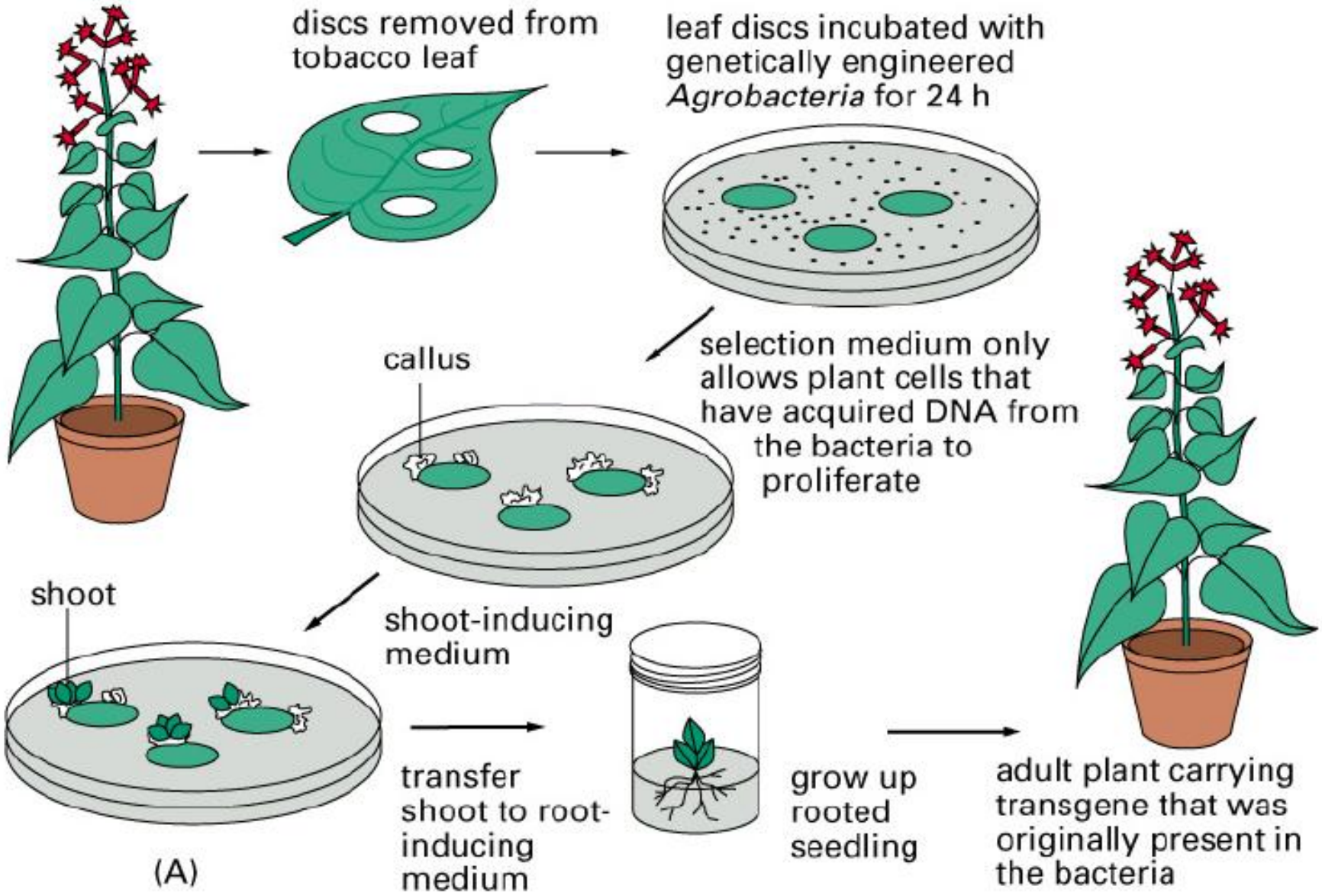


Figure 8–72 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Plant transformation

Problem:

We want to transform the whole organism, not one cell!!!

This is done by:

- Transforming plant cells in culture, selecting transformed cells and regenerating the entire plant from the transformed cell (eg. tobacco)

- *In planta* transformation of *Arabidopsis*
  - Dip flowering plants into *Agrobacterium* suspension
  - Harvest seed and select for transformants – (they are hemizygous!)

Systemic infection in *Arabidopsis* was accomplished by transformation of female gametes!

Direct DNA Transfer

- Protoplasts
- Electroporation
- BIOLISTIC - Particle bombardment

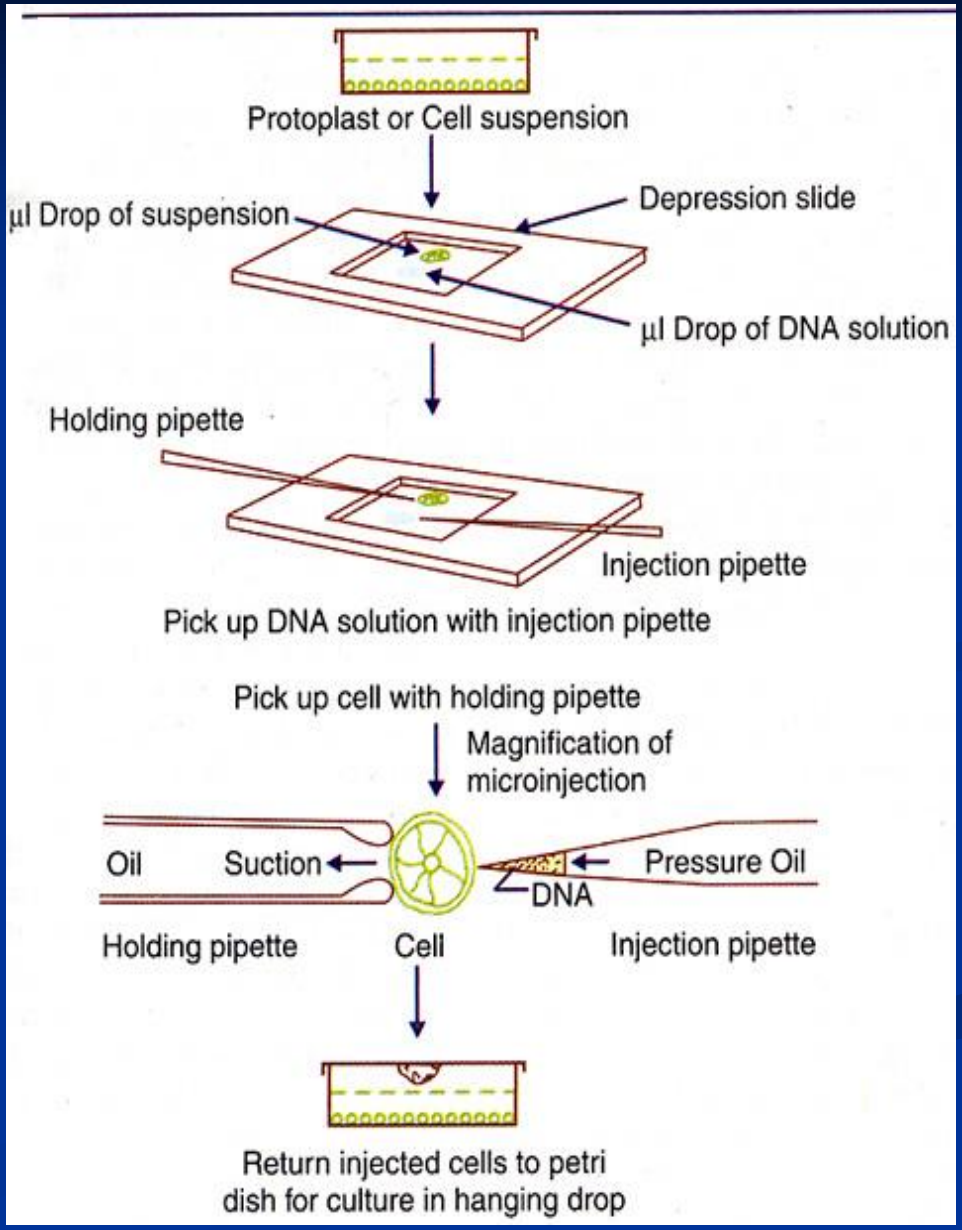
Polyethylene glycol mediated transformation of sugarbeet stomatal guard cell protoplasts

Why guard cell protoplasts?

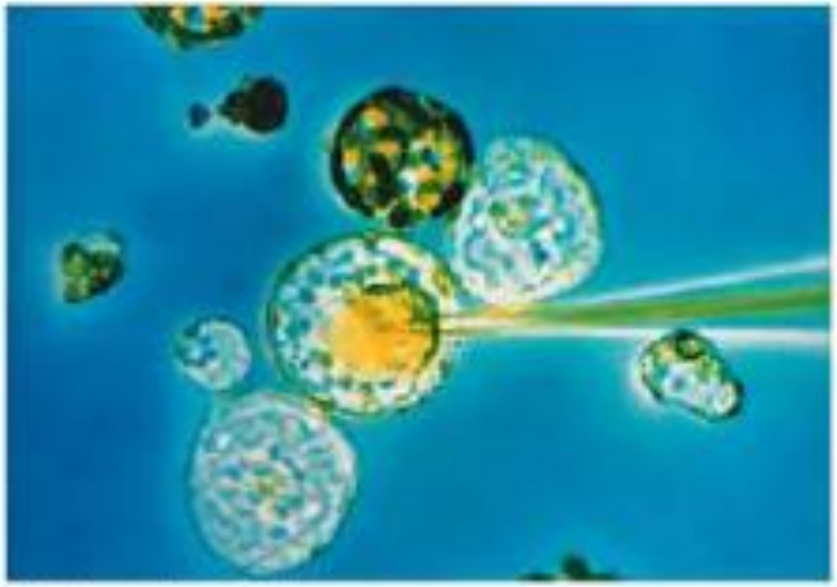
Only totipotent cell type

Transformation with Microinjection

Protoplasts attached to slide by embedding in agarose using a holding pipet
DNA injected using injection pipet

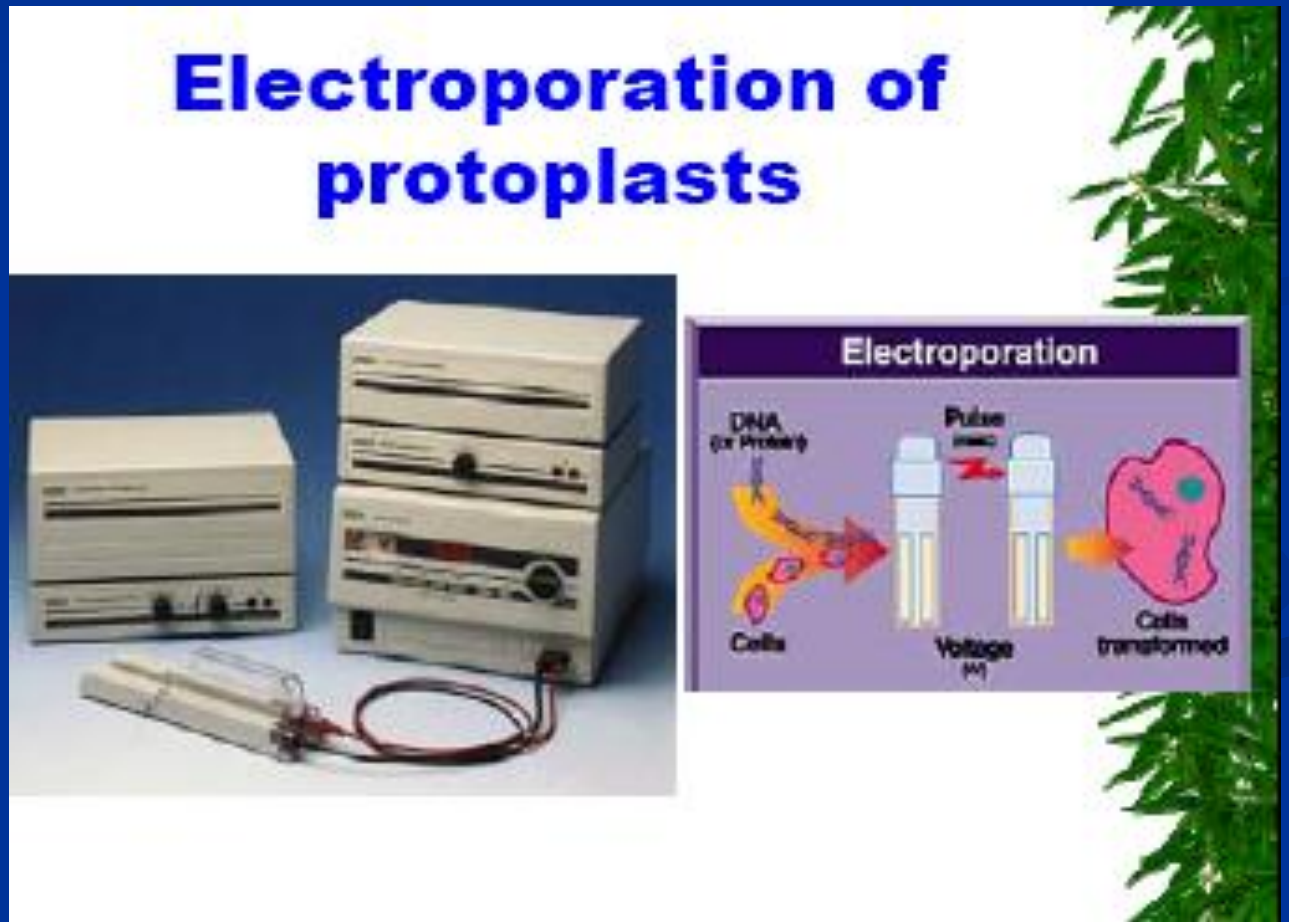


Microinjection of protoplasts

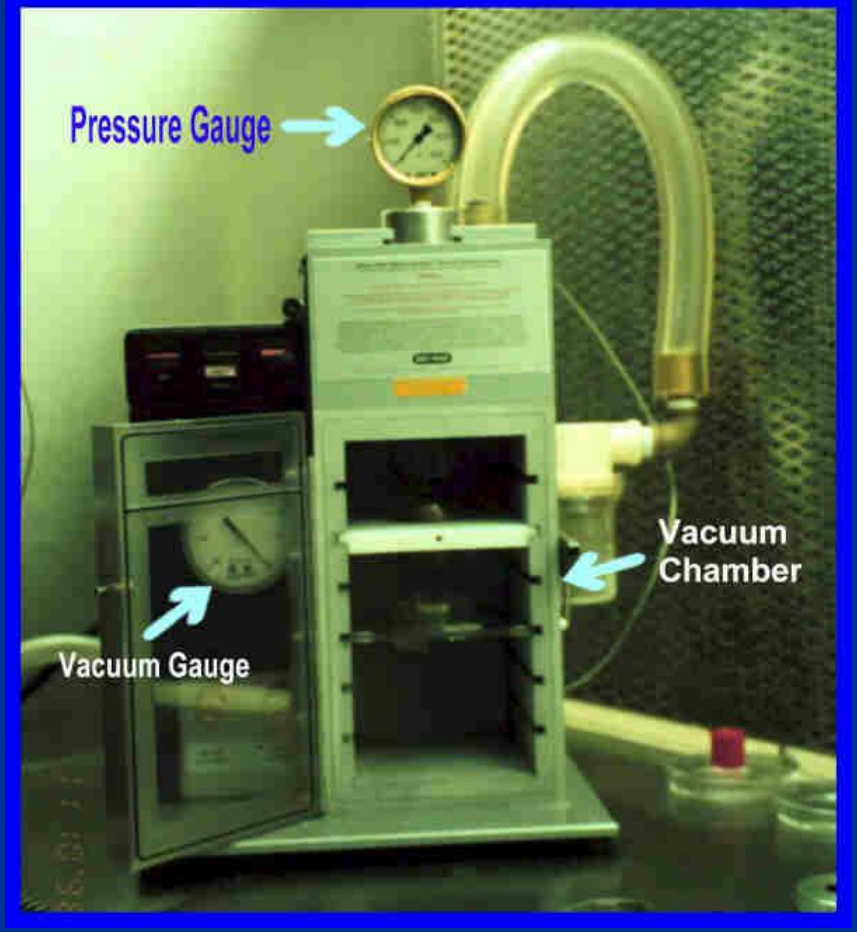


Transformation with Electroporation

- Can transfer chimeric genes with a brief electric pulse
- Transient expression of foreign DNA

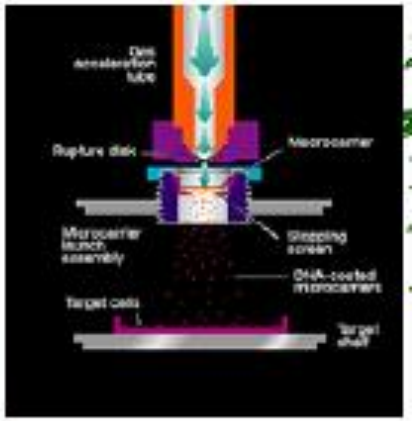


BIOLISTIC TRANSFORMATION

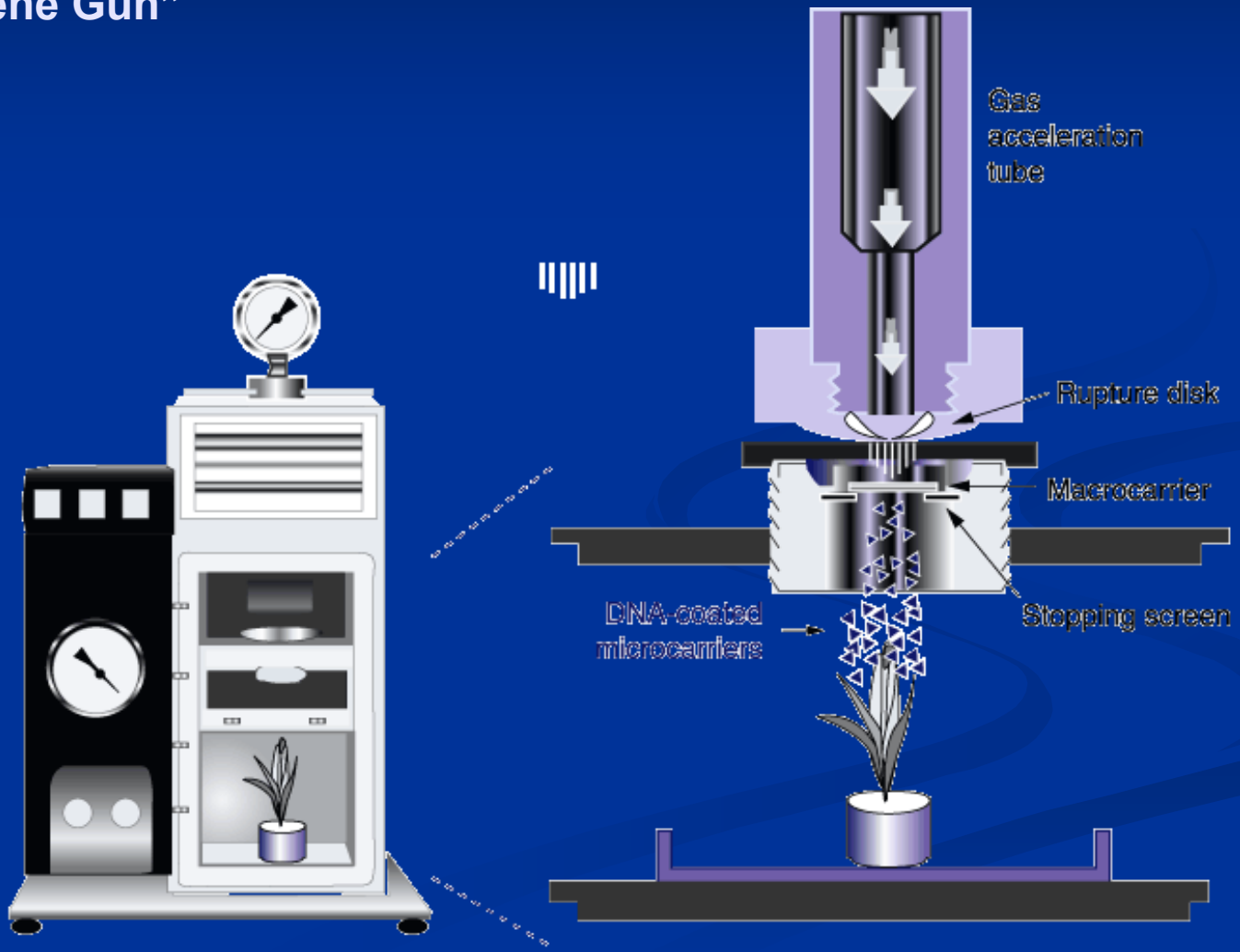


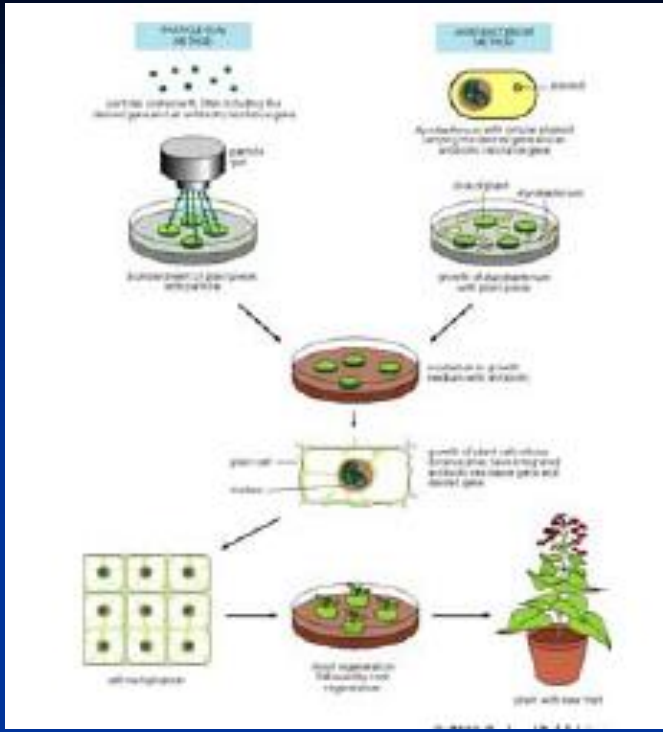
Gene Gun (animation)

- Invented by Cornell researcher, John Sanford



Microparticle Bombardment :The Gene Gun”



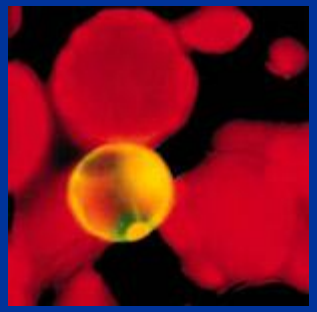


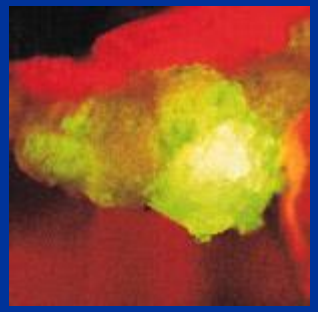
Gene gun set up in the hood

Marker genes

- NPT II- Kanamycin resistance
- GUS

- GFP – Green Fluorescent Protein





GENES of interest

- ipt
- BAR – BASTA resistance
- SOD

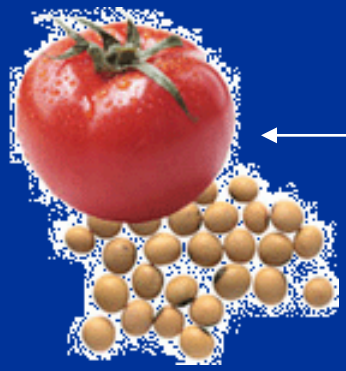
Concept Based on the Term *Transgene*

Examples: – modified EPSP synthase gene (encodes a protein that functions even when plant is treated with Roundup)

Ex. – Roundup Ready Crops

Why are transgenics important?

We can develop organisms that express a “novel” trait not normally found in the species



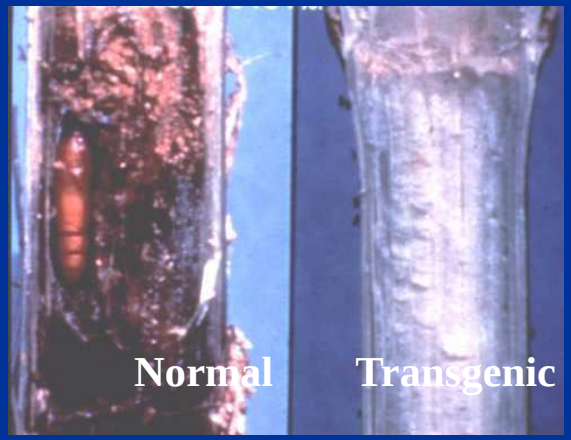
Extended shelf-life tomato (Flavr-Savr)

Herbicide resistant soybean (Roundup Ready)

Agriculture Transgenics On the Market

Insect resistant cotton – Bt toxin kills the cotton boll worm

- ***transgene = Bt protein***



Insect resistant corn – Bt toxin kills the European corn borer

- ***transgene = Bt protein***

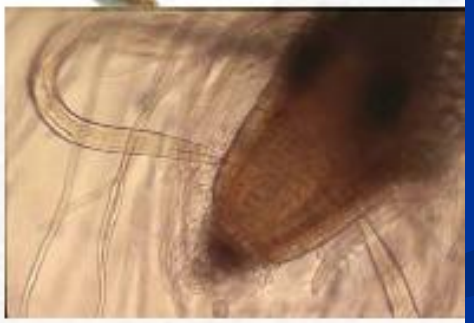
Diverse insects affected by Bt toxins

Cabbage looper

Cabbage looper

Heliocoverpa worms

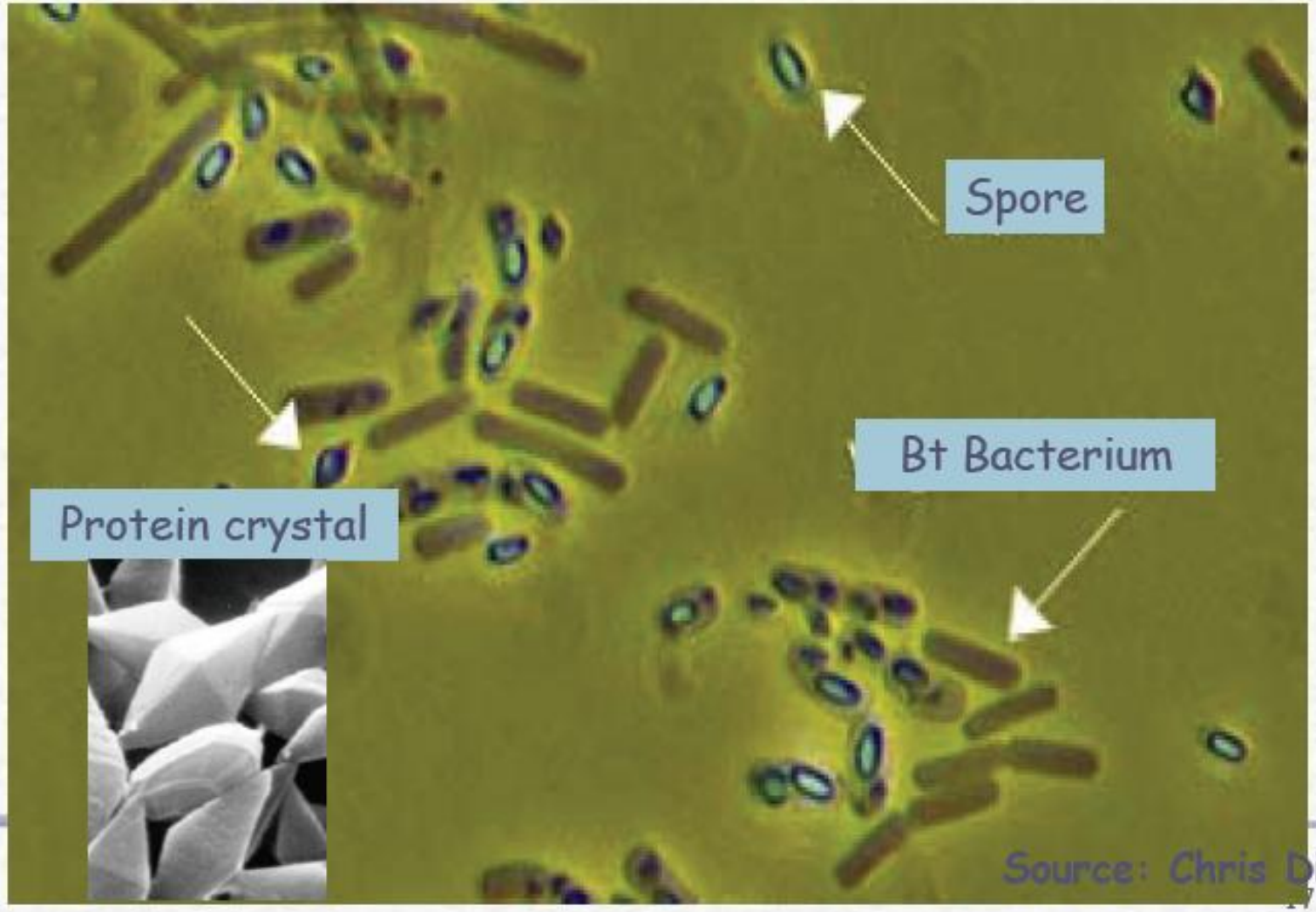
Mosquito



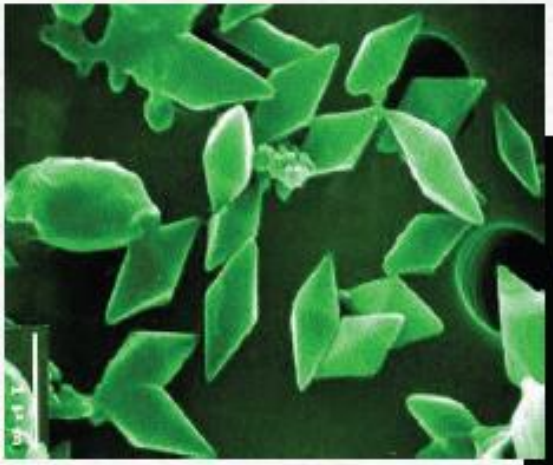
Nematodes

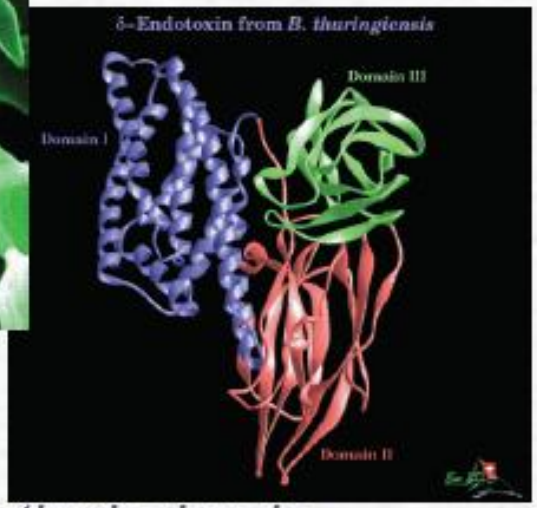
Bacillus thuringiensis (Bt)

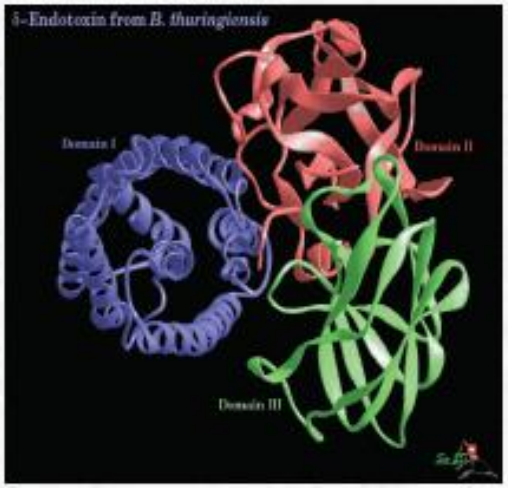
- Common soil bacteria
- When it sporulates, it produces protein crystals



The Bacillus thuringiensis toxin



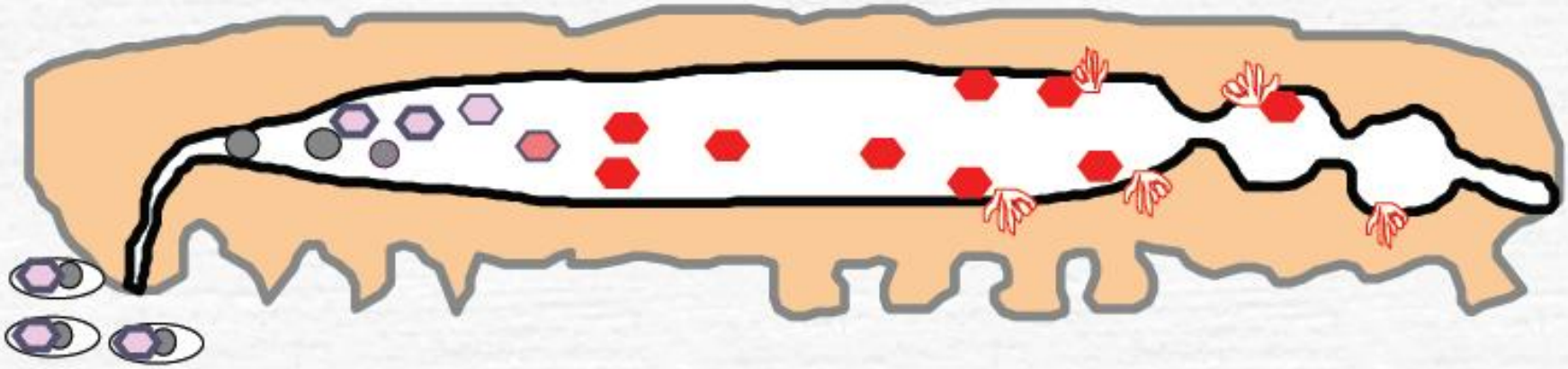




- Crystal produced by *B. thuringiensis*
- The crystal controls insect species
  - Lepidoptera,
  - Diptera
  - Coleoptera

that have a larvae stage in their life cycle

Bt mode of action



Ingestion of spores, crystal proteins (= protoxins)

Activation of toxin:
- crystals solubilized under high pH
- protein cleaved by gut proteases

Toxin binding to gut receptors:
- paralyzes gut
- insects stops feeding
- gut membrane leaks
- bacteria multiply in body
- septicemia

Source: Chris DiFonzo

The Bt toxin confers resistance against many economic pests

In cotton, it controls:
cotton bollworm,
pink bollworm

In maize, it controls:
European corn borer
corn rootworm (Diabrotica).

Insect Resistant Cotton

- Bollgard and Bollgard II cotton
- *Bacillus thuringiensis* (Bt) gene
- Bollworm resistance
- Reduced pesticide use and spraying time
- Increased yields and net return*?*

The Next Test Is The Field

Herbicide Resistance

Non-transgenics

Transgenics

Final Test of the Transgenic

Consumer Acceptance

RoundUp Ready

Before **After**

Source: Monsanto

Herbicide resistant crops

Now: soybean, corn, canola

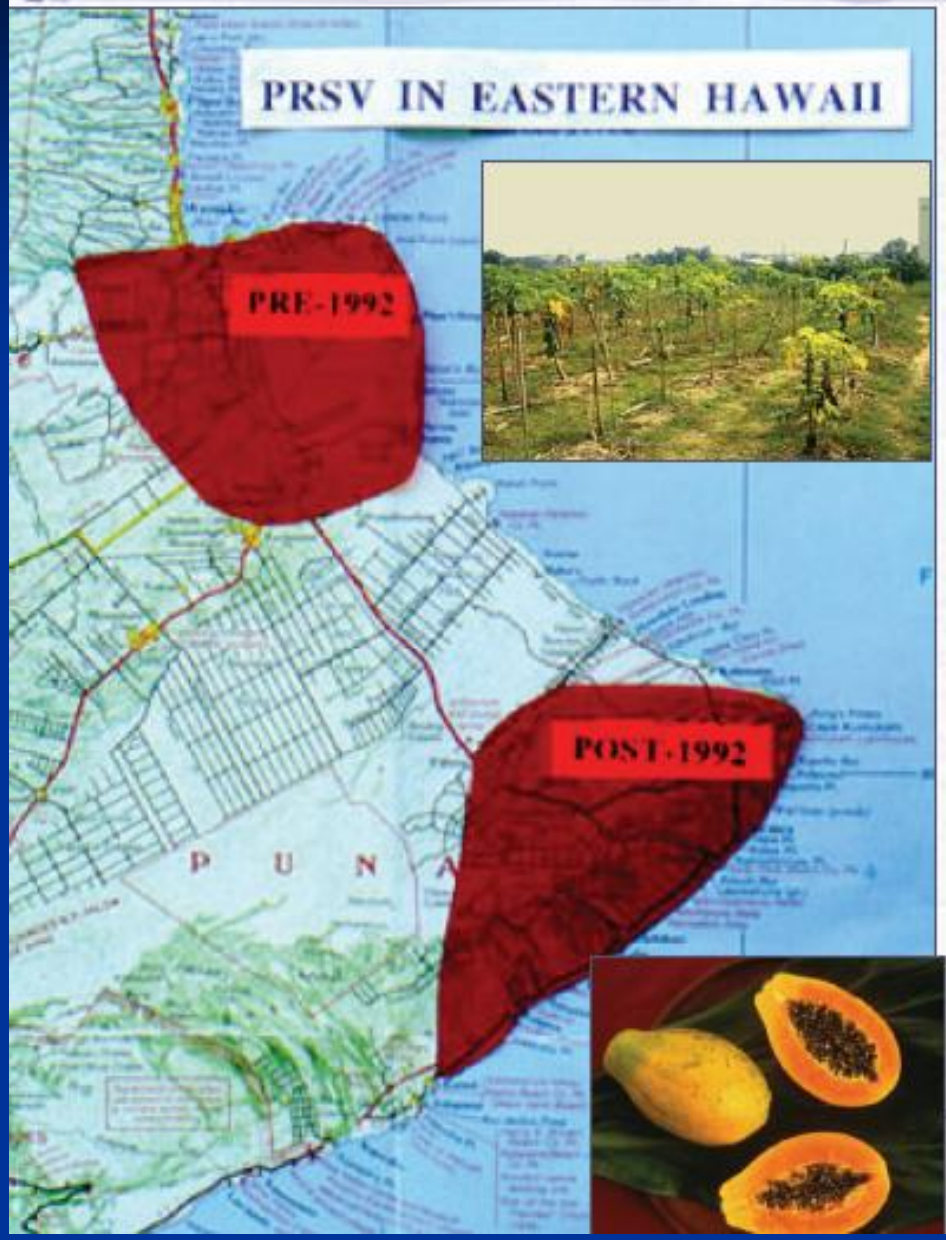
Coming: sugarbeet, lettuce, strawberry alfalfa, potato, wheat

- ***transgene = modified EPSP synthase or phosphinothricin-N-acetyltransferase***

Virus resistance - papya resistant to papaya ringspot virus

- ***transgene = virus coat protein***

Why was Virus Resistant Papaya developed?



Threat from Papaya Ring Spot Virus (PRSV) in Hawaii

1940 – PRSV discovered

1950s –

1. Eliminated large productions from Oahu Island

2. Papaya Industry relocated to Puna District (free of PRSV)

Why was Virus Resistant Papaya developed?

1980s –

1. 95% production established in Puna

2. Research started on resistance through transgenic approach

1992 –

1. PRSV infected Papaya fields in Puna
2. Small scale field trial with the transgenic lines

1998 –

1. GM papaya commercialized

Source: Chr. Hansen

Biotech chymosin; *the enzyme used to curdle milk products*

- ***transgene = genetically engineered enzyme***

Source: Rent Mother Nature

bST*;* ***bovin somatotropin****; used to increase milk production*

- ***transgene = genetically engineered enzyme***

Some Ag Biotech Products Are Discontinued

Why???

Poor Quality
- *FlavrSavr* tomatoes (Calgene)

Negative Consumer Response
- Tomato paste (Zeneca)

Negative Corporate Response
- NewLeaf (Monsanto)

Universal Negative Publicity
- StarLink corn (Aventis)

Next Generation of Ag Biotech Products

Golden Rice – increased Vitamin A content
(but not without controversy)
transgene = three pathway enzymes

Sunflower – white mold resistance
transgene = oxalate oxidase from wheat

Turfgrass – herbicide resistance;
slower growing (= reduced mowing)

Bio Steel – spider silk expressed in goats; used to make soft-body bullet proof vests (Nexia)

Biotechnology is Not Just on the Farm

Disease Treatment

Diagnostics

Environmental Cleanup

Human Applications

Human Applications

- **Pharmaceutical products**
  New solutions to old problems
- **Disease diagnosis**
  Determine what disease you have or may get
- **Gene therapy**
  Correcting disease by introducing a corrective gene

Biotechnology and Health

Product	Use
Insulin	Diabetes
Interferon	Cancer
Interleukin	Cancer
Human growth hormone	Dwarfism
Neuroactive proteins	Pain

The genes for these proteins are:

- Cloned
- Inserted into bacteria
- ***Product isolated using biofermentation***

Environmental Applications

***Bioremediation* -** cleanup contaminated sites; uses microbes designed to degrade the pollution

Indicator bacteria – contamination can be detected in the environment

Future Health-related Biotech Products

Vaccines – herpes, hepatitis C, AIDS, malaria

Tooth decay – engineered ***Streptococcus mutants***, the bacteria that destroys enamel

Edible Vaccines

Transgenic Plants Serving Human Health Needs

- Works like any vaccine
- A transgenic plant with a pathogen protein gene is developed
- Potato, banana, and tomato are targets
- Humans eat the plant
- The body produces antibodies against pathogen protein
- Humans are ***"immunized"*** against the pathogen
- Examples:
  - ✓Diarrhea
  - ✓Hepatitis B
  - ✓Measles

A Popular Term We Need To Know

GMOs - Genetically modified organisms

- **GMO -** an organism that expresses traits that result from the introduction of foreign DNA

- Originally a term equivalent to ***transgenic organism***

The GMO Ruse

- ✓ Some claim any improved biological product is a GMO
- ✓ They feel this will
  - ease the publics fear
  - pave the way for product acceptance
- ✓ For example, some call plant varieties biotechnology products
- ✓ This is a false claim

Let's Be Up Front

- **Biotechnology adds traits** not available in the species

  - ✓ Soybean does not have a gene to breakdown Roundup
  - ✓ The gene comes from bacteria

- **Breeding ≠ Biotechnology**

  - ✓ ***Breeding only exchanges genes found in the species***
  - ✓ Breeding can transfer the transgene to other breeding materials
  - ✓ **BUT** this does not make it a biotechnology procedure

Important Plant Improvement Methods

Source: USDA

- **Breeding**

  Crossing two individuals from the same species; produces a new, improved variety; ***not a biotechnology procedure***

Source: USDA

- **Transformation**

  Adding a gene from another species; the ***essential biotechnology procedure*** to produce transgenics

PLANT BIOTECHNOLOGY IN AGRICULTURAL UNIVERSITY- PLOVDIV

PLANT BIOTECHNOLOGY LABORATORY

- Is educational center for the BSc, MSc and PhD students from AU and other research institutions

and also research center
Main Projects developed during the last 10 years

- **Plum (*Prunus domestica*)**

  Cellular and molecular approaches for PPV (Plum Pox Virus) resistance induction in plum - Joint Project - AU, IGE and Fruitgrowing Res. Institute -Plovdiv;

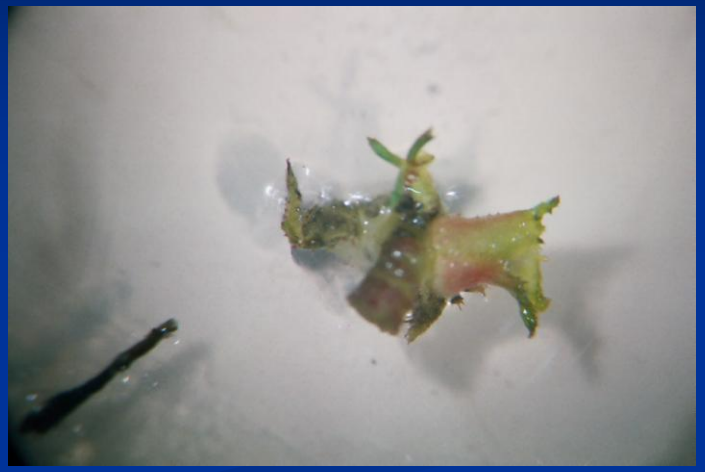
  Creation of efficient regeneration system and gene transfer as approach for the breeding improvement of standard and Bulgarian native plum varieties.

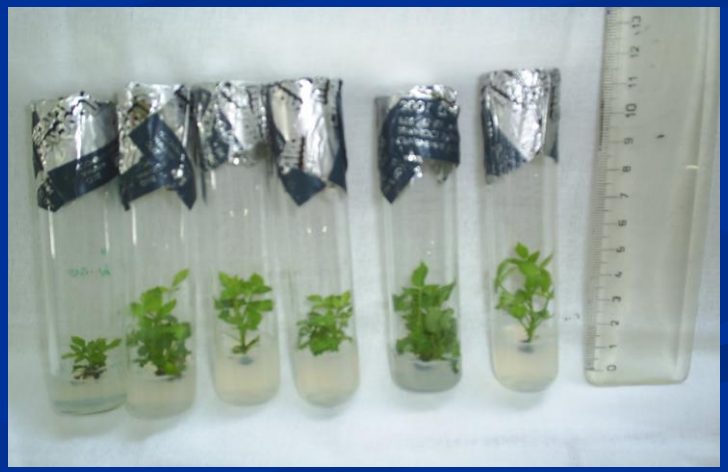
- **Carnation**

Participation in Joint Research Project of CPRO-DLO Wageningen and five Dutch Flower companies for carnation regeneration and transformation.

- **Rose** - Joint Research Project between Agricultural University and IGE-

  for propagation, plant regeneration and gene transfer of new Bulgarian hybrid breeding lines and rose cultivars for cut flower.

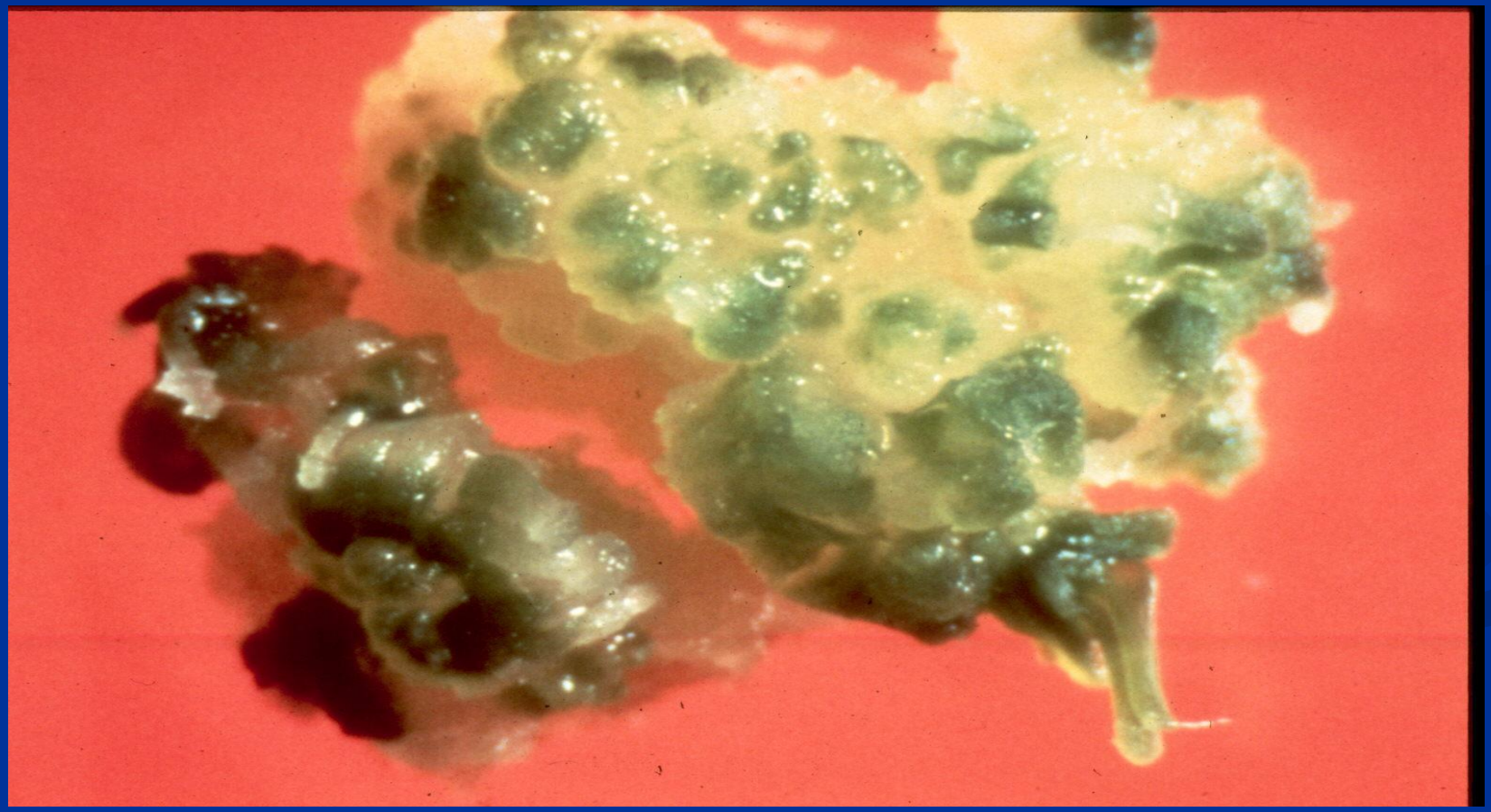




- **Bean** (Phaseolus vulgaris) –

Application of Biotechnological methods in the bean breeding process- plant regeneration via somatic embryo, organogenesis of Bulgarian standard and new mutation varieties as a prerequisite for gene transfer.

Research project supported by Ministry of Education and Science.

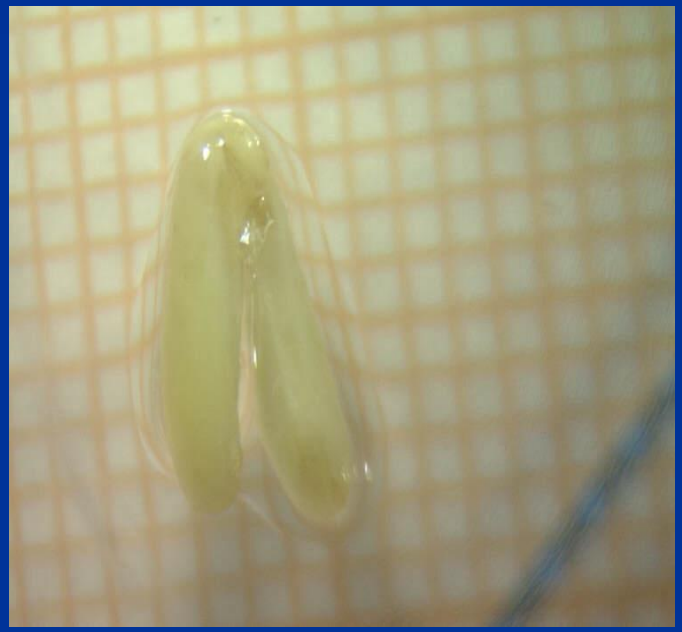


Development of new variety « Plovdiv 11M » of common bean, *Phaseolus vulgaris*

By Prof. DSc D. Svetleva

- **Barley**

Research project for crop improvement by creation of somaclonal variation - plant regeneration from anthers, immature and mature embryos.

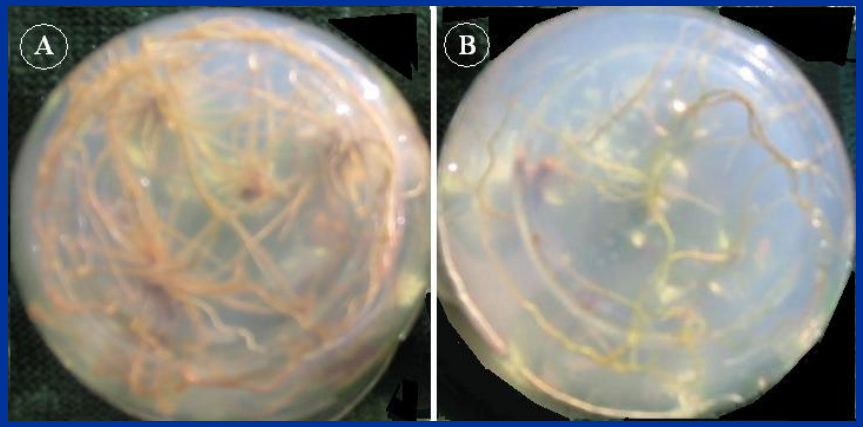


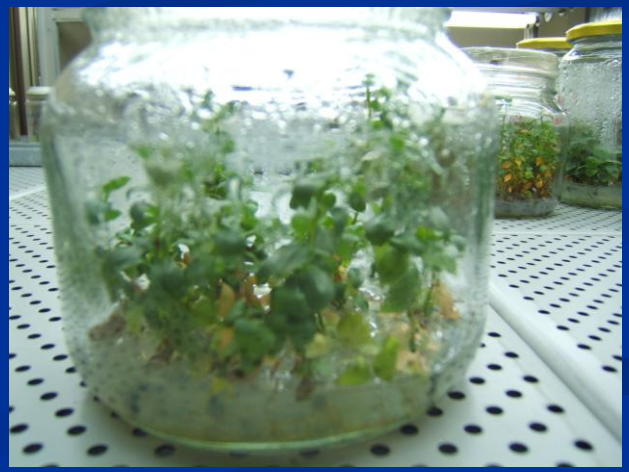
- **Strawberry**

- Plant regeneration from different explants and gene transfer.
- Development of regeneration systems by somatic embryo-, organogenesis from leaf protoplasts, leaf and root segments

Black chokeberry (Aronia ssp.)

Development of technology for clonal micropropagation





Adaptation, acclimatization and transfer to the field

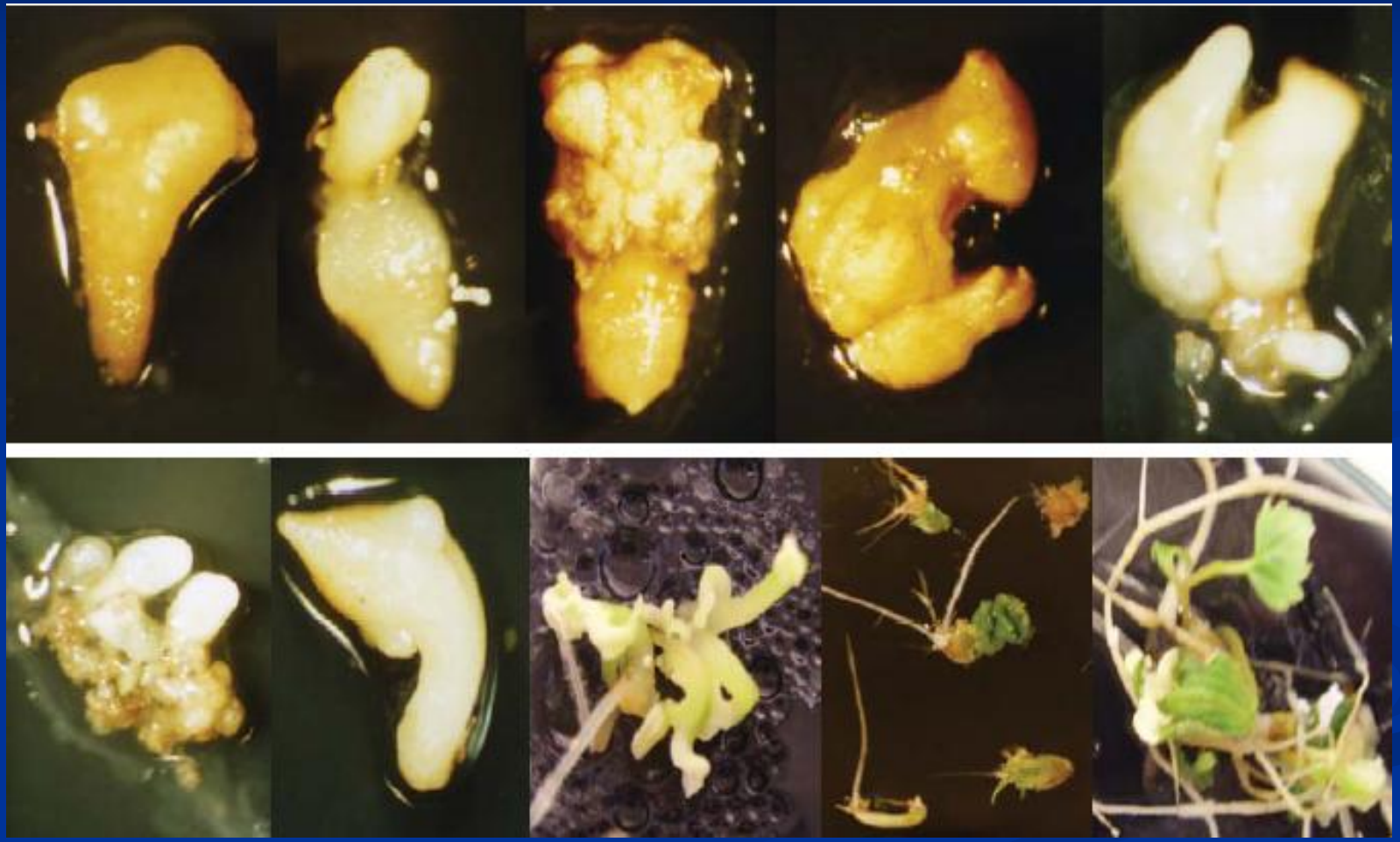
Grapevine

- Micropropagation of grapes
- Molecular analysis for pathogens detection in Bulgarian grape varieties
- Application of molecular markers for grape cultivar and hybrid identification.
- Somatic embryogenesis in seedless cultivars and hybrid combinations for crop improvement.

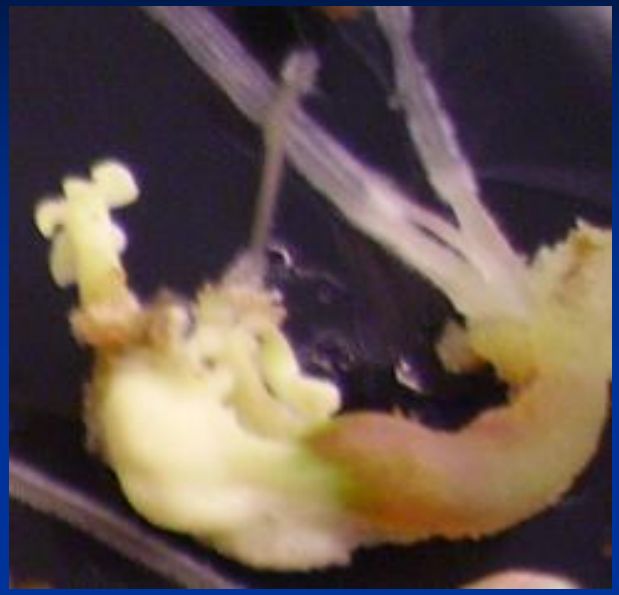
Factors influencing regeneration

- Genotype
- Culture medium- liquid or solid
- Growth regulators – concentration and type
- Carbohydrates
- Explant age
- Cultural conditions

Induction and subsequent development of grape embryos at different stages with converting to green plants.



Repetitive somatic embryo formation



Grape plants obtained from somatic embryos:

a.) following acclimatization

b.) growing in the field conditions for further investigation.

Present in vitro study demonstrated the obtaining of plants from inbreed seedless grapes and hybrids of crosses between seedless and seeded forms and supported the classical breeding program of seedless grapes with additional possibility to overcome the

limitations and difficulties like seed abortion and incompatibility.

- **Apple** -

  Plant regeneration, somatic organo- and embryogenesis, gene transfer and molecular techniques.

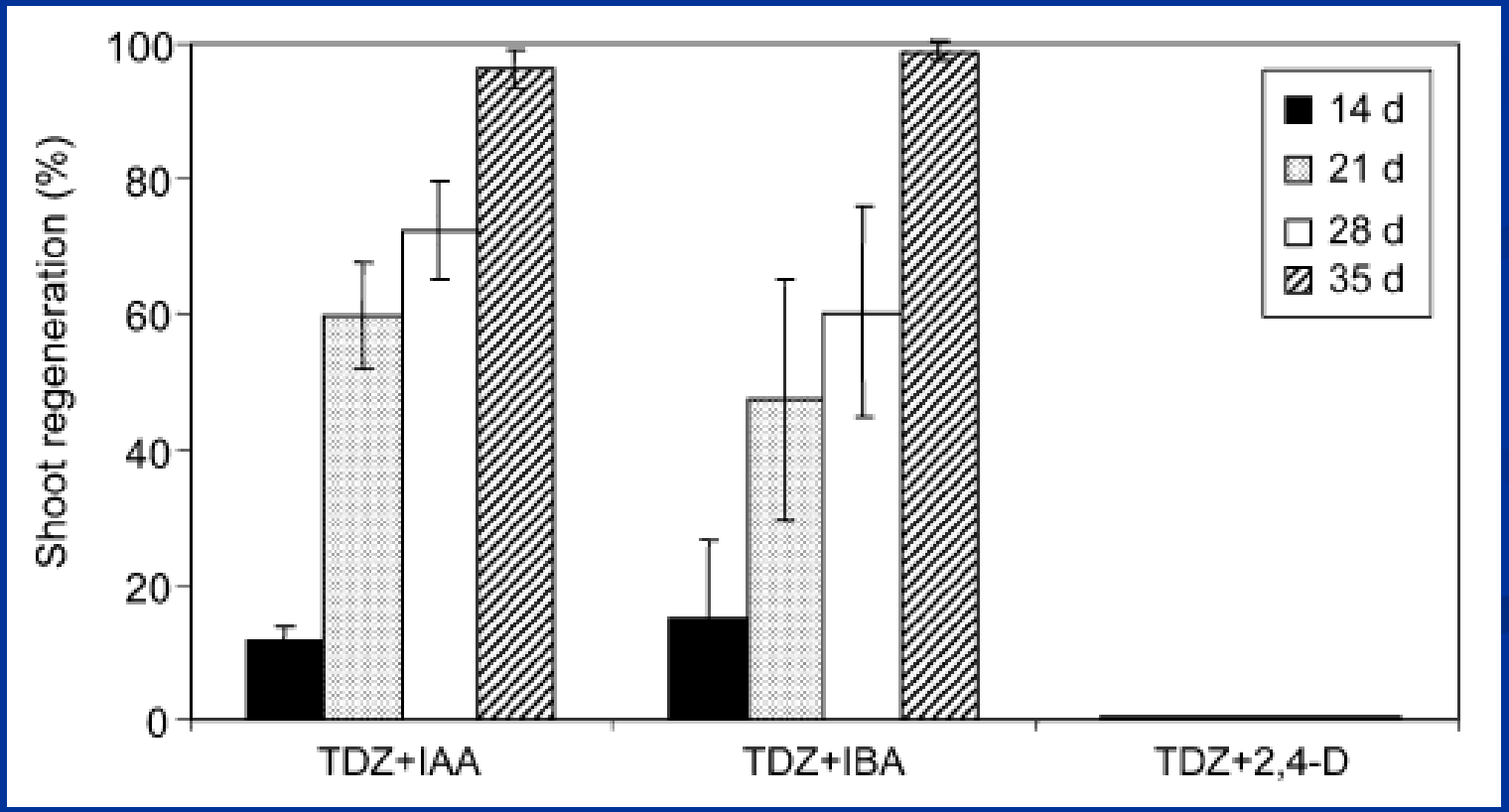
  Joint research with ARO- Volcani Center, Israel

The present study examined

- the ontogeny of in vitro apple shoot organogenesis, with the aim to illuminate the early events in shoot meristem development,
- and the role of different types of auxin in the progress or arrest of such developmental plans.

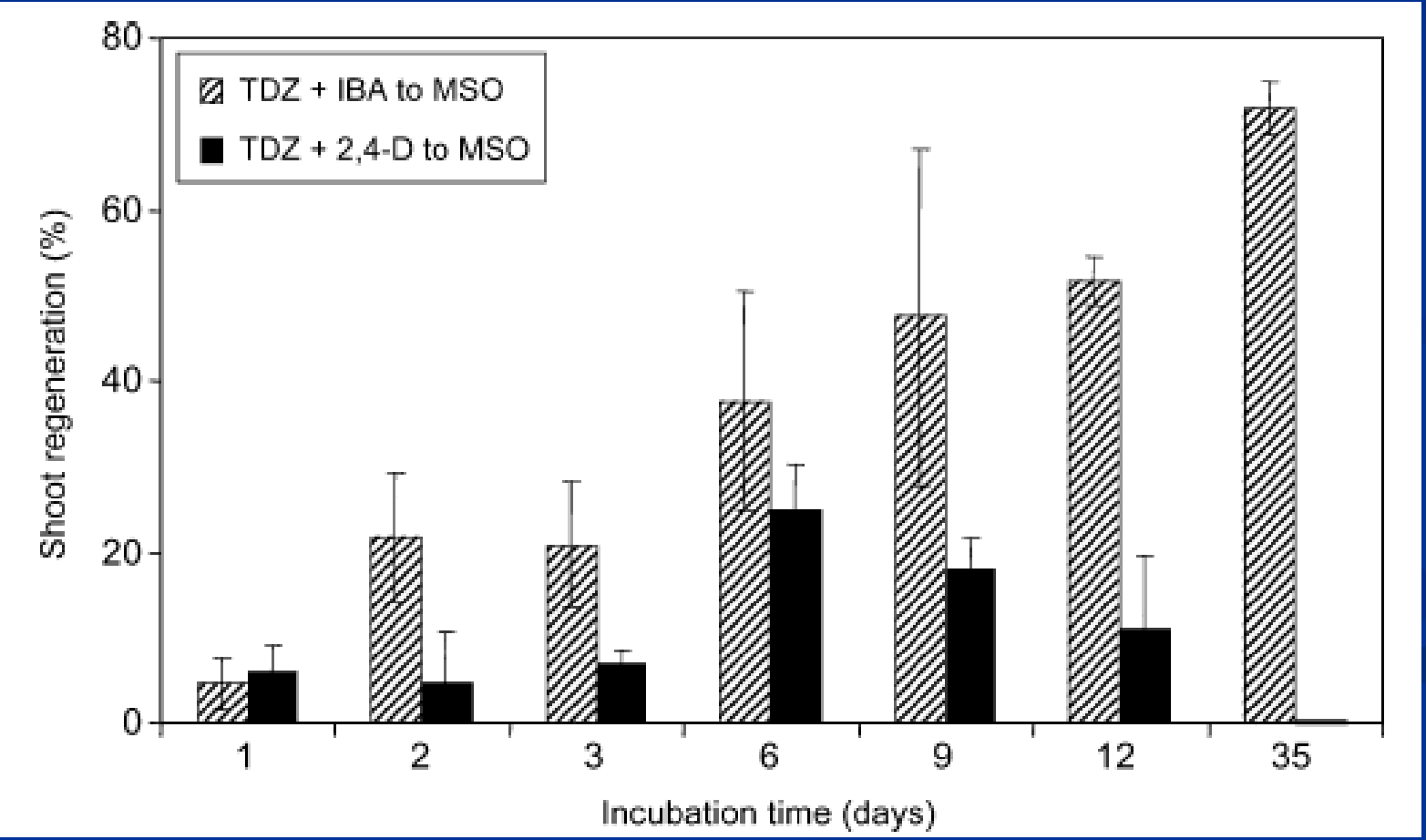
Effect of different auxins on differentiation

Three combinations of growth regulators,
the cytokinin TDZ (5 mg /l) with the auxins-IAA, IBA and 2,4-D
(all the auxins at aconcentration of 1 mg /l) were compared.
Control treatments with only TDZ (5 mg /1), IBA (1 mg /1) and 2,4-D
(1 mg /1) were performed.

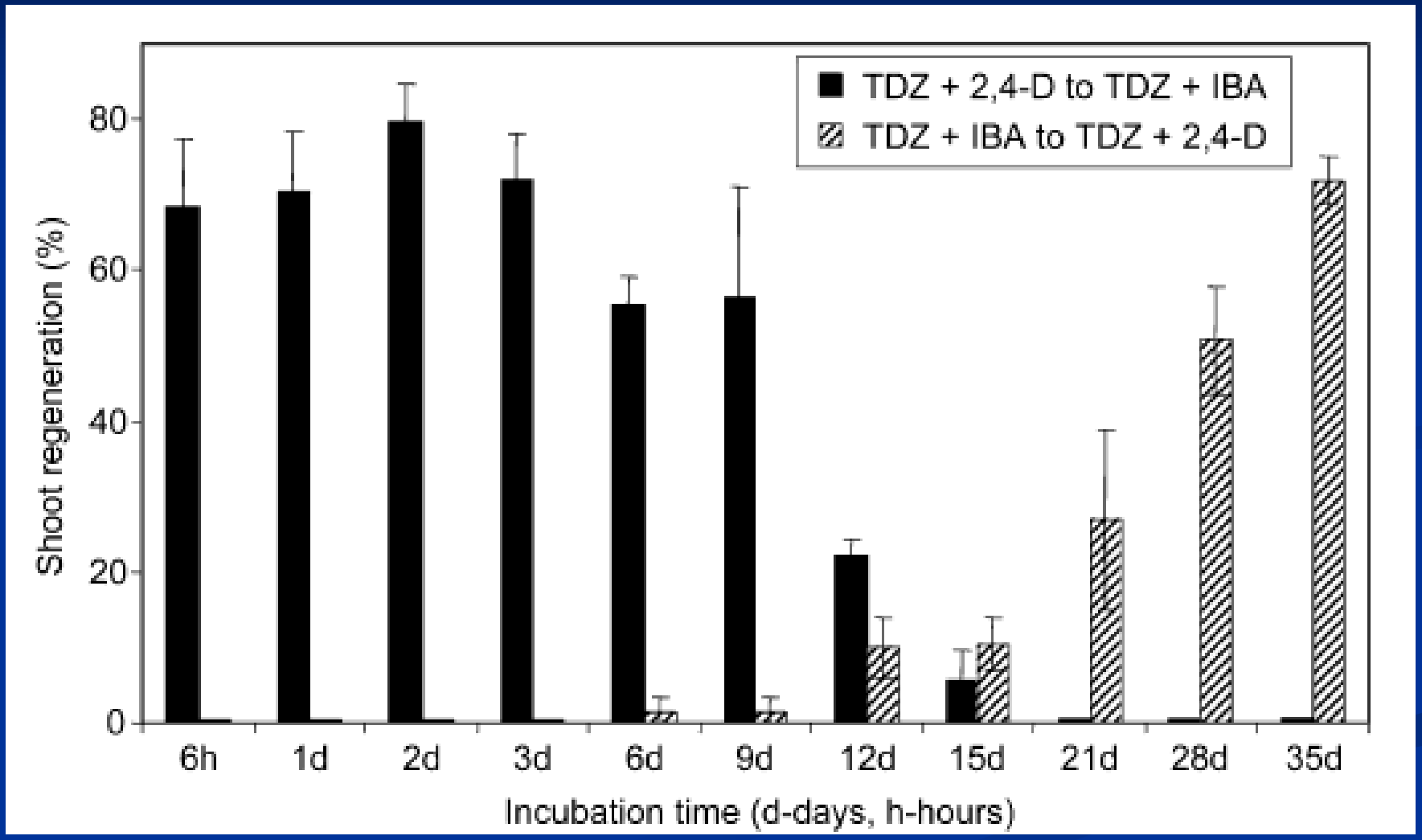


Determination for shoot organogenesis

Competence and time of determination for shoot organogenesis

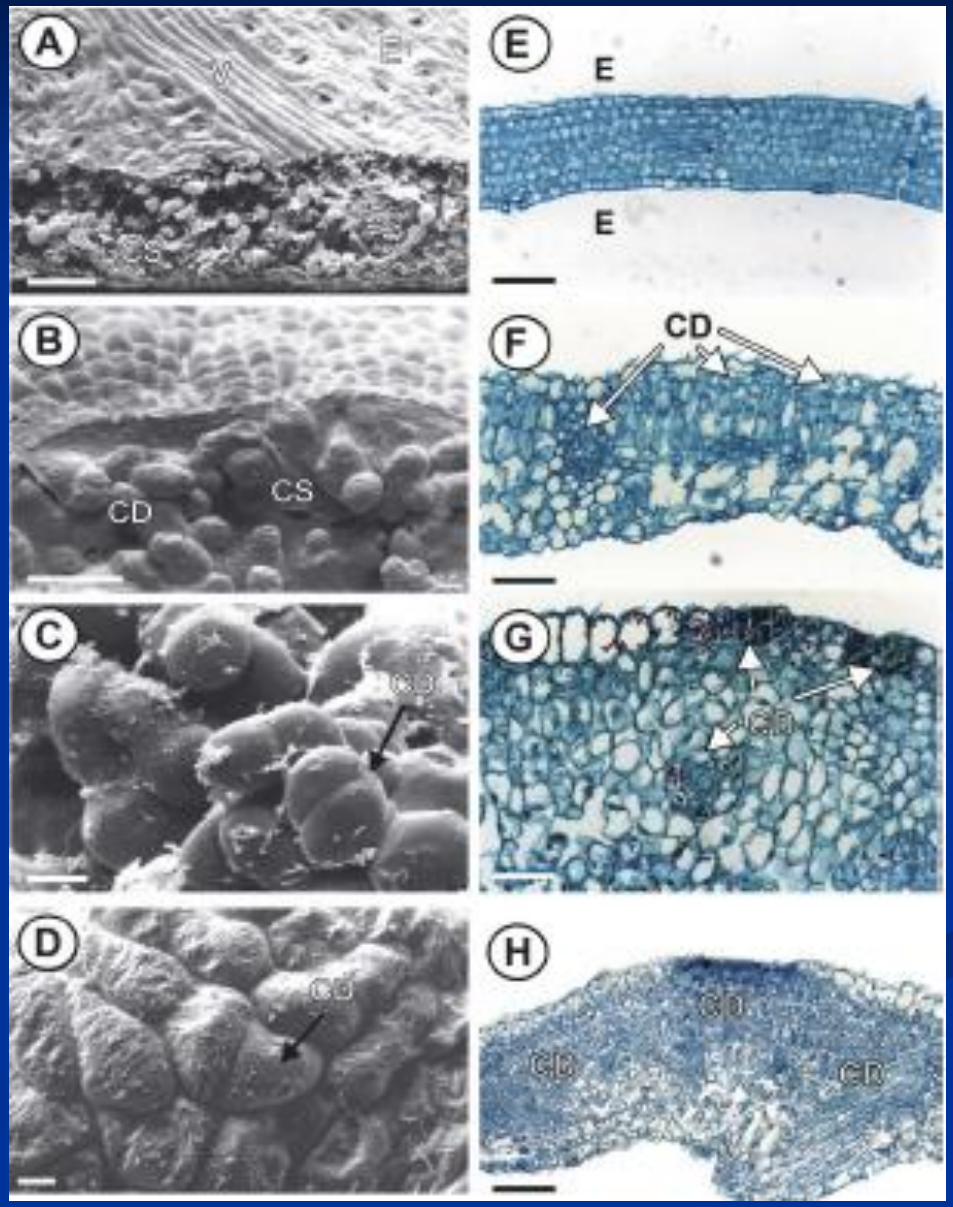


Auxin interactions

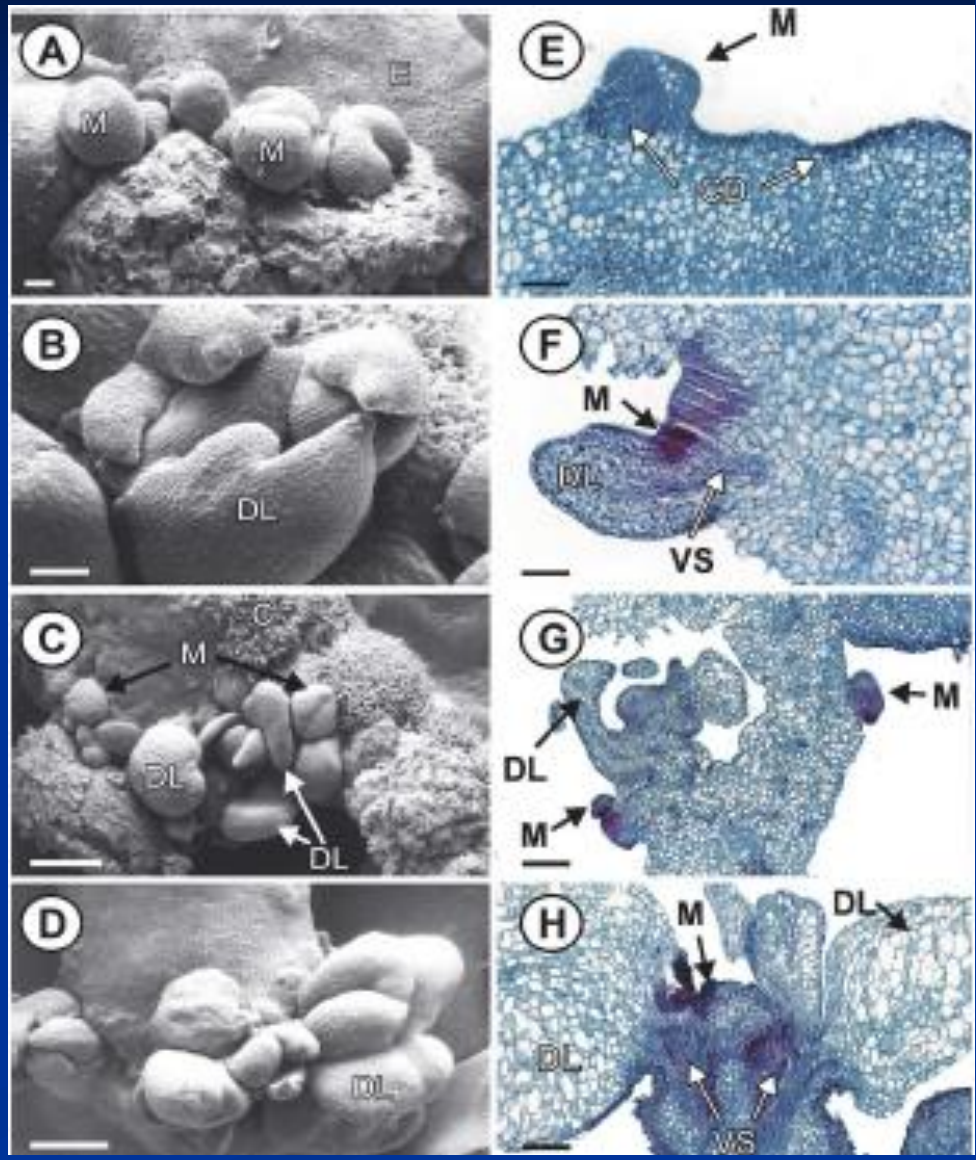


Development of plant regeneration. SEM and histological observations

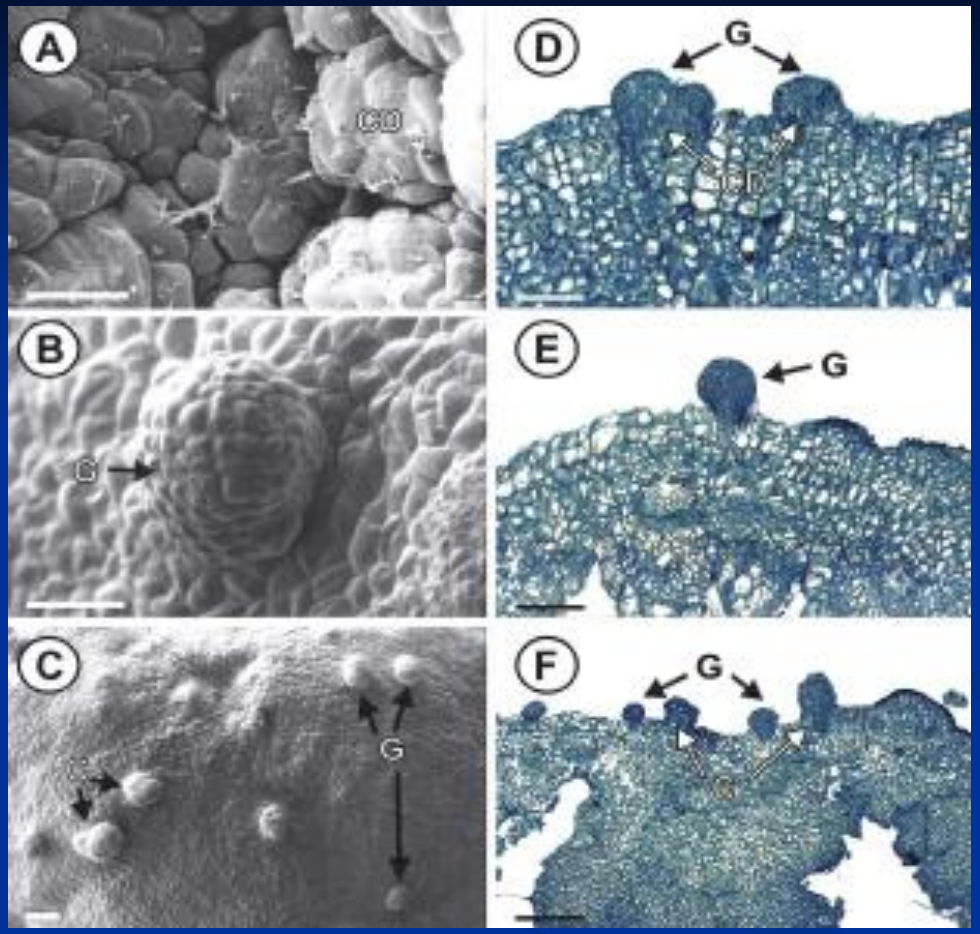
Early effects of the type of auxin IBA or 2,4-D (1 mg l1) with TDZ (5 mg l1) on apple shoot organogenesis determination. SEM (A/D) and light microscopy (E/H) observations. (A) Time 0 (control)*/leaf surface and the cut made before culture. Bar/50 mm. (B) After 6 days (TDZ/IBA)*/explant expansion and first cell divisions on the cut surface. Bar/50 mm. (C) After 6 days (TDZ/IBA)*/cell divisions on the leaf explant surface. Bar/10 mm. (D) After 6 days (TDZ/2,4-D)*/cell expansion and first divisions. Bar/10 mm. (E) Time 0 (control)*/young leaf consists of 8 cell layers before culture. Bar/60 mm. (F) After 3 days (TDZ/IBA)*/expanding cells, some divisions had already been initiated in the first and second layer of palisades. Bar/95 mm. (G) After 6 days*/cell divisions in individual cells of the abaxial epidermis and in the phloem and xylem parenchyma. Bar/60 mm. (H) 2 days (TDZ/2,4-D)*/active cell divisions in all cell layers. Bar/95 mm; CD, cell division; CS, cut surface; E, epidermis; V, vein.



Developmental pattern of apple shoot regeneration with application of IBA (1 mg l1) and TDZ (5 mg l1) observed by SEM (A/D) and light microscopy (E /H). (A) After 9 days*/meristematic domes appear on the abaxial epidermis. Bar/100 mm. (B) After 14 days*/shoot primordia develop. Bar/100 mm. (C) After 25 days*/multiple shoot formation from the explant surface with calli surrounding the shoots. Bar/500 mm. (D) After 25 days*/shoot development from the proximal end of the petiole with no callus formation. Bar/500 mm. (E) After 9 days*/the first meristematic domes, formed by anti- and periclinaly dividing epidermal cells, appeared on the surface of the explant. Bar/95 mm. (F) After 12 days*/adventitious buds and leaf primordia on the abaxial epidermis. Bar/95 mm. (G) After 14 days*/adventitious shoots with developing leaves. Bar/235 mm (H) After 25 days*/adventitious shoots with differentiated primary leaves and apical meristems. Bar/235 mm; C, callus; CD, cell division; DL, developing leaves; E, epidermis; M, meristem; VS, vascular system.



SEM (A/C) and light microscopy (D/F) observations of leaf explants cultured with TDZ/2,4-D. (A) After 9 days*/ clusters consisted of sister cells of the same origin initiating globular embryo- like structures on the adaxial epidermis. Bar/50 mm. (B) After 12 days*/globular embryolike structures as single domes on the epidermis. Bar/50 mm. (C) After 21 days*/ multiple globular structures on the adaxial epidermis and calli continuing to divide actively. Bar/100 mm. (D) After 9 days*/cell divisions with columnar orientation and beginning of globular structure formation. Bar/95 mm.



(E) After 12 days*/structures with the shape of typical globular somatic embryo, consisting of one tunica layer and anticlinaly dividing internal corpus cells. Bar/95 mm. (F) After 21 days*/multiple globular structures on the adaxial epidermis and large calli formation. Bar/235 mm; C, callus; CD, cell division; G, globular structure.

The present study examined apple shoot organogenesis from leaf explants from a developmental point of view on the basis of the current understanding of the concept of tissue competence, determination and differentiation. Considerable attention has been given to the effect of the type of auxin on growth kinetics and development of the regeneration process using detailed histology and SEM.

The process of organogenesis has three main phases.

- In the first phase, the cells of the explant have to acquire organogenic 'competence'/defined as the ability to respond to hormonal signals.
- In the second phase, competent cells are determined for specific organ formation under the influence of exogenous hormonal signals.
- In the third phase, the morphogenesis proceeds independently of the exogenously supplied growthregulators.

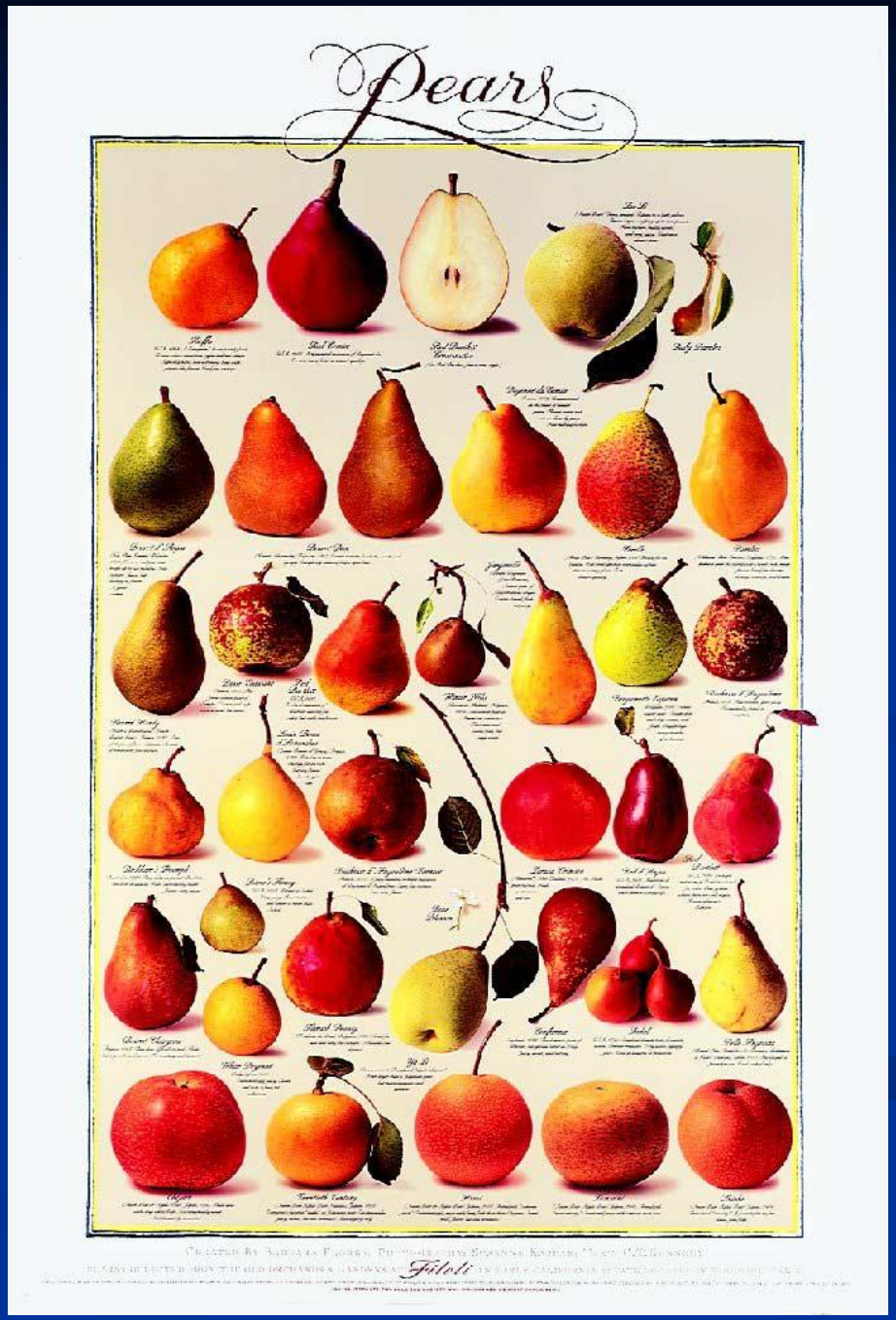
- In our regeneration study we established that in apple some cell determination accured after only one day of exposure to TDZ/IBA or TDZ/2,4-D, prior to transfer to hormone free medium.
- The breaking point for cell fate determination and critical stage for the activation and progression of the plant-cell developmental program started to occur on the sixth day- ‘window’ of cell determination.
- Regeneration ability follows a different developmentalpattern, when auxin interactions have to be overcome.

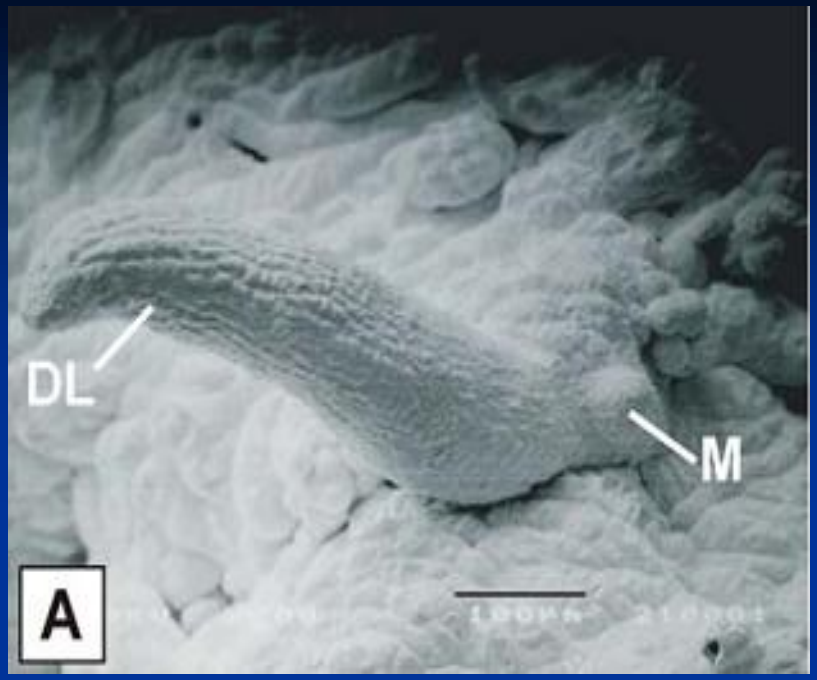
We conclude that in apple, the type of auxin and the length and timing of its application are critical for the activation and progression of the plant-cell developmental program.

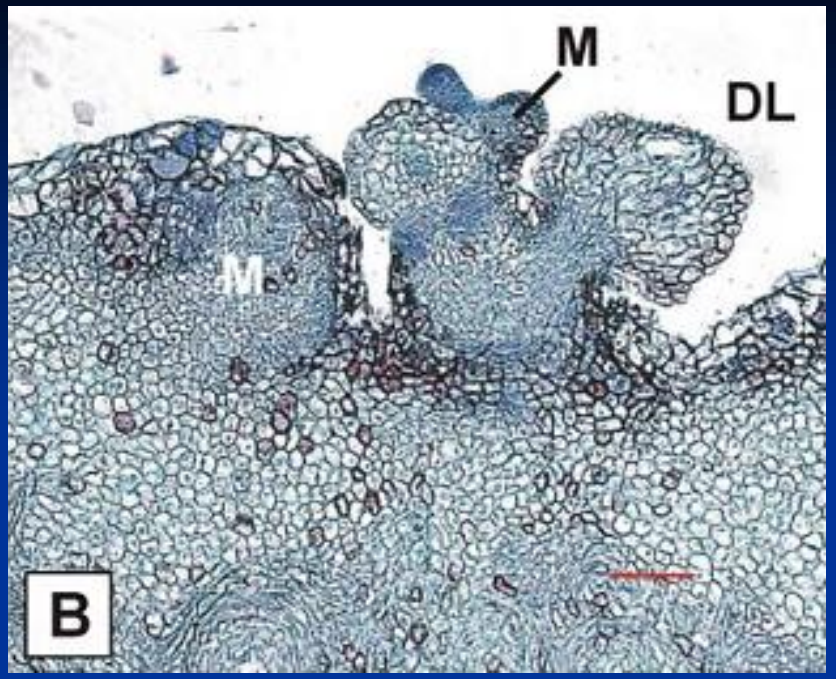
Pear -

Plant regeneration, genetic transformation and molecular analysis.

Joint research with ARO-Volcani Center, Israel







Adventitious shoot formation from Spadona leaf explants.
(A) Scanning electron microscopy of the emerging adventitious shoots after 23 days of culture on regeneration medium (bar =100 μm) (B) Histological section of leaf explant after 23 days showing adventitious shoots with apical meristems and developing primary leaves (bar = 100 μm). (C) Stereomicroscope view of multiple shoot formation from leaf explant after 6 weeks in culture (0.63×1.25)

GENETIC TRANSFORMATION OF PEAR with NPT II and GFP gene

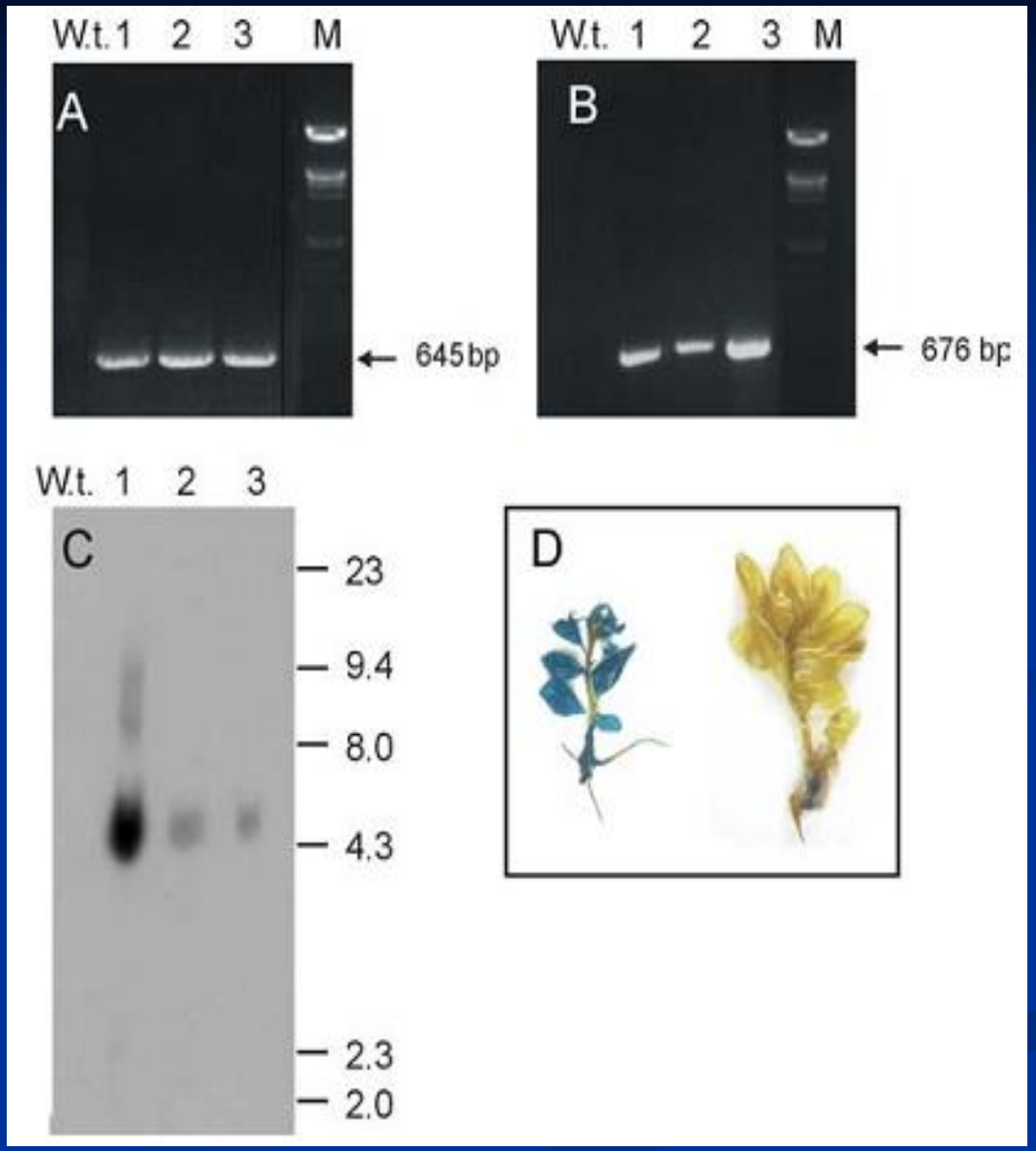
- The use of green fluorescent protein (GFP) improves Agrobacterium-mediated transformation of pear (Pyrus communis L.)
- Agrobacterium tumefaciens cv. EHA105 (Hood et al. 1993) harboring either

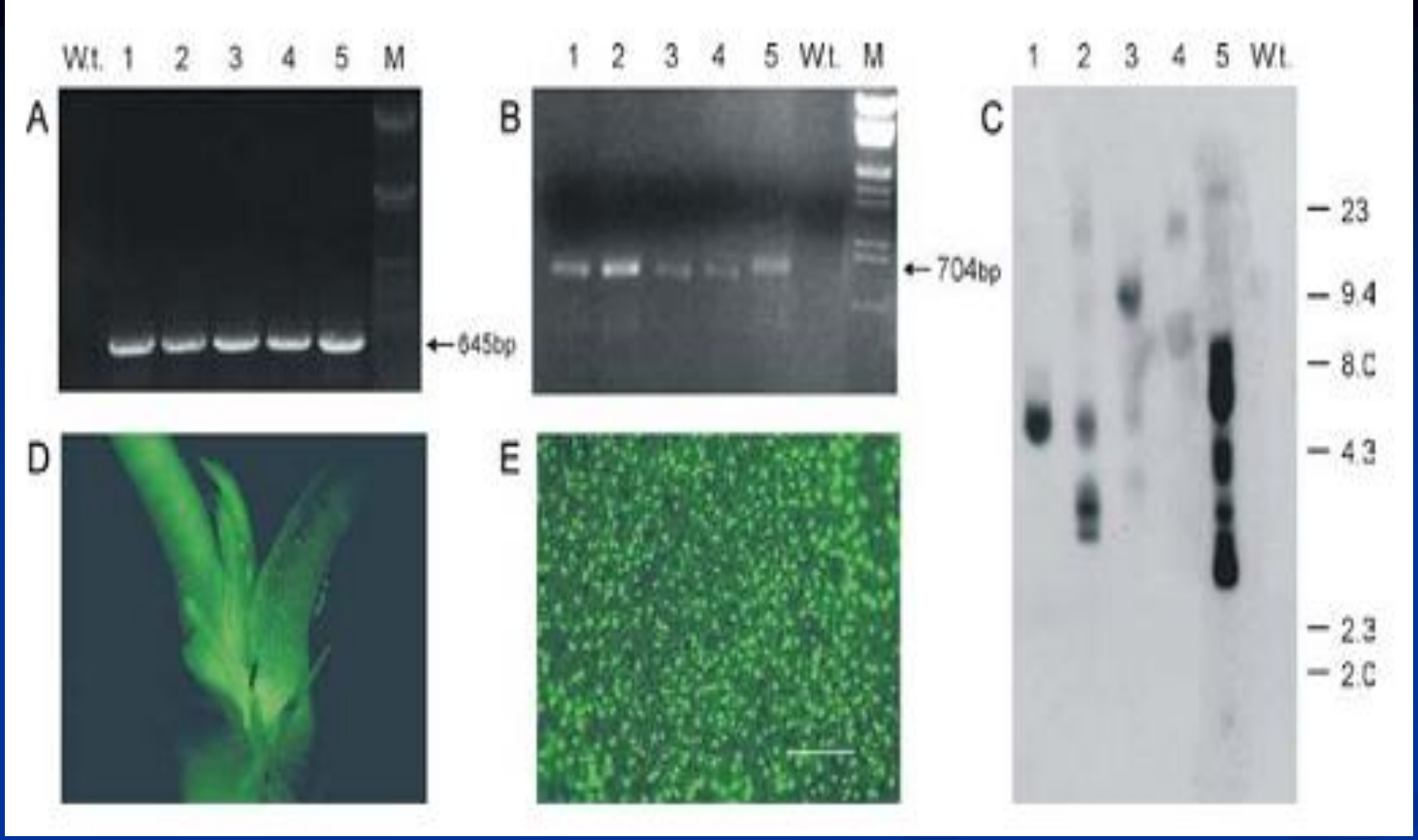
  - pME504 carrying the nptII and the uidA-intron genes (Vancanneyt et al. 1990),

  - or the PZP plasmid carrying both - nptII and GFP genes were used.

  The GFP- HP1γ fusion protein is targeted to heterochromatic nuclear regions (Fass et al. 2002).

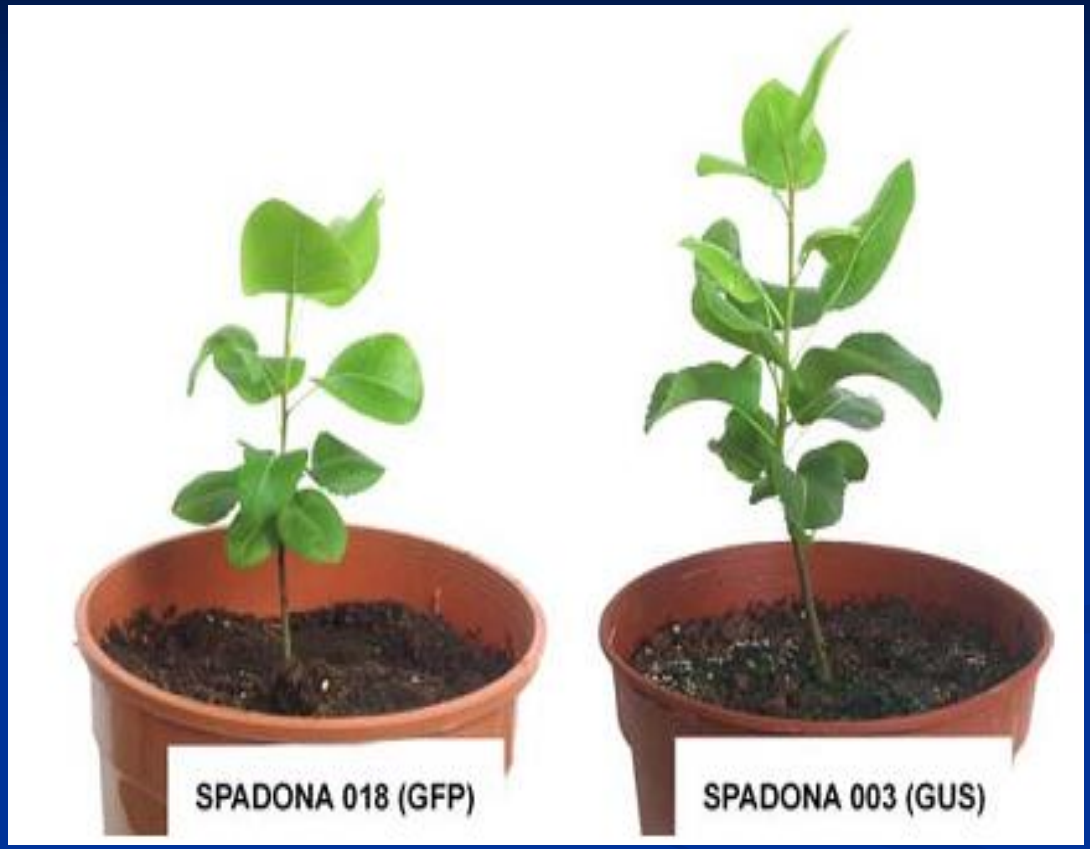
Molecular and histochemical analysis of pear clones selected after transformation with pME504. Molecular analysis of transgenic lines: agarose gel electrophoresis of the PCR-amplified 645 bp specific fragment of the nptII gene (A) and of the 676 bp fragment of the GUS gene (B). DNA was isolated from pear clones selected after transformation with pME504. Lanes: w.t., wild-type; 1–3, transgenic lines Spd 001–003. Southern hybridization analysis of the nptII gene.
Total DNA (5 µg) was digested with HindIII, separated by 0.8% agarose gel electrophoresis, and transferred to a nylon membrane. Hybridization was carried out with nptII PCR-amplified fragment as a probe. Lanes: w.t., wild-type; 1–3, transgenic lines Spd 001–003. Histochemical GUS assay of transgenic clone Spd 001 (C)





Molecular and histological analysis of pear clones selected after transformation with pPZP. PCR amplification of (A) 645 bp specific fragment of the nptII gene and (B) 704 bp of the GFP gene. (C) Lanes: w.t, wild type; 1–5, transgenic lines Spd 004–008; M, DNA marker. Southern hybridization analysis of the nptII gene. Total DNA (5 μg) was digested with HindIII, separated by 0.8% agarose gel electrophoresis, and transferred to a nylon membrane. Hybridization was carried out with nptII PCR-amplified fragment, as a probe. Lanes: 1–5 transgenic lines Spd 015–019, w.t.—wild-type (D) Fluorescent image of transgenic clone (Spd 016) under GFP3 filter (0.63×1.25). GFP expression is detectable in the shoot apex, stem, and leaves. (E) Fluorescent image of the abaxial leaf surface. The expression of GFP is strictly localized to the guard cells with no diffusion of the fluorescence into the cytoplasm (bar=100 μm)

Under the selective conditions employed here, Spadona explants regenerated at the frequencies of 3% and 5% with pME504 and pPZP (GFP), respectively.
The presence of the GFP visible marker enables early detection and recovery of shoots, yielding transformation frequencies of 0.3–0.8 % for the plasmid pME504, and 3.0–4.0% for pPZP (GFP).



Rooting of transgenic Spadona pear plants. Potted transgenic plants in the greenhouse 6 months after acclimatization

CONCLUSION

* GFP is a useful marker that does not interfere with subsequent plant development neither in culture nor in the greenhouse. The early visualization of GFP can significantly decrease labor, time, and cost constraints in transformation, and as such it represents a improvement over existing kanamycin and GUS-based selection systems.

* Utilization of the GFP gene as visible marker could be a useful approach to facilitate selection in transformation experiments involving recalcitrant and kanamycin-sensitive species, and for the generation of antibiotic-free plants.

Fig (Ficus carica L.)

Plant regeneration

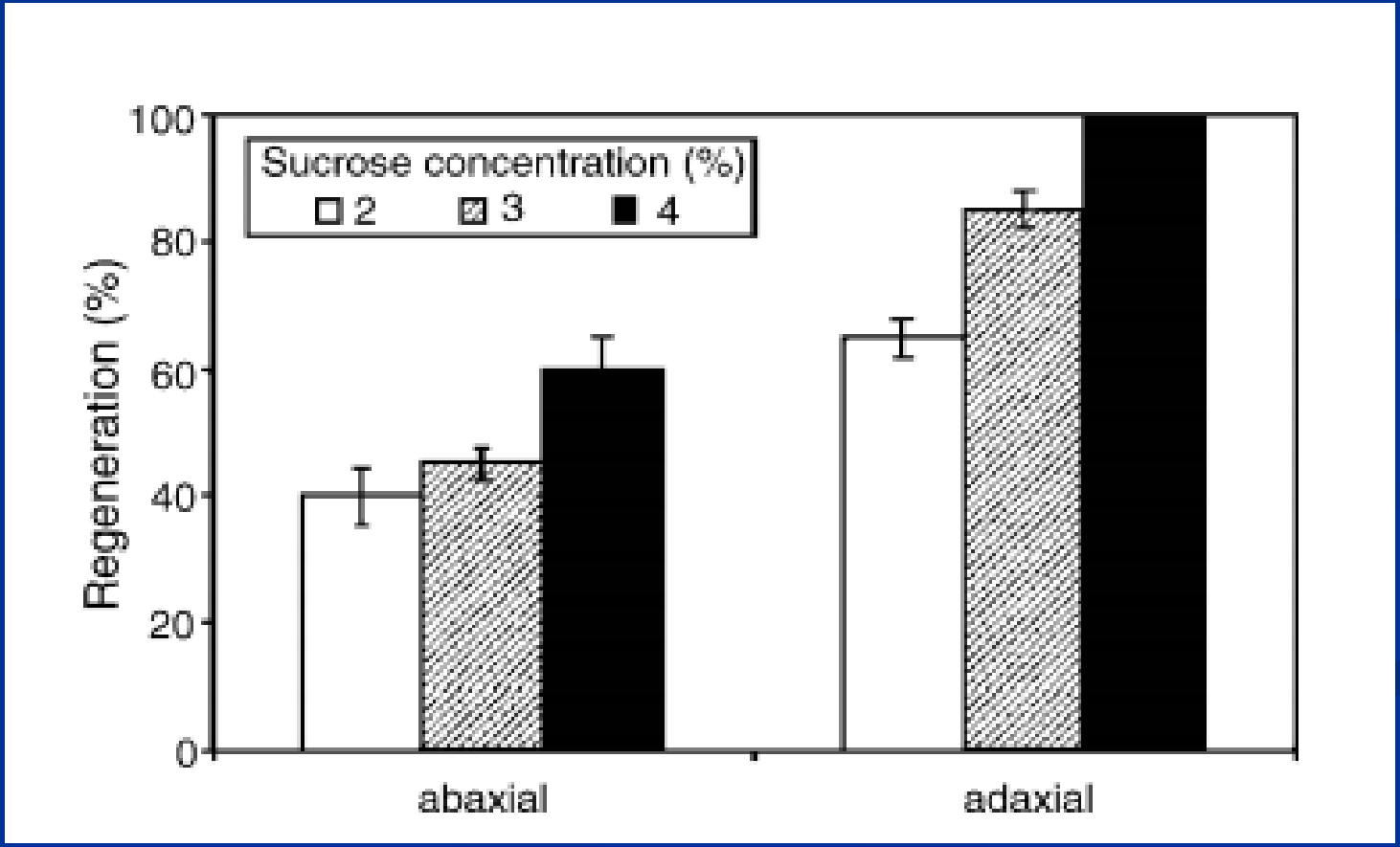
and gene transfer.

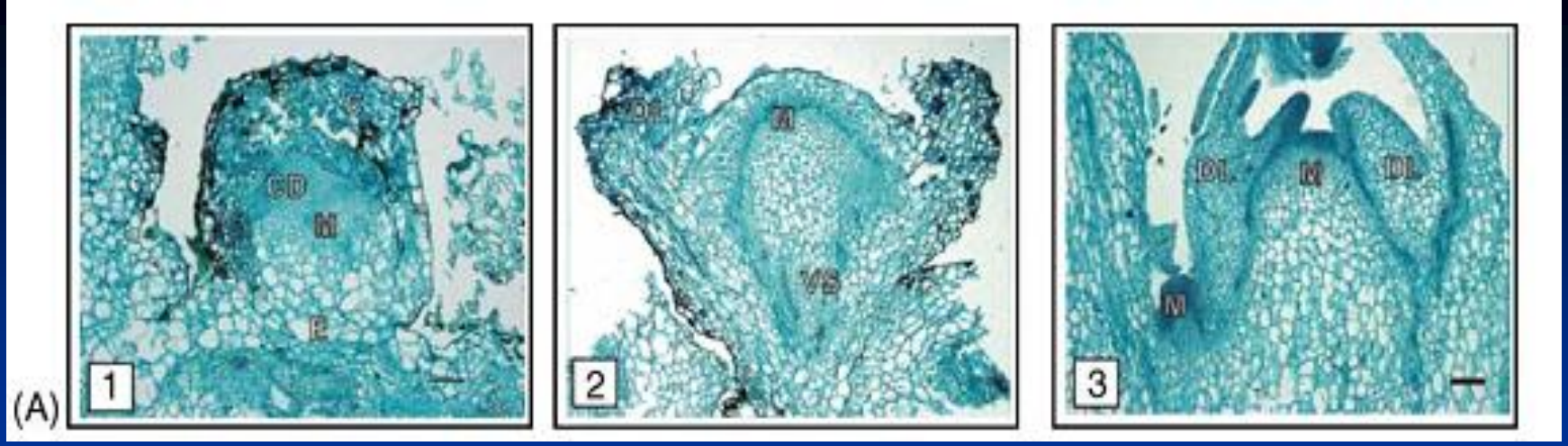
Joint research with ARO- Volcani Center, Israel

- An efficient and reproducible system for regeneration (up to 100%) was developed.
- Agrobacterium-mediated transformation of the common fig (Ficus carica L.) cultivars Brown Turkey (fresh consumption) and Smyrna (dry consumption) was achieved.

Influence of sucrose concentration and orientation of the leaf surface on adventitious shoot regeneration.

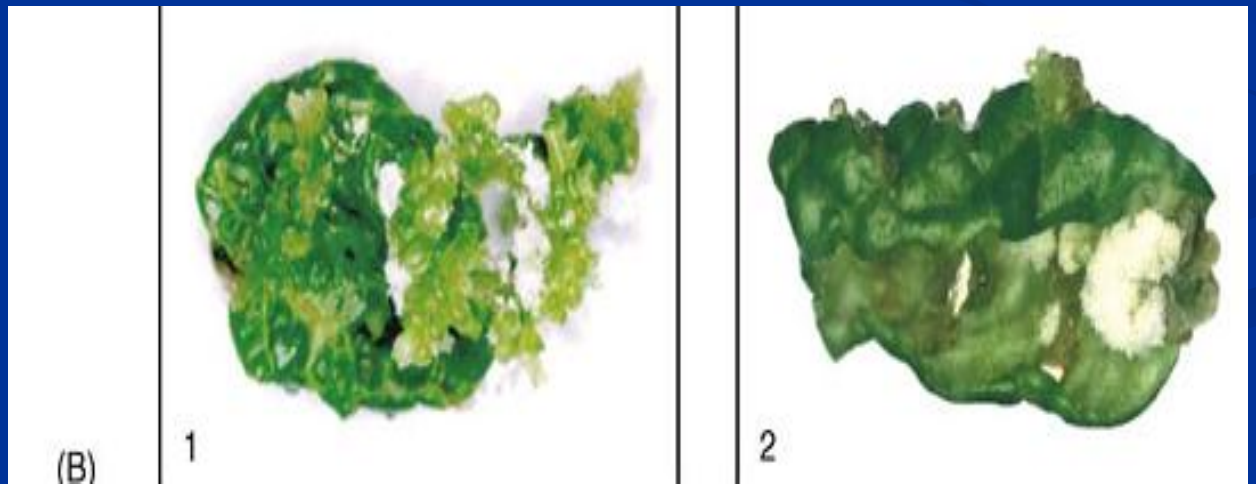
Leaf explants of cv. Brown Turkey were cultured on medium with TDZ (2 mg/l) and IBA (2 mg/ l). Vertical bars indicate standard error (S.E.).





(A)Light microscopy observations of shoot organogenesis in fig.

(B)(1) After 15 days, the first meristematic domes appeared on the adaxial surface of the explant. Bar = 100 mm. (2) After 21 days, appearance of adventitious buds and leaf primordia on the adaxial epidermis. Bar = 200 mm. (3) After 28 days, appearance of adventitious shoots with differentiated apical and axillary meristems and developing leaves. Bar = 100 mm; C – callus, CD – cell division, DL – developing leaves, E – epidermis, M – meristem, VS – vascular system.

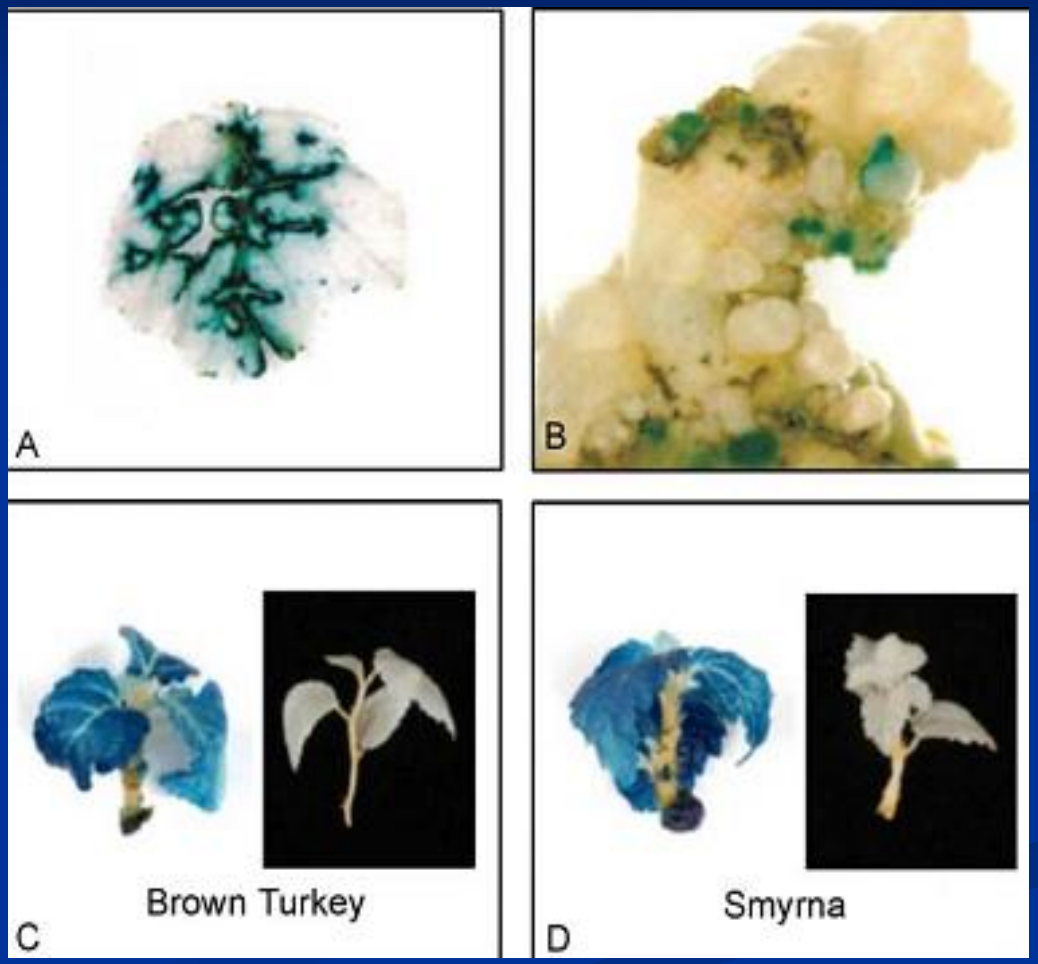


(C) Rooting and acclimatization of plants from cv. Brown Turkey. (1) In vitro rooted plant (bar, 1 cm). (2) Plants in rooting cylinders. (3) Potted plants in the greenhouse 1 month after acclimatization

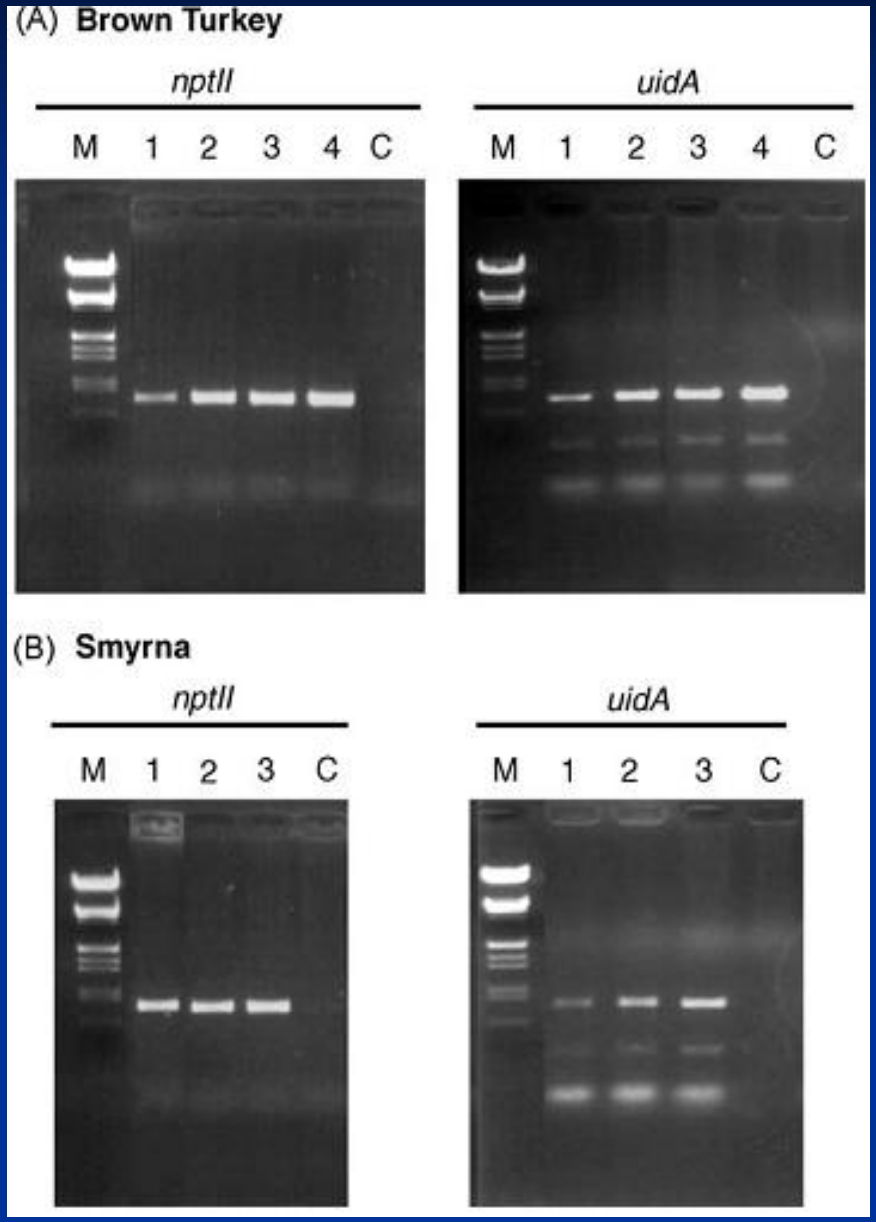
GENETIC TRANSFORMATION

Histochemical GUS analysis:

Stereomicroscopic observations.
(A) Transient GUS expression 3 days after inoculation of leaf explants of cv. Brown Turkey (0.63 X 1.0).
(B) GUS staining of regenerating leaf explants after 4 weeks culture in the presence of 50 mg/l kanamycin and 150 mg/l ticarcillin (0.63 X 2.5).
(C) (D) GUS expression detected in an isolated putatively transformed shoots cultured on PM with 100 mg/l kanamycin and. 150 mg/l ticarcillin (0.63 X 2.0).

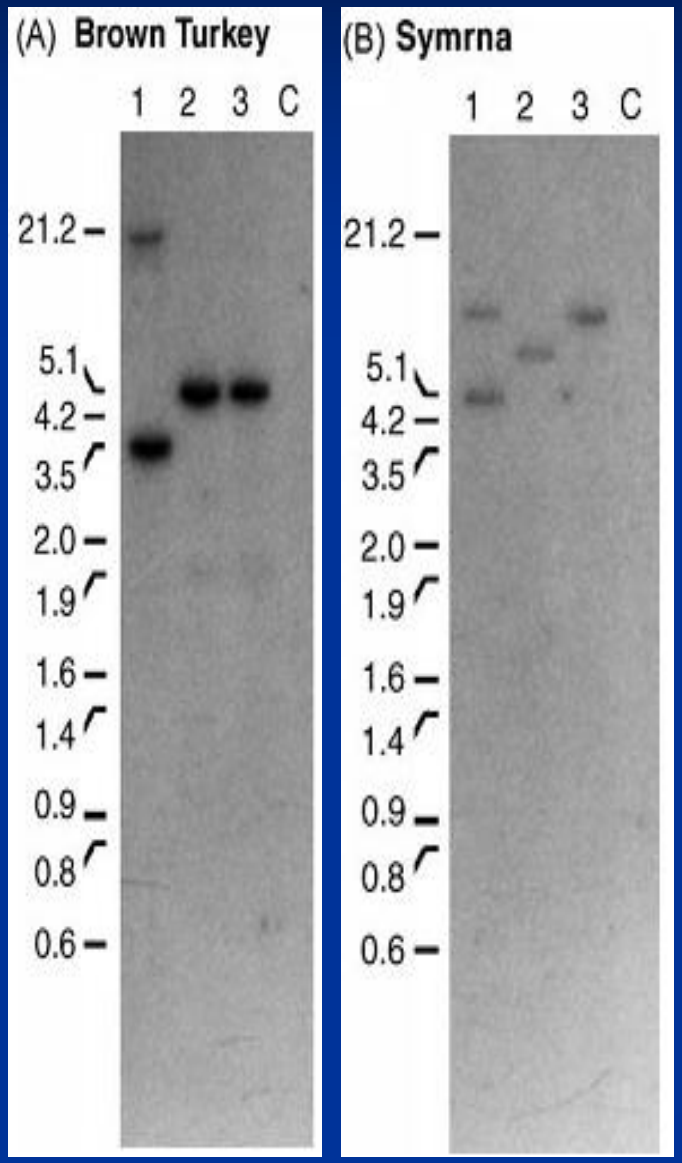


Molecular confirmation of transformation



PCR analysis of the putative transgenic shoots showed the predicted bands for the nptII gene and uidA-intron (GUS) gene in the cvs. Brown Turkey (A) and Smyrna (B).
For Brown Turkey (A): M – lambda DNA/EcoRI + HindIII marker; lanes 1–4, transgenic clones; C, untransformed plants.
For Smyrna (B): M – lambda DNA/EcoRI + HindIII marker; lanes 1–3, transgenic clones; C, untransformed plants.

Molecular confirmation of transformation



Demonstration of the T-DNA integration in the cvs. Brown Turkey (A) and Smyrna (B) genome by Southern blot analysis. Total DNA was digested with HindIII and hybridized with the nptII probe. Lanes 1–3, transgenic clones; C, untransformed plants.

CONCLUSION

- The present study describes for the first time the successful transformation of the commercially important fig cultivars Brown Turkey and Smyrna
- Transformation frequencies varied in Brown Turkey from 1.7 to 10.0% and in Smyrna from 2.1 to 7.8%.
- This technology should pave the way for the development of transgenic Ficus varieties with improved agronomic performance characteristics.
- It also provides a new experimental system for studying gene expression and function in these species, and a means for the production of foreign proteins in edible parts of *Ficus* species, such as the production of edible vaccines.

Breeding Problems and Limitations:

- GMO and Public acceptance - ?
- Lack of knowledge
- Potential risk

GENERAL CONCLUSION

- Traditional breeding methods require a long-term effort for improving traits as well as for production of new varieties.
- Biotechnology methods present a possibility for solving number of problems and overcome some limitations of the classical breeding.

Study for the TEST!