Plant Biotechnology

Summary

- **Introduction**
- **Plant tissue culture**
- **Applications of Plant Genetic Engineering**
  - Crop improvement
  - Herbicide resistance
  - Insect and virus resistance
  - Plants as bioreactors
- **Genetically Engineered Foods**
  - Public concern
- **Production of Pharmaceuticals Using Plants**
- **Regulation of Plant Biotechnology**

Introduction

- Biotechnology is being used as a tool to give plants new traits that benefit agricultural production, the environment, and human nutrition and health.
- The ability to move genes into plants from other organisms, thereby producing new proteins in the plant, has resulted in significant achievements in plant biotechnology that were not possible using traditional breeding practices.

Plant Biotechnology

- Increasing the efficiency of pulp production from trees
- Modifying fatty acids and oils for paints and manufacturing;
- Creating plastics from corn for use in consumer packaging;
- Introducing pigment-producing genes to make flowers bloom in colors not possible through other breeding methods;
- Producing spider silk from the milk of goats;
- Turning plants into biosensors that can detect or monitor hazardous materials in the environment; and
- Modifying turf grass to increase its tolerance to drought, salt and cold.

Introduction

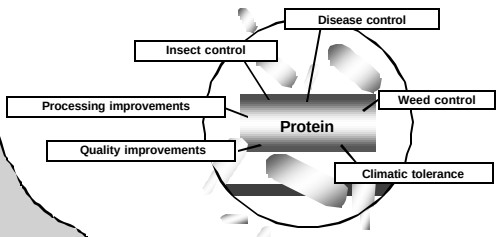
- Plant breeding
  - selection of superior plants
    - seeds from superior plants saved for planting next season, mass selection, line breeding, family breeding, etc.
  - genetic variability
    - germplasm collections
    - mutagenesis, polyploidy
    - sexual hybridization
- Plant biotechnology
  - provides powerful new tools to help in crop improvement
- Genetic modification of our domestic plants and animals has been practiced for 10,000 years

Biotechnology is an Extension of Traditional Plant Breeding

- Method of transferring genetic information to improve crops
  - more precise and efficient
  - not dependent on sexual plant reproduction
  - allows transfer of genetic information from one species to another
  - provides enhancements in plant quality, plant protection and yield

Genes Control Plant Traits

Genes code for production of proteins
Proteins influence specific plant characteristics

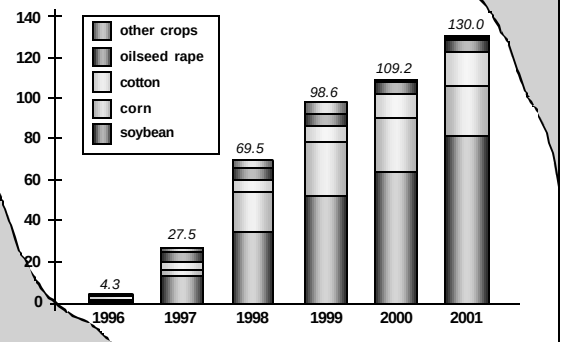


More than 50 biotech food products have been approved for commercial use in the United States

- Canola
- Corn
- Cotton
- Papaya
- Potato
- Soybeans
- Squash
- Sugarbeets
- Sweet corn
- Tomato

Products on the market

Biotech Global Plantings (Million Acres)



Plant tissue culture

- Regeneration of whole plants from cells or tissues, due to the **totipotency** ability of somatic plants cells.
- Cloning plant by micropropation
- Meristematic tissue
- Aseptic conditions

Plant tissue culture

Terminologies and Techniques

3. **Nutrient medium:** mixture of substances on/in which cells, tissues, or organ can grow.
- Inorganic nutrients
  - Macronutrients
    - N, P, K, Ca, Mg, Cl, Na
  - Micronutrients
    - Cu, Zn, Mn, Fe, Bo, Mo, Co, I
- Organic nutrients
  - Vitamins
  - Amino acids
  - Complex organic supplements
    - Coconut milk, yeast extract
- Growth regulators (hormone)
- Carbon source – sucrose
- With or without agar (semi-solid or liquid medium)

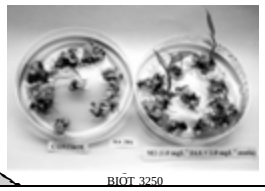
Plant tissue culture

- Expand the genetic variability that breeders can use
  - **somaclonal variation** - genetic variability via tissue culture
  - **embryo culture** - facilitates wide hybrid crosses
  - **somatic hybridization** - facilitates wide hybrid crosses
  - **regeneration of fertile plants** - for gene transformation

PLANT TISSUE CULTURE

1. Callus culture

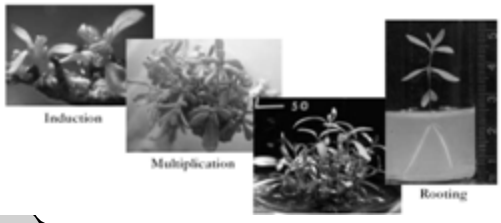
- creation of genetic variants via somaclonal variation and/or *in vitro* selection
- elimination of pathogens esp. viruses
- gene transfer (eg via *Agrobacterium*, microprojectiles)



Plant tissue culture
Growth regulators: phytohormones

- Auxins: a group of plant hormones (natural or synthetic) which induce cell elongation, or in some cases cell division. Often induce adventitious roots and inhibit adventitious shoot formation.
- Cytokinins: a group of plant hormones (natural or synthetic) which induce cell division and often adventitious shoots, and in most cases inhibit adventitious root formation.
- General rules for hormonal action:
  - Auxin : Cytokinin = ~1 • = Callus
  - Auxin : Cytokinin < 1 • = Shoot
  - Auxin : Cytokinin > 1 • = Root

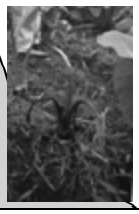
PLANT TISSUE CULTURE



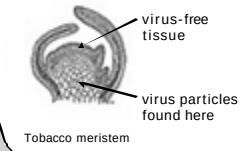
PLANT TISSUE CULTURE

2. Meristem culture

- clonal propagation
- elimination of pathogens
- germplasm collection and long-term storage (eg cryopreservation)



Virus elimination



Tobacco meristem

- why does the meristem have low/no virus titre?
  - virus spreads primarily through vascular system - this is not developed in the meristem
  - mitosis and chromosome replication compete with virus replication
  - high auxin in meristem inhibits virus replication
  - a virus "inactivating" system has greatest activity in meristem

Virus elimination

- virus elimination most useful for vegetatively reproduced species - banana, sugarcane, potato, strawberry, sweet potato
- chemotherapy and/or thermotherapy may also be used
- disease-free planting material may be rapidly reinfected by insect vectors, contaminated soil or implements
- the only long-term solution is **genetic resistance**

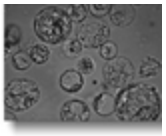
PLANT TISSUE CULTURE

3. Embryo culture
   - shortening breeding cycles
   - creating wide hybrids (interspecific/intergeneric)
   - haploid production via chromosome elimination

4. Anther/pollen/ovule culture
   - production of haploids for genetic analysis and shortening breeding programmes
   - creation of genetic variants via gametoclonal variation

PLANT TISSUE CULTURE

5. Protoplast culture
   - gene transfer
   - creating wide hybrids via somatic hybridization



Plant protoplasts

Transgenic Plant

Genomics Methods: DNA markers throughout the genome

- **RFLP**: restriction fragment length polymorphism
- **RAPD**: random amplified polymorphic DNA
- **STS**: sequence tagged sites
- **EST**: expressed sequence tags
- **SCAR**: sequence characterized amplified region

What traits have been engineered into plants?

Trait	Modified Plants	Gene Source
Insect resistance (Bt)	corn, cotton, potato, tomato	soil bacterium
Herbicide resistance	corn, soybeans, cotton, canola, sugarbeets, rice, flax	various bacteria, tobacco (modified)
Virus resistance	squash/zucchini, papaya, potato	plant viruses
Delayed fruit ripening	tomato	tomato, soil bacterium, or virus
Altered oil content	canola, soybeans	bay or soybeans
Pollen control	corn, chicory	soil bacterium

Structural Genomics Projects for Plants

Completed

Arabidopsis thaliana

In Progress:

Brassica napus
Hordeum vulgare
Glycine max
Gossypium hirsutum
Lotus japonicus
Medicago sativa
Oryza sativa
Phaseolus vulgaris
Pinus sp.
Populus sp.
Saccharum sp.
Solanum tuberosum
Sorghum bicolor
Triticum aestivum
Zea mays

http://wit.integratedgenomics.com/GOLD/eukaryagenomes.html

Transgenic Plant Methods

- Delivery of gene clone into target cells "transgene" or "gene of interest"
- Selection of transgenic cells "selectable marker gene"
- Regeneration of fertile transgenic plants
- Expression of gene clone in transgenic plants

Transgenic Plant Methods :Delivery of gene clone into target cells

- Biolistics – ("Gene Gun") gene transfer via particle bombardment

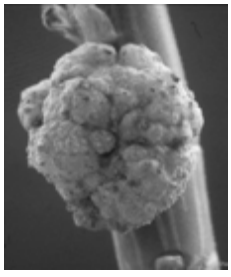
plastid gene transformation

nuclear gene transformation

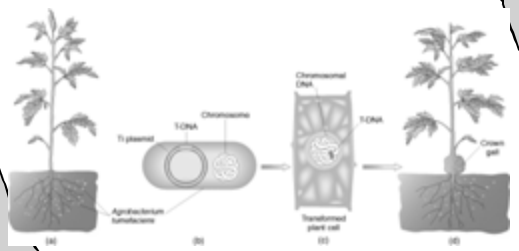
- *Agrobacterium tumefaciens* gene transfer nuclear gene transformation

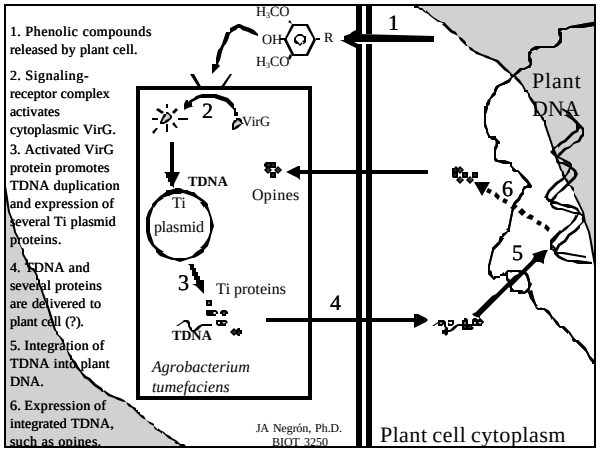
Agrobacterium tumefaciens

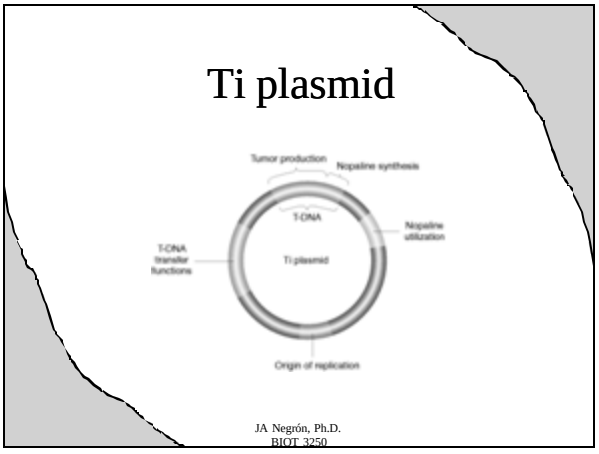
- *A. tumefaciens* is capable to transfer a particular DNA segment (T-DNA) of the tumour-inducing (Ti) plasmid into the nucleus of infected cells where it is subsequently stable integrated into the host genome and transcribed, causing the crown gall disease

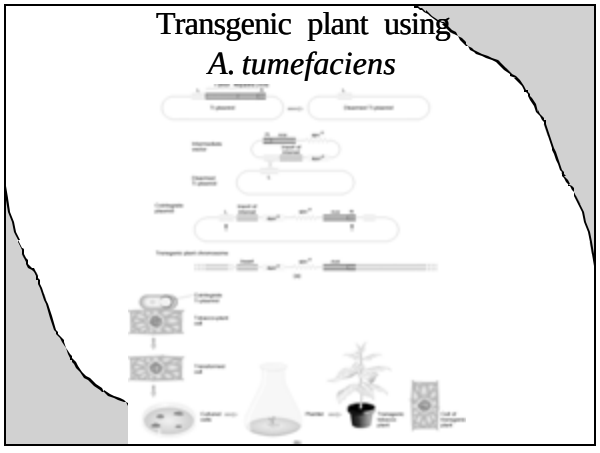


Ti plasmid vector from *Agrobacterium tumefaciens*









Recombinant *Agrobacterium* can be used to produce transgenic plant

- Clone gene in *E. coli*
- Gene clone transferred into *Agrobacterium*
- *Agrobacterium* transfers gene into plant cells

JA Negrón, Ph.D. BIOT 3250

Some commercial releases of transgenic plants

Crop and release date	Name	Company	Novel properties
Tomato (1994)	**Flavr Savr**	Calgene	Vine-ripened flavour, shelf life
Tomato (1995)		Zeneca	Consistency of tomato paste
Cotton Potato Maize (1996-97)	**Bollgard** **NewLeaf** **YieldGuard**	Monsanto	*Bacillus thuringiensis* toxin for insect resistance
Soybean Canola Cotton (1995-96)	**Roundup Ready**	Monsanto	Glyphosate herbicide resistance

Promoters for expression of nuclear transgenes

- Constitutive = continuous expression
  - CaMV 35S, actin, ubiquitin
- Developmentally regulated
  - alpha-amylase
- Chemically inducible
  - glutathione-S-transferase

Genes Control Plant Traits

Bt gene ⇨ Bt toxin expression ⇨ pest control

Bacillus thuringiensis *Transformed Plant* *Lepidopteran Control*

Bt crops

- **Pesticidal** crops are commonly called **Bt crops** because they contain a toxin produced by the bacterium *Bacillus thuringiensis* (Bt).
- **Bt corn** was designed to target the European corn borer, but it is also toxic to some butterflies and moths, including the monarch.

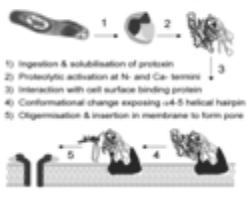
Bt corn- A Corn protected from corn borer damage, a GMO, which has bacterial gene transferred in the laboratory

Bt crops resist certain insects

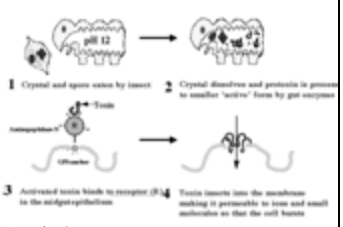
Cry gene designation	Toxic to these insect orders
CryIA(a), CryIA(b), CryIA(c)	Lepidoptera moths, like European corn borers, and butterflies
CryIB, CryIC, CryID	Lepidoptera
CryII	Lepidoptera Diptera flies, including mosquitoes
CryIII	Coleoptera beetles, like Colorado potato beetle and corn rootworm
CryIV	Diptera
CryV	Lepidoptera, Coleoptera

Bacillus thuringiensis

Proposed mode of action of Cry toxins



MECHANISM OF TOXIN ACTION



Constraints

- Potential of genetically modified organisms (GMOs) to cause unacceptable impacts on the environment.
- Possibility that the transgenic crops with new traits, such as pest or pathogen resistance, could gain weedy characteristics.
- Transfer of the GMOs trait by natural hybridization may produce hybrid progeny that are more aggressive or more difficult to control.

Monarch butterfly and Bt crops

- "**Bt**" is short for *Bacillus thuringiensis*, a soil bacterium whose spores contain a crystalline **(Cry)** protein.
- In the insect gut, the protein breaks down to release a toxin, known as **delta-endotoxin,** that causes insect death.

References on Bt issue

- **Transgenic pollen harms monarch larvae.**
  Losey, J. E. , Rayor, L. S. & Carter, M. E. (1999)
  ***Nature (London)* 399, 214**
- **Impact of Bt corn pollen on monarch butterfly populations: A risk assessment**
  PNAS -**October 9, 2001**-vol. 98-no. 21- **11937–11942**

Maine Food Co-ops Unite for a statewide Moratorium to

"Keep Maine Free From Genetically Engineered Crops"

"The main health concern with genetically modified (GM) food is that there's **little independent safety testing**." - *Friends of Earth, UK*

No genetic manipulation of nature!

"Consumers want real food and the right to know and to choose. Genetically engineered food must be segregated and labeled."

Other concerns:

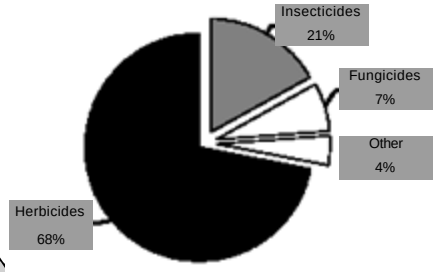
Unpredictable effects of GM

Who is in control?

– Greenpeace International

Pesticides

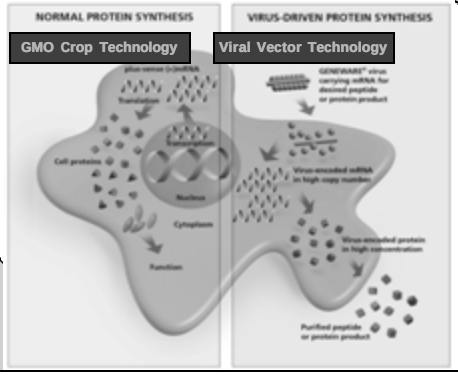
Crop protection pesticide sales in USA in 1997



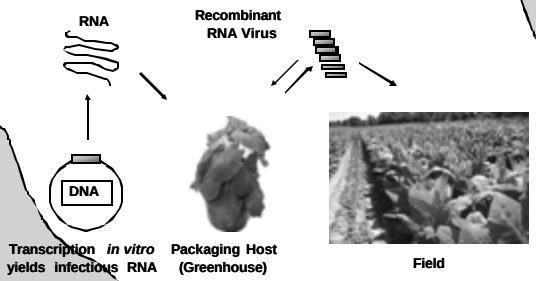
Production of Pharmaceuticals Using Plants

- VIRAL VECTOR vs TRANSGENIC PRODUCTION
  - EXAMPLES OF PRODUCTS OF VIRAL VECTORS
  - GFP
  - α-Galactosidase
  - scFv
  - Parvo Virus Vaccine

COMPARISON AT CELLULAR LEVEL



FIELD PRODUCTION USING VIRAL VECTORS

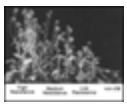


Examples of proteins produced in plants virus driven

GENE PRODUCT	MW (kD)	USE	Additional Information
Green Fluorescent Protein	18	Demonstration Marker	Plants Fluoresce Brightly
a-Galactosidase	50	Enzyme Therapy	Glycosylated, Complex
Single Chain Antibodies	30	Disease Therapy	Active; In Clinical Trials

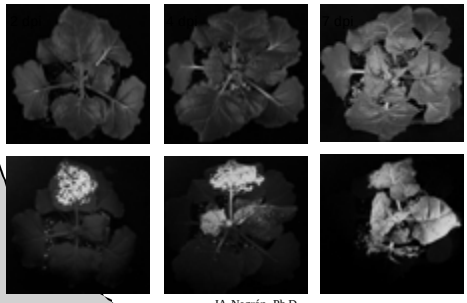
Phytoremediation: Using Plants To Clean Up The Environment

- Phytoremediation is the use of certain plants to clean up soil, sediment, and water contaminated with metals and/or organic contaminants such as crude oil, solvents, and polyaromatic hydrocarbons.

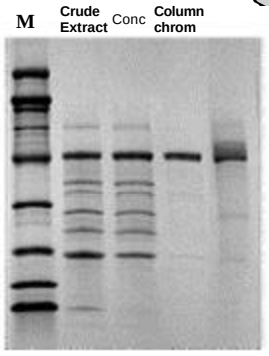




Application	Media	Contaminants	Typical Plants
1.Phytovolatization	Soil, groundwater, Landfill leachate, land application of wastewater	Herbicides (atrazine, alachlor); Aromatics (BTEX); Chlorinated aliphatics(TCE); Nutrients; Ammunition wastes(TNT,RDX)	Phreatophyte trees(poplar,willow, cottonwood,aspen); Grasses(rye, Bermuda, sorghum, fescue); Legumes (clover, alfalfa, cowpeas)
2.Microorganism stimulation	Soil, sediments, Land application of waste water	Organic contaminants(pesticides aromatic, and polynuclear aromatic hydrocarbons	Phenolics releasers(mulberry, apple,osage orange); Grasses with fribous roots(rye,fescue,bermuda); Aquatic plants for sediments
3.Phytostabilization	Soil, sediments	Metals(Pb,Cd,Zn,As,Cu,Cr, Se,U), Hydrophobic Organics(PAH,PCB,DDT,di eldrin)	Phreatophyte trees to transpire large amounts of water(hydraulic control); Grasses to stabalize soil erosion; Dense root systems are needed to sorb/bind contaminants
4.Phytoaccumulation/ extraction	Soil, Brownfields, sediments	Metals(Pb,Cd,Zn,As,Cu,Cr, Se,U) with EDTA addition for Pb Selenium	Sunflowers; Indian Mustard; Rape seed plants; Barle, Hops; Crucifers; Serpentine plants; Nettles, dandelions
5.Degradation	Soil, groundwater, Landfill leachate, land application of wastewater	Herbicides (atrazine, alachlor); Aromatics (BTEX); Chlorinated aliphatics(TCE); Nutrients; Ammunition	Phreatophyte trees(poplar,willow, cottonwood,aspen); Grasses(rye, Bermuda, sorghum, fescue); Legumes

VIRAL VECTOR GFP EXPRESSION



GFP from plants: purification profile

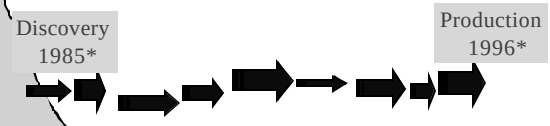


Regulating Plant Biotechnology

- U.S. government commissions NIH, USDA, EPA and FDA to secure food and environmental safety of biotechnology derived-products.
- These 4 organizations see that each biotech innovation goes through this 10 step process which takes $2-4 million and in completed in 7-8 years.
- USDA, EPA and FDA each have the authority to recall products from the food chain if new science-based information identifies a public or environmental health hazard.

Regulating Plant Biotechnology

10 Steps to Safety



* Actual discovery to production period for insect resistant Bt Cotton.

Steps

1. National Institutes of Health (NIH) Biosafety Review
2. USDA Greenhouse Approval
3. USDA Field Trial Authorization
4. USDA Authorization to Transport Seed
5. USDA Permission to Commercialize
   - Environmental Effects, Wildlife Effects, Weediness
6. EPA Experimental Use Permit
7. EPA Food Tolerance or Exemption
   - Product characterization, Allergenicity, Toxicology, Environmental Fate, Non-target Organisms, Potential Pest Resistance
8. FDA Review Process
9. EPA Product Registration
10. After Commercialization

References

- http://www.comm.cornell.edu/gmo/traits/herbres.html (herbicide resistance)
- http://www.comm.cornell.edu/gmo/traits/bt.html (insect resistance)
- http://www.comm.cornell.edu/gmo/traits/virusres.html (virus resistance)
- http://www.comm.cornell.edu/gmo/traits/fruitrip.html (delayed fruit ripening)
- http://www.comm.cornell.edu/gmo/traits/altoil.html (oil content)
- http://www.comm.cornell.edu/gmo/traits/polcont.html (pollent control)
- http://wit.integratedgenomics.com/GOLD/eukaryagenomes.html
- http://www.biotech.iastate.edu/publications/bt_curriculum/

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- *Scientific American, April 2001* – GM foods
- http://www.monsanto.com/
- http://biotech.dupont.com/