Plant Biotechnology

Plant Tissue Culture

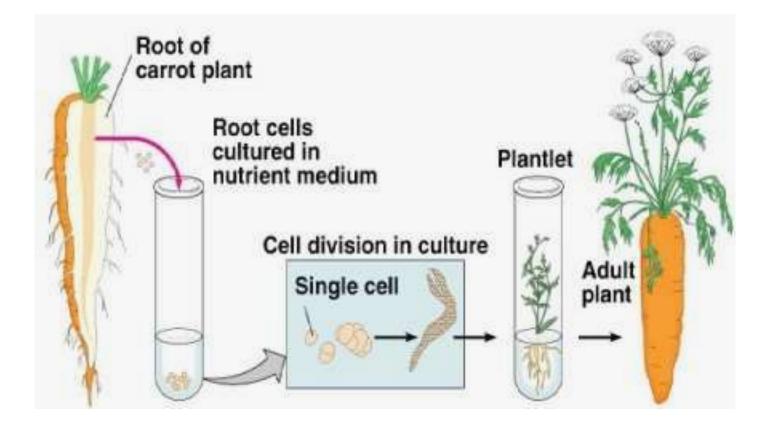
Plant cells differ from animals cells in that they are totipotent

A totipotent cell is one that can develop into specialized cell types & regenerate an entire organism

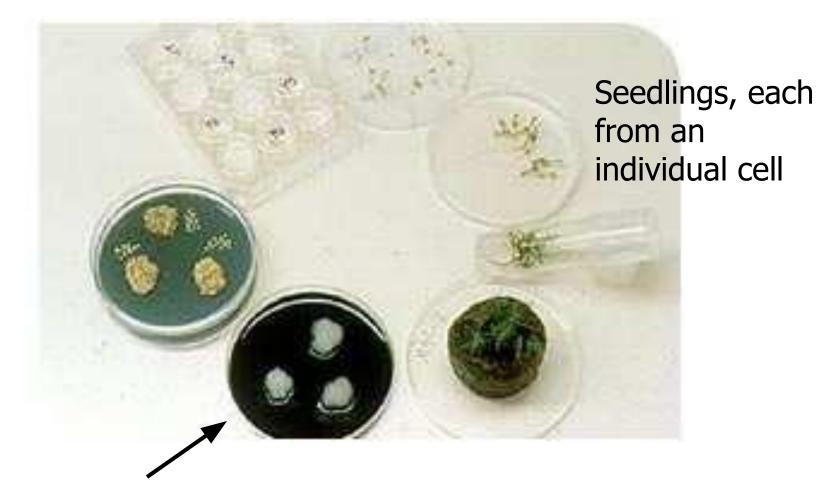
Tissue culture of plants and the regeneration of complete plants from cells has been done since 1930s

This allows large-scale **clonal** propagation of plants

Plant Cloning



Micropropagation



Callus, undifferentiated mass of plant cells

http://catf.bcresearch.com/biotechnology/tissueculture_research.htm

How Do They Engineer Plants?

Methods of producing transgenic plants





Plant Genetic Engineering

Ti plasmid of Agrobacterium tumefaciens

A. tumefaciens is a soil microbe that induces crown gall

Crown gall is a 'cancerous' mass which forms at the site of infection in plants



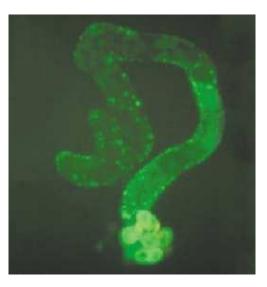
http://www.colostate.edu/programs/lifesciences/TransgenicCrops/how.html

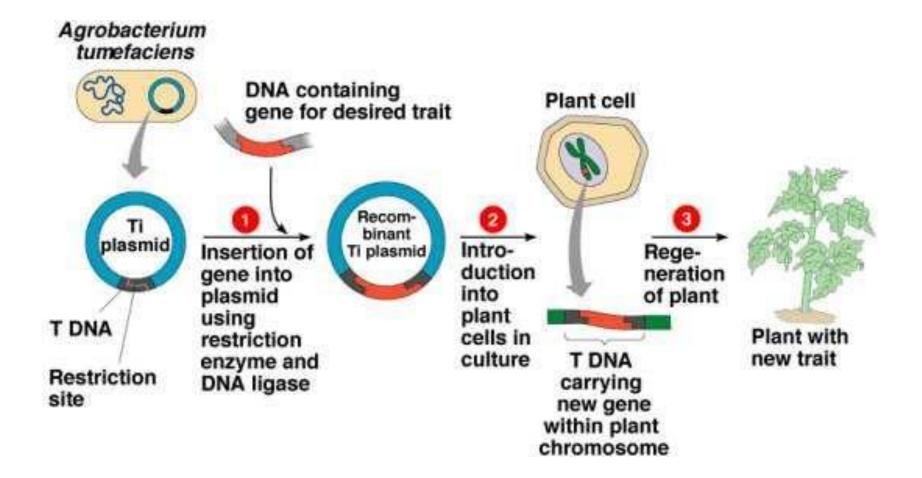
T-DNA portion of the Ti plasmid contains genes responsible for the disease

T-DNA becomes incorporated into the genome of the plant

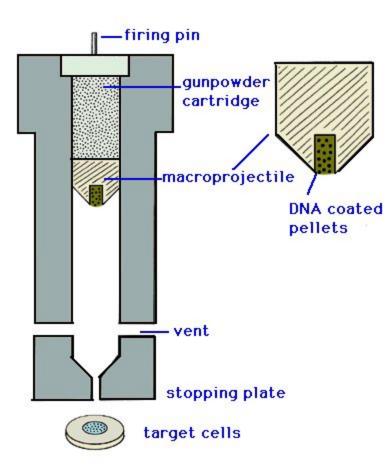
Part of the T-DNA may be replaced with a foreign gene and used to incorporate this gene into the plant's genome

A marker is also added to determine which cells have the recombinant gene





Biolistics (Biological Ballistics)



Useful for engineering corn, rice, wheat, barley, & other crops

Helios Gene Gun

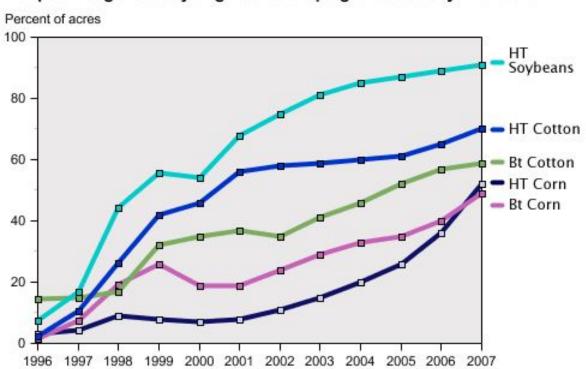


Uses an adjustable burst low-pressure helium to sweep DNA- or RNA-coated gold pellets from the inner wall of a small plastic cartridge directly onto a target

Genetically Engineered Plants

- Herbicide tolerance
- Insect resistance
- •Crop improvements
- •Functional foods
- Plants as bioreactors
- •Biofuels
- •Timber improvements
- Bioremediation

Genetically Modified (GM) Crops



Adoption of genetically engineered crops grows steadily in the U.S.

Note: Data for each crop category include varieties with both HT and Bt (stacked) traits. Source: 1996-1999 data are from Fernandez-Cornejo and McBride (2002). Data for 2000-07 are available in the ERS data product, Adoption of Genetically Engineered Crops in the U.S., tables 1-3.

Currently 215 million acres of GM crops grown worldwide

How Much of What We Eat Is GM?

- >60% of the foods we purchase have GM ingredients
- 95% of canola is biotech herbicide-tolerant
- 50% of corn is biotech herbicide-tolerant
- 35% of corn is biotech insect-resistant
- 61% of cotton is biotech herbicide-tolerant
- 52% of cotton is biotech insect-resistant
- 93% of soybean is biotech herbicide-tolerant

(2005 Data, Source :GM Crops: The First 10 Years -- Global Socio-economic and Environmental Impacts; PG Economics Limited)

Who Produces GM Food?

BASF Inc.

Aventis Cropscience

Bayer Cropscience

Syngenta Seed Inc.

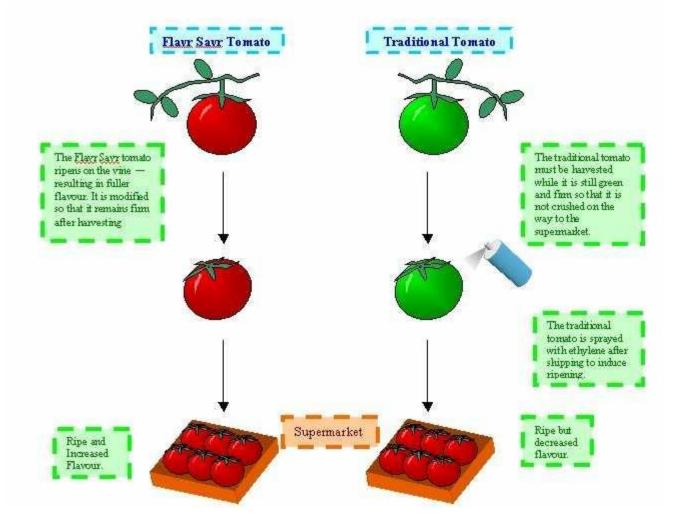
Pioneer Hi-Breed International Inc.

Dow Agroscience LLC

Monsanto Company

FLAVR SAVR, The First GM Food

The first GM food was the FLAVR SAVR tomato Introduced in 1994 it had delayed ripening characteristics



http://resources.emb.gov.hk/envir-ed/globalissue/images/ModifiedTomato.jpg

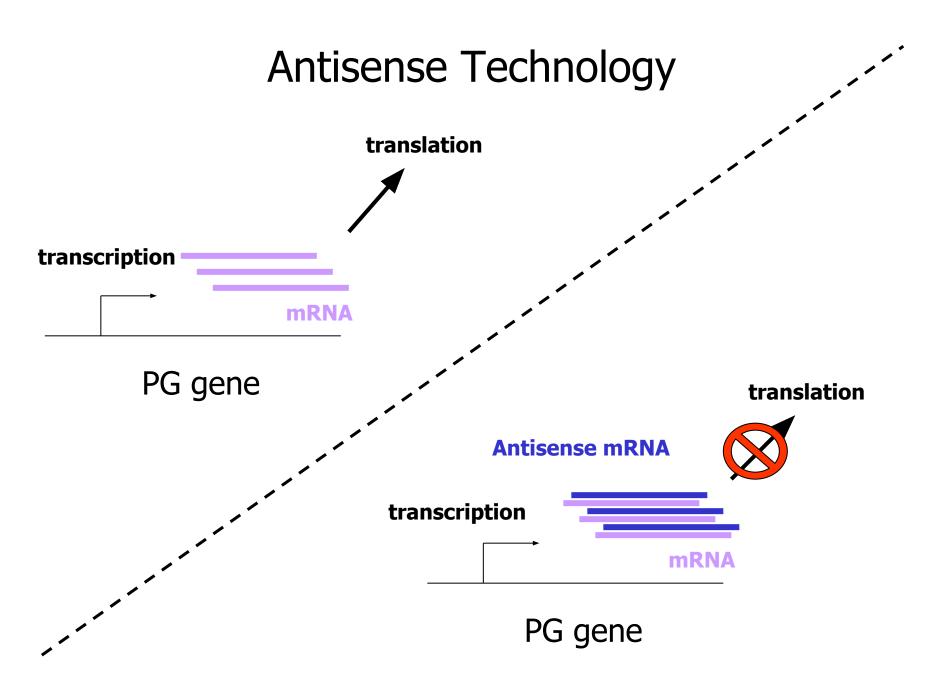
Fruit softens because polygalacturonase degrades pectin

Antisense technology was used to turn off (silence) the polygalacturonase (PG) gene

Gene encoding antisense RNA was inserted into tomato cells

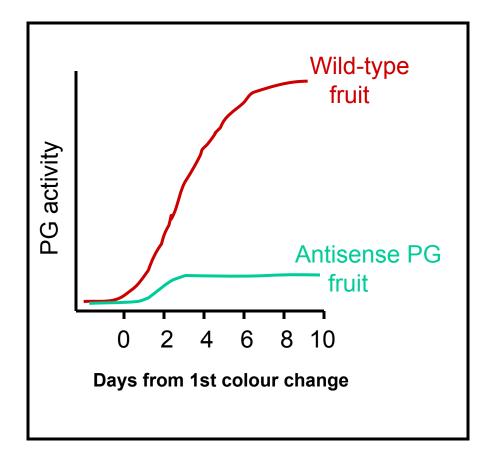
The antisense RNA finds the normal RNA and hybridizes

The cell then degrades this complex, preventing the normal RNA from being translated



Altering Fruit Ripening with Antisense RNA

Polygalacturonase (PG) is an enzyme that breaks down pectin in ripening fruit walls



Plants with an antisense PG transgene produce less PG. Walls soften more slowly

Many genes manipulated in the same way to answer basic questions:

- what is the role of hormones in ripening?
- what do particular enzymes do in fruit walls?



Most GM tomatoes were used only in canned puree By 1997 FlavrSavr was no longer marketed

http://www.wachstumshormon.info/kontrovers/gentechnik/flavrsavr.html?gfx=2

Current technologies aid the farmer not the consumer

- •Herbicide Resistance
- •Virus Resistance
- Insect Resistance

Herbicide Tolerance

Methods used to promote crop growth also promote weeds Weeds often outgrow crops and reduce farm output Even though there are about 100 chemical herbicides, weeds still reduce crop productivity by ~12% Problem is that many herbicides kill both crops & weeds This has led to the creation of herbicide tolerant crops

Herbicide Tolerance

Herbicides are used for weed control



Weeds drastically reduce crop yield and quality



Soybean with no

Soybean after

Roundup - Glyphosphate

Herbicide - chemical structure:

но-с-сн₂-N-сн₂-Р-он

Mode of action: blocks synthesis of certain amino acids (aromatic amino acids produced by the shikimic acid pathway)

 \rightarrow Toxic to most plants, but not to animals

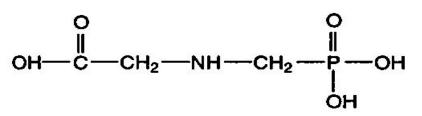
Note: can still be toxic to animals, not just the active chemical but other components of the formulation

Monsanto Chemical Company – major moneymaker – while under patent protection

Non-selective herbicides (Roundup Ultra and)

Roundup[®] (chemical name: glyphosate)

GLYPHOSATE (Roundup®)



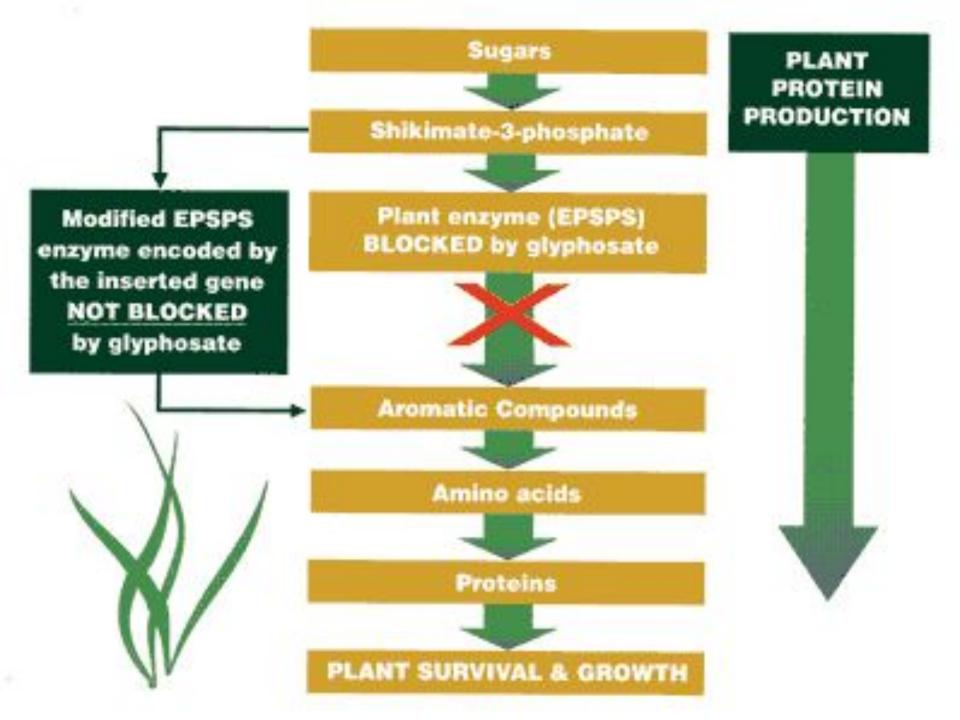
N-(phosphonomethyl)glycine (isopropylamine salt Breaks down quickly in the soil, eliminating residual carry-over problems

and

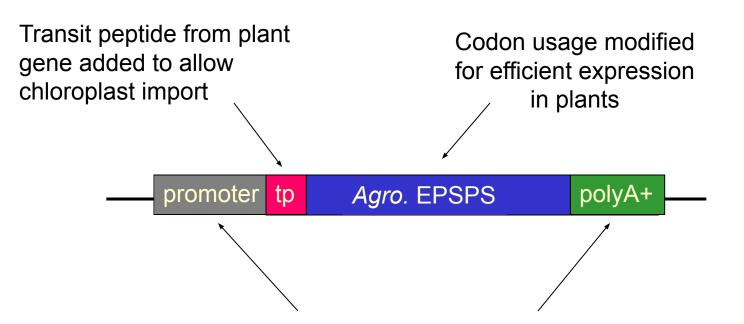
reducing environmental impact.

Roundup Ready®

transgenic varieties of common crops completely resistant to those herbicides

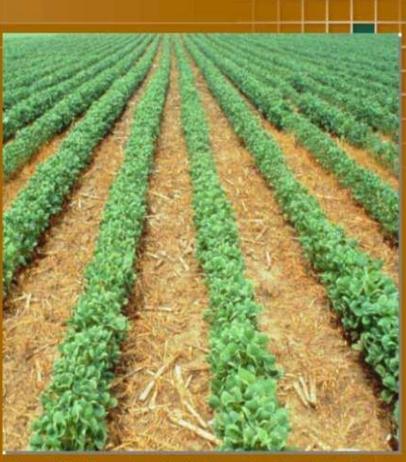


EPSPS Transgene Introduced into Plants



Regulatory sequences recognised by plant (either from plant gene or plant virus gene). In this case 35S CaMV promoter





Unmatched weed control in Roundup Ready Soybeans



MONSANTO

Benefits of Glyphosate Tolerance in Crops

Can use at any time

- can wait until there is a problem

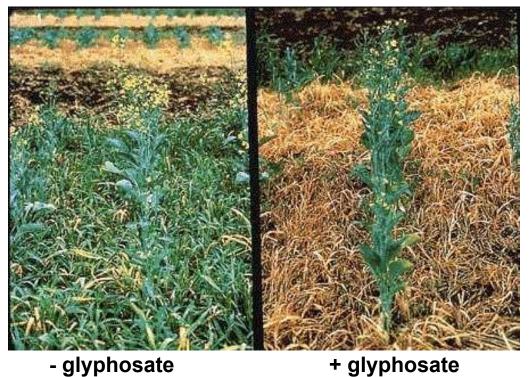
Very effective

- Weeds very sensitive
- GM crop very resistant

Move to greener herbicide

Reduced herbicide use

GM canola surrounded by weeds



Roundup[®] Ready Crops Corn Alfalfa Soybeans Canola Sorghum Cotton Tomato Potato Wheat

1996 Roundup® Ready Gene Agreement

Terms:

- The farmer must pay a \$5 per bag "technology fee"
- The farmer must give Monsanto the right to inspect, monitor and test his/her fields for up to 3 years
- \bullet The farmer must use only Monsanto's brand of the glyphosate herbicide it calls ${\rm Roundup}^{\rm (R)}$

- The farmer must give up his/her right to save and replant the patented seed (replanting seed is a practice as old as agriculture)
- The farmer must agree not to sell or otherwise supply the seed to "any other person or entity."
- The farmer must also agree, in writing, to pay Monsanto "...100 times the then applicable fee for the Roundup[®] Ready gene, times the number of units of transferred seed, plus reasonable attorney's fees and expenses..." should he violate any portion of the agreement.

Concerns of Roundup® Ready Crops Spread of resistance genes to weeds Problems with quality of crops e.g.. Cotton bolls falling off prior to harvest Farmers required to purchase seed annually The herbicide is still toxic at high doses Benefits of Roundup[®] Ready Crops Fields no longer need tilling Reduction in weed management costs of up to 37% Decrease in herbicide use by >1lb/acre Overall 74% increase in farmer profits¹ Roundup[®] patent recently expired

Researchers have designed new method of resistance

Sorted thru 100s of microbes to find a detoxifying enzyme

Found 3 genes in *Bacillus licheniformis* which encode glyphosate N-acetyltransferase (GAT)

Using directed evolution generated an enzyme 10000x more efficient

~5yrs to market

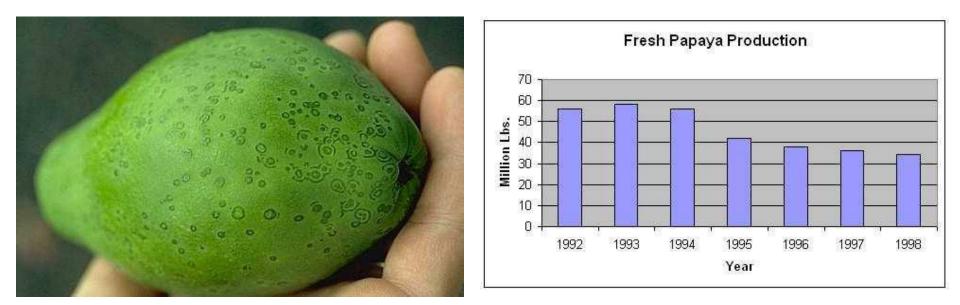
There are also varieties of various crops resistant to: Glufosinate Bromoxyil Sulfonylurea

Virus Resistance

- Yellow Squash resistant to three different viruses were developed by Asgrow Seed
- Resistance was then transferred to zucchini
- Virus resistant papaya were developed in the mid '90s
- This was after a outbreak of papaya ring spot virus destroyed 40% of the Hawaiian crop
- The varieties called Rainbow $^{\ensuremath{\mathbb{R}}}$ & SunUp $^{\ensuremath{\mathbb{R}}}$ are provided free to farmers

Increased virus resistance: Papaya ringspot virus (PRV)

Virus has had huge impact on papaya industry in Hawaii - reduction of fresh fruit production directly related to spread of PRV



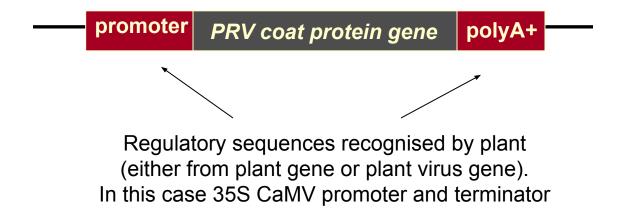
No naturally occurring resistance genes - without GM, papaya industry in Hawaii would be destroyed

Transgenic PRV-resistant papaya has been grown commercially in Hawaii since 1996

Papaya Resistant to PRV

PRV coat protein gene expressed from 35S CaMV promoter.

Papaya transformed by particle bombardment



Confers partial resistance to PRV in one variety ('Rainbow') and complete resistance in another ('SunUp')

Growers have to sign up to careful crop management - minimize virus pressure on transgenics to maintain resistance

Insect Resistance

Various Cry genes (CryIA(b), CryIA(c), & Cry9C) have been inserted crops such as corn, cotton, potatoes, & rice

- Pest must ingest a portion of the plant for the toxin to be effective
- Within hours the gut breaks down and the pest dies

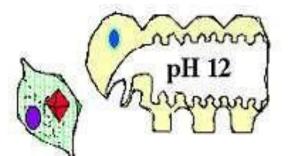


Wt Corn

Bt Corn

What is BT doplnit z prednasky roslinna biotechnologie

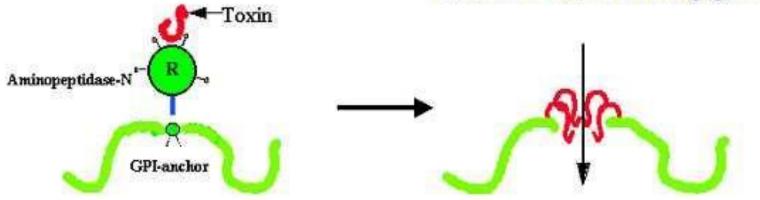
Molecular basis of the Bt action

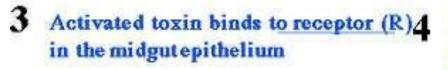


Crystal and spore eaten by insect



2 Crystal dissolves and protoxin is processo to smaller 'active' form by gut enzymes





Toxin inserts into the membrane making it permeable to ions and small molecules so that the cell bursts

Bt Corn & Monarch Butterflies



Cry toxin is expressed in all of the plant as well as pollen

- Corn pollen can blow onto milkweed growing near corn fields
- Monarch caterpillars feed exclusively on milkweed
- An early study showed a possible toxic effect of Bt pollen on monarch caterpillars





 $http://homepages.ihug.co.nz/\sim mostert/land\% 20 photography/Insects/insects/monarch\% 20 butterfly.jpg$

Biodiversity / NTO Studies



 Monarch Butterfly, symbol of nature and "wildness" in North America. The reports of Bt effects on Monarch butterflies have fueled much emotional debate on the use of biotech erops.

Potential Concerns

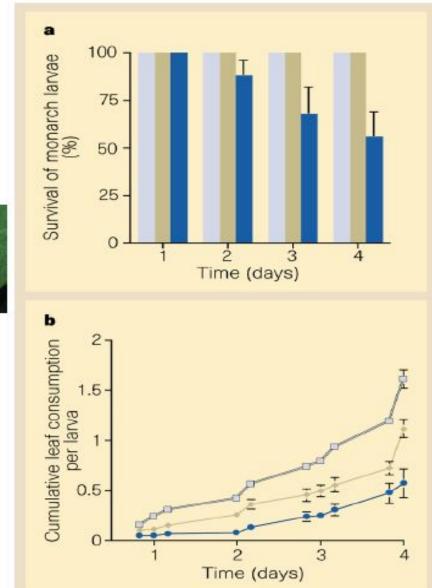
- Effect on target organisms
 - Development of resistance
- Effect on non-target organisms
 - Lack of resistance
- Escape of transgenes from transgenic cultivars to other cultivars or to wild relatives
 - Reduction in genetic diversity
 - Development of resistance

Effect on Bystanders?

 Monarch butterfly studies



- Losey et al. 1999
 - Pollen sprinkled on milkweed leaves
 - Criticisms:
 - pollen dosage?
 - lab study



Effects of Bt pollen: Lethal doses vs. Actual doses (Hellmich et al. 2001; Pleasants et al. 2001; Stanley-Horn et al. 2001)

- Lethal doses: linked to expression levels/events
 - Bt11, MON810 and TC1507
 - > 1,000 grains/cm²
 - 176
 - ≈ 10 grains/cm²
- Actual doses:

Average poll	en density lev	els on milkw	eed leaves (/	cm²)
Within field	0 m from edge	1 m from edge	2 m from edge	4-5 m from edge
161	63	35	14	8

Unanswered concerns about Bt crops (Scriber 2001)

- Long-term resistance management (refugia)
- Fate of Bt in the environment
 - Aerial sprays against gypsy moth: 4-6 weeks effect on non-target
- Escape of transgenes into wild relatives
- Effects of sublethal levels of Bt toxin
- Multitrophic interactions; ecosystem level interaction

Effect of pesticides

(Pimentel and Raven 2000)

- 115 x 10⁶ kg/yr of pesticides on corn:
 - corn rootworm: 45% yield reduction; other control: rotation
 - European corn borer: 20% yield reduction; difficult to control when inside the plant --> Bt
- Effect of pesticides:
 - pesticide poisonings, cancer
 - 35% of food samples have pesticide residues
 - a 70 x 10⁶ birds/yr killed
 - 10⁹s of insects, including beneficials: pollination, biological control
- Reasons for decline of monarch: insecticide use, loss of habitat

Bt Corn & Allergies

- Bt corn is approved for human consumption
- However Starlink[®] brand corn is approved *only* for animal feed
- Contains Cry9C which may be a potential allergen
- In 2000 Starlink[®] was found in Taco Bell-brand taco shells
- EPA determined that no one who ate the food was allergic, but they found that it had a moderate potential allergenicity
- All the products were recalled and Starlink[®] corn is not approved for human consumption

Benefits of Bt Corn Crop yield increases by up to 33% 39% less insecticide used Increase in monetary gains by 18%¹

Concerns associated with GM crops

1. Possible production of allergenic or toxic proteins not native to the crop

2. Adverse effects on non-target organisms, especially pollinators and biological control organisms

3. Loss of biodiversity

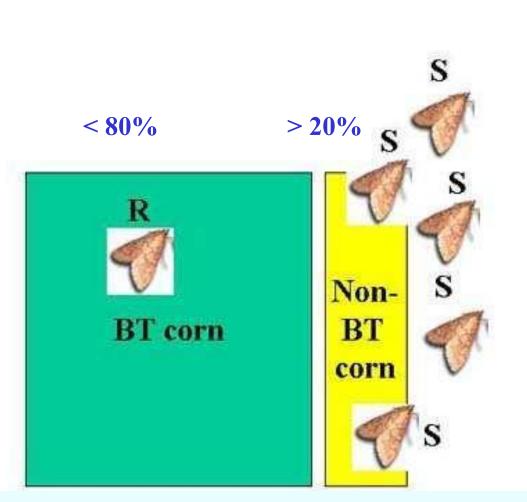
4. Genetic pollution (unwanted transfer of genes to other species)

5. Development of pest resistance

6. Global concentration of economic power and food production

7. Lack of "right-to-know" (i.e., a desire for labeling transgenic foods)

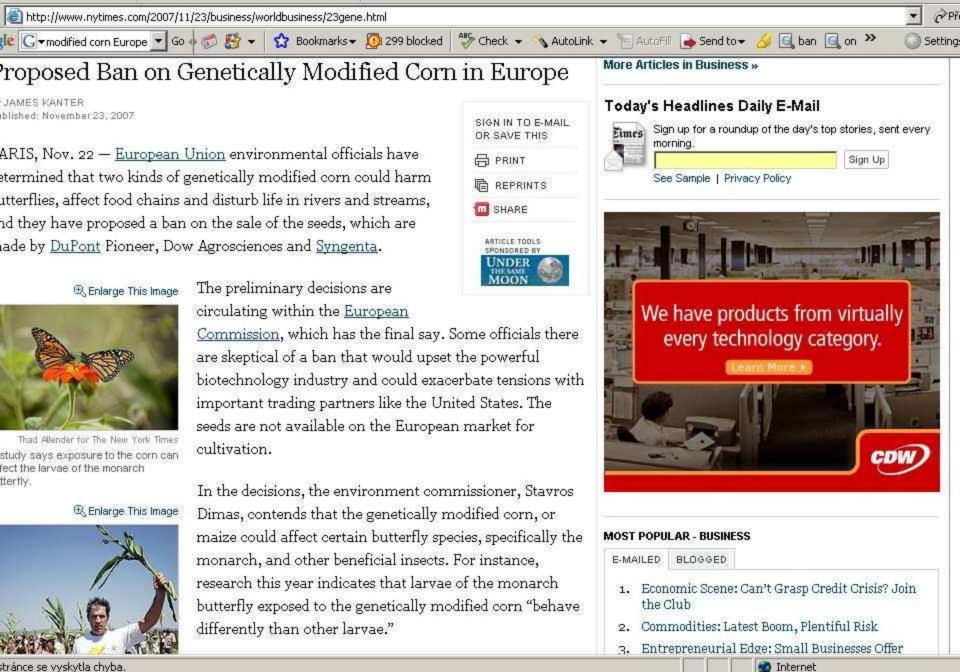
How to prevent development of Bt resistance in insects?



Strategy will not work if resistance is dominant !!!

at least 20% of a farm's corn acreage must be planted to non-BT corn. R = resistant European borer; S = susceptible borer.

few Bt-resistant insects surviving in the Bt field would likely mate with susceptible individuals that have matured in the non-Bt refuge. Thus, the resistance alleles would be swamped by the susceptible alleles.



stránce se vyskytla chyba

Microso...

rt

Microso... S Doruče... Don Se... Propos... 🔁 Najît zp...

AMB

(e) http://...

CS O.

Crop Improvements

Current research into crop improvements include:

- Increased growth rate
- Increased salt tolerance
- Increased drought resistance
- Modification of seed oil content

Drought / Salinity Resistance

Trehalose is a protectant against many environmental stresses; freezing, osmotic pressure (salinity), heat and dessication.

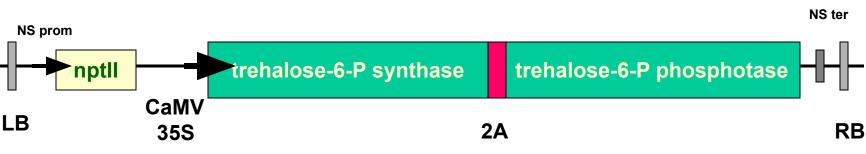
Trehalose (1- α -D-glucopyranosyl-glucopyranoside) is synthesised in a two-step process in yeast.

Zygosaccharomyces rouxii is one of the most highly osmo-tolerant yeasts – especially to salt

Kwon, S.J., Hwang, E.W. & Kwon, H.B. (2004). Genetic engineering of drought resistant potato plants by co-introduction of genes encoding trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase of *Zygosaccharomyces rouxii*. *Korean J. Genet.* <u>26</u>, 199-206.



Figure 4. Drought stress tolerance of ZrTPS2-2A-ZrTPS1 transgenic plants. One month old plants rooted in the soil mixture were not watered for 3 weeks. (A) is the empty vector transgenic control plant, and (B~D) are ZrTPS2-2A-ZrTPS1 transgenic lines.



Transgenic potatoes morphologically identical to parents.

Modification of Seed Oil Content

Plant Seed Oils

Oils either for cooking or industrial uses are usually extracted from seeds such as,

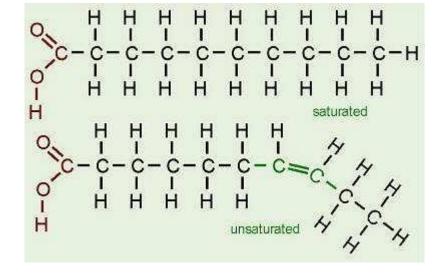
Corn, safflower, sunflower, canola, coconut, flax

Seed rely on their stored oil as an energy & carbon sources for germination

Most seed oils are made up of unsaturated fatty acids

Some tropical oils such as palm & coconut have significant levels of saturated fatty acids

Fatty Acids



Triglyceride

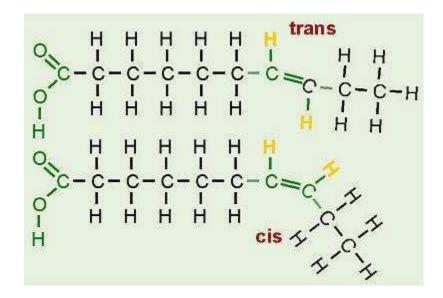


Canola Oil -10°CCoconut Oil 20-25°CLard 40.5°C5% Saturated85% Saturated100% Saturated

http://food.oregonstate.edu/images/fat/lard1.jpg http://www.plattsalat.de/Gawang.html http://www.aces.edu/dept/extcomm/newspaper/feb23b01.html Cis-fatty acids, naturally occuring unsaturated acids

Trans-fatty acids, artifically generated to keep unsaturated fatty acids from going rancid

Trans-fatty acids increase LDL (bad cholesterol) increasing the risk of heart disease

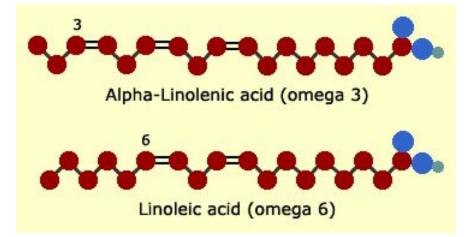


Soybeans with Modified Oil Content

Soybeans normally have high amounts of the unsaturated fatty acid linolenic acid

Vistive by Monsanto is Roundup Ready soybeans bred to have low amounts of linolenic acid

This eliminates the need to hydrogenate the oil



Biotechnology...what's down the road

Healthier soybean oil
Improved flavor
Improved digestibility



The cloudy hydrogenated soybean oil on the left contains unhealthy trans-fatty acids while the clear 1% linolenic soybean oil on the right contains no trans-fatty acids. "Food manufacturers Bask scrambling to eliminate trans fat from their products" Harvard Health Watch

Market Need

Trans fat labeling on foods mandated by FDA in 2006

Features

- Linolenic acid < 3%</p>
- A more stable soy oil requiring less hydrogenation: reduce/eliminate trans fats
- Better flavor profile
- Full agronomic and yield performance

Product Linolenic 18:3 Linoleic Oleic Sats Std Soybean Low Lin Soy

Technical Status

- 2005 prototype testing
- 2006 commercial launch

Likely Uses

 Substitute for hydrogenated oil and blends for low trans solutions
MONSANTO Over 60 million tons of seed oil are used for edible purposes

About 15 million tons is employed in industrial usage

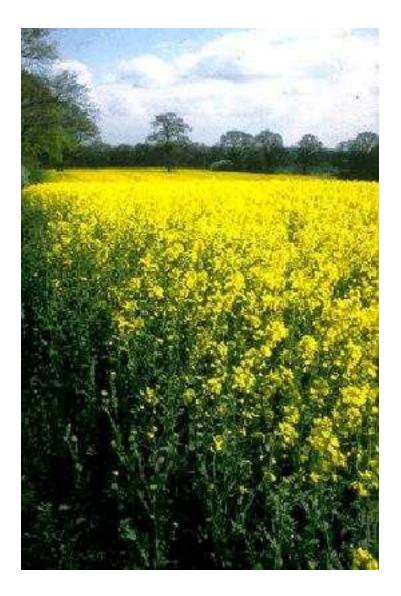
One of the first plants modified was Rape (*Brassica napus* oleifera)

A member of the mustard family, Rape has been grown for centuries as animal feed and natural lubricant

In the 1970s selective breeding led to strains of Rape lacking erucic acid

The removal of erucic acid made the oil fit for human consumption, LEAR (low-erucic acid rapeseed)

Rape field full of brillantly yellow flowers



http://www.tiscali.co.uk/reference/encyclopaedia/hutchinson/m0011652.html

The first transgenic crop with modified oil content was a high lauric oil rapeseed.

Rapeseed oil normally contains about 0.1% lauric acid

Modified rapeseed contains ~40% lauric acid

Lauric acid from GM rapeseed would be used in place of oils from palm or coconut

The primary use of lauric acid is in detergents

Sodium Lauryl Sulfate a.k.a SDS

Other varieties of GM rapeseed could provide:

- Steric acid as a substitute for hydrogenated oils
- Jojoba waxes for use in cosmetics and lubricants
- Various acids for use in biodiesel
- Phytases for animal feed
- Novel peptides for pharmaceuticals

Why is rapeseed so versatile?

One reason is because it is related to...

Arabidopsis thaliana (Cress)



Arabidopsis is a model organism used by scientists to investigate plant development and genomics

The Arabidopsis genome was recently completed

Brassica (Mustard) Family



Nutritional Enhancement

Improving Protein Quality

Animals and humans are incapable of making 10 'essential' amino acids - must obtain in diet

Nutritional value of seed storage proteins is often limited - may lack one or more amino acid essential to human health e.g. legume seeds lack cysteine and methionine; other seeds can lack lysine

Amino acid balance in seeds has been manipulated in laboratory experiments using a number of strategies:

- introduce seed storage protein from another species
- alter sequence of seed storage protein gene in vitro

- manipulate amino acid biosynthetic pathway to increase abundance of particular amino acids

Similar strategies have been used to improve protein content and composition in non-seed food crops...

'Increased nutritive value of transgenic potato by expressing a nonallergenic seed albumin gene from Amaranthus hypochondriacus' Chakraborty et al., PNAS 97, 3724-3729 (2000)

Potato is the fourth most abundant global crop and used for food, animal feed and production of starch and alcohol

Limited in lysine, tyrosine, methionine and cysteine

Transformed potato with seed albumin from *Amaranthus hypochondriacus* which has good amino acid balance

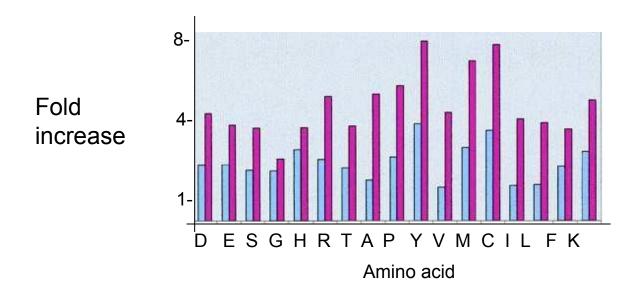


Expression in tuber 5-10 fold higher with GBSS promoter than with 35S promoter

Changes in protein quality in Amaranthus albumin potatoes

5-8 fold higher essential amino acids in pSB8G transgenics

Total protein content also increased (35-45%)



Golden Rice

Inserted genes from other plants & bacteria to produce β -carotene

Vitamin A deficiencies affect >124 million children worldwide

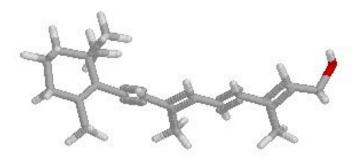


 $http://www.princeton.edu/{\sim}fecelik/GMFoods/impactshumanconsumptionpros.html$

Vitamin A

Vitamin A (retinol) is essential to human growth

Our bodies cannot make vitamin A,



All carotenoids that contain a β -ring can be converted into retinol, and one of the most important carotenoid pro-vitamins is β -carotene

 β -carotene is a pigment required for photosynthesis

- produced in all plant green tissues

Vitamin A deficiency

400 million people are at risk of vitamin A deficiency (VAD), particularly in Asia and Africa

implicated in up to 2.5 million deaths annually in children under 5



VAD makes children especially vulnerable to infections

0.5 million children go blind each year because of VAD

Supplementation programmes have reduced child mortality by up to 50% in target areas

supplementation not universal; expensive; misses remote areas

VAD is most serious in regions where rice is the staple food ; up to 70% children under 5 affected



Vaccine Foods

In the early 1990's tomatoes, bananas, & potatoes were proposed as delivery vehicles for vaccines

Touted as a simple method of delivering vaccines especially to developing countries

Studies have shown plant-produced oral vaccines to increase immunity in mice

Potatoes containing Hepatitis B vaccine have been shown to boost immunity in humans There are concerns about dosing when these crops are directly consumed.

Would a dose be? 2 bananas and a tomato

What if a person eats too many vaccine potatoes?

Also there is concern if the vaccine foods enter the food supply of people who are vaccinated the traditional way

Plants as Bioreactors

- Plants (crops or cell culture) can be used to produce proteins currently produced by microbes or animal cells
- The advantage over microbes:
- The proteins are more like human proteins
- The advantage over animal cells:
- Plants cannot become contaminated with mammalian pathogens

Large Scale Biology Corp. (LSBC) uses tobacco plants for drug manufacturing

LSBC uses an engineered tobacco mosaic virus (TMV)

Recombinant gene is inserted into TMV which infects & replicates in the plants

During replication large amounts of the drug are generated

The drug accumulates in the leaves which are harvested

a-galactosidase A purified from tobacco is as effective in treating Fabry's disease as the animal cell derived drug

More and More Plants Are Being Used to Produce Proteins

- •USDA just approved the use of rice to produce lactoferrin and lysozyme
- •Sigma-Aldrich now sells aprotinin and typsin made in tobacco
- •Duckweed is being used to produce interferon-a

Plant BioFuels

Cellulosic Ethanol

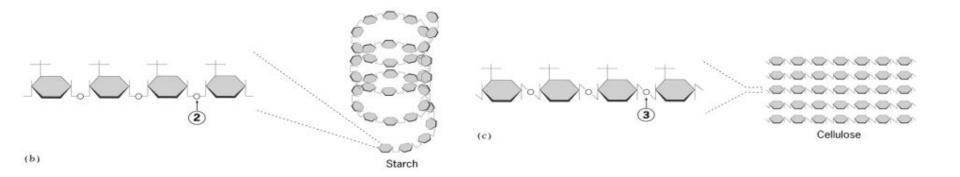
Has higher yield due to the fermentation of sugar released from cellulose

Requires the addition of cellulase or acid

Agricultural plant wastes (corn stover, cereal straws)

Plant wastes from industrial processes (sawdust, paper pulp)

Crops grown specifically for fuel production (switchgrass)

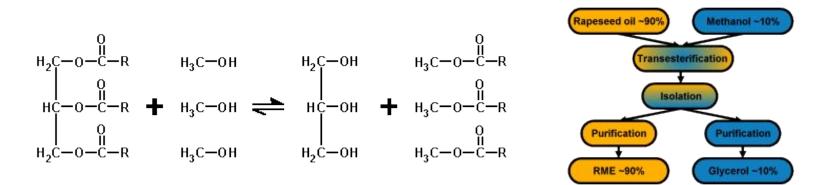


BioDiesel

1900 Rudolph Diesel runs his engine on peanut oil

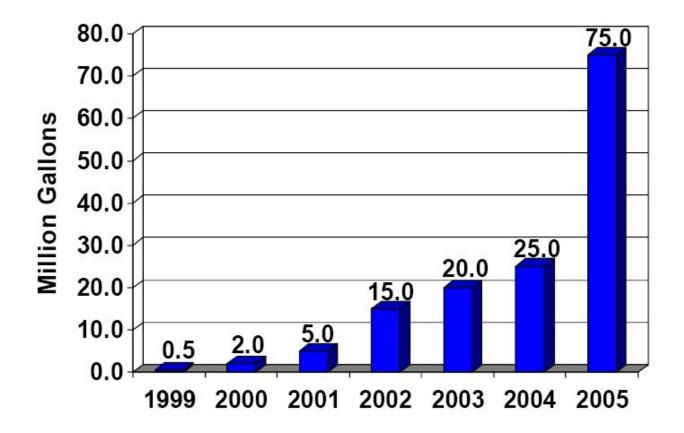
Biodiesel is defined as "a fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats"

Transesterification converts triglycerides into methyl esters of fatty acids



http://www.campa-biodiesel.de/caengnof/caenkra2.htm

Estimated US Biodiesel Production



U.S. consumes 40 billion gallons of diesel/yr

http://www.biodiesel.org/pdf_files/fuelfactsheets/Production_Graph_Slide.pdf

Timber Biotechnology



http://www.wired.com/wired/archive/11.04/genetics.html?pg=1&topic_set=

Reduction in generation time

Trees can take years to flower

- Those overexpressing the *LEAFY* (*LFY*) gene can flower in as little as 7 months.
- This is of particular value in fruit bearing trees
- Also allows for rapid analysis mature traits

Alteration in tree size or form

Altered expression of a gene involved in hormone synthesis can give wildly differing results

Overexpression of GA 20-oxidase gives faster growing trees both in height and diameter and longer wood fibers

Inhibition of GA 20-oxidase gives dwarf trees

Six Weeks Old Hybrid Aspen



From left to right:

antisense-GA 20-oxidase, wild type, & GA 20-oxidase overexpressing

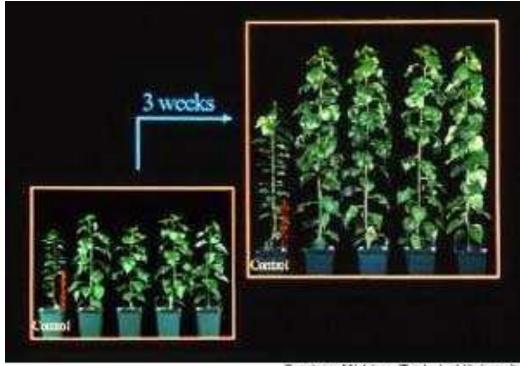
www.upsc.se/tmoritz.htm



Leaves from GM poplar

http://stacks.msnbc.com/news/947076.asp?0cl=cr&cp1=1

Trees engineered to produce lower amounts of lignin These trees grow faster and have greater cellulose content Lignin is a glue-like compound that must be chemically removed from pulp prior to papermaking



Courtersy Michigan Technical University

Bioremediation

Bioremediation using bacteria has limitations

The engineered or specialized bacteria used are often unable to compete with indigenous soil bacteria

An alternative is **phytoremediation**, the use of plants to mop up toxic waste

A standard technique for treating soils contaminated with heavy metals (lead or cadmium), or organic pollutants (pesticides) in a cost-effective way









NO FOOD SHALL BE GROWN THAT WE DON'T OWN

MONSANTO

