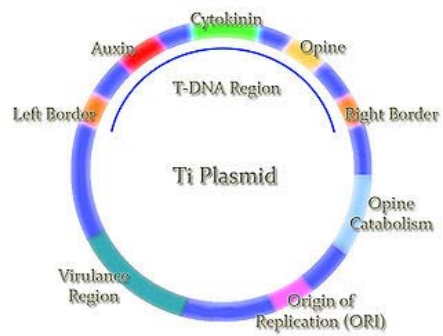


Genetic Engineering of Plants – lecture #2



Roman Sobotka

The next generation of GM crops

- **Modification of plant nutritional content** (vitamins, iron, protein/oil quality...)
- **Improvement of plant quality for industry** (starch, oil, cellulose, lignin...)
- **Resistance against biotic and abiotic stress** (viruses, salinity, drought ...)
- **Phytoremediation** (sequestering of hazardous substances from the environment)
- **Modification of taste and appearance** (sweetness, colour, flowers ...)
- **Plants as bioreactors**
- **Plants producing vaccines**
- Nitrogen fixation, CO₂ concentrating mechanisms around Rubisco
(large EU frameworks)

Higher pre-vitamin A content - the Golden rice story

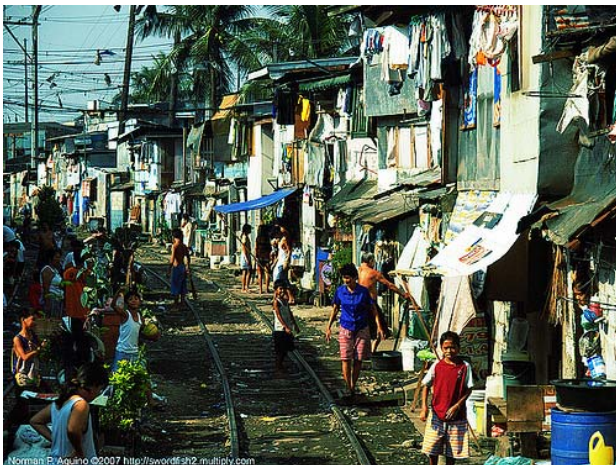
'Golden rice' is the result of an effort to develop rice varieties that produce provitamin-A (β -carotene) **in endosperm** as a means of alleviating vitamin A (retinol)

Vitamin A deficiency is a big health problem - causes blindness, influences severity of diarrhea, measles...

>100 million children suffer from the problem, 1-2 mil deaths per year, more than HIV, malaria, TB..

For many countries, the infrastructure doesn't exist to deliver vitamin pills

Improved provitamin A content in widely consumed crops is an attractive alternative

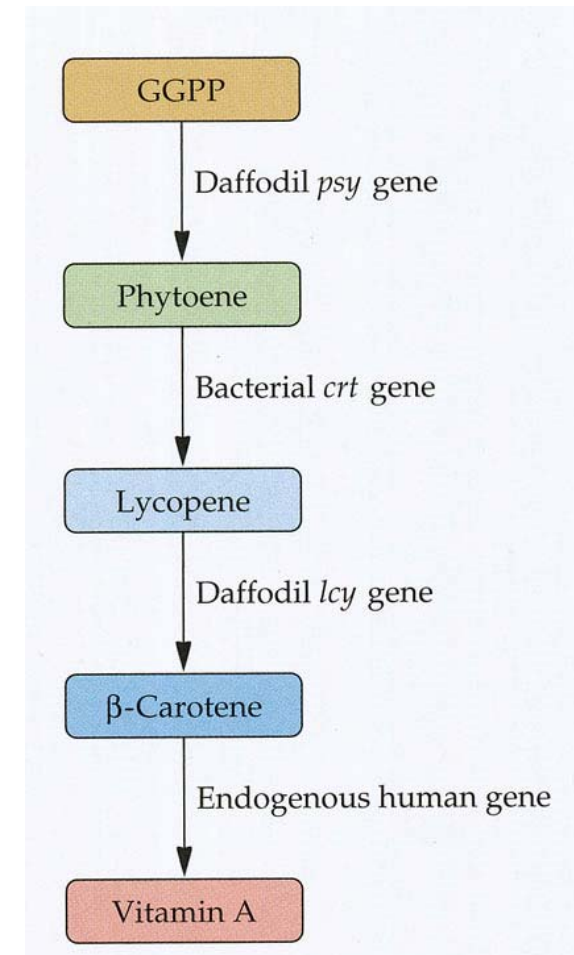
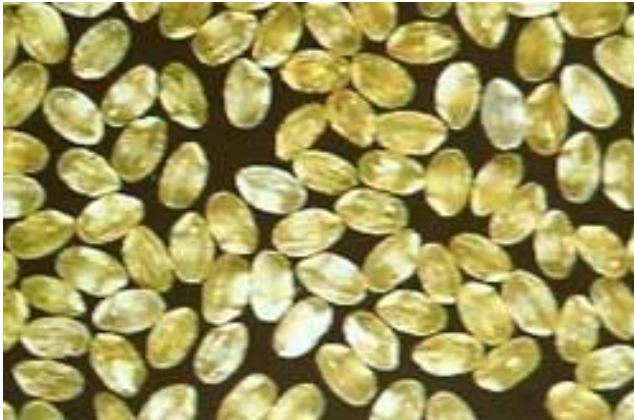


The Golden rice story

Strategy: carry out the four steps required for the production of β -carotene in rice endosperm

Ver. 1: two daffodil (*Narcissus*) genes, one bacterial catalyzing two steps

low β -carotene content – 3kg of rice/day to reach minimal daily requirement



The Golden rice story

2005 – **Golden rice ver. 2**: daffodil phytoene synthase replaced by maize genes – 23-fold higher level of β -carotene, effective source of vitamin A

Introducing golden rice 2 into subspecies *indica* - grow better in the field in Asia

2016 - **Golden rice ver. 3**: plus high iron and zinc, vitamin E



The Golden rice story

Developed as a humanitarian tool under Humanitarian Use Licenses – free for farmers

A significant opposition from anti-GMO organizations (The Golden rice is a trojan horse...) , destroying test fields.. yield still not satisfactory.

" This golden rice is a technological fix that may generate new problems. For example, the single-crop approach of GE 'Golden' rice could make malnutrition worse because it encourages a diet based solely on rice, rather than increasing access to a diverse diet of fruits and vegetables."

'Golden' rice does not address the primary causes of VAD, which are poverty and lack of access to a healthy and varied diet.

Greenpeace



The Golden rice story – an opposition to anti-GMO activists

Patric Moore (Greenpeace co-founder).. denying Golden Rice to malnourished dying children is "*a crime against humanity*"

A current realistic German study says that ~1.5 mil people would not die if the Golden rice would be planted for the last decade

151 Nobel laureates have signed a letter urging Greenpeace to end its opposition to genetically modified organisms. The letter asks Greenpeace to cease its efforts to block introduction of a genetically engineered strain of rice that supporters say could reduce Vitamin-A deficiencies causing blindness and death in children in the developing world.



The Golden rice story is slowly moving on ...

2018 - Golden Rice received successive positive food safety evaluations - Australia, New Zealand, Canada, United States.

2019 – approved for direct use (food) in Philippines

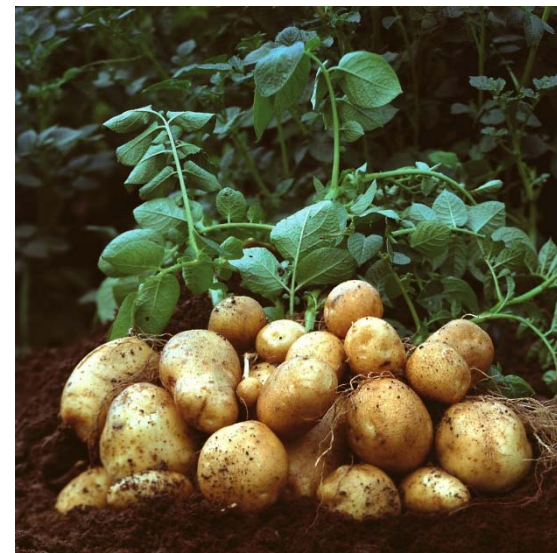
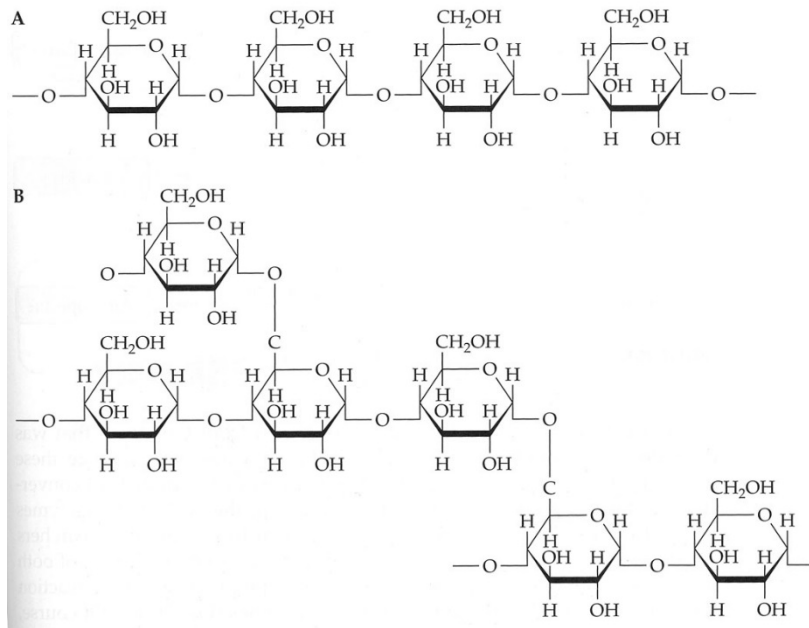
Field trials completed in Philippines and in Bangladesh

2021 - Philippines becomes first country to approve Golden Rice for planting

Better starch quality - Amflora potatoes

The starch in conventional potatoes consists of two different components: amylopectin (70-80%) and amylose (20-30%).

In many applications (the paper, textile and adhesives industries...), the amylose is not desirable but separating out the different kinds of starch is uneconomical.



Better starch quality - Amflora potatoes

Amflora is a starch potato developed specifically for industrial use by BASF, produces pure amylopectin

Granule-bound starch synthase (*gbss*) - the gene responsible for synthesizing amylose is deactivated by antisense technology

Amflora was approved for industrial applications in the European Union market on March 2010

Czech Republic – 150 hectares in 2010

Amflora potatoes - epilogue

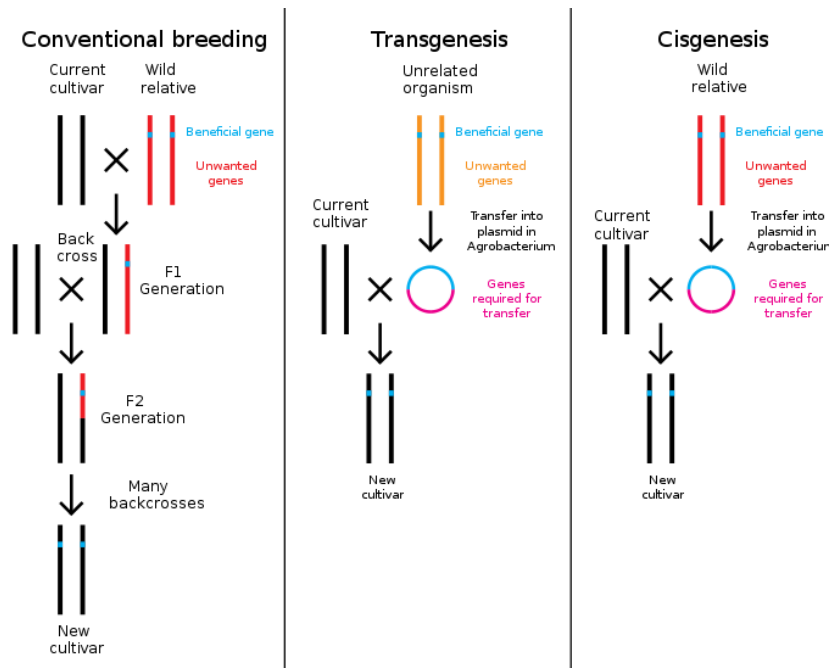
2012 - BASF Plant Science decided to stop Amflora commercialization

The relocation of BASF plant science research from Germany to the USA –
loss of 140 jobs in Europe

BASF ... there is still a lack of acceptance for this technology in many parts of Europe from the majority of consumers, farmers and politicians. Therefore, it does not make sense to continue investing in products exclusively for cultivation in this market ..

Fortuna potatoes – a product of cisgenesis

Blight (*Phytophthora infestans*) resistant potato, two genes transferred from Mexican wild potatoes



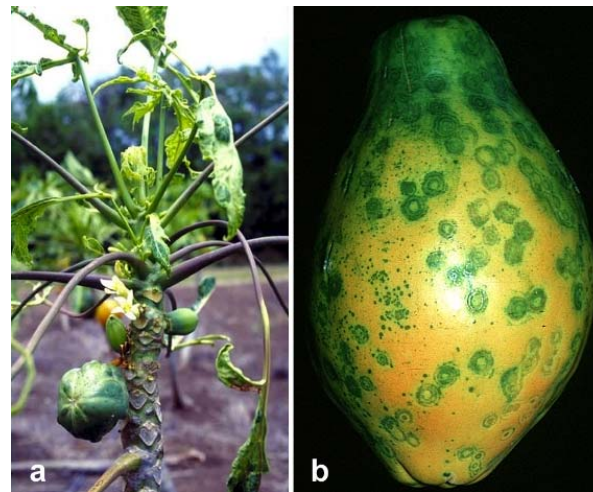
Cis-genesis = GMO, 2012 The EFSA concluded that "similar hazards can be associated with cisgenic and conventionally bred plants ... "

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

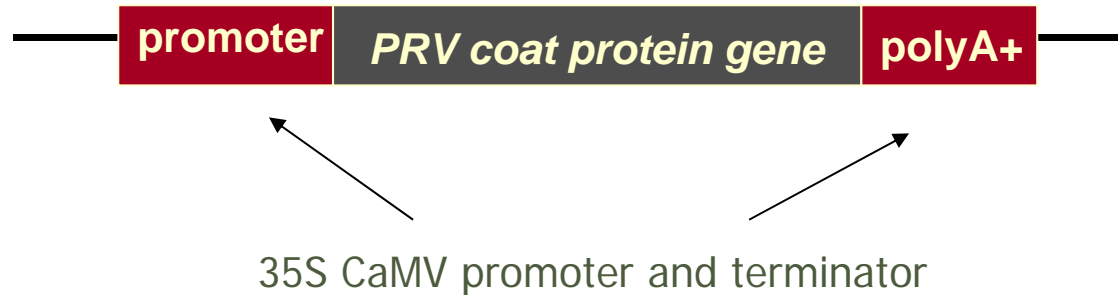
No naturally occurring resistance genes - without GM, papaya industry in Hawaii would be destroyed

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



Virus resistant GM papaya

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')

The varieties called Rainbow & SunUp are provided free to farmers

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

Innate potatoes

In 2014, the company Simplot received US approval for a potato that is genetically engineered to **resist bruising** and **reduce asparagine** (an amino acid that reacts with some sugars to oxidize into acrylamide, a possible carcinogen, especially during high-temperature frying).

Second generation of Innate potatoes (2016) contains reduced bruising and black spots; reduced asparagine; resistance to late blight pathogens; and enhanced cold storage capability.



Arctic apples

No-browning, commercialized in US, Canadian company, Specialty Fruits. Made by Cisgenosis, an anti-sense construct

In 2019, 3.6 million kg of Arctic apples were produced for the United States market



Plenish and Vistive Gold soybeans

Commercialized 2015. These soybeans would allow for the production of soybean oil without the introduction of trans-fats (the oil is more stable and therefore does not need the hydrogenation that produces trans-fat).

Plenish (Pioneer – DuPont), Vistive Gold (Monsanto) .

2016 (after 8 years) European Commission approved the Plenish high-oleic soybean single trait for import, food, feed use and processing.



Drought / salinity tolerance

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

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

Trehalose-6-P synthase and Trehalose-6-P phosphatase transformed into targeted plant

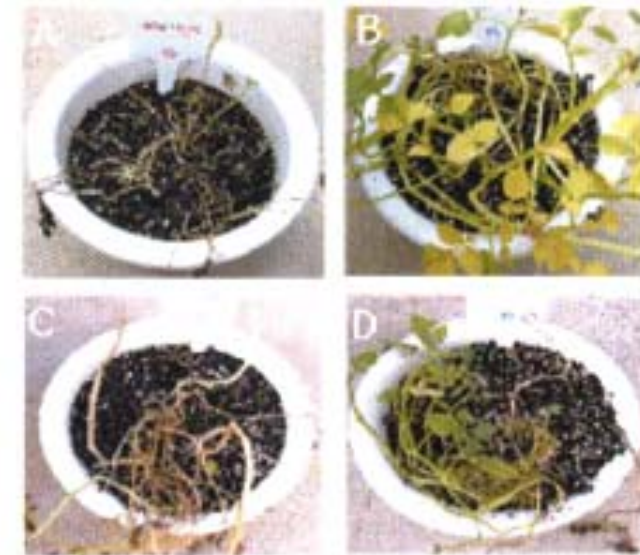
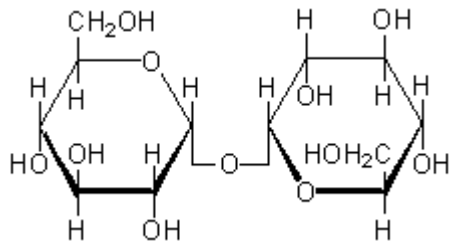
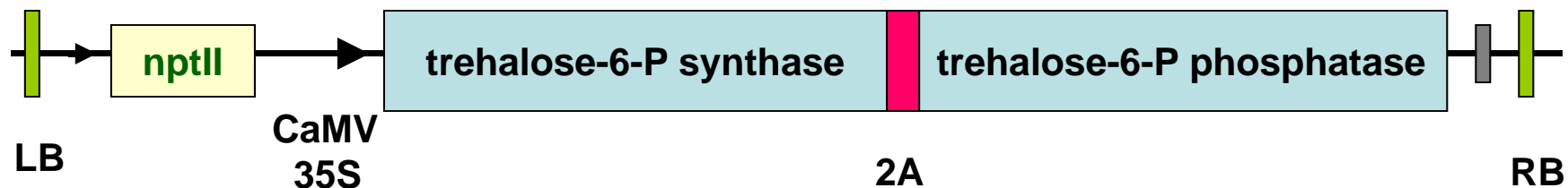


Figure 4. Drought stress tolerance of ZrTPS2-2A-ZrTPS1 transgenic plants. One month old plants rooted in the soil mixture were not watered for 3 weeks. (A) is the empty vector transgenic control plant, and (B~D) are ZrTPS2-2A-ZrTPS1 transgenic lines.



WEMA project

The **Water Efficient Maize for Africa project** (WEMA) is a public/private partnership, led by the Kenyan-based African Agricultural Technology Foundation (AATF) and funded by the Bill and Melinda Gates Foundation, Howard G. Buffett Foundation, and USAID.

.. the efforts of developing more drought-tolerant maize varieties and will also be available royalty-free. Kenya, Mozambique, South Africa, Tanzania and Uganda.



Drought-tolerant GM maize

Monsanto's drought-tolerant GM maize approved in U.S. in 2011

Express anti-freeze CspB protein from *Bacillus subtilis*

The CspB protein is a RNA chaperone - allowing the RNA to fold neatly into their proper shape

Field trials of this GM maize began in Kenya and Uganda in 2010, released ???



GM poplar tree, eucalyptus

GM poplar trees that produce less **lignin** and more **cellulose**

Lignin is a glue-like compound that must be chemically removed from pulp prior to papermaking or for conversion to biodiesel

Higher yield due to the fermentation of sugar released from cellulose

Initial field test (2011) - ethanol yield up 81% over non-modified trees

2015: FuturaGene's eucalyptus is approved for commercial use in Brazil (after 10 years). Approximate 20% increase in yield compared to its equivalent conventional variety.

Current situation with GM trees:

"People have stopped doing research, because the signals from the marketplace and regulators are so extraordinarily hostile. It's almost completely shut down"



Edible vaccines

An edible vaccine doesn't need sterile syringes, costly refrigeration, or multiple injections

Works like any vaccine

A transgenic plant with a pathogen protein gene is developed; potato, banana, and tomato are targets



Edible vaccines

Four plant vaccines were successful in phase I clinical trials

Vaccine antigen	Plant(s) or vector
Hepatitis virus B surface proteins	Tobacco, potato, yellow lupin,
Malaria parasite antigen	Virus
Rabies virus glycoprotein	Tomato
Human rhinovirus 14 and human immunodeficiency virus (HIV) epitopes	Virus
<i>E. coli</i> heat-labile enterotoxin	Tobacco, potato
Norwalk virus capsid protein	Tobacco, potato
Diabetes-associated autoantigen	Tobacco, potato, carrot
Mink enteritis virus epitope	Virus
Rabies and HIV epitopes	Virus
Foot and mouth disease VP1 structural protein	<i>Arabidopsis</i> , alfalfa
Cholera toxin B subunit	Potato
Human insulin–cholera toxin B subunit fusion protein	Potato
Human cytomegalovirus glycoprotein B	Tobacco
Dental caries (<i>S. mutans</i>)	Tobacco
Respiratory syncytial virus	Tomato

Edible vaccines - concerns

- 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?
- What if the vaccine foods enter the food supply of people who are vaccinated the traditional way

Plants as bioreactors - Bio-Pharming

- 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

Plants as bioreactors - Bio-Pharming

Protein	Plant(s)	Application(s)
Human protein C	Tobacco	Anticoagulant
Human hirudin variant 2	Tobacco, canola, Ethiopian mustard	Anticoagulant
Human granulocyte-macrophage colony-stimulating factor	Tobacco	Neutropenia
Human erythropoietin	Tobacco	Anemia
Human enkephalins	Thale cress, canola	Antihyperanalgesic by opiate activity
Human epidermal growth factor	Tobacco	Wound repair, control of cell proliferation
Human α -interferon	Rice, turnip	Hepatitis C and B
Human serum albumin	Potato, tobacco	Liver cirrhosis
Human hemoglobin	Tobacco	Blood substitute
Human homotrimeric collagen I	Tobacco	Collagen synthesis
Human α 1-antitrypsin	Rice	Cystic fibrosis, liver disease, hemorrhage
Human growth hormone	Tobacco	Dwarfism, wound healing
Human aprotinin	Corn	Trypsin inhibitor for transplantation surgery
Angiotensin-1-converting enzyme	Tobacco, tomato	Hypertension
α -Tricosanthin	Tobacco	HIV therapy
Glucocerebrosidase	Tobacco	Gaucher disease
Human muscarinic cholinergic receptors	Tobacco	Central and peripheral nervous system
Human interleukin-2 and interleukin-4	Tobacco	Immunotherapy
Human placental alkaline phosphatase	Tobacco	Children with achondroplasia or cretinism
Human insulin	Safflower	Diabetes
Trout growth factor	Tobacco	Fish growth
Lipase	Corn	Cystic fibrosis
Lactoferrin	Rice	Diarrhea

HIV, human immunodeficiency virus.

Plants as bioreactors - Elelyso

The U.S. Food and Drug Administration approved Elelyso (taliglucerase alfa) for long-term enzyme replacement therapy to treat a form of Gaucher disease.

- a genetic disease in which lipid accumulates in cells and certain organs

About 1 in 100 people in the United States are carriers of the most common type of Gaucher disease

Elelyso - glucocerebrosidase enzyme produced in carrot cell culture, the first drug approved produced via plant genetic engineering

GM crop/food regulatic

Figure 1. Global Area of Biotech Crops, 1996 to 2016: Industrial and Developing Countries (Million Hectares)

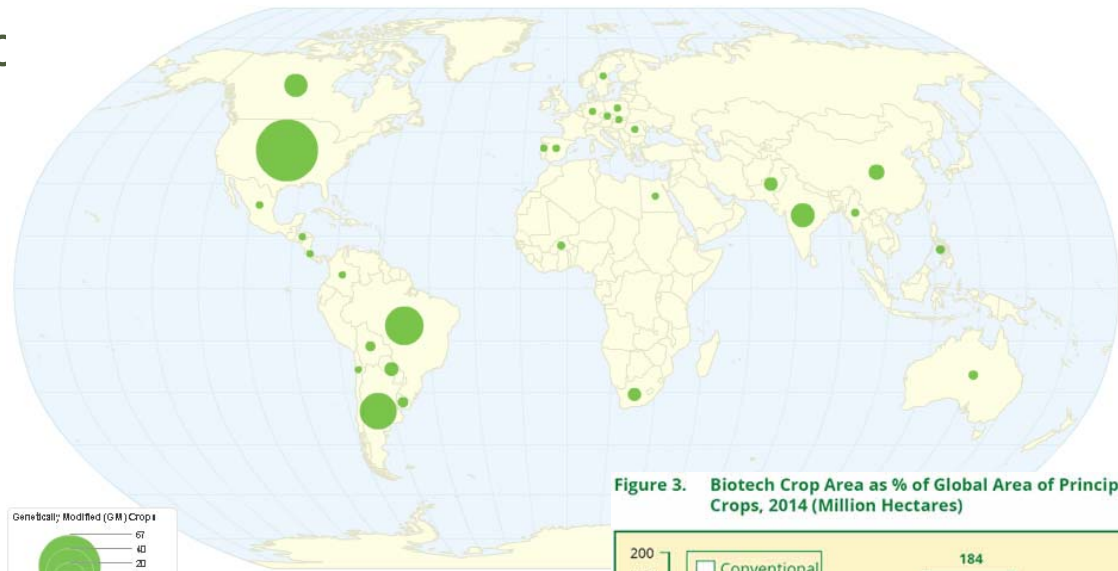
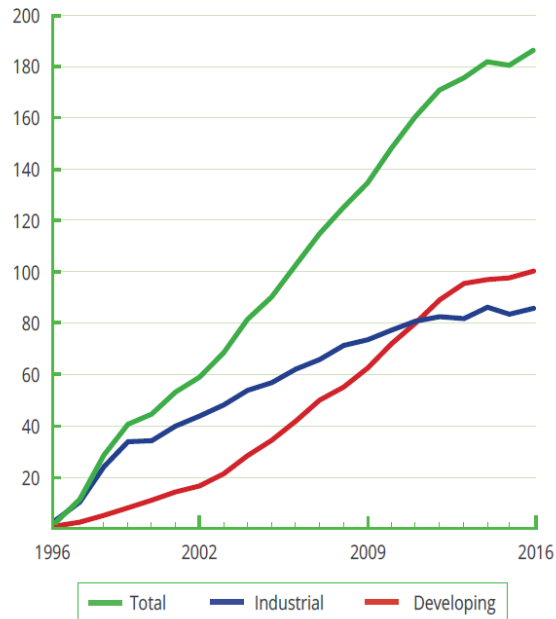
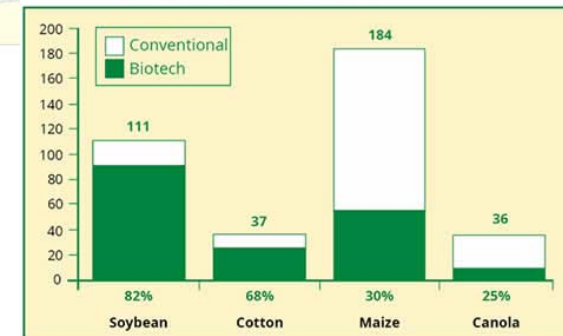


Figure 3. Biotech Crop Area as % of Global Area of Principal Crops, 2014 (Million Hectares)



Source: Clive James, 2014.

Currently (2019) 190.4 million hectares of GM crops grown worldwide
26 countries. More visible public sector (government-distributed seeds).

~ 300 new GM cultivars in process of approval (ISAAA database)

Term GMOs as used in regulatory documents:

plants, animals and microorganisms which have had DNA introduced into them by means other than by combination of an egg and a sperm or by natural bacterial conjugation.

EU - *genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination (1991)*

GMO regulation in the U.S.

NIH (National Institutes of Health) initially responsible for biotechnology regulation,
Established safety protocols for biotech labs

1986 Coordinated Framework for Regulation of Biotechnology - primary blueprint
for the regulation of biotechnology Redistributed responsibility for biotechnology
regulation to FDA and USDA

FDA - Food and Drug Administration

USDA - United States Department of Agriculture

1992 Statement of Policy

Foods Derived from New Plant Varieties, said GM products are 'GRAS' - **Generally
recognized as safe**

GMO regulation in the U.S.

FDA Responsibility (2001-): oversees the safety of all foods and animal feeds for consumption, including both GM and non-GM products

2001 - new proposal to expand the FDA's regulatory control by making *pre-market consultation mandatory*

Food producers must *notify* the FDA at least 120 days in advance of their intent to market GM product

Must show that the GM product is as *safe as its conventional counterpart*

A GM food will be considered to be “**substantially equivalent**” to the natural product if after a comparison of several different characteristics, no difference is shown.

United States Department of Agriculture Responsibility (USDA):

Oversees field testing of GM seeds and plants

Places inspectors in biotechnology plants

Labeling GM Foods (The US Position)

Labeling of GM products necessary only when the product is materially different from its non-GM equivalent:

e.g. whether the use of biotechnology has changed the:

- quality
- safety
- nutritional composition

Mandatory Labeling of Genetically Engineered Food Initiative

-2012, voting in California - the final result was **No 53.7%** and Yes 46.3%

-2013 Washington State - **55% No** and 45% Yes.

- labelling required in Vermont (**2016**). It doesn't require GMO labels on milk or cheese (an important sector in Vermont)

GM food regulation in the EU

Directives 90/119 and 90/220 of 1990:

regulates the unintentional and deliberate release of GMO including GM crops into the environment

No central European Food Safety Authority similar to the FDA or USDA

No labelling requirements

European Regulation 178/2002: The European Food Safety Authority (**EFSA**) is an agency of the European Union that provides independent scientific advice and communication on existing and emerging risks associated with the food chain

Labeling GM foods in EU

EU directives 1829 and 1830/2003

Food and feed made from GMOs fall under their own regulation that came into effect across the EU; the threshold $> 0.9\%$ of GMOs

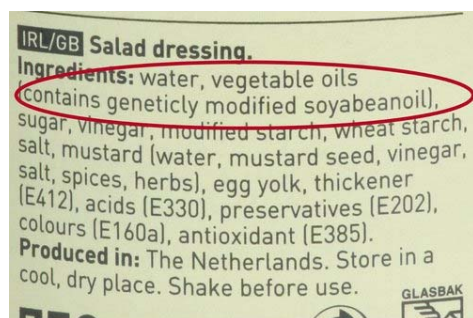
All food products that make direct use of GMOs at any point in their production are subjected to labelling requirements, regardless of *whether or not GM content is detectable in the end product*

- Each stakeholder that produces or trades GM raw materials, ingredients, or foods is obligated to pass information on to subsequent stakeholders in the food supply chain.
- Documentation must be retained for five years.
- It must always be possible to trace the route of a GMO from the farm to the final product.
- Upon authorisation, every GMO is assigned an ID number that can be used to identify it at all times.

Labeling GM foods in EU.... 'Kosher food' practice

EU directive 1830/2003

*It may be the case that physically identical products have different labelling requirements. Under this labelling regulation, it doesn't matter if the differences become impossible to measure. **The main principle is the use of a GM product at some point in the processing and production ...***



Expensive, bureaucratic, consumers believe that GMO labels are meant to notify of health hazards -> no GMO labeled goods in EU

What about cheese?

About 80% of commercially made cheeses in EU are made using recombinant Chymosin approved in the mid of 90's and replacing rennet from baby calf. Today, the most widely used is produced either by the fungus *Aspergillus niger* and commercialized under the trademark CHY-MAX by the Danish company Chr. Hansen, or produced by *Kluyveromyces lactis* and commercialized under the trademark MAXIREN by the Dutch company.

According to EU system, it is GM food but the labeling is not required – exempt from labeling requirements

"vegetarian cheese" is the term that usually refers to GM rennet

From a vegetarian forum: "*GMO rennet is made from animal chromosomes. So, it is a form of animal rennet.*"



Concerns associated with GM crops/food



OCCUPY MONSANTO

FRIDAY SEPTEMBER 21
9:00AM

Syngenta Flowers Inc.
2280 Hecker Pass
Highway, Gilroy, CA 95020

Monsanto's genetically engineered GMO crops, weed killers, and pest killers are a danger to our planet!

We will occupy at any Bio-tech facilities in order to keep genetic biohazards out of our food supply

Contact for Carpool Meet Up!

<http://occupy-monsanto.com/>
gcu_san_jose@occupy-monsanto.com

Concerns associated with GM crops/food

Healthy issues: possible production of allergenic or toxic proteins not native to the crop, antibiotic resistance, an effect of CaMV promoter

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

Genetic pollution (unwanted transfer of genes to other species)

Development of pest, herbicide resistance - superweeds

Loss of biodiversity

Global concentration of economic power and food production
Patent issues

GM crops/food – healthy issues

GM crops - the most intensively tested plants ever

... and planted for 20 years (~75% of any food in US contains GMO)

500 independent research groups have received **300 million** € from the EU to study the risks. The conclusion in a summary of the results is that “GMOs are not per se more risky than conventional plant breeding technologies”

Antibiotic-resistant organisms in human guts and in soil are so common that they overcome any potential influence of the corresponding genes in engineered crop plants.

a few studies with a negative effect of transgenesis, usually obscure journals but often media exposed ..

GM crops/food – Arpad Pusztai's study

Ewen SW, Pusztai A. Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. *Lancet*, 1999 354:1353-4.



- rats fed a diet of potatoes expressing a gene coding for a snowdrop sugar-binding protein (agglutinin) showed stunted growth and reduced immune function

- Pusztai jumped to the conclusion that these differences must be attributable to the fact that the plants were transgenic – and he went public with his conclusion (before accepting for publication).

Conclusion –> all GM crops are potentially unhealthy as the method (transgenesis) is not safe to produce new crops

Problem of the Pusztai's study -> no appropriate controls

- Headlined in mass media –> accepted in Lancet to prevent speculations/conspiracy theories

What method of breeding is more/less safe?

Induced mutagenesis (chemical mutagenesis, X-ray bombardment..)

– *e.g.* many cultivars of spring barley based on semidwarf-mutant Diamant (Czechoslovakia 1965), used in every barley beer, whisky..

Interspecific cross - completely new species like triticale (wheat x rice)

Generation of polyploids – many cultivars of vegetables ...

Transfer of a single antisense gene - SavrFlavr tomato, Amflora potato

Site-directed mutagenesis – TALEN, CRISPR/CAS

What method of breeding is more/less safe?

2007-2012, Swiss national research programme - "Benefits and Risks of the Deliberate Release of Genetically Modified Plants"

... plant biotechnology does not pose risks to health or the environment

.. risk assessments should be based on the specific characteristics of the plant variety rather than on the breeding method used to produce it

... one can now assume that many conventional breeding methods potentially pose a greater risk to health and the environment than the new genetic engineering methods

Global concentration of economic power and food production?

Who Produces GM seeds? Big companies..

Bayer → *acquired Monsanto Company in 2018*

BASF

Aventis Cropscience

Syngenta Seed -> acquired with China's state-owned ChemChina

Pioneer Hi-Breed International

Dow Agroscience merged with Du Pont - > DowDuPont

But ...

- not so different is the situation with conventional seeds ..
- regulation/testing of GM crops is very stringent (long) – it is **very expensive** to obtain all permissions for new product
- > **only big companies can do through all the process**

Global concentration of economic power and food production?

The first GM crop approved - FlavrSavr tomato

1987 - Calgene (a small company) identified and cloned a tomato fruit PG gene,

1992 - the U.S. Department of Agriculture determined that the PG-antisense tomato lines no longer required permits for field testing or transport.

1994 - the U.S. Food and Drug Administration approved the FlavrSavr tomato for market

The last GM crop approved (in EU) - Amflora potatoes (BASF)

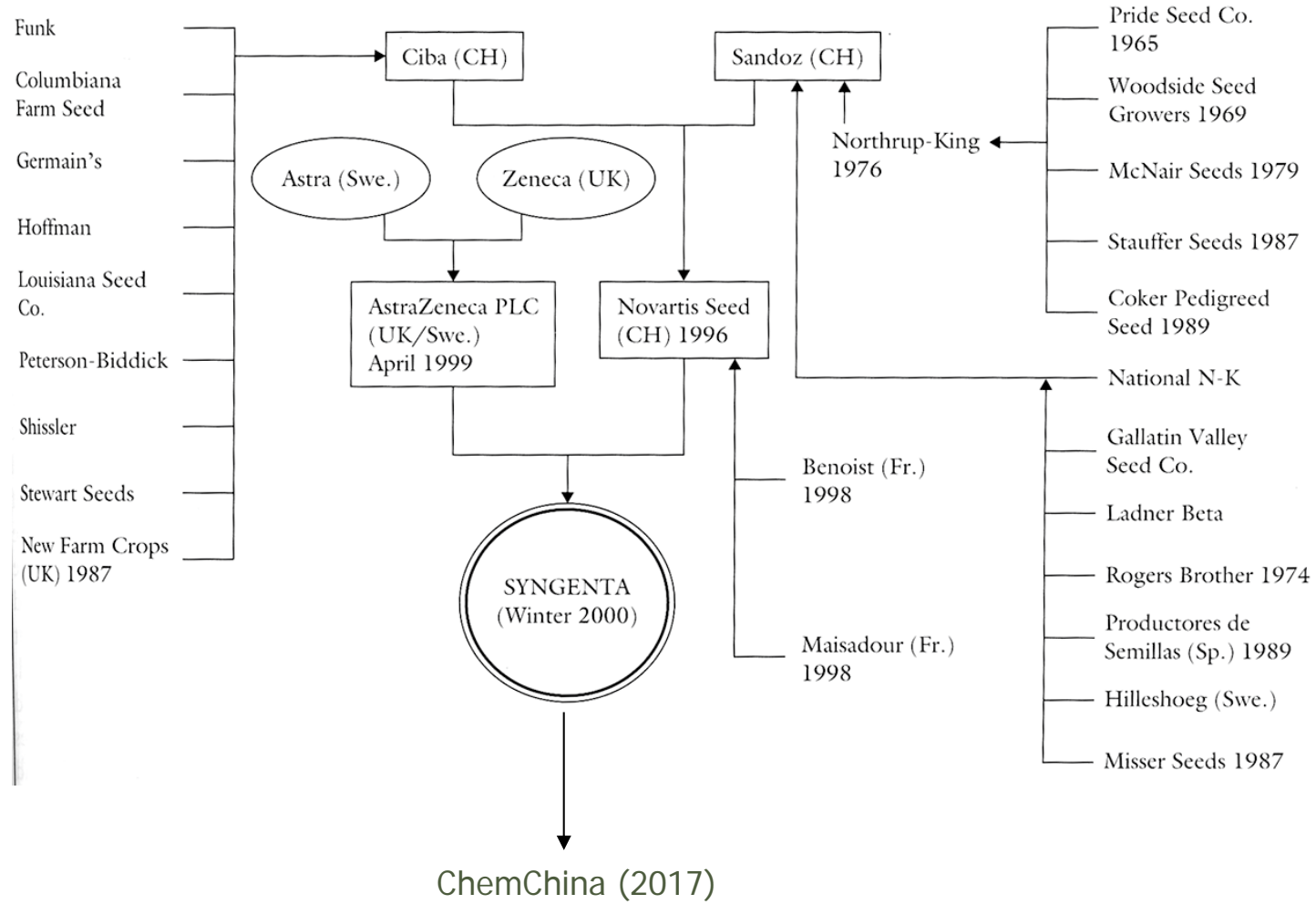
1996 - approval process was initiated

– **13 years testing** (effect of antibiotic resistance, CaMV promoter ...)

2010 - approved for commercial cultivation in EU

Only a few multinational companies are able to take these costs and therefore give the impression of a monopoly

GM crops and the formation of monopolies



GM crops and public sector

Bt Mustard – released 2017
India, Delhi University's Centre

Bt Brinjal – Bangladesh
Agricultural Research Institute
released 2013

Table 43. Crops and Traits under Field Testing by the Public Sector in 2016

Country	Crop	Trait	Developer
Australia	Banana	Fusarium wilt resistance	Queensland University of Technology
	Wheat	Disease resistance, drought tolerance, altered oil content and altered grain composition	Commonwealth Scientific and Industrial Research Organization (CSIRO)
New Zealand	Ryegrass	Better nutritional quality and energy system; high metabolizable energy	AgResearch, New Zealand
United Kingdom	Wheat	Yield and biomass	Rothamstead Agricultural Research,
European Union	Potato Maris Piper	Blight and nematode resistant, less bruising and less acrylamide	The Sainsbury Laboratory (TSL)
European Union	Camelina	Omega-3LC-PUFAS (Omega-3 long chain polyunsaturated fatty acids)	Rothamstead Research
Malawi	Bananas	Bunchy top virus	Byumbwe Research Station
Uganda	Potato varieties Desiree and Victoria	Late blight resistance	Kachwekano Zonal Agricultural Research and Development Institute
India	Indian Mustard	Barnase-barstar system to induce heterosis	Delhi University South Campus
	Chickpea	Insect resistance with cry7Ac, Cry1Aabc	ICAR-Indian Institute of Pulses Research
	Pigeonpea	Insect resistance with cry7Ac, Cry1Aabc	ICAR-Indian Institute of Pulses Research
	Sugarcane	Drought tolerance with DREB gene	Sugarcane Research Institute, UP Council of Sugarcane (UPCSUR), Shahjahanpur
Philippines, Bangladesh	Rice	β-carotene	IRRI, PhilRice, BARI

GM crops and patent issues

What was covered by patents (hundreds patents):

- Vectors for transforming plants
- Genes of the vectors
- Transgene
- Methods for making vectors
- Methods for making *Agrobacterium* with engineered vector
- *Agrobacterium* containing engineered vectors.
- Improved *Agrobacterium* strains for transformation
- Methods of preparing plant tissue for transformation
- Methods of transforming specific plants
- Transformed plants and plant cells.

But plant transgenesis is an old technology – 1982, patent expiration ~25 years

GM crops and patent issues

Key patent expiration:

Binary vector – 2007

Callus transformation - 2014

Embryo transformation – 2014

Monocot transformation – 2014

Using of many promoters including CaMV - expired

...

Floral, seed, pollen transformation – 2018-20

Cotton transformation- 2019

Maize MON810 approved in EU – all Monsanto patent expired 2014..

but it does not mean the MON810 seed can be freely replicated (Bowman vs. Monsanto case, US, 2013)

Eat or not to eat?

Red grapefruit

18 century - a hybrid of an orange and a pummelo
intra-genus crossing -> not natural, GMO according to EU rules

~1950 radiation mutagenesis -> Rio Red grapefruit

EarthOpenSource: chemical/radiation mutagenesis is as risky as genetic modification and should be banned ...



TomTat™

Hand-grafted plants producing potatoes AND tomatoes are available to UK home gardeners

All natural - not GMO -> no testing needed