CG020 Genomics

Lesson 11

Genomics – practical applications

Markéta Pernisová

Functional Genomics and Proteomics of Plants,
Mendel Centre for Plant Genomics and Proteomics,
CEITEC - Central European Institute of Technology, Masaryk Univerzity, Brno
marketa.pernisova@ceitec.muni.cz, www.ceitec.muni.cz

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky

Outline

1. Genomics in medicine
2. Biotechnology
3. Genetically modified organisms
4. GMO – pros and cons
5. Discussion

GENOMICS IN MEDICINE

GENOMICS IN MEDICINE

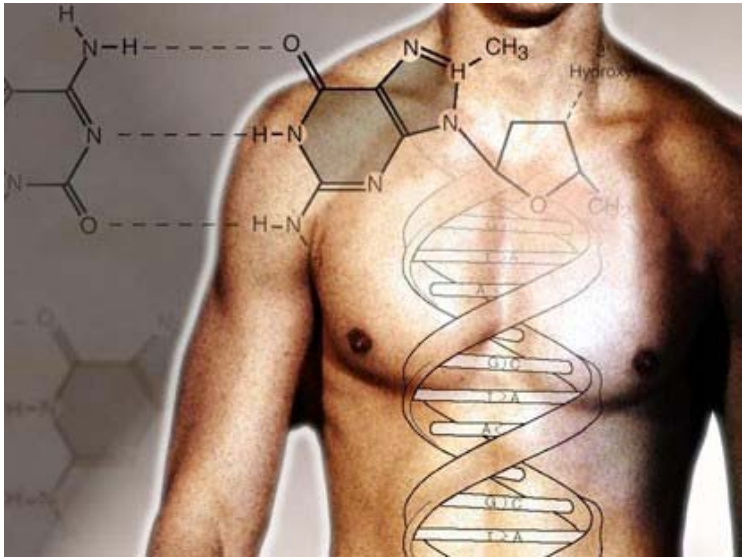
- **Biotechnology** and **gene technology** are crucial technologies in medicine
- Improvement in practice:
  - faster identification and analysis of new pathogens
  - faster development and production of vaccines and reliable diagnostic tools
  - genetic tests for screening of heritable diseases or severe genetic defects in the embryo or foetus
- many drugs produced in laboratories:
  - bacteria, yeast and mammalian cell cultures produce human proteins available as drugs
  - developing of e.g. edible vaccines produced by transgenic plants
  - 1st biotech drug: insulin, 1982

GENOMICS IN MEDICINE

- Influence of genomics on medicine:
  - knowledge of the genome can lead to understanding the origin of disease - mutant gene – misfunction of the protein
  - knowledge of the gene will allow screening for the disease
  - understanding gene influence on drug effects and origin of side effects
  - assessing individual genetic predisposition becomes possible
  - improving the effectiveness of new approaches to cure diseases
  - knowledge of bacterial and viral genomes helps easier identification of the mechanism of infection and improve prevention, treatment, as well as accelerate vaccine development
  - genome research is helping us better understand the aging process and improving the quality of life for older people

GENOMICS IN MEDICINE

- The use of genomics in medicine:
  - genetic diagnosis
  - individualized medicine
  - gene therapy
  - regenerative medicine
  - xenotransplantation
  - molecular *in vitro* systems to study human diseases



GENETIC DIAGNOSIS

- test performed to **identify specific genetic traits** of an individual
- do not need to know the sequence of the entire genome
- used for:
  - clarifying whether a disease appeared as a result of changes in specific genetic traits
  - confirming and refining standard medical diagnosis of an already apparent disease
  - identification of hereditary diseases before they become evident
  - pre-implantation screens of embryos fertilized *in vitro*
  - assessment of individual predispositions of a person for developing certain diseases later in life
  - DNA fingerprinting in criminal investigations and paternity disputes

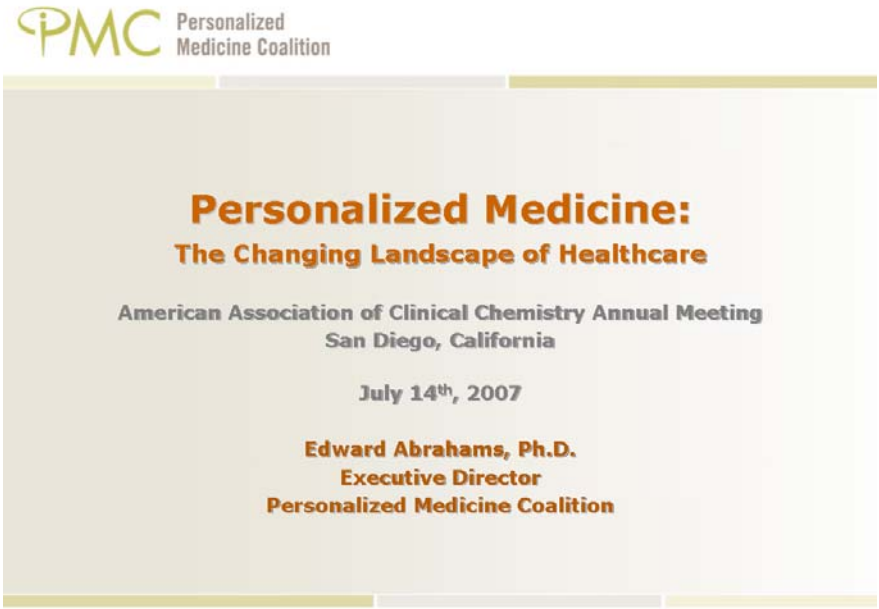
PERSONALIZED MEDICINE

The medical approach that emphasizes the systematic use of information specific to an individual patient to set up an optimized preventive and therapeutic plan for each patient.

- Presumptions:
  - detailed knowledge of the genome of the patient, ideally the entire genome sequence
  - knowledge of the function of individual genes, including functional differences of individual alleles represented in the population
  - correlation of sequences with the prognosis of the disease, and with the success rate of therapeutic procedures ...

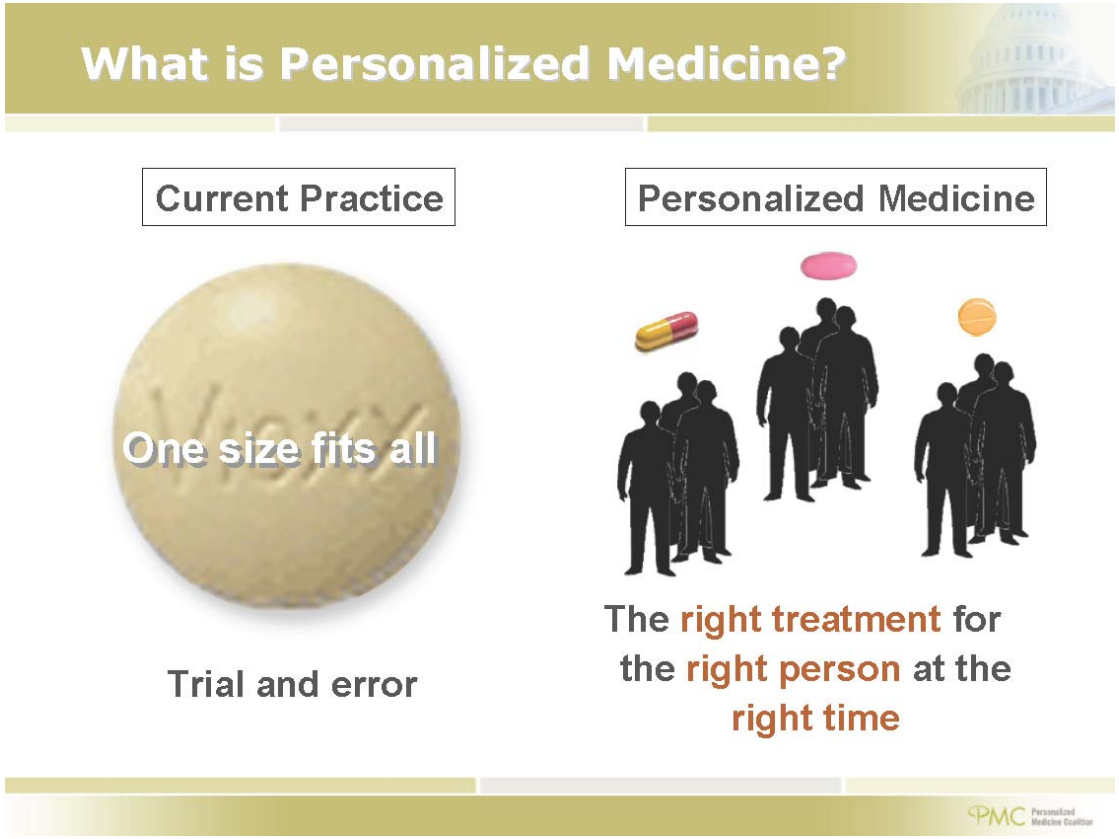
PERSONALIZED MEDICINE

- uses knowledge of the genome for:
  - prediction of health risks
  - diagnosis
  - selection of the most appropriate type of treatment
  - minimizing the side effects of treatment
  - prevention



http://www.personalizedmedicinecoalition.org/

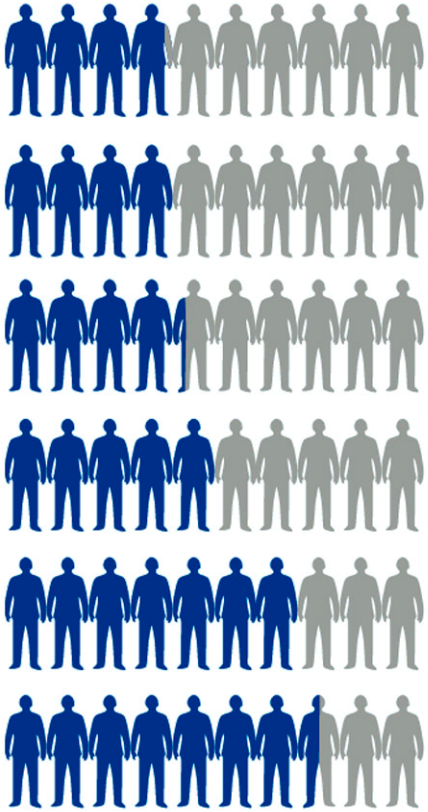
PERSONALIZED MEDICINE



PERSONALIZED MEDICINE

PERCENTAGE OF THE PATIENT POPULATION FOR WHICH A PARTICULAR DRUG IS INEFFECTIVE, ON AVERAGE

ANTI-DEPRESSANTS (SSRIs)	38%
ASTHMA DRUGS	40%
DIABETES DRUGS	43%
ARTHRITIS DRUGS	50%
ALZHEIMER'S DRUGS	70%
CANCER DRUGS	75%



- Just in hospitals: about 6.7% of patients (2.2 million) experience serious adverse drug reactions

Serious adverse drug reactions in even smaller percentages of treated populations have led to the withdrawal of several drugs from the market

Zelnorm Vioxx Cylert

"Are good drugs going to the wrong people?"

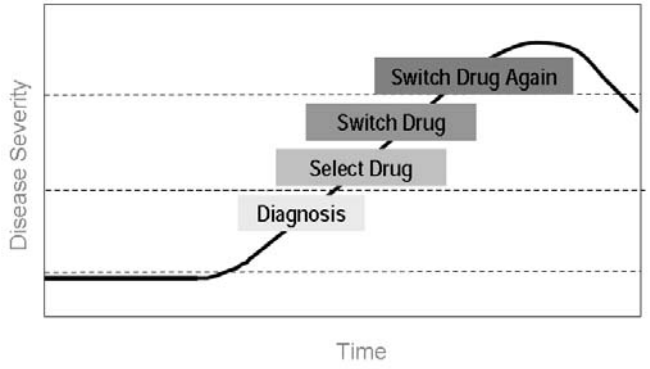
Rezulin Baycol Lotronex*

Source of data: Brian B. Spear, Margo Heath-Chiozzi, Jeffery Huff, "Clinical Trends in Molecular Medicine," Volume 7, Issue 5, 1 May 2001, Pages 201-204.

PERSONALIZED MEDICINE

The Old Paradigm: Treatment of Disease

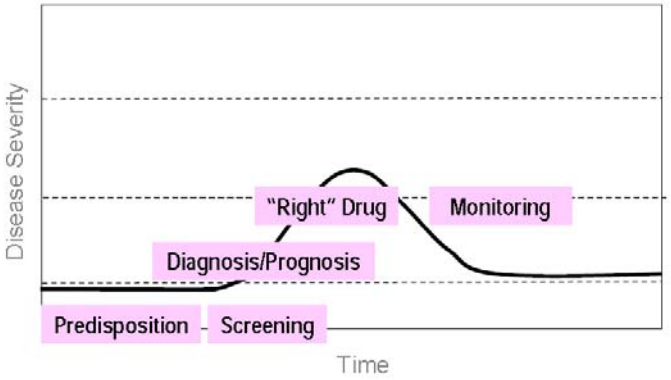
Reactive Medical Care



Diagnose Disease; Treat Symptoms; Costly, Trial and Error Treatment

Personalized Medicine Paradigm: Health Management

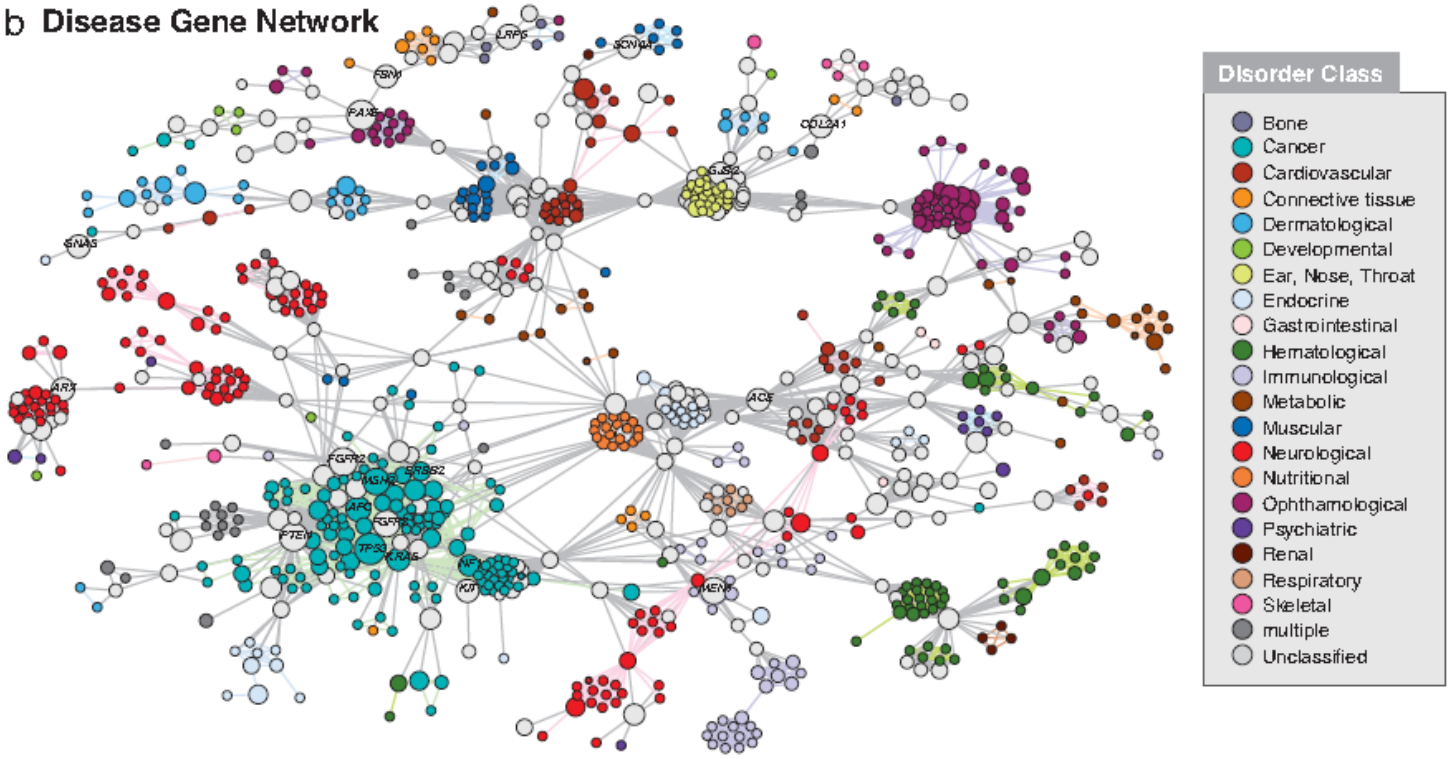
Efficient Medical Care



Health Management; Molecular Screening; Early Detection; Rapid Effective Treatment; Improved Quality of Care

PERSONALIZED MEDICINE

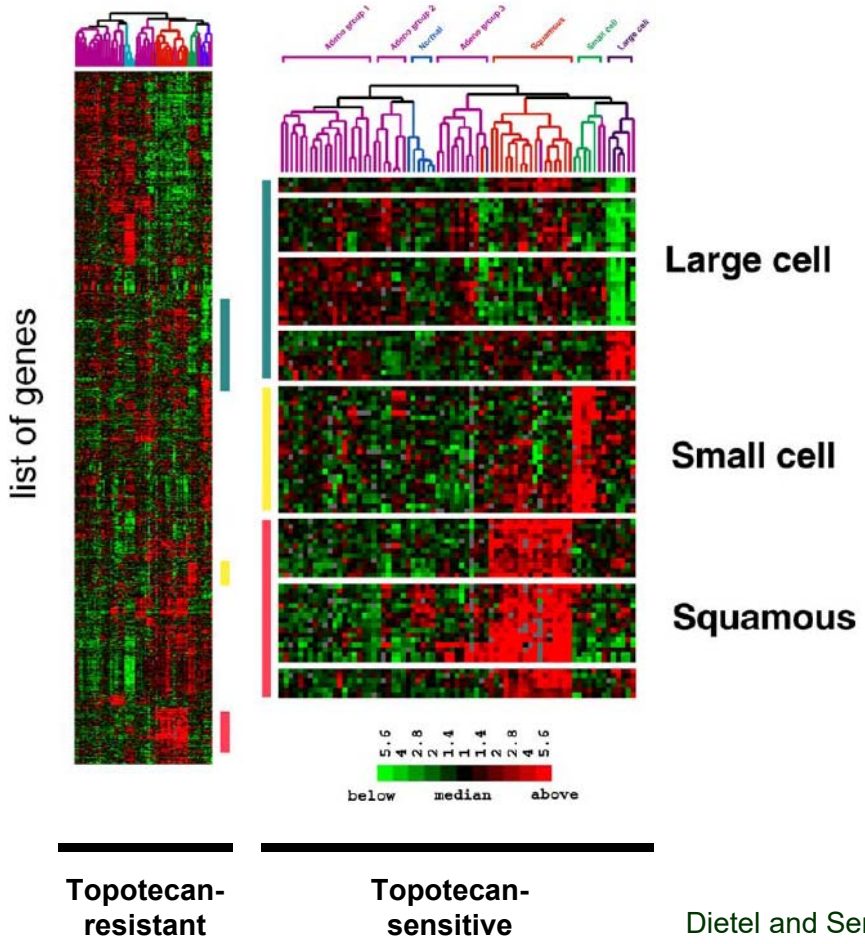
- Problem:
  - multigene conditionality of most human diseases



Goh *et al.*, 2007

PERSONALIZED MEDICINE

- Problem-solving:
  - systems biology - uses e.g. gene clustering to identify genes involved in the observed phenomenon



Dietel and Sers, 2006

PERSONALIZED MEDICINE

- Problem-solving :
  - biomarkers
  - tests

Table: Selected Personalized Medicine Drugs, Treatments and Diagnostics as of September 2011*

Indications in quotes and otherwise unattributed, are cited from the therapeutic or diagnostic product label.

Therapeutic product labels contain pharmacogenomic information as:

- *Information only*
- *Recommended*
- *Required*
- *Unhighlighted products have no pharmacogenomic information, recommendations or requirements in the label.*

THERAPY	BIOMARKER/TEST	INDICATION
Mivacron® (mivacurium)	Cholinesterase gene	**Anesthesia adjunct**: "Mivacron is metabolized by plasma cholinesterase and should be used with great caution, if at all, in patients known to be or suspected of being homozygous for the atypical plasma cholinesterase gene."
Ansaid® (flurbiprofen)	*CYP2C9*	**Arthritis**: "*In vitro* studies have demonstrated that cytochrome P450 2C9 plays an important role in the metabolism of flurbiprofen to its major metabolite, 4'-hydroxy-flurbiprofen."
Depakote® (divalproex)	UCD *(NAGS; CPS; ASS; OTC; ASL; ARG)*	**Bipolar disorder**: "Hyperammonemic encephalopathy, sometimes fatal, has been reported following initiation of valproate therapy in patients with urea cycle disorders [UCDs]...particularly ornithine transcarbamylase deficiency [OTC]."
Aromasin® (exemestane) Arimidex® (anastrozole) Nolvaldex® (tamoxifen)	Estrogen Receptor (ER)	**Breast cancer**: Exemestane is indicated for adjuvant treatment of post-menopausal women with ER-positive early breast cancer. Anastrozole is for treatment of breast cancer after surgery and for metastases in post-menopausal women. Tamoxifen is the standard therapy for estrogen receptor-positive early breast cancer in pre-menopausal women.
Chemotherapy	Mammostrat®	**Breast cancer**: Prognostic immunohistochemistry (IHC) test used for postmenopausal, node negative, estrogen receptor expressing breast cancer patients who will receive hormonal therapy and are considering adjuvant chemotherapy.
Chemotherapy	MammaPrint®	**Breast cancer**: Assesses risk of distant metastasis in a 70-gene expression profile.
Chemotherapy	Onco*type* DX® 16-gene signature	**Breast cancer**: A 16-gene signature (plus five reference genes) indicates whether a patient has a low, intermediate, or high risk of having a tumor return within 10 years. Low-risk patients may be treated successfully with hormone therapy alone. High-risk patients may require more aggressive treatment with chemotherapy.
Chemotherapy	CompanDx® 31-gene signature	**Breast cancer**: The test predicts "time to event" for metastasis of breast cancer, following surgery or biopsy.
Faslodex® (fulvestrant)	Hormone Receptor *(HR)*	**Breast cancer**: Fulvestrant is indicated for the treatment of hormone receptor positive metastatic breast cancer in post-menopausal women with disease progression following anti-estrogen therapy.
Herceptin® (trastuzumab) Tykerb® (lapatinib)	HER-2/neu receptor	**Breast cancer**: "…for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER-2 [Human Epidermal growth factor Receptor 2] protein and who have received one or more chemotherapy regimens for their metastatic disease." High levels of HER-2 expression have been associated with increased disease recurrence in breast cancer, but show a better response to trastuzumab.
Pharmaceutical and surgical prevention options and surveillance	*BRCA 1/2*	**Breast cancer**: Guides surveillance and preventive treatment based on susceptibility risk for breast and ovarian cancer.
Nolvadex® (tamoxifen)	Breast Cancer Index℠ *(HOXB13, IL17BR)*	**Breast cancer**: Calculates a combined risk analysis for recurrence after tamoxifen treatment for ER-positive, node-negative breast cancer.

The Case for Personalized Medicine, 3rd edition

PERSONALIZED MEDICINE

- Other problems
  - Ethical issues:
    - the condition is genetic testing or knowledge of the genome - easily abused
    - risk: insufficient data security
    - in some countries, employers or insurance companies do not have access to such data
  - High costs - risks:
    - medicine could be divided into first-class and low-class services
    - globalization gap could grow even larger - poor countries would not be able to afford this
  - Privacy:
    - crucial and complex issue
    - what information about oneself can/should be considered private?

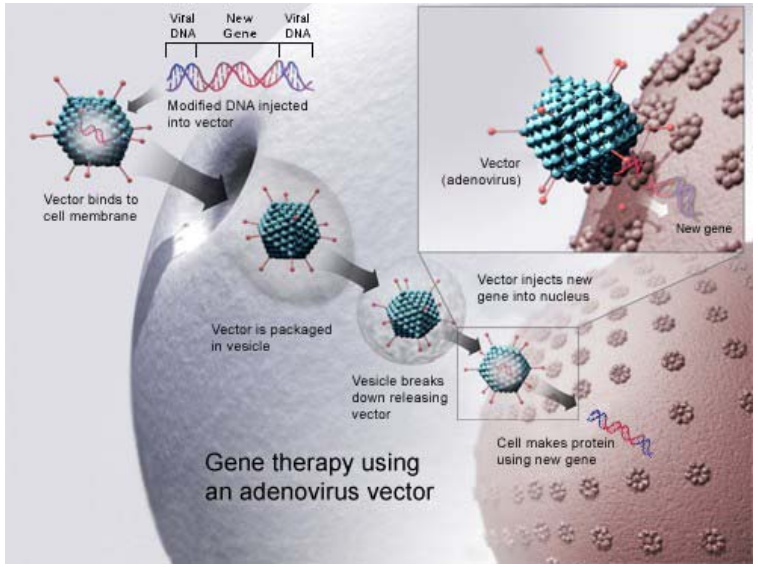
GENE THERAPY

Procedure in which the DNA sequence is inserted into the patient genome to replace or supplement an original gene

- Options:
  - replace the mutated gene
  - repair the mutation
  - deliver DNA encoding a therapeutic protein
  - antisense therapy
- In the future useful for treating e.g. hereditary diseases
- Types:
  - somatic gene therapy
  - gene therapy of germ cells

GENE THERAPY

- Methods:
  - viral vectors
    - retroviruses
    - adenoviruses
    - herpes simplex virus
    - vectors capable of replication
  - non-viral methods
    - injection of plasmid DNA into muscle
    - increased efficiency of DNA delivery:
      - electroporation
      - sonoporation
      - „gene gun"
      - magnetofection
  - hybrid methods



REGENERATIVE MEDICINE

The aim is to **recover** diseased or injured **organs** or tissues.

- to overcome the problems of transplantation:
  - shortage of donors
  - risk of rejection
  - severe immunosuppresive course
- therapeutic cloning - cell therapy that uses stem cells to produce healthy copies of cells/tissues of the patient
- potential medical applications: treatment of degenerative diseases such as Parkinson's disease, apoplexy, organ damage, diabetes, burns ...

REGENERATIVE MEDICINE

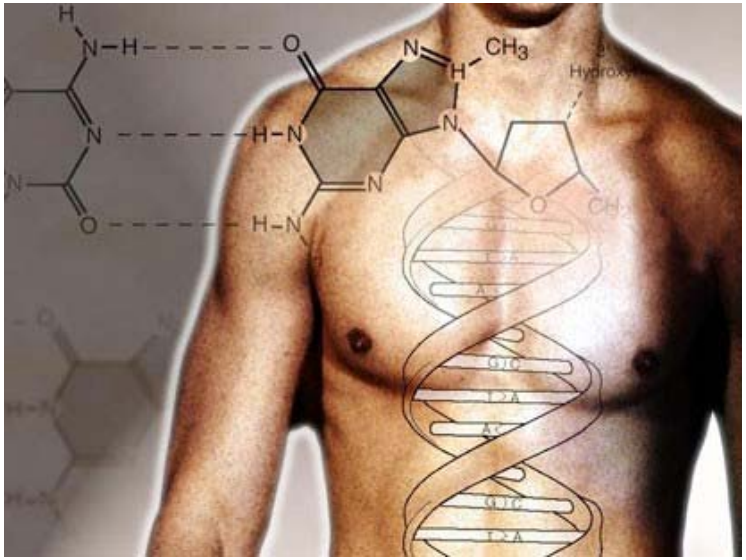
- embryonic stem cells from animals have been successfully programmed to develop into neural, muscle and other cells - promising results

Ethical issues: egg cell is used to form an embryo that does not develop into an organism. Instead, after 5-6 days of development, embryonic stem cells are extracted, which destroys the embryo. Depending on the definition of the point from which you must protect human life, it can be regarded as creating human life to destroy it, and is therefore prohibited.

- consequence of ethical debate: increased support for research on adult stem cells

GENOMICS IN MEDICINE

- The use of genomics in medicine:
  - genetic diagnosis
  - individualized medicine
  - gene therapy
  - regenerative medicine
  - xenotransplantation
  - molecular *in vitro* systems to study human diseases



BIOTECHNOLOGY

BIOTECHNOLOGY

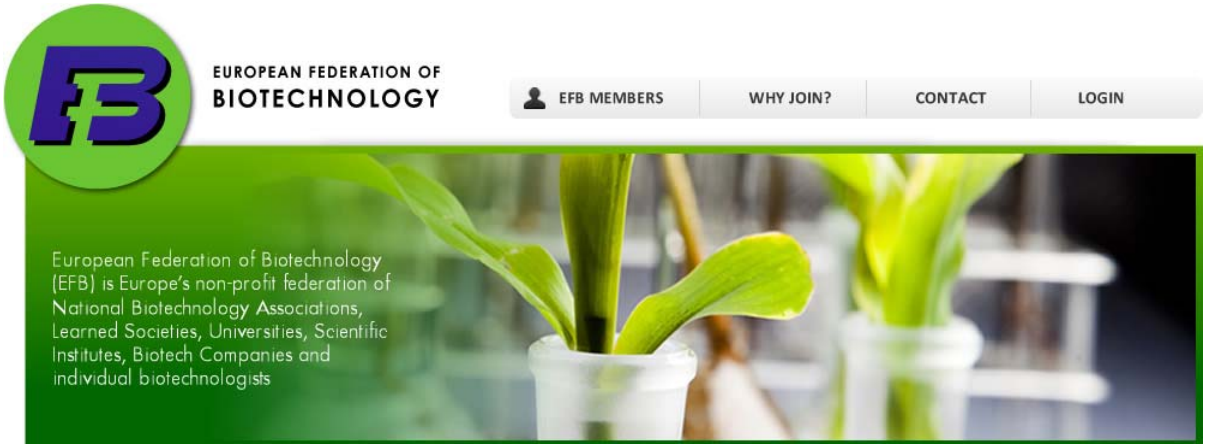
- It uses living organisms, cells or parts of cells (enzymes) for research, leading to new products and applications in medicine, agriculture, food, environmental protection
- Also used in developing better/sustainable production methods for the chemical industry and other industrial processes.
- An **interdisciplinary approach** requiring knowledge of chemistry, biology, physics, material sciences, engineering and informatics.
- The origin of biotechnology can be traced back 4,000 years, when the Sumerians (although not knowingly) used microbes for the production of alcoholic beverages.

BIOTECHNOLOGY

- **modern biotechnology** often changes the genetic status of cells and organisms to optimize processes, e.g. by chemical or physical treatment, cell fusion or genetic engineering.
- **genetic engineering** modifies isolated nucleic acids
- concepts of modern biotechnology and genetic engineering are often used as synonyms
- genetic engineering is actually only one branch of the biotechnology industry
- genetic engineering - GMO

BIOTECHNOLOGY

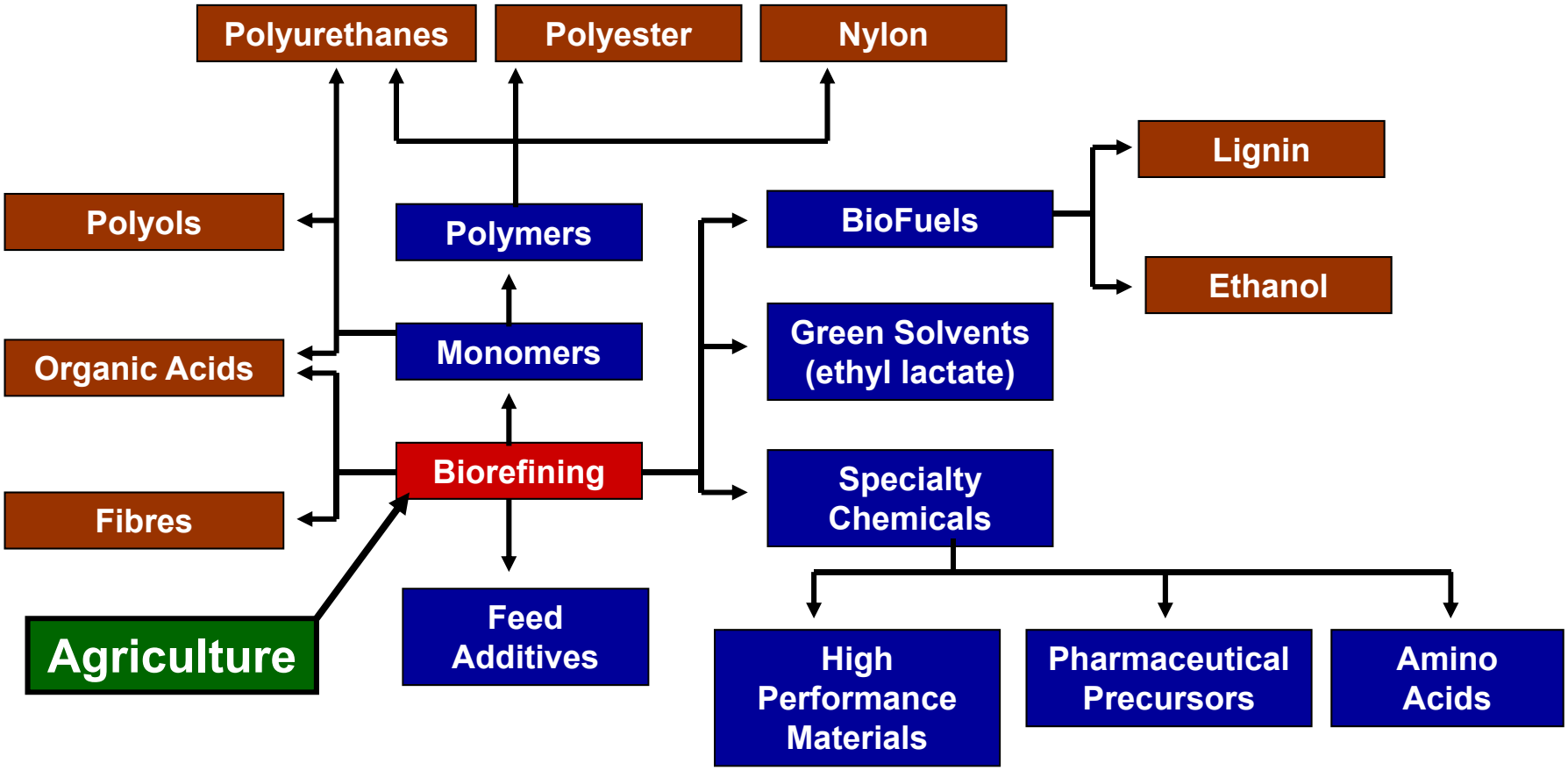
- Examples:
  - effective utilization of plant biomass for fuel production
  - acquisition of starting material (monomers) for the production of polymers from living organisms instead of from fossil sources
  - phytopharmaceutics – using plants to produce new vaccination methods such as expression of antibodies, or antigens suitable for immunization
- European Federation of Biotechnology



http://www.efb-central.org

PLANT BIOTECHNOLOGY

The Biorefinery platform using Agricultural Feed Stocks

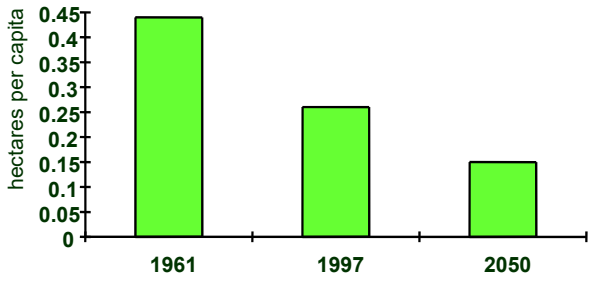


Marc Van Montagu, ACPD, Praha 2009

PLANT BIOTECHNOLOGY

- Examples:
  - production of spider filaments
  - production of degradable biopolymers
  - production of elastin-like polypeptides (component of animal tissues)
  - production of vaccines and antibodies:
    - human (HIV TNF)
    - animal (veterinary)
  - immunomodulation of plant hormones

FACTS TO THINK ABOUT



Source: UN Millennium Ecosystem Assessment

- Our civilization is built on farming, the surface area needed for feeding people has decreased by 90% over 10,000 years
- To prevent collapse, it is necessary to reduce this area from the current 0.45 ha/person to 0.2 ha/person by the year 2050
- Return to original methods of agriculture would be a return to the original demands on area and therefore would be unsustainable
- Intensive farming = conversion of water and oil into food
- The **goal of plant biotechnology** is to use all the available scientific knowledge to **breed varieties with higher yield** with lower inputs (of land, water, fertilizers, sprays ...)

GENETICALLY MODIFIED ORGANISMS

BREEDING

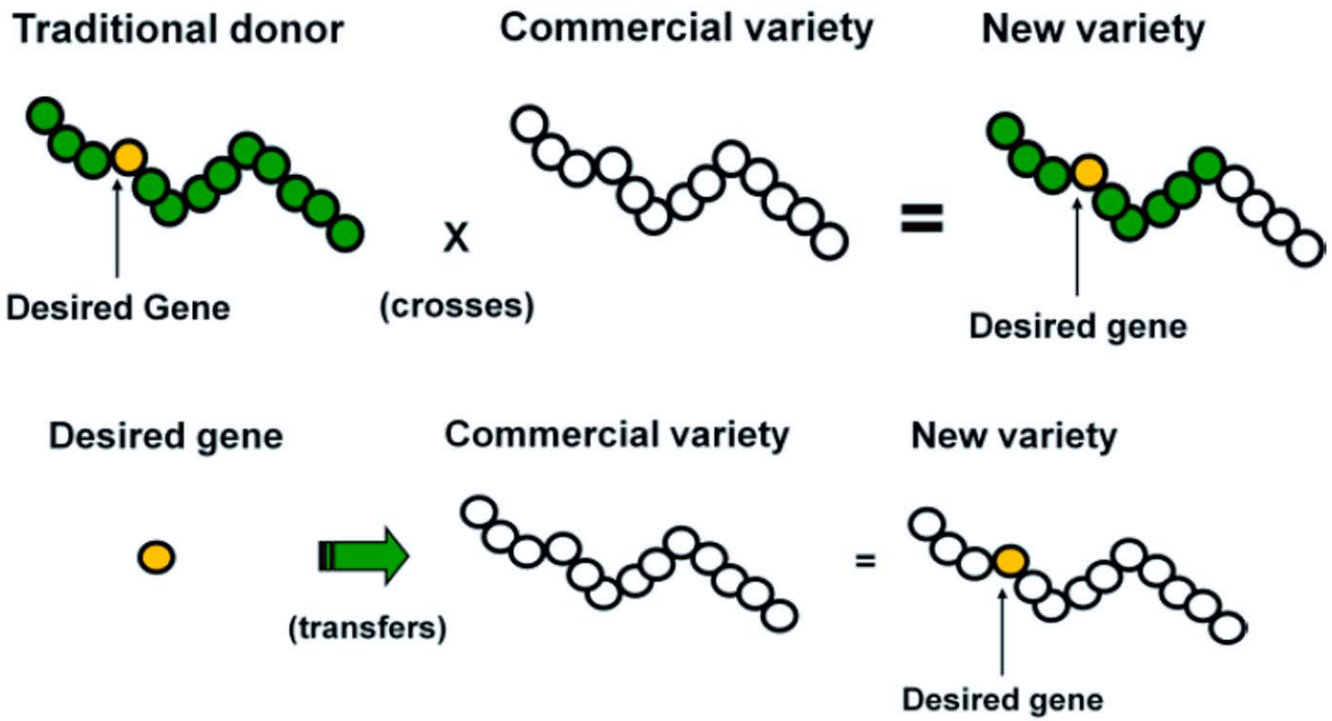
- genetic basis of organisms naturally varies due to mutations
- before the era of genetic engineering - **question of chance**
- breeding tools:
  - selection and crossing
- selection:
  - positive or negative
- results were incidental
- modern breeder learned to change hereditary information
- chemicals, radiation ...

Success
is not always visible at a glance

GENETIC ENGINEERING

- Discovery of:
  - DNA structure (1953)
  - restriction enzymes
  - plasmids
- **targeted change** ("targeted breeding")
- 1973 - bacteria produce frog protein
- recombinant DNA technology = "gene cut" = genetic engineering
- ability to transfer genes = transgenosis
- according to the law: genetic modification
- result: **GMO**
- the first practical application: production of human insulin in bacteria - 1978

BREEDING vs. GENETIC ENGINEERING



GENETICALLY MODIFIED ORGANISMS (GMOs)

- Organisms carrying modified genetic information – either own or foreign (from another organism), enabling targeted changes in the organism and its use for specific purposes
- GMO:
  - plants
  - bacteria
  - animals

http://www.gmo-compass.org/

GMO PLANTS

- Use:
  - resistance to pests
  - herbicide resistance
  - resistance to drought
  - resistance to cold
  - resistance to salinity
  - more efficient nitrogen utilization
  - increasing nutritional quality

http://ipbo.vib-ugent.be/

Bt PLANTS

- resistance to insect pests
- corn, cotton, rice
- genes from *Bacillus thuringiensis* (**Bt**)
- express delta-endotoxins (Cry proteins)
- increasing yields, reducing the amount of chemical sprays

Corn borer damage

European corn borer damage and fungal infection in non-Bt (left) and Bt hybrids (right)

Ht PLANTS

- resistance to systemic herbicides
- glyphosate
  - interferes in the synthesis of aromatic amino acids; animals without the appropriate enzymatic apparatus = harmless
  - blocks the enzyme 5-enolpyrovylshikimate-3-phosphate synthase (EPSPS) in chloroplasts – affects green plants
  - ineffective for bacterial EPSPS - evolutionarily divergent
  - soya, maize, sugar beet, canola, cotton, alfalfa - added enzyme for tolerance
  - company Monsanto: Roundup
- glufosinate (phosphinothricin)
  - prevents processing of ammonium - toxic
  - *Streptomyces hygroscopicus* synthesizes and transforms it: acetylation by the enzyme phosphinothricin acetyltransferase – coding gene isolated in 1987 - named *bar*
- trade names: Basta, Liberty, Finale, Radical ...

MULTIRESISTENT PLANTS

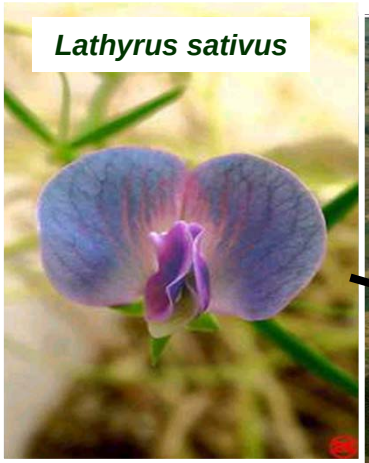
- Bt resistance + herbicide
- multiresistant corn - the majority of total production in the USA
- example of multiresistant corn:
  - three Bt genes for resistance to air pests
  - three Bt genes for resistance against soil pests
  - two genes for herbicide resistance

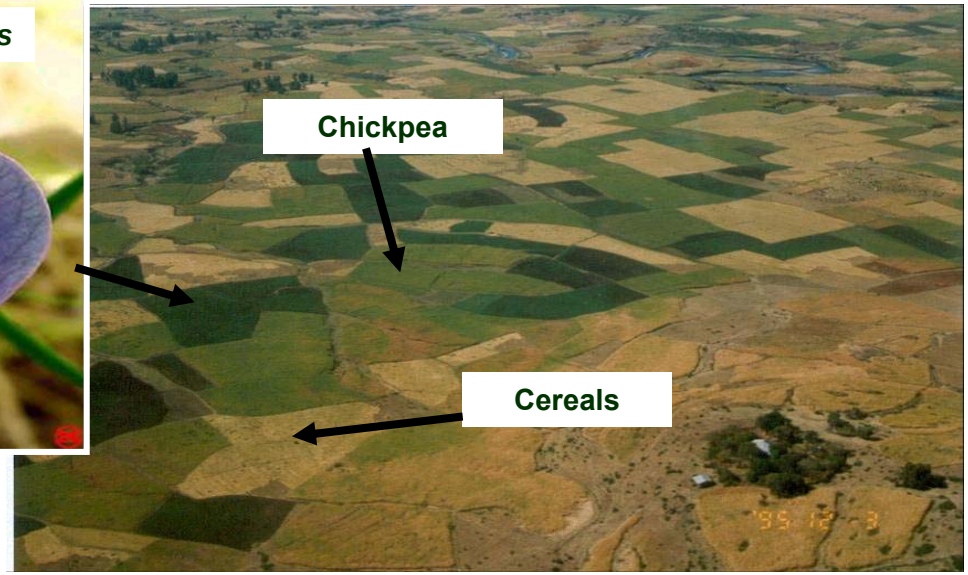
DISEASES TOLERANT PLANTS

- viruses - no chemical agents available
- gene encoding non-infectious viral envelope protein - increases resistance to viral infection
- banana; papaya - Hawaii, Southeast Asia
- cassava - a basic food ingredient for more than 500 million people + animal feed

Left: Papaya with Papaya ringspot disease
Right: Biotech Papaya resistant

DROUGHT TOLERANT PLANTS





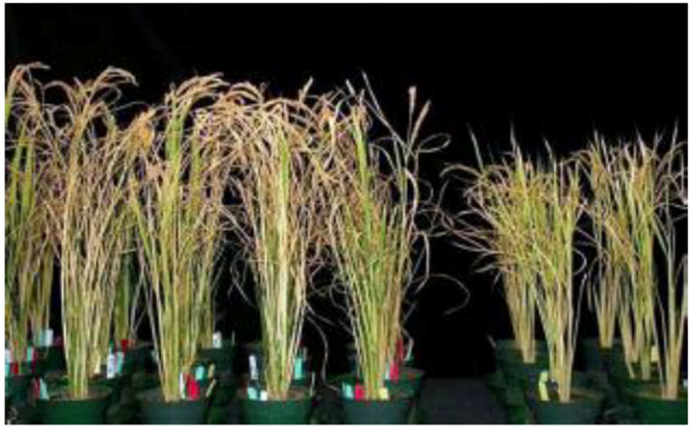
Drought in Ethiopia

New drought-tolerant maize (right) needs less water.

- chickpeas - more resistant to drought, but toxic
- corn resistant to drought: commercially utilizable in 2016

NITROGEN USAGE INCREASE

- use of nitrogen from fertilizers
- gene from barley - 3x higher nitrogen utilization under oxygen deficiency



The effect of Nitrogen Use Efficiency (NUE) in rice growth with reduced N applications. Left: rice engineered

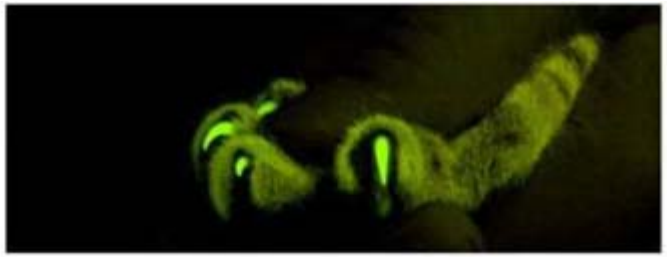
INCREASING NUTRITIONAL QUALITY

- golden rice
  - several genes from maize encoding enzymes for the biosynthesis of β-carotene (precursor of vitamin A)
  - avoid problems with your eyes in a large part of the population in India and China
- canola and soybean
  - improved oil properties: stable, resistant to high temperatures, long storage

Golden Rice

GMO ANIMALS

- Transgenic cats
  - lentiviruses are sensitive to restriction factors
  - specific restriction factor: rhesus macaque TRIMCyp + eGFP
  - uniform expression, no mosaicity and no silencing in F1 generation
  - lymphocytes of transgenic animals resistant to replication of FIV



Wongsrikeao *et al*., 2011, Nature Methods

GMO – PROS AND CONS

GMO – PROS AND CONS

- **GMO plants**
  - increased yields without insecticides, pesticides, fungicides

Economic impacts and impact dynamics of Bt (*Bacillus thuringiensis*) cotton in India

Jonas Kathage[1] and Matin Qaim[1]

Department of Agricultural Economics and Rural Development, Georg-August-University of Goettingen, D-37073 Goettingen, Germany

Edited by Calestous Juma, Harvard University, Cambridge, MA, and approved May 15, 2012 (received for review March 2, 2012)

Despite widespread adoption of genetically modified crops in many countries, heated controversies about their advantages and disadvantages continue. Especially for developing countries, there successful farmers may have higher crop yields and profits anyway, this can result in inflated benefit estimates. Third, most available studies focus on agronomic impacts of Bt, such as yield

  - 24% yield gain per acre
  - 50% yield improvement for small farmers

Yield Gains from

Bt Maize	Bt Cotton
5% U.S. & Canada	9.6% United States
6.3% Uruguay	11.8% Mexico
7.4% Spain	24.3% South Africa
7.8% Argentina	28.6% Argentina
15.3% South Africa	54.8% India
24.1% Philippines	

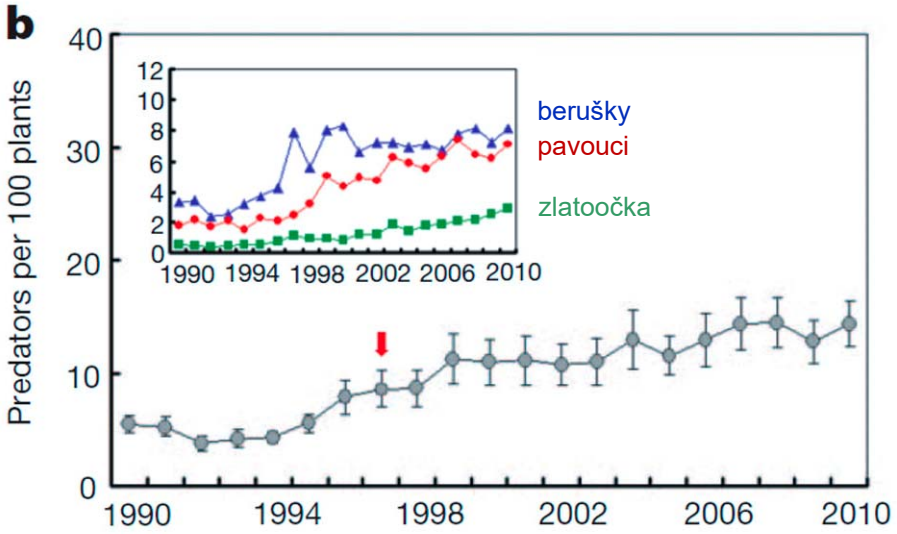
Ohio State University, December 2011

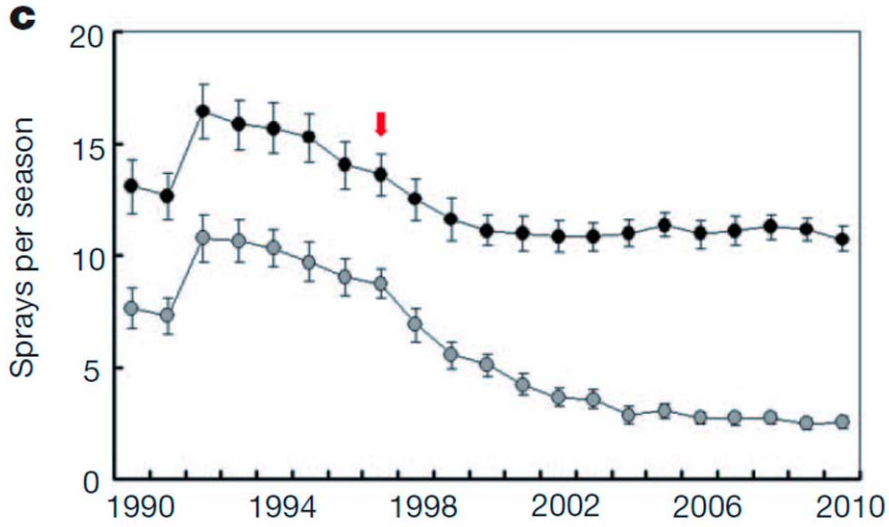
GMO – PROS AND CONS

- **GMO plants**
  - reduce pesticide use - the negative impact of strong chemicals currently used in agriculture
  - increase nitrogen utilization from mineral fertilizers - the negative impact of excessive fertilizer use on water quality
  - increase the nutritional quality of crops
  - reduce cultivation area (increased yield per unit area)
  - pollen transfer to other species (hybridization) was detected in frequencies between 0.05 to 0.53%

GMO – PROS AND CONS

- **GMO plants**
  - Bt crops: negative impact on other than pest insects ???
  - affect some butterflies and beetles
  - more insects on Bt cotton





Lu *et al.*, 2012, Nature

GMO – PROS AND CONS

- **GMO plants**
  - risk of allergies
    - GMO plants always contain only one or a few transgenes and are very thoroughly tested
    - on the other hand, new varieties created using strong mutagens, e.g. X-rays are far less tested. With these methods tens or hundreds of new mutations occur at once
  - ban on GMO cultivation in Switzerland was decided by referendum
  - 30% of the EU population believes that the only transgenic plants have genes and refuse to eat them ...

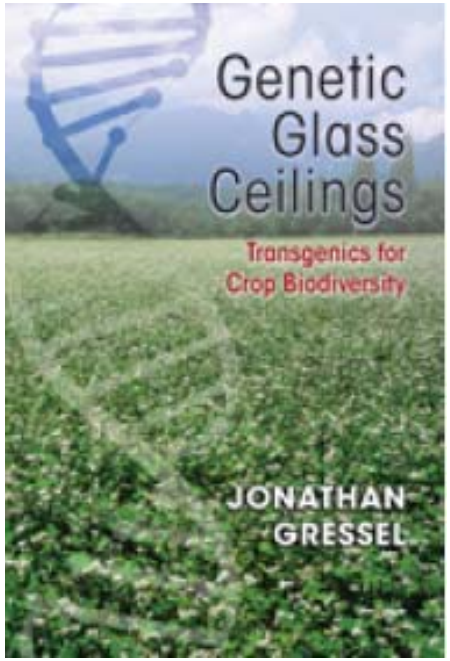
GMO – PROS AND CONS

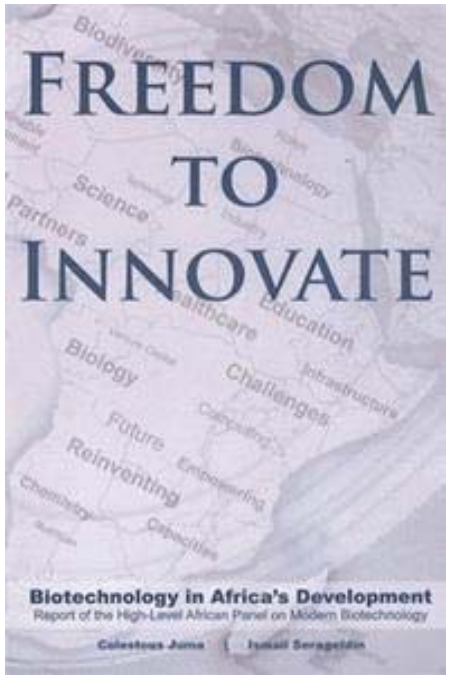
Our task:

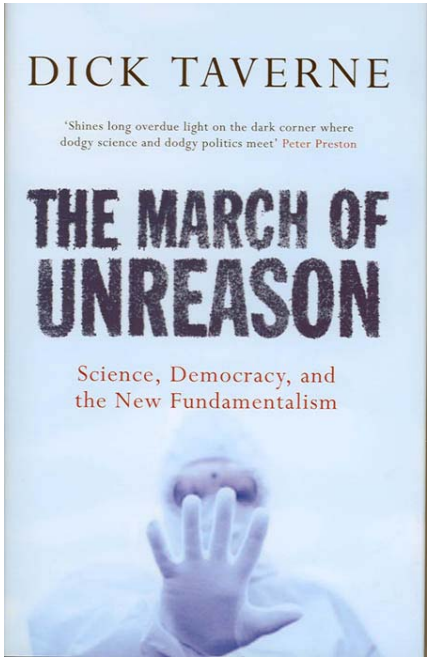
- tirelessly to explain the rational use of scientific knowledge, including genomics, it is necessary both for advances in medicine and for the preservation of our civilization
- discussions with the general public about the importance and benefits of GMOs for human society
- need to defend GMO plants – crops for 21st century
- no technology is without risk, including GMOs, but there is not cause to demonize GMOs and target them - financial benefit of some companies ...

LITERATURE

- see lecture







GMO – PROS AND CONS

Discussion