LOSCHMIDT LABORATORIES

11. Molecular Biotechnology in Agriculture

Outline

- Definition of green biotechnology
- Genetic engineering of plants
- Genetic engineering of animals
- Biopharming
- GMO benefits and controversies

Green (agricultural) biotechnology

- green biotechnology applied to **agricultural processes**
- **environmentally-friendly** solutions as alternative

  to traditional agriculture, horticulture, and animal breeding
- modification of **plants** and **animals** increasing value in agriculture
  - **traditional** agriculture – selective crossbreeding and hybridization
  - **modern** molecular biotechnology – transgenesis (rDNA)
- **transgenic organism** - altered by addition of exogenous DNA
- **transgene** – DNA that is introduced

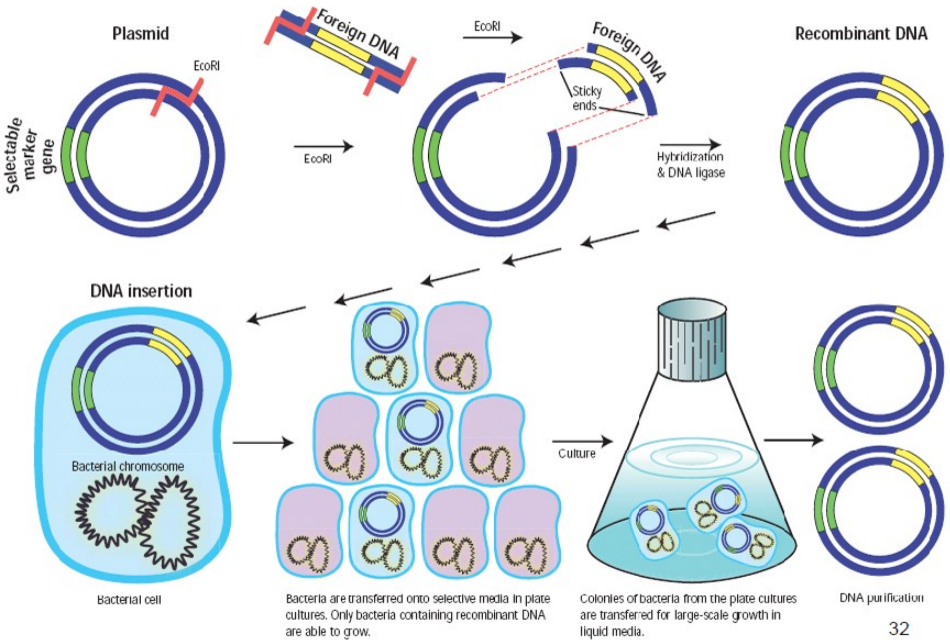
Genetic engineering of plants

- > 150 different plant species in 50 countries worldwide
- **DNA sequence** of *A. thaliana* (2000), rice (2005), cotton (2006), corn (2009), potato (2011), tomato (2012), etc.
- transgenic plants engineered to
  - **overcome biotic and abiotic stress**
    - pesticides (herbicides)
    - pests and diseases (insects, viruses, bacteria, fungi)
    - environmental stress (salt, temperature, cold and drought)
  - **improved crop quality**
    - improved nutritional quality
    - enhance taste, appearance and fragrance
    - increase shelf-life
  - **biopharming**
    - plants as bioreactors for production of useful compounds (e.g., therapeutics, vaccines, antibodies)
  - **phytoremediation**

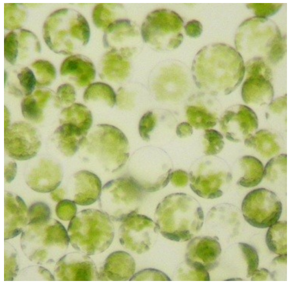
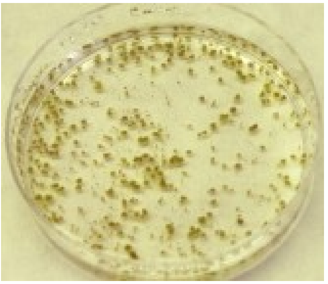
Genetic engineering of plants

- plant **transgenesis** procedure

  1. **construction** of vector/plasmid (restriction digests, ligation)
  2. **propagation** in *E.coli*
  3. **transformation**
  4. **culture** and **selection**

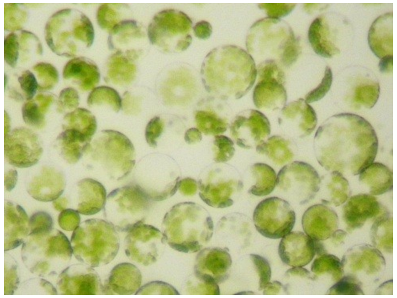


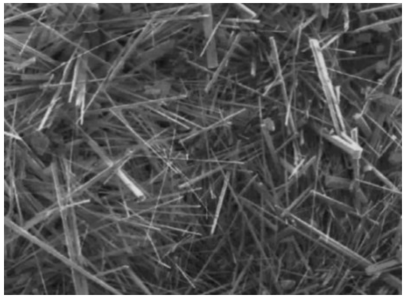
- **totipotency** - entire plant generated from a single, non-reproductive cell


Methods of plant transformation

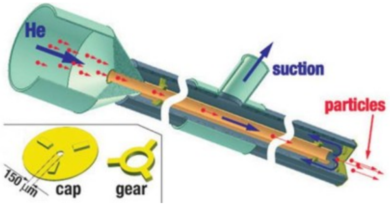
- **direct methods**
  - **protoplast polyethylene glycol (PEG) method**
    - first technique for plant transgenesis
    - PEG induces reversible permeabilization of the plasma membrane
  - **protoplast electroporation**
    - intensive electrical field leads to pores on plasma membrane
  - **silicon carbide fibers**
    - fibers punch holes through plant cells during vortexing
  - **protoplast microinjection**

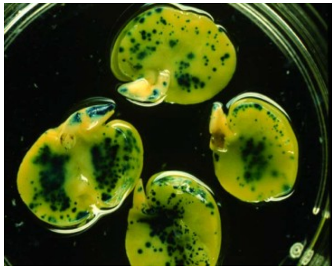




Methods of plant transformation

- **direct methods**
  - **particle bombardment**
    - most common technique for direct transformation
    - „particle gun” or „gene gun”
    - DNA precipitated onto tungsten or gold particles
    - particles shot into the plant tissue/cells

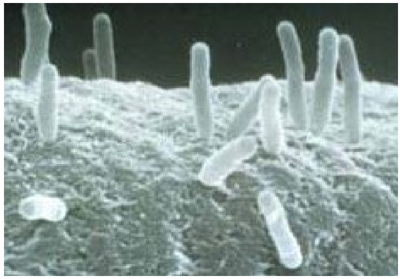


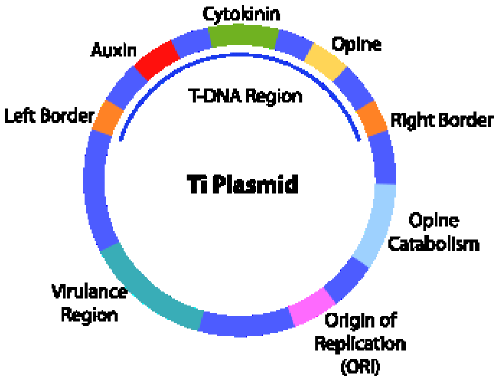


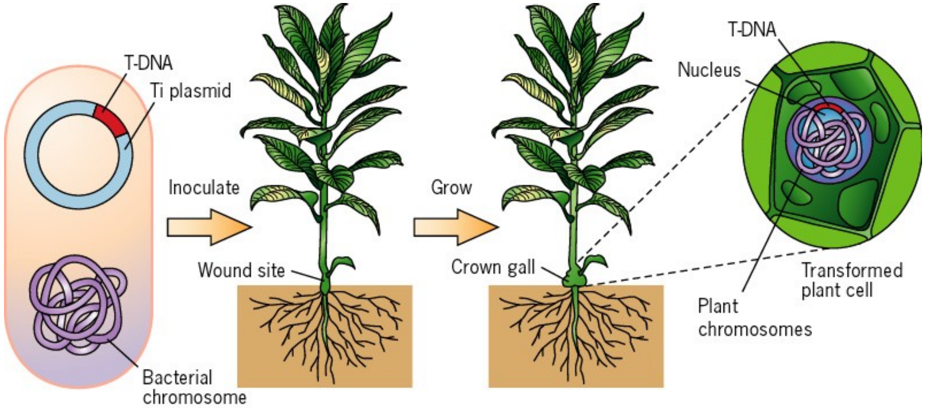
Methods of plant transformation

❑ indirect methods (vectored)

- ***Agrobacterium*-mediated transformation**
  - *A. tumefaciens* plant pathogenic bacteria causes Crown gall (tumors)
  - tumor inducing (Ti) plasmid
  - T-DNA transferred and integrated into plant cell

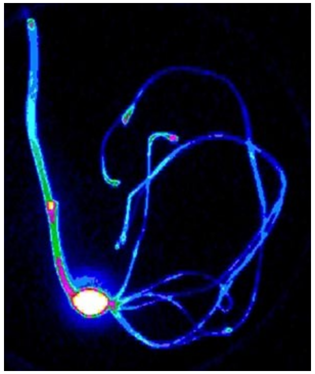






Markers and selection

- transformation frequency is low (less than 3%)
- without **selective advantage** transformed cells overgrown by non-transformed
- **selection markers**
  - antibiotics resistance (Kanamycin, Geneticin)
  - herbicides resistance (Phosphinothricin)
- **reporter genes**
  - GUS (β-glucuronidase)
  - GFP (green fluorescent protein)
  - LUC (luciferase)

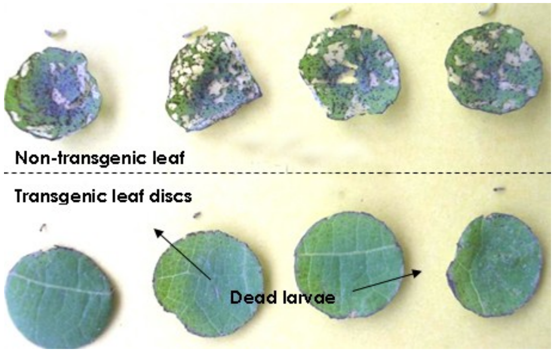


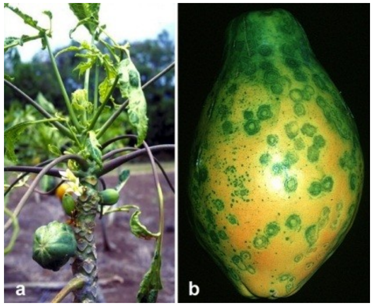
Application of transgenic plants

- **pest and disease resistance**
  - **toxin gene** from *Bacillus thuringiensis*
    - Bt-corn resistant to European corn borer
    - Bt-cotton resistant to cotton bollworm
    - Bt-peanut resistant to cornstalk borer
  - Papaya ringspot **virus resistance**

    inserting gene from pathogen into crop

    affords the crop plant resistance

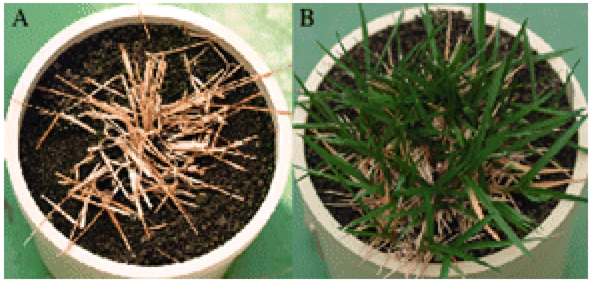


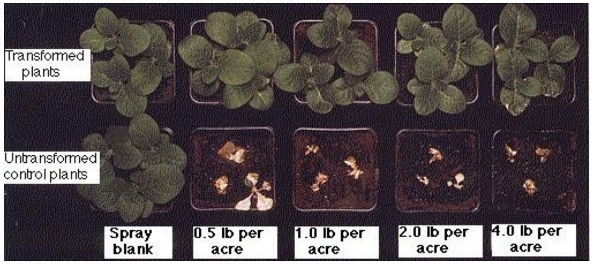


Application of transgenic plants

❑ herbicide resistance

- herbicide target modification
- herbicide target overproduction
- herbicide detoxification (enzymatic)





❑ EXAMPLES

- **sulfonylurea resistance**

  blocking the enzyme for synthesis Val, Leu, isoLeu

  mutated gene transferred from resistant tabaco

- **bromoxynil resistance**

  transgene encoding enzyme bromoxynil nitrilase

- **glyphosinate resistance**

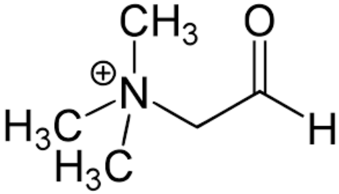
  bacterial transgene protein inactivating herbicide

Application of transgenic plants

- **resistance to environmental stress**
- marginal land or climate change induced drought
- crucial ways of securing the world's food supply

- **drought tolerance**

  - gene from *Xerophyta viscosa* - unique protein in cell membrane
  - gene for production of protective waxy cuticle on leaves
  - gene for expression of trehalose (stabilization of biomolecules)



- **salt tolerance**

  - gene for enhanced glycinebetaine production

Application of transgenic plants - idea

- crucial ways of securing the world's food supply
  - **salt tolerance**

Application of transgenic plants

- **improved crop quality**
  - **higher nutrition value**
    - **golden rice** (beta – carotene genes)

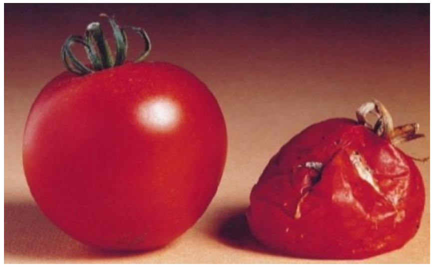
      120 million children suffers from vitamin A deficiency

      healthy vision and prevents night blindness
    - **black tomato** (anthocyanin antioxidant gene)

      prevent heart disease, diabetes and cancer
  - **improve shelf life**
    - delayed fruit ripening (FlavrSavr tomato)

      antisense gene blocking pectinase
  - **improved appearance**
    - delphinine gene from pansy cloned to rose
  - **biopharming**






The case of the FLAVR SAVR tomato

- First commercially grown genetically engineered food to be granted a license for human consumption
- Developed by californian company Calgene in the 1980s.

1998

- Dr. Arpad Pusztai - *persona non grata*

Del/Ros1N – purple tomatos

- John Innes Centre (UK)

nature biotechnology

Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors

Eugenio Butelli[1], Lucilla Titta[2], Marco Giorgio[2], Hans-Peter Mock[3], Andrea Matros[3], Silke Peterek[3], Elio G W M Schijlen[4], Robert D Hall[5], Arnaud G Bovy[4], Jie Luo[1] & Cathie Martin[1]

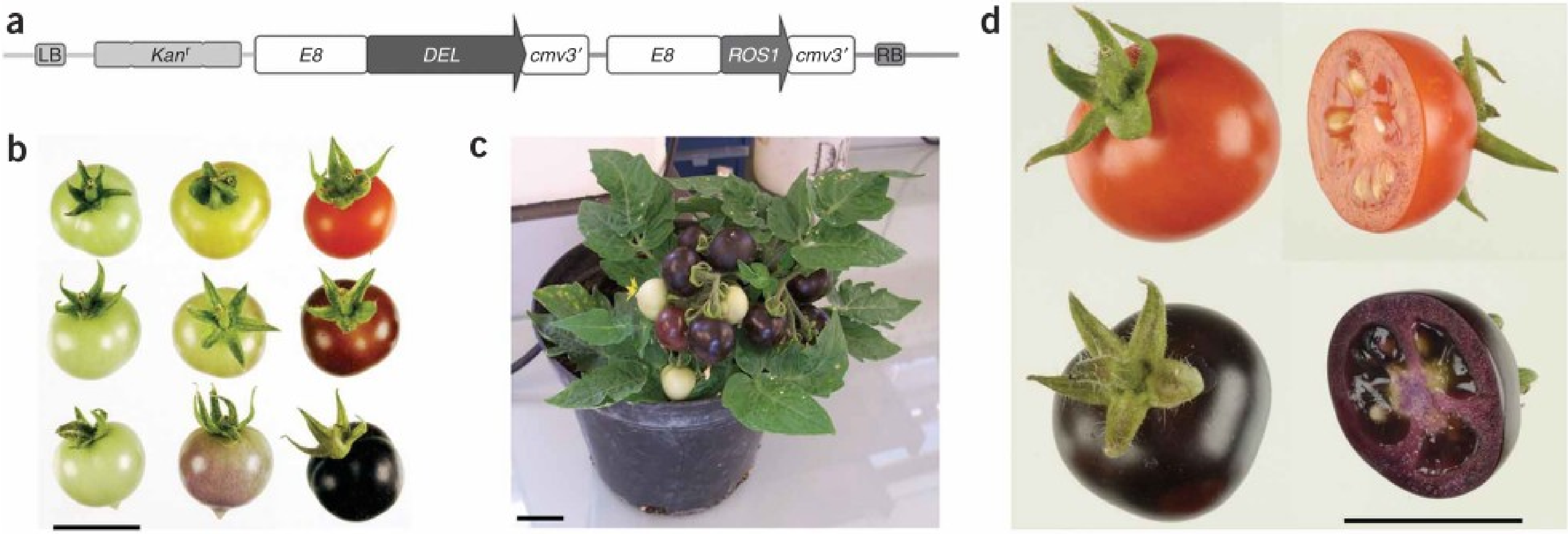


Figure 1 Fruit-specific phenotypes of T1 generation tomatoes (cv. MicroTom) expressing both *Del* and *Ros1* under the control of the *E8* promoter. (**a**) Map of T-DNA region of the binary vector used for transformation. LB, left T-DNA border region; RB, right T-DNA border region; Kan^r, *nptII* gene conferring kanamycin resistance under the control of the *nos* promoter; cmv3′, terminator region of cauliflower mosaic virus. (**b**) Phenotypic analysis of wild-type (upper row), *Del/Ros1*C (middle) and *Del/Ros1*N (lower) tomato fruit harvested at the green (left column), breaker (middle) and red (right) ripening stages. (**c**) *Del/Ros1*N tomato plant showing fruit at different stages of ripening. (**d**) Whole and cross-section of ripe wild-type and *Del/Ros1*N tomato fruit. All scale bars, 2 cm.

Break 5 min

Genetic engineering of animals

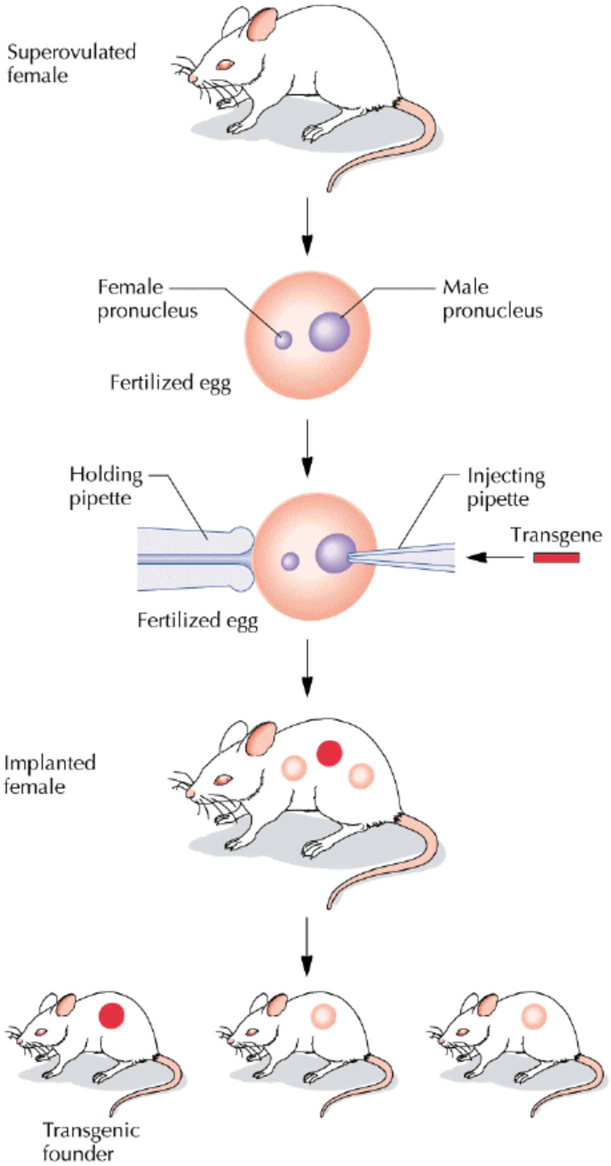
- **selective breeding**
  - time consuming and costly
  - limited number of properties available
  - difficult to introduce new genetic traits / lines

- **transgenic animals**
  - fast generation lines carrying **desired properties**
    - increased growth
    - improved disease resistance
    - improved nutritional quality
    - increased wool quality
  - **model animals** for human disease research
  - **biopharming** - production of useful molecules
  - **biosensors** for environmental pollution

Genome targeting technologies

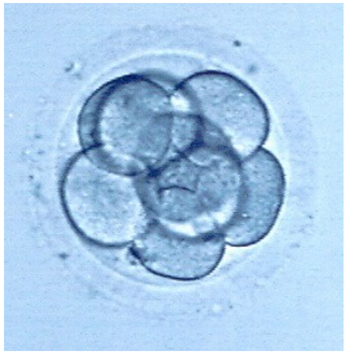
- **direct microinjection (pronucleus method)**
  - injection of desired DNA to male pronucleus
  - most popular, commercial available
  - success range from 10 to 30%
  - transfer of large genes possible
  - no theoretical limit for gene construct size
  - **random insertion of the transgene**

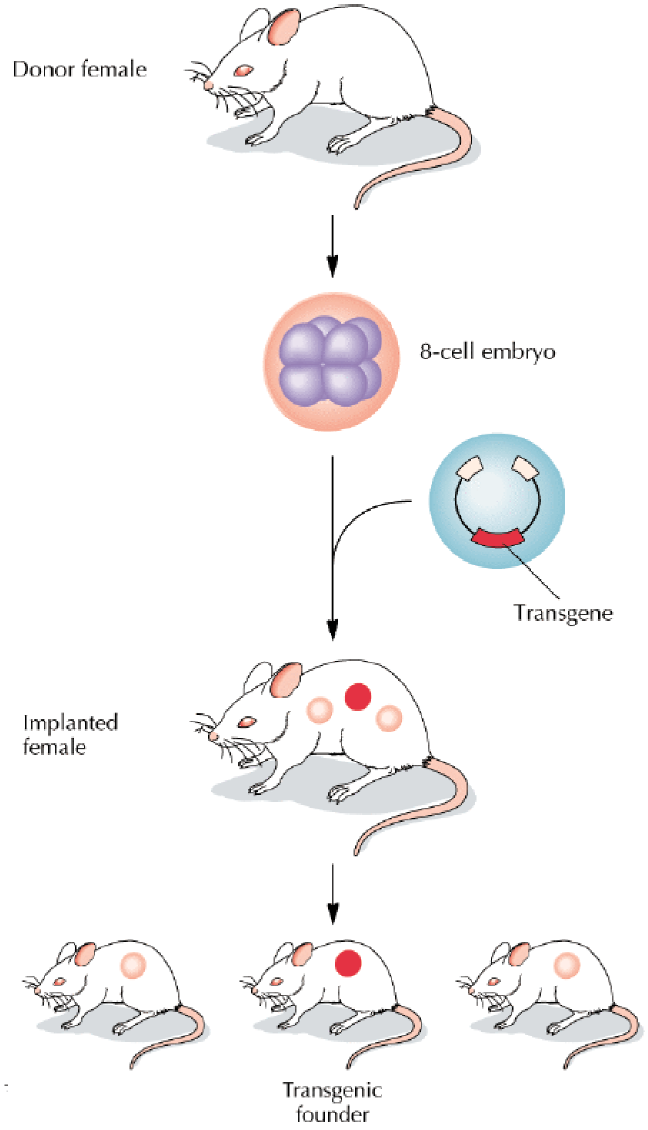
    (affecting other genes and expression patterns)



Genome targeting technologies

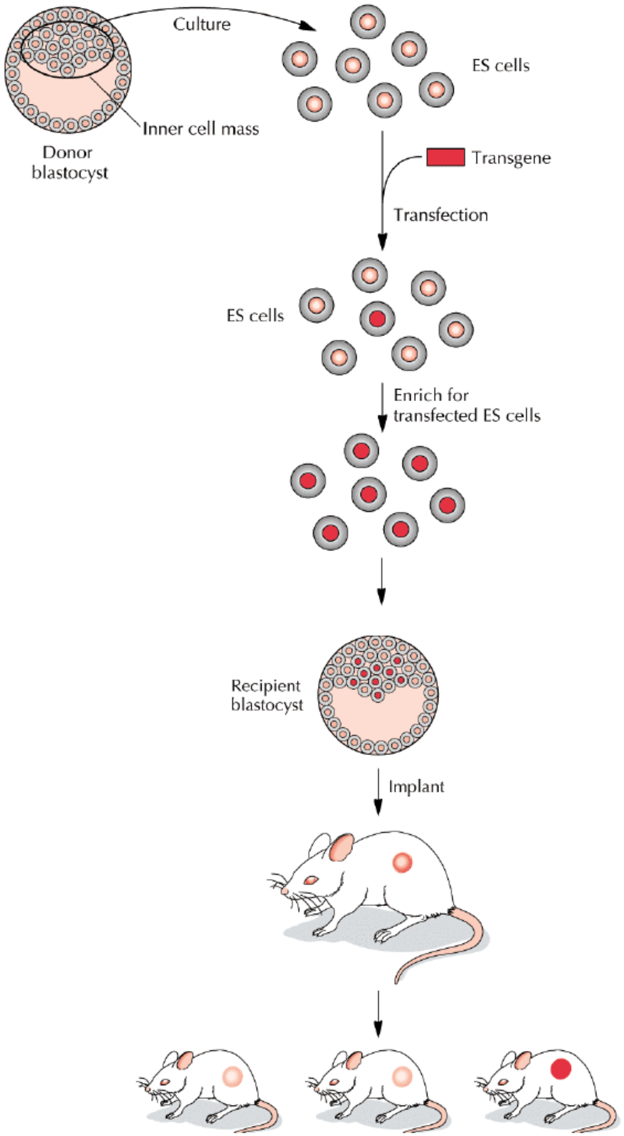
- **retrovirus mediated gene transfer**
  - retroviruses used as vectors (gene therapy)
  - virus gene is replaced by transgene
  - replication defective virus infect host cells (e.g., ES cells, embryo cells)
  - efficient mechanism of transgene integration
  - **transfer of genes < 8 kb only possible**
  - **random insertion of the transgene**





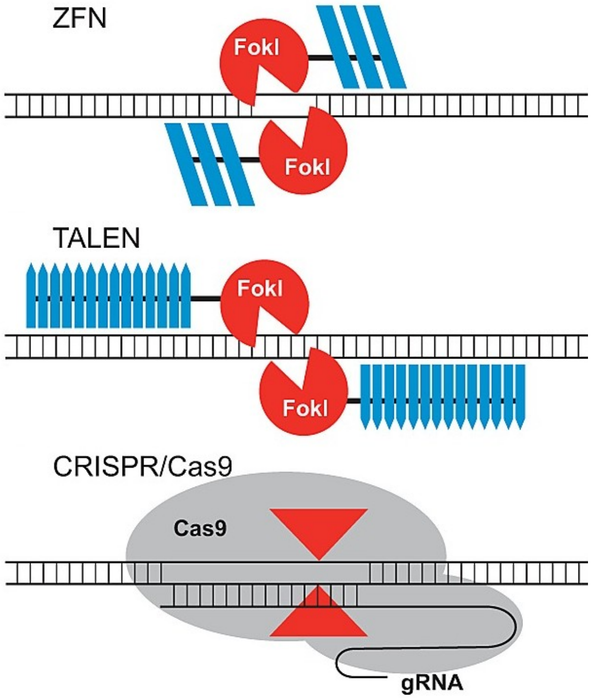
Genome targeting technologies

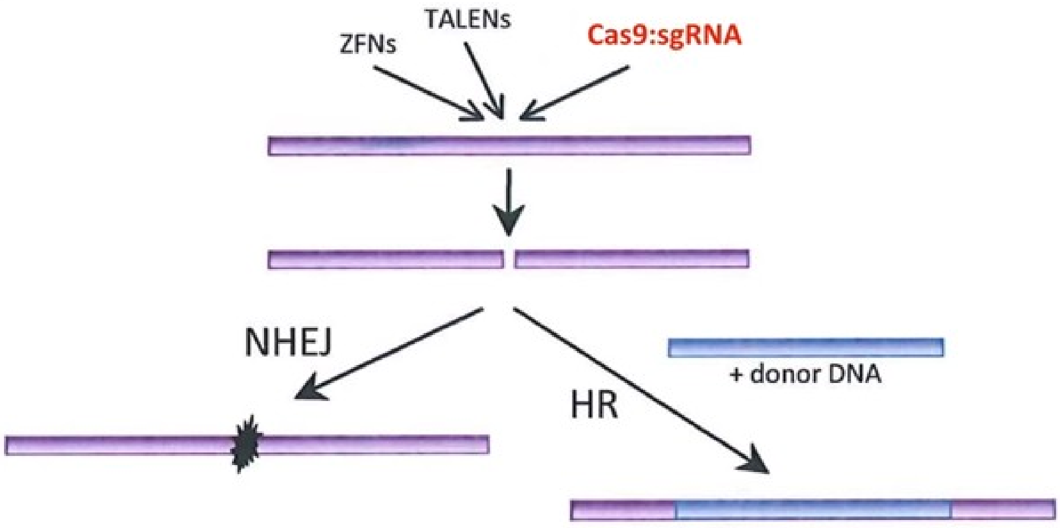
- **embryonic stem cell method**
  - transfection of gene construct into *in vitro* culture of embryonic stem (ES) cells
  - ES recombinant cells incorporated into embryo at blastocyst stage
  - **1 in a million incorporated at desired position**
  - **ES cell lines not available in farm animals**
  - **random insertion of the transgene**



Genome targeting technologies

- **engineered nucleases, „molecular scissors"**
  - site-specific double stranded breaks
  - Zinc finger nucleases (ZFNs)
  - transcription-activator like effector nucleases (TALEN)
  - RNA-guided DNA endonuclease (CRISPR-Cas9)

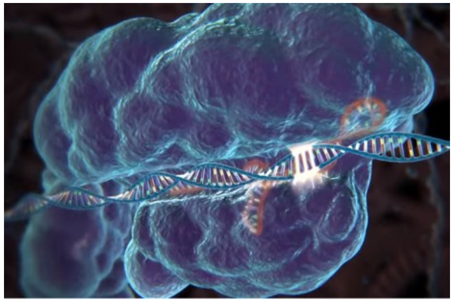
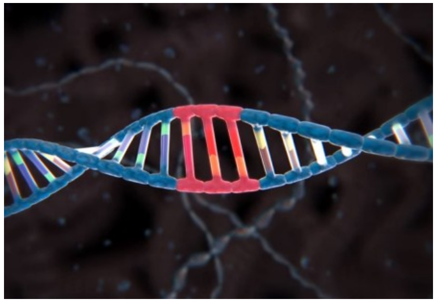
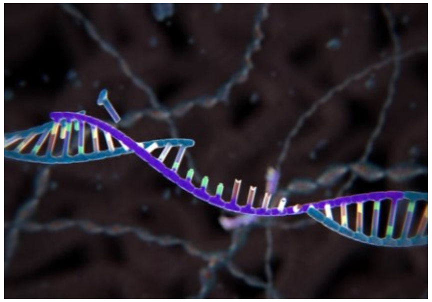
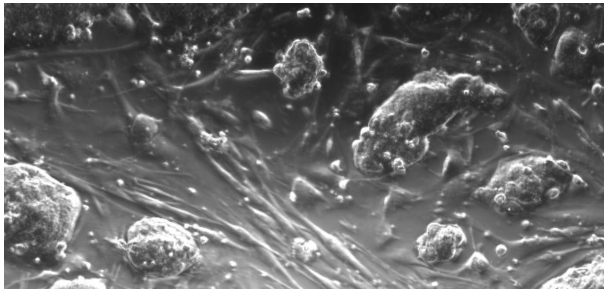
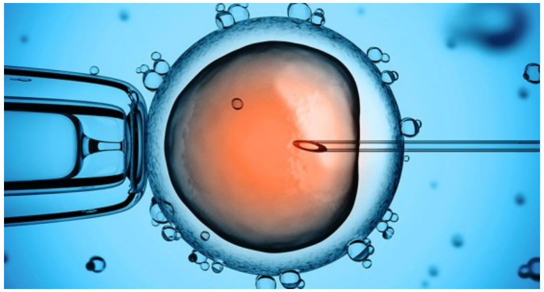




Genome targeting technologies

CRISPR-Cas9

- synthetic guide RNA (gRNA)
- delivering Cas9 nuclease complexed with gRNA into a cell
- *in vivo* (nucleus), stem cells, fertilized egg
- can target several genes at once

Application of transgenic animals

- **disease-resistant livestock**
  - *in vivo* immunization - overexpress genes encoding monoclonal antibodies
  - eliminate production of host cell components interacting with infectious agent
- **improving milk quality**
  - increase casein contents let to increase cheese production
  - decrease lactose content by overexpress lactase
  - abolish lacto globulin expression (for milk allergic consumer)
- **improving animal production traits**
  - transgenic fish - enhanced growth 3-5 times (growth hormone)
  - transgenic pig - production of omega-3-fatty acids (roundworm gene)
  - transgenic poultry - lower cholesterol and fat in eggs
- **biopharming**

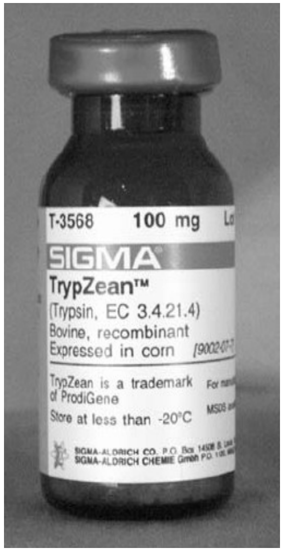
Biopharming

- use of plants or animals for the production of useful molecules
- **industrial products**
  - proteins (enzymes)
  - fats and oils
  - polymers and waxes
- **pharmaceuticals**
  - recombinant human proteins
  - therapeutic proteins and pharmaceuticals
  - vaccines and antibodies

Biopharming

- **industrial products from plants**
  - cheap and easy to produce
  - free of animal viruses
  - risk of food supply contamination
  - environmental contamination

- EXAMPLES (transgenic corn, Sigma):
  - **trypsin**
    - traditionally isolated from bovine pancreas
    - first large scale transgenic plant product
    - worldwide market = US$120 million
  - **avidin**
    - medical diagnostics
  - **β-glucuronidase**
    - visual marker in research labs



Biopharming

- **edible vaccines from plants**
  - no purification required
  - no hazards associated with injections
  - may be grown locally where needed
  - no transportation costs
  - no need for refrigeration or special storage
- EXAMPLES:
  - HIV-suppressing protein in **spinach**
  - rabies virus G protein in **tomato**
  - vaccine for rotavirus or hepatitis in **potato**

Biopharming

- **plant-made antibodies**
  - **plantibodies** - monoclonal antibodies produced in plants
  - free from potential contamination of mammalian viruses
  - plants used include tobacco, corn, potatoes, soya and rice
  - EXAMPLES: cancer, herpes simplex virus
- **plant-made pharmaceuticals**
  - therapeutic proteins and intermediates
  - EXAMPLES: proteins to treat cystic fibrosis, HIV, hypertension

Biopharming

- **production of pharmaceuticals in milk**
  - easy to purify - few other proteins in milk
  - dairy cattle produce 10,000 liters of milk/year (35 g protein/liter)
  - only few transgenic cows can meet worldwide demand
  - risk of food supply contamination
- EXAMPLES:
  - COW: human serum albumin, human lactoferrin
  - SHEEP: alpha-1-antitrypsin
  - GOAT: human antithrombin III (FDA approved), tissue plasminogen activator, malaria antigen

- **production of materials in milk**
  - BioSteel from spider silk (Nexia Biotech)

GMO benefits

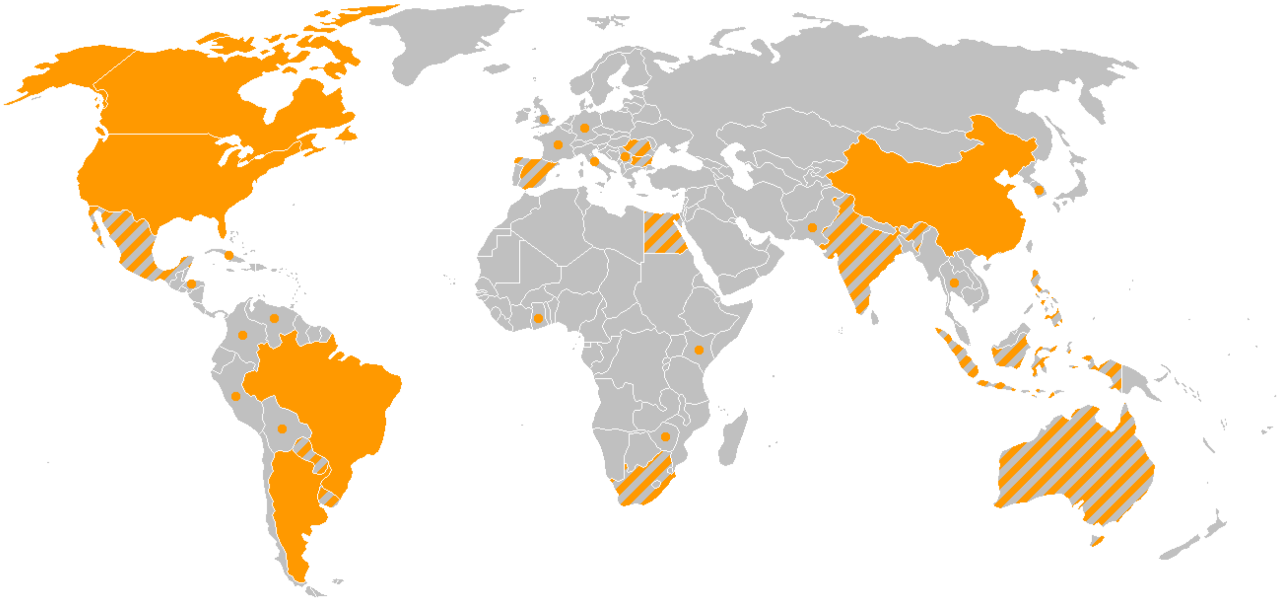
- **crops**
  - increased stress tolerance
  - improved resistance to disease, pests and herbicides
  - increased nutrients, yields, enhanced taste and quality
- **animals**
  - improved animal health, resistance, productivity and feed efficiency
  - better yields of meat, eggs, and milk
- **environment**
  - more efficient processing
  - conservation of soil, water, and energy
  - better natural waste management
- **society**
  - increased food security for growing populations
  - climate change induced drought

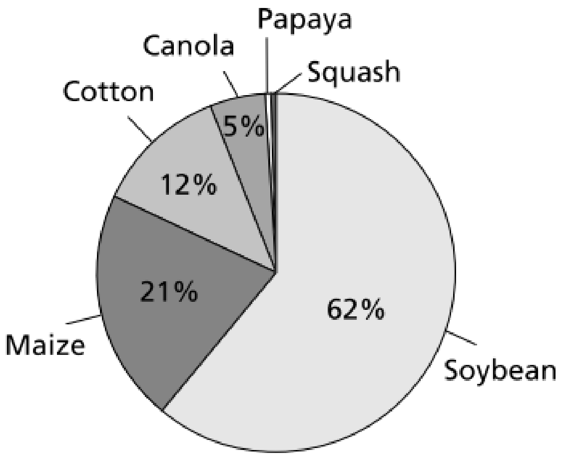
GMO controversies

- **safety**
  - human health – toxicity, allergens, antibiotic resistance, unknown effects
  - environment - unintended transfer through cross-pollination, unknown effects on other organisms, loss of biodiversity
- **ethics**
  - tampering with nature by mixing genes among species / cloning
  - violation of natural organism´s intrinsic values
  - stress for animals
- **access and intellectual property**
  - domination of world food production by few companies
  - increasing dependence on industrialized nations by developing countries

GMO future

- GMO crop first commercialized in 1996
- 17.3 million farmers grew biotech crops on 170 million hectares
- 90% of new users are small resource-poor farmers in developing countries
- EU research on risk of GMOs over the past two decades unable to detect any risks that have not yet been known from conventional agriculture*





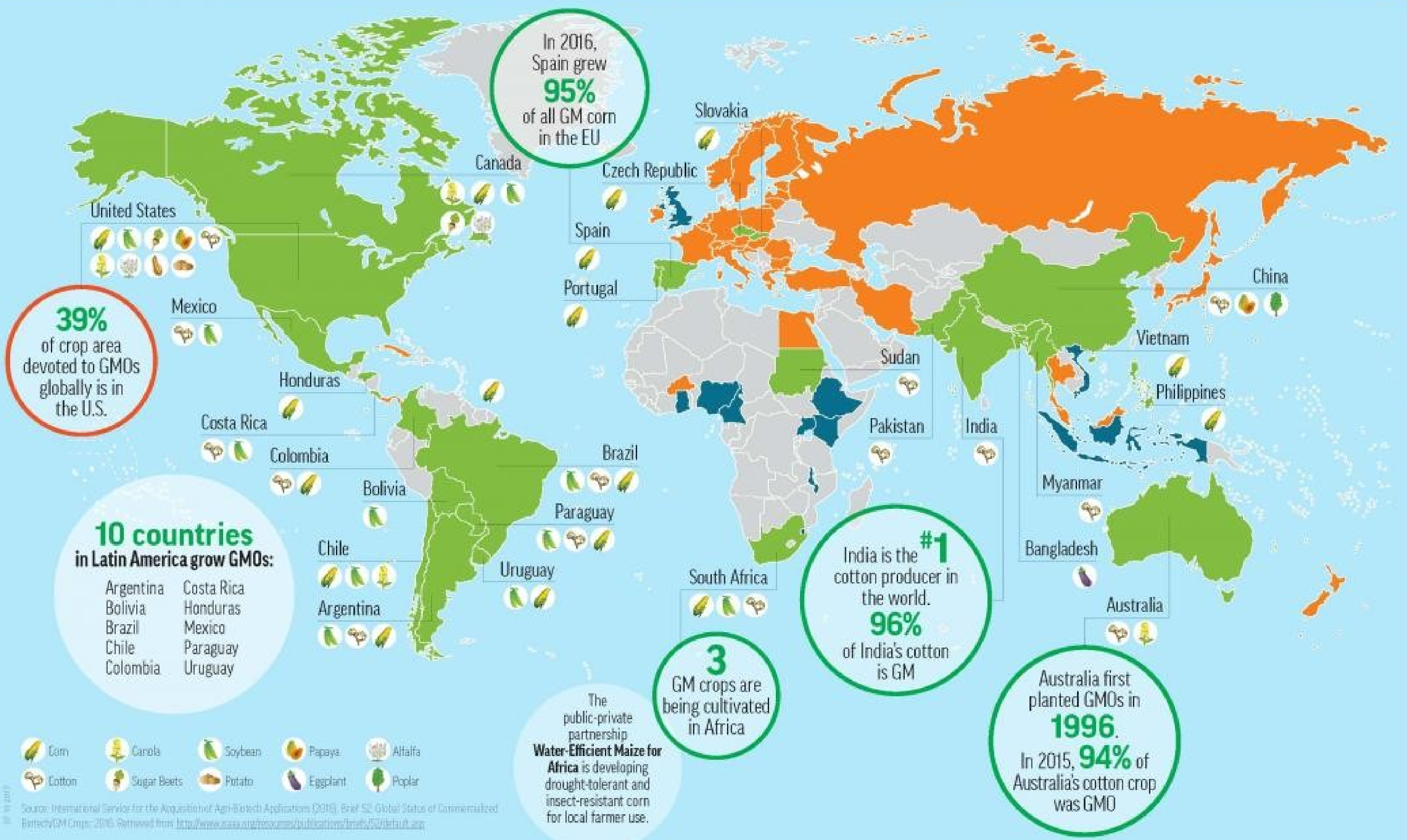
* EU Commission (2012): A Decade of EU-funded GMO Impacts Research

GMOs Around the **World**

gmo answers

18 million farmers grew GMO crops in 2016. Most were from small farms in developing countries.

26 countries grew GMOs in 2016 | **19** developing countries grew GMOs | **7** industrialized countries grew GMOs



As of 2016, GMOs are **GROWN**, **IMPORTED**, and/or used in **FIELD TRIALS** in more than **75 countries**.

GROWING BIOTECH AND GRANTING IMPORT APPROVALS

Argentina, Australia, Bangladesh, Bolivia, Brazil, Canada, Chile, China, Colombia, Costa Rica, Czech Republic, Honduras, India, Mexico, Myanmar, Pakistan, Paraguay, Philippines, Portugal, Slovakia, South Africa, Spain, Sudan, United States, Uruguay, Vietnam

GRANTING IMPORT APPROVALS

Austria, Belgium, Bulgaria, Burkina Faso, Croatia, Cuba, Cyprus, Denmark, Egypt, Estonia, France, Finland, Germany, Greece, Hungary, Iran, Ireland, Italy, Japan, Latvia, Lithuania, Luxembourg, Malaysia, Malta, Netherlands, New Zealand, Norway, Panama, Poland, Romania, Russia, Singapore, Slovenia, South Korea, Sweden, Switzerland, Taiwan, Thailand, Turkey

APPROVING RESEARCH FIELD TRIALS

Cameroon, Ethiopia, Ghana, Indonesia, Kenya, Malawi, Nigeria, Swaziland, Uganda, United Kingdom

https://gmoanswers.com/gmos-and-modern-agriculture-feeding-growing-world

Questions

Reading

- U.S. Agency for International Development, Agricultural Biotechnology Support Project II, and the Program for Biosafety Systems
- *How are Biotech Crops & Foods Assessed for Safety?, Developing a Biosafety System* (**BRIEF #5 and #6**)

BRIEF #5

agricultural biotechnology

How are Biotech Crops & Foods Assessed for Safety?

Commercially available foods and crops made using biotechnology have been subjected to more testing and regulation than any other agricultural products, and have all been found safe. This note is an introduction to the assessment processes that have been used in determining the food and environmental safety of these products.

FOOD SAFETY ASSESSMENT

Although biotechnology broadens the scope of genetic changes that can be introduced into plants used for food, it does not inherently result in foods that are less safe than those produced by other techniques. This means that previously established principles for assessing food safety still apply for products of biotechnology. Moreover, these products can be judged on their individual safety, allergenicity, toxicity and nutrition rather than their method of production