LOSCHMIDT LABORATORIES

7. Molecular Biotechnology in Agriculture

Bi7430 Molecular Biotechnology

Outline

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

Green (agricultural) biotechnology

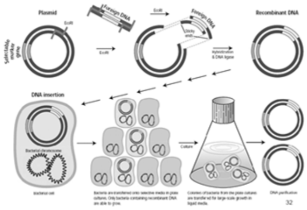
- green biotechnology applied to **agricultural processes**
- environmentally-friendly solutions as alternative to traditional agriculture, horticulture, and animal breeding processes
- modification of **plants and animals** increasing value in agriculture
  - **traditional** agriculture – selective 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

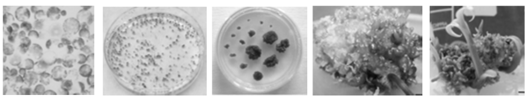
- > 150 different plant species in 50 countries worlwide
- **DNA sequence** of *A. thaliana* (2000), rice (2005), cotton (2006), corn (2009), potato (2011), tomato (2012) …
- transgenic plants engineered to
  - **overcome biotic and abiotic stress**
    - pesticides (herbicides)
    - pests and diseases (insects, viruses, bacteria, fungi)
    - environmental stress (salt, 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**

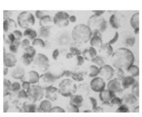
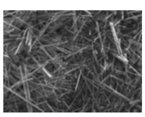
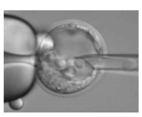


- **totipotency** - entire plant generated from a single, non-reproductive cell



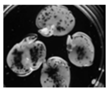
Methods of plant transformation

- DIRECT METHODS
  - **protoplast polyethyleneglycol (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**

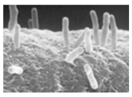
Methods of plant transformation

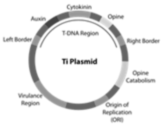
- DIRECT METHODS
  - **particle bombardment**
    - most common technique for direct transformation
    - „particle gun" or „gene gun"
    - DNA precipitated onto tungsten or gold particles
    - particles shot into the plant tissue/cells

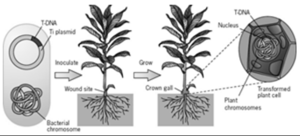


Methods of plant transformation

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

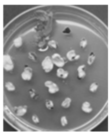


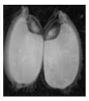


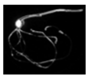


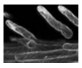
Markers and selection

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



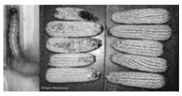


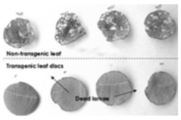


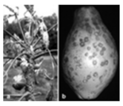


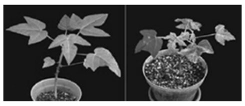
Application of transgenic plants

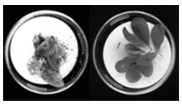
- **pest and disease resistence**
  - **toxin gene** from *Bacillus thuringiensis*
  - ***Bt-corn*** resistant to european corn borer
  - ***Bt-cotton*** resistant to cotton bollworm
  - ***Bt-peanut*** resistant to cornstalk borer
  - **Papaya ringspot virus resistence**
    inserting gene from pathogen into crop
    affords the crop plant resistance









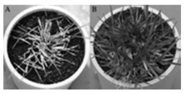


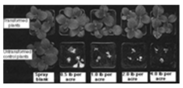
Application of transgenic plants

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

EXAMPLES:

- **sulfonylurea resistance**
  blocking the enzyme for synthesis Val, Leu, isoLeu
  mutated gene transfered from resistent tabaco
- **bromoxynil resistance**
  transgene encoding enzyme bromoxynil nitrilase
- **glyphosinate resistence**
  bacterial transgene protein inactivating herbicide



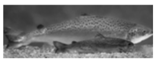


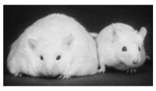
Application of transgenic plants

- **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 tomatoe** (anthocyanin antioxidant gene)
    - prevent heart disease, diabetes and cancer
  - **improve shelf life**
    - delayed fruit rippening (FlavrSavr tomatoe)
      antisense gene blocking pectinase
  - **improved appearance**
    - delphinidin gene from pansy cloned to rose
  - **biopharming**

Genetic engineering of animals

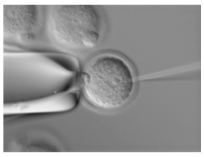
- **selective breeding**
  - time **consuming** and **costly**
  - **limited** number of properties available
  - **difficult** to introduce new genetic traits to established line
- **transgenic animals**
  - fast generation lines carrying **desired properties**
    - increased growth
    - increased muscle mass
    - improved disease resistance
    - improved nutritional quality
    - increased wool quality
  - **model animals** for human disease research
  - **biopharming** - production of useful molecules
  - **biosensors** for environmental polution

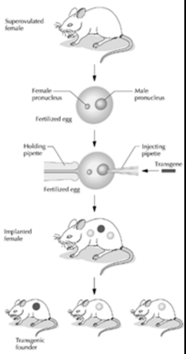




Methods to produce GE animals

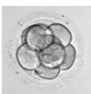
- **direct microinjection (pronucleus method)**
  - injection of desired DNA to male pronucleus
  - most popular, commercial available
  - success range from 10 to 30%
  - transfer of large genes possible, no theoretical limit for gene construct size
  - random insertion of the transgen

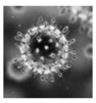


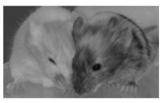


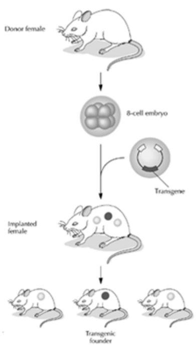
Methods to produce GE animals

- **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 transgen integration
  - transfer of genes < 8 kb possible
  - random insertion of the transgen



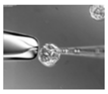


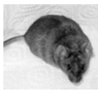


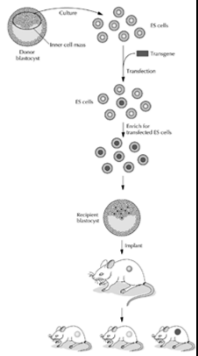


Methods to produce GE animals

- **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
  - targeted **insertion** or **knockout** of gene(s) prior to microinjection
  - ES cell lines not available in farm animals
  - has revolutionized genetics, development, immunology and cancer research in mice







Application of transgenic animals

- **disease-resistant livestock**
  - ***in vivo* immunization**: overexpress genes encoding monoclonal antibodies
  - eliminate production of host cell components that interact with infectious agent
- **improving milk quality**
  - **increase casein** contents let to increase cheese production
  - **decrease lactose** content by overexpress lactase
  - abolish lactoglobulin 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 (roundwarm gene)
  - **transgenic poltry**: 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):

- **avidin**
  - medical diagnostics
- **β-glycuronidase**
  - visual marker in research labs
- **trypsin**
  - traditionally isolated from bovine pancreas
  - first large scale transgenic plant product
  - worldwide market = US$120 million

Biopharming

EDIBLE VACCINES FROM PLANTS

- no purification required
- no hazards associated with injections
- may be grown locally, where needed most
- no transportation costs
- no need for refrigeration or special storage

EXAMPLES:

- HIV-suppressing protein in spinach
- rabies virus G protein in tomato
- human vaccine for hepatitis B 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, respiratory syncytial virus

PLANT-MADE PHARMACEUTICALS

- therapeutic proteins and intermediates

EXAMPLES: proteins to treat CF, HIV, hypertension, hepatitis B

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**
  - enhanced taste and quality, reduced maturation time
  - increased nutrients, yields, and stress tolerance
  - improved resistance to disease, pests, and herbicides
- **ANIMALS**
  - better yields of meat, eggs, and milk
  - improved animal health, resistance, productivity, and feed efficiency
- **ENVIRONMENT**
  - more efficient processing
  - conservation of soil, water, and energy
  - better natural waste management
- **SOCIETY**
  - increased food security for growing populations

GMO controversies

- **SAFETY**
  - human health : allergens, antibiotic resistance, unknown effects
  - environment: unintended transfer through cross-pollination, unknown effects on other organisms, loss of biodiversity
- **ACCESS AND INTELLECTUAL PROPERTY**
  - domination of world food production by few companies
  - increasing dependence on industrialized nations by developing countries
- **ETHICS**
  - violation of natural organism´s intrinsic values
  - tampering with nature by mixing genes among species
  - objections to consuming animal genes in plants and vice versa
  - stress for animals

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-funded risk research on 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.