UBAYA
UNIVERSITAS SURABAYA

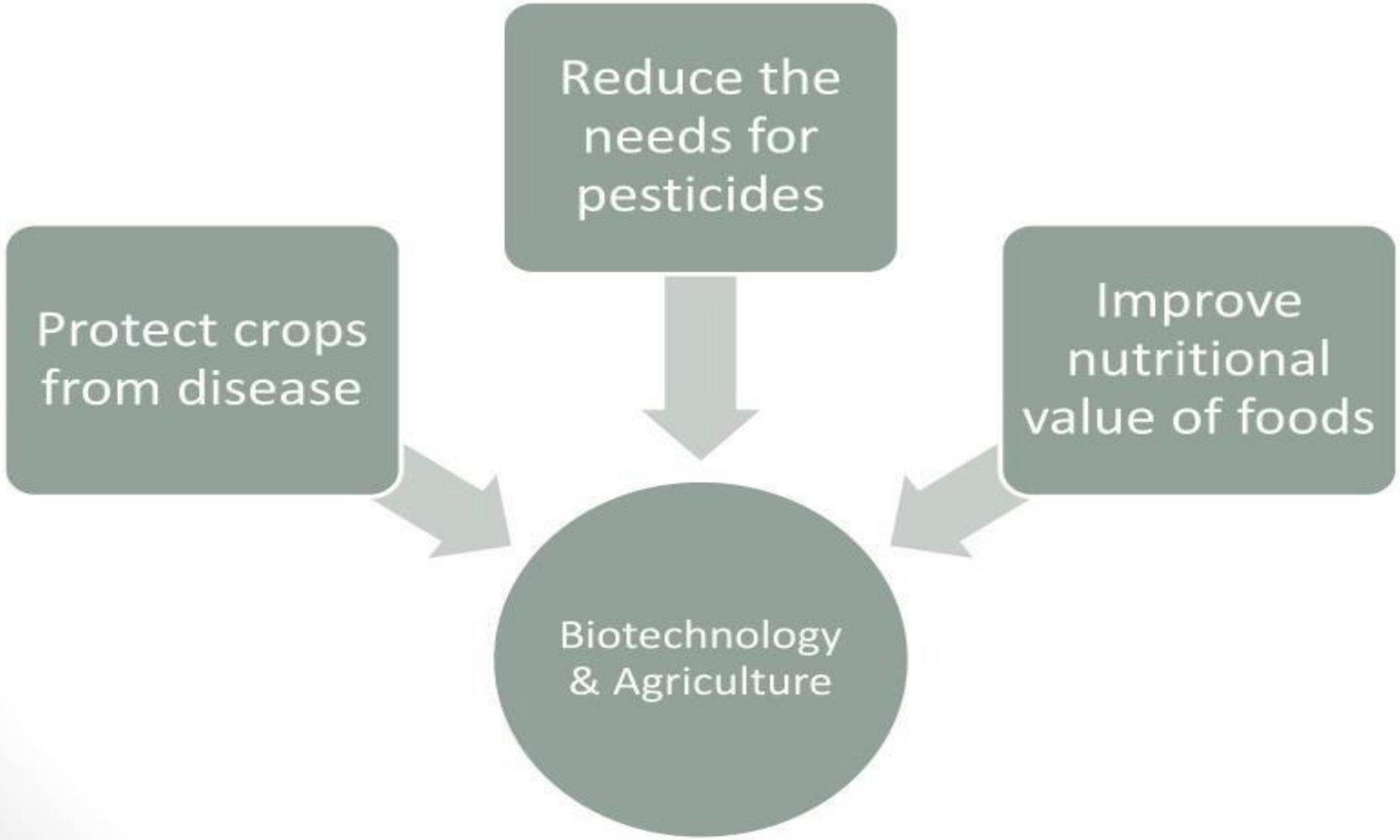
Introduction of Plant Biotechnology

Johan Sukweenadhi, Ph.D.
Faculty of Biotechnology

Chapter Contents

1. Agriculture: The Next Revolution
2. Methods Used in Plant Transgenesis
3. Practical Applications in the Field
4. Health and Environmental Concerns

Biotechnology and Agriculture



1. Agriculture: The Next Revolution

- **Plant Transgenesis** – transferring genes to plants directly
  - Development of plant vaccines, plants that produce their own pesticides and are resistant to herbicides
- For eg: thru classical breeding, the average strength of cotton fiber increasing by about 1.5%. By inserting a single gene, the strength increased by 60%.
- 17 countries are growing more than 200 million acres of crops improved through biotechnology (GMO)
- Besides food and feed crops, plant-produced vaccines, bioplastics and enhanced phytoremediation plants are also explored.
- Focus of considerable controversy
- Hunger in some part of the world- more productive and nutritious crops
- Some sectors concerned that experimentation could be harmful to the environment and human health.
-

2. Methods Used in Plant Transgenesis

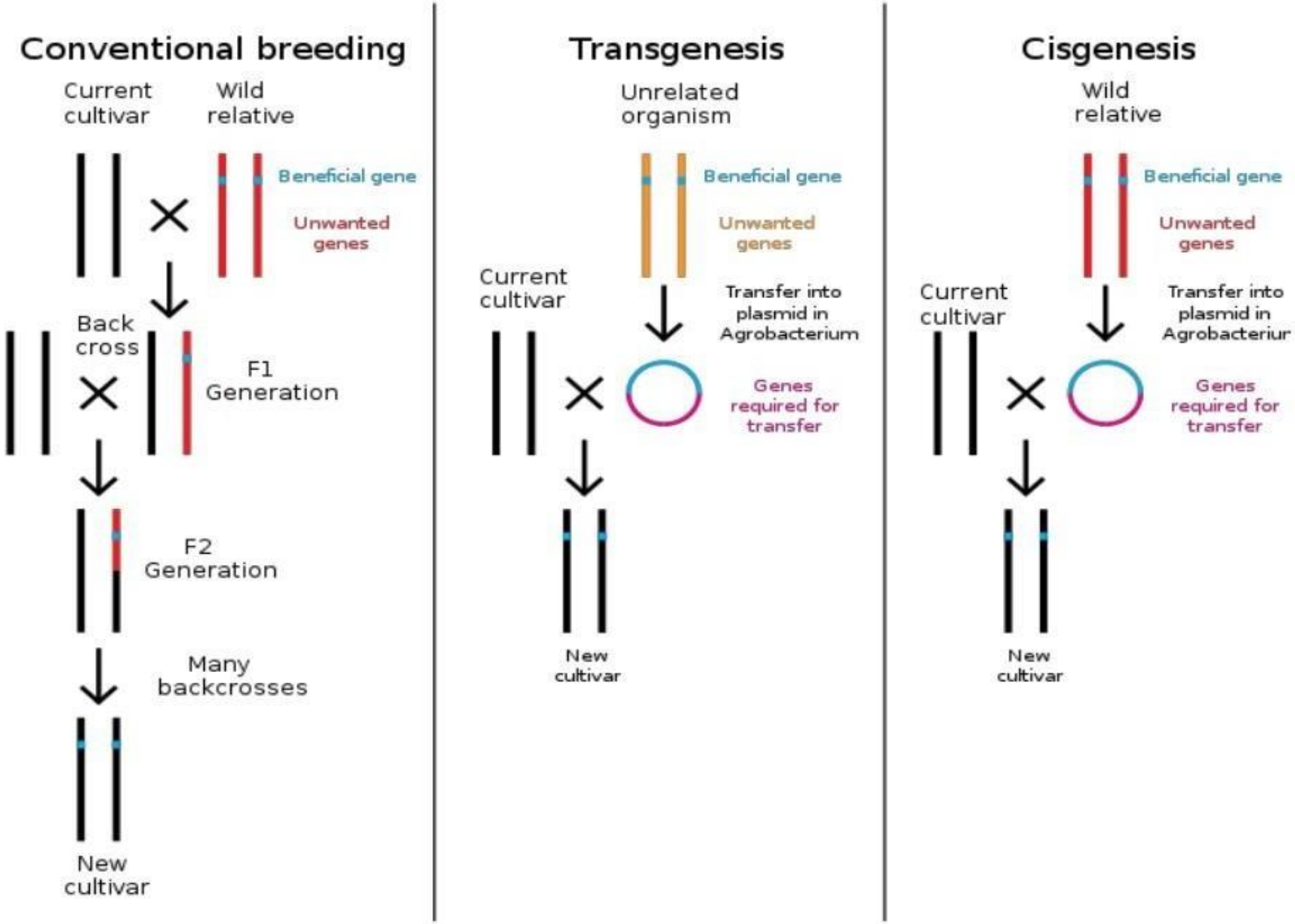
- Conventional Selective Breeding and Hybridization
- Cloning
  - Protoplast fusion
  - Leaf fragment technique
  - Gene guns
  - Chloroplast engineering
  - Antisense technology

- **Definition:** Transgenesis is the process of inserting a gene from one source into a living organism that would not normally contain it. The gene may be transfered from within the same species or come from another species

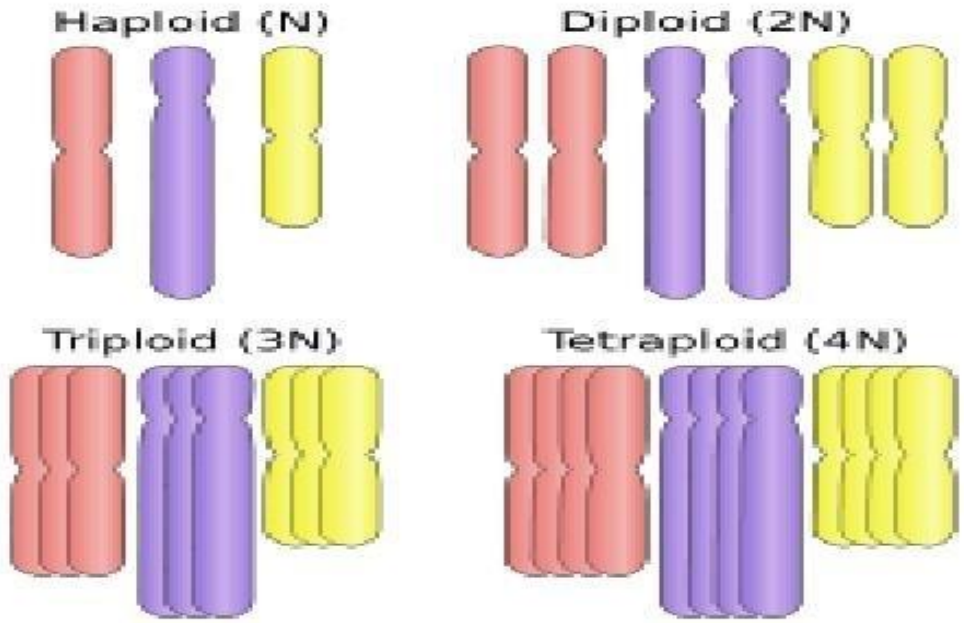
2. Methods Used in Plant Transgenesis

1. Conventional Selective Breeding and Hybridization

- Sexual cross between two lines and repeated backcrossing between hybrid offspring and parent
  - Take very long time
- Polyploid plants (multiple chromosome sets)
  - Increases desirable traits, especially size
  - Whole chromosomes can be transferred rather than single genes


Conventional breeding
Current cultivar
Wild relative
Beneficial gene
Unwanted genes
Back cross
F1 Generation
F2 Generation
Many backcrosses
New cultivar
Transgenesis
Unrelated organism
Beneficial gene
Unwanted genes
Current cultivar
Transfer into plasmid in Agrobacterium
Genes required for transfer
New cultivar
Cisgenesis
Wild relative
Beneficial gene
Unwanted genes
Current cultivar
Transfer into plasmid in Agrobacteriur
Genes required for transfer
New cultivar

Polyploidy



2. Methods Used in Plant Transgenesis

2. **Cloning** – growing plants from a single cell

2.1. **Protoplast fusion** is the fusion of two protoplast cells from different species

- Protoplast cell is a callus cell whose cell wall has been dissolved by the enzyme cellulase
- Fusion of the two protoplast cells creates a cell that can grow into a hybrid plant
- Examples include broccoflower

Somatic hybridization technique

1. isolation of protoplast

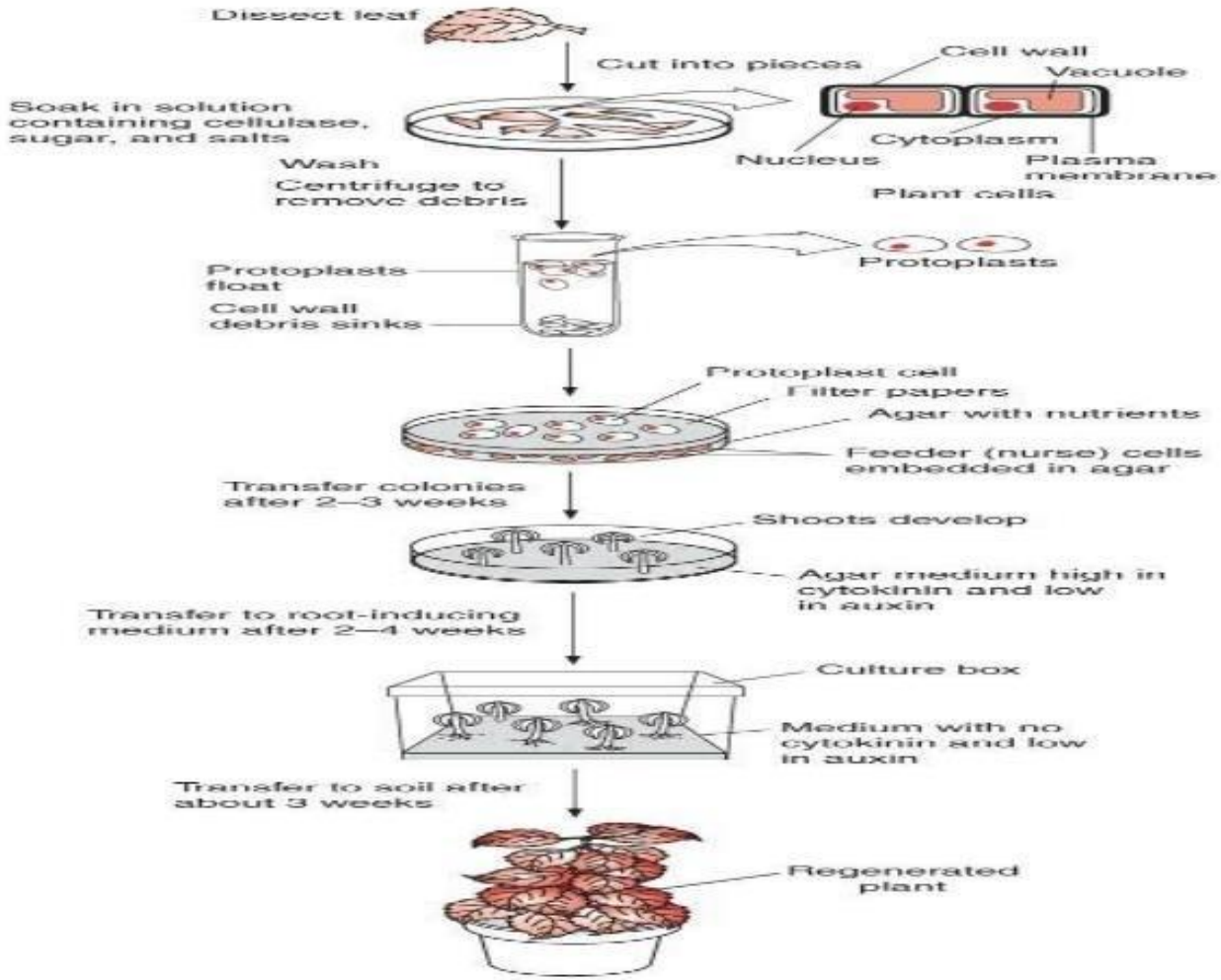
2. Fusion of the protoplasts of desired species/varieties

3. Identification and Selection of somatic hybrid cells

4. Culture of the hybrid cells

5. Regeneration of hybrid plants

2. Methods Used in Plant Transgenesis



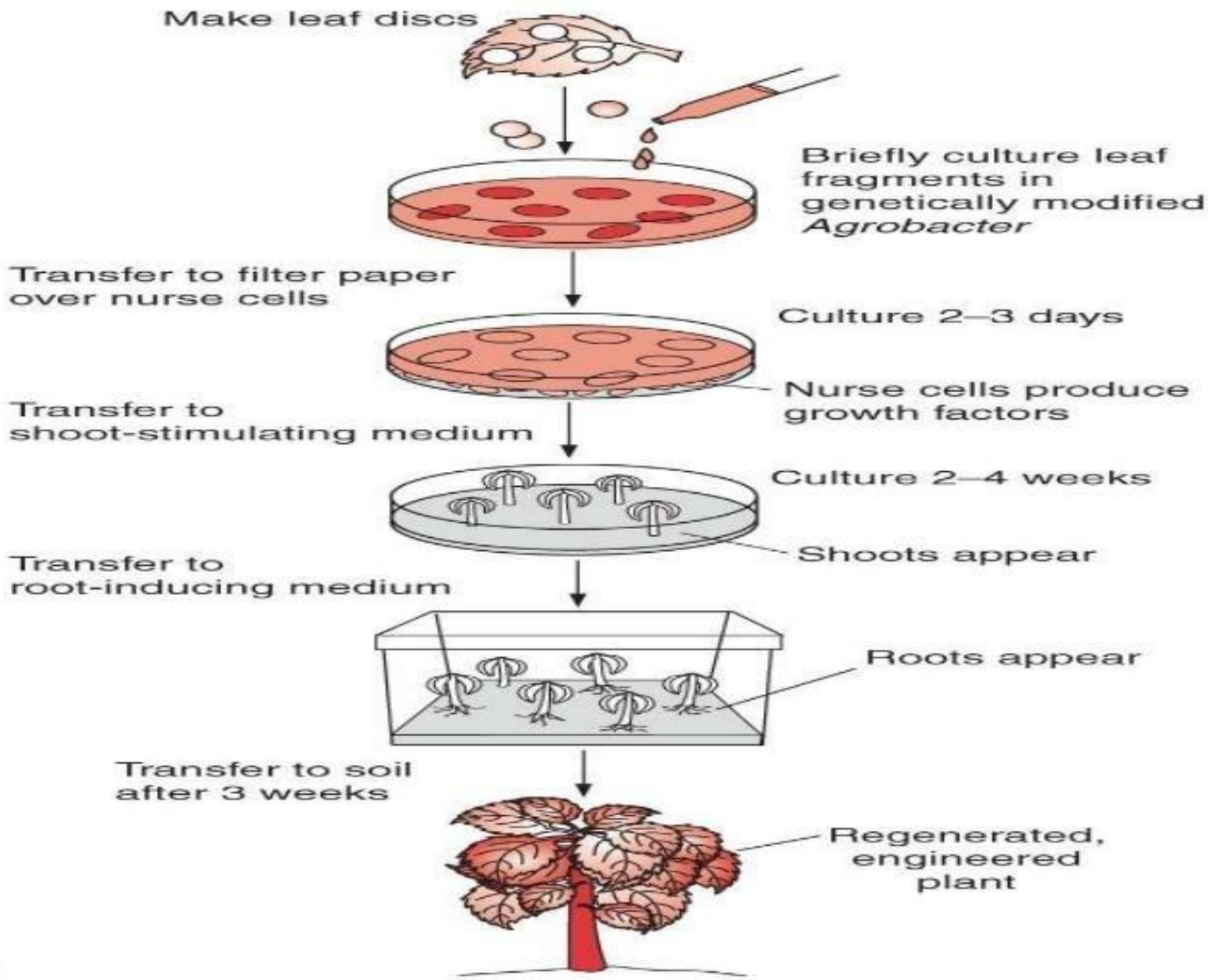
2. Methods Used in Plant Transgenesis

2. Cloning

2.1. Leaf fragment technique

- Small discs are cut from leaf
- Cultured in a medium containing genetically modified *Agrobacter (Agrobacterium tumefaciens)*
  - A soil bacterium that infects plants
  - Bacterium contains a plasmid, the TI plasmid, that can be genetically modified
  - DNA from the TI plasmid integrates with DNA of the host cell
- Leaf discs are treated with plant hormones to stimulate shoot and root development

2. Methods Used in Plant Transgenesis



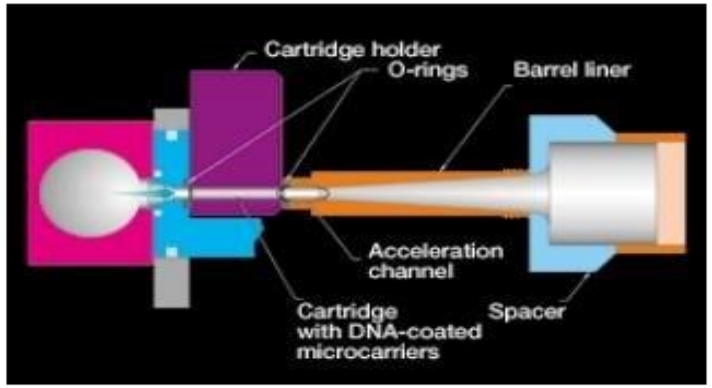
2. Methods Used in Plant Transgenesis

2. Cloning

3.2. Gene Guns

- Used to blast tiny metal beads coated with DNA into an embryonic plant cell
- Aimed at the nucleus or the chloroplast
- Use marker genes to distinguish genetically transformed cells
  - pesticides resistance

- **Gene Guns**


Cartridge holder
O-rings
Barrel liner
Acceleration channel
Cartridge with DNA-coated microcarriers
Spacer

How the Gene Gun Works:

- The gene gun is part of a method called the biolistic (bioballistic) method
- DNA (or RNA) become “sticky,” adhering to biologically inert particles such as metal atoms (usually tungsten or gold).
- By accelerating this DNA-particle complex in a partial vacuum and placing the target tissue within the acceleration path, DNA is effectively introduced.

2. Methods Used in Plant Transgenesis

2. Cloning

2.4. Chloroplast engineering

- DNA in chloroplast can accept several new genes at once
- High percentage of genes will remain active
- DNA in chloroplast is completely separate from DNA released in pollen – no chance that transformed genes will be carried on wind to distant crops

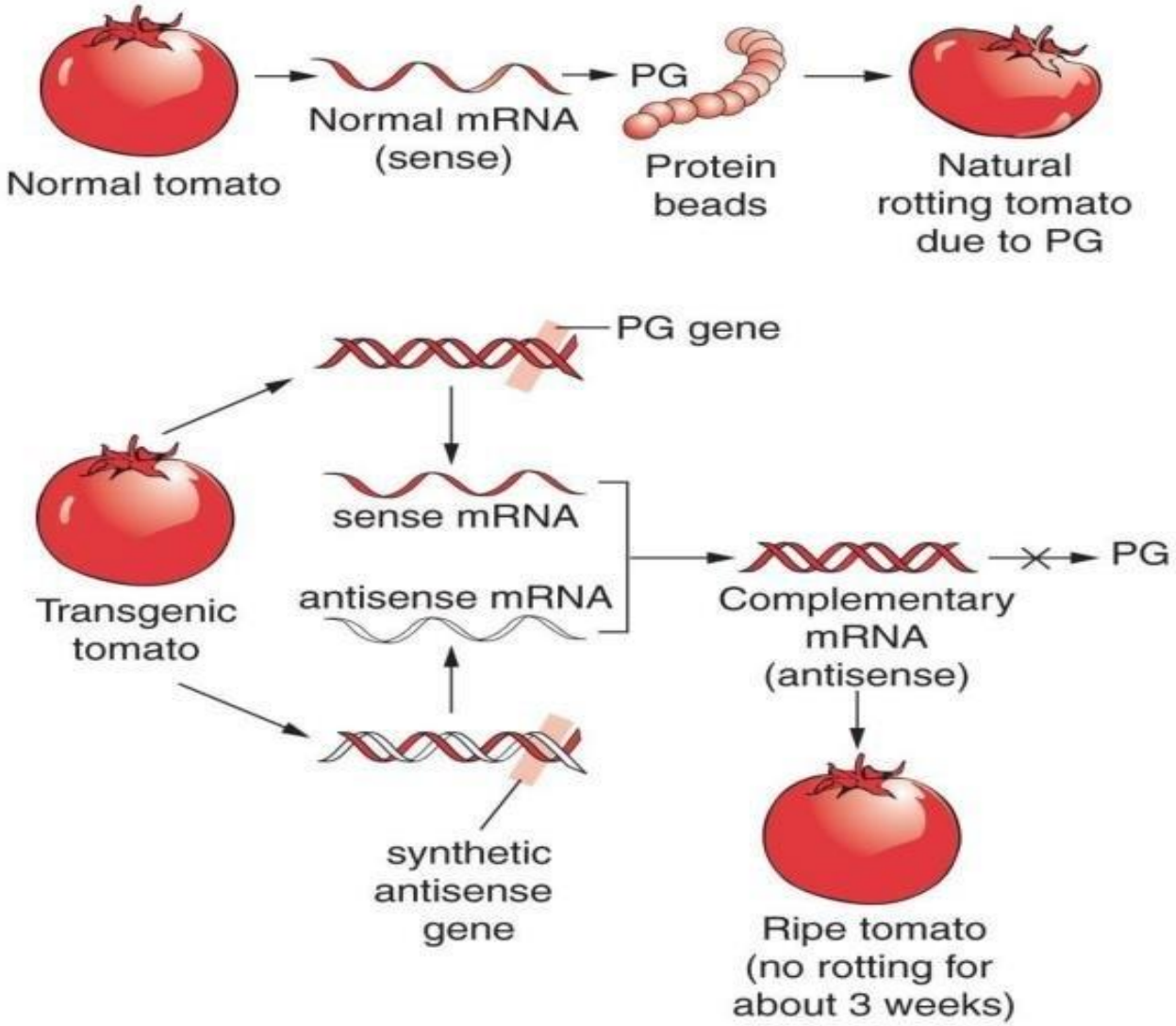
2. Methods Used in Plant Transgenesis

2. Cloning

2.5. Antisense technology

- Process of inserting a complementary copy of a gene into a cell
- Gene encodes an mRNA molecule called an antisense molecule
- Antisense molecule binds to normal mRNA (sense molecule) and inactivates it
- Example is *Flavr Savr* tomato

6.2 Methods Used in Plant Transgenesis



3. Practical Applications in the Field

1. Vaccines for plants
2. Genetic pesticides
3. Safe storage
4. Herbicide resistance
5. Stronger fibers
6. Enhanced nutrition
7. The future: from pharmaceuticals to fuel
8. Metabolic engineering

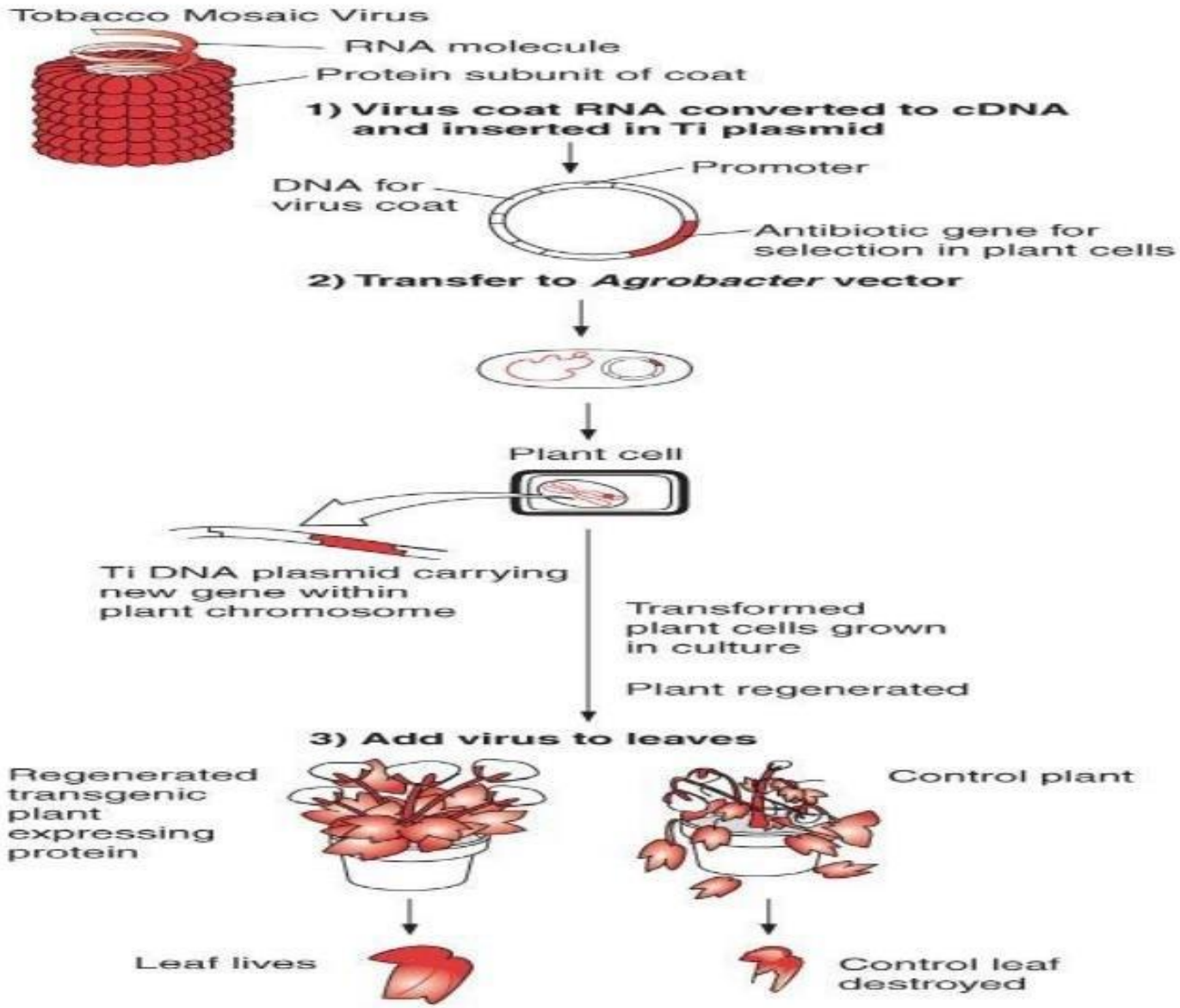
3. Practical Applications in the Field

1. **Vaccines for Plants**
   - Crops are vulnerable to wide variety of plant viruses. Infections: reduced growth rate, poor crop yield & low crop quality.

   Solution:
   - Vaccine is encoded in a plant's DNA.
   - For example, a gene from Tobacco Mosaic Virus (TMV) inserted into tobacco plants
     - Protein produced from the viral gene stimulates the plant's immune system
     - Plant is immune to the virus.
- Genetic vaccines have already proven in a wide variety of crops.

- Disease and pest-resistant potatoes offer many advantages to both consumers and farmers.

3. Practical Applications in the Field



3. Practical Applications in the Field

2. Genetic Pesticides

- *Bacillus thuringiensis* (Bt) is a bacterium that produces a protein that kills harmful insects and their larvae
- The crystalline protein (from *Chry* genes) deteriorates the cementing substance that fuses the lining cells of digestive tracts of certain insects.
- The insects die in short period of time due to 'auto-digestion'.
- The *Cry* gene that caused this event is used in the insect-resistant genetically engineered plants.
- Bt genes can be inserted into a plant's DNA.
  - Creates a built-in defense against certain insects.
  - Have been successfully introduced into tobacco, tomato, corn and cotton.

3. Practical Applications in the Field

3.Safe Storage

- Millions of dollars are lost every year due to insect infestations of crops during storage.
- Such damage is devastating in developing country with scarce food supplies.
- Transgenic corn highly resistant to pests during storage
  - **Avidin** found in egg whites – the protein blocks the availability of biotin, a vitamin required by insects to grow

3. Practical Applications in the Field

4. Herbicide Resistance

- Traditional weed killers or herbicides tend to kill desirable plants along with the weeds.
- Genetically engineer crops to be resistant to common herbicides such as **glyphosphate**.
- This herbicide works by blocking an enzyme required for photosynthesis.
- Thru bioengineering, scientists have developed transgenic crops that produce alternate enzyme that is not affected by glyphosphate.
- Allows farmers to control weeds with chemicals that are milder and more environmentally friendly than typical herbicides.
- Successful in soybeans and cotton.

3. Practical Applications in the Field

5. Stronger Fibers

- Classical breeding has only been able to increase the average strength by 1.5%
- Biotechnology increased the strength of one variety of cotton by 60%
- Stronger fiber = Softer, more durable clothes for consumers
- Greater profits for farmers

3. Practical Applications in the Field

6. Enhanced nutrition

- the most potential Benefits of biotechnology: opportunity to save millions of life from the effects of malnutrition.
- Potential crop against malnutrition- golden rice (genetically modified to produce large amounts of β- carotene, a vitamin that the body converts to vitamin A).
- According to recent estimates- 500,000 children in the world will become blind because of vitamin A deficiency.
- Adding the nutrient to food supply would be more efficient and in theory more effective.
- However, there are limitations- vit A dissolved in fats therefore the golden rice must dissolve in fats before can be used by the body. Those who do not get enough fat in their diets may not be able to reap the full benefit of golden rice.

3. Practical Applications in the Field

3. Practical Applications in the Field

- The Future: From Pharmaceuticals to Fuel
  - Plants can be ideal protein factories- a single field of transgenic crop can produce a large amounts of proteins.
  - Used to grow medicines
    - Vaccines for humans, antibodies, human insulin
    - Plants expresses the proteins and it is eaten with the plant.
  - Plant-based petroleum for fuel, alternatives to rubber, nicotine-free tobacco, caffeine-free coffee, biodegradable plastics, stress-tolerant plants.
  - Lignocellulose materials → biofuel

3. Practical Applications in the Field

- Metabolic Engineering
  - Manipulation of plant biochemistry to produce non-protein products or to alter cellular properties
- Products:
- Alkaloids such as quinine (drug still in common use)
- lipids- long chain polyunsaturated fatty acids to reduce cholesterol in foods
- Polyterpenes- new kind of rubber or aromatic components such as S-linalool (the enticing aroma in fresh tomatoes)
- pigment production- blue delphinidin in flowers, and
- biodegradable plastics
  - Challenges: Involves transfer of more than one gene and more finite regulation

4. Health and Environmental Concerns

4. Health and Environmental Concerns

- People have been worried about potential harmful effects of transgenic crops to human and environment.
- For eg: In 2000, people stop buying the GM-potatoes (engineered to be pest resistance) although there are no reports stated that they are dangerous.
- This GM-potatoes look and taste just like normal potatoes with less usage of chemicals to get the grow.
- **Concerns on Human Health**
  - Opponents fear the effects of foreign genes, bits of DNA not naturally found in plants
  - Soybeans that contain the gene from brazil nut could trigger allergic reaction to people who were sensitive to brazil nut.
    - Antibiotic-resistance marker genes could spread to disease-causing bacteria in humans
    - Cause cancer
  - To date, science has not supported any of these concerns

4. Health and Environmental Concerns

- Environmental Concerns
  - Genes for pest or herbicide resistance could spread to weeds
  - Since many crops like sunflowers and canola are close relatives to weeds, crossbreeding occasionally occurs, allowing the genes from one plant to mix with the genes of the other.
  - Few experts predict this will happen; further studies are needed.
  - However: Biotechnology can reduce the use of chemical pesticides. According to National Center for Food and Agriculture Policy: farmers who planted biotechnologically enhanced cotton were able to cut back their use of chemical pesticides by more than 1 million pounds.

4. Health and Environmental Concerns

- Regulation
  - FDA regulates foods on the market
  - USDA oversees growing practices
  - EPA controls use of Bt proteins and other pesticides

Benefits gained from the applications of genetically modified organisms (GMO) in environment, consumers and farmers.

Environmental benefits

- Reduce the needs of pesticides since plants already have the ability to protect themselves against pests and diseases.
- Decreases of water usage, soil erosion and greenhouse gas emissions thru more sustainable farming practices.

Consumer benefits

- Reduction of undesirable qualities in food such as saturated fats in cooking oils
- Elimination of allergens
- Increase in nutrients to help reducing the chronic diseases in some part of the world
- Softer, more durable fiber for clothes

Farmer benefits

- Increasing productivity due to pest-resistant. Less occurrence of diseases
- Greater profits for farmers – do not need to spend much on chemical pesticides.

  For eg: US cotton farmers spent 300 million per year on harsh chemicals for their plants.

Genetically Modified/ Transgenic Crop

- **Definition**: Genetically modified (GM) crops/plants are crops/plants that have had their DNA changed through genetic engineering.
  - transgenic crop = genetically modified (GM) crop = genetically engineered crop = genetically modified organism (GMO)
  - GM products include medicines, vaccines, foods, feeds, and fibers.

Common Examples of Transgenic Crops (GM)

- There are a number of common genetically modified (GM) crops currently planted by farmers.
- Types of GM crops being grown are;
  - Tolerate to herbicides
  - Tolerate to pest attack
  - Enhanced nutrition
  - Long storage life
  - Strong fibers

- **Herbicide Tolerance Crop**: a crop is engineered to tolerate a herbicide that kills other plants and weeds.
  - GM crop does not have to compete with other plants for nutrients, water and light.
  - Higher yields from the GM crops when competition with weeds is eliminated.

- **Insect Tolerance Crop**: a crop is engineered to produce a specific toxin that kills the pests who feed on it.
- This is commonly done for non-food plants such as cotton.

Common Examples of Transgenic Crops

1. Soybeans (HT)

- Resistant to glyphosate and glufosinate herbicides
- Herbicide resistant gene taken from bacteria inserted into soybean
- 77% area planted in in the world is HT soybeans.
- Percent planted in the USA – 93%

Common Examples of Transgenic Crops

2. Corn (field corn/maize)

- It is engineered to be resistant to glyphosate and glufosinate herbicides
- Also is engineered to tolerate to insect attack through producing Bt proteins,
- New gene from the bacterium *Bacillus thuringiensis* transferred into plant genome.
- Percent planted in the in world - 26%
- Percent planted in the USA – 86%

Common Examples of Transgenic Crops

3. Cotton (cottonseed oil)

- Pest-resistant cotton
- Bt gene added/transferred into plant genome
- Percent planted in the USA – 93%
- World – 49%
- Bt cotton plants produce a chemical that kills the cotton bollworm.
- It is also reduces insect attack in neighboring fields planted with corn, soybeans, and other crops.

Common Examples of Transgenic Crops

4. Canola

- Resistance to herbicides (glyphosate or glufosinate)
- New genes added/transferred into plant genome
- Percent use in the USA - 93%
- World - 21%

5. Hawaiian papaya

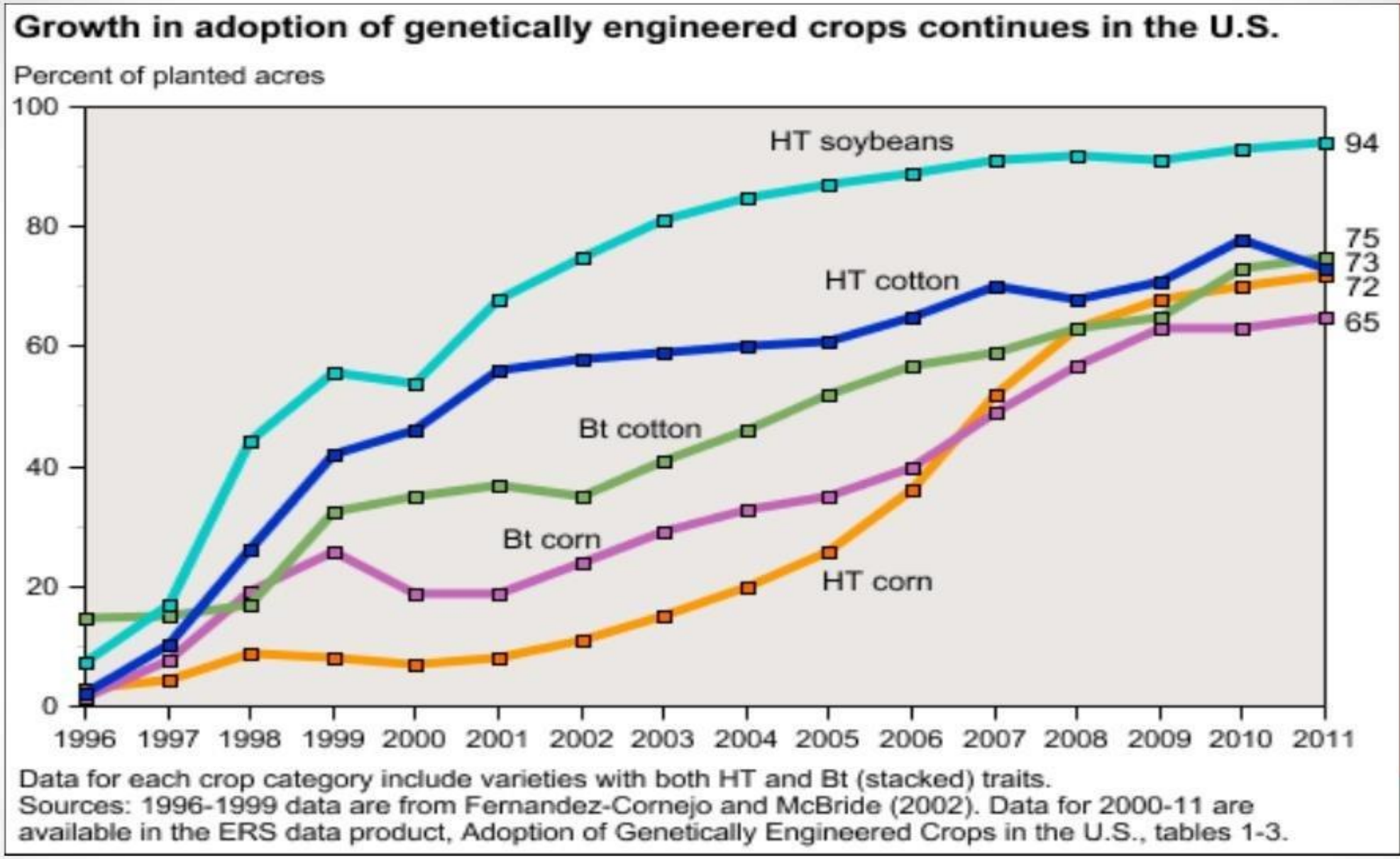
- Variety is resistant to the papaya ringspot virus.
- New gene added/transferred into plant genome
- Percent the in USA - 80%
- Percent in the world- ?

Common Examples of Transgenic Crops

6. Rice

- Golden Rice: genetically modified to contain beta-carotene (a source of vitamin A)
- Current version of Golden Rice under development contains genes from maize and a common soil microorganism
- Previous prototype version contained three new genes: two from daffodils and the third from a bacterium
- Forecast to be on the market in 2013.

Growth in adoption of genetically engineered crops continues in the U.S.



Data for each crop category include varieties with both HT and Bt (stacked) traits.
Sources: 1996-1999 data are from Fernandez-Cornejo and McBride (2002). Data for 2000-11 are available in the ERS data product, Adoption of Genetically Engineered Crops in the U.S., tables 1-3.

Advantages & Benefits of GM Plants to Farmers

Utilization of GM crops/plants can benefit farmers and agricultural industry in the following ways;

1. Less pesticide is used due to insect pest resistant plants.
2. Environmental friendly as pesticides do not go into the air, soil, and water, especially freshwater supplies.


Thank you