Genetically modified food

Introduction

- For years, farmers have been selecting for certain features in crops producing plants that are easier to grow, tastier and bigger without knowing the exact mechanism of how this occurs.
- Recognizing valuable traits and incorporating them into future generations is very important in plant breeding.
- Advances in plant biotechnology have made it possible to identify and modify genes controlling specific characteristics.
- Now a day scientists can transfer genes from one organism to another unrelated organism, producing what is now known as “genetically modified organism” or “transgenic animal/ plant”.
- Any food produced this way is called Genetically Modified food. In India, a total area of 8.4 million hectares is used in the production of GM crop especially cotton. The foods obtained by added or deleted gene sequence is called genetically modified foods.

Why Genetically Modified Plants?

By genetically modifying the plant in the laboratory it is possible to have stricter control over the genes. With GM, the desirable genes can be separated from the undesirable genes. It is also possible by GM to cross species that would not breed together naturally.

The following are some reasons for genetic modifications:

- Improved yields
- More resistant to disease
- Less likely to be damaged by insect
- Tolerance to herbicides
- Better nutritional value

- Increased shelf life
- Better climatic survival by increasing tolerance to draught, flood or frosty conditions to allow the use of previously inhospitable land
- Higher crop yields
- Reduced farm costs
- Increased farm profit
- Improvement in health and environment

What is Genetically Modified Food?

- Food that contain an added gene sequence
- Food that have a deleted gene sequence
- Animal products from animals fed GM Feed
- Products produced by GM organisms

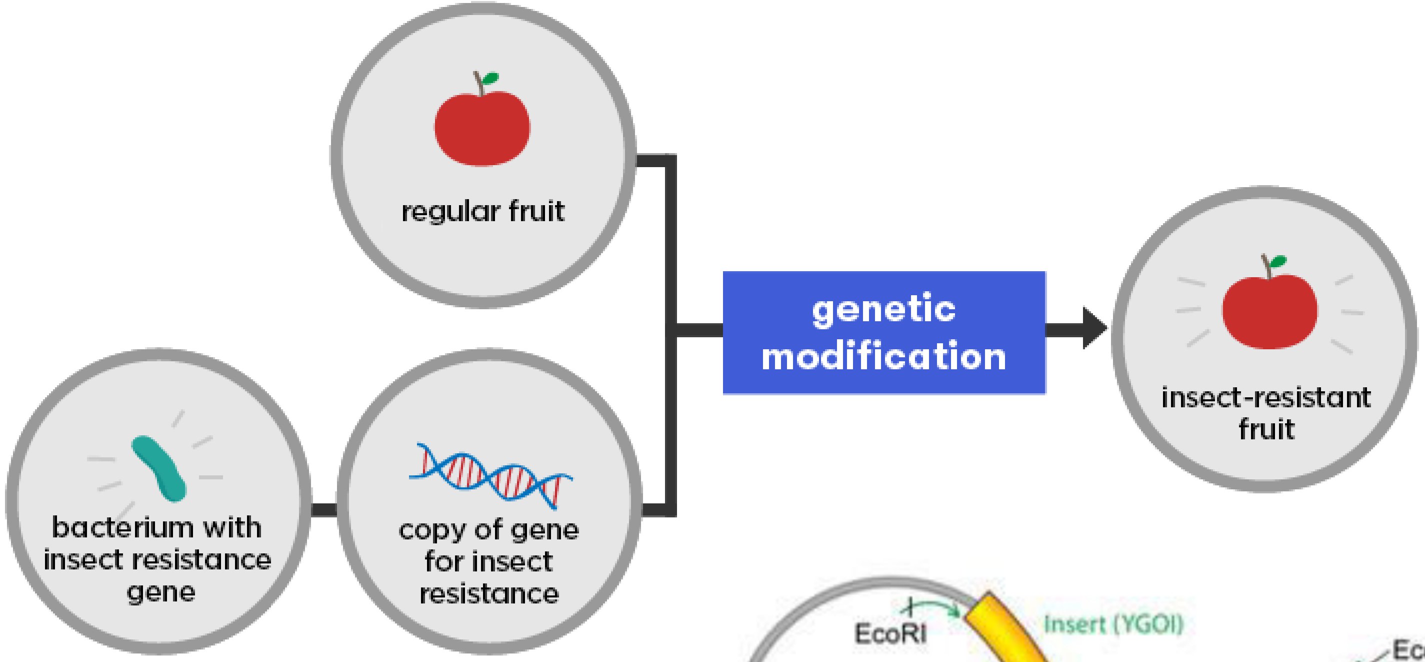
Why Produce GM Food?

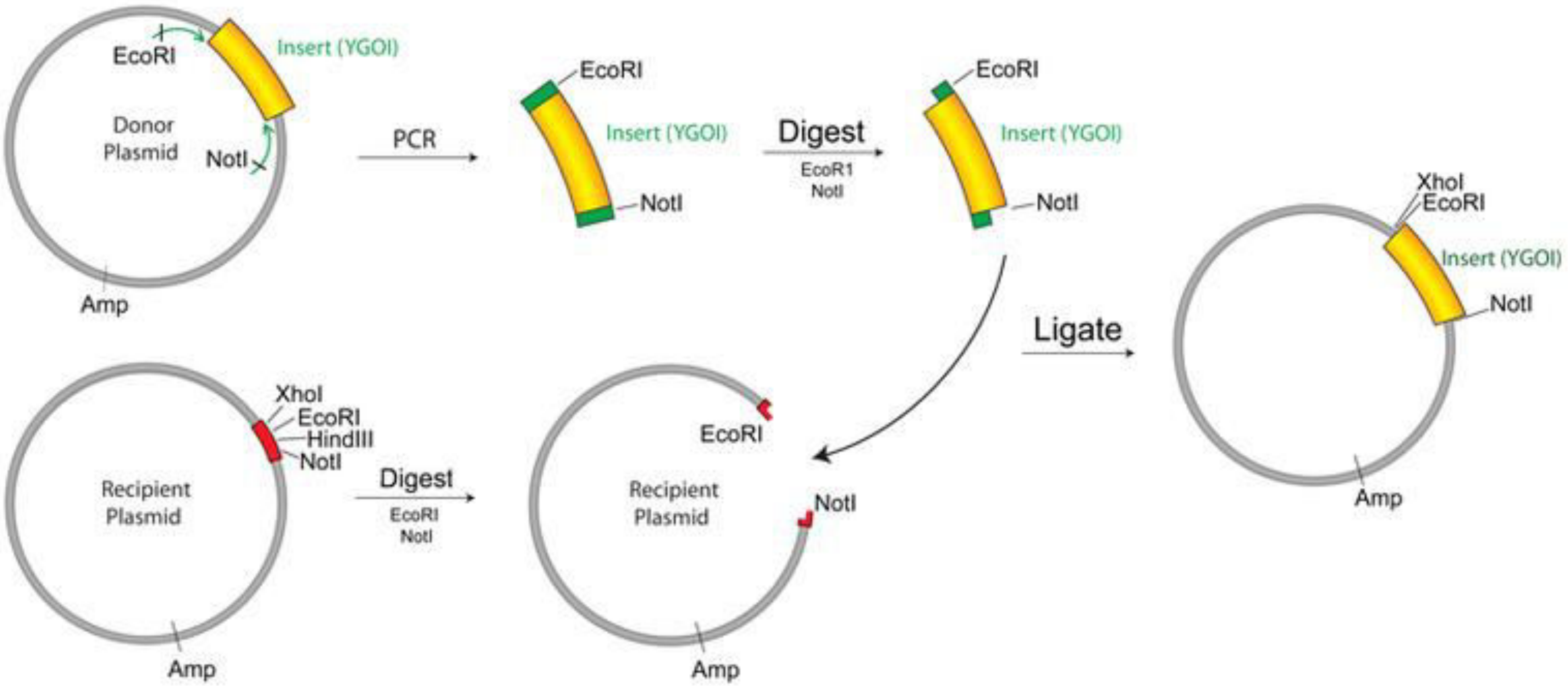
- Traditionally, combining the desirable genes in one plant is a tough task that utilizes longer time and so much attention, involving crossing one plant to another plant of the same species or related species.
- From economical and agricultural standpoints, it is advantageous to grow crops that have higher yield or improved quality, pest or disease resistance, or tolerance to heat, cold and drought.
- Desirable genes may provide means for plants to combat these conditions.
- The development of transgenic technology allows useful genes from various living sources to be brought together in a

How is Genetic Modification Possible?

The component of DNA is same in all organisms in the form of amino acid sequence that are responsible for genetic make-up of the organism. This amino acid sequence can be altered or moved from one organism to another by recombinant technology, molecular cloning and genetic engineering. There are two enzymes which play major role in the process:

1. **Restriction Enzyme:** are used to “CUT” DNA Segment from one Genome.
2. **DNA Ligases:** are used to “PASTE” them into another genome.


regular fruit
bacterium with insect resistance gene
copy of gene for insect resistance
genetic modification
insect-resistant fruit


EcoRI
Insert (YGOI)
Donor Plasmid
NotI
Amp
PCR
EcoRI
Insert (YGOI)
NotI
Digest
EcoR1
NotI
EcoRI
Insert (YGOI)
NotI
Ligate
XhoI
EcoRI
Insert (YGOI)
NotI
Amp
Recipient Plasmid
XhoI
EcoRI
HindIII
NotI
Amp
Digest
EcoRI
NotI
EcoRI
Recipient Plasmid
NotI
Amp

Shifting of DNA from one organism to other

- Find an Organism with the desired trait.
- Isolate the gene sequence that Code for the desired trait.
- Insert the gene sequence into the genome of the plant cell.
- Allow the genetically engineered cell to grow into a plant.
- Allow the Plant to propagate.
- A vector can carry DNA. The vector can be transfer in a organism by a gene gun, viruses and bacteria also can be utilized to transfer gene.

Transformation method

Transformation system should allow for stable integration of DNA into the host genome without structural alteration and the whole process comprises of three basic steps as follows:

- Integration of a distinct number of copies of the transforming DNA
- Stability of the new phenotype over several generations
- Character regulation of the inserted gene

Methods of transformation:

1) *Agrobacterium tumefaciens*
2) Electroporation
3) Polyethylene Glycol
4) Silicon carbide fiber
5) Gene Gun
6) Microinjection

Agrobacterium tumefaciens

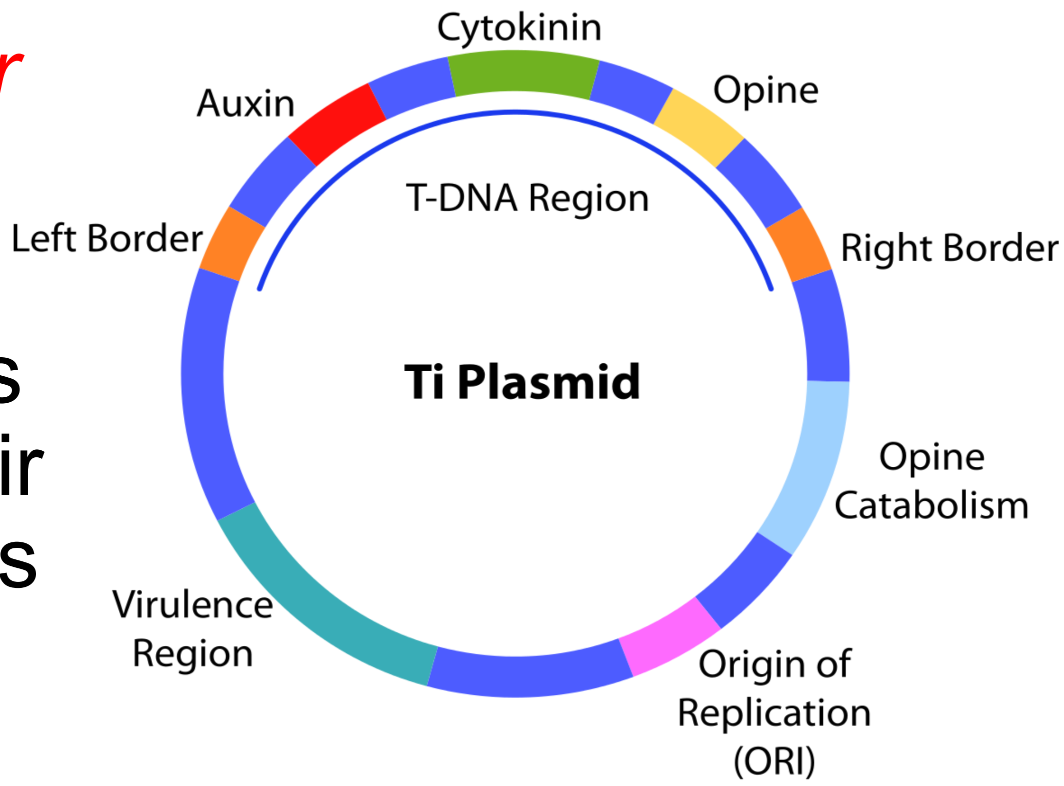
Plant transformation mediated by *Agrobacterium tumefaciens* Virulent Strain of *Agrobacterium tumefaciens* & *Agrobacterium rhizo* genes contains a large plasmid known as Ti plasmid. These bacteria possess the ability to transfer T-DNA, Causing the Crown of Gall Disease & Hairy root. The initial results of T-DNA transfer process
to plant cells demonstrate three important features-

- Tumor formation is the results of the integration of T-DNA into plant cells.
- The T-DNA genes are transcribed only in plant cells.
- Every DNA sequence can be transferred to plant cells

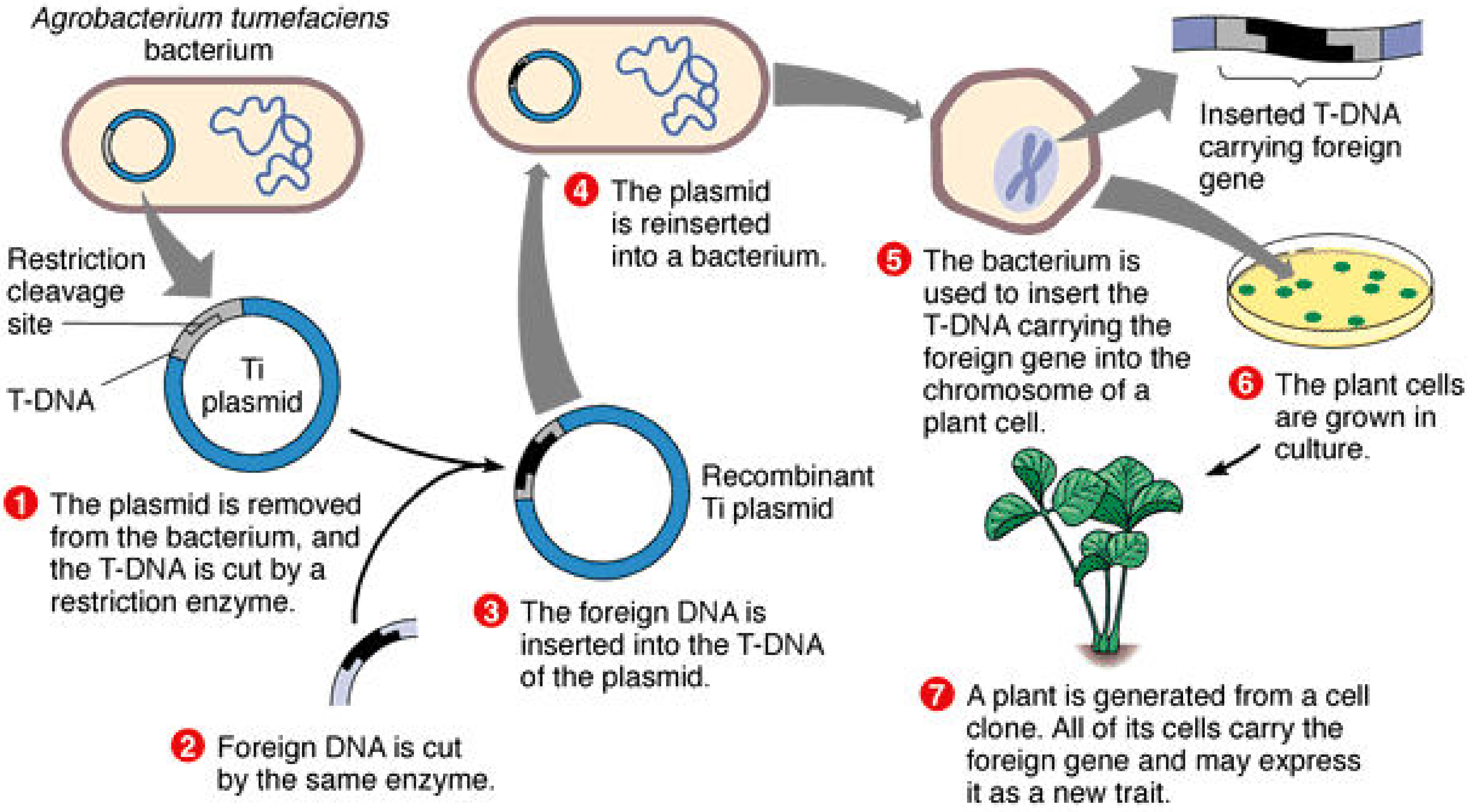
Ti Plasmid

- The Tumour inducing or Ti plasmid is present in the bacterium *Agrobacterium tumifaciens.*
- *A. tumefaciens* is responsible for crown gall disease of dicotyledonous plants.
- There are different kinds of Ti plasmids based on the different genes they possess, which code for different opines (arginine derivatives).
- The opines synthesized are usually either nopaline or octopine depending on the strain of *A. tumefaciens*.
- These opines are used as energy sources by the infecting bacteria.

- Ti plasmid has two components the T-DNA (Transferred DNA) and the *vir* region, which are essential for the transformation of plant cells.
- T DNA: In nopaline-type Ti plasmids the T-DNA is a 23,000-nucleotide-pair segment that carries 13 known genes including genes encoding enzymes that catalyze the synthesis of phytohormones (the auxin indoleacetic acid and the cytokinin isopentenyl adenosine).
- These phytohormones are responsible for the tumorous growth of cells in crown galls



- The vir (for virulence) region: It contains the genes required for the T-DNA transfer process.
- These genes encode the DNA processing enzymes required for excision, transfer, and integration of the T-DNA segment.
- During the transformation process, the T-DNA is excised from the Ti plasmid, transferred to a plant cell, and integrated into the DNA of the plant cell.



Agrobacterium tumefaciens bacterium
Restriction cleavage site
T-DNA
Ti plasmid
1 The plasmid is removed from the bacterium, and the T-DNA is cut by a restriction enzyme.
2 Foreign DNA is cut by the same enzyme.
Recombinant Ti plasmid
3 The foreign DNA is inserted into the T-DNA of the plasmid.
4 The plasmid is reinserted into a bacterium.
5 The bacterium is used to insert the T-DNA carrying the foreign gene into the chromosome of a plant cell.
Inserted T-DNA carrying foreign gene
6 The plant cells are grown in culture.
7 A plant is generated from a cell clone. All of its cells carry the foreign gene and may express it as a new trait.

Electroporation: In this, Material is incubated in a buffer solution containing DNA and subjected to high voltage electric pulses. DNA migrates through high voltage, introduces pores in the plasma membranes & integrates into the genome. Mainly Used for- Rice, Wheat and Maize.

Polyethylene glycol: Plant Protoplast can be transformed with naked DNA by treatment with Polyethylene Glycol in the presence of divalent Cation (Ca_2+). They both help in destabilizing the plasma membrane of plants and make it convenient for DNA penetration. Once the DNA enters the nucleus, it gets integrated into the genome.

Silicon carbide fiber: In this, plant material is introduces into a buffer containing DNA and silicon carbide fibers. The fibers penetrate the cell wall and plasma membranes allowing the DNA to gain access to inside of the cell.

Microinjection: This method involves the use of a fine needle to inject the DNA material in the form of a solution into developing embryo.

Gene gun method: A particle gun is used to shoot small bits of metals coated with the gene into the plant.

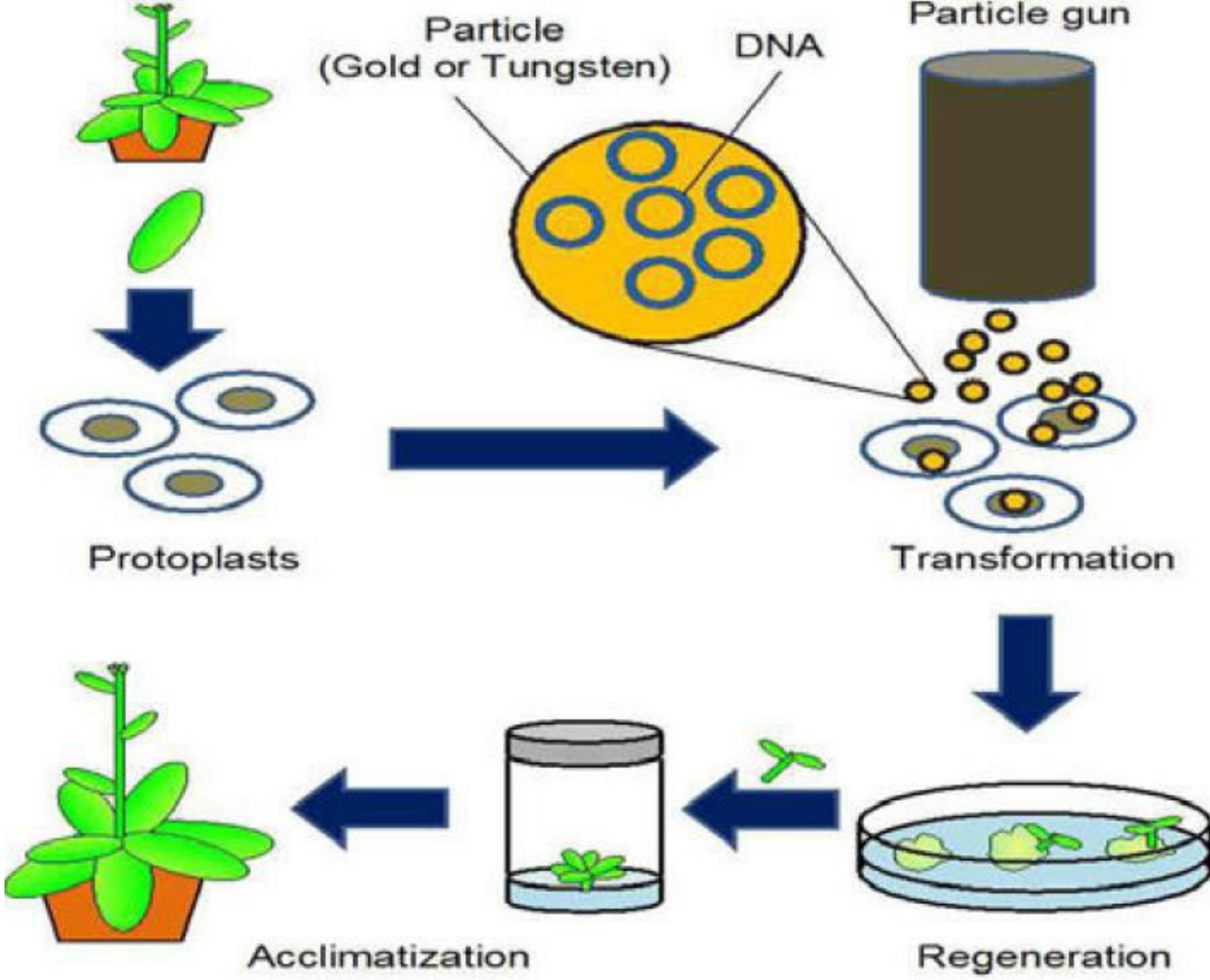


Figure 5: Plant transformation process using particle bombardment includes following steps:
(1) Isolate protoplasts from leaf tissue. (2) Inject DNA-coated particles into protoplasts using particle gun. (3) Regenerate into whole plants. (4) Acclimate the transgenic plants in a greenhouse.

Uses of Genetic Modification

Genetic modification in the laboratory was first reported in the early 1970s. Since then, there has been a wide range of research and applications of genetic engineering in agriculture, medicine, the environment, food production and the manufacturing industry. A number of examples are listed below:

In agricultural area:

- Herbicide tolerant crops
- Insect resistant crops
- Virus resistant crops

In the field of medicine:

- Insulin to treat diabetic mellitus
- Production of human growth hormone
- Production of blood clotting factors VIII and IX
- The treatment of cystic fibrosis
- Research into human and animal diseases

In other areas:

- Environmental clean-up of soil spills
- Treatment of contaminated land and water
- Manufacture of useful chemicals such as enzymes
- Plants providing renewable sources of industrial chemicals

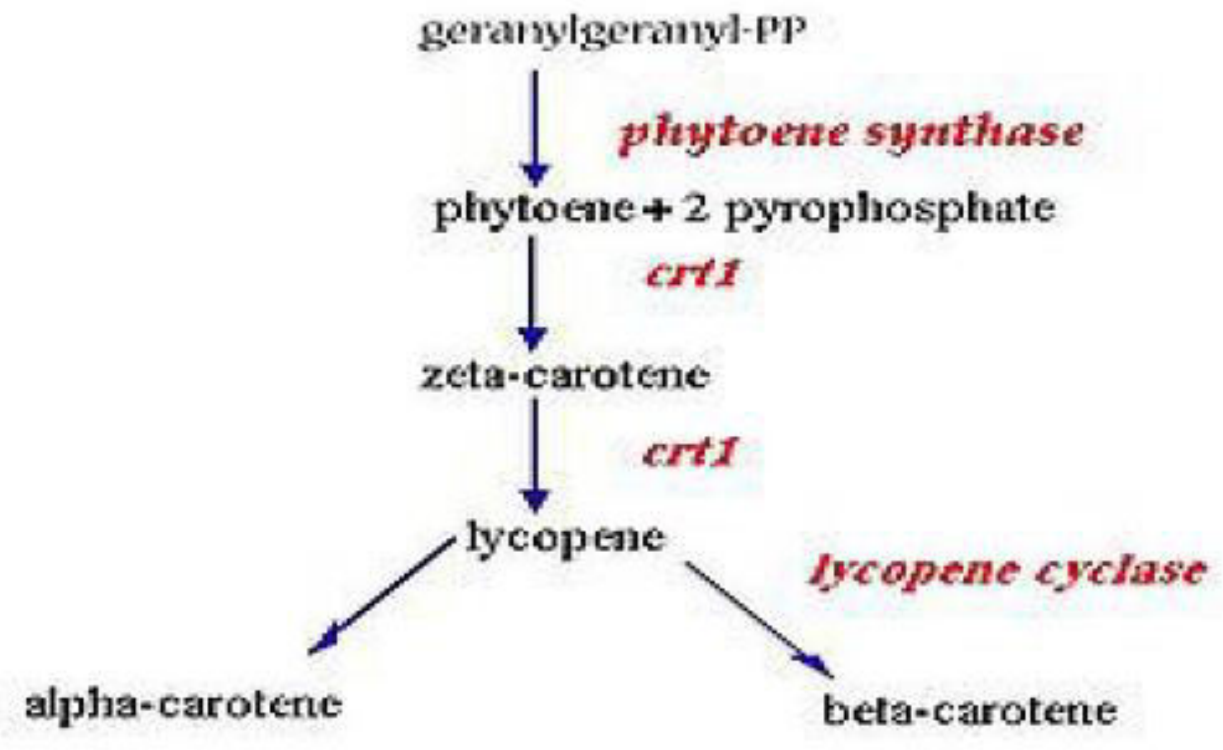
Common Genetically Modified Food

1) **Soybean, Corn & sugar-beet:-** resistant to glyphosate by inserting herbicide resistant gene
2) **Cottonseed oil:-** by inserting pest resistant Bt crystal protein gene
3) **Tomato:-** by removing the gene that codes for polygalacturonase, responsible for softening of fruits after harvesting
4) **Potatoes:-** Amylopectin rich variety by switching off of GBSS (granule bound starch synthase) gene, responsible for amylose production.
5) **Rapeseed (canola):-** with high oleic acid content by adding new gene.

Golden rice

- It was created by Ingo Potrykus.
- Golden rice is a variety of rice produced through genetic modification to biosynthesize the precursors of beta-carotene (pro-vitamin A) in the edible parts of rice (endosperm).
- More than 120 million children in the world suffer from vitamin A deficiency.
- Golden Rice has the potential to help prevent the 1 to 2 million deaths each year caused by a deficiency in this vitamin.
- However, the plant does not normally produce the pigment in the endosperm since photosynthesis does not occur in the endosperm.
- Golden rice was created by incorporating rice with two beta carotene biosynthesis genes.
  - I. Psy (Phytoene synthase)
  - II. Lyc (lycopene cyclase)

The psy and lyc genes were transformed into the rice nuclear genome and placed under the control of an endosperm specific promoter, so that they are only expressed in the endosperm. The end product of the engineered pathway is lycopene, but if the plant accumulated lycopene the rice would be red. Recent analysis has shown that the plant's endogenous enzyme process the lycopene to beta carotene in the endosperm, giving the rice the distinctive yellow colors for which it is named



Iron enriched rice

As cereal grains are deficient in certain essential mineral nutrients, including iron, several approaches have been used to increase the iron accumulation and alter iron metabolism. Since ferritin is a general iron storage protein in all living organisms, the ferritin genes has been added to rice and wheat plants to increase their iron content.