

# Chapter 5

# Agricultural Biotechnology



# Introduction

- Extension of traditional plant breeding
- Allows for the transfer genetic information in a more precise and controlled manner
- Plant biotechnology allows for the transfer of only one or a few desirable genes
- Allows plant breeders to develop crops with specific beneficial traits and without undesirable traits

# Plants are suitable for genetic engineering

- 1. Long history of plant breeding : Have wealth of strains that can be exploited to the molecular level
- 2. Plant produce large number of progeny, so rare mutations and recombination can be found more easily
- 3. Better regenerative ability than animal cell
- 4. Species boundaries and sexual compatibility are no longer an issue

# Methods used in plant transgenesis

1. Somatic fusion or protoplast fusion
  - 2 distinct species of plants are fused together to form a new hybrid plant with the characteristics of both
  - Production of somatic hybrid
  - Hybrids have been produced either between the different varieties of the same species

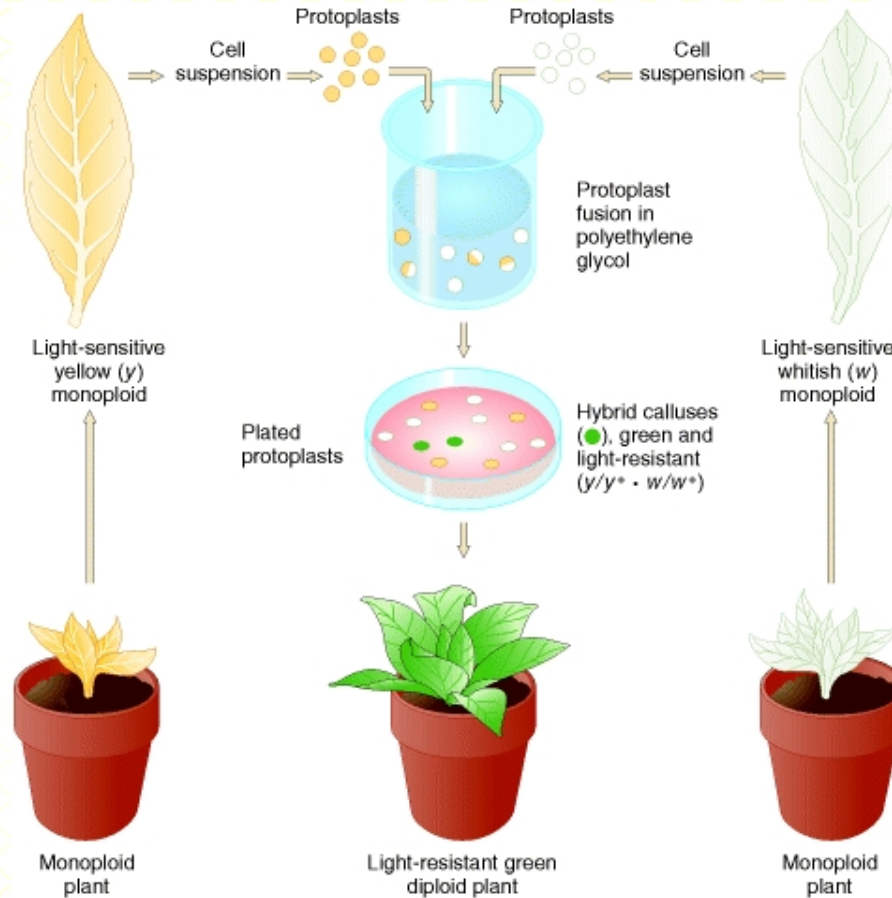
# Protoplast cell

- Cell walls are removed and cytoplasmic membrane
- Removal cell wall by lytic enzymes
- Protoplast fusion for fusion two or more protoplasts





# Protoplast fusion



# Chloroplast Engineering

- C h l o r o p l a s t transformation require:
  - A chloroplast expression vector
  - DNA delivery method through double membrane of the chloroplast
  - An efficient selection

# Advantage of chloroplast transformation

- 1. Transgene escape: Natural containment because of lack of pollen transmission
- 2. Low expression level: High level of transgene expression because of high copy number of plastomes
- 3. Gene silencing: Absence of position effect due to a compact chromatin structure and efficient transgene integration



# Chloroplast genome versus nuclear genome

Chloroplast genome	Nuclear genome
Polyploidy result in an abundant transgene transcript & high accumulation of protein	Gene regulation determine the rate of transcription & accumulation of protein is often a limitation
Genes are often arranged in operons & transcribed in into polysistronic mRNA so that multiple gene can be introduced and expressed in single transformation event	Each transgene is independently inserted into nuclear genome and transcribed into monosistronic mRNA
Gene silencing not reported	Both transcriptional and post transcriptional gene silencing reported
Uniform gene expression	Highly variable expression
Maternal gene inheritance in most crop plants results in natural gene containment	Paternal gene inheritance results in out-crossing between crop and weed
Chloroplast form disulfide bonds and correctly fold human protein, making them ideal for edible vaccine	For disulfide bond formation, protein are target to the RE

# Application of chloroplast engineering

- Abiotic stresses : The unsaturated level of fatty acid has several implication for the stress tolerance of higher plant as well as for their nutritional value
- Agronomic traits

# Gene guns

- Widely accepted technique. Called as direct-transformation method
- The use of high velocity, DNA-coated microprojectiles for gene transfer.
- Other name: particle bombardment, microprojectile bombardment, particle acceleration, gene-gun method, biolistic or the particle gun method.

# Microparticles

- Gold and tungsten powders
- Small differences in size influence the ability of particles to penetrate the cell wall

Gold particle	Tungsten particle
Uniform	Heterologous
Biological inert	May be toxic to certain cell
Unstable	Stable



# Biolistics/ Microprojectile/ Bombardment

- The PDS-1000/He device is powered by burst of helium gas that accelerated a macrocarrier, upon which millions of DNA-coated microcarriers have been dried

# Advantages of PDSHe-1000

- Cleaner and safer
- Allows better control over bombardment parameters
- Distributes microcarrier more uniformly over target cells
- Less disruptive to target cells
- More consistent between bombardments
- Yield 4-3000 fold transformants in several species

# Mechanism

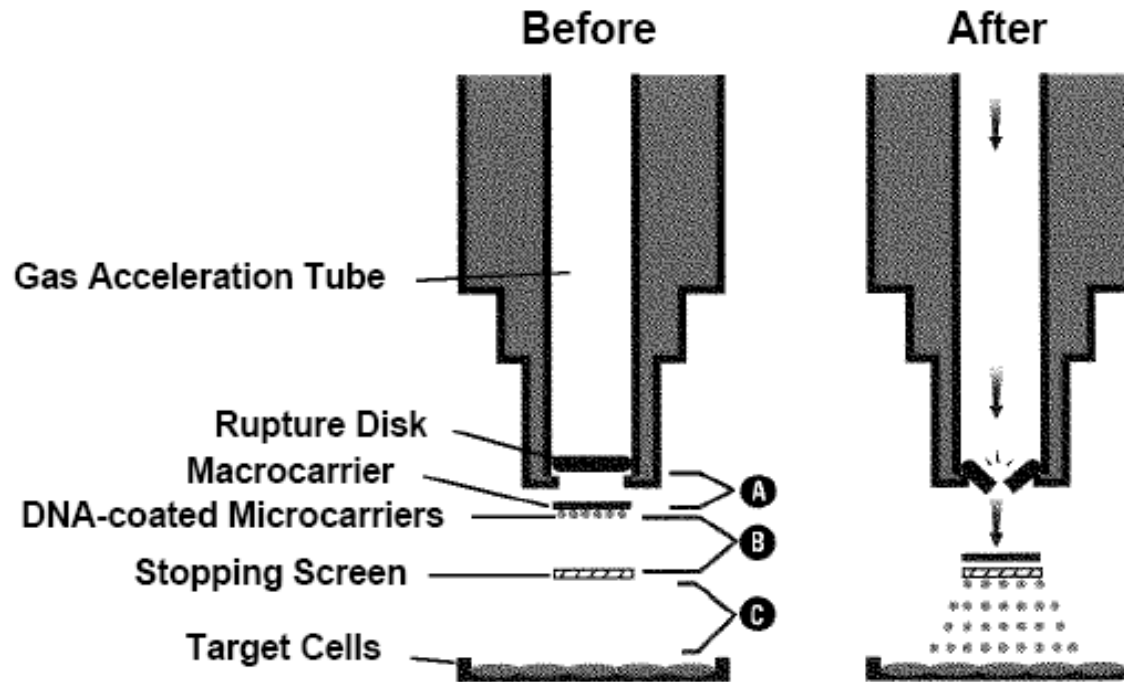


Fig. 1.2. The Biolistic bombardment process.

# Advantages

- 1. Universal delivery system while transformation of recalcitrant crop species in important agronomic crops (rice, cotton, maize etc), can be achieved when conventional methods are restricted
- 2. Versatility
- 3. Does not rely on biological limitations of any single of microorganisms
- 4. Both single cells and organized tissues can be used as transformation targets



# *Agrobacterium* sp

- Soil phytopathogen
- Causing crown gall tumors
- Affects most dicotyledoneous plants
- Transfers a discrete portion of its DNA (the Transferred or T-DNA) through the wounded cell and stably integrates it into nuclear genome of the host plant.
- *A. tumefaciens* (common for transformation) and *A. rhizogenes*



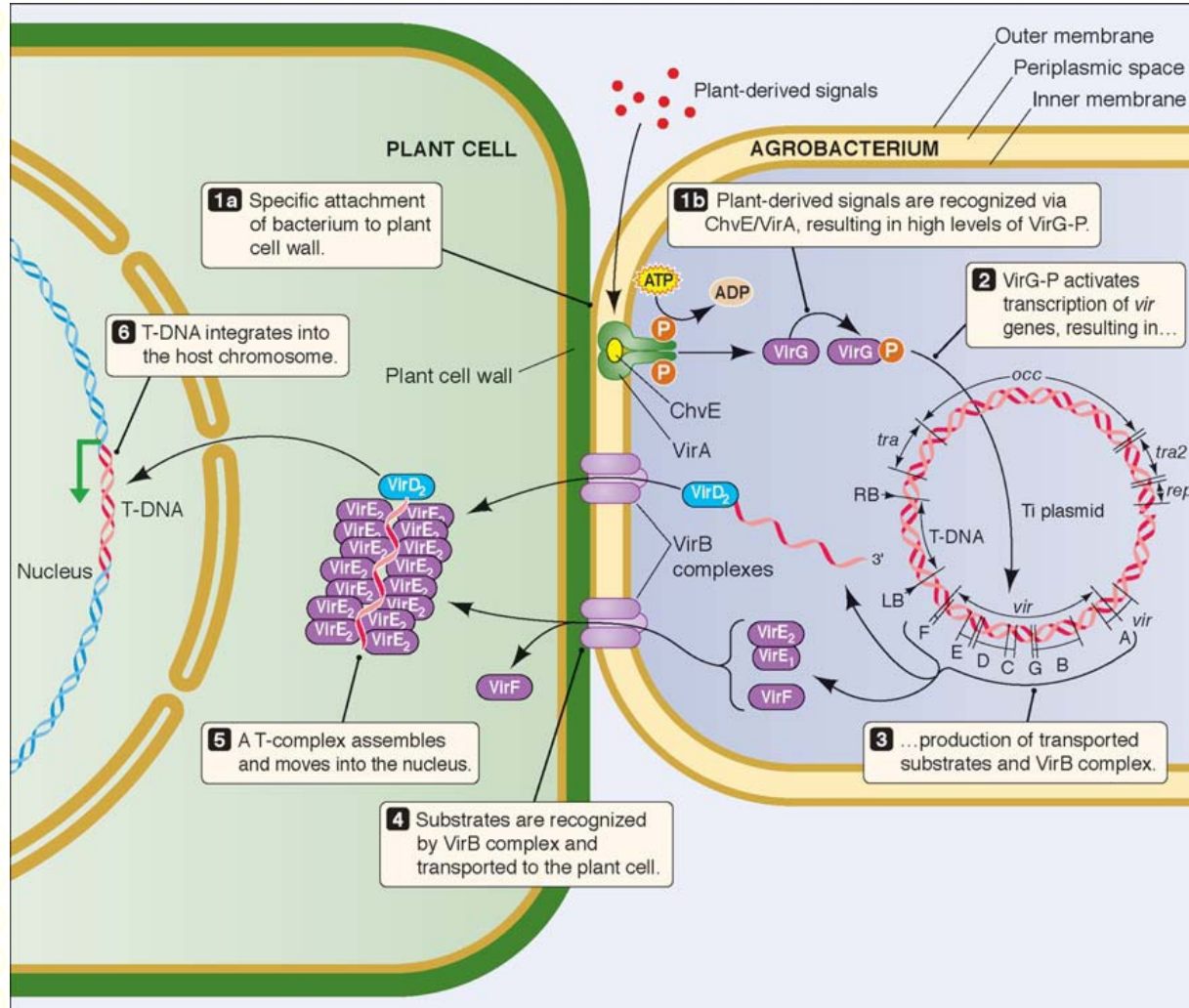
# Unique of *Agrobacterium* sp

- T-DNA transfer resides on a tumor-inducing (Ti) plasmid
- Ti contained T-DNA, delimited by 25 bp imperfect repeats (RB and LB, right and left border respectively) that define the boundaries of the T-DNA and ~30kb virulence (*vir*) genes, clustered into a *vir* region

# Ti plasmid

- Vir (Virulence) gene: 30kb
- Six operons
  - virA, virB, virD and virG : essential for T-DNA transfer
  - virC and VirE : increasing of transfer efficiency
- The opines are unique amino acid derivatives, different from normal plant products
- Provide *Agrobacterium* with a unique food source that other bacteria cannot use

# Integration of T-DNA into plant cell





# Advantages

- (1) The simplicity of *Agrobacterium* gene transfer
- (2) A precise transfer and integration of DNA sequences with defined ends
- (3) A linked transfer of genes of interest along with the transformation marker

- (4) The higher frequency of stable transformation with many single copy insertions. *Agrobacterium* based methods led to integration of one T-DNA copy per genome in 30 to 60% transgenic plants
- (5) Reasonably low incidence of transgene silencing.
- (6) The ability to transfer long stretches of T-DNA (>150kb)