TRANSGENIC PLANTS

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terms to know

TRANSGENE- It is a foreign gene or genetic material that has been transferred naturally or by any of a number of genetic engineering techniques from one organism to another.

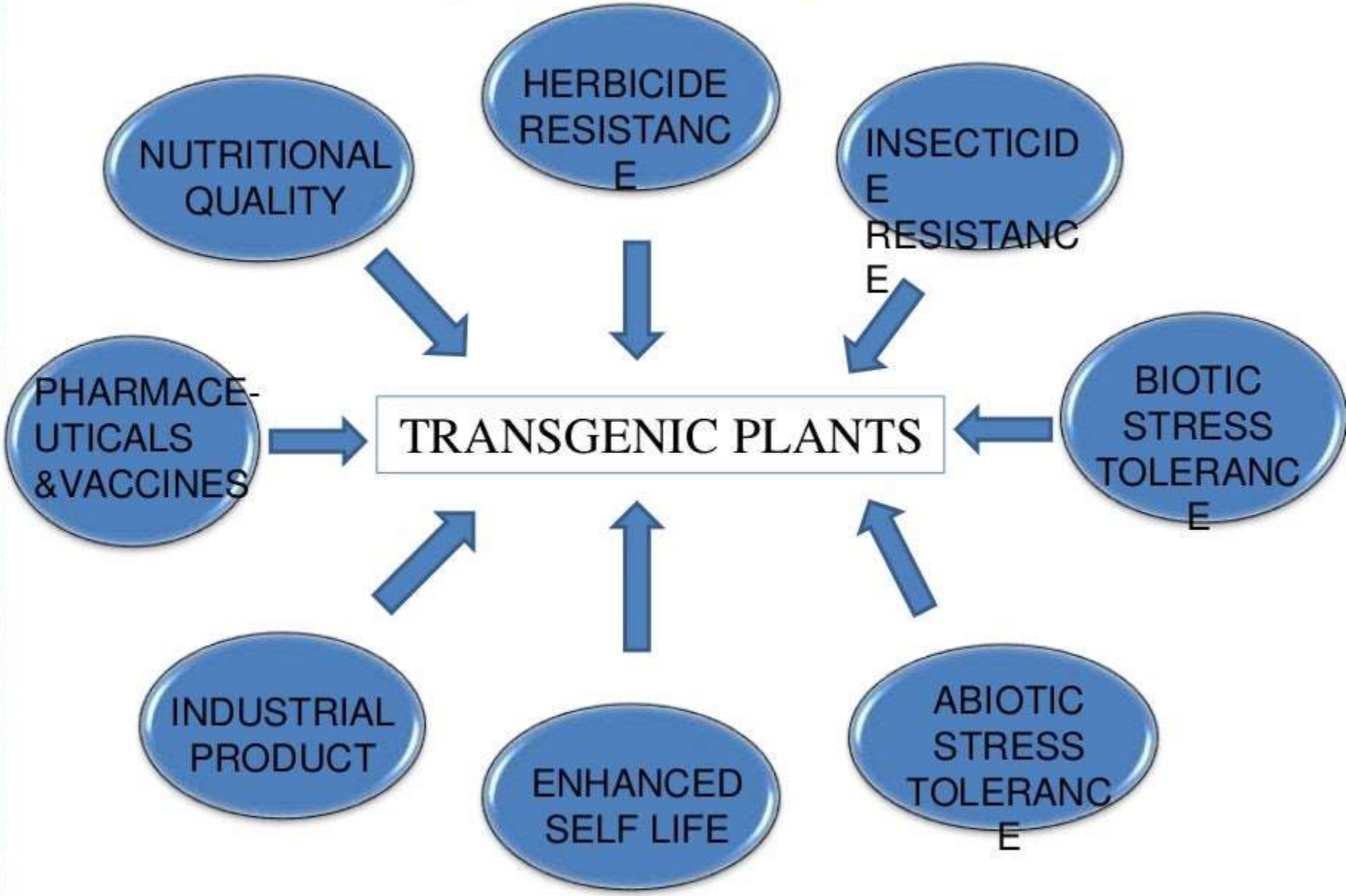
TRANSGENESIS- The phenomenon of introduction of exogenous DNA into the genome to create and maintain a stable and heritable character.

TRANSGENIC PLANTS- The plant whose genome is altered by adding one or more transgenes are known as transgenic plants.

history

1982	• 1st transgenic plant produced which is an antibiotic resistance tobacco plant.
1984	• 1st successful plant genetic engineering experiments using caulimovirus vector.
1994	• 1st genetically modified crop approved for sale in U.S. was FlavrSavr tomato.
1995	• 1st pesticide producing crop, Bt Potato was approved by U.S. Environmental Protection Agency
1996	• 1st genetically modified flower Moondust, bluish colored carnation, was introduced.
2000	• Golden rice with β- carotene developed with increased nutrient value.
2013	• 1st genetically engineered crop developed by Robert Fraley, Marc Van Montagu &Marry Dell Chilton were awarded World Food Prize.

Why transgenic plants?



GENE TRANSFER METHODS

BIOLOGICAL METHODS

- Agrobacterium mediated gene transfer
- Plant virus vectors

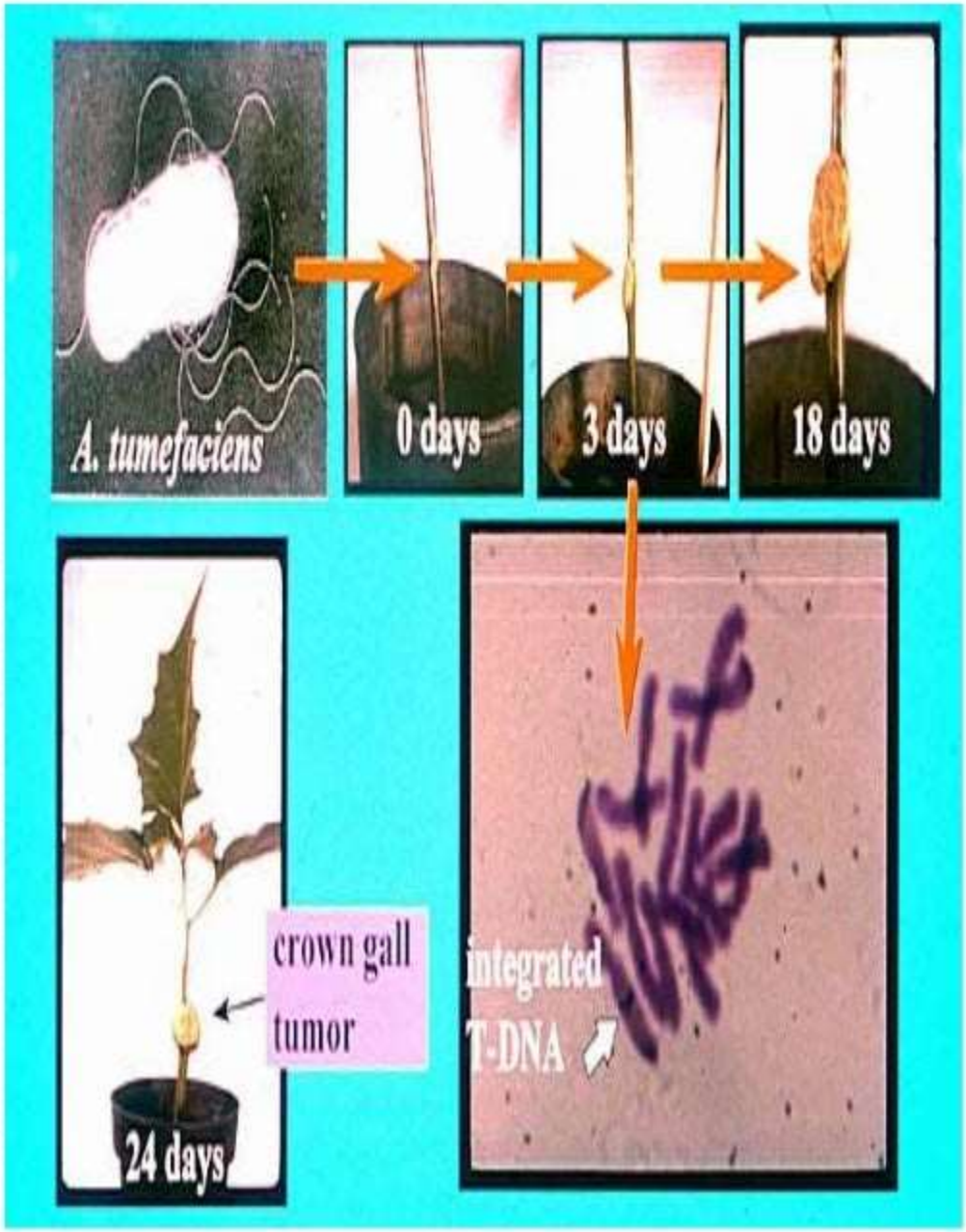
PHYSICAL METHODS

- Electroporation
- Microprojectile
- Microinjection
- Liposome Fusion

CHEMICAL METHODS

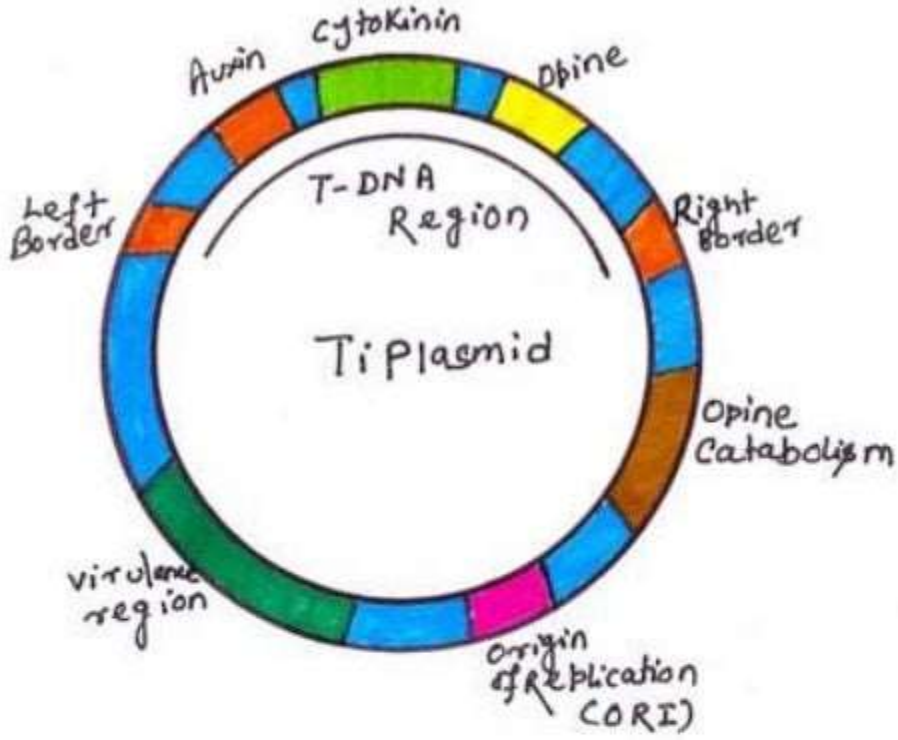
- Polyethylene glycol mediated
- Diethylaminoethyl dextran mediated

AGROBACTERIUM MEDIATED GENE TRANSFER



- Agrobacterium is treated as **nature's most effective plant genetic engineer.**
- ***A.tumifaciens*** infects wounded or damaged plant tissues results in the formation of plant tumor called crown gall.
- The bacterium releases Ti plasmid into the plant cell cytoplasm which induce crown gall.
- Several dicots are affected by this disease e.g. grapes, roses, etc.

ORGANIZATION OF Ti PLASMID



- The size of Ti plasmid is approx. 200 kb.
- The Ti plasmid has three important regions:
  (i) **T-DNA region**
  (ii) **Virulence region**
  (iii) **Opine catabolism region**
- There is ori region that is responsible for the origin of DNA replication.

T-DNA transfer and integration

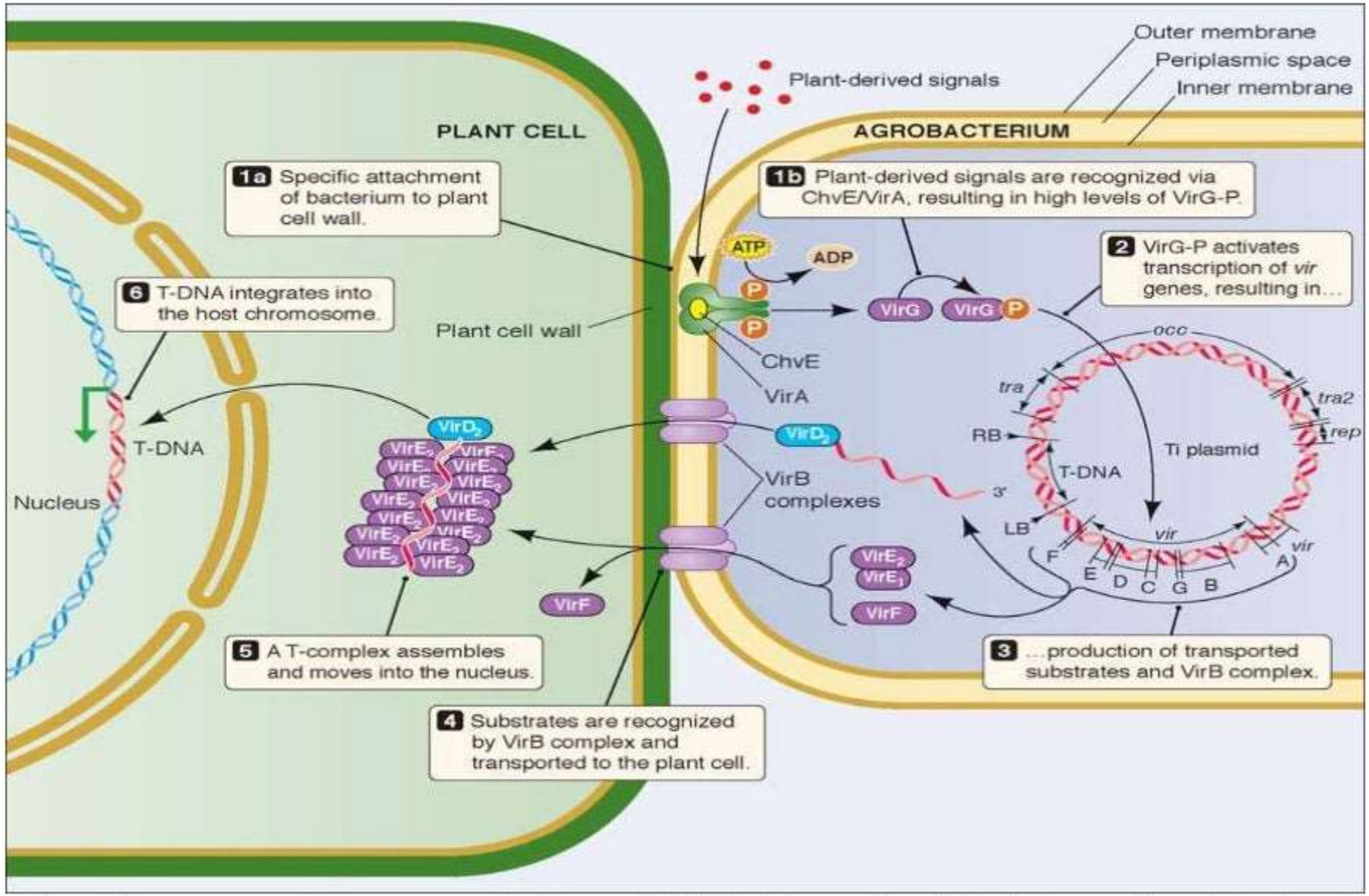
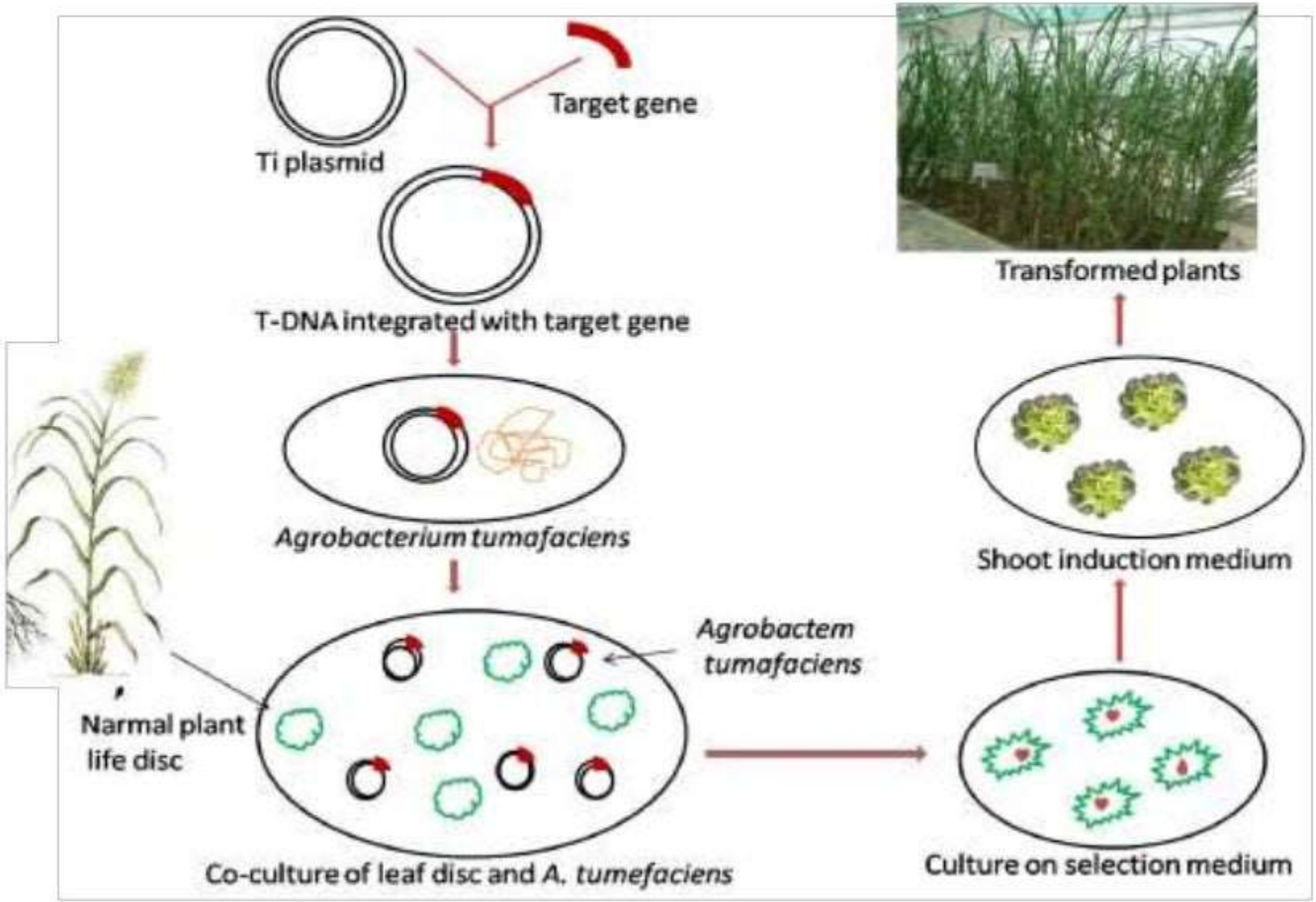


Fig: Diagrammatic representation of T-DNA transfer &its integration into host plant cell genome

TRANSFORMATION TECHNIQUE USING AGROBACTERIUM MEDIATED GENE TRANSFER



PLANT VIRus VECTORS

- Plant viruses are considered as efficient gene transfer agents as they can infect the intact plants and amplify the transferred genes through viral genome replication.
- It has some limitations like that vast majority of plant viruses have genome not of DNA but of RNA. Two classes of DNA viruses are known to infect higher plants, caulimovirus and geminivirus & neither is ideally suited for gene cloning.

i) CAULIMOVIRUS:

They contain circular dsDNA and are spherical in shape. The caulimovirus group has around 15 viruses & among these Cauliflower Mosaic Virus(CaMV) is the most important for gene transfer.

CaMV infects many plants & can be easily transmitted. The infection is systemic. It's genome does not contain any non-coding regions.

It has certain limitations-

i) CaMV vectors has a limited capacity for insertion of foreign genes.

ii) Infective capacity of CaMV is lost if more than a few hundred nucleotides are introduced.

iii) Helper viruses cannot be used since the foreign DNA gets expelled and wild-type viruses are produced.

II) GEMINIVIRUS:

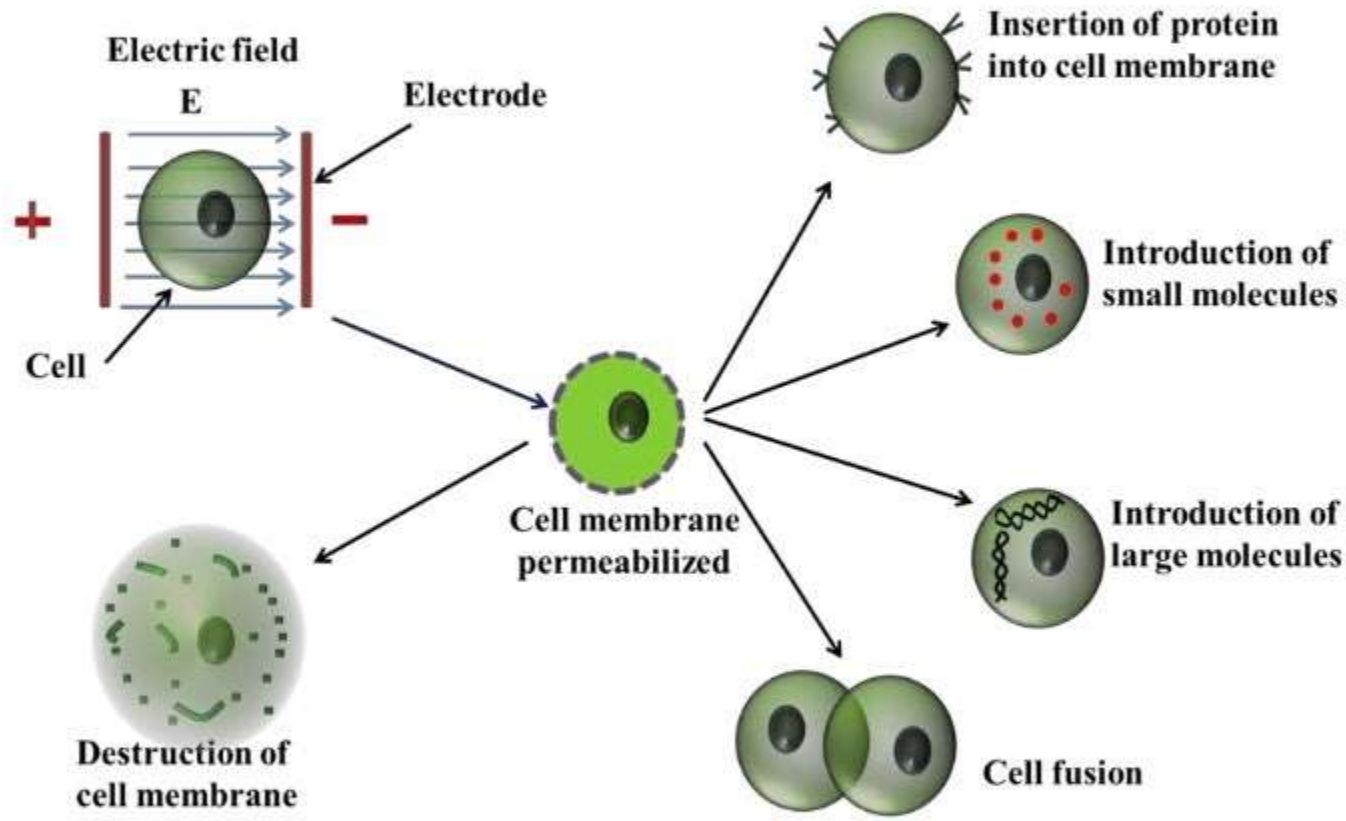
They contain one or two circular ssDNA. They are particularly interesting because their natural host include plant size such as maize & wheat. They could therefore be potential vector for these and other monocots.

They have their own set of difficulties-

It is very difficult to introduce purified viral DNA into the plants.

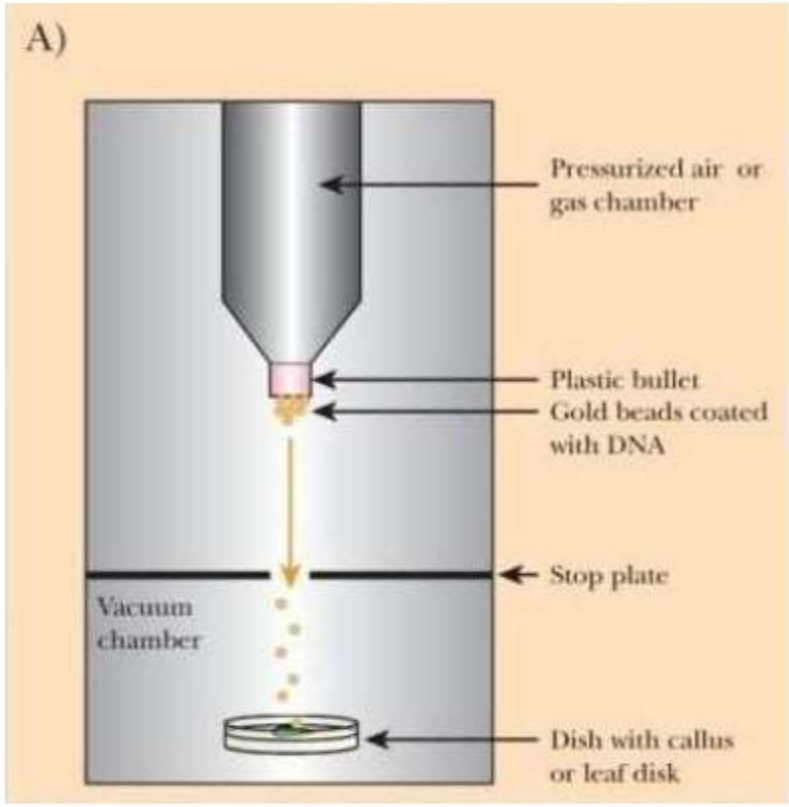
PHYSICAL GENE TRANSFER METHODS

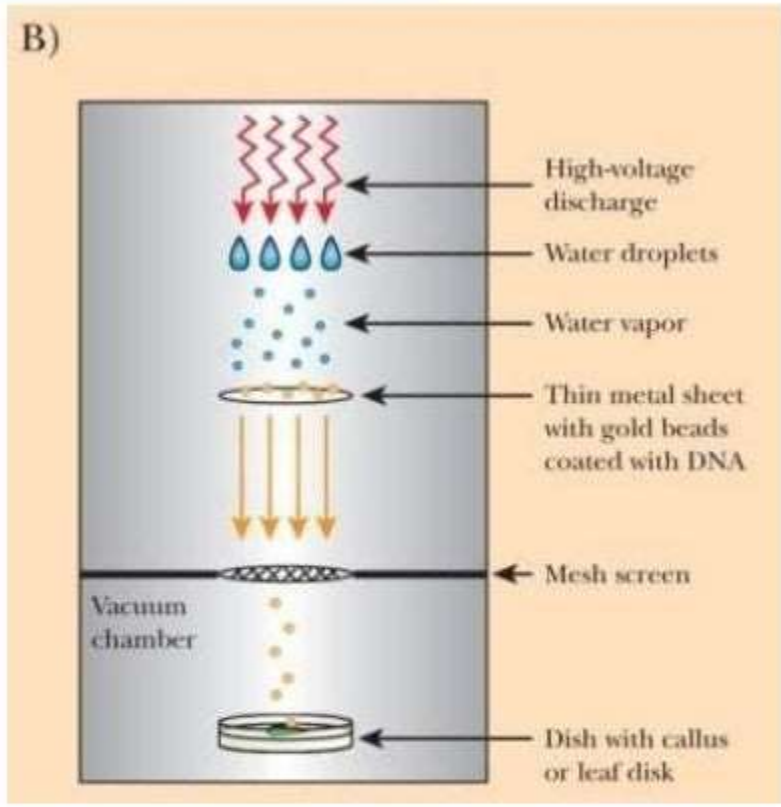
1) ELECTROPORATION



❖Electroporation involves the creation of pores in the cell membrane using electric pulse of high field strength. If DNA is present in the buffer solution at sufficient concentration, it will be taken up through these pores.

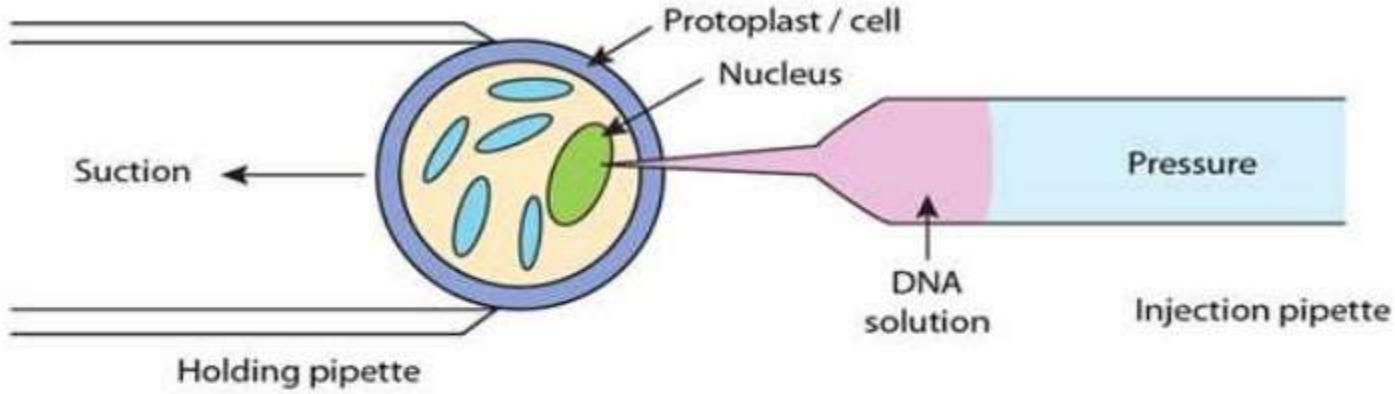
2) PARTICLE BOMBARDMENT





- It is also known as microprojectile bombardment, biolistics, gene gun, etc.
- Foreign DNA coated with high velocity gold or tungsten particles to deliver DNA into cells.
- This method is widely being used because of its ability to transfer foreign DNA into the mammalian cells and microorganisms.

3) MICROINJECTION



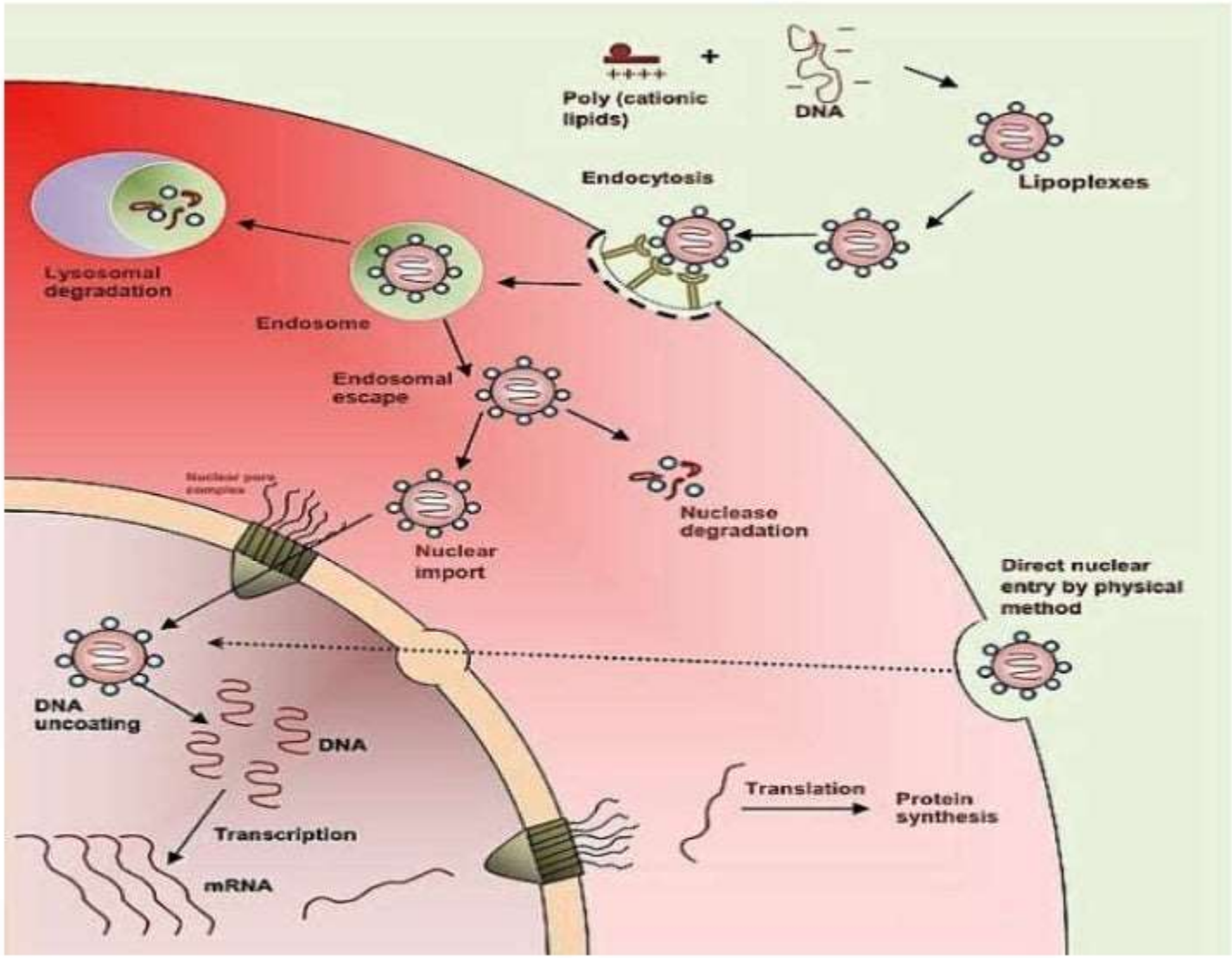
- Microinjection is a direct physical method involving the mechanical insertion of the desirable DNA into a target cell.

- The technique of microinjection involves the transfer of the gene through a micropipette into the cytoplasm or nucleus of a plant cell or protoplast.

- The most significant use of this is the introduction of DNA into the oocyte and the eggs of animals, either the transient expression analysis or to generate transgenic animals.

- The major limitations of microinjection are that it is slow, expensive, and has to be performed by trained and skilled personnel.

4) LIPOSOME MEDIATED TRANSFORMATION



❖Liposome mediated transformation involves adhesion of liposomes to the protoplast surface, its fusion at the site of attachment and release of plasmids inside the cell.

CHEMICAL GENE MEDIATED TRANSFER

1) POLYETHYLENE GLYCOL- MEDIATED TRANSFORMATION

- Polyethylene glycol (PEG), in the presence of divalent cations, destabilizes the plasma membrane of protoplasts and renders it permeable to naked DNA.
- A large number of protoplasts can be simultaneously transformed.
- This technique can be successfully used for a wide variety of plant species.
- It has certain limitations:

  i) The DNA is susceptible for degradation and rearrangement.

  ii) Random integration of foreign DNA into genome may result in undesirable traits.

  iii) Regeneration of plants from transformed protoplasts is a difficult task.

2) DEAE-DEXTRAN MEDIATED TRANSFER

- The desirable DNA can be complexed with a high molecular weight polymer diethyl amino ethyl(DEAE)dextran and transferred.
- The major limitation of this approach is that it does not yield stable transformants.

Marker genes for plant transformation

- Some methods for selecting the transformed plant materials have been devised by using a set of genes referred to as marker genes.

- These marker genes are introduced into the plant material along with the target gene. The marker genes are of two types:

i) Selectable marker genes- The selection is based on the survival of transformed cells when grown on a medium containing a toxic substance (antibiotic, herbicide, antimetabolite). This is due to the fact that the selectable marker gene confers resistance to toxicity in the transformed cells, while the non-transformed cells will get killed.

Some of them are given below:

Antibiotic resistance genes(Hygromycin phosphotransferase, hpt gene)
Herbicide resistance genes(Enolpyruvylshikimate phosphate synthase, epsps)
Antimetabolite marker genes(Dihydrofolate reductase, dhfr gene)

ii) Reporter genes- An assay for the reporter gene is carried out by estimating the quantity of the protein it produces or the final products formed.

Some of the important ones are given below:

Opine synthase (ocs), β-Glucuronidase (gus), Bacterial luciferase (luxA), Firefly luciferase (luc)

APPLICATIONS

- Transgenic plants have various applications -:

RESISTANCE TO BIOTIC STRESS

1) INSECT RESISTANCE

2) VIRUS RESISTANCE

3) FUNGAL AND BACTERIAL RESISTANCE

RESISTANCE TO ABIOTIC STRESS

1) HERBICIDE RESISTANCE

2) GLYPHOSATE RESISTANCE

IMPROVEMENT OF CROP YIELD & QUALITY

1) EXTENDED SELF LIFE OF FRUITS

2) IMPROVED NUTRITION

3) IMPROVED COLORATION

PRODUCTION OF LOW-COST PHARMACEUTICALS

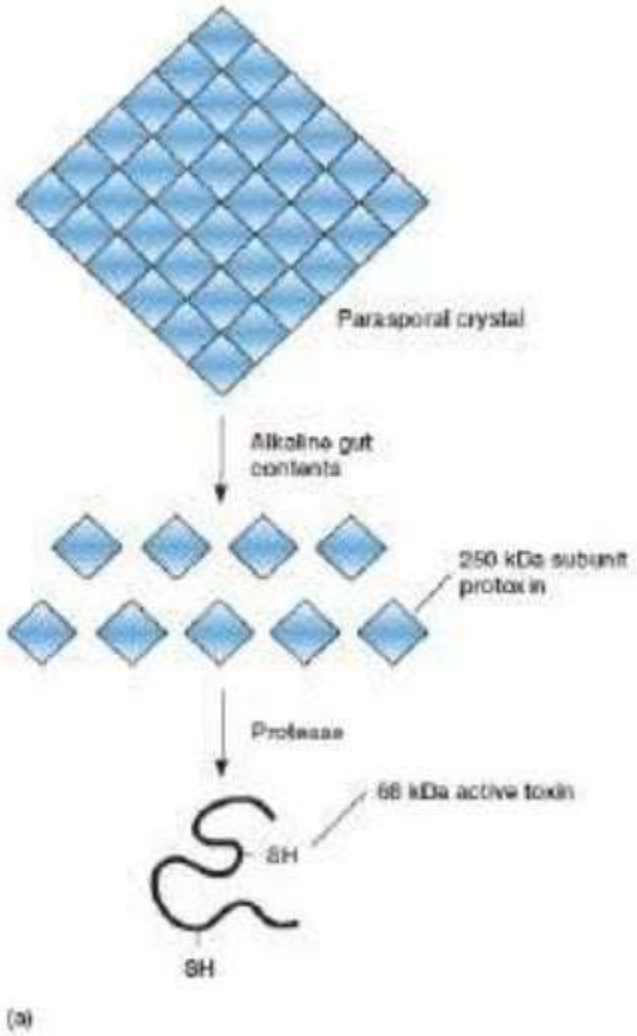
1) EDIBLE VACCINES

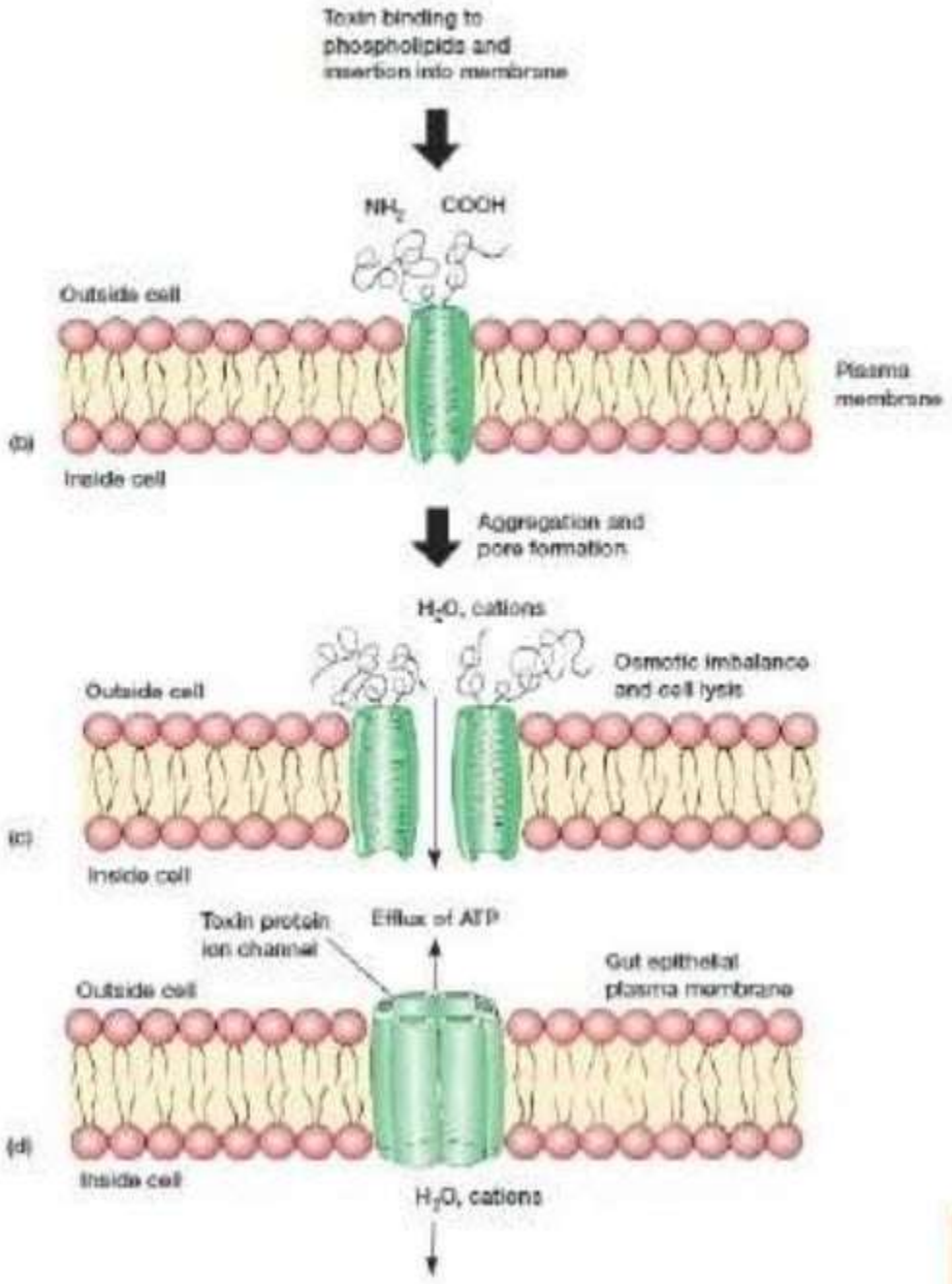
2) ESSENTIAL PROTEINS

Insect resistant plants

- It is estimated that about 15% of the world's crop yield is lost due to insects or pests.
- *Bacillus thuringiensis* was first discovered by Ishiwaki in 1901. It is a gram negative soil bacterium.
- Most of the Bt toxins are active against Lepidopteron larvae, while some of them are specific against Dipterans and Coleopteran insects.
- Different cry protein produced by Bacillus:
  Cry I : kills butterflies and moths
  Cry II : kills butterflies and flies
  Cry III : kills beetles
  Cry IV : kills only flies
- Plant made only low levels of toxin because they are designed to express well in bacteria and not in plants as they are produced from bacterium.
- Insect toxin gene was altered by changing many bases of the third position of the redundant codon to improve its toxicity.

Action of Bt toxin





Virus resistant plants

- Plants may be engineered with genes for resistance to viruses, bacteria, and fungi.
- Virus-resistant plants have a viral protein coat gene that is overproduced, preventing the virus from reproducing in the host cell, because the plant shuts off the virus protein coat gene in response to the overproduction.
- Coat protein genes are involved in resistance to diseases such as cucumber mosaic virus, tobacco rattle virus, and potato virus X.

Herbicide resistance

- Weeds are unwanted and useless plants that grow along with the crop plants. To tackle these, herbicides are used.

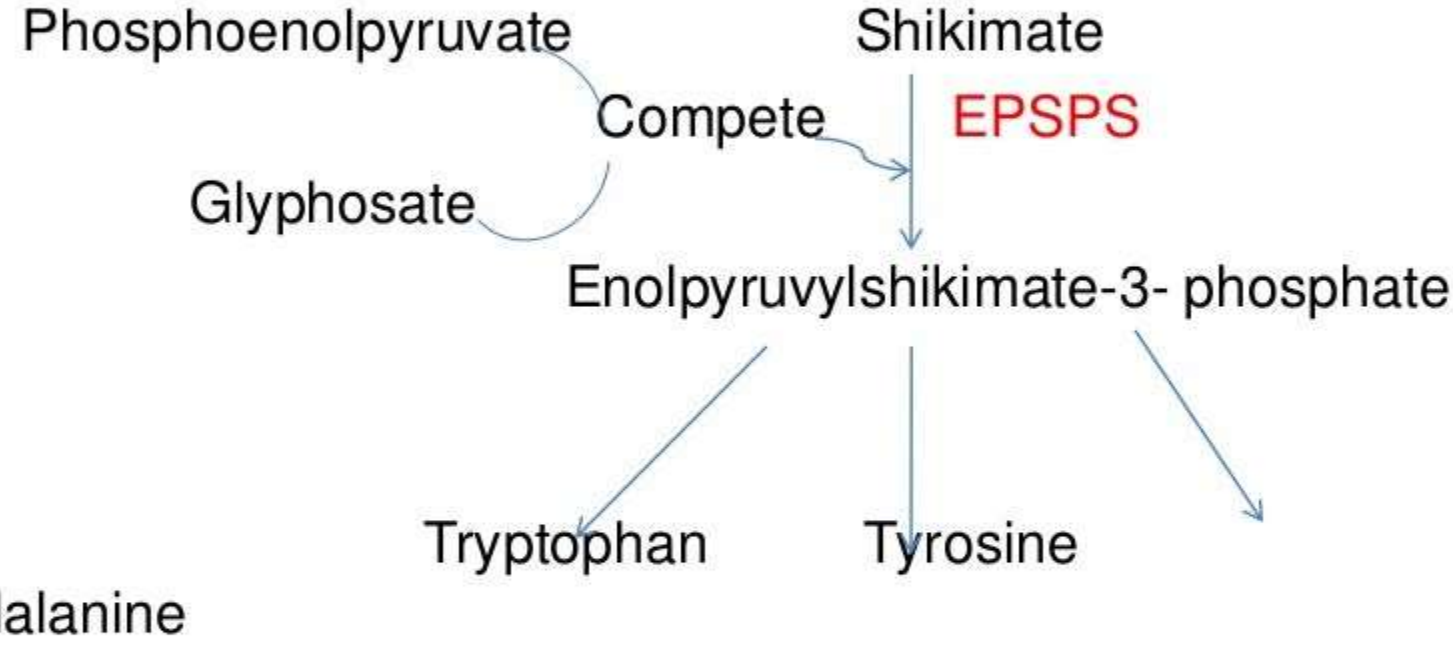


Fig: Glyphosate competes with the phosphoenolpyruvate in the EPSPS catalyzed synthesis of enolpyruvylshikimate-3- phosphate and inhibits synthesis of tryptophan, tyrosine and phenylalanine.

- EPSPS- Enolpyruvylshikimate-3- phosphate synthase

- The 1st crops to be engineered for glyphosate resistance were produced by Monsanto Co. and called "Roundup Ready".

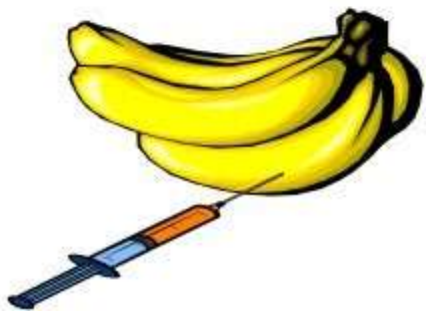
Vaccine production

- Potatoes have been studied using a portion of the *E. coli* enterotoxin in mice and humans and then transgenic potatoes were produced. Ingestion of this transgenic potato resulted in satisfactory vaccinations and no adverse effects.

- Other candidates for edible vaccines include banana and tomato, and alfalfa, corn, and wheat are possible candidates for use in livestock.

- Edible vaccines are vaccines produced in plants that can be administered directly through the ingestion of plant materials containing the vaccine. Eating the plant would then confer immunity against diseases.

One focus of current vaccine effort is on hepatitis B. Transgenic tobacco and potatoes were engineered to express hepatitis B virus vaccine.



GOLDEN RICE

- Transgenic technology produced a type of rice that accumulates β-carotene in rice grains.
- When it is consumed, β-carotene is converted into vitamin-A.
- It contains 37 mg/g of carotenoid of which 84% is β-carotene.

Normal rice

Golden rice

FLAVR-SAVR TOMATO

This is produced by antisense technology.

The polygalactouronase gene, which is responsible for fruit decay is silenced.

Biopolymers and plants

a) Plant seeds may be a potential source for plastics that could be produced and easily extracted.

b) A type of PHA (polyhydroxylalkanoate) polymer called "poly-beta-hydroxybutyrate", or PHB, is produced in *Arabidopsis* or mustard plant.

c) PHB can be made in canola seeds by the transfer of three genes from the bacterium *Alcaligenes eutrophus*, which codes for enzymes in the PHB synthesis pathway.

d) A polymer called PHBV produced through *Alcaligenes* fermentation, which is sold under the name Biopol.

TEARLESS ONION

Fig: Produced by Gene Silencing

COLOURFUL CAULIFLOWER

PURPLE TOMATOES

PURPLE ROSE

COLOURFUL CORNS

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