GOVERNMENT DEGREE COLLEGE, SHADNAGAR

GOVT. DEGREE COLLEGE
SHADNAGAR.
T. S.
DIST: RANGA REDDY

DEPARTMENT OF BOTANY

PROJECT WORK

GOVERNMENT DEGREE COLLEGE, SHADNAGAR

DEPARTMENT OF BOTANY

DECLARATION

We the following students studying B.Sc. II Yr. at Government Degree College, Shadnagar during the academic year 2021-22 here by declared that it is our original project work on **Genetically Modified Crops** submitted under the guidance of Dr. T. Uttara Phalguni.

Sr. No.	Name of the Student	Hall Ticket No.	Sign. of the Student
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2	B. Ramesh	20033067 445 003	B. Ramesh
3	G. Manasa	20033067 445 006	G. Manasa
4	K.Pavani	20033067 445 008	K. Pavani
5	K. Supriya	20033067 445 010	K. Supriya
6	M. Santosh Kumar	20033067 445 011	M. Santoshkumar
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Guided by

Dr. T. Uttara Phalguni

Department of Botany.

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CERTIFICATE

This is to certify that

1. B.Sravani --- 20033067 445 002
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3. G. Manasa --- 20033067 445 006
4. K.Pavani --- 20033067 445 008
5. K. Supriya --- 20033067 445 010
6. M. Suresh --- 20033067 445 013
7. M. Santosh Kumar--- 20033067 445 011

Have successfully completed their project work on **Genetically Modified Crops.**

Signature of Supervisor

Signature of Principal

Principal
GOVT. DEGREE COLLEGE
SHADNAGAR
Ranga Reddy Dist.

TRANSGENESIS :=

Is the process of introduc-ing an Exogenous gene called a Transgene into a living organism. So that the organism will exhibit a new property and transmit that property to its offspring.

HISTORY :=

In 1983 the First genetically engineer-ed plant- Michael W Bevan. Richard B Flavell and Mary Dell chilton.

They infected tobacco with agrobacteriu-m transformed. With an antibiotic resista-nce gene and through tissue culture techniqu-es were able to grow a new plant containing the resistance gene.

In 2000. vitamin A-enriched golden rice. was the First plant developed with increased nutrient value.

TRANSGENIC PLANTS :=

→ Genetically modified plants in which foreign-source genes have been introduced - inserted into desired - ~~target~~ -ed plants

→ Generation of transgenic plants are referred as Transformation (i.e. uptake of foreign DNA by plants cells.) and this techniq -ue is known as Transformation technique

→ It is also known as genetic engineering (or) genetic modification

→ 3 STEPS OF GENETIC ENGINEERING

a) Isolation of gene

b) Finding a vector

c) Placing the vector

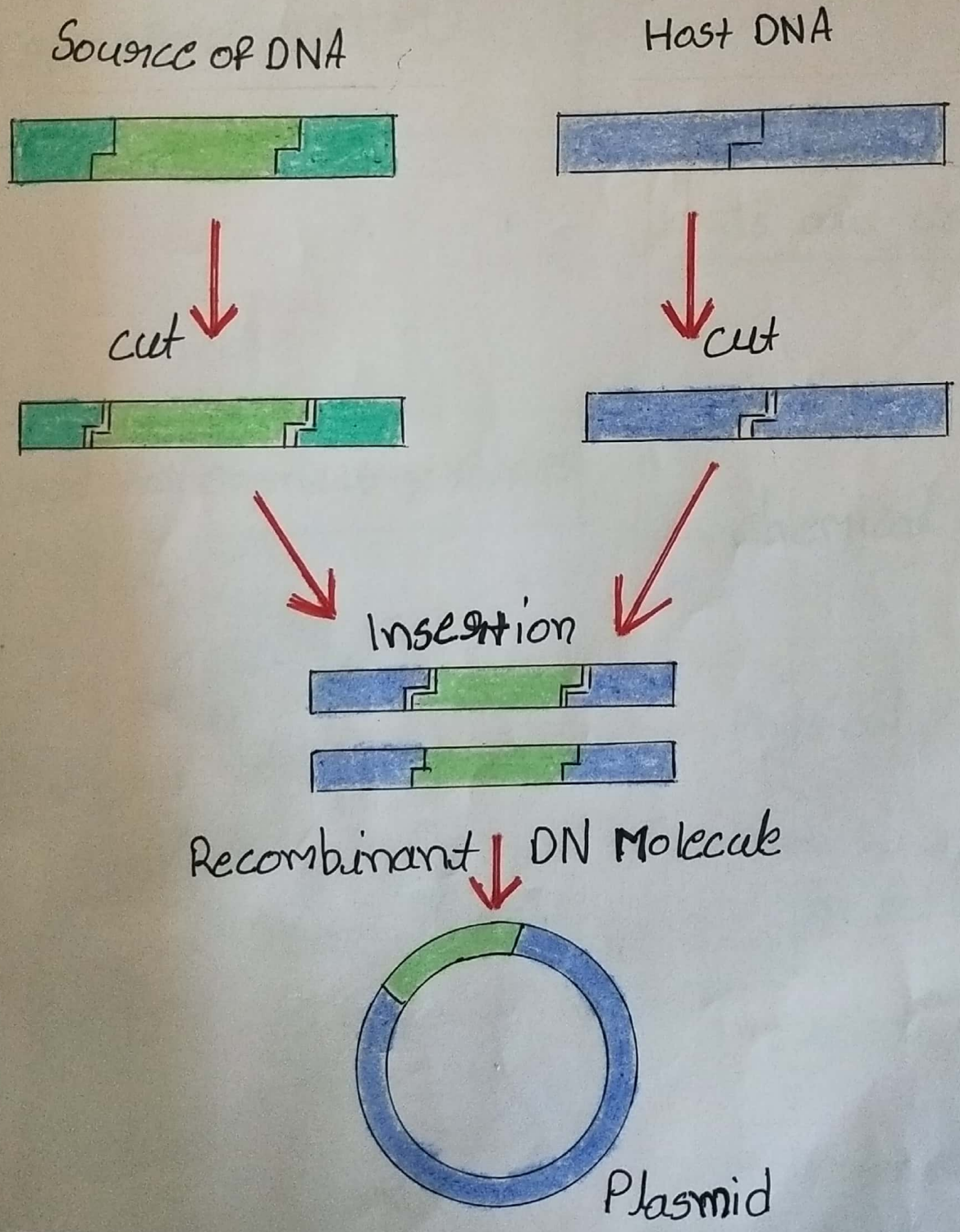
⇒ Digesting DNA by means of Restriction Endonuclea -se carryout M-DNA directed DNA synthesis through reverse transcriptase (C-DNA)

⇒ Binary vector - plasmid

⇒ Inserting the DNA into the vector - open plasmid and then introduce the foreign

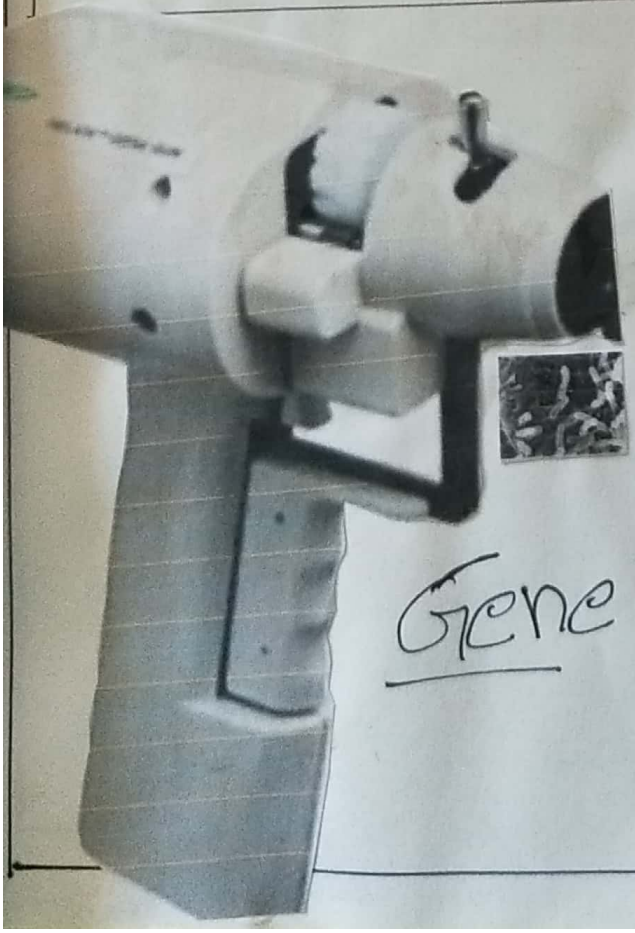
DNA and now plasmid is ready ~~is ready~~ for introduction into the host cell

Cloning



TRANSFORMTION TECHNIQUE

- Agrobacterium Mediated gene Transfer (INDRECT)
  - Used for engineering DICOTS
- Direct gene transfer
  - For MONOCOTS methods are used
  - → chemical
  - → Physical
    - → Micro-Macro injection
    - → Biolistis 6
    - → Electroporation



Gene gun

· On the methods used for producing transgenic plants. can be categorized as.....

I INDIRECT

a) BIOLOGICAL

→ Agrobacterium Mediated

→ Virus Mediated

II DIRECT

d) PHYSICAL

→ Gene gun/biolistics

→ Micro/Macro injection

→ Electrophoresis

→ Pressure

→ Laser Mediated

→ Using pollen tubes

→ silical/carbon fibers

c) CHEMICAL

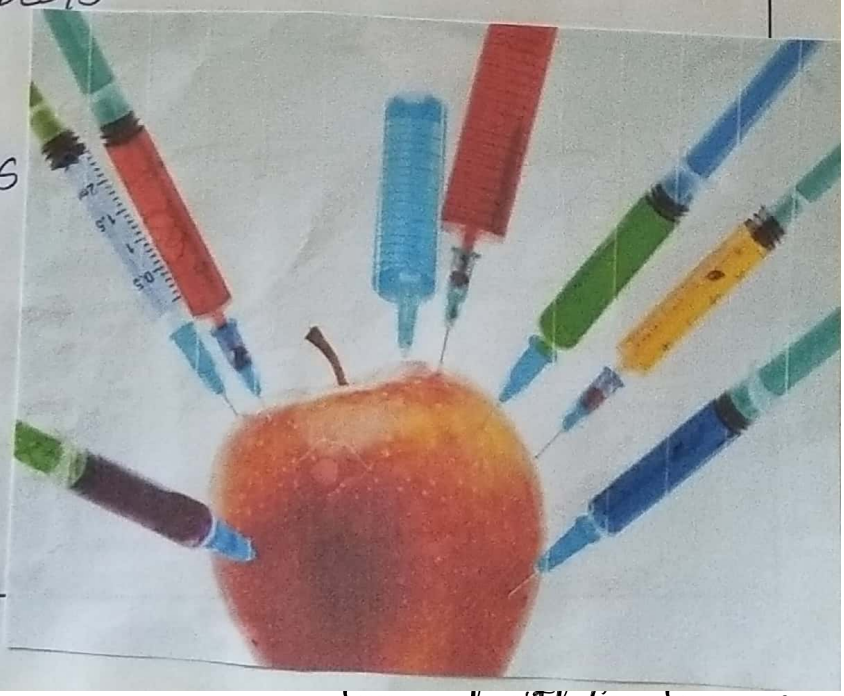
→ Artifical lipids

→ PEG

→ Protcins

→ Dendrimers

→ Dextran



TRANSFORMATION TECHNIQUE

I INDIRECT GENE TRANSFER

a) USE OF AGROBACTERIUM SPECIES.

Agrobacterium - a self styled natural genetic engineer.

- A. Tumefaciens. A. Righogenes & A. Vitis are 3 gram negative soil bacteria often found near the soil level
- A. Tumefaciens. causes crown gall disease disease

A. Tumefaciens

- A. Righogenes. cause hairy root

A. Rihogenes.

→ Agrobacterium-Mediated T-DNA transfer is widely used as a tool in Biotechnology.

→ Agrobacterium mediated transformation is the easiest and most simple plant transformation.

→ It contain Ti and Ri plasmid.

→ It has an ability to integrate new genetic material called as T-DNA into plants

→ Foreign gene used for inserting into the Ti-plasmid has similar function to the already present gene but with different DNA sequences.

Ti-PLASMID (pTi)

→ Plasmid is a small DNA molecule within a cell - Replicate independently

→ Ti plasmid - Tumour inducing plasmid of Agrobacterium tumefaciens / A. species which aids in the development of modified plants

→ The Ti plasmid is lost when Agrobacterium is grown above 28°C - such cured bacteria do not induce crown galls - become avirulent

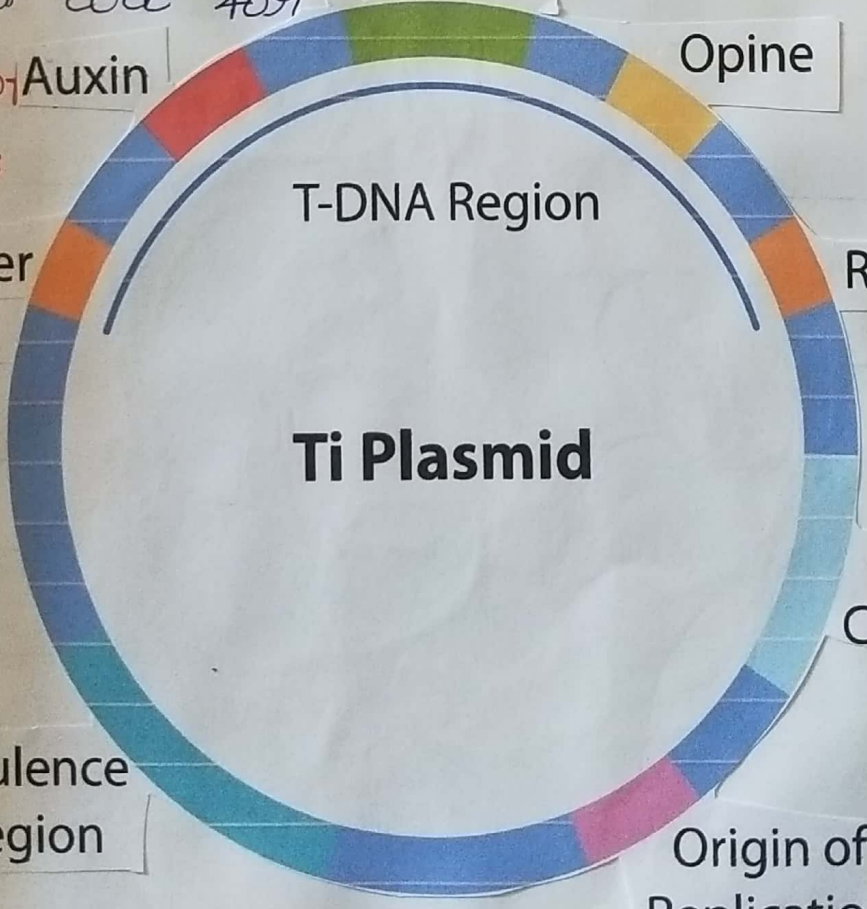
→ size of the plasmid ~ 25K bp & it has 196 genes that code for

Cytokinin

Auxin

Opine

Proteins

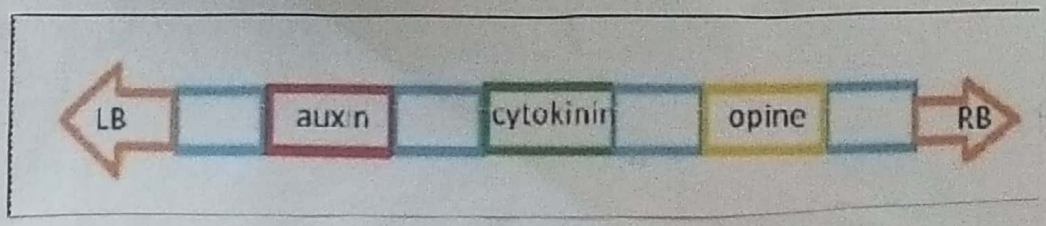


pTi is a circular DNA. contains;

→ T-DNA (has gene for phytohormones)
→ Virulence region (has gene for T-DNA transfer)
→ Origin of replication
→ Opine catabolism (has gene for opine utilization)

T-DNA

→ Is the transferred DNA of the tumor-inducing (pTi) plasmid of some species of Agrobacterium
→ This T-DNA is responsible for crown gall formation in plants
→ This T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the right border and terminated at the left border and requires the vir genes of the Ti plasmid
→ The bacterial T-DNA is about 24,000 base pairs long and contains genes that code for enzymes synthesizing opines and Phytohormones.



T-DNA

- → Auxin & cytokinin gene induces cell division & proliferation
- → Opine synthesize opine-amino acid
- → LB & RB are required for transfer

VIR REGION

- → Transfer the T-DNA to plants
- → Acetosyringone (As) - Flavonoid released by wounded plant cells. activate vir genes.
- → vir region organized into 8 operons - vir A-H
- → Has approximately 25 genes

OPINES

- → Derivatives of amino acid synthesized by T-DNA
- → pTi are categorized based on the type of opine produced by their genes octopine.

agropine, succinamopine and leucinopine

LEFT & RIGHT BORDER SEQUENCE

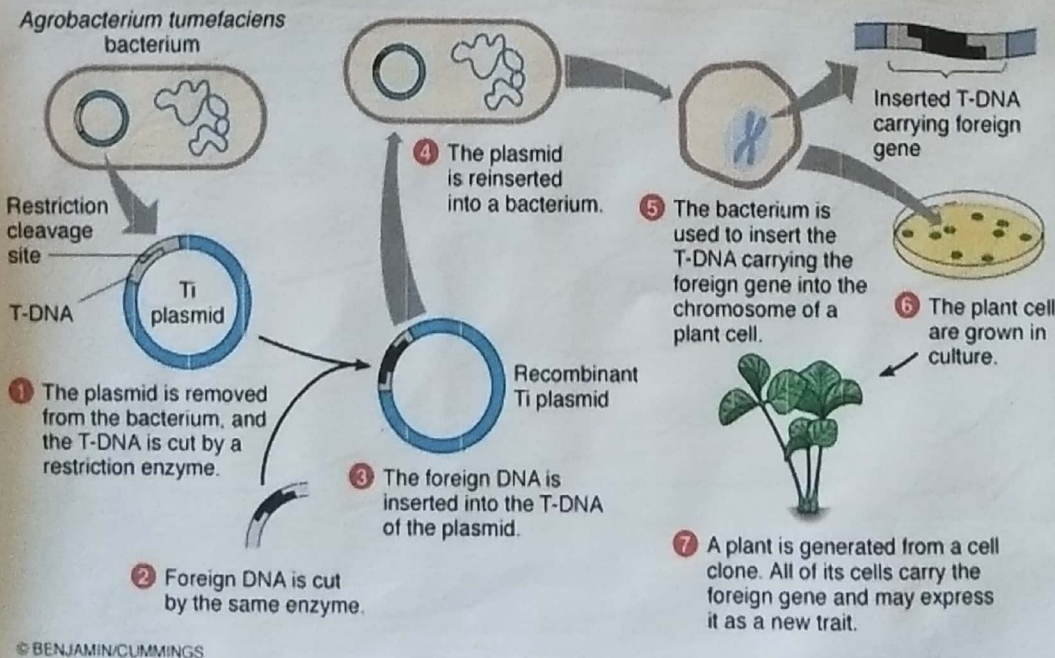
→ Required for T-DNA integration

→ RB enable LB to produce single stranded DNA

PROCEDURE

→ Plant tissue (often leaves) are cut into small pieces, e.g. 10x10mm. and soaked for 10 minutes in a fluid containing suspended Agrobacterium

→ The bacteria will attach to many of the plant cells exposed by the cut



VIRAL TRANSFORMATION

→ Viral transformation. Is the change in growth phenotype. or indefinite reproduction of cells caused by the introduction of inheritable material.

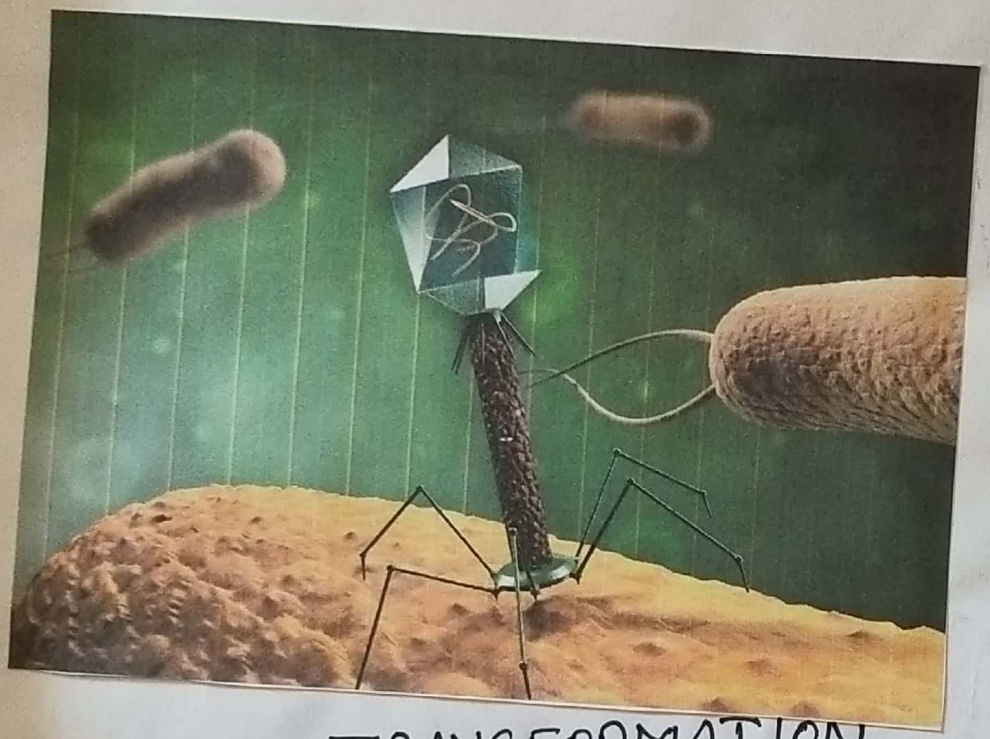
→ Through this process. a virus causes harmful transformations of an in vivo cell or cell culture.

→ The term can also be understood as DNA transfection USING A VIRAL VECTOR

→ In order for a cell to be transformed by a viral. the viral DNA must be entered into the host cell. The simplest consideration /e.g: is viral transformation of a bacterial cell. This process is called lysogeny.

→ A bacteriphage(Entero bacteriophage/lambda phage) lands on a cell and pins itself to the cell. The phage can then penetrate the cell membrane and inject the viral DNA into the host cell.

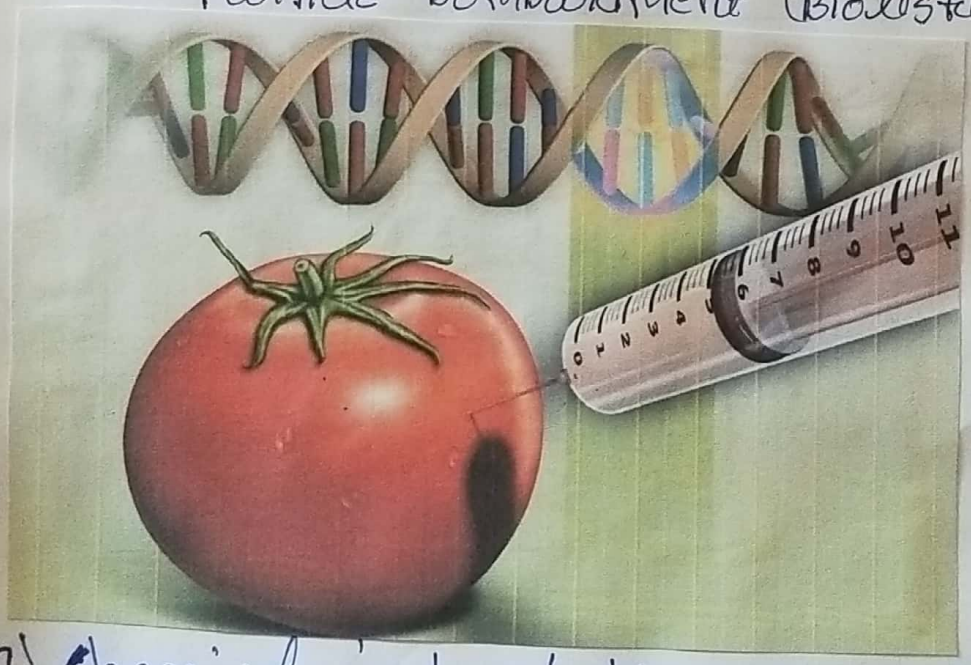
→ The viral DNA can then either lay dormant until stimulated by a source such as UV light



VIRAL TRANSFORMATION

II. DIRECT GENE TRANSEER

→ Is a vector less DNA transfer systems
→ Naked DNA is introduced into the plant/animal cells
→ DNA can be introduced by the following methods;
  a. Chemical
  b. Microinjection
  c. Electroporation
  d. Particle bombardment (Biolistic)



Direct gene tran - SFER

a) Chemical-induced transformation
→ Usually one cell lacking walls are used.
→ Protoplast are incubated with a solution of DNA and PEG (in case of PEG mediated transfer)

→ Catechal was the most potent inducing transformation at concentrations of 1-30uM Followed by hydroquinone (3-30uM) Phenol (10-100uM) and benzene (only at 100 uM)

b. Micro injection.

→ Introduction of cloned genes into cells by means of very fine needles or glass micropipettes (dia 0.5 µM)

→ The microinjection technique is a direct physical approach. and therefore independent, for introducing substance under microscopical control into defined cells without damaging them.

→ Is a limited technique only one cell can be can be injected at a time

→ These two facts differentiate this technique from other physical approaches, such as biolistic transformation and Macroinjection

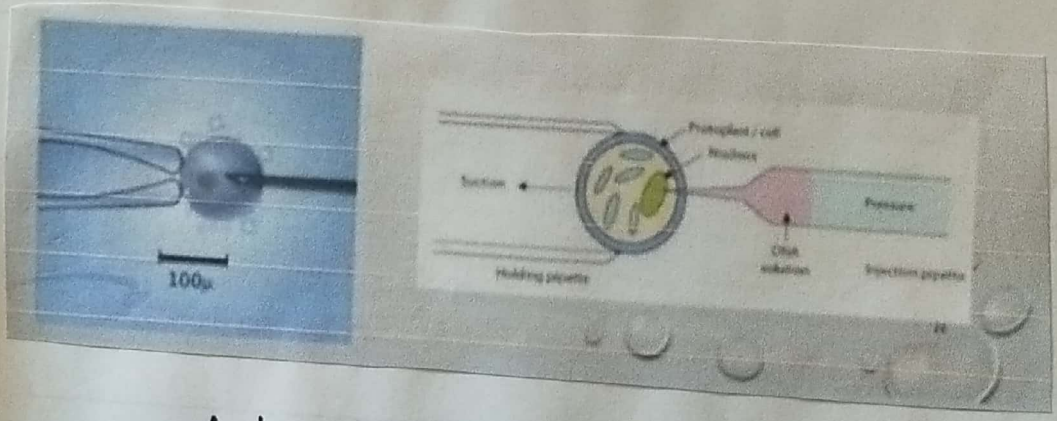
Advantages :-

→ Frequent stable integration of DNA is far better when compared to other methods

→ Method is effective in transforming primary cells as well as cells in establish-ed cultures

→ The DNA injected in this process is subjected to less extensive modifications

→ Mera precise integration of recombinant gene in limited copy no. can be obtained.



Advantages :-

C. Electroporation

→ Thus electrical pulses [high intensity electric field] to produce transient pores in the plasma membrane by allowing DNA in to the cells

→ These pores are known as electropores

→ When the electric field is purified off. the pores the membrane reseal enclosing the DNA inside

Advantages.

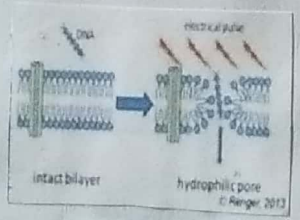
→ Easy to perform

→ high efficiency

→ Can be used for wide range of cell type

Disadvantages

→ cell Mortality [if using suits. optimal condition]



Electroporation

d. Gene Gun Method

→ A biolistic particle delivery system. Originally designed for plant transformation.

→ Device for delivering exogenous DNA to cells

→ It was invented and used by John C. Sanford. Ed Wolf and Nelson at Cornell University. and Ted Klein of Dupont. between 1983 and 1986. to transform epidermal cells of Allium cepa.

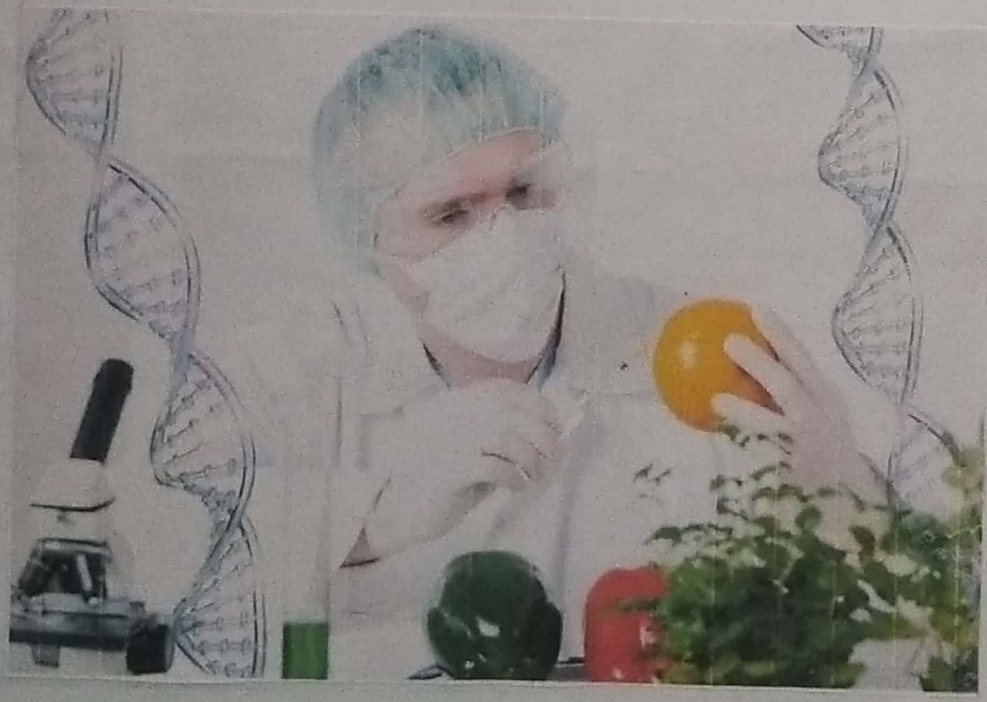
→ This Method is mainly used for cereal transformation

GENE GUN

GENE GUN METHOD

APPLICATION :-

- Herbicide resistance
- Insect resistance
- Virus resistance
- Altered oil content
- Delayed fruit ripening
- Drought. cold, salinity resistance.
- Pollen control
- Enhanced shelf life
- Pharmaceutical & edible vaccines
- Biotic & Abiotic stress tolerance
- Nutritional quality.



APPLICATION

1. HERBICIDE RESISTANCE

a. Bromoxynil Resistance :-

→ A gene enoding the enzyme Bromoxynil nitrilase (BXN) is transferred from Klebsiella pneumoniae bacteria to plants

→ Nitrilase inactivates the bromoxynil before it kills the plant.

b. Sulfonylurea :=

→ kills plants by blocking an enzyme needed for synthesis of the amino acids valine, leucine and isoleucine.

→ Resisance generated by mutating a gene in tobaco plants, and transferring the mutated gene into crop plants.

2. INSECT RESISTANCE.

→ The Bt toxin isolated from Bacillus thuringie-nsis has been used in plants. The gene has been placed in corn, cotton, and has been Marketed

→ Alkaline protein degrades gut wall of lepidopteran larvae

1. Corn borer catepillars
2. Cotton bollworm catepillars
3. Tobacco, hornworm catepillars
4. Gypsy Moth larvae

→ Sprayed onto plants - but will wash off

3. VIRUS RESISTANCE

→ Chemicals are used to control the insect vectors of viruses, but controlling the disease itself is difficult because the disease spreads quickly.

→ 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.

4. ALTERED OIL CONTENT

→ Oil content in plants are altered by modifying an enzyme in the fatty acid synthesis pathway.

→ Varieties of canola and soybean plants have been genetically engineered to produce oils with better cooking and nutritional properties

→ Genetically engineered plants may also be able produce oils that are used in detergents, soaps, cosmetics, lubricants, and paints.

5. DELAYED PRUTT RIPENING

→ Allow for crops, such as tomatoes, to have a higher shelf life

→ Tomatoes generally ripen become soft during shipment to a store.

→ Tomatoes are usually picked and sprayed with the plant hormone ethylene to induce ripening although this does not improve tasic

→ Tomatoes have been engineered to produce less ethylene so they can develop more tasic before ripeing shipment to markets.

6. POLLEN CONTROL

→ Hybrid crops are created by crossing two distantly relad varieties of the same crop plant

→ The method may generate plants with favorible traits, such as tall soybean plants that make more seeds and are resistant to environmental pressures.

→ For success. plant pollination must be controlled This is usually done by removing the make flower parts by hand before pollen is released.

Also sterilized plants have been genetically engineered with a gene from the bacteria Bacillus amyloliquefaciens. This gene is dominant gene for male sterility.

RISKS OF GMO/GMCs.....

→ Can be dangerous & cause allergies

E.g.... soybean containing gene of brazil nut

→ Indirectly promote Antibiotic resistance

→ Weed shows herbicide resistance & resistance to viral disease

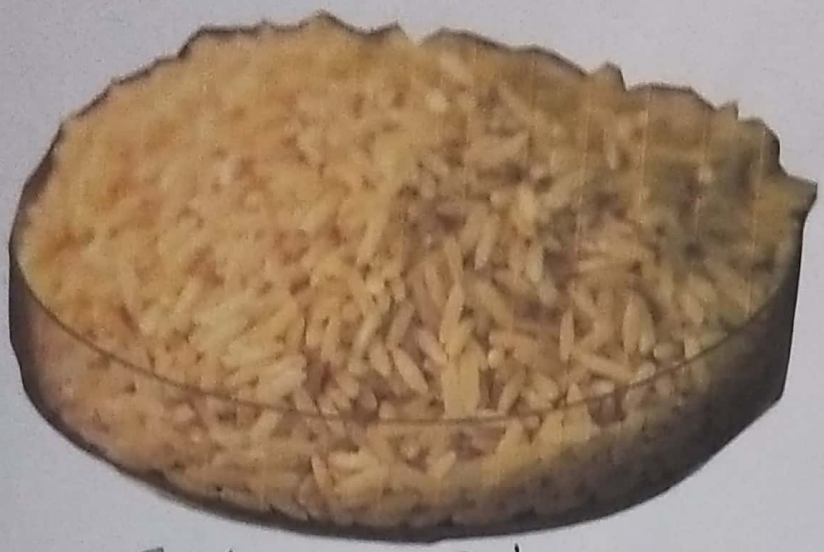
→ change in chemistry of soil

→ Genetically engineered plant cross pollinate non-engineered plants

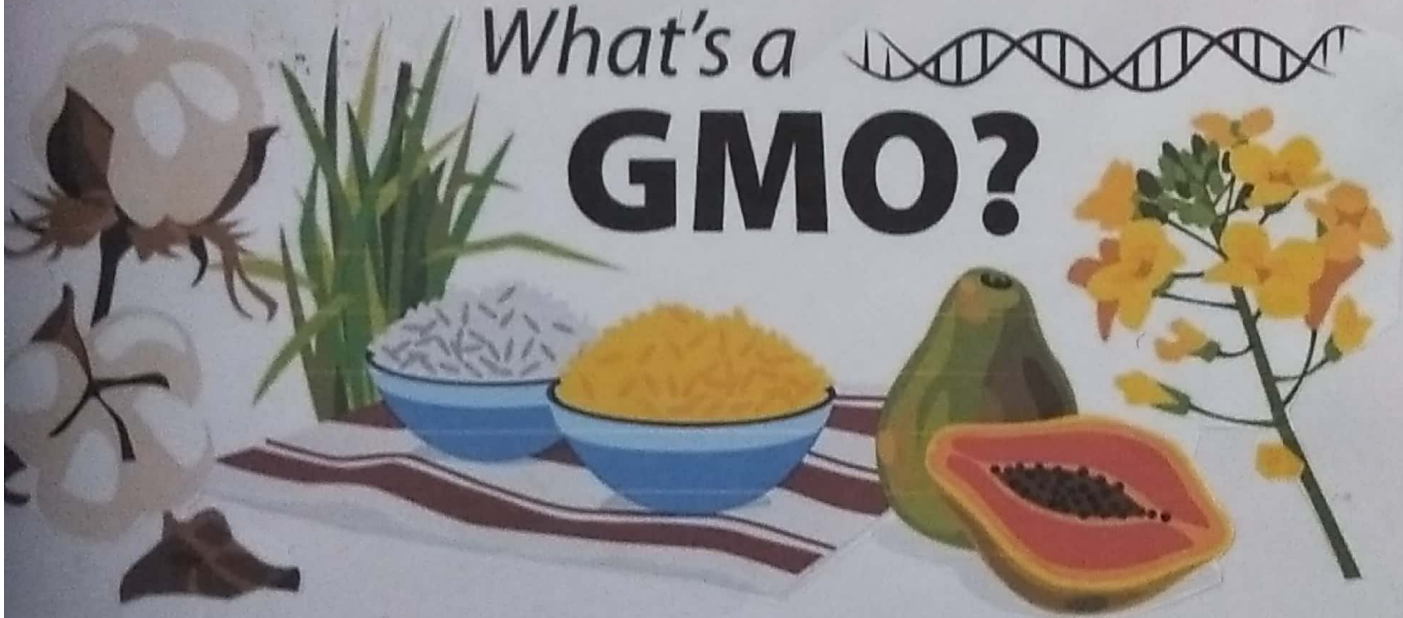
EXAMPLES GM CROPS.....

1. Soybeans.
2. Corn
3. Canola.
4. Cotton.
5. Papaya. rice.
6. Tomato
7. sugar beet. and
8. Red heart chicory
9. Golden rice

Transgenic technology produced a type of rice that accumulates beta-carotene in rice grains. Once inside the body. beta-carotene is ~~convert~~ converted to vitamin A



Golden Rice



Examples GM Crops