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Zoology Assignment

→ Shivaji College, (DU)

Topic:- Production and Application of Transgenic Plant

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V-Sem-'A'

21/23034.

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INTRODUCTION OF TRANSGENIC PLANTS.

- Transgenic plants are plants in which one or more genes from another species have been introduced into the genome using genetic engeneering processes.
- The aim is to introduce a new trait to the plant which doesn't occur naturally in the species.
- A transgenic plant consist a gene or genes that have been artifically inserted. The inserted gene sequence is known as transgene, it may come from an unrelated plant or from a completely different species.
- Techniques involve the biolist method - in which a heavy metal is coated with plasmid DNA is shot into cell - and Agrobacterium teumefaciens mediated transformation.
- The word 'Transgenic' stands for any external genetic features artifically introduced into the genome of another organism to get desired features.
- The 'flavr savr' tomato was the first transgenic plant that made it appearance in the market, developed by Celegene.
- With the advent of new molecular biology techniques such as crispr - cas 9, the future of transgenic plants has unlimited potential.
- 1st transgenic plant produced which is an antibotic resistance tobacco plant.
- 1st successful plant genetic engineering experiment using the caulimovirus vector.
- 1st pesticide producing crop, Bt potato was approved by U.S. Environmental Protection Agency.
- Glocden rice - with β-carotene developed with increased nutrient value.
- 1st genetically engineered crop developed by Robert fraley,

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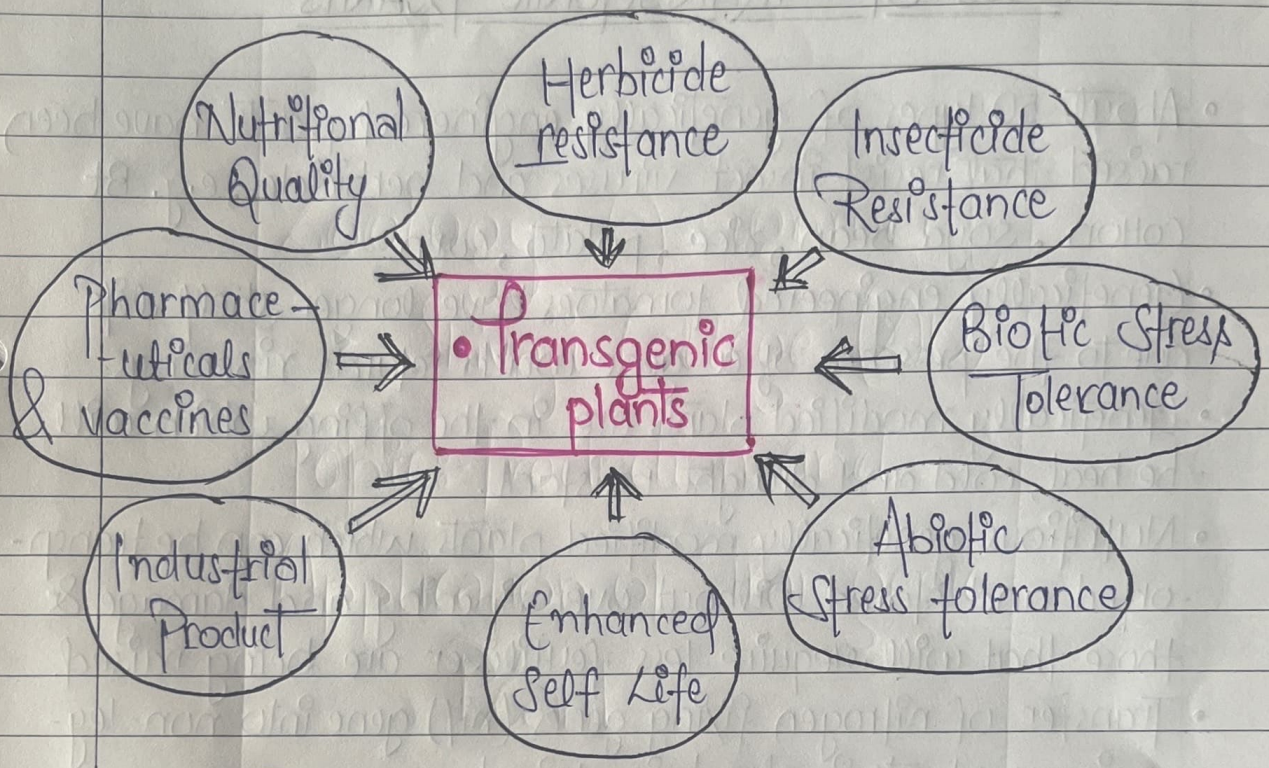
Marc Van, Montagu and Marry Dell chilton was awarded World food prize.

WHY TRANSGENIC PLANTS ?



PRODUCTION OF TRANSGENIC PLANTS

- So to create a transgenic plant, first, we need to identify a helpful gene (this acts as our gene of interest) in an organism we want to insert into our plant.
- This gene is then extracted with the help of restriction endo-nuclease enzymes (molecular scissors) and inserted into the traget genomes.
- These Intsertions can be carried out by several method such as:
  - → particle Gun or gene gun or biolistics,
  - → PEG (polyethylene glycal mediated transformation).
  - → electroporation.
  - → Agrobacterium-mediated gene transfer.

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Generally, a small section of cells take up the gene of interest, and thus they are selected and regenerated into transgenic plants via tissues culture techniques.

• Uses of Transgenic Plants

- About 50 types of genetically enginered plants have been raised that resist insect viruses and herbicides, e.g. Bt cotton, soya bean, rice, potato, corn.
- Genetically engineered tomatoes have longer shelf life due to the presence of a gene which retards ripenings.
- Genetically modified plants are in the offing which will be heat, cold and draught resistant.
- Nutritious genetically modified plants which can be transported and stored without over ripening and damage & those that will require less feutilizer are being raised.
- Transfer of nitrogen fixing genes (nif) gene into non-leguminous plants is also being teired.
- Plants are also being genetically engineered to produce in their seeds proteins (eg. Hormones) which are of interest to humans
- A weed name-mouse-eared cress has genetically engineered to produce a biodegradable plastic poly hydroxyl butylrate or PHB inside its cell granules.

• Advantages of Transgenic Plants.

for the producers of the new varities.
— A high efficiency in plants is obtained.

for farmers

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→ Process of press destroying is simplified, therefore less damage.
→ Production output is increased as well as profits of the transgenic culture, even the obtaining cost of the GMO is rather high.

for industry
→ As in the case of modified starch, low lignin content wood (in this case paper manufacturing is less pollutant), human protein production (for therapeutic aim).

for consumers
→ More nutritious
→ fruits and vegetables are delayed maturation can easily be stored, with minimum losses.
→ In future, transgenic plants content high contain of vitamins, minerals, essential amino acids by using the vaccine products of plant, the rice enriched in pro vitamin A, etc.

for the environment and human future
→ Imply lower pollution
→ Higher agriculture productions.

• DISADVANTAGES OF TRANSGENIC PLANTS

Damage to human health
→ Allergies
→ horizontal transfer and antibiotic resistance.
→ eating foreign DNA
→ Changed nutrient values.
→ Large genomes
(polyploid → presence of many genomes in one cell)

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Damage to the natural environment
→ Crop to weed gene flow
→ leakage of GM protein into soil
→ reductions in pesticides spraying : are they real?

Disruptions of current practices of farming and food production in developed countries.
→ Crop-to-crop gene flow

Disruptions of traditional practices and economics in less developed countries.
→ Lack of research on consequence of transgenic crops.

PLANT GENE TRASFER METHODS

INDIRECT METHODS (Vector-based)
- Agrobacterium mediated transformation.

DIRECT METHOD (Vector-less)
- Physical methods
  - → particle bombardment
  - → electroporation
  - → Microinjection
  - → Liposome mediated DNA transfer
  - → Silicon Carbide fibre mediated DNA transfer.
- Chemical method
  - → PEG-mediated DNA transfer.

IN-PLANTA TRANSFORMATION
- Floral Dip
- Vacuum infiltration
- Agro infection

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AGROBACTERIUM - MEDIATED TRANSFORMATION

→ Below are the basic steps in the Agrobacterium-mediated Plant Transformation Process:

1. Isolate genes of interest from the source organism.
2. Create a functional transgenic construct that include:
   (a) The gene of insert
   (b) Expression promoters
   (c) Codon optimization that increase protein production
   (d) Markers genes to track gene expression in the host plant
3. Insert transgene into Ti plasmid.
4. Insert T-DNA containing plasmid into Agrobacterium
5. Mix newly transformed agro cells with plant cells to allow transfer of the T-DNA into the plant chromosomes
6. Regenerate the transformed cells into genetically modified plants
7. Test at various stages to ensure trait performance.

- Agrobacterium tumefaciens and Agrobacterium rhizogens are soil-borne, Gram-negative bacteria. These are phytopathogen (that cause infection in plants) and as they are treated as the nature's most effective plant genetic engineer. A. tumefaciens induce crown gall disease and A. rhizogenes that induces hairy root disease in plant.

Crown Gall Disease- Ti plasmid.

- A. tumefaciens infects wounded or damaged plant tissues, it induces the formation of a plant tumor called crown gall.
- The entry of bacterium into the plant tissues is facilitated by the release of certain phenolic compound by wounded sites.
- It is a rod-shaped, Gram-negative soil bacterium, the casual agent of crown gall disease in over 140 species of dicots.

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Formation of a crown Gall Tumor.

- Crown gall formation occurs when the bacterium releases its Ti plasmid into the plant cell cytoplasm. A fragment of Ti plasmid, referred to as T-DNA, is actually transferred from the bacterium into the host where its integrated into the plant cell chromosomes. Thus, crown gall disease is a naturally evolved genetic engineering process.
- The T-DNA carries gene that code for proteins involved in the biosynthesis of growth hormones (auxin and cytokinin) and novel plant metabolites namely opines- amino acid derivatives and agropines- sugar derivatives.
- The growth hormones cause plant cells to proliferate and form the gall while opines and agropines are utilized by A. tumefaciens as sources of carbon and energy. Thus, A. tumefaciens genetically transforms plant cell and creates a biosynthetic machinery to produce nutrients for its own use.
- As the bacteria multiple, & continue infections, crown gall develops which is a visible mass of the accumulated bacteria and plant material. Crown gall formation is the sequence of the transfer, integration & expression of gene of T-DNA (or Ti plasmid) of A. tumfaciens in the infected plant.

Organization of Ti plasmid:

- The Ti plasmids (approx 200 Kb each) exist independent replicating circular DNA molecules with the Agrobacterium cell. The T-DNA is variable in length in range of 12 to 24 Kb which depend on the bacterial strain from which Ti plasmids come.

→ Nopaline strains of Ti plasmid have one T-DNA with length of 20 Kb while octopine strains have two T-DNA regions referred to as TL and TR that are respectively 14 Kb & 7 Kb in length.

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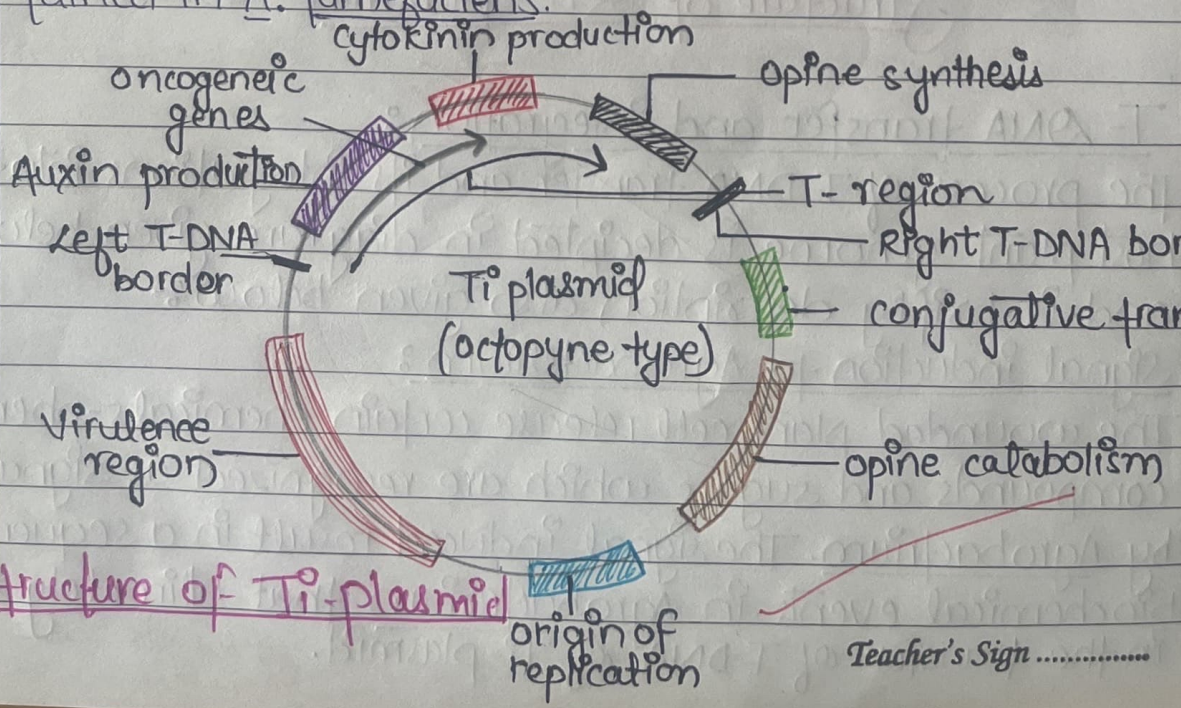
The Ti plasmid has three important regions:-

1. T-DNA regions: This regions has the genes for the biosynthesis of auxin (aux), cytokinin (cyt) and opine (ocs) and is flanked by left and (cytokinin) left borders.
   - These three genes - aux, cyt and ocs are referred to as oncogenes, as they are the determinants of the tumor phenotype.
   - T-DNA borders - A set of 24Kb sequences present on either side (right and left) of T-DNA are also transferred to plant cell.
   - It is now clearly established that the right border is more critical for T-DNA transfer and tumori-genesis.
2. Virulence region or vir region: The genes responsible for transfer of T-DNA into the host plant are located outside T-DNA and the region is referred to as vir or virulence region.
   - Vir region codes for proteins involved in T-DNA transfer. Atleast nine vir-gene operons have been identified.
   - These include vir A, vir G, vir B1, vir C1, vir D1, D2, D4 and vir E1 and E2.
3. Opine catabolism region: This region codes for proteins involved in the uptake and metabolism of opines. Besides the above three, there is ori region that is responsible for the origin of DNA replication which permits the Ti plasmid to be stable maintained in A. tumefaciens.



Structure of Ti-plasmid

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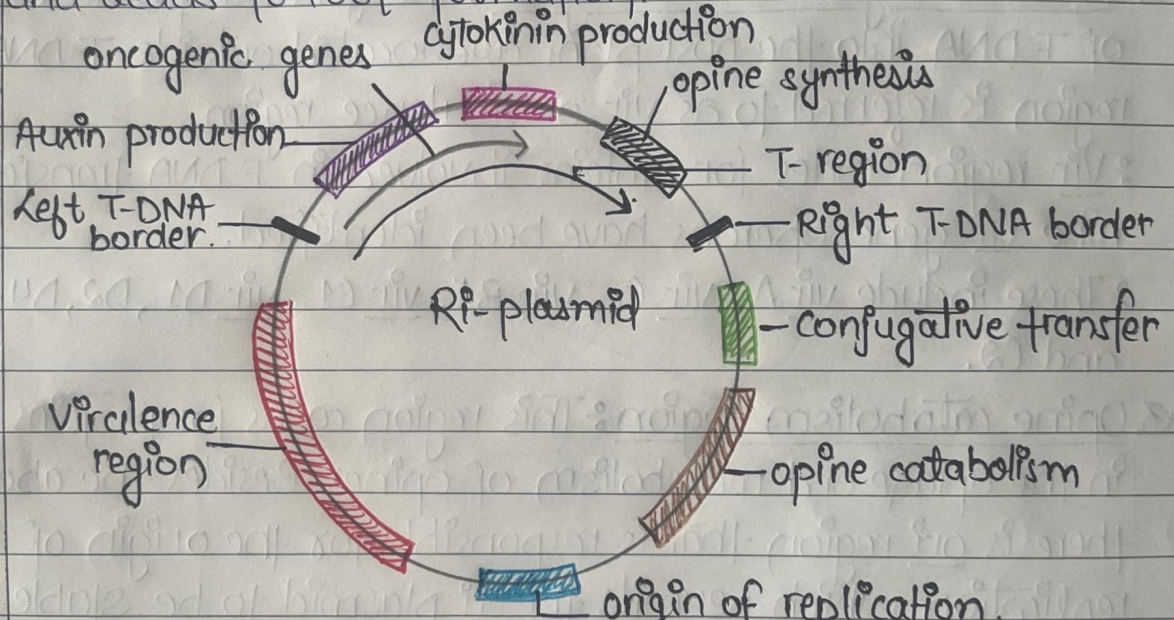
Hairy Root Disease of A. Rhizogenes - Ri Plasmids:

- Agrobacterium rhizogenes can infect plants that cause hairy root disease. The plasmids of A. rhizogens are referred to as Ri plasmid (Root-inducing plasmids). These are of different types. Some of the Ri plasmid strain possess gene that are homologous to Ti plasmid eg. auxin biosynthesis genes.
- Instead of virulences genes, Ri plasmid gene contain a series of open reading frames on the T-DNA.
- The product of these genes are involved in the metabolic of plant growth regulators which gets sensitized to auxin and leads to root formation.



Structure of Ri- plasmid

T-DNA transfer and integration:

The process of T-DNA transfer and its integrations into the host plant genome is decipted in diagram given below and is brifly description also given below:-

1. Signal induction to Agrobacterium:

The wounded plant cell release certain chemicals-phenolic compounds and sugars which are recognized as signals by Agrobactrium. The signal induced result in a sequence of biochemical events in Agrobacterium that ultimetely help in the transfer of T-DNA of Ti-plasmid. Teacher's Sign .....

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2. Attachment of Agrobacterium to plant cell:
- They attach to the plant cell through polysaccharides, particularly cellulose fibres produced by the bacterium.

3. Production of virulence proteins:
- As the signal induction occurs in the Agrobacterium cells attach to plant cell, a series of events that result in the production of virulence protein. To start with, signal induction by phenolics stimulates vir A which is turn activates (by phosphorylation) vir C. This induces expression of virulence gene of Ti-plasmid to produce the corresponding virulence protein (D1, D2, D3, E2, B) Certain sugars (eg. gulcose, galactose, xylose) that induce virulence gene have been identified.

4. Production of T-DNA Strand:
- The right and left borders of T-DNA are recognized by vir D1/ vir D2 proteins. These proteins are involved in the production of single-standard T-DNA, its protection and export to plant cells. The ss T-DNA gets attached to vir D2.

5. Transfer of T-DNA out of Agrobacterium:
- The ss T-DNA-vir-D2 complex in association with vir G is exported from the bacterial cell. Vir B product from the transport apparatus.

6. Transfer of T-DNA into plant cell and integration:
- The T-DNA-vir-D2 complex cross the plant plasma membrane. In the plant cell, T-DNA gets covered with vir E2. This covering protects the T-DNA from degradation by nucleases. Vir D2 and vir E2 intreact with a variety of plant proteins which influence T-DNA transport and integration.
- The T-DNA-vir D2-E2 plant protein complex enter the nucleas through nuclear complex pore. Within the nucleus, the T-DNA gets integrated into the plant chromosome

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through a process referred to illegitimate recombination.

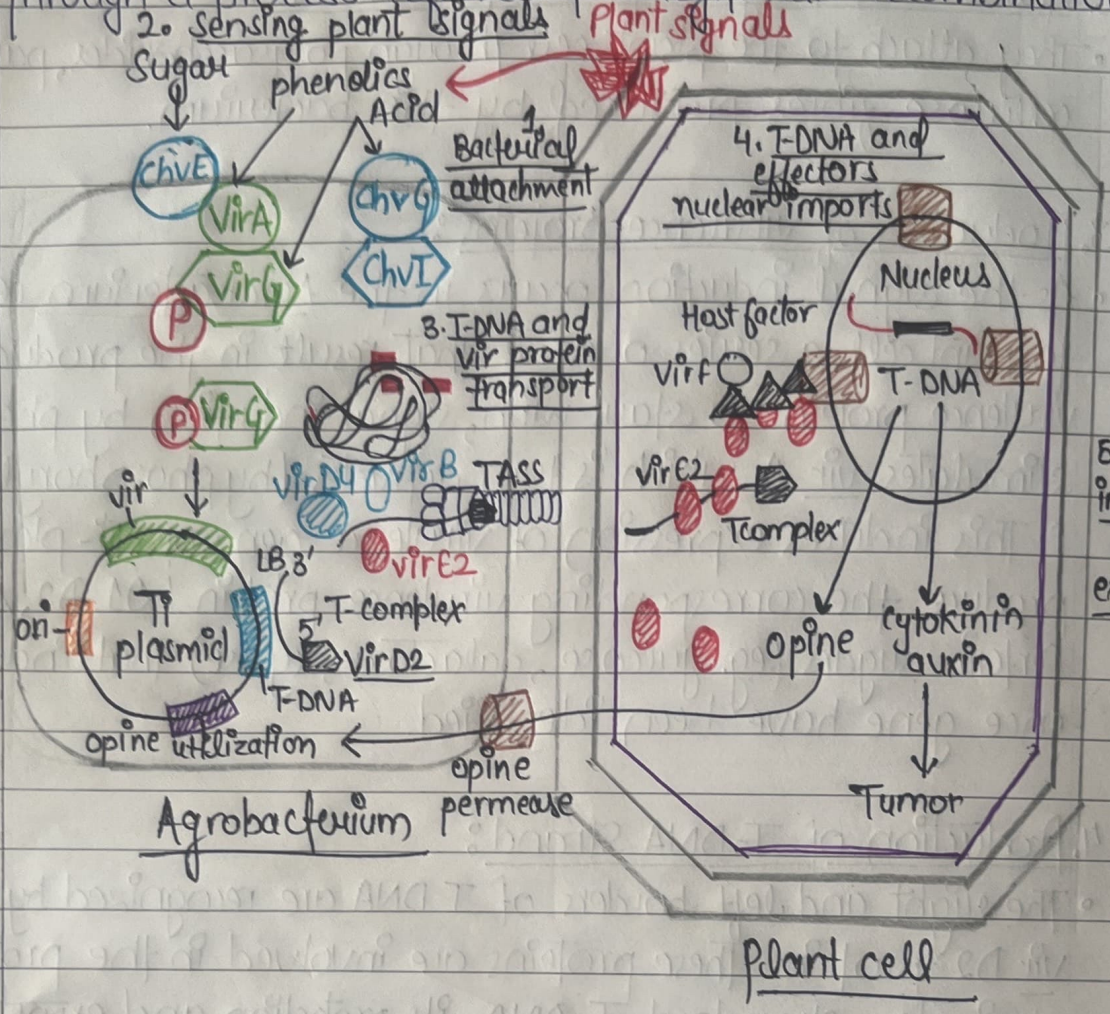

fig:- Process of T-DNA transfer and its integration into host plant genome

Modification of Ti plasmid:

- Two types of Ti-plasmid-derived vectors are used for genetic transformation of plants.

(i) Co-integrate vector
(ii) Binary vectors
(iii) Co-integrate vectors in in the system, the disarmed and the modified Ti-plasmid combines with an intemediate cloning vector to produce a recombinant Ti-plasmid.

Production of disarmed Ti plasmid:

In these Ti plasmid, the oncogenes located in the T-DNA region have been replaced by exogenous DNA.

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- Examples of these vectors include:

1. SEV series:- The right border of the T-DNA together with the phytochromone gene coding for cytokinin and auxin are removed and replaced by a bacterial kamanyin resistance gene while the left border and a small part of the left segment (TL) of the original T-DNA (referred to as left inside Homology (LIH)) are left intact.
2. pGV series: The pytochromone gene are excersied and substitute by part of pBR322 vector sequence. The left and right border sequence as well as the nopaline synthase gene of the Ti plasmid are conserved.

Construction of Intermediate vectors

- These are small pBR322-based plasmids consist a T-DNA region. They are used to overcome the problem derived from the large size of disarmed Ti plasmids and their lack of unique restriction sites.
- Intermediate vector are replicated in E. coli and are transferred into Agrobacterium by conjugation.
- They cannot replicate in A. tumefaciens and therefore, carry DNA segment homologous to the discharmed T-DNA to permit recombination to form a co-integrated T-DNA structure.

Helper vectors

- These are small plasmids maintained in E. coli that contain transfer (tra) and mobilization (mob) genes, which allow the transfer of the conjugation-deficient intermediate vector into Agrobacterium
- A resulting co-integrated plasmid assembled by in vitro manipulation normally contains:

1. the vir genes
2. the left and right border, TDNA.

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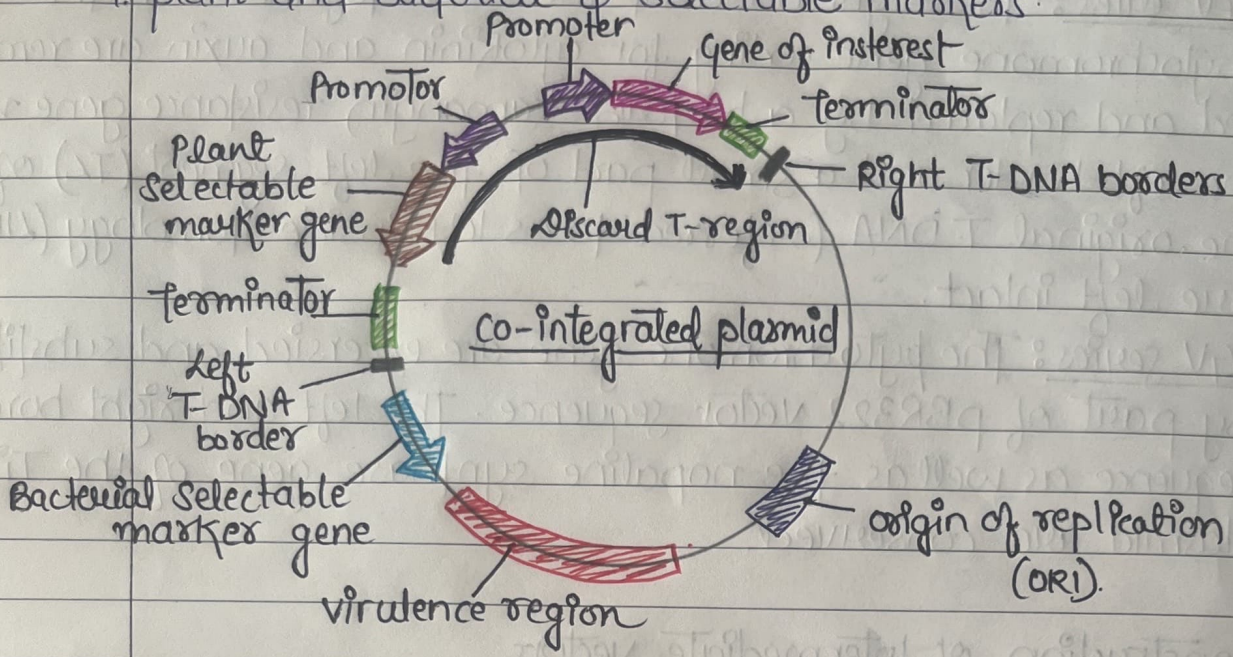
3. an exogenous DNA sequence between the two T-DNA border.
4. plant and bacterial selectable markers.



Binary Vector

- The binary vector system consist of an Agrobacterium strain along with a discard Ti plasmid called vir helper plasmid
- It may be noted that both of both them aren't physically linked. A binary vector with T-DNA can replicate in E.coli and Agrobacterium
- The binary vector has the following components:

→ Left and right border that delimit the T-DNA regions.
→ A plant transformation marker (PTM) e.g. npt II that confer kanamyin resistance in plant transformed cells
→ A multiple cloning sites (MCS) for introducing target/foreign genes
→ A bacterial resistance marker eg. tetracyline resistance gene for selecting binary vector colonies in E.coli and Agrobacterium
→ oriT sequence for confugal mobilization of the binary vector from E.coli to Agrobacterium.
→ A broad host range origin of replication such as RK2 that allow the replication of binary vector in Agro-bacterium.

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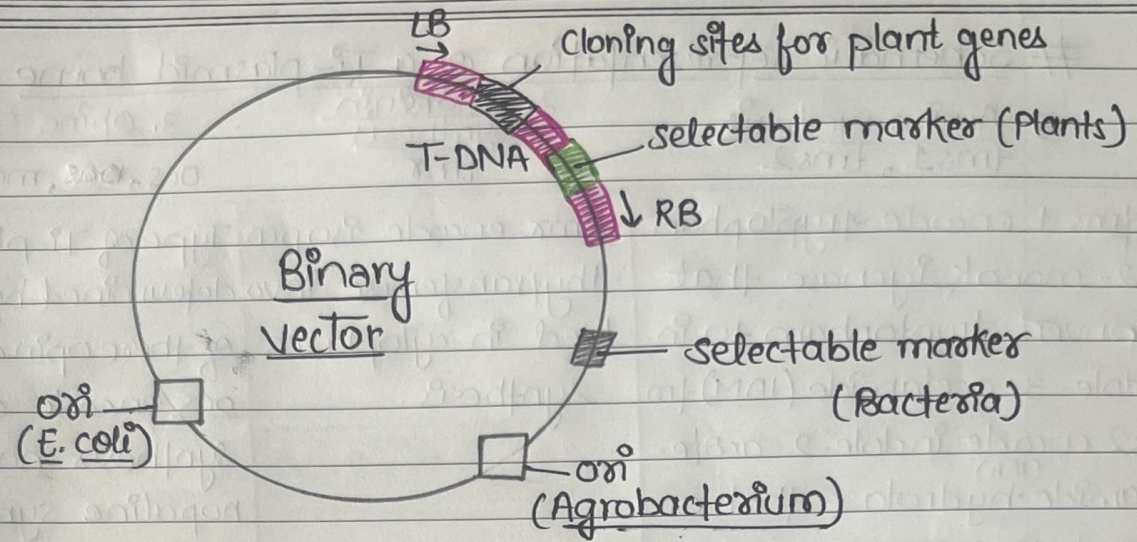
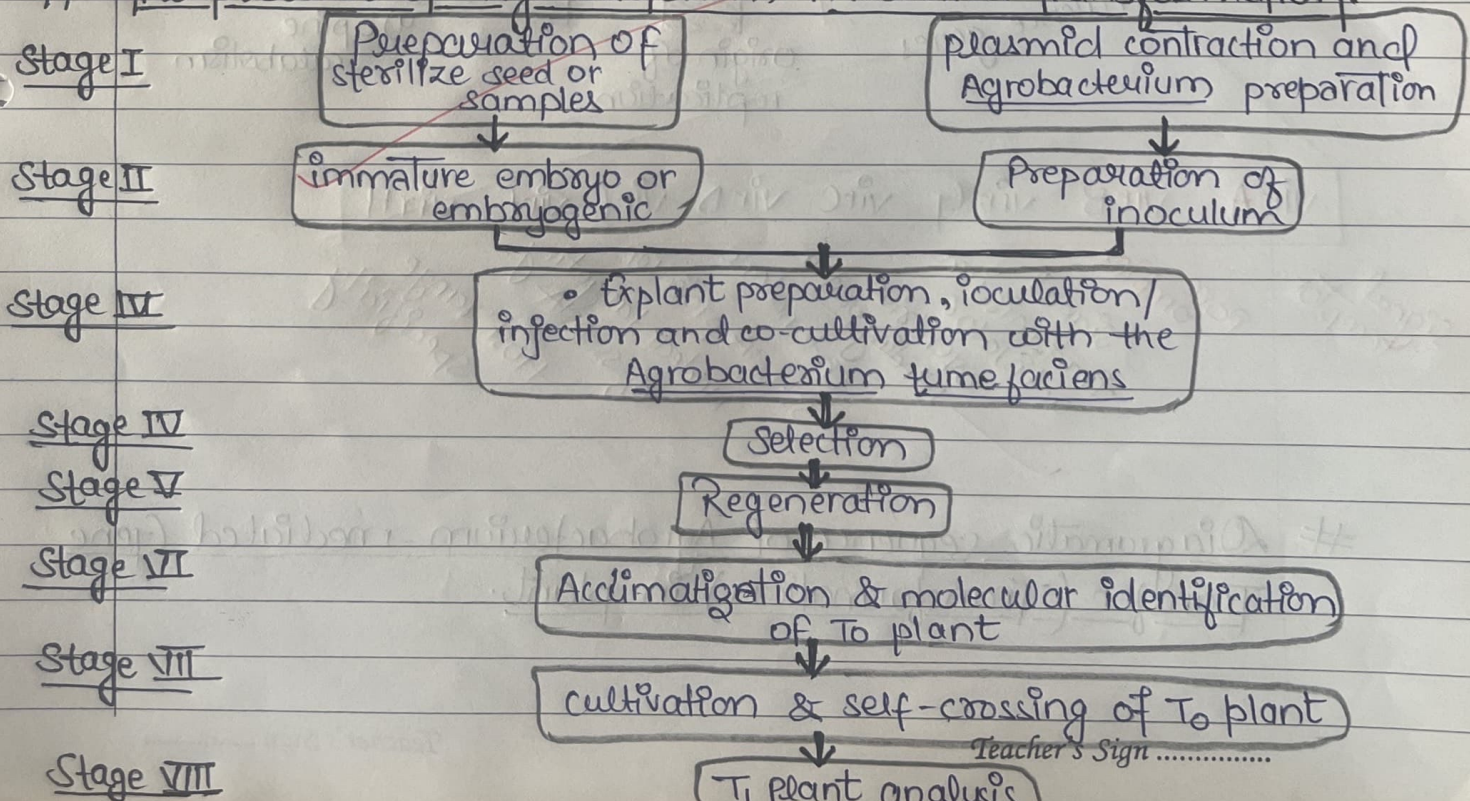


Advantages of Agrobacterium mediated gene Transfer

1. Simple and comparatively less expansive
2. High transformation efficiency
3. Transgenic crops obtained have better fertility percentage
4. Protocols for both dicotyledons and monocotyledons are available
5. Relatively large length DNA segment can be transferred.

The protocol of Agrobacterium-mediated transformation.



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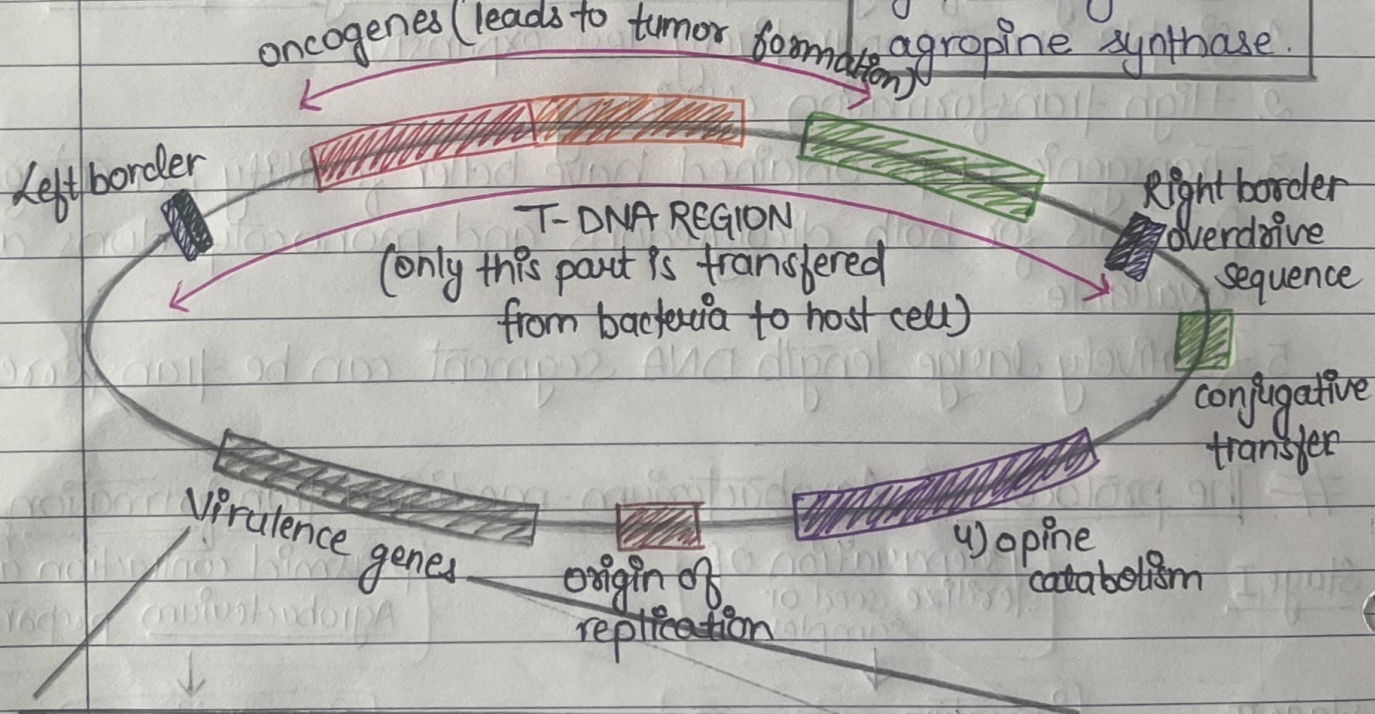
Schematic representation of a Ti-plasmid borne.

1. Auxine synthesis tms1, tms2
2. cytokinin synthesis tmr
3. opine synthesis ocs, nos, mas, ags.

tms 1 encode tryptophan 2-monoksigenase that convert tryptopham to indole-3-acetamide (IAM) tms2 encode indole-2-acate-amide hydrolase (IAA) that convert IAM to IAA.	tmr encode isopenthytransferase involved in cytoplasm synthesis.	Type of Ti-plasmid are determined by the type of there opine production ocs encode octopine synthesis nos encode nopaline synthase mas encode mannopine synthase ags encode agropine synthase.



VirA VirB virG virC virD virE virF virH

signal receptor — pore complex — signal transmission activator — overdrive binding protein — Endonuclease — ss-DNA protection — Nuclear targeting of ss-DNA — Host range specificity

Diagramatic sequence of Agrobacterium-mediated Gene Transfer (Transformation) in plant.

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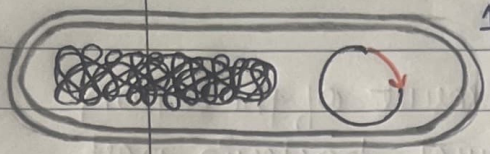
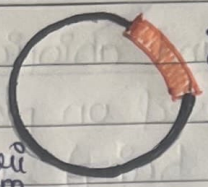
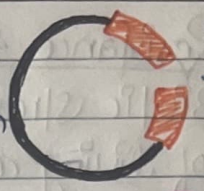


1. T-DNA plasmid removed from bacterium

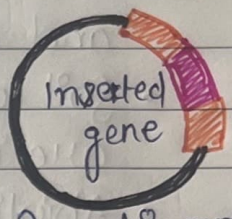


2. Plasmid is cut using a restriction enzymes.



3. Foreign DNA cut with same RE. & inserted into plasmid

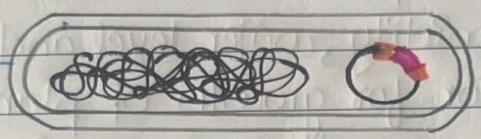
DNA fragment



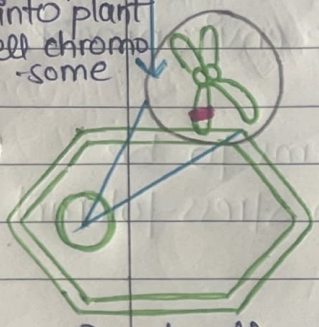
Inserted gene

Recombinant plasmid

Recombinant Ti-plasmid reinserted into bacterium

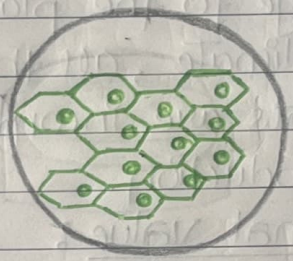


4. T-DNA carrying inserted gene incorporate into plant cell chromo-some

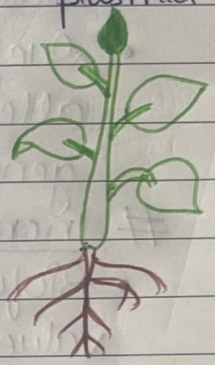


Plant cell

5. plant cell cultured



6. A transgenic plant expressing insert gene is generated



APPCICATION OF TRANSGENIC PLANTS

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 Pharmaceutical**
  1. Edible vaccines
  2. Essential proteins.

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Resistance to biotic and abiotic stress:

- Biotic stress is imposed on plant as a result of the action of living cell (living being) such as viruses, bacteria, pest and pathogens.
- To believe the plant from such stress they are incorporate with disease-resistance gene, which gives a better yield and quality to the crops.
- Soil composition, humidity, water level and temperature of are important factor for growth of plant
- Abiotic stress, as the result of change in the environment, causes great damage to the plant
- Due to change in climate, all the factor seems to be altered. Thus, plants are incorporated with stress-tolerant genes for better production.

Increased nutritional value:

- Biofortification is the process of increasing the nutritional value of a crop.
- Malnutrition is a common problem in developing countries
- As a solution, plant are engineering to produce crops of better nutritional value.

factories of production of recombinant proteins.

- Recombinant (DNA) human protein have been produced using animal and microorganism, system but due to some shortcoming it has been shifted to the plant system.
- Vaccines and antibotics have been obtained from transgenic plant
- However, this application is still in the development stage and has not been commercialised yet.

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INSECT RESISTANCE PLANTS.

- Its is estimated that about 15% of the world's cup crop yield is lost due to insect 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 the Coleopteran insects.
- Different cry proteins produced by Bacillus:
  Cry I : kill butterflies and moth.
  Cry II : kill butterflies and flies.
  Cry III : kill beetles.
  Cry IV : kill 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 reducdant codon to improve its toxicity.

Bt toxin.

- The soil bacterium Bacillus thuringiensis, Bt is a gram-negative aerobic spore forming bacterium found wirld-wide. It produce protein toxic to various herbivorous insects especially to larvae of insect order Lepidoptera, which include cotton boilworms, moths, butterflies, bettles and flies.
- It is harmful to mammals include human, birds, fishes, or the benefical insects. The toxic protein is produced in inactive crystalline form and is converted to active toxin form called delta endo-toxin only when it is consumed by insects.
- It is known as cry protein as it is produced as crystalline protein inclusion during sporulation. The protein bind with certain

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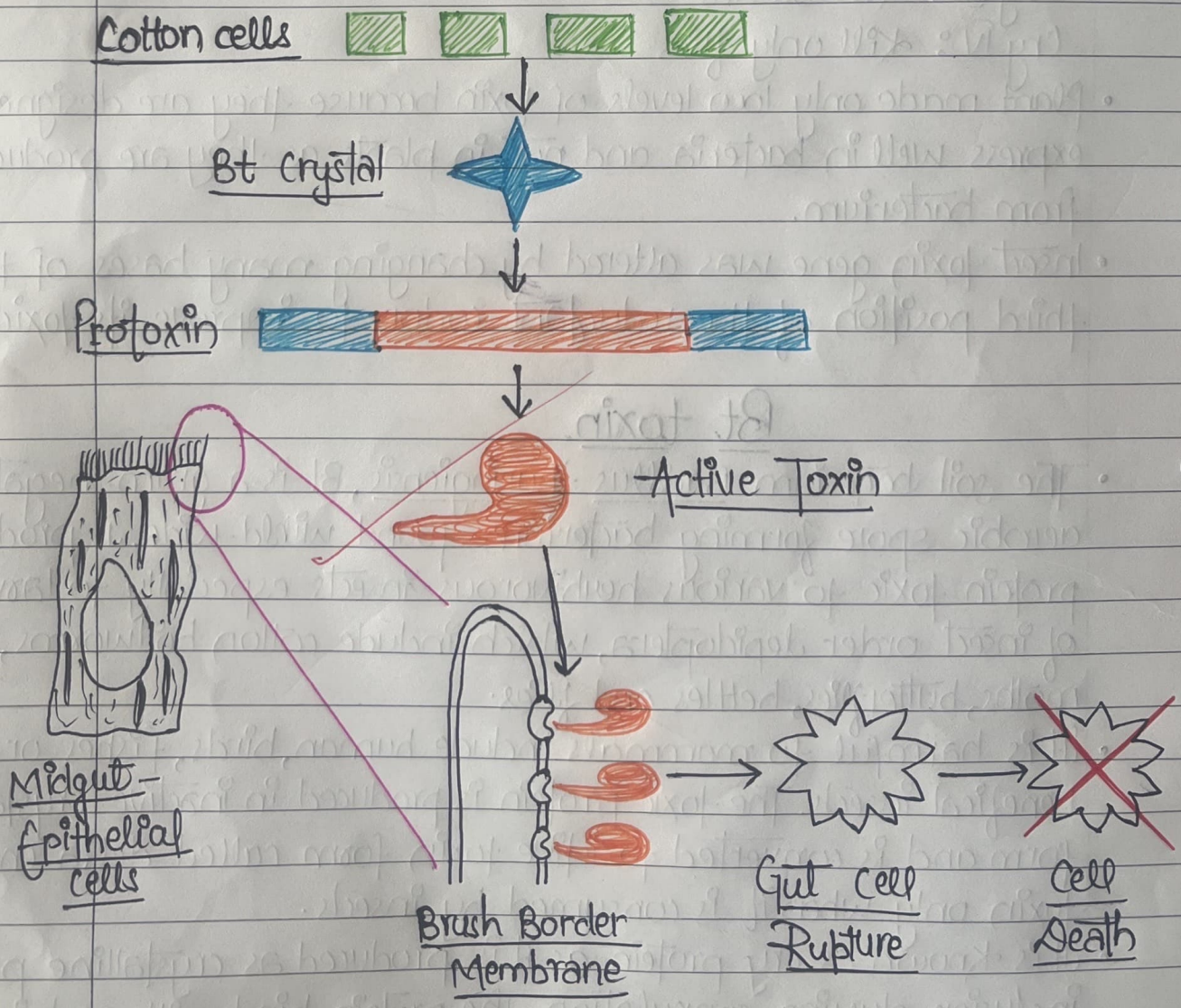
receptors (aminopeticlase N (APN) receptor & cadherin-like receptors) in insect's interstine and causes death of insects. Wounding of epithelial midgut cell.

- The toxin distroy gut of insect, ultimetely rear leading to death of insect. The toxic protein is also known as Bt protein as it is produced by Bacterium Bacillus thuringiensis.
- In USA, it is registered as a biopesticide but the performance of Bt insecticide on cotton plant was limited. The insecticide can be degraded as light, heat, UV, high pH and desiccation.
- Even the area where the prest of cotton (boilworms) feeds are difficult to treat. The insect must eat sufficient treated plant to accumulate lethal dose of toxin.



Mechanism of action of Bt toxin

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B. thuringiensis X Soyabean pest

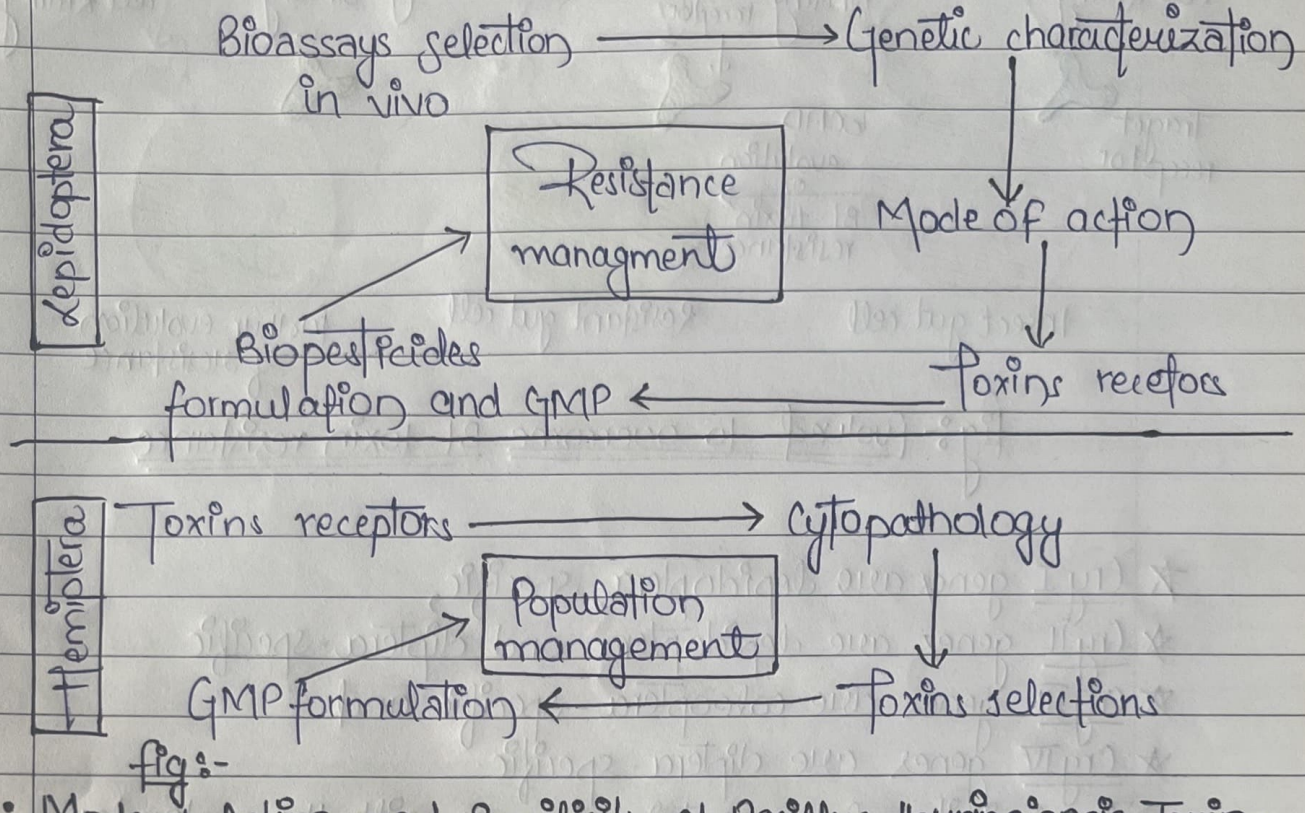
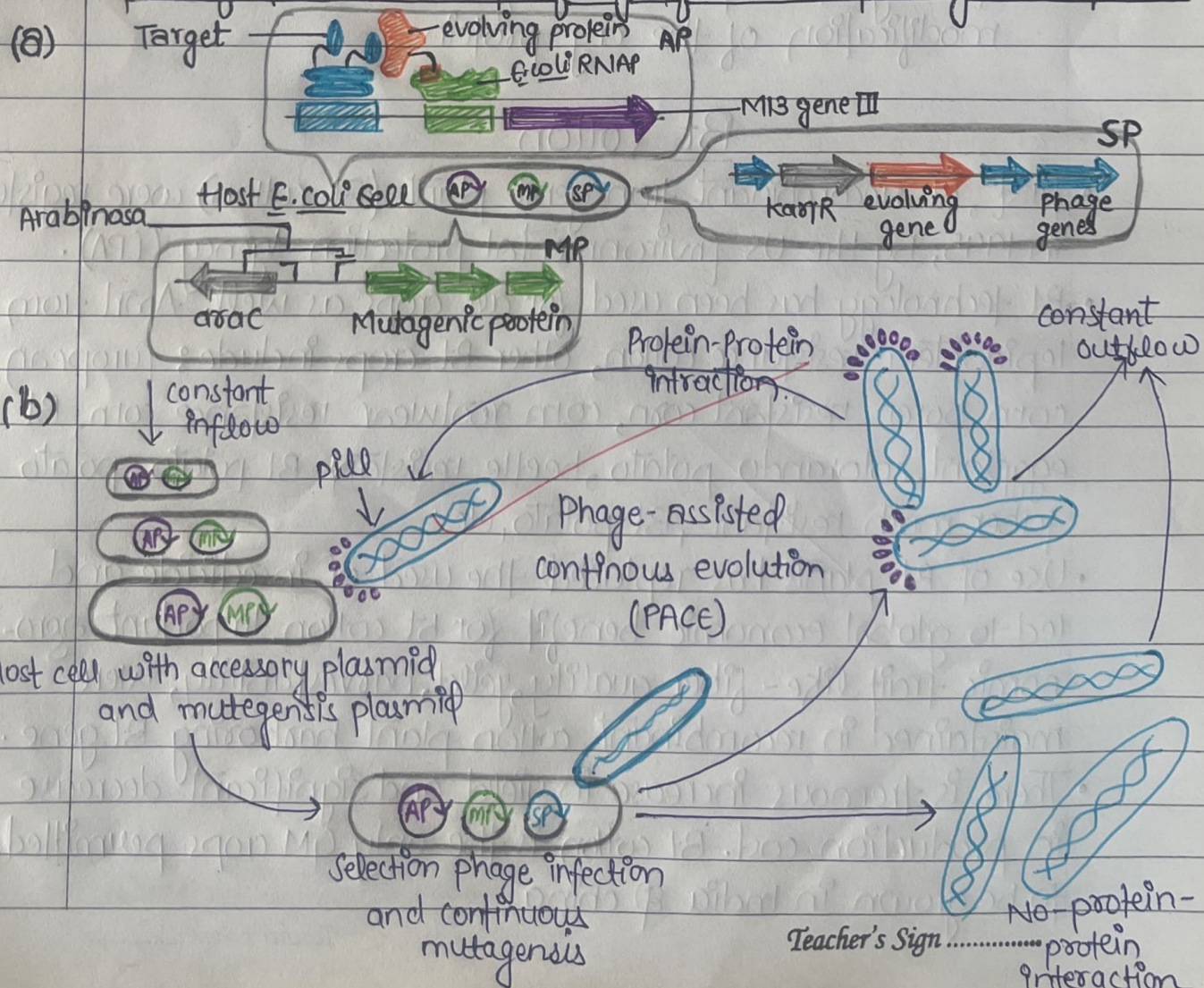


fig:-

∴ Mode of Action and Specificity of Bacillus thuringiensis Toxins



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Topic: Bt toxin ..... Date .....

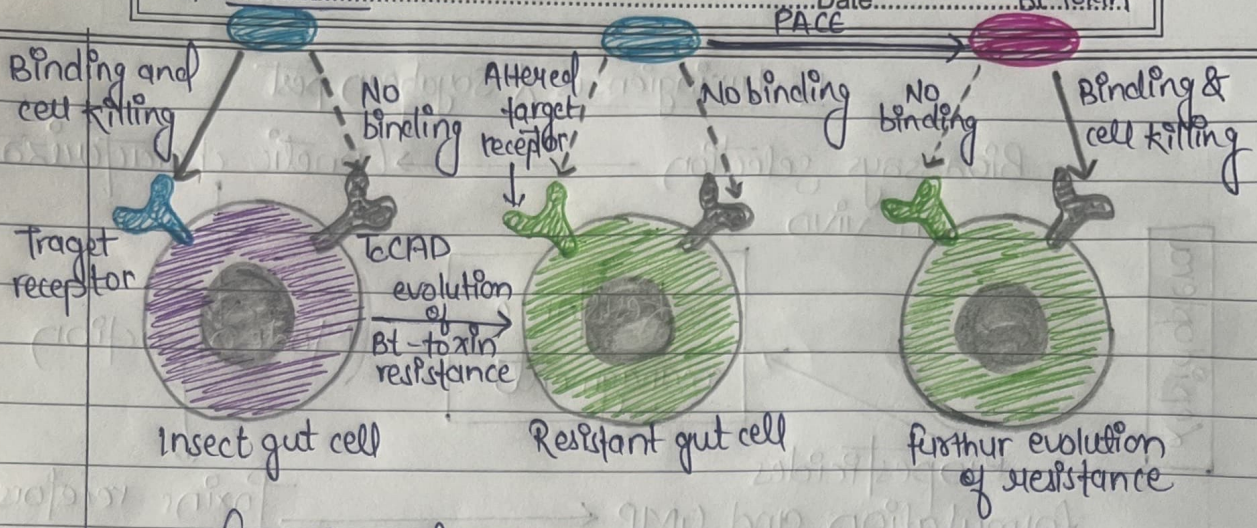

fig:- Evolved to overcome Bt-toxin resistance

- ★ Cry I genes are lepidoptera specific,
- ★ Cry II genes are lepidoptera and diptera-specific
- ★ Cry III genes are coleoptera-specific and
- ★ Cry IV genes are diptera-specific.

→ The toxicity of the protein changes with the molecular modification of the cry protein.

Bt Cotton

- First, Bt recombinant transgenic plant (Bt cotton) were registered in 1995, by the US Environmental protection Agency (EPA).
- Bt technology has been used for all crops as well. Apart from lepidoptera resistance cotton, other Bt crops include: European corn borer resistant corn, corn rootwear resistance corn, Bt eggplant, colorando potato, beetle resistant Bt potato, potato tuber moth resistant, Bt potato, etc.
- Use of Bt plants has replaced the used of insecticide and led to global economic benefit for bt cotton. Important agronomic trait like - fiber quality, yeild, harvestability, were maintained in recombinant cotton plant harboring Bt gene.
- There is tremous increase in yield and significant decrease in production cost. Bt cotton is the only GM crops permitted to be grown in India & china.

Teacher's Sign .....

Topic.....Date.....

- Bt cotton is a cotton variety that has an inbuilt mechanism to protect itself from caterpillar pests, as known as the bollworm.
- The protection corner from a scientific process known as genetic modification (GM). The inbuilt mechanism is from a common soil bacterium also known as Bt, which stands for Bacillus thuringenesis.
- Bt produce a protein that is harmful for the digestion system of a capillary pest. When the capillary feed on the Bt cotton plant, is digestive system is weekend, making it unstable to feed and it eventually dies.
- However, Bt is specific and not harmful for human and other animals. It has been used in organic farming as a spray for over 50 years to control insect pests.

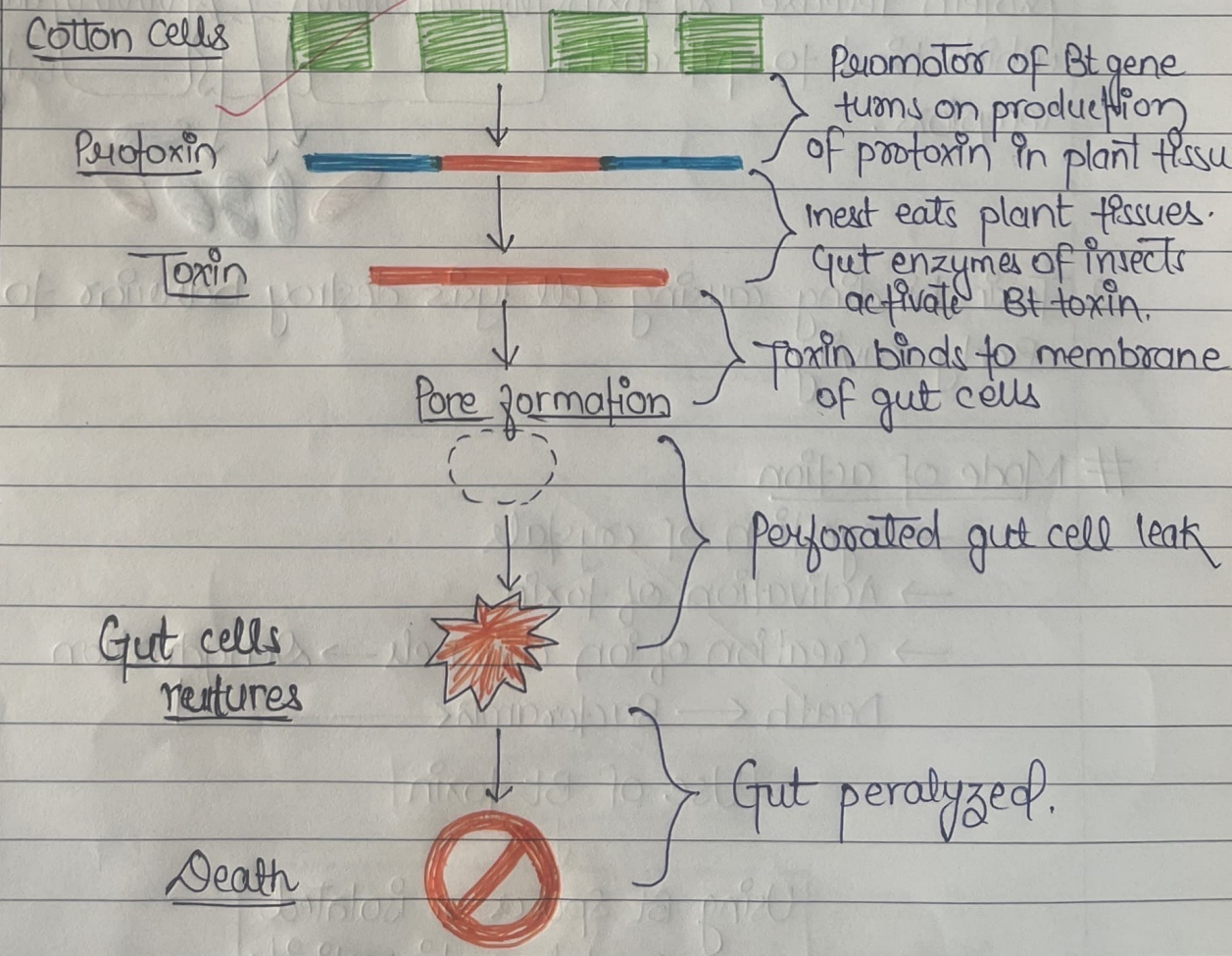

Fig: Mechanism of action of Bt Cotton

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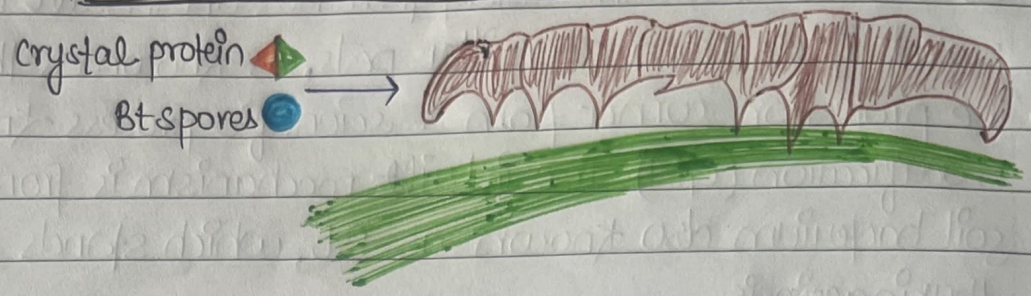
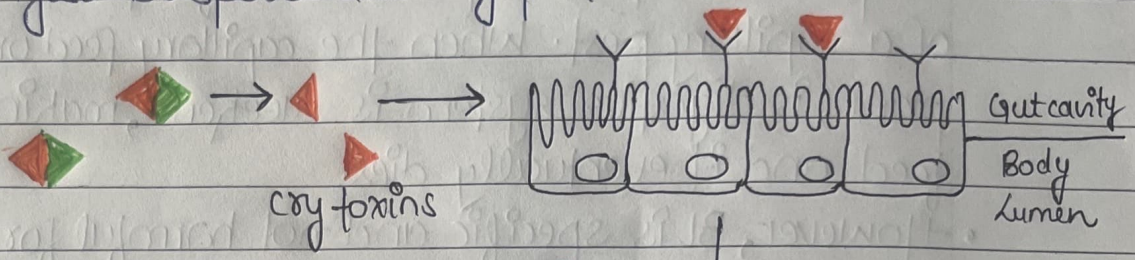
Topic.....Date.....

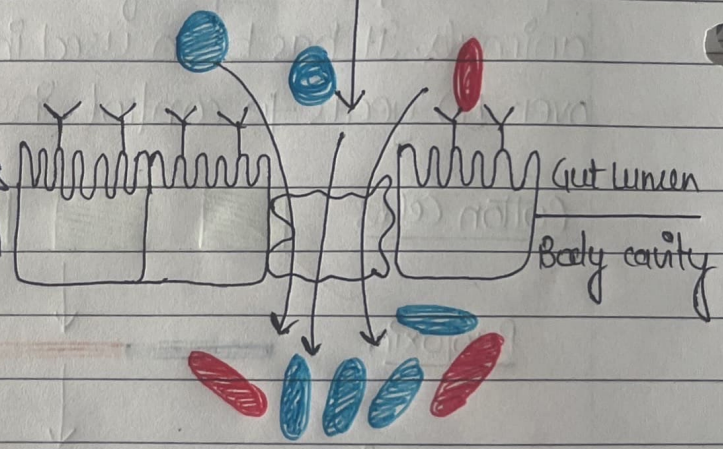
→ How does Bt cotton kill bacteria?



1. Larvae ingest Bt spore and cry protein



2. In larval midgut, proteolytic digestion of protein release cry toxins which bind to epithealial receptors.



3. Toxin binding causing cell lysis destroy barrier to Body cavity.

Mode of action

→ Ingestion of crystals
→ Activation of toxin
→ creation of ion channels → Dehydration
Death ← Bacteramia ←

Use of Bt toxin

Using Bt spores or isolated Crystals as biopesticide

Topic..... Date.....

EDIBLE VACCINES

- Edible vaccines are vaccines produced in plant that can be administrated directly through that ingestion of plant material containing the vaccines. Eating the plant would then confer immunity against disease
- One focus on current vaccines effort is on hepatitis B. Transgenic tobacco and potato were engeineered to express hepatitis B. virus vaccine
- Potato has been studied using a portion of the E.coli entero-toxin in mice and human and then transgenic potatoes were produced.
- Other candidates for edible vaccines include banna and tomato
- In the edible vaccines, Transgenic plants are used as vaccine production systems.
- The genes encoding antigens of bacterial and viral pathogens can be expressed in plants in a form in which they retain native immunogenic properties.
- Initially throught to be useful only for preventing infectious disease, it has also found application in preventing of autoimmune disease, birth control, cancer therapy, etc.
- Edible vaccines are currently being developing for a no. of human and animal disease
- As hippocrates said, 'Let the food be thy medicine'.

Mechanism of action

- The goal of oral vaccination is to stimulate the musocal and systemic immunity against pathogen.
- Edible vaccines when taken orally undergoes the mastication process and the majority of plant cell degradation occur in the digestion of interstine as a result of action of bacterial enzymes on edible vaccine.

Teacher's Sign .....

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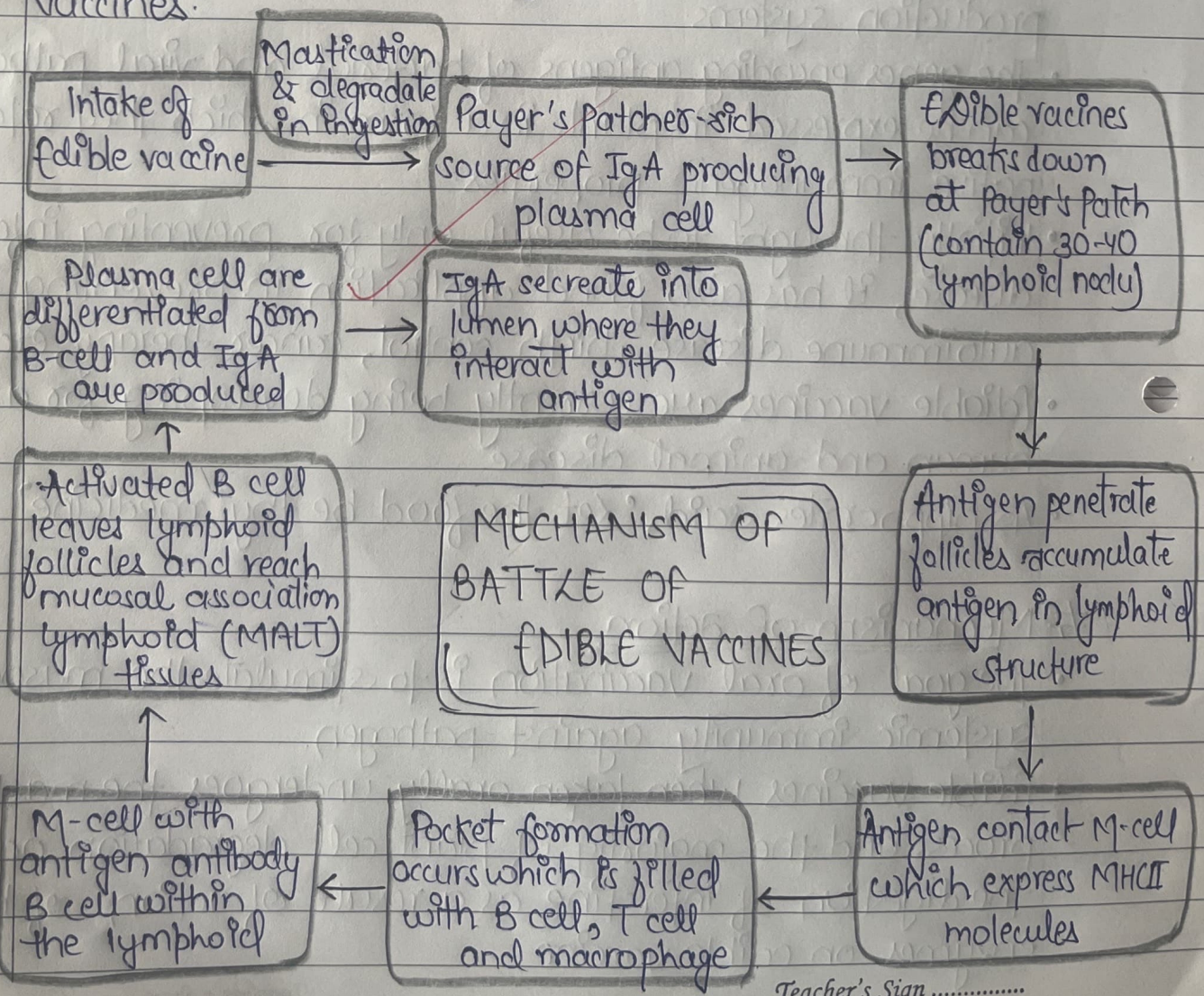
- Peyer's patches (PP) are an enriched source of IgA producing plasma cell and have the potential to populate musocal tissues and serve as muscoal immune effector site.
- The breakdown of edible vaccine near PP, consisting of the 30-40 lymphoid nodules on the outer surface of interestine and contain follicles.
- These follicles act as the site from which antigen penitrate the interestinal epithelium, thereby antigen accumilation antigen within organized lymphoid structure.
- Then antigen comes in contact with M-cell
- M cell passes the antigen to macrophages & B cell
- These B cell activates the T cell to produce immune response
- In this way, the immunity is activated by the edible vaccines.



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Developing an edible vaccines.

- There are Two ways of developing edible vaccines:-

→ In one case, the entire structural gene is inserted in plant transformation vector between 5' and 3' regulatory element, the will allow the transcription and accumulation of encode sequence in the plant.

→ In second case, entipode within the antigen are identify, DNA fragment encoding these can be used to construct gene by fusion with a protein coat gene from plant virus. e.g. TMV.

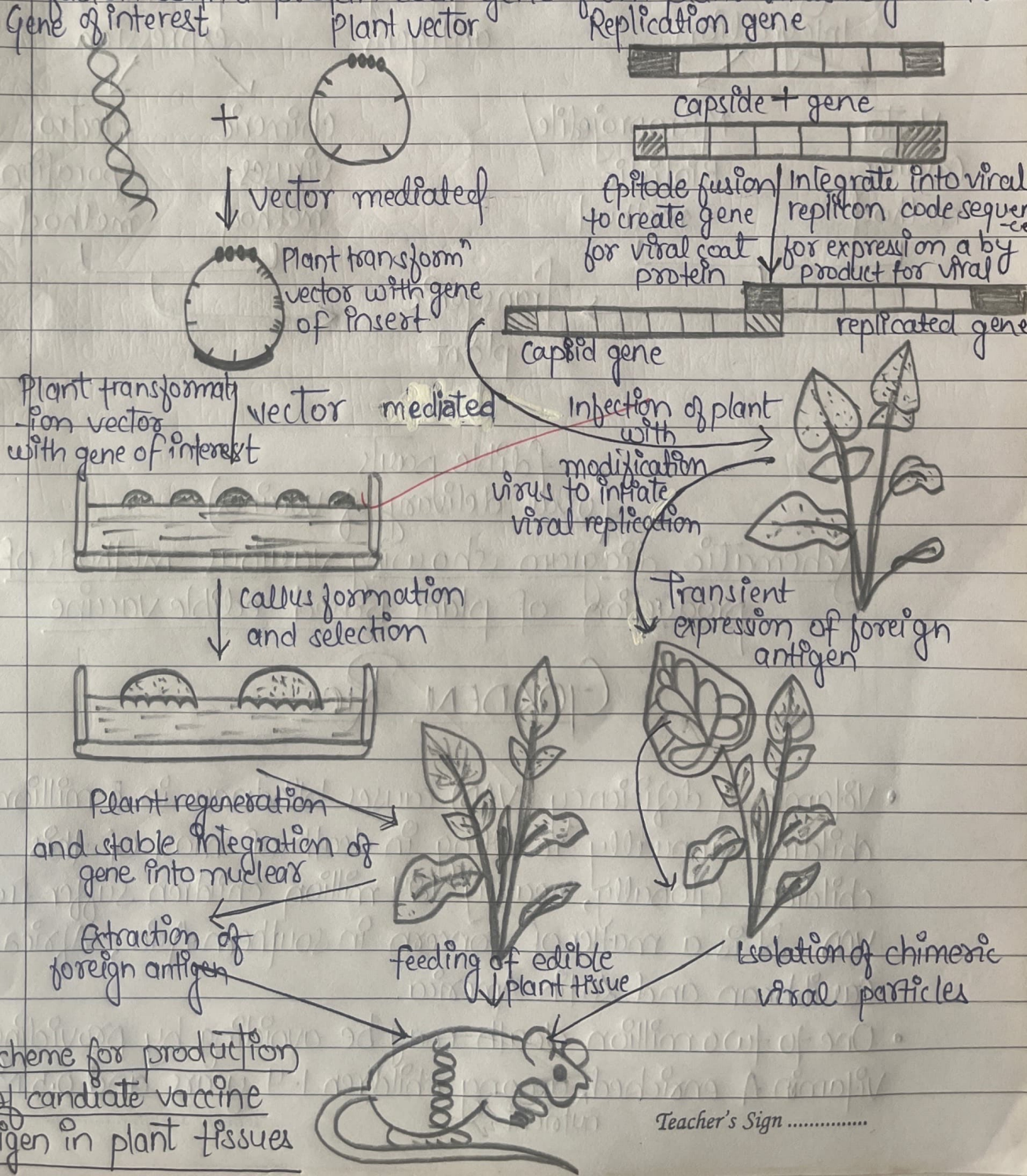

fig: Scheme for production of candiate vaccine antigen in plant tissues

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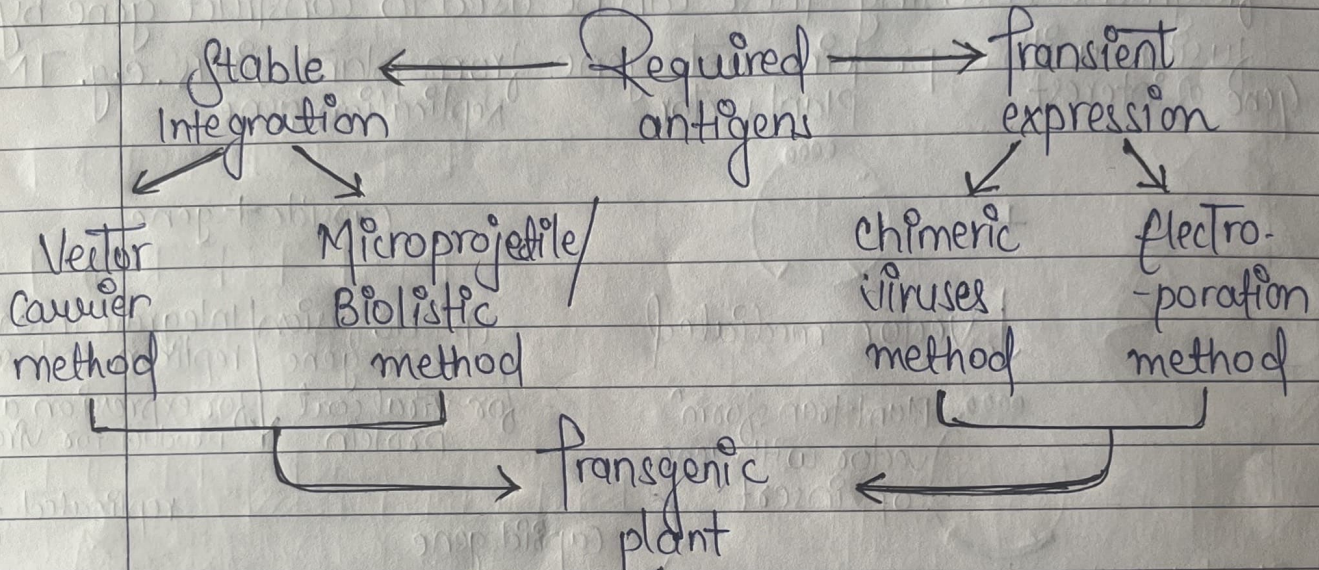
Topic.....Date.....

IDEAL VACCINES

- It should not be toxin or pathogenic
- Low level of side effects
- It should not contaminated the environment
- It should not cause problem in individual
- Technique of vaccine should be simple
- It should be cheap.



Schematic diagram showing various methods of production of plant based edible vaccine

GOLDEN RICE

- Vitamin A deficiency (VAD) causes blindness in 5 million children annually resulting in death of most of this children. Globally, nearly 124 million children suffer from VAD. It is a matter of concern in southern Asia, Africa, Caribbean and Latin America.
- One to two million death may be avoiding by providing Vitamin A enriched, among children 1-4 years and 0.5 M. nutrient

Teacher's Sign.....

Topic..... Date.....

during high age group of children. One approch providing vitamin A capsules to children and new mothers and alter-native provitamin A can be provided in the form of b-carotene in rice which is one of the best known examples of nutritional improvement of a food crop.

- Carotenoids are compound belonging to a class of plant metabolise called terpenoids or isoprenoids. Carotenoid are produced from it precursor by a biochemical pathways located in plasmid.
- The 40-carbon backbone of b-carotene (pro vitamin-A) is known as phytoene and it assemble by two 20-carbon geranylgeranyl diphosphate (GGPP) molecules by the enzyme phytoene synthase.
- Double bond in phytoene are added by denaturation steps producing an antioxidant compound called lycopene, give red colour to tomatoes which is later converted to b-carotene by the enzymes lycopene cyclase
- Within the aim of fortified food production, research was carried out to grow & consume variety of rice in areas where VAD is found to be common.
- A variety of Oryza sativa was produced through genetic engineering capable of synthesizing beta carotene, a precursor of vitamin A in the endosperm of rice (transgenic). The variety known as 'Golden rice', differ from the parental variety by having two additional beta-carotene synthesis genes.

☆ Golden rice produced by transforming rice with two beta-carotene synthases genes:-

(1) psy (phytoene synthase) from Daffodil
(2) crt I (carotene desaturase) from a soil bacterium.

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Topic.....Date.....

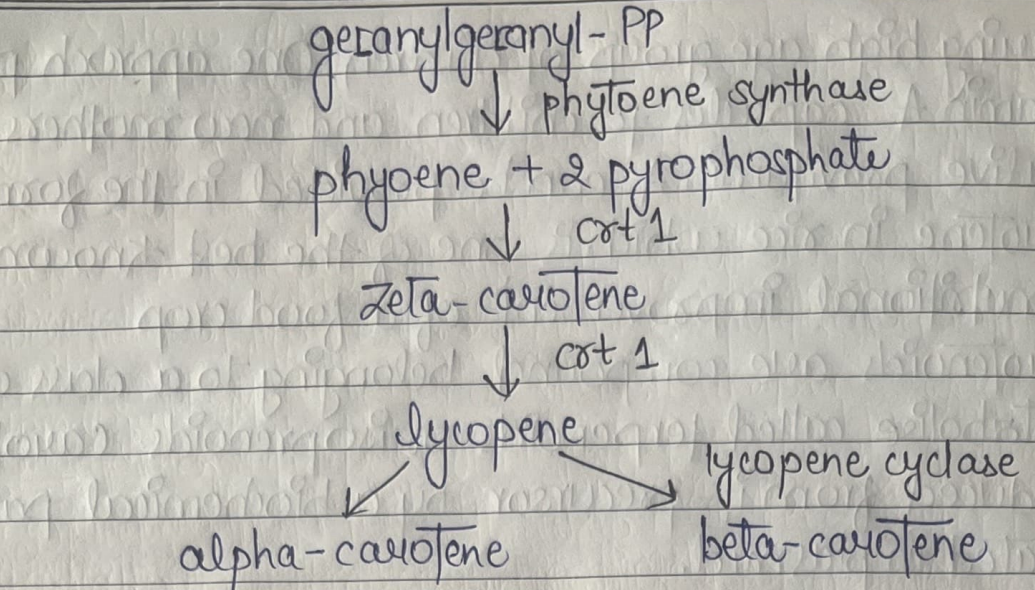

Fig :- Carotenoid biosynthesis pathways in Golden rice

- Both these gene are inserted into rice nuclear genomere under the control of an endosperm specific promoter to express them only in endosperm. The end product of this pathway to lycopene, but plant don't accumulate it other wise the rice would have beenred.
- An endogenous enzymes ingide the rice convert lycopene to beta-carotene giving yellow colour after which it is named. The detail of golden rice is first published in sci in 2000. This is product of an eight year project by Ingo Potrykus of swiss Pedrual Institute of Technology and Peter Beyer of University of freiburg.
- In the year 2005, a new variety of rice, called golden rice 2, producing 23 times more beta carotene in comparison of original golden rice, was announced.

Topic..... Rice endosperm ..... Date.....

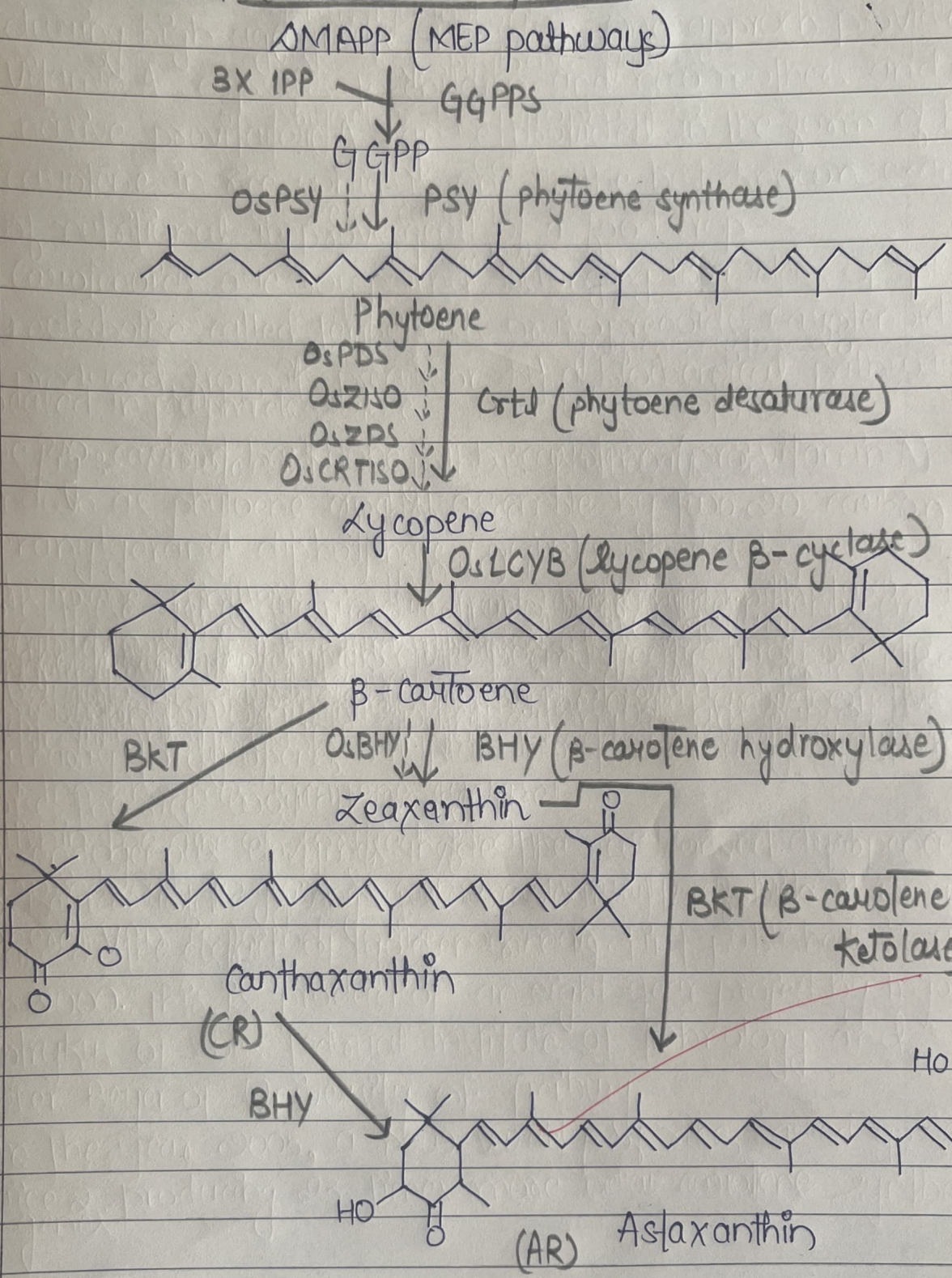

Fig:- flow diagram for chemical pathways in golden rice

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