Ch.6

Plant biotechnology

Team alpha

Plant biotechnology

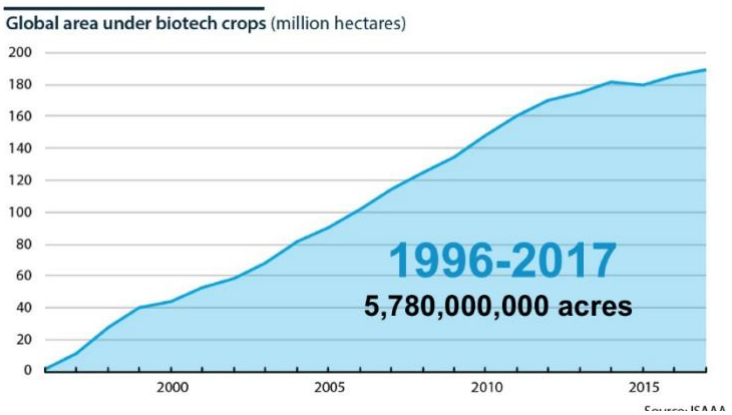
The world population has nearly doubled in the past 40 years, while arable land has only increased by 10%. The increase in human population means increase in water and food usage.

• Improved crop breeding through traditional methods has allowed us to feed so many people

• Recently, development of new, more productive crops has been accelerated by direct transfer of genes, to grow food more quickly and efficiently.

Biotech crops → genetically engineered plants. (whether its foods, corn and meat, or biofuel or cotton, etc.). → can be done by plant transgenesis or gene editing (CRISPR).

Plant Transgenesis – the direct transfer of genes to plants .



- Development of **plant vaccines**, plants that produce their own pesticides and are resistant to herbicides.

• By 2008, 13.3 million farmers in 25 countries planted transgenic crops

- 90% in developing countries

• By 2009, a significant portion of several key crops worldwide, were transgenic

- 70% of soybeans, 40% of corn, 10% of cotton

This figure shows how biotech crops are taking over the conventional crops (grown in traditional ways).

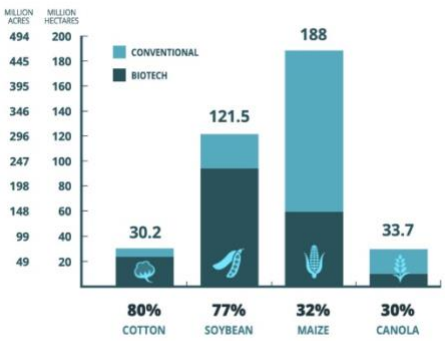


FIGURE 3. GLOBAL ADOPTION RATES (%) FOR TOP 4 BIOTECH CROPS (MILLION HECTARES)

Source: ISAAA, 2017

Methods used in plant transgenesis :

1- Conventional selective breeding and hybridization
2- Cloning: - protoplast fusion
    - Leaf fragment technique
    - Gene guns
    - Chloroplast engineering
    - Antisense technology

Conventional selective breeding

Selective breeding: sexual cross between two lines and repeated backcrossing between hybrid offspring and parent, Can take years .

– Polyploid plants (multiple chromosome sets greater than normal)

- Increases desirable traits, especially size
- Whole chromosomes can be transferred rather than single genes

Ex; - kiwi → hexaploid (6n)

- Banana and watermelon → triploid (3n)
- Large strawberries → octaploid (8n)
- Potato and wheat → tetraploid (4n)

Cloning:

Growing plants from a single cell. (different from cloning previously taken).

1- Protoplast fusion:

Protoplast fusion is the fusion of two protoplast cells from different species

• **Protoplast cell** is a callus cell whose cell wall has been dissolved by the enzyme **cellulase** (enzyme that dissolves the cell wall of cells).

Callus cell: undifferentiated cell.

• Fusion of the two protoplast cells creates a cell that can grow into a hybrid plant, Examples include broccoflower.

Start with a leaf cut into small pieces, soak in solution containing cellulase , to dissolve the cell wall of the cells, you end up with a bunch of protoplasts that will float to the top of the solution, and the debris will soak to the bottom.

Fuse together protoplasts to produce regenerated plants.

Take the fused protoplasts and place them on a filter paper in a petri dish, with agar and nutrients and **feeder cells** (nurse cells) embedded in the agar. Each of those protoplasts can be used to grow a whole plant when providing it with hormones that allow growth of shoots.

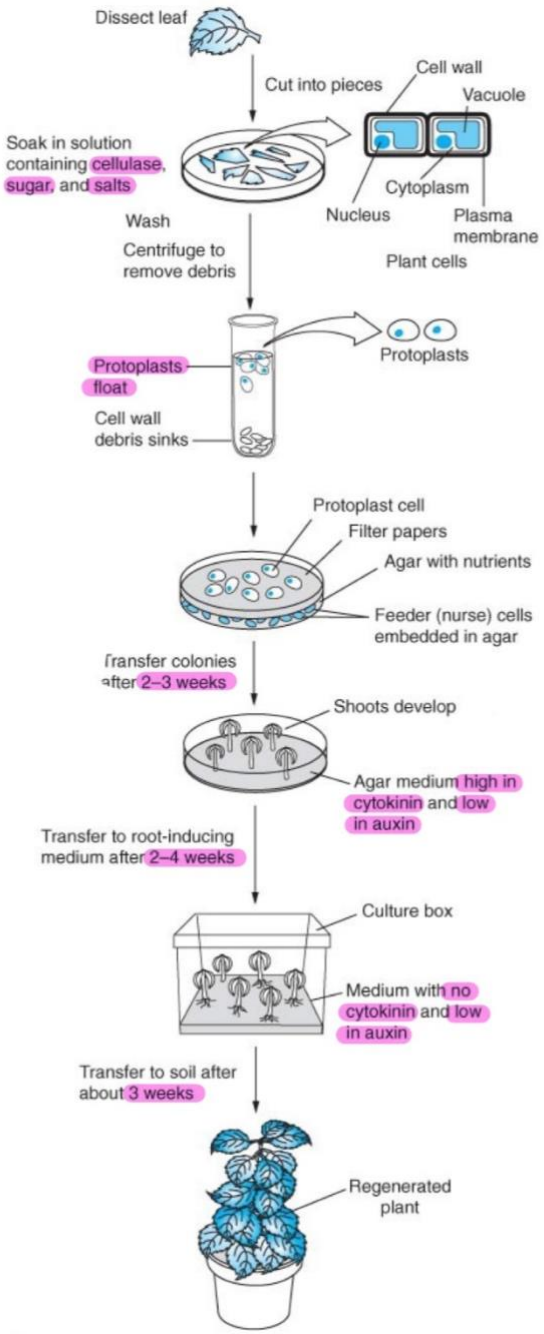
Shoots: first part to appear from growing plants.

Transfer cells into another petri dish, with agar **medium high in cytokinin** and **low in auxin**, (plant hormones that stimulate division and growth), shoots start to develop.

Then transfer to root-inducing medium after 2-4 weeks to start growing roots.

Root-inducing medium has **no cytokinin** and **no auxin**.

Then transfer to soil after 3 weeks and grow the regenerated plant.

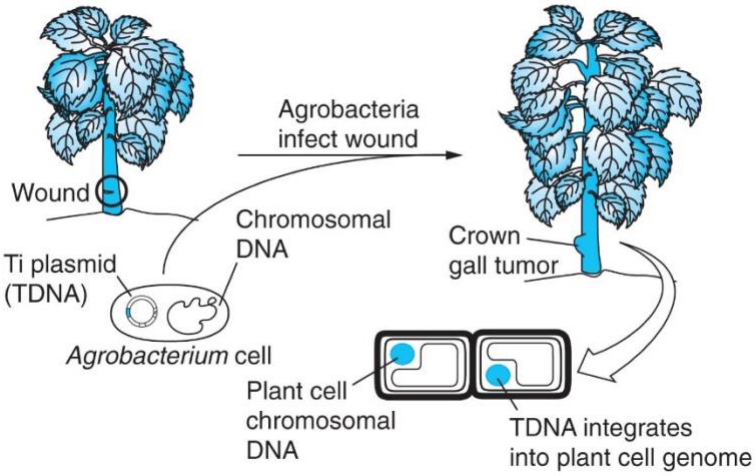


2- Leaf fragment technique:

Small discs are cut from leaf, Cultured in a medium containing genetically modified Agrobacter (Agrobacterium tumefaciens)

–(Agrobacterium tumefaciens): A soil bacterium that infects plants, contains a plasmid, (the TI plasmid), that can be genetically modified.

– DNA from the TI plasmid integrates with DNA of the host cell then Leaf discs are treated with plant hormones to stimulate shoot and root development.



When plant is wounded, it releases substances that attract bacteria, such as agrobacterium tumenfacines.

This bacteria infects the plant cells, it forces the plant cells to divide non-stop, and forms a tumor called **crown gall tumor.**

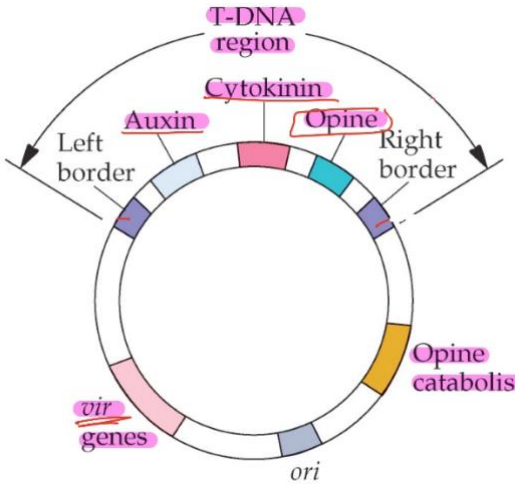
The presence of the plasmid is what makes the bacteria harmful.

The T-DNA will be transferred from the bacteria to the plant cells, and integrated with the DNA of the plant.

This T-DNA region has genes that produce plant proteins (like auxin and cytokinin), when integrated to the plant cells, will start producing those proteins in large quantities, and stimulate division of cells, this causes the tumor to grow.

In addition to auxin and cytokinin, there is opine ;

- produces products if combines a.a + keto acid or a.a +sugars.



- Rich in Nitrogen and carboxylic acid groups.
- Can be used as N and C source for the bacteria only.

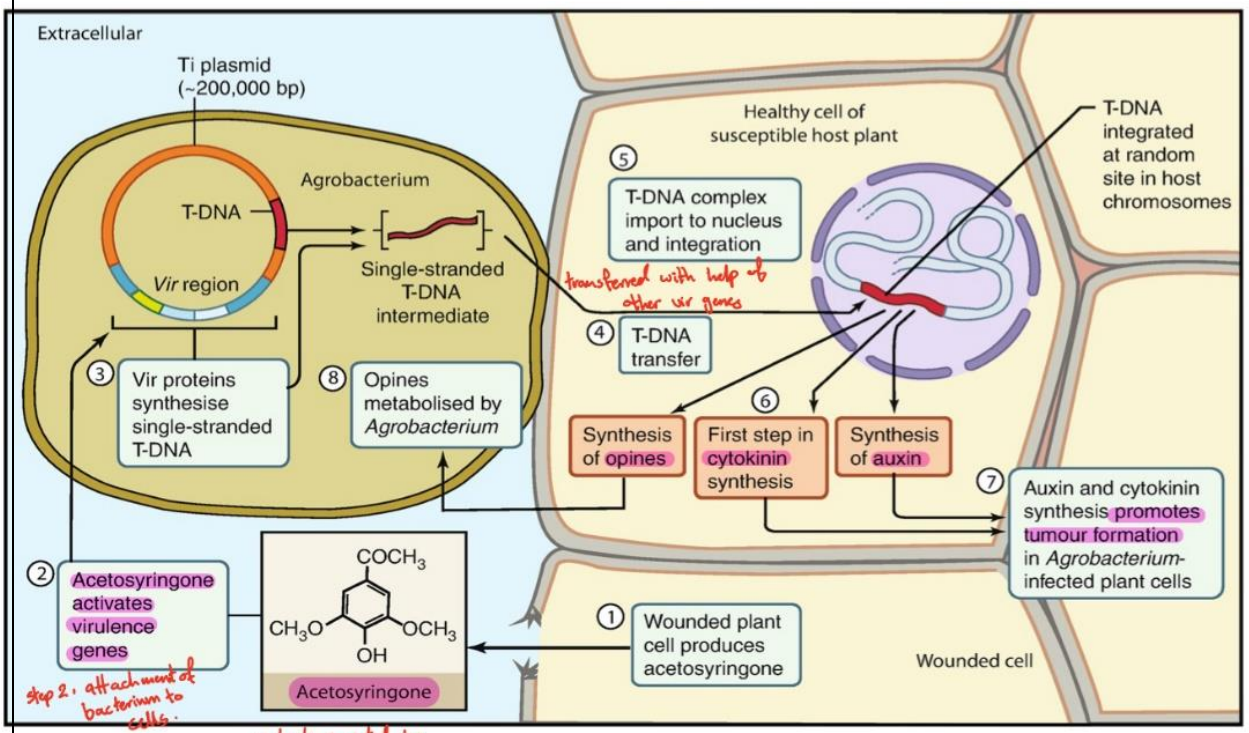
Any wounded plant secretes phenolic compounds that attract the bacteria that goes inside the plant and transfers its T-DNA into the plant cells. once inside the plant cell the gene carried on the T-DNA will be expressed.

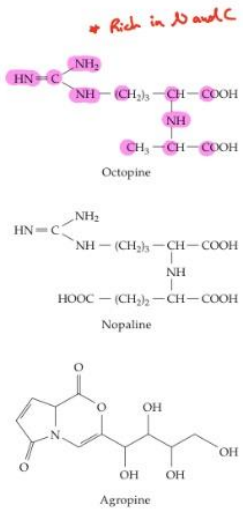
Only T-DNA genes are expressed in the plant cells.

- Cytokinin + auxin → division of cells, formation of crown gal tumor.
- Opine → N and C sources for bacteria.

Bacteria hijacks the plant cells and uses it as factories to produce opine, and only bacteria cells use the opine as food; because its plasmid has opine catabolism genes that degrades the opine, that is why plant cells doesn't use products of opines.

The bac plasmid also contains vir genes, that are necessary for **removal of T-region** and **transport of T-DNA to plant cells**.





Buchanan B, Gruissem W, Jones R (2015) Biochemistry and molecular biology of plants

Vir genes ant their fuctions:

1- virA: detecting molecule secreted by wounded plant (acetosyringone), transports acetosyringone onto the bacterium, and activates virG.

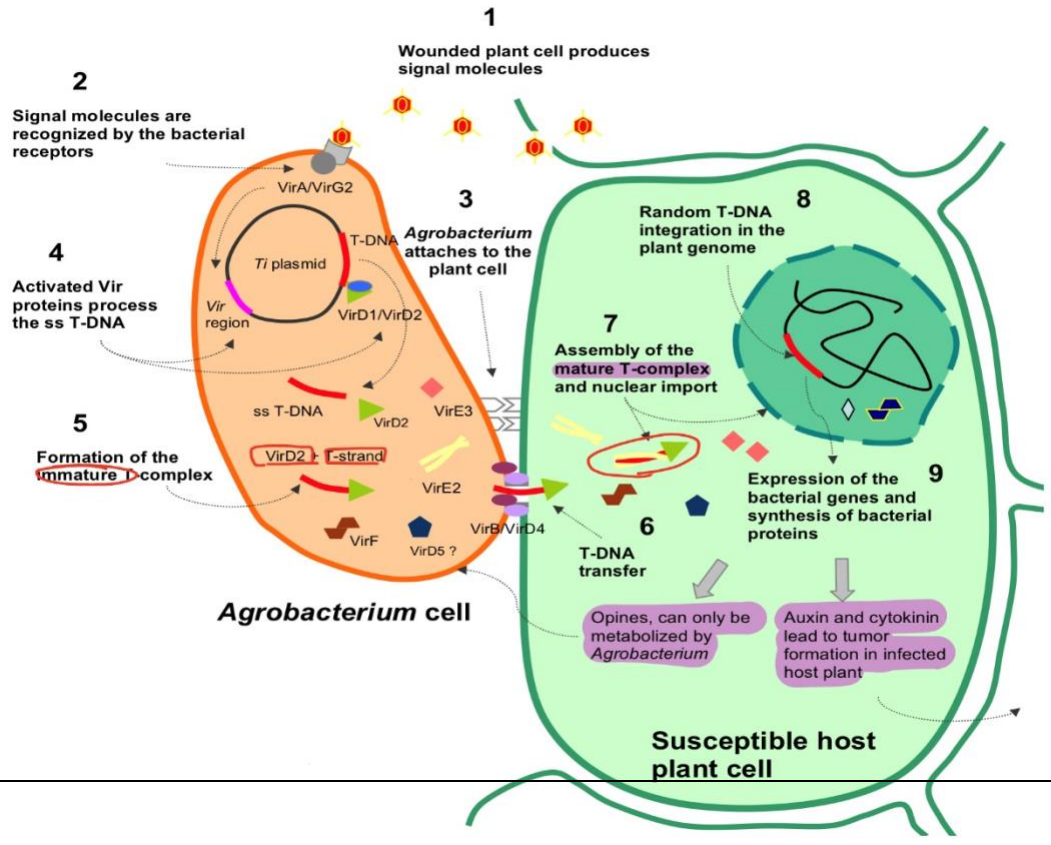
Once the acetosyringone binds to the virA proteins on the surface of the bacteria cell, virG is activated.

2- virG: promotes the expression of all vir genes. (transcription factor).
3- virD2: endonuclease, cuts the bacterial plasmid at the boarders of the T-DNA, but **only cuts one strand of the plasmid**.
4- virE2: forms channels in membranes **of plant cells**, and protects the T-DNA inside the plant cells.
5- virE1: chaperone for vir2.
6- VirD2 + virE2: have **NLSs (nuclear localization signals)**, get the T-DNA to the **nucleus of the plant cell**.
7- virB: **operon for 11 proteins**, gets T-DNA through **the bacterial membrane**.

- Note: virE2 → membranes of plant cells
  virB: → membranes of bacteria
- immature T-complex → T-DNA + virD2
  mature T-complex → T-DNA +virD2+ virE2

- **VirB** moves the **immature T-complex** from bacteria → plant cell, **virE2** moves the **mature** T-complex to the nucleus.

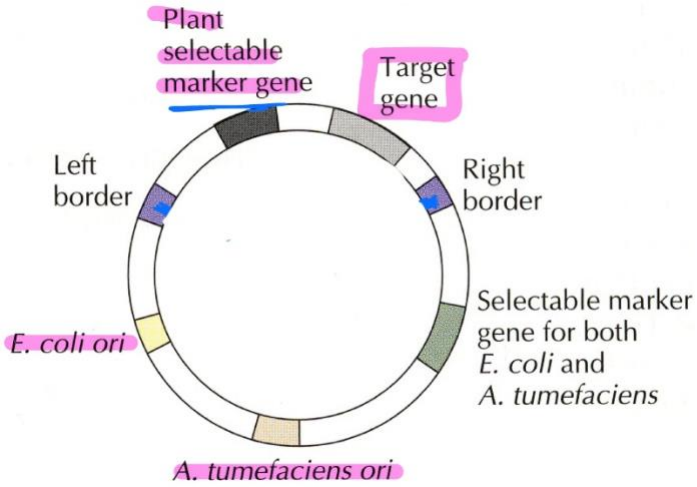
Once T-DNA is integrated into the nucleus of the plant cell, genes will be expressed, auxin, cytokinin and opines.



- **Binary Ti plasmid system**: involves the usage of two plasmid molecules, one small T-DNA, and one disarmed plasmid.

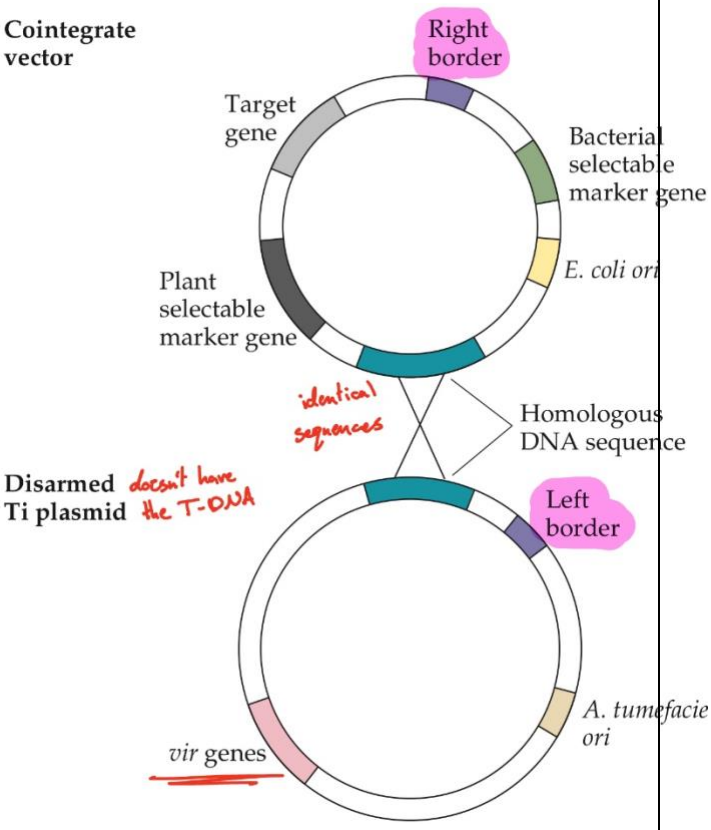
Disarmed plasmid: without T-DNA, but has vir genes.

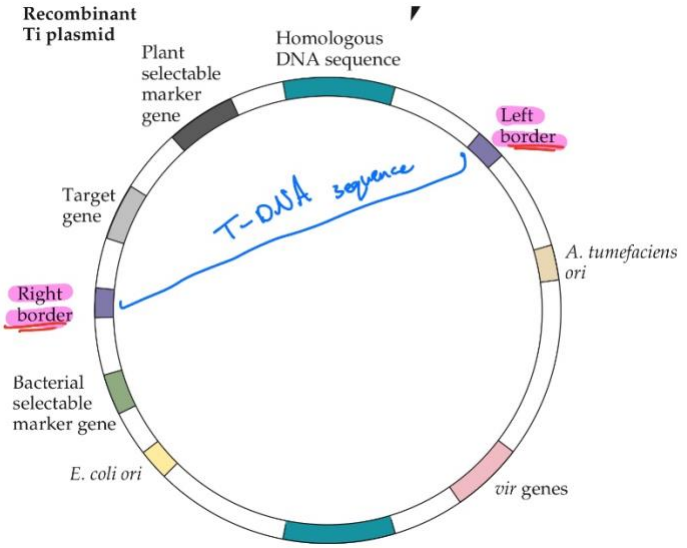
we use the disarmed for the vir genes, and the small T-DNA for the T-DNA region.



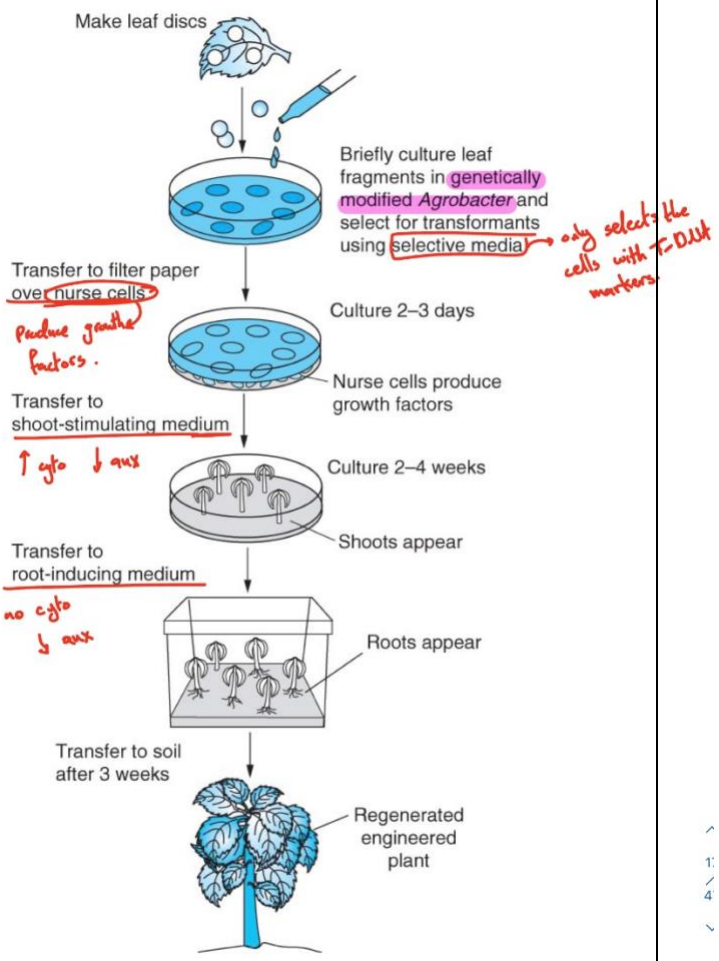
- **The co-integration plasmid system:** usage of two plasmids, one with T-DNA and one boarder only, And the other one is disarmed (no T-DNA) and has vir genes and the other boarder needed.
  - **Both have a homologous (identical) sequence, useful to do recombination between the two.**

We do recombination to get both boarders in one plasmid, between them well have the T-DNA sequence.





- **Using this method to make regenerated plants → leaf fragment technique:**



- Culture leaf discs on plate with genetically modified agrobacteria and do selection using selective media. (select using T-DNA markers).

Note: one marker commonly used is neomycin phosphotransferase genes, it encodes enzymes that enables the cells with integrated DNA to survive in the selective media.

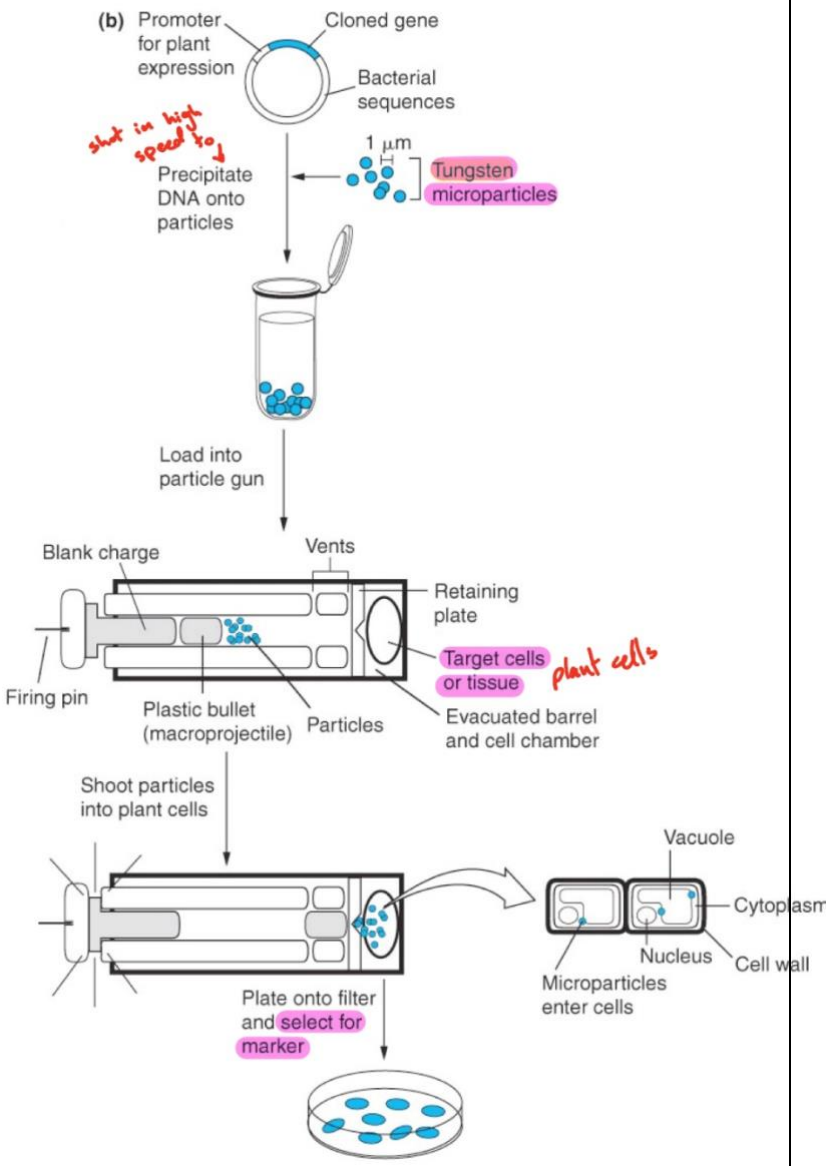
- Transfer transformed cells and culture over nursing cells for 2-3 days.
- Transfer to **shoot-stimulating** media, (high cytokinin, low auxin). culture for 2-4 weeks.
- Transfer to **root-inducing** medium, (no cytokinin, low auxin). grow for 3 weeks.
- Plant in soil and grow regenerated plant.

3- Gene guns:

A gun- like device used to introduce genes directly to the cells. It uses tiny metal beads covered with DNA, and shoots them onto the embryonic plant cells.

- Aimed at the nucleus or chloroplasts.

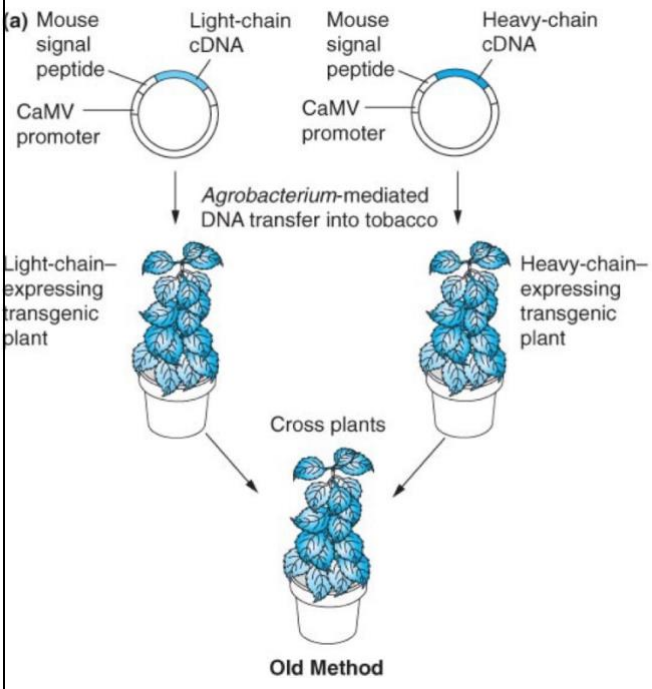
Then select cells for markers and culture cells then plant.

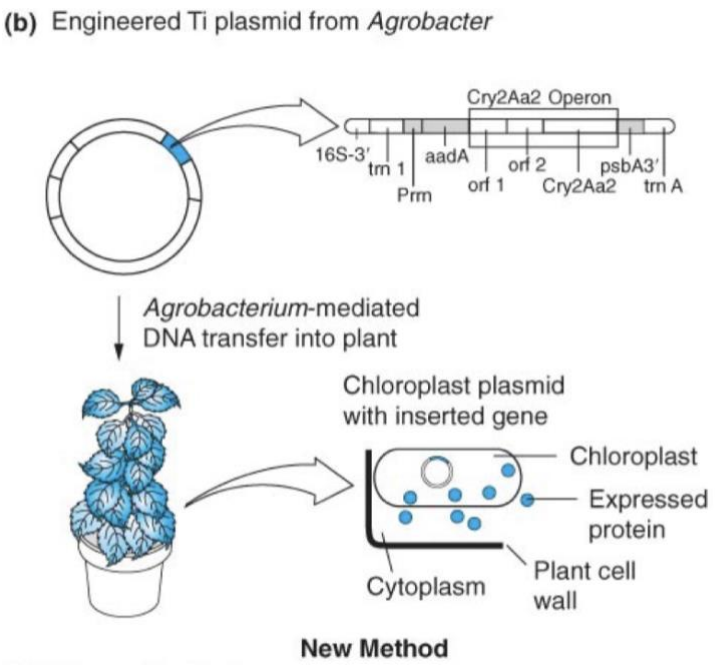


4- Chloroplast engineering:

- Plant chloroplasts contain DNA, and is used to engineer plants because it can accept several new genes at once.
- High percentage of genes introduced to the chloroplast remains active, thats what makes it a useful method in plant transgenisis.

We sometimes don't want certain genes to be carried on to other plants, whoch can happen if sexual reproduction occurred between plants (wind carrying pollen from one plant to another). Therefore , we use chloroplast engineering because the DNA in the chloroplast is completely separated from the DNA released in pollen; no chance that transformed genes will be carried on to other plants.



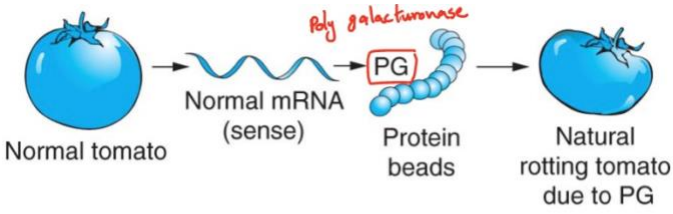


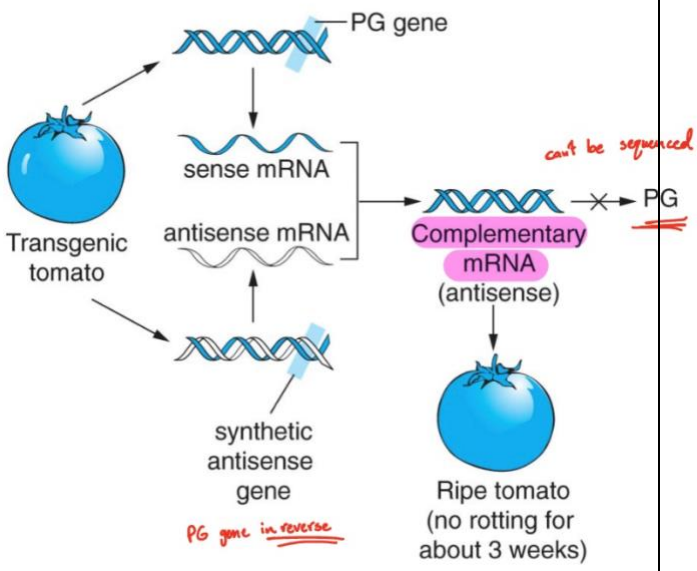
5- Antisense technology:

Inserting a complementary copy of a gene into a cell. this gene will encode an mrna molecule called antisense molecule, that binds to normal mrna and inactivates it.

Example; flavr savr tomato

Enzyme **PG** (poly galacturonase): degrades **pectin** which is a polysaccharide found in the cell wall of plant cells.





Applications of CRISPR–Cas in agriculture and plant biotechnology

• Gene targeting technology in plants relies on HDR, which enables precise genome editing. However, the low editing efficiency achieved with HDR has limited its application in plants.

• Deaminase-mediated base editing and reverse transcriptase-mediated prime editing technologies are alternative genome editing technologies

– These technologies do not involve DSB formation

– Do not require donor DNA (like HDR)

– These CRISPR–Cas-based tools induce precise sequence editing (cant be used to introduce point mutation, or change nucleotide into another) and are more efficient than HDR in plants.

Here we are going to talk about introducing modifications (mutation, deletion), not introducing genes.

We have number of gene targeting technology in plants that relies on crispr-cas, but not on homology direct repair (HDR), because the efficiency of gene editing using HDR is very low, why?

Because in general HDR is not very efficient

That's why we are using crispr to introduce gene editing deaminase enzyme

• CRISPR– Cas-based molecular platforms used for precise genome editing:

1. Cytosine base editing (CBE)

2. Adenine base editing (ABE)

3. Dual base editing, saturated targeted endogenous mutagenesis editor (STEME)

4. CBE-based precise DNA deletion. The APOBEC–Cas9 fusion-induced deletion system (AFID)

5. Prime editing

All of these are based on using cas9 and the guide RNA but there are other enzymes involved

Cytosine base editing (CBE)

• CBE is composed of:

1. Cas9 nickase (nCas9): catalytically defective (Mutation D10A) Cas9 variants that cut only one strand of the target DNA

2. Cytidine deaminase: deaminates cytidines to uridines in the non-target strand

3. Uracil DNA glycosylase (UDG) inhibitor (UGI): prevents UDG from deaminating cytidines to apyrimidinic (AP) sites

• CBE introduces C:G>T:A base transitions directly into DNA sites targeted by single guide RNA (sgRNA)

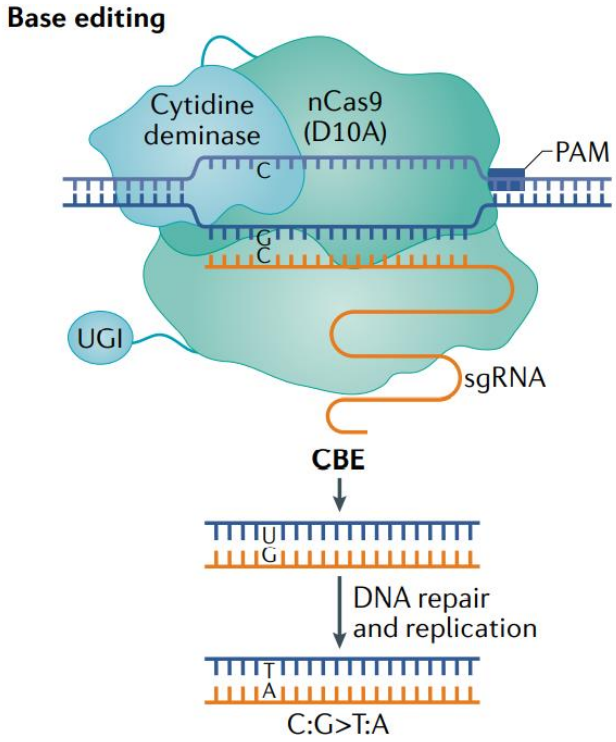
• When nCas9 (D10A) induces a nick on the target strand, the DNA mismatch repair pathway is activated and preferentially

resolves the U:G mismatch into the desired U:A, and following DNA replication a T:A product, thereby generating a C:G>T:A base transition.

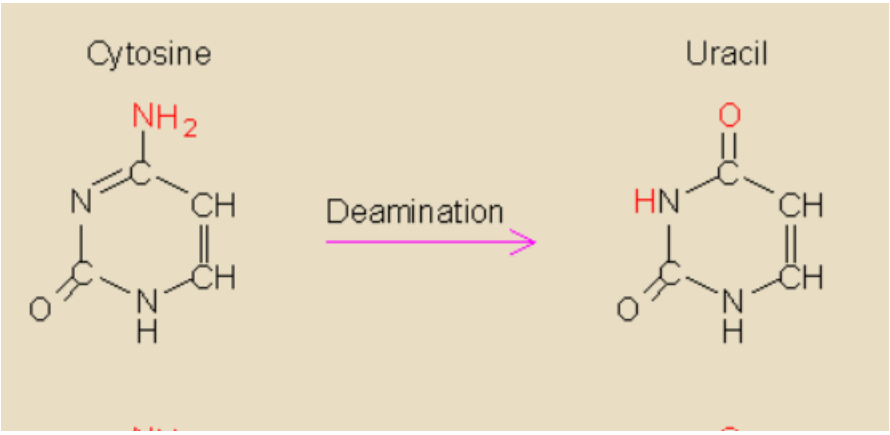
CBE is used to convert a nucleotide bp from C → T
G A

This requires the use of 3 proteins:

1- nCas9 is not like the normal Cas9, the normal Cas9 can introduces DSB, this one can introduce a cut in one of the strands not the two, (D10A) amino acid number 10 was converted from aspartic acid into amylin (in the nCas9)
2- cytidine deaminase
3- Uracil DNA glycosylase (UDG) inhibitor (UGI): the UGI inhibit the UDG enzyme



If we need to change the C to T first, we design guide RNA to bind to specific location, then cytidine deaminase from its name it removes amino group, and this convert the cytosine to uracil and then after several round of replication the DNA repair system the U will be converted to T because normally the uracil will not be found in DNA



Adenine base editing (ABE)

• ABEs expand base editing to include A:T>G:C substitutions using

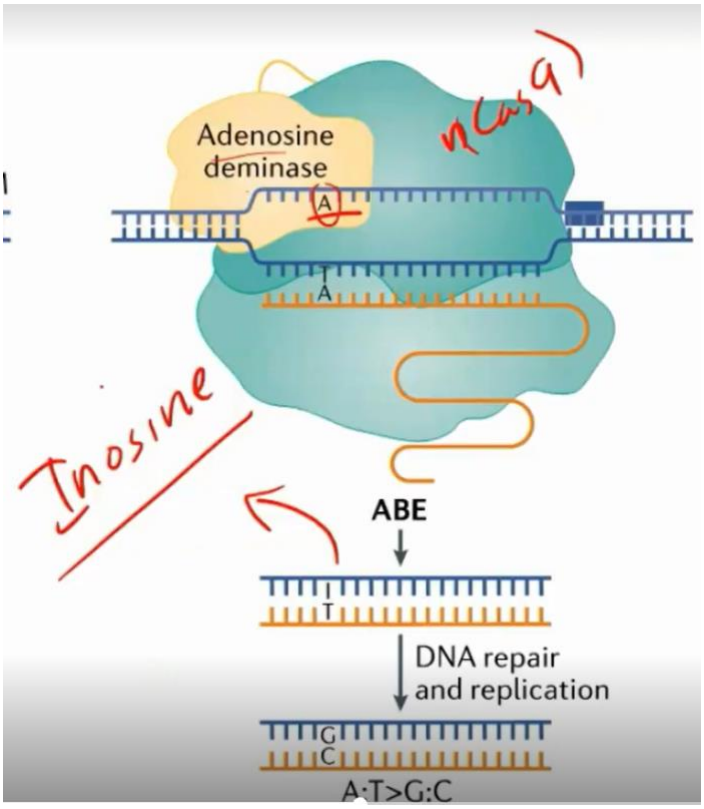
1. Cas9 nickase (nCas9): catalytically defective (Mutation D10A) Cas9 variants that cut only one strand of the target DNA

2. Adenosine deaminase: deaminates adenosines to inosines, which are recognized as guanosines by DNA polymerase during DNA repair and replication.

• Although there is no natural adenosine deaminase for deaminating ssDNA, such an enzyme has been evolved from Escherichia coli tRNA-specific adenosine deaminase (ecTadA)

Use the same enzyme but instead of cytidine deaminase we will use Adenosine deaminase, this is used to convert A → G

T → C



To do these modifications we do the same thing as the previous one but here the Adenosine deaminase will convert the A into I and after that the repair system like the previous one will convert the I into G

Dual base editing, saturated targeted endogenous mutagenesis editor (STEME)

The dual base editing can carry out the cytosine base editing and the adenine base editing both at the same time

• A cytosine and adenine dual base editor has been created to simultaneously perform C:G>T:A and A:T>G:C editing using a single sgRNA19 and the following proteins:

1. Cytidine deaminase

2. Adenosine deaminase (ecTadA–ecTadA*)

3. nCas9 (D10A)

4. UGI fusion

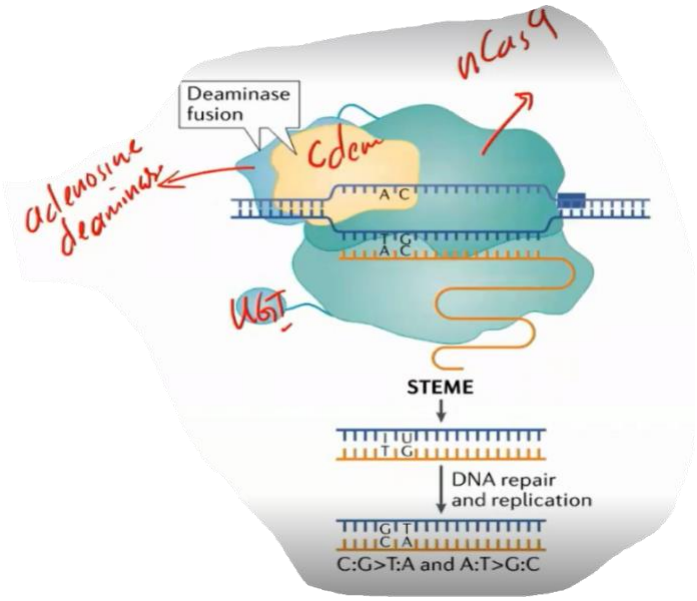
• The STEME system deaminates cytidines to uridine and adenosines to inosines in the editing window of the protospacer, and these are then copied by DNA repair and replication

• These dual base editors facilitate directed evolution of endogenous plant genes in situ.

• STEME might also be used to change cis elements in regulatory regions and genome-wide screening in a high- throughput manner in plants.

Here the four proteins will fuse together

So, lets say that we want to change A to G and C to T this system can do it



CBE-based precise DNA deletion

• Using the following proteins:

1. Cas9

2. Cytidine deminase

3. Uracil DNA glycosylase

4. Apyrimidinic lyase

• In CBEs, uridine generated by deaminating cytidines are preserved by the UGI, which inhibits the activity of the cellular UDG.

• The opposite situation, in which UDG is overexpressed, should trigger base excision repair and lead to excision of the uridines and generation of AP sites, which can be nicked by AP lyases

• The combination of such a nick with the formation nearby of a DSB by Cas9 should produce a specified and precise deletion between the deaminated cytidine and the Cas9 cleavage site

Here we are going to use the normal Cas9 not the nCas9, and we used to produce double strand break, also we will use cytidine deaminase, Uracil DNA glycosylase as we said before this is the enzyme not the inhibiter, and we will also use AP lypase that will be found in the plant cells so they will not be attached to the Cas9

So what will happen here is that we want to delete a DNA fragment and this DNA fragment contain C so we add the three enzyme (Cas9,UDG,cytidine deaminase), now the guide RNA will lead the three enzyme into the target sequence, the cytidine deaminase will convert the C into U ,and the UDG is part of the repair system and this will lead to excision repair (removal one of the strand) and that will happen by the conversion of U into apyrimidinic base by the UDG and AP lypase will introduce cut along with the Cas9 causing the removal of the target sequence

Prime editing

• This technology can produce all 12 kinds of base substitutions, precise insertions of up to 44 bp, deletions of up to 80 bp and combinations of these edits.

• Prime editor uses a

1. nCas9 (H840A): can cut the non-target strand

2. Reverse transcriptase

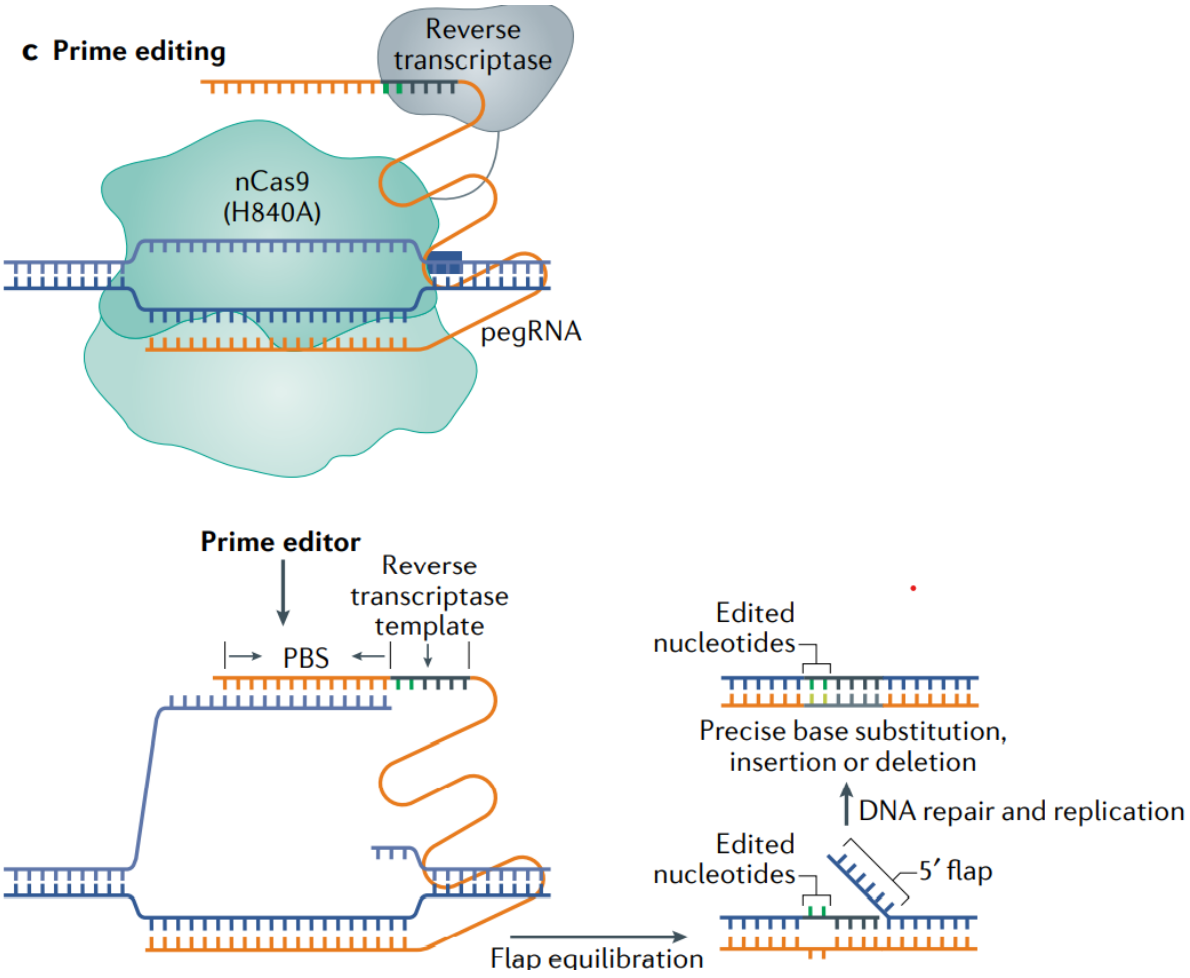
3. Prime editing guide RNA (pegRNA)

• The pegRNA is composed of a reverse transcriptase template and a primer-binding site at the 3’ end of the sgRNA.

• The reverse transcriptase template contains the genetic information for the desired mutations, and the primer-binding site pairs with the nCas9 (H840A)-nicked ssDNA strand, thereby priming reverse transcription and incorporating the genetic information from the reverse transcriptase template into the genome

• This is then followed by equilibration between the edited 3′ flap and the unedited 5′ flap, ligation and repair, which generate the desired edit.

• As prime editor generates base substitutions and short insertions and deletions at a relatively wide range of positions



As we can see the reverse transcriptase is attached to the nCas9 but here this nCas9 have different mutation (H840A), this nCas9 can introduce a cut in one of the strands and this strand would be the non-target strand (which means that this is the strand that is not complementary to the guide RNA)

The pegRNA is a guide RNA which means it would be complementary to the target sequence we want to change

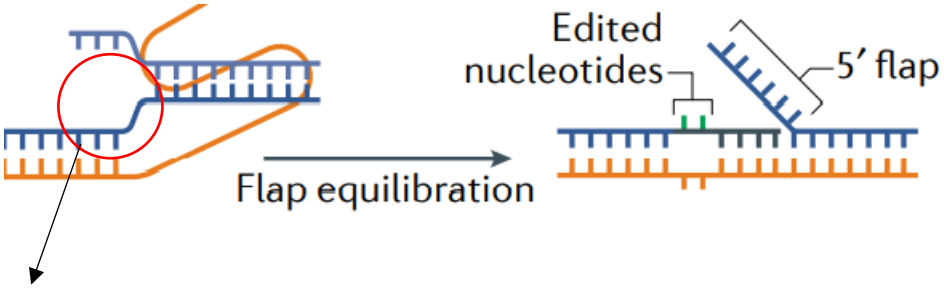
Just a reminder that here we can substitute nucleotide with another one, delete or add nucleotide depending on the sequence of the guide RNA

We have along guide RNA that consist of a sequence that will allow the binding of the cas to the target sequence we want to change, but it also contains a sequence that we want to introduce

Here the binding of the pegRNA with the cas to the target sequence, will cause the nCas to cut the non-target strand, now the other end of the strand (the upper one) will be designed that the first couple of the nucleotide will be complementary to the cut strand, then we will have a couple of nucleotides that are designed based on what you want to do, change one nucleotide to another, substitution, insertion, deletion

Now the reverse transcriptase can extend the DNA strand and introduce what ever change you want

So, let’s say we want to substitute AT into GC, we will design the pegRNA to have GC instead of AT, now when the nCas cut the other strand (non-target strand) as we said that it will be complementary to the other end of the pegRNA, now the reverse transcriptase will extend the other strand, while its extending it will add the edited nucleotide



This part will be called 5`flap and will be removed by the DNA repair system

Now the replication will result in the precise base substitution

Practical Applications in the Field

- Vaccines for plants
- Genetic pesticides
- Herbicide resistance
- Enhanced nutrition
- The future: from pharmaceuticals to fuel

• Vaccines for Plants

– Vaccine is encoded in a plant's DNA

– For example, a gene from Tobacco Mosaic Virus (TMV) inserted into tobacco plants

• Protein produced from the viral gene stimulates the plant's immune system

• Plant is invulnerable to virus

Just like animal plants can be infected by disease, and this disease could be cause by viruses or bacteria, and of course just like animal they have their own immune system just like animal, but their immune system is different than animal

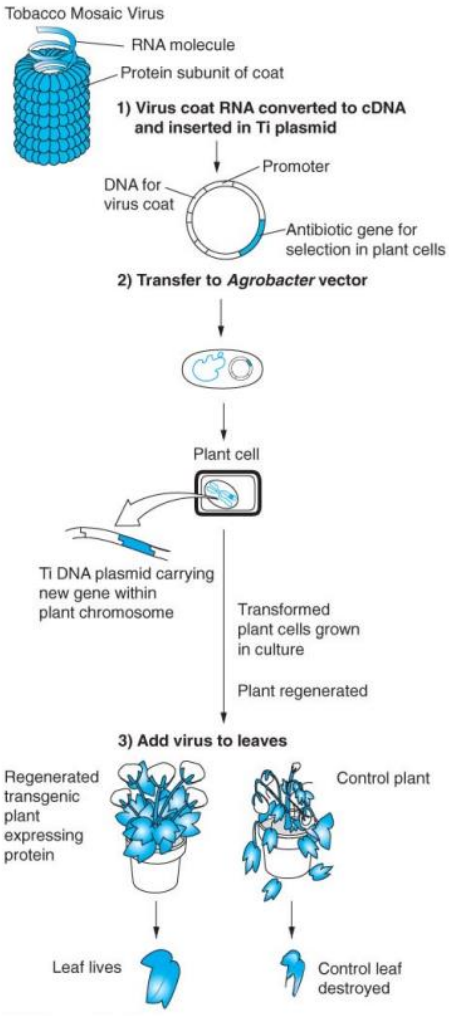
There are two ways to increase the production of food from plants

1- Genetically engineered plants that could produce large amounts of food
2- To protect the plants from pathogens or peats or environment

Because if the plants are damaged by insects, viruses, environment no food will be produced

Now some plants are genetically engineered to produce vaccines that could protect them from pathogens

So, the tobacco mosaic are genetically engineered (transgenic plants) because they can express one of the protein from the tobacco mosaic virus, which will yield immunity against virus just like we use vaccines against viruses



• Genetic Pesticides

– Bacillus thuringiensis (Bt) is a bacterium that produces a protein that kills harmful insects and their larvae

– It has been used as a natural pesticide for over 50 years

– Bt genes can be inserted into a plant's DNA

• Creates a built-in defense against certain insects

– Controversy surrounding Monarch butterflies

Some plants have been genetically engineered to be resistant to pesticides or insect

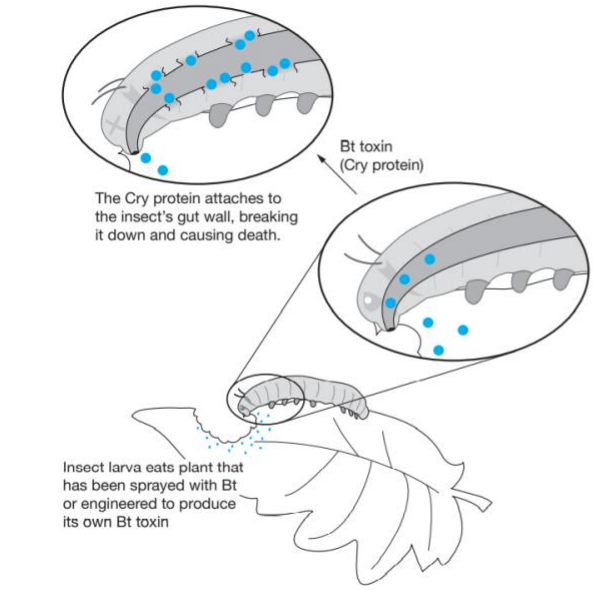
Bacillus thruringensis bacteria have a gene called Cry gene now these gene were taken from the bacteria and introduced to plants like cotton plants, corn and many other by that the plants can be genetically engineered

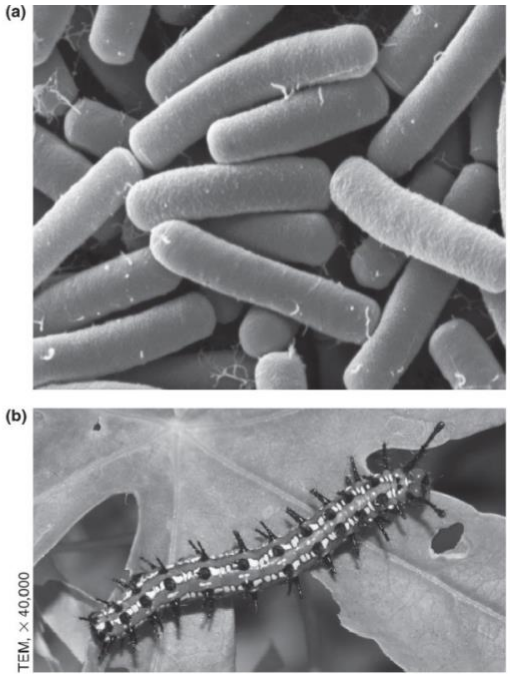
Many plants could be damaged by insect larvae because they fed on plants, so plants with the Cry gene can produce Bt toxin (protein), now whenever the larvae start eating the leave of the plant, they will inject the toxin causing sevar deliria to these larvae and these larvae will die

Note

The Cry gene stand for crystal, because this protein form like crystal inside the BT

Some people agree to the use of genetically engineered plants but to increase the amount of food produced, other don't be cause they think it will be harmful for us and for the ecosystem





- Safe Storage

– Millions of dollars are lost every year to insect infestations of crops during storage

– Transgenic corn that expresses avidin is highly resistant to pests during storage

- Avidin blocks the availability of biotin, a vitamin required by insects to grow

Another application in the field of plant biotechnology is to protect plants the produce corps for us, and also to protect the plant after being harvested

One of the major challenges in the crops industry is that after the crop is harvested and stored some types of insects, pests might damage the corps, or they might start feeding on the crops and causing damage to the crops

So, there is a strategy to protect the harvested crops even after being collected from plants

For example, there are a transgenic corn that expresses avidin (protein) this protein is known to bind tightly to a type of vitamin called biotin, so insects without biotin they will die, so what will happen is that avidin will limit the availability of biotin for insect, so when the insect start eating this transgenic corn they will not benefit from the biotin in the corn because its attach to avidin

Herbicide Resistance

– Traditional weed killers kill desirable plants along with the weeds.

– Can genetically engineer crops to be resistant to common herbicides

– Allows farmers to control weeds with chemicals that are milder and more environmentally friendly than typical herbicides

Sometimes that plants are made herbicide resistance, now herbicide is used to kill weeds but sometimes they may kill our plant, so we need to make our plant resistance to herbicide, so it kills only the harmful weeds, one way to do that is to make

transgenic plants that contain genes that will allow them to be resistance to herbicide

– One example is resistance to glyphosate, which blocks then enzyme EPSPS, which functions in a key biochemical pathway

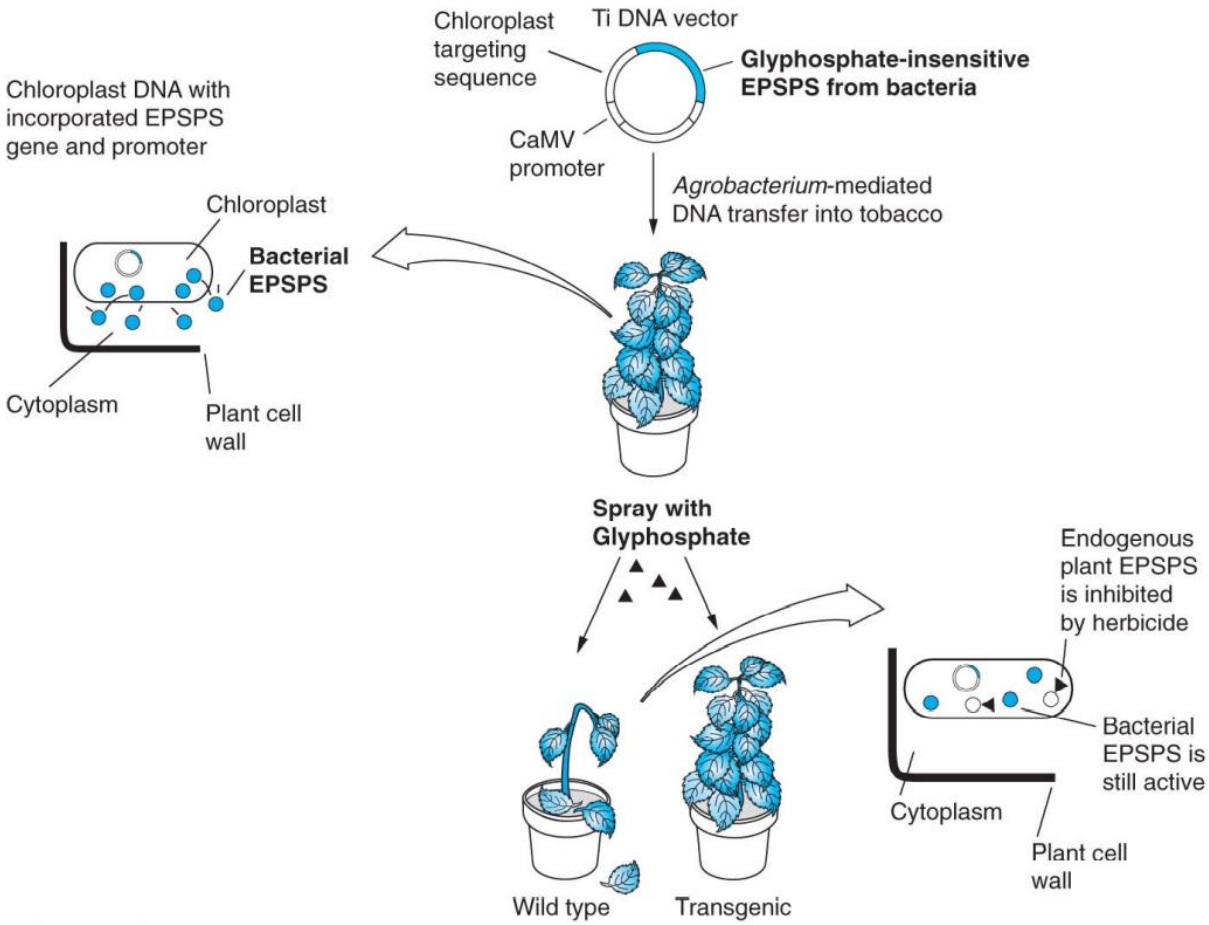
– Crops that transgenically produce an alternative enzyme not affect by glyphosate have been developed

– Most soybeans grown today contain herbicide resistance genes

– Unfortunately, glyphosate resistant weeds have evolved

The glyphosate(chemical) is a herbicide that is also called (roudup) contain a phosphate group, now this compound is toxic to weeds because this herbicide target an enzyme important for plants EPSPS, this enzyme is called 5`-enolypyruvateshikimate-3`phosphate synthase this enzyme is important in cooperate in shikimate pathway, and this pathway is a pathway that will be found on plants and it's important for the biosynthesis of aromatic amino acids like phenylalanine, tryptophan and tyrosine

The glyphosate can block the activity of this enzyme which means weeds can't synthesis the aromatic amino acids that's why they will die, but if we make our plant resistance to the glyphosate this means we can protect our plants and also still kill the weeds



Now the idea here is that we use a gene from bacteria that will encode for the same enzyme but there enzyme is not sensitive to glyphosate which means that this enzyme will not be inhibited using glyphosate, so we can clone the gene encoding the enzyme in Ti plasmid and introduce this gene by agrobacterium, now plants will start expressing the bacterial enzyme, and this bacterial enzyme could be expressed by a gene encoded and introduced to the chloroplast for example to prevent having this gene being transferred by mistake into other plants or into weeds, now when this plant is sprayed with glyphosate the plant will not be killed and the glyphosate will kill only the weeds or any plants that doesn't have the bacterial enzyme

– Glufosinate is a naturally occurring broad

-spectrum herbicide produced by Streptomyces soil bacteria

– Works by interfering with the synthesis of glutamine and with ammonia detoxification.

– Plants have been engineered to resist this herbicide by using two genes first isolated from Streptomyces bacteria:

1. "bialaphos resistance" or "bar" gene

2. "phosphinothricin acetyltransferase" or "pat" gene

– Crops that are resistant to the herbicide glufosinate have been engineered for resistance to multiple herbicides, permit- ting growers to use a mixed group of two to four different chemicals that combat herbicide resistance

• Enhanced Nutrition

– Golden rice has been engineered to contain large amounts of beta carotene, which the body converts to vitamin A

– However, as of 2011, no farmers have planted golden rice due to concerns voiced by environmental organizations

• The Future of Plant Biotechnology in Pharmacology

– Engineered crops could be used as miniature factories for producing pharmaceutical proteins and industrial chemicals (called "biopharming")

– Used to grow medicines

• Inexpensive edible vaccines that do not require refrigeration

• "Molecular pharming" of phytochemicals that produce chemicals useful to human health

– Phytochemicals, antibodies, blood products, cytokines, growth factors, hormones and recombinant enzymes

Many companies and researchers are using plants as a protein factory because plants could be optimized to produce protein in large quantities, to produce for us some drugs in the fruit so all we have to do is to eat the fruit, one example of that is to produce vaccine for use so all we have to do is to eat the fruit or the leaves of the plant and that will produce for us edible vaccine, and these vaccine that will be produced from the plants doesn't require the use of refrigeration or any special storage

We have another concept in plant biotechnology and its molecular farming allowing plants to produce for us other drugs

we have phytochemical (phyto: plant-based), so phytochemical these are chemical produced by plant cell that are useful for human health

so molecular forming is the use of plants to produce for us pharmaceutical products

• The Future of Plant Biotechnology: Fuels

– Biofuels are fuels produced from biological products, such as plants

– The need for alternatives to fossil fuels in increasing

– However, it takes 7 gallons of gasoline to produce 10 gallons of kernel corn ethanol

• In the future, want to convert plant wastes, such as husks and stems, to sugars that can be converted to ethanol

• Algae may be the next alternative to petroleum

Plant waste (the green parts that are not being harvested, husks, stems) could be used because they are rich in cellulose that could be converted to sugar by certain types of bacteria and this sugar will be converted to ethanol and this ethanol is actually called biofuel

So far there are 4 generation of biofuel:

1- The first generation was made from crops like:
   a- Sugarcane
   b- Corn

   They were used to produce ethanol, and ethanol could be burnt to produce energy

They also used soybean to produce to biodiesel

Many people around the world opposed the use of food crops to produce energy, because many people around the world don't have food to eat

2- The second generation were produced from lignocellulotic woody plants, and these are plants that are not used for the production of food, but they still have a lot of cellulose in their woody part, so we have lignocellulotic biomass and this biomass is rich in cellulose and lignin and as we said this cellulose will be converted to sugar and these sugar will produce energy

There are some types of tree plants like poplar trees, they are rich in lignocellulotic tissue, and these tissue have been used or grown to produce biofuels

3- The third generation were produced from genetically engineered algae, that were engineered to produce large amounts of fats and these fats could be used to make biofuel

In the first two generation we were talking about the using of sugars to produce biofuel, but here in the third generation we will have high lipid content that would be used to make biofuel

4- There is a fourth generation of biofuel, that relay mainly in the use of genetically engineered microorganisms like cyanobacteria, now these bacteria can capture Co2 from the atmosphere and produce molecule that could be used to produce biofuel

The cyanobacteria can be called photobiological solar cells so they can capture solar light and Co2 from atmosphere and converted into product to produce biofuel

Sunlight+H2o+Co2→fuel

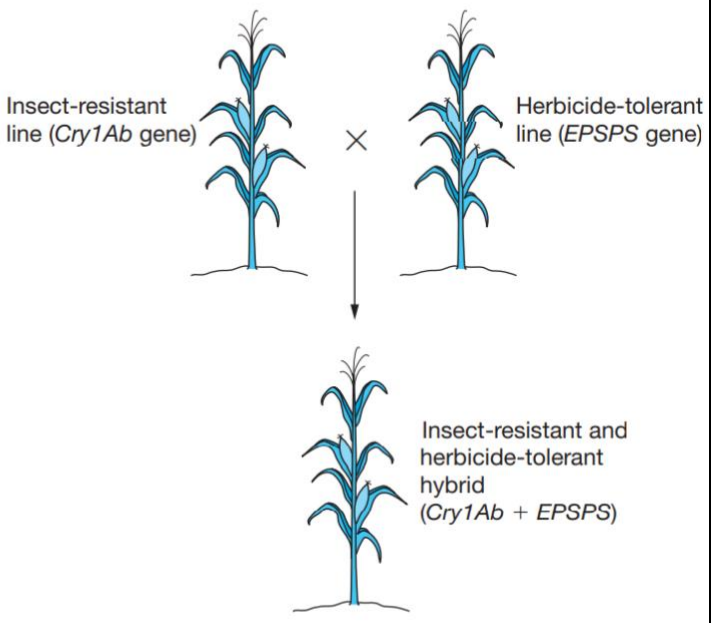
So, they clean the environment form Co2 that cause the global warming, and also produce fuel

Note:

There are already algae refineries that produce fuel from the algae in the USA, where large areas are used to grow algae and these algae will be harvested and the high level of lipid will be used to make fuel, an example of company that sell the fuel from algae is Exxon Mobile

Gene Stacking

- Gene stacking
  - For both conventional breeders and genetic engineers, the goal is often to move more than one desired gene into a plant
  - Such combinations of two or more inserted genes are called gene stacks.
  - A common way to stack genes is to cross parental lines that each have one of the genes, then select offspring that inherit both. This process is called hybrid stacking.



Good luck ☺

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