Department of BSBE
Indian Institute Of Technology Guwahati

Transgenic plants and animals

Dr. Sanjukta Patra

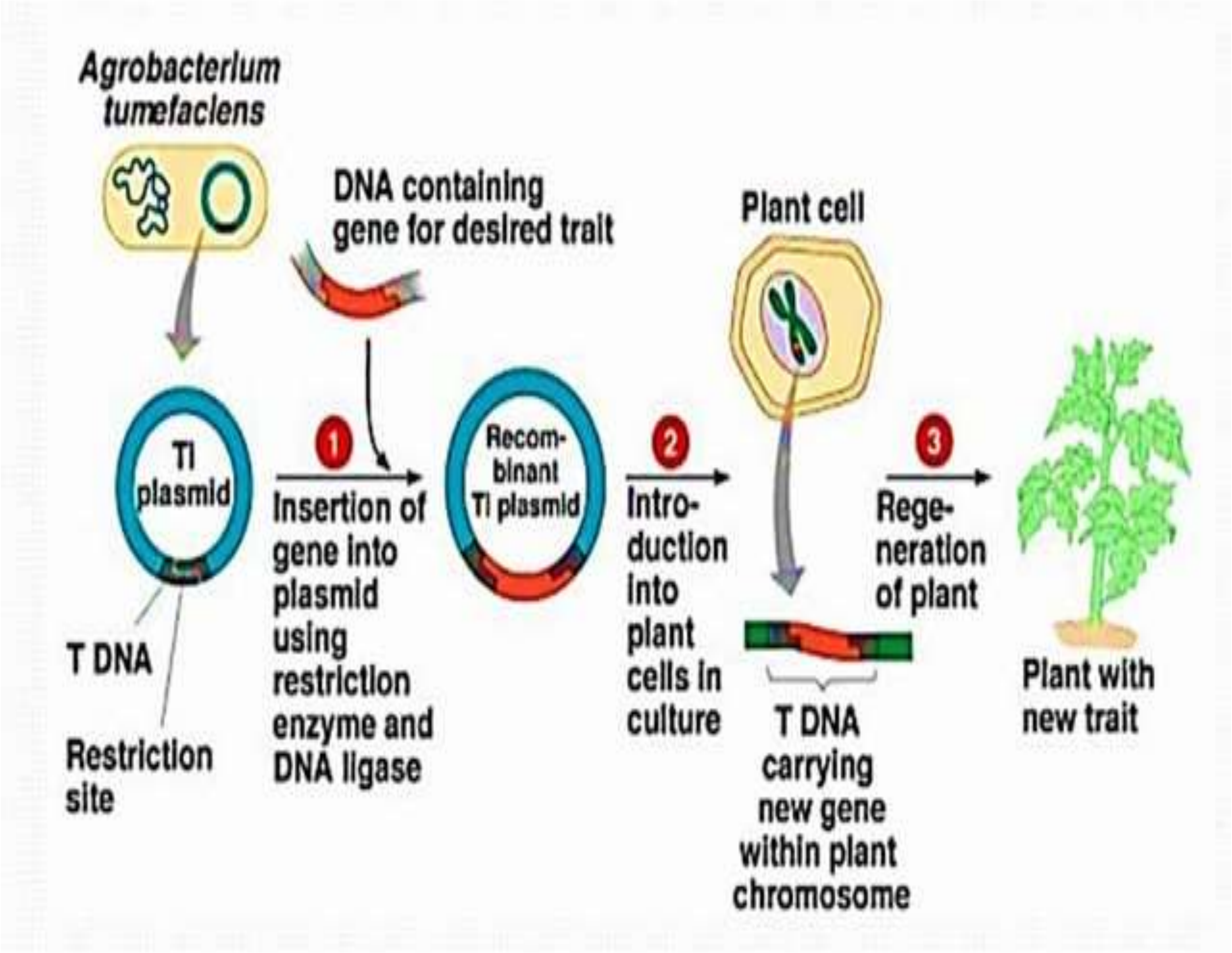
BT 207

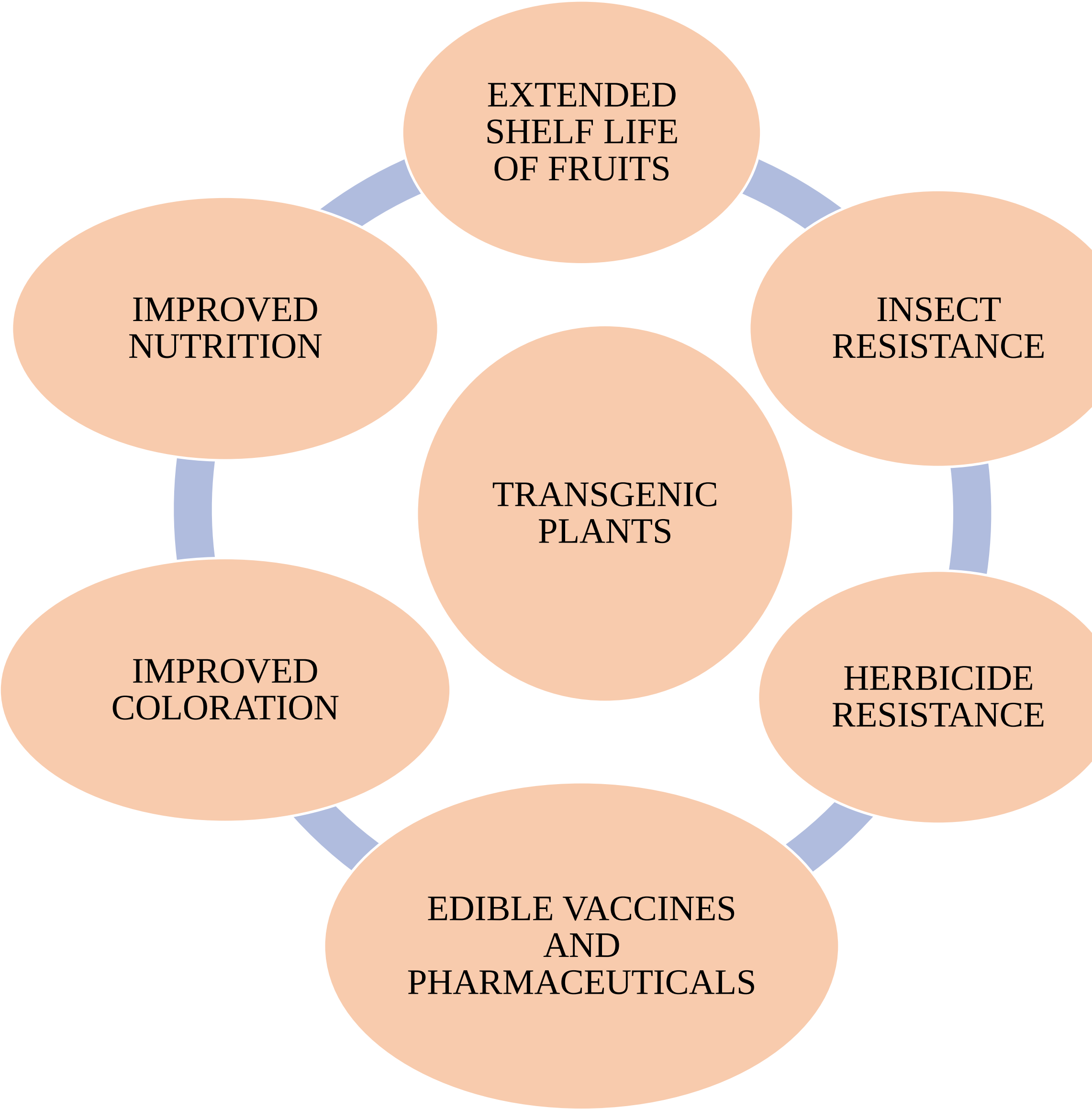
Genetic Engineering

Jan-April 2023

Transgenic plants

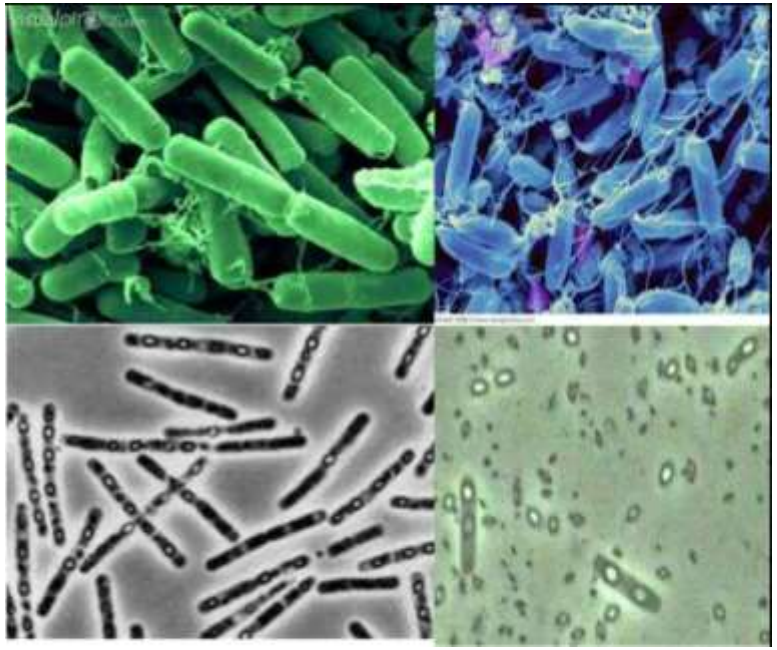
TRANSFORMATION TECHNIQUE USING AGROBACTERIUM MEDIATED GENE TRANSFER





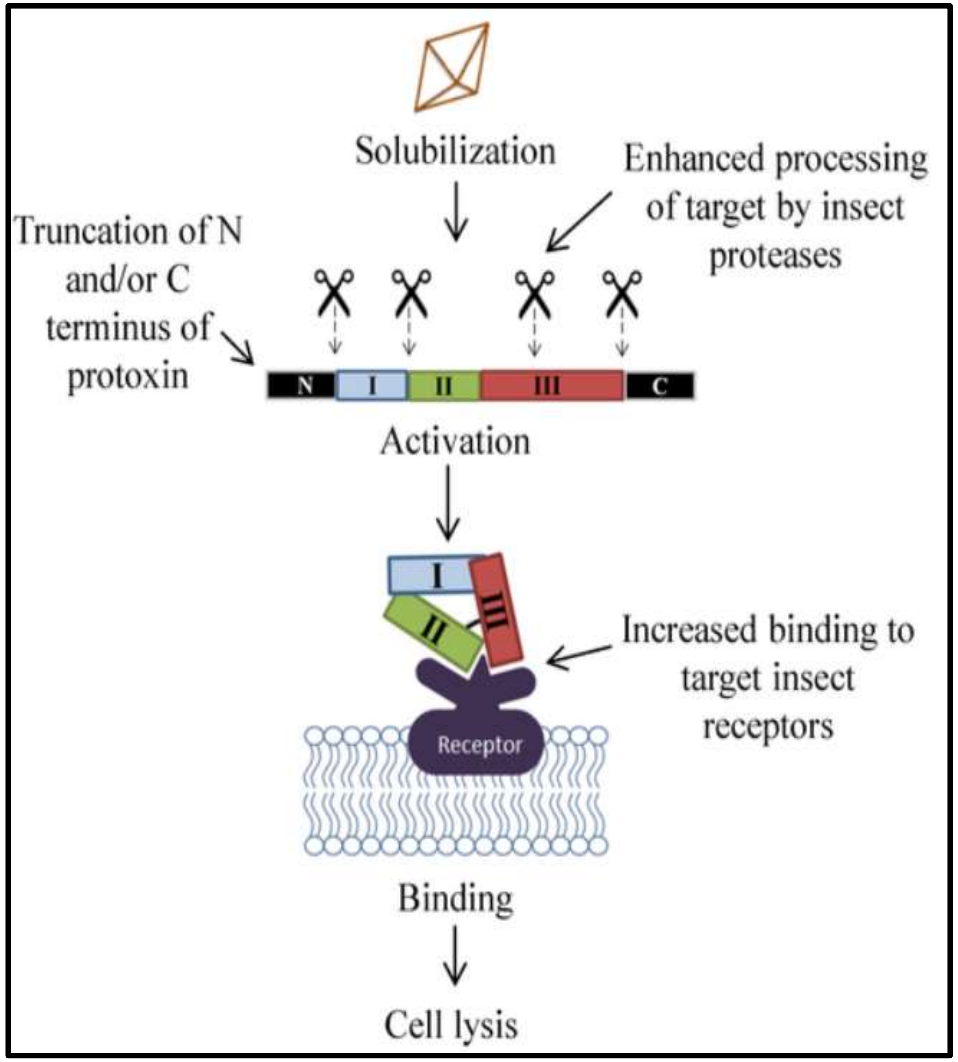
Bacillus thuringenesis- Transgenic crops (Bt crops)

- Shigetane Ishiwatari (1901), first isolated *Bacillus thuringiensis*.
- *Bacillus thuringiensis* (Bt), is a gram-positive, facultative aerobic, rod-like, motile and sporulating bacterium.
- Bt is a naturally-occurring soil borne bacterium.
- Ubiquitous in nature.
- Produces crystals of endotoxin (Cry protein or delta endotoxin) –also known as Insecticidal Cry Proteins (ICP)
- ICPs are toxic to insects mainly in their larval stage, thus they act as insecticides.

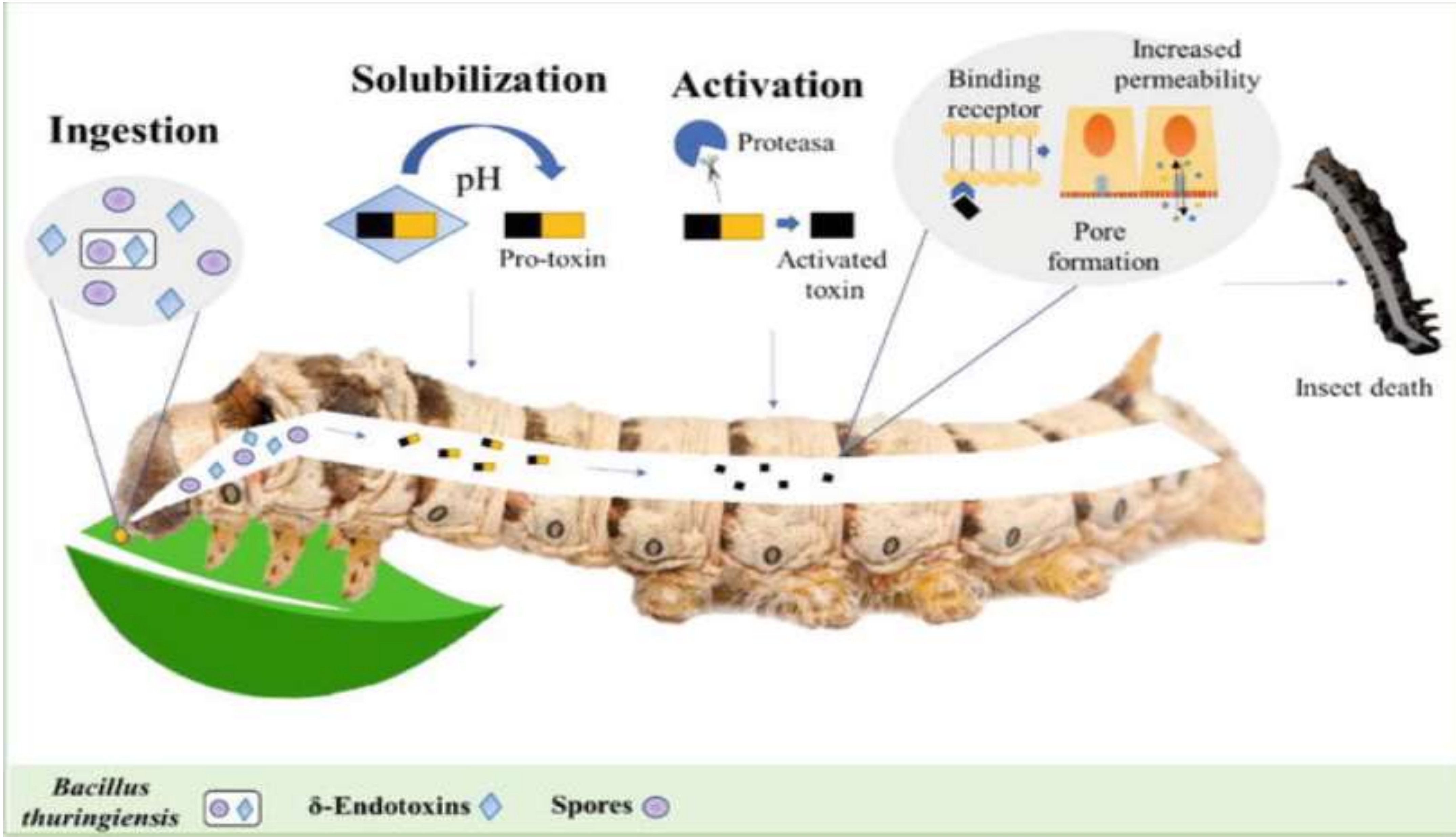


Cry Protein- Mode of Action

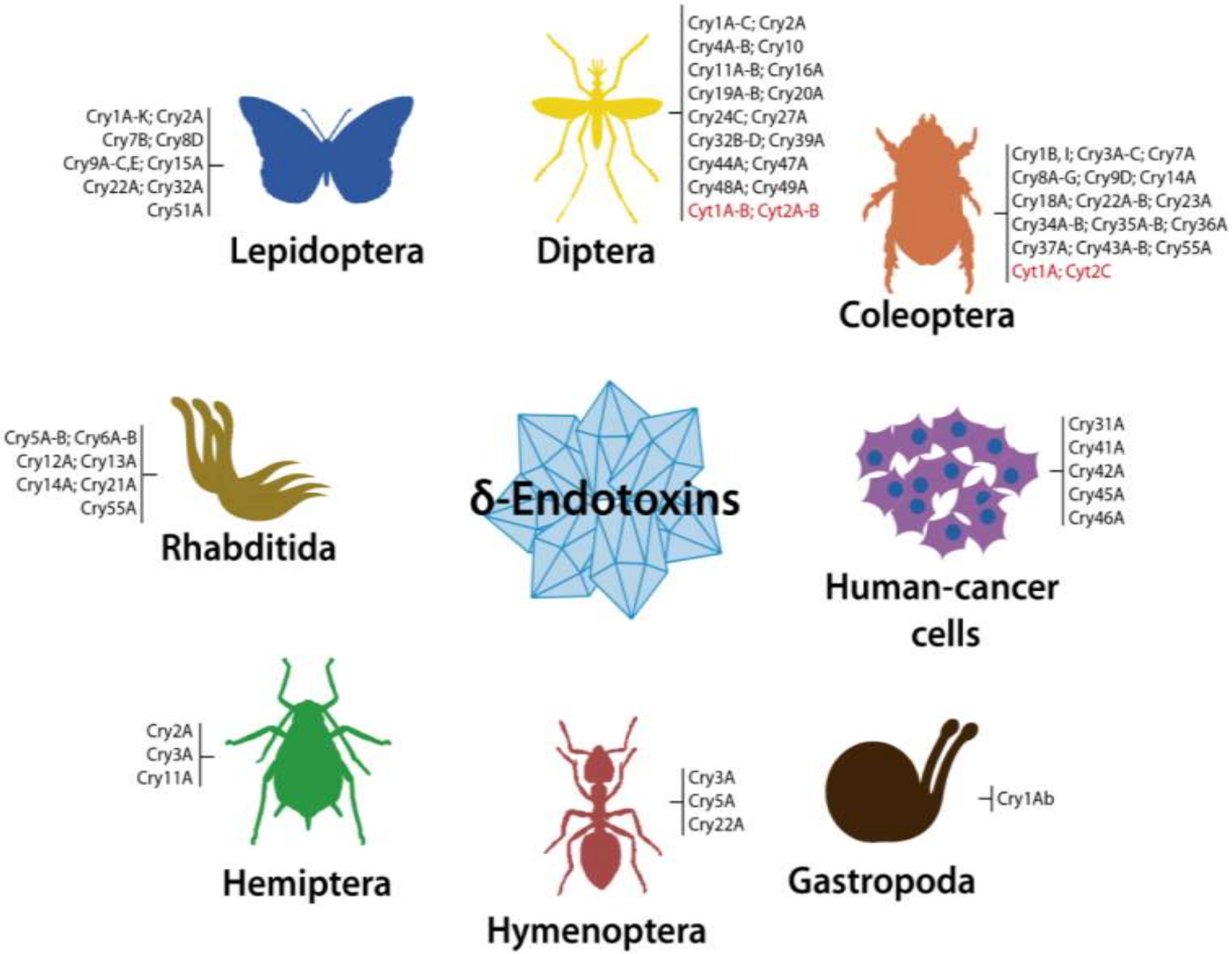
- Bt crystals, sometimes referred as insecticidal crystal proteins (ICP), are protein crystals formed during sporulation in some Bt strains coded by *cry* genes.
- The Cry protein is made as an inactive protoxin. It has to be eaten to cause mortality.
- Conversion of the protoxin (130 kDa) into the active toxin (68 kDa) called delta endotoxin requires the combination of a slightly alkaline pH (7.5-8) and the action of a specific protease(s) found in the insect gut.
- The active toxin binds to protein receptors on the insect gut epithelial cell membrane.
- The toxin forms pores in the insect gut.



Cry Protein- Mode of Action



Targets of different Cry toxins



Insects targeted by modified Cry toxins

Cry2A:
Truncated N-terminus increases efficacy against *Helicoverpa armigera* [16]

Cry4Ba:
Changes in loop 3 of Domain II broadens toxicity against *Culex quinquefasciatus* [17]

mCry3A:
chymotrypsin/cathepsin cleavage site in Domain I increases efficacy against *Diabrotica virgifera virgifera* [18]

Different types of Bt crops

Bt Cotton

- Bt cotton is an example of a transgenic organism. In the 1990s, CSIRO scientists in collaboration with the US company Monsanto extensively trialed the use of its Ingard GM cotton, also known as Bt cotton, which is a transgenic species.

- Over the years, traditional pesticides used on cotton plants had to be made stronger and be applied more frequently to eradicate insect pests such as the caterpillar of the *Helicoverpa zea* moth, a pest which destroys hundreds of millions of dollars worth of cotton each year

ADVANTAGE OF BT COTTON

- Increases yield of cotton due to effective control of three types of bollworms, viz. American, Spotted and Pink bollworms.
- Insects belonged to Lepidoptera (Bollworms) are sensitive to crystalline endotoxic protein produced by Bt gene which in turn protects cotton from bollworms.
- Reduction in the cost of cultivation and lower farming risks.
- Reduction in environmental pollution by the use of insecticides rarely.
- Bt cotton exhibit genetic resistance or inbuilt resistance which is a permanent type of resistance and not affected by environmental factors. Thus it protects crop from bollworms.
- Bt cotton is ecofriendly and does not have adverse effect on parasites, predators, beneficial insecticides and organisms present in soil.
- It promotes multiplication of parasites and predators which help in controlling the bollworms by feeding on larvae and eggs of bollworm.
- No health hazards due to rare use of insecticides (particularly who is engaged in spraying of insecticides).
- Bt cotton are early in maturing as compared to non Bt cotton.

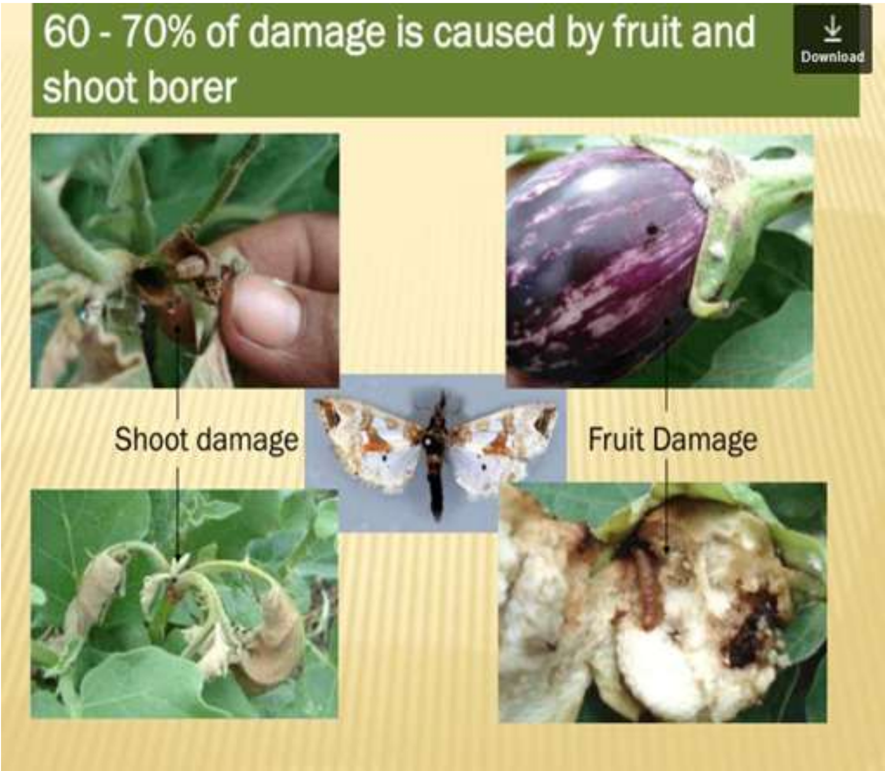
Bt Brinjal

- Bt Brinjal is a transgenic brinjal created by inserting a gene cry1Ac from the soil bacterium *Bacillus thuringiensis* into Brinjal.
- This is said to give the Brinjal plant resistance against lepidopteran insects like the Brinjal Fruit and Shoot Borer *Leucinodes orbonalis* and Fruit Borer *Helicoverpa armigera*
- Mahyco, an Indian seed company based in Jalna, Maharashtra, has developed the Bt brinjal.
- But it was discontinued in India due to -

1) The insufficient data by Mahyco on its impact on human health.

2) Impact on lactation period of cows, delayed food consumption in rabbits and health impacts on rat were observed.

3) Brinjal belongs to Solanacae family and highly cross -pollinated crop, so the gene can easily transferred to other varieties leading great threat to bio diversity.



GOLDEN RICE PROJECT

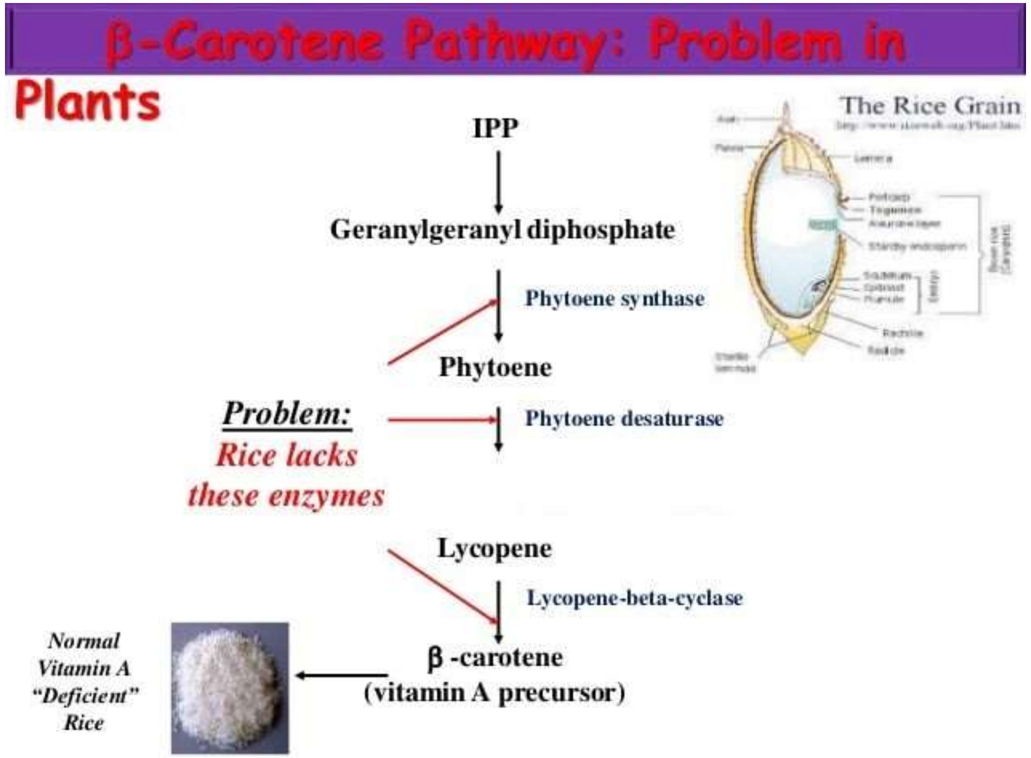
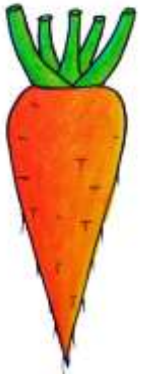
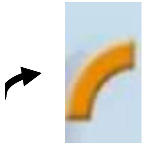


Fig 1: Golden Rice (*Oriza sativa*) was created by transforming rice with three beta-carotene biosynthesis genes –
Phytoene synthase from daffodil
Lycopene beta-cyclase from daffodil
Phytoene desaturase from soil bacterium *Erwinia uredovoia*

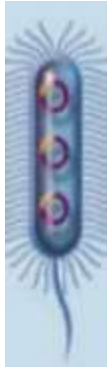
VITAMIN RICH TOMATO





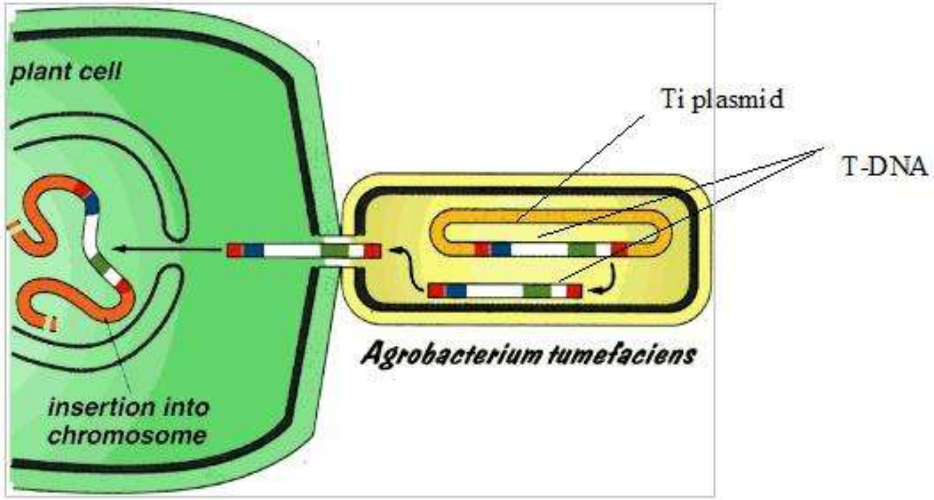
Beta- carotene producing gene from carrot inserted into Ti plasmid

Ti plasmid



The plasmid is introduced into *Agrobacterium*

***Agrobacterium* transfers the carrot gene into the plant cells**



Plant with new trait- vitamin rich tomato

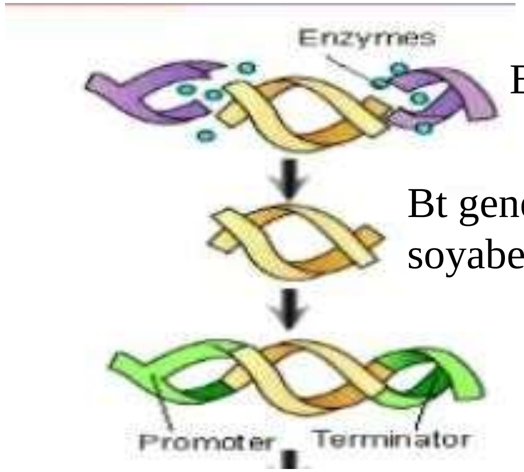
- **Protein enriched potatoes-** *AmA1* gene encoding for a tuber specific seed albumin protein from *Amaranthus hypochondriacus* inserted in potato to increase the total protein content and amino acids in transgenic tubers

Amaranthus hypochondriacus

AmA1 gene encoding for a tuber specific seed albumin protein

Inserted into potato via *Agrobacterium* mediated transfer

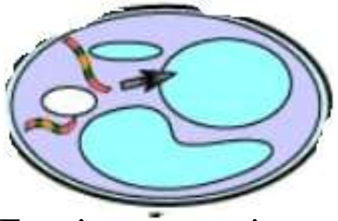
- **Bt soybean-** *Bacillus thuringiensis* gene is incorporated into soybean which has insecticidal protein that maintains the yield of the crop.



Bt gene

Bt gene insertion into soyabean expression cassette

Vector in transgene multiplied in bacteria.



Foreign genes inserted into soyabean cell genome

Insect-resistant, transgenic soybeans against the velvet bean caterpillar and the soybean looper.

- **Ring spot virus disease resistant Papaya**

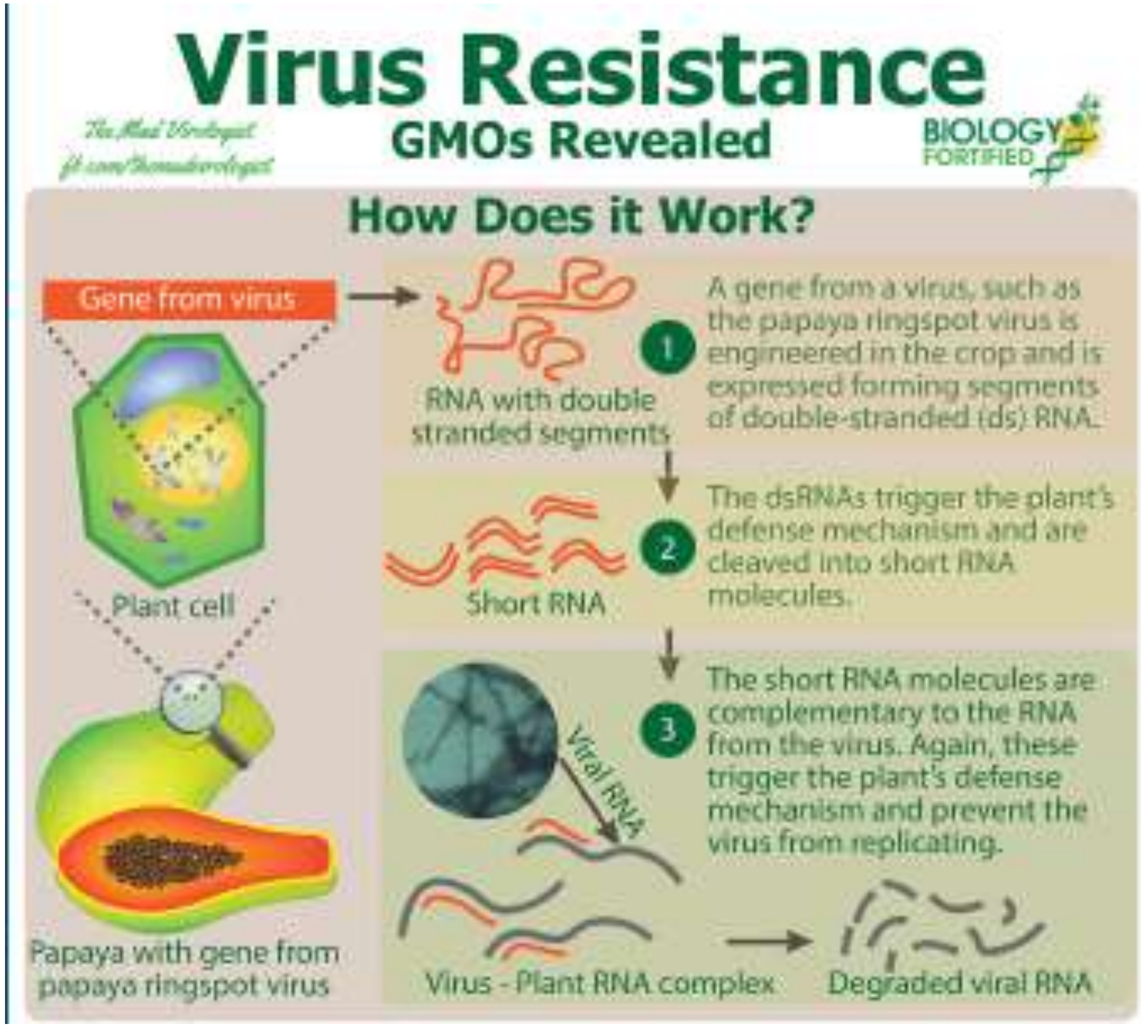
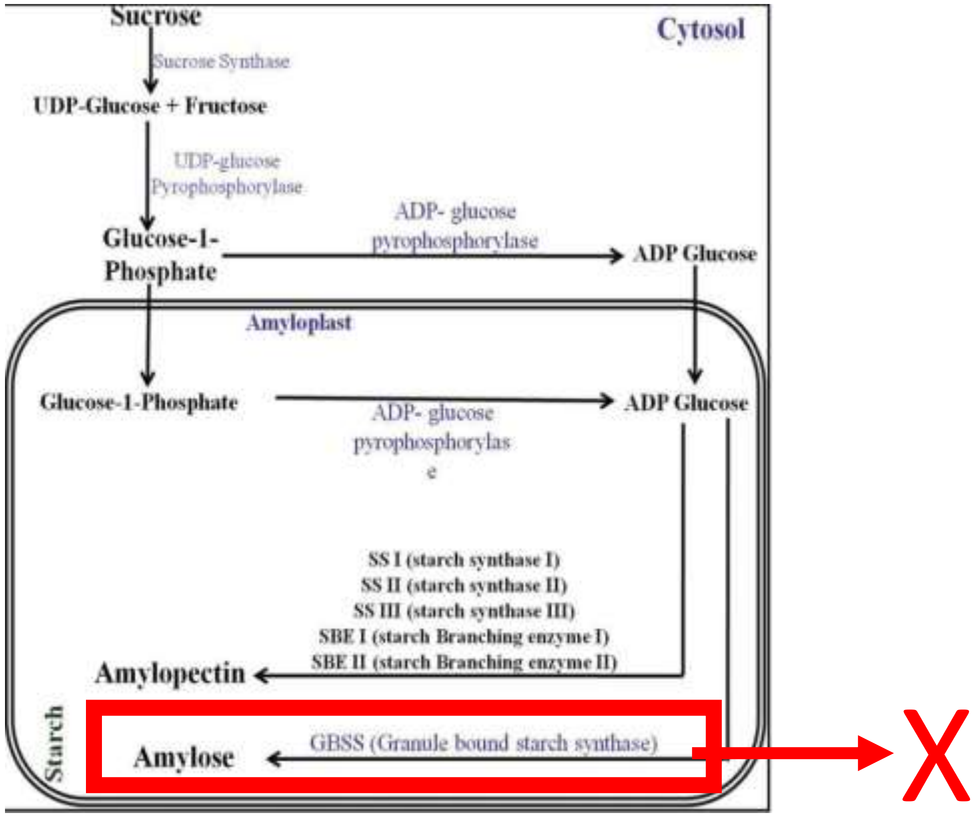


Fig 2: Certain viral genes encoding capsid proteins were transferred to the papaya genome which elicit an "immune response" from the papaya plant To increase the yield of papayas which was previously threatened by the papaya ring spot virus, a disease that sharply lowers the fruit yield.

- **Amylopectin rich sweet potato**

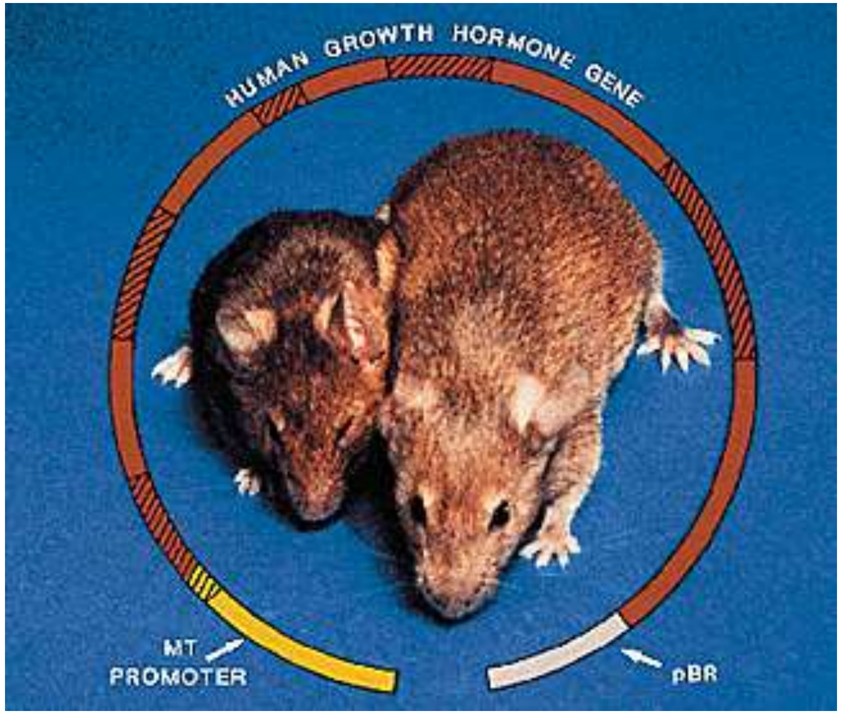
The gene encoding for granule-bound starch synthase I (GBSSI) –key enzyme involved in formulation of amylose is switched off.Amylose free or low amylose content sweet potatoes can be used to develop new industrial materials or might provide new demands in food materials.

Starch synthesis pathway in sweet potato



Transgenic animals

- A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome.
- Transgenesis is the process by which mixing up of genes takes place.
- Foreign genes are inserted into the germ line of the animal, so it can be transmitted to the progeny.
- Transgenic technology has led to the development of fishes, live stock and other animals with altered genetic profiles which are useful to mankind.
- First transgenic animal was a 'Supermouse' created by Ralph Brinster (U Pennsylvania) and Richard Palmiter (University of Washington) in 1982.
- It was created by inserting a human growth hormone gene in mouse genome.
- The offspring was much larger than the parents.
- Mouse – common transgenic expt.
- Other animals include pig, goat, cow, sheep, fish etc.



Production of transgenic animals

Step 1 – Construction of a transgene

- Transgene made of 3 parts:
- Promoter
- Gene to be expressed
- Termination sequence

Step 2 – Introduction of foreign gene into the animal

- Pronuclear microinjection method
- Embryonic stem cell method.

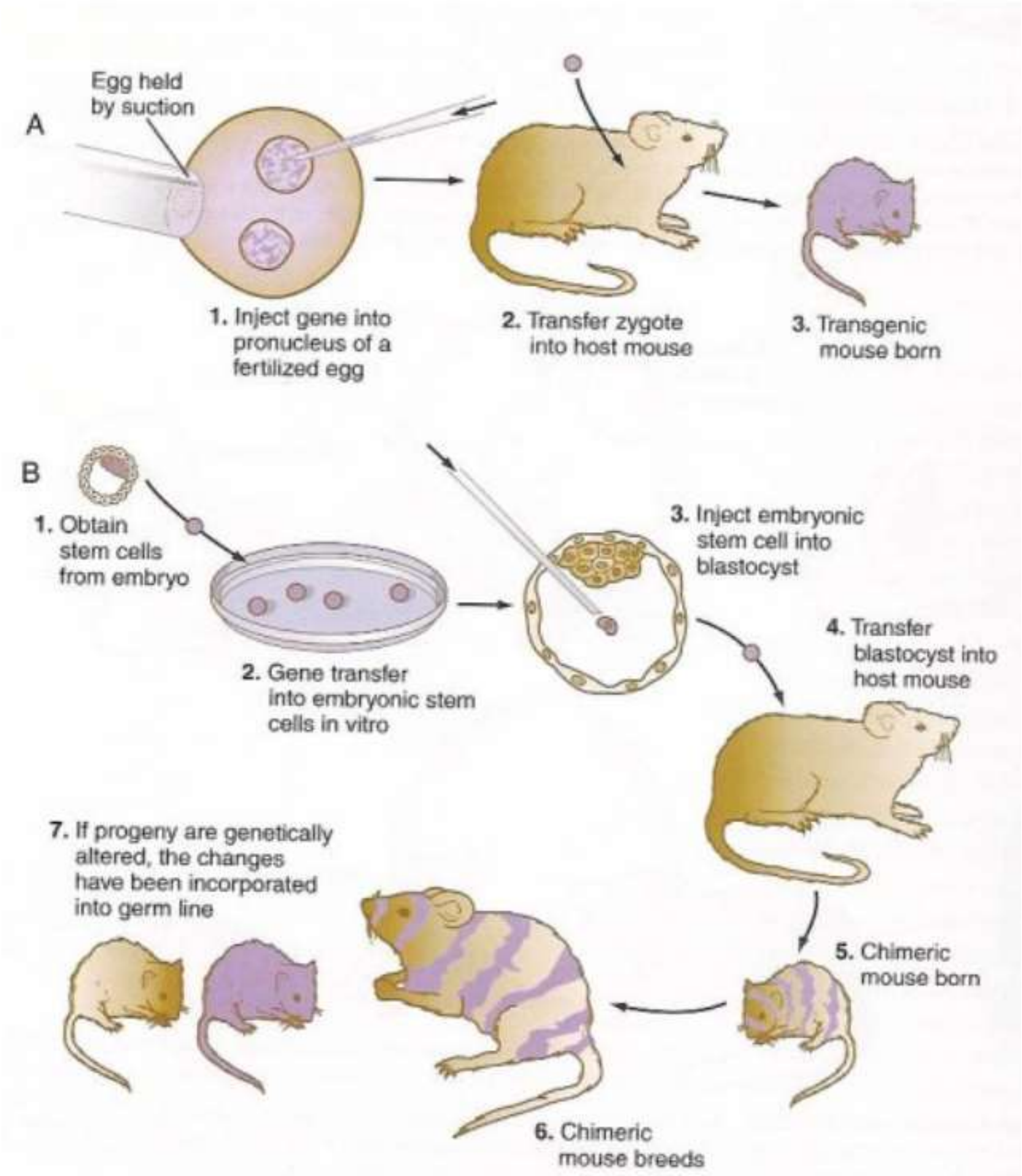
Methodology contd...

Step 3: Screening for transgenic positives

- Transgenic progenies are screened by PCR to examine the site of incorporation of the gene
- Some transgenes may not be expressed if integrated into a transcriptionally inactive site.

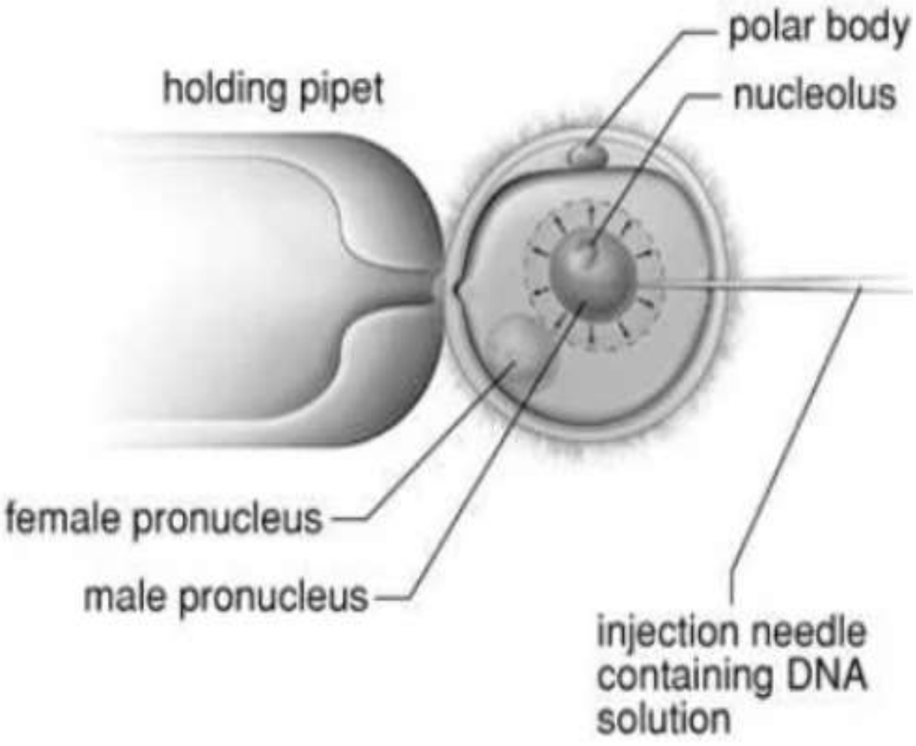
Step 4: Further animal breeding is done to obtain maximal expression.

- Heterozygous offsprings are mated to form homozygous strains.



A. Microinjection
B. Embryonic stem cell method

- A female animal is superovulated and eggs are collected.
- The eggs are fertilized in vitro.
- The transgene containing solution is injected into the male pronucleus using a micropipette.
- Eggs with the transgenes are kept overnight in an incubator to develop to a 2 cell stage.
- The eggs are then implanted into the uterus of a pseudo - pregnant female (female which has been mated with a vasectimized male the previous night)



- Transgenic animals can be created by manipulating embryonic stem cells.
- ES cells are obtained from the inner cell mass of a blastocyst.
- Transgene is incorporated into the ES cell by
    - Microinjection
    - By a retro virus
    - By electroporation
- Transgenic stem cells are grown in vitro.
- Then they are inserted into a blastocyst and implanted into a host's uterus to grow normally.

1.The GloFish is a patented and trademarked brand of genetically engineered fluorescent fish.

They have been created from several different species of fish: Zebrafish (*Danio rerio*) were the first GloFish available in pet stores, and recently tetra (*Gymnocorymbus ternetzi*), tiger barbs (*Puntius tetrazona*), Rainbow Shark (*Epalzeorhynchos frenatum*), and most recently betta have been added to the lineup.

They are the first genetically modified animals to become publicly available.

The rights to GloFish are owned by Spectrum Brands, Inc., which purchased GloFish from Yorktown Technologies, the original developer of GloFish, in May 2017.

Chemicals that mimic natural estrogens have well-documented effects on the reproductive systems of vertebrates, typically acting as endocrine disruptors, and GloFish fluorescence is being used to detect levels of estrogenic chemicals.

Investigators found that muscles such as the heart are more affected by estrogen than the liver.

Using the GloFish may thus give insights into endocrine disrupting chemical actions.

Fluorescent zebrafish also have been used for other experimental research. The alterations in the zebrafish's genes have given the organism the ability to fluoresce as a bio-indicator. This genetic ability has been used to detect pollution and other chemicals

GloFish

2.OncoMouse

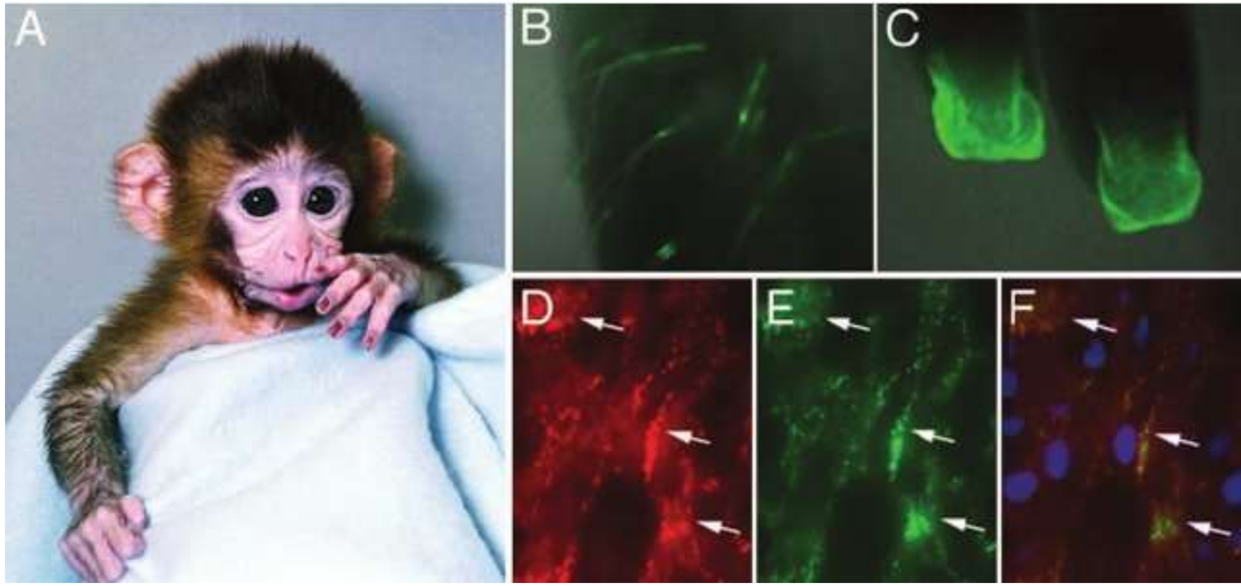
The OncoMouse or Harvard mouse is a type of laboratory mouse (*Mus musculus*) that has been genetically modified using modifications designed by Philip Leder and Timothy A Stewart of Harvard University.

It carries a specific gene called an activated oncogene (v-Ha-ras under the control of the mouse mammary tumor virus promoter).

The activated oncogene makes the mouse more susceptible to developing a tumor.

Hence making this model perfect for cancer research.

ANDi



- ANDi was the first transgenic monkey, born in 2000.
- “ANDi” stands for “inserted DNA” spelled backwards.
- An engineered virus was used to insert the harmless gene for green fluorescence protein (GFP) into ANDi’s rhesus genome.
- ANDi proves that transgenic primates can be created and can express a foreign gene delivered into their genome.

AquAdvantage Salmon

- AquAdvantage salmon were developed in 1989 by the addition of a single copy of the opAFP-GHc2 construct, which consists of a promoter sequence from ocean pout directing production of a growth hormone protein using the coding sequence from Chinook salmon.
- The continuous expression of this transgene allows the fish to grow all year-round instead of only during spring and summer.
- These GM fish were back-crossed to wild-type Atlantic salmon, and the genetically modified EO-1α gene sequence was identical in the second through fourth generations, indicating that the insertion is stable.

Problems encountered during production

- Multiple insertion – too much proteins
- Insertion into an essential gene – lethality
- Insertion into a gene leading to gene silencing
- Insertion into a different area can affect the gene regulation

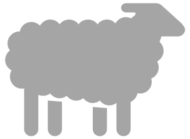
Importance of transgenic animals

Medical importance

Disease model

Bioreactors for pharmaceuticals

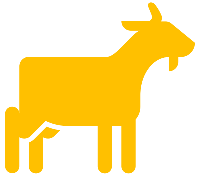
Xenotransplantation



Agricultural importance

Disease resistant animals

For improving quality and quantity of milk, meat, eggs and wool production



Industrial importance

Toxicity sensitive transgenic animals to test chemicals.

Spider silk in milk of goat

Issues with transgenic technology

- Blurring the lines between species by creating transgenic combinations.
- There may be health risks associated with transgenics.
- There may be long term effects on the environment when transgenic animals are released into the field.
- Various bioethicist argue that it is wrong to create animals that would suffer as a result of genetic alteration.

Questions