BIOTECHNOLOGY AND ITS APPLICATIONS
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Biotechnology is mainly employed for industrial scale production of biopharmaceuticals and biologicals using genetically modified microbes, fungi, plants and animals. The application of biotechnology include therapeutics, diagnostics and genetically modified crops for agriculture, processed food, bioremediation, waste treatment and energy production.

Three research areas of biotechnology are as follow.

(i) Providing the best catalyst in the form of improved organism; generally a microbe or pure enzyme.

(ii) Creating optimal conditions through engineering for a catalyst to act.

(iii) Downstream processing technologies to purify the protein / organic compound.

BIOTECHNOLOGICAL APPLICATION IN AGRICULTURE:

Three options can increase the food production.

(i) Agrochemical based agriculture.

(ii) Organic agriculture.

(iii) Genetically engineered crop-based agriculture.

Green Revolution

In 20th century significant increase in agricultural productivity of grains (particularly wheat and rice) was observed, resulting from

(i) Introduction of improved crop varieties i.e high-yielding varieties.

(ii) Better management practices (irrigation, mechanisation and soil conservation technique).

(iii) Use of agrochemicals (fertilisers or pesticides) its early dramatic success was in Mexico and the Indian subcontinent.

The green revolution succeeded in tripling the food supply but yet it was not enough to feed the growing human population.

Difficulties: Green Revolution was not a complete success in developing world due to various factors.

- For farmers in devolping world, agrochemicals are often too expensive.
- Further increases in yield with existing varieties are not possible using conventional breeding.
- The new varieties required large amounts of fertilizers, and pesticides to produce high yields, raising concern about cost and potentially harmful effects.
- minimising the use of fertilizers and chemicals so that their harmful effects on the environment are reduced.

For overcoming the above stated problems use of genetically modified crops is a possible solution

Gene cloning provides a new dimension to crop breeding by enabling directed changes to be made to the genotype of a plant, eliminating the random processes inherent in conventional breeding.

Two general strategies have been used:

(a) Gene addition: in which cloning is used to alter the characteristics of a plant by providing it with one or more new genes.

(b) Gene subtraction: in which genetic engineering techniques are used to inactivate one or more of the plants, existing genes.

Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called genetically modified organism (GMO) or transgenic organisms.

TRANSGENIC PLANTS

Genetic modified crops or GM Crops:

They represent crops that have one or more useful foreign genes or transgenes.

GM crops has two advantages.

(a) Any gene of any organisms or a synthetic gene can be incorporated.

(b) Genotypic change is precisely control.

This technique is superior to breeding programmes because in breeding only the already present genes are reshuffled and that changes would occur in all traits for which the parents are different.

Application of DNA Technology in the production of Transgenic Plants:

GM plants have been useful in many ways:

(i) Made crops more tolerant to abiotic stresses (Cold, drought, salt heat).

(ii) Reduced reliance on chemical pesticides (pest-resistant crops).

(iii) Helped to reduce post harvest losses.

(iv) Increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil).

(v) Enhanced nutritional value of food, e.g. Vitamin 'A' enriched rice.

In addition to these uses, genetic engineering has been used to create tailor- made plants to supply alternative resources to industries, in the form of starches, fuels and pharmaceuticals.

GENE ADDITION

- Recombinant DNA techniques are being used to develop transgenic plants.
- Plasmid of **Agrobacterium tumefaciens** is widely used as **vector** to introduce new genes into dicot plants. The former is called **Ti plasmid (Tumor inducing plasmid)**, so called because in nature, it induces tumors in broad leaf plants such as tomato, tobacco and soyabean. It does not infect cereals.
- Agrobacterium initiates formation of cancerous growth called a **crown gall tumor**. For genetic engineering purposes, its strains are developed in which tumor-forming genes are eliminated. These transformed bacteria can still infect plant cells.
- The part of Ti plasmid transferred into plant cell DNA, is called the **T-DNA**. This T-DNA with desired DNA spliced into it, is inserted into the chromosomes of the host plant where it produces copies of itself.

- Such plant cells are then cultured, induced to multiply and differentiate to form plantlets. Transferred into soil, the plantlets grow into mature plant, carrying the foreign gene, expressed throughout the new plant.

  Agrobacterium tumefaciens is called natural genetic engineer because it integrates its plasmid's gene into plant genome and these genes carried by its plasmid produce effect in several parts of the plant.

(i) Insect Resistant transgenic plant:

Bt Cotton –

- **Bacillus thuringiensis bacterium form crystal protein (Cry protein)** contain a toxic insecticidal protein. That kill certain insects like **Lepidopterans (tobacco budworm, armyworm), coleopteran (beetles) and dipterans (flies, mosquitoes).**

  The Bt toxin proteins exist as inactive **protoxins** but once an insect ingests the inactive toxin it is converted into an active form of toxin due to the alkaline pH of the alimentary canal that solublizes the crystals. The activated toxin binds to the surface of midgut epithelial cells and create pores which cause cell swelling and lysis and finally cause death of the insect.

- **Bt toxin** genes were isolated from **Bacillus thuringiensis** and incorporated into the several crop plants like **cotton**. The toxin is coded by a gene named **cry**.
- **cry I Ab** gene has been incorporated in **Bt corn** to protect the same from **corn borer**.
- Two **cry genes IAc and cry II Ab** have been introduced in cotton. **The genetically modified crop** is called **Bt cotton** that shows **resistance** against **cotton bollworms**.

THE ENDOTOXINS OF BACILLUS THURINGIENSIS

Insects not only eat plants; bacteria also form occasional part of their diet. In response, several types of Bacteria have evolved defense machanisms against insect predation, an example being B. thuringiensis which, during sporulation, forms-intracellular crystalline bodies that contain an insecticidal protein called the endotoxin.This toxin is encoded by a gene named **cry**. The endotoxin that accumulates in the bacterium is an inactive precursor. After ingestion by the insect, this protoxin is cleaved by proteases (alkaline conditions in gut), resulting in shorter versions of the protein that display the toxic activity, by binding to the inside of the insects mid gut and damaging the surface epithelium by creating pores that cause swelling and lysis. So, that insect is unable to feed and consequently starves to death. The choice of gene depends on the crop and the targeted pest, as most Bt toxins are insect group specific.

This toxin called Bt toxin as produced by Bacillus thuringiensis has been cloned in bacteria and been expressed in plants to provide resistance to insects without the need for insecticides in effect created a bio-pesticide. Examples are Bt cotton, corn, rice, tomato, potato and soya bean etc.

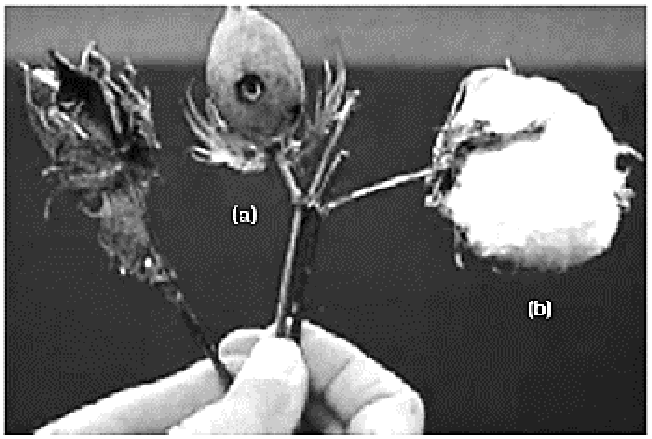

Figure - Cotton boll: (a) destroyed by bollworms; (b) a fully mature cotton boll

(ii) Golden Rice:

Golden rice was developed by **Ingo Potrykus** and **Peter Beyer** to combat vitamin A and Iron deficiency as this could accumulate more β-carotene.

Golden rice is a transgenic variety of rice (Oryza sativa) which contains good quantities of β-carotene **(provitamin A -** inactive state of vitamin A). It is required by all individuals as it is present in retina of eyes. Deficiency of vitamin A causes night blindness and skin disorders. β-carotene is a principal source of vitamin A. Due to β-carotene the grains (seeds) of this GM rice are pale yellow instead of pearly white in colour, thus the rice is commonly called **golden rice**.

It is a transgenic variety of rice with genes for synthesis of β–carotene taken from **Daffodil** (Narcissus pseudonarcissus) and inserted into the genome of a temperate strain of rice using Agrobacterium tumefaciens, as the vector to effect the transfer.

Golden rice required the introduction of three genes encoding the enzymes phytoene synthase, Carotene desaturase and lycopene β-cyclase.

Isoprenoid units

↓ Sequential addition

geranyl geranyl diphosphate

↓ Phytoene synthese (psy)

phytoene

↓ Carotene desaturase (crt 1)

Lycopene

↓ Lycopene β - cyclase (β–lcy)

β –Carotene (provitamin A)

(iii) Production of hirudin from transgenic Brassica napus seeds

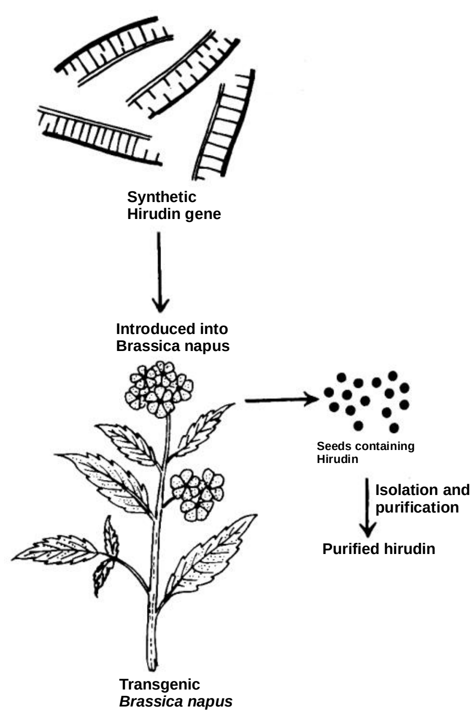

Fig :- Diagrammatic representation of the production of hirudin from transgenic *Brassica napus* seeds.

Hirudin is a naturally occurring, anticoagulant produced and secreted in the salivary glands of the medicinal leech, Hirudo medicinalis, with a number of desirable properties. Hirudin has been made in variety of microorganisms including yeast and E. coli but these entail significant costs associated with fermentation. Therefore, the gene-encoding hirudin was chemically synthesized and introduced into Brassica napus using Agrobacterium-mediated transformation. The resulting transgenic plant yielded seeds in which hirudin accumulates. The hirudin is purified and used as medicine.

(iv) Herbicide resistant transgenic plants

The herbicide **glyphosate** [**Roundup** (Trade name)] inhibits photosynthesis. It blocks the activity of **enolpyruvylshikimate phosphate synthase (EPSPS)**, a key enzyme involved in the biosynthesis of phenyl alanine, tyrosine and tryptophane. The genes epsps/aroA confer resistance to transgenic plants which can be selected. This gene obtained from mutant strain of Agrobacterium. Thus Roundup ready transgenic plants has been produce and commercialised. Herbicide tolerence has been developed in Maize, Cotton**,** Soyabean and Tobacco etc.

Gene Subtraction

This is another method of changing the genotype of a plant by either inactivation of the gene or sometimes **knockout of the gene**. There are several possible strategies for inactivating a chosen gene in a living plant, the most successful so far in practical terms being the use of anti-sense RNA.

The strategy adopted to prevent this infestation is based on the process of RNA interference (RNAi): RNAi was first discovered in 1998 by Andrew Fire and Craig Mello in the nematode worm **Caenorhabditis elegans** and later found in a wide variety of other organisms, including mammals.The importance of this discovery is reflected by the fact that 2006 Nobel prize for medicine was awarded to Fire & Mello.

RNAi is a naturally occurring mechanism that leads to the "silencing" of genes. In consequence, the respective protein is no longer synthesized. In nature this mechanism is used for the regulation of specific genes and is also applied as a defence against viruses. This technique has been used for **loss of function** studies where a gene (responsible for parasitism) is specifically silenced. It takes place in all eukaryotic organisms as a method of cellular defense.This method involves silencing of a specific mRNA due to formation of dsRNA molecule formed by binding of complementary RNA (anti-sense RNA) molecule to original mRNA, thereby preventing translation of the original mRNA (silencing). The source of this complementary RNA could be from infection by viruses having RNA genomes or mobile genetic elements (transposons) that replicate via an RNA intermediate.

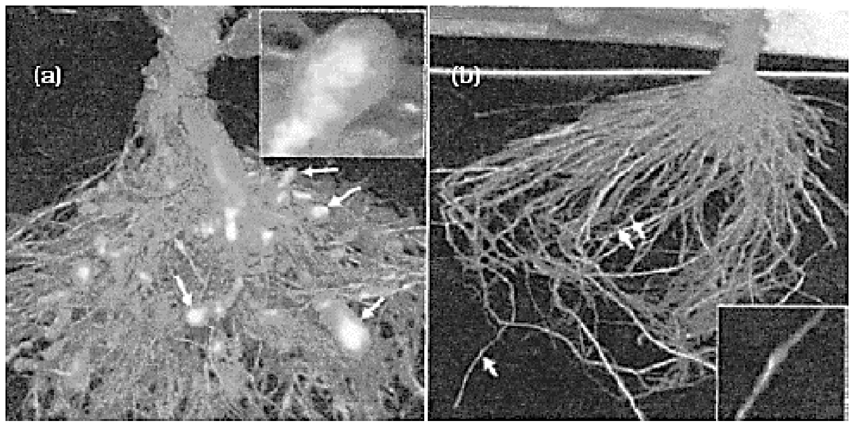

Fig. Host plant-generated dsRNA triggers protection against nematode infestation: (a) Roots of a typical control plants; (b) transgenic plant roots 5 days after deliberate infection of nematode but protected through novel mechanism.

USE OF ANTI-SENSE RNA IN CREATING PEST RESISTANT PLANTS:

Several nematodes parasite have wide variety of host plants and animals including human beings. They attack nearly every food and fibre crop grown by invading the plants roots. It feeds on the roots cells causing roots to grow into large galls/knots, damaging the crop and reducing its yield. Hence, called root-knot nematode.

A nematode Meloidegyne incognita infects the roots of tobacco plants causing a great reduction in yield. The most cost effective tactic for preventing such damage was to bioengineer resistant plants that prevent the nematode from feeding on the roots.

Using Agrobacterium vectors, nematode specific genes (responsible for parasitism) were introduced into the host plant. The introduction of DNA was such that it produced both sense and anti-sense RNA in the host cells forming dsRNA. These two RNA's being complementary to each other formed a dsRNA that was taken up by the parasitic nematode and initiated RNAi, thus silenced the specific mRNA of the nematode. The consequence was that the parasite could not survive in a transgenic host expressing specific interfering RNA. The transgenic plant therefore got itself protected from the parasite.

DIFFERENT STEPS INVOLVED IN MAKING TOBACCO PLANT RESISTANT TO NEMATODE ARE BRIEFLY DESCRIBED BELOW:

1. Double-stranded RNAs are processed into approximately 21-23 nucleotide RNAs with two nucleotides. An RNase enzyme called **Dicer** cuts the dsRNA moelcules (from a virus, transposon, or through transformation) into small interfering RNAs (siRNAs).
2. Each siRNA complexes with ribonucleases (distinct from Dicer) to form an RNA-induced silencing complex (RISC).
3. The siRNA unwinds and RISC is activated.
4. The activated RISC targets complementary mRNA molecules. The siRNA strands act as guides where the RISCs cut the transcripts in an area where the siRNA binds to the mRNA. This destroys the mRNA.
5. When mRNA of the parasite is destroyed no protein was synthesized. It resulted the death of the parasite (nematode) in the transgenic host. Thus the transgenic plant got itself protected from the parasite.

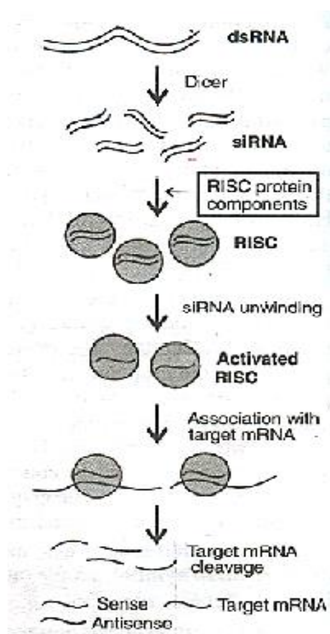

Fig. The steps in RNA interference (RNAi)

Flavr Savr-tamato (post harvest losses and delayed fruit ripening):

The tomato variety Flavr Savr developed by Calgene, presents an example where an expression of a natural tomato gene has been blocked.

In 'Flavr Savr' transgenic tomato, expression of a native tomato gene for **polygalacturonase** has been blocked. This gene produces enzyme **polygalacturonase** which promotes softening of fruit. The production of this enzyme was reduced in the Flavr Savr transgenic tomato. The non-availability of this enzyme prevents over-ripening because the enzyme is essential for degradation of cell walls.

Transformation was carried out by introducing recombinant plasmid into **Agrobacterium tumefaciens** and then allowing the bacteria to infect tomato stem segments. The transformed fruits, although undergoing a gradual softening could be stored for a prolonged period before beginning to spoil.

Advantages-

- longer shelf life of fruit
- Good flavour
- superior taste
- higher quantity of total soluble solids.

S.No.	Transgenic plants	Useful application
1	Bt Cotton	Pest resistance, herbicide tolerance and high yield. It is resistant to boll worm infestation.
2	Wheat	Resistant against the herbicide.
3	Brassica napus	A gene encoding hirudin (a protein that prevents blood clotting) is synthesized chemically and then transferred into Brassica napus, This gene is activated in the plant and starts synthesizing hirudin which acccumulates in seeds. The hirudin is then extra

4	Tobacco	CPTI (Cow pea trypsin inhibitor) gene has been introduced in tobacco to show resistance against pests.
5	Flavr Savr Tomato	Increased shelf-life (delayed ripening) and better nutrient quality.
6	Golden Rice	Vitamin A-rich
7	Potato	Higher protein content
8	Corn, Brinjal	Insect resistance
9	Soyabean, Maize	Herbicide resistance

Advantage of Transgenic Plants:

Due to genetic modification GM plants have been useful in many ways:

1. **Pest Resistance Crops:** Growing GM crops can help to reduce the use of chemical pesticides, e.g. Bt Cotton.
2. **Tolerance:** GM crops have made more tolerance to abiotic stresses (cold, drought, salt, heat, etc.)
3. **Reduction in Post-harvest Losses**: They have helped to reduce post harvest losses, e.g. Flavr Sarv transgenic tomato.
4. **Prevention of Early Exhaustion of Fertility of Soil:** Increased efficiency of mineral usage by plants prevents early exhaustion of fertility of soil.
5. **Increasing Nutritional Value of Food:** GM plants enhance nutritional value of food, e.g. golden rice is rich in vitamin A.
6. **Herbicide Resistance:** Herbicides (weed killers) do not harm the GM crops.
7. **Alternative Resources to Industries:** GM plants have been used to create alternative resources to industries in the form of starches, fuels and pharmaceuticals. Researchers are working to develop edible vaccines, edible antibodies and edible interferons.
8. **Disease Resistance:** Many viruses, bacteria and fungi cause plant diseases. Scientists are working to create genetically engineered plants having resistance to these diseases.
9. **Phytoremediation:** Plants such as popular trees have been genetically engineered to clean up heavy metal pollution from contaminated soil.

Disadvantages of Transgenic Plants:

1. **Environmental hazards**

(i) **Unintended harm to other organisms**: Pollen from Bt corn caused high mortality rates in monarch butterfly caterpillars. Monarch caterpillars consume milkweed plants, not corn, but the fear is that if pollen from Bt corn is blown by the wind on to milkweed plants in neighbouring fields, the caterpillars could eat the pollen and perish.

(ii) **Reduced effectiveness of pesticides:** Just as some populations of mosquitoes developed resistance to the now-banned pesticide DDT, many people are concerned that insects will become resistant to Bt or other crops that have been genetically modified to produce their own pesticides.

(iii) **Gene transfer to non-target species**: Another concern is that crop plants engineered for herbicide tolerance and weeds will cross-breed, resulting in the transfer of the herbicide resistance genes from the crops into the weeds. These "superweeds" would then be herbicide tolerant as well. Other introduced genes may cross over into non-modified crops planted next to GM crops.

2. **Human health risks:**

(i) **Allergies**: The transgenic food may cause toxicity and or produce allergies. The enzyme produced by the antibiotic resistance gene can cause allergies, because it is a foreign protein.

(ii) **Effect on Bacteria of Alimentary canal:** The bacteria present in the human alimentary canal can take up the antibiotic resistance gene that is present in the GM food. These bacteria can become resistant to the concerned antibiotic and will be difficult to manage.

3. **Economic concerns:** Bringing a GM food to market is a lengthy and costly process and of course agro-biotech companies wish to ensure a profitable return on their investment