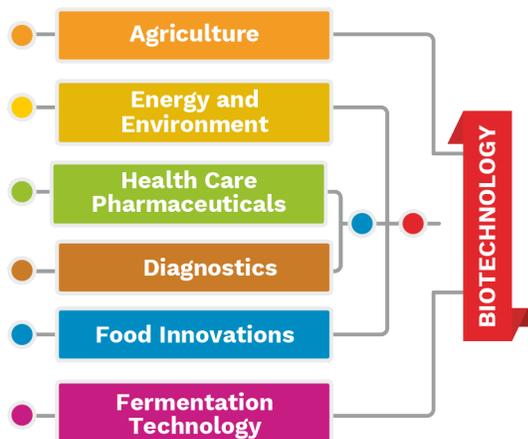


Biotechnology and Its Applications

INTRODUCTION

- Biotechnology is a field in which DNA is isolated from an organism and integrated with a vector to make multiple copies. This DNA with the desired gene of interest is then inserted into the organism and the organism expresses the phenotype of the inserted DNA. This method of biotechnology has been explored in the past few years and have been used in the different fields to modify organisms for the benefit of the mankind.
- The applications of biotechnology are in the field of:



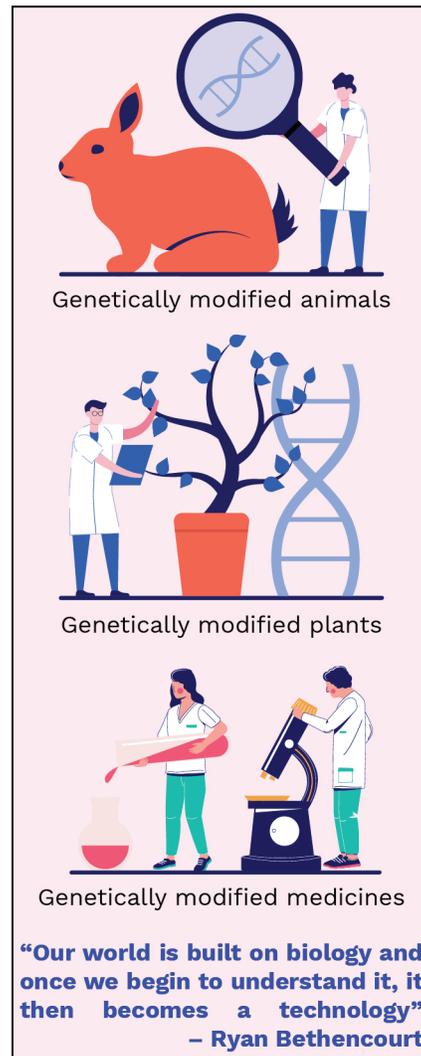
- Three research areas of biotechnology are:
 - Improving an organism through the process of biotechnology to act as the best catalyst.
 - Creating optimum conditions for growth and action of the microbes.
 - Downstream technologies to purify the compounds being formed.

APPLICATIONS OF BIOTECHNOLOGY IN AGRICULTURE

- The main consideration that has been for the past many years is the increase in the quantity and quality of the food crops.
- This has been achieved by
 - agro-chemical based agriculture

Definition

Biotechnology: The integration of natural science and organisms, cells, parts therefore, and molecular analogues for products and services.





- organic agriculture
- genetically engineered crop-based agriculture
- Green Revolution took place in the late sixties. **Norman Borlaug** is known as the Father of Green Revolution. He tried to increase the agricultural productivity in the world.
- In India **M.S. Swaminathan** initiated Green Revolution and encouraged modern methods and technology for agriculture.
 - It aimed at increasing the yield of the crops.
 - This was done by providing
 - ◆ improved variety of seeds to the farmers (high yielding variety)
 - ◆ improving irrigation facilities
 - ◆ providing good use of agrochemicals.
- For the farmers of developing country it is difficult to afford agrochemicals for their farming practices.
- The normal breeding technique was not giving high yield of crops and thus it was necessary to think about other alternative ways to increase the yield of crops and to fight against the pests.
- Are there ways that can be used to increase the yield of the crops but also to reduce the dependency on fertilizers, pesticides and insecticides?
- The answer to the above question was found in the field of biotechnology and it was applied to different plants, animals and microbes.
- The plants, animals and microbes that are genetically modified by biotechnology are known as **Genetically Modified Organisms**.
- Genetically modified plants and crops have been useful. They are being used by the farmers. The following are the advantages of genetically modified plants:

Previous Year's Question



Golden rice is a transgenic crop of the future, with the following improved trait

- (1) insect resistance
- (2) high lysine (essential amino acid) content
- (3) high protein content
- (4) high vitamin – A content

Definition

Genetically Modified Organisms (GMO): Plants microbes, fungi and animals whose genes have been altered by manipulation are called GMO.

Gray Matter Alert!!!

Golden Rice: Ingo Potrykus and Peter Beyer genetically produced golden rice. It is a rice variety which has a high quantity of beta carotene, which is a provitamin A. Beta carotene converts into vitamin A when metabolised by humans. The colour of rice grain is yellow.

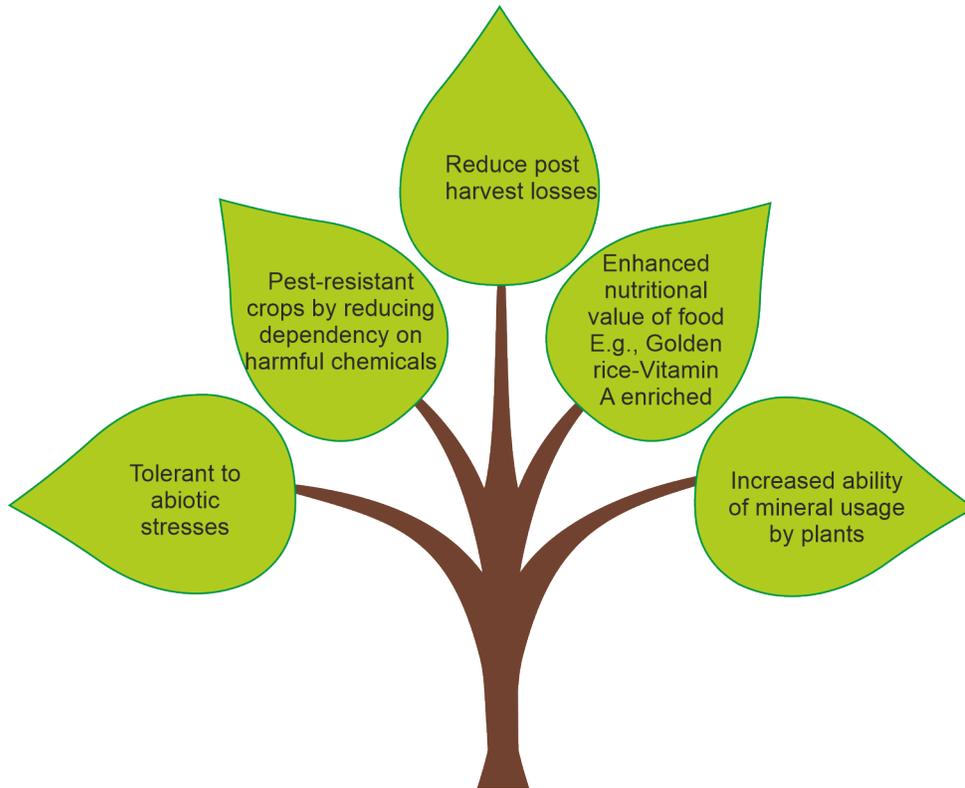
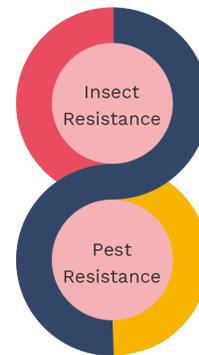


FIG. ADVANTAGES OF GENETICALLY MODIFIED PLANTS

Production of pest resistance plants

- *Bacillus thuringiensis* has a gene that produces a toxin which has pesticide properties. This gene can be isolated from the bacteria and integrated with different plants to provide them resistance against certain pests.
- The *cryI* gene of *B. thuringiensis* produces protein, that is responsible for the insecticidal activities of the bacteria.
- The protein kills lepidopterans (tobacco budworm, armyworm), coleopterans (beetles) and dipterans (flies, mosquitoes).
- The bacteria *Bacillus thuringiensis* produces protein crystals which contain the insecticidal proteins.
- The crystal does not kill the bacteria as the Bt toxin protein exists as **inactive protoxins** in the



TRANSGENIC PLANTS

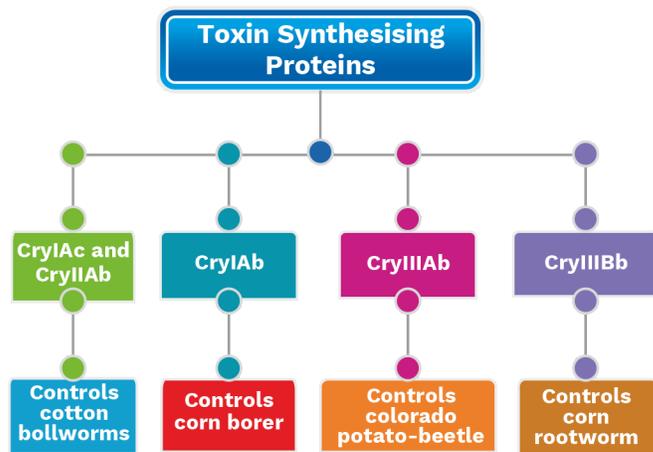
Gray Matter Alert!!!

Flavr Savr: It is a transgenic tomato. It has been made resistant to rotting and thus has high shelf value.



bacteria and **alkaline pH** is required to activate the crystals.

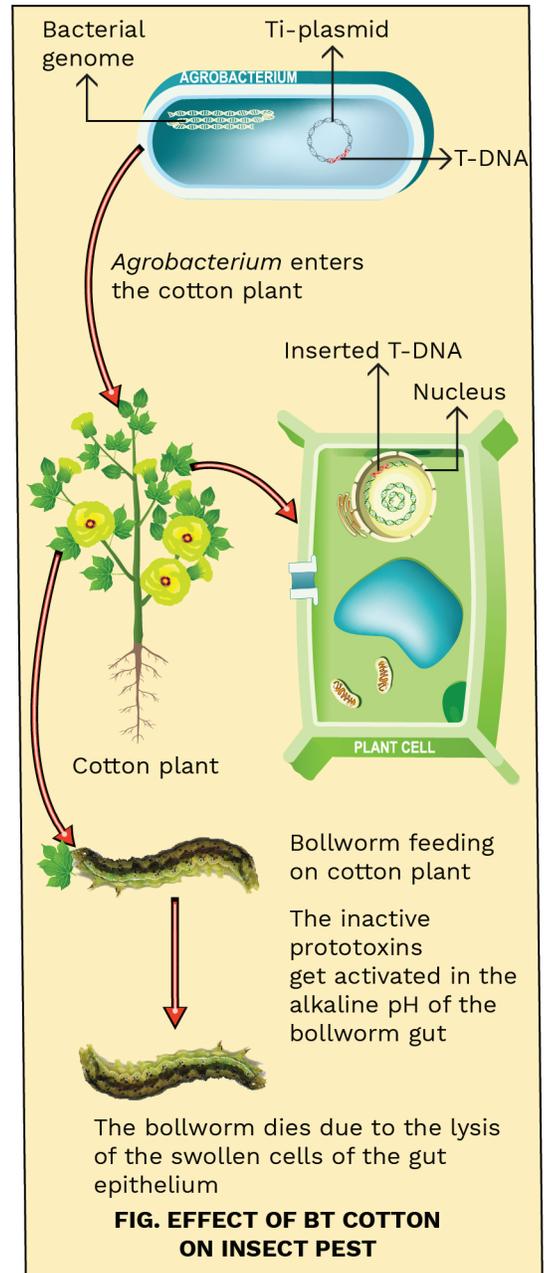
- Once inside the body of the insect, the alkaline pH of the gut activates the protoxin and the crystal becomes soluble in the insect's gut.
- The toxin binds to the epithelial lining of the gut creates pores and causes the cell to swell and then lyses. This kills the insect.
- The **Cry proteins** into four main groups, *Cry-I*, *Cry-II*, *Cry-III* and *Cry-IV*, on the basis of their insecticidal activities. The choice of genes depends upon the crop and the targeted pest. The proteins encoded by the genes *cryIAc* and *cryIIAb* control the cotton bollworms, that of *cryIAb* controls corn borer.



There are many insect species that are not sensitive to Cry proteins; this could be due to a high affinity of midgut proteases of these species to the Cry proteins. In such insect species, the Cry proteins seem to be cleaved into several fragments.

Pest resistance in Plants-RNAi Mediated Interference (RNA)

- Roots of Tobacco plant is infected by a nematode *Meloidegryne incognitia*. It affects the yields of the leaves.



Rack Your Brain



Why are the proteins coded by *cryIAc*, *cryIAb* and *cryIIAb* are slightly different in their control?

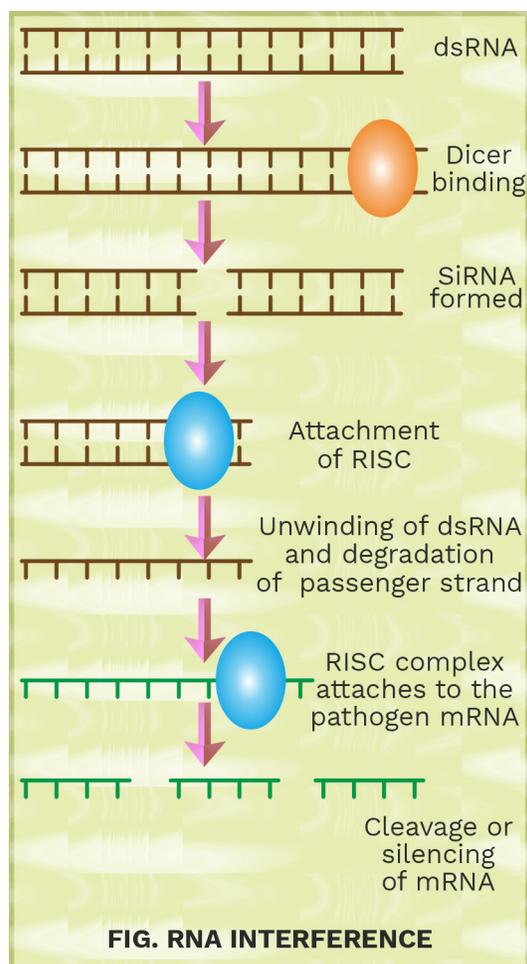
- A strategy was developed based on RNA interference (RNAi). RNAi takes place in all eukaryotic organisms as a means of cellular defense.
- Silencing of gene expression triggered by double-strands RNA (dsRNA) is called RNA-mediated interference or RNA interference (RNAi)
- In this, a specific mRNA molecule is silenced using a complementary dsRNA. An infection of RNA virus or mobile genetic elements i.e., transposons could be the source of the complementary RNA.
- By using *Agrobacterium* vectors, nematode-specific genes are introduced into the host plant.
- The introduced DNA produces both **sense** and **anti-sense RNA** which together form dsRNA molecule.
- RNA enzyme dicer cuts the dsRNA into short sequences of nucleotides known as small interfering RNA i.e siRNA.
- **siRNA** binds with ribonuclease and forms **RNA-inducing silencing complex** (RISC).
- This RISC binds to the mRNA of the nematode and cut it into smaller fragments thus making it inactive.
- The parasite is later killed as it cannot make any more proteins in the infected tobacco plant.
- RNAi can also serve as an antiviral defence mechanism and is used for analysis of gene function in invertebrates, plants, and mammals.
- Disadvantages of RNAi is that:
 - RNAi based on exogenous siRNAs is short-lived.
 - longer dsRNA molecules trigger antiviral response and a general suppression of gene expression.

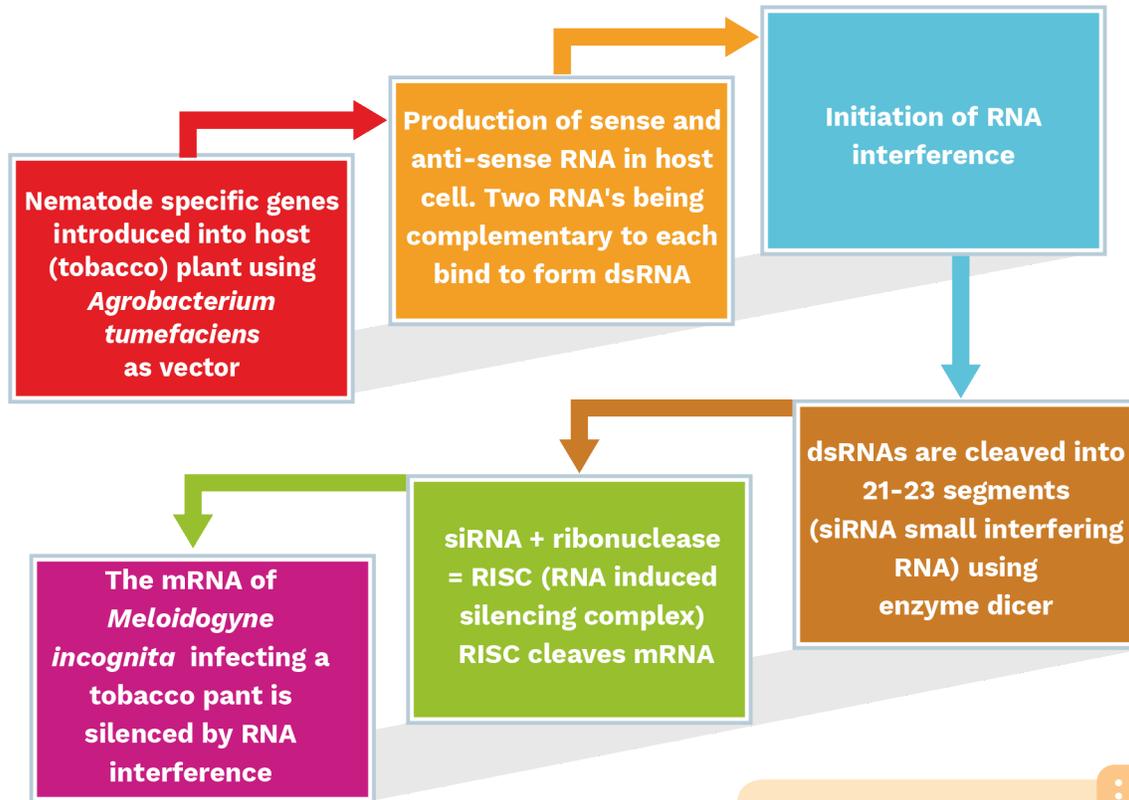
Previous Year's Question



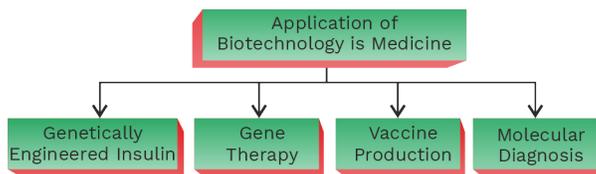
The bacterium *Bacillus thuringiensis* is widely used in contemporary biology as

- (1) insecticide
- (2) agent for production of dairy products
- (3) source of industrial enzyme
- (4) indicator of water pollution.





APPLICATION OF BIOTECHNOLOGY IN MEDICINE



- Recombinant DNA technology is used in the field of healthcare and has led to the development of many techniques and drugs that has helped the humans. It is used in the following-
 - Genetically Engineered Insulin
 - Gene therapy
 - Vaccine production
 - Molecular diagnosis

Definition

Transposons: Units of DNA which can move from one DNA molecule to another.



Previous Year's Question

- RNA interference involves
- (1) synthesis of cDNA and RNA using reverse transcriptase
 - (2) silencing of specific mRNA due to complementary RNA
 - (3) interference of RNA in synthesis of DNA
 - (4) synthesis of mRNA from DNA



Genetically Engineered Insulin

- Many people in the world are suffering from diabetes. Thus they need to be treated with medicine or insulin.
- Insulin was isolated from the pancreas of cattle and pigs and given to the people suffering from insulin deficiency.
- This was causing a lot of problems. A large number of cattle were being killed and in some patients, the insulin extracted from the cattle was causing side effects.
- This was a matter of concern and thus other alternative techniques were researched in which insulin could be manufactured. It then was thought that insulin could be prepared by rDNA but the structure of Insulin caused a problem.
- Human insulin is a dimer comprising one chain of 21 amino acids (A chain) and the other of 30 amino acids (B chain) linked together by disulphide bridges.
- In mammals, insulin is synthesised as a pro-hormone which contains an extra stretch called the C peptide.
- This **C peptide** is removed from the pro-hormone and then the mature Insulin is formed.
- The main challenge was to assemble the two chains of insulin by rDNA technology.

Previous Year's Question



Which part of the tobacco plant is infected by *Meloidogyne incognita*?

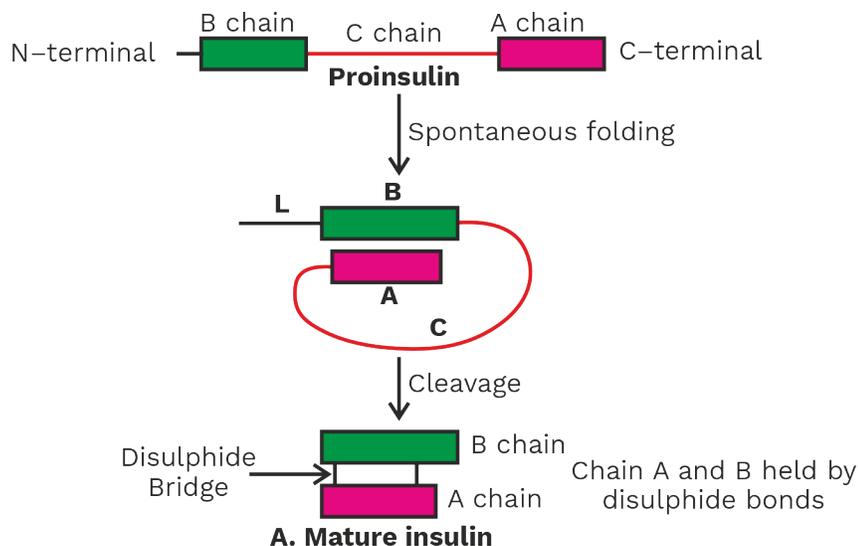
- (1) Stem
- (2) Root
- (3) Flower
- (4) Leaf

Previous Year's Question



Human insulin is being commercially produced from a transgenic species of

- (1) *Rhizobium*
- (2) *Saccharomyces*
- (3) *Escherichia*
- (4) *Mycobacterium*





- In 1983, an American company Eli Lilly prepared the DNA sequences of the two chains A and B and then introduced them into pBR322 of *E.coli* to produce the insulin chain.
- Chains A and B were produced separately and combined together with **disulphide bonds**.

Gene Therapy

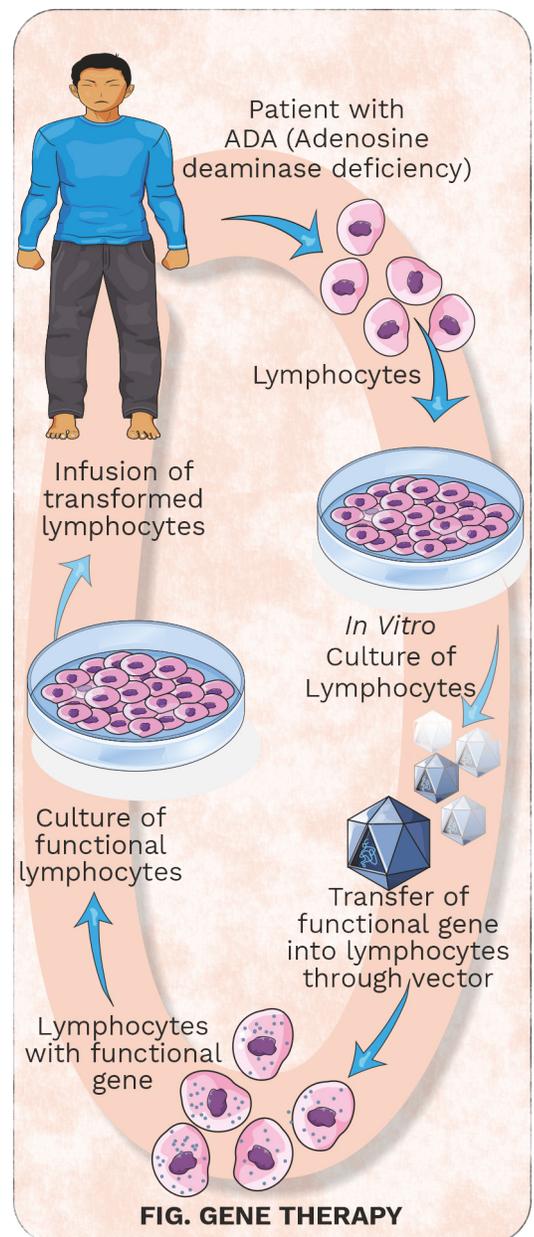
- Gene therapy is a set of ways through which gene defects in the embryo or child can be corrected after diagnosis.
- The first clinical gene therapy was given in 1990 to a four-year old child who was suffering from **Adenosine deaminase (ADA) deficiency**.
- This disorder was caused due to the deletion of gene for adenosine deaminase.
- This disorder in some can be cured by bone marrow transplant.
- In others it can be treated with enzyme replacement therapy in which a functional enzyme (ADA) is injected into the patient.
- In gene therapy normal ADA gene copies were produced by cloning. These genes were packed into a defective retrovirus.
- **Lymphocytes** were isolated from the patients and with the help of retrovirus functional ADA cDNA were introduced.
- The modified lymphocytes were injected back into the patients.
- This method is not a permanent cure as lymphocytes die after a few days and thus new genetically modified lymphocytes need to be inserted again into the patient.
- **A permanent cure could be when genes are isolated from the bone marrow and introduced into the developing embryo.**

Vaccine Production

- Vaccines are produced by recombinant DNA technology by the following steps:

Definition

Gene Therapy: A collection of methods that allow correction of a gene defect that has been detected in an embryo or in a child.



- **Generating the antigens:** Antigens are generated from the microbes. Antigens can be a part of the disease causing microbe or a part of the protein which is weakened or in an inactive form.
- **Isolation of the antigens:** Antigens are isolated from the culture in which they are generated.
- **Adding preservatives and adjuvant:** Preservatives are added to the vaccine to facilitate multi-dose use while the adjuvant increase the immune response to the vaccine antigens. Stabilisers increase the storage life.

Molecular Diagnosis

- It is important to detect infection of pathogens in the body in the early days as its cure become easy and possible. But with the traditional ways in which the serum, blood or urine is analysed, it becomes difficult to detect in early stages of infection.
- The early detection of an infection is possible by:
 - Recombinant DNA technology
 - Polymerase Chain Reaction
 - Enzyme Linked Immuno-Sorbent Assay (ELISA)

Recombinant DNA technology

- Cells are cloned from the patient.
- **Probe** a single-stranded DNA or RNA labelled with radioactive molecule which is allowed to hybridize with the complementary DNA of the cloned cells.
- The cloned cells having the mutated genes will not appear on the photographic plate. The probe will not have complementarity with the mutated genes.

Polymerase Chain Reaction

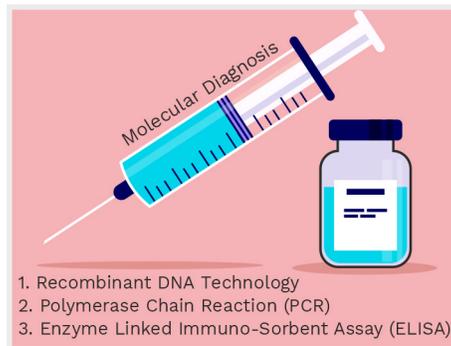
- Presence of a pathogen can only be detected when it becomes of high number in the body of an individual. But by that time the infection would have spread a lot in the body.

Previous Year's Question



Which one of the following vectors is used to replace the defective gene in gene therapy?

- (1) Adenovirus
- (2) Cosmid
- (3) Ri plasmid
- (4) Ti plasmid



1. Recombinant DNA Technology
2. Polymerase Chain Reaction (PCR)
3. Enzyme Linked Immuno-Sorbent Assay (ELISA)

Rack Your Brain



Name any one recombinant vaccine being used currently in the vaccination programme?

KEY POINTS



- ◆ Green Revolution
- ◆ Genetically Modified Organisms
- ◆ *Bacillus thuringiensis*
- ◆ RNA interference
- ◆ Anti-sense RNA



- Through PCR technique we can amplify the DNA or RNA of the **pathogen detected in the body in low quantity** and once the genome is amplified, the detection of the pathogen in the early stages becomes easy.

Enzyme Linked Immuno-Sorbent Assay (ELISA)

- It is based on the **principle of antigen-antibody interaction**.
- It can detect even small amount of the protein with the help of the enzyme.
- Infection by pathogen can be detected by the presence of antigens.
- Like proteins, glycoproteins or by detecting the antibodies synthesised against the pathogen.

Note:

Stem cell: These are undifferentiated cells that are able to develop into any type of tissue and perform a specialise function. Stem cells can be obtained from bone **marrow**, **umbilical cord**, **inner cell mass of embryos** and **amniotic fluid**.

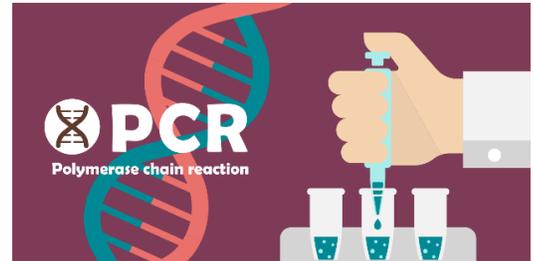
They can be used for programmed stem cell culture. With the help of genetic engineering, cells are isolated and reprogrammed to act as embryonic cells. Reprogrammed stem cells can be used for developing organs for transplantation. Stem cells can also be used for generating transgenic animals.

TRANSGENIC ANIMALS

- Animals whose DNA have been modified to express the foreign DNA are known as transgenic animals.
- Some animals that have been genetically modified are rats, rabbits, pigs, sheep, cow and fish.

Definition

Transgenic Animals: Animals whose DNA has been modified and they express the phenotype of the foreign gene.



Rack Your Brain



Which technique is based on antigen-antibody interaction to detect a virus?

Rack Your Brain



Which extra nutritional element is present in the milk of Rosie, which makes its milk more nutritional?



STEPS TO PRODUCE TRANSGENIC ANIMALS

- 1 Identification and isolation of the desirable gene
- 2 Selection of the vector
- 3 Insertion of desirable gene into the vector with the help of enzyme ligase
- 4 Introduction of vector into the cell, tissue, or embryo
- 5 Expression of the foreign gene in the transgenic animal

- The modification of the animals are been done to study-

- **Normal physiology and development:**

- ◆ Transgenic animals are used to study the normal working of the genes in the normal growth and development of the the animals.
- ◆ They are also used to study the effect of complex factors like insulin, growth factors etc. on the growth of the organism.
- ◆ Genes from other species can be introduced and the effects of the introduced gene can be studied in the animals.

- **Study of disease:**

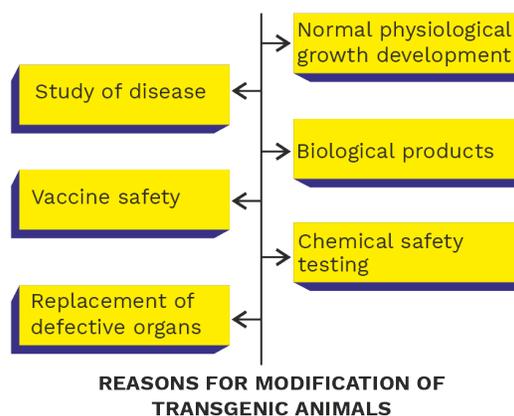
- ◆ Transgenic animals help us to study the diseases and through them, we can find a cure to the diseases.
- ◆ They serve as models for human diseases. Today transgenic animals are used to find a cure for **cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's.**

Previous Year's Question



The transgenic animals are those which have

- (1) foreign RNA in all of its cells
- (2) foreign DNA in some of its cells
- (3) foreign DNA in all its cells
- (4) both (1) and (2)



Gray Matter Alert!!!

First Transgenic Plant: Antibiotic-resistant tobacco plant.

First Transgenic Animal: Green fluorescent protein containing mice.



o **Biological products:**

- ◆ Transgenic animals are made to produce useful biological products such as **human protein** (α -1-antitrypsin) used to treat emphysema. Attempts are being made for treatment of phenylketonuria (PKU) and cystic fibrosis.
- ◆ In 1997, the first transgenic cow, Rosie, produced human protein-enriched milk (2.4 gm/litre). The milk contained the human **alpha-lactalbumin** and was nutritionally a balanced product for human babies than natural cow-milk.

o **Vaccine safety:**

- ◆ Animals are being developed for use in testing the safety of vaccines before they are used on humans.
- ◆ Transgenic mice are being used to test the safety of the polio vaccine, presently even monkeys are being used.
- ◆ If the mice prove to be safe, then in the years to come they will replace the use of monkeys.

o **Chemical safety testing:**

- ◆ This is known as toxicity/safety testing.
- ◆ Some transgenic animals carry genes which make them more sensitive to toxic substances than non-transgenic animals.
- ◆ They are then exposed to the toxic substances and the effects studied. It will help us to save time and obtain results fast.

ETHICAL ISSUES

- The modification of the living organisms needs to be monitored, otherwise it can cause havoc.
- The modification of the animals need to be regulated and checked that the modified plant or animal products and medicines are fit for human usage.

Previous Year's Question



Read the following four statements (A–D) about certain mistakes in two of them.

- (A) The first transgenic buffalo, Rosie produced milk which was human alpha-lactalbumin enriched.
- (B) Restriction enzymes are used in isolation of DNA from other macromolecules.
- (C) Downstream processing is one of the steps of rDNA technology.
- (D) Disarmed pathogen vectors are also used in transfer of rDNA into the host.

Which of the two statements have mistakes?

- (1) B and C
- (2) C and D
- (3) A and C
- (4) A and B

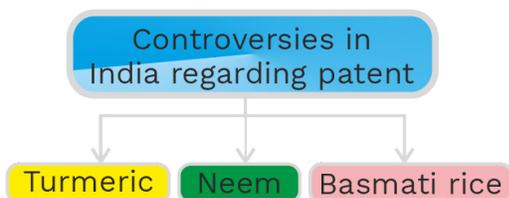
Previous Year's Question



Which body of the Government of India regulates GM research and safety of introducing GM organisms for public services?

- (1) Genetic Engineering Approval Committee
- (2) Research Committee on Genetic Manipulation
- (3) Biosafety committee
- (4) Indian Council of Agricultural Research

- In India, **GEAC (Genetic Engineering Approval Committee)** makes decisions regarding the validity of GM and the safety of genetically modified organisms for public services.



- The modification and usage of genetically modified resources have led to a problem with the patent. There have been many incidences where multinational companies have exploited the resources that have long been identified and used by farmers and indigenous people of a specific country.
- India has also faced patent issues related to Neem, turmeric and basmati rice.
 - US patent of office granted a patent to University of Mississippi Medical Center for use of turmeric in healing the wound. Dr. R.A. Mashelkar, an Indian scientist challenged the patent. The patent was revoked as it was established that the use of turmeric as a healing agent was used in India for many centuries.
 - The European Patent Office, Munich granted a patent to W.R. Grace and Co for fungicidal uses of neem oil. The patent was given for the process of extraction of the oil. Vandana Shiva and Ajay Phadke challenged the patent and finally, it was revoked.
 - Basmati rice** has many different varieties. Texas patented Basmati rice grains by US Patent and Trademark Office. The patent prevented people from selling Basmati rice. The patent act caused a diplomatic crisis between India and America. Later the patent was revoked. This was a case of biopiracy.

Gray Matter Alert!!!

The Patent Act, 1970: It came into force in 1972. It gives in a clear understanding of inventions related to a product on a process that is new and involves inventive steps is industrial applications that can be patented in India.



Previous Year's Question

Production of a human protein in bacteria by genetic engineering is possible because

- (1) the human chromosome can replicate in bacterial cell
- (2) the mechanism of gene regulation is identical in humans and bacteria
- (3) bacterial cell can carry out the RNA splicing reactions
- (4) the genetic code is universal

Gray Matter Alert!!!

Criteria for Grant of Patent:

Novelty: The invention should be new.

Non-obviousness: The Invention may not be documented but it is otherwise well-known.

Utility: The invention or product should be of use to humans.



- **Biopiracy** refers to the use of bio-resources by multinational companies and other organisations without proper authorisation from the countries and people concerned without compensatory payment.
- Industrialised countries are poor in bio-resources and traditional knowledge while developing countries are rich in bio-resources. There have been many cases of no proper compensation and benefit sharing between developed and developing countries. So now nations are developing laws to prevent such unauthorised exploitation of their bio-resources and traditional knowledge.
- The Indian Parliament has recently cleared the second amendment of the **Indian Patents Bill**, that takes such issues into consideration and try to prevent such exploitation.

Definition

Biopiracy: Use of bio resources by multinational companies and other organizations without proper authorization from the countries and people concerned without compensatory payment.

Gray Matter Alert!!!

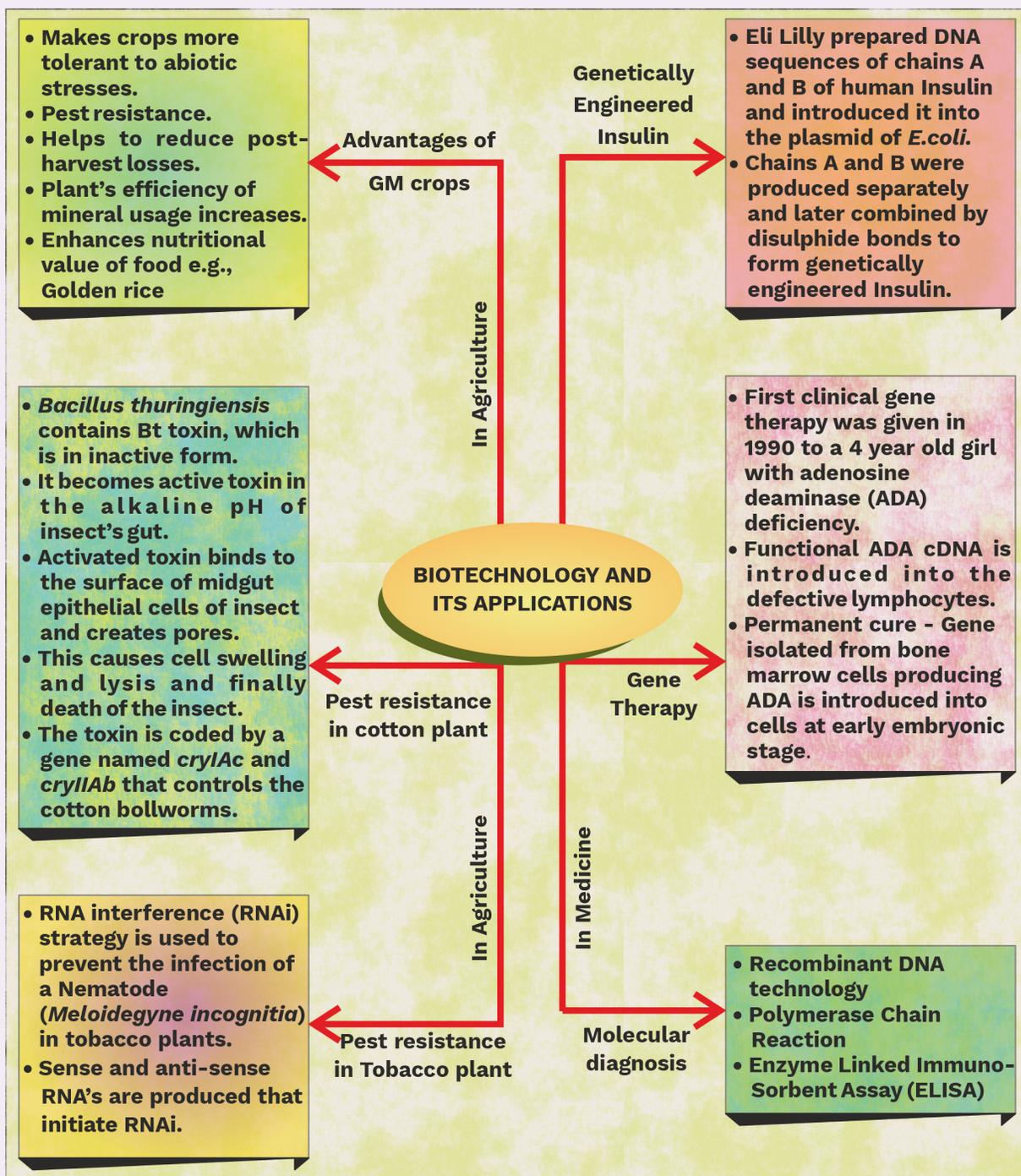
Biological Warfare: The use of infectious agents like bacteria, fungi, viruses, insects to harm or kill the humans, animals and plants.

KEY POINTS

- ◆ Transgenic animals
- ◆ Human alpha-lactalbumin
- ◆ GEAC
- ◆ Biopiracy
- ◆ Indian Patents Bill

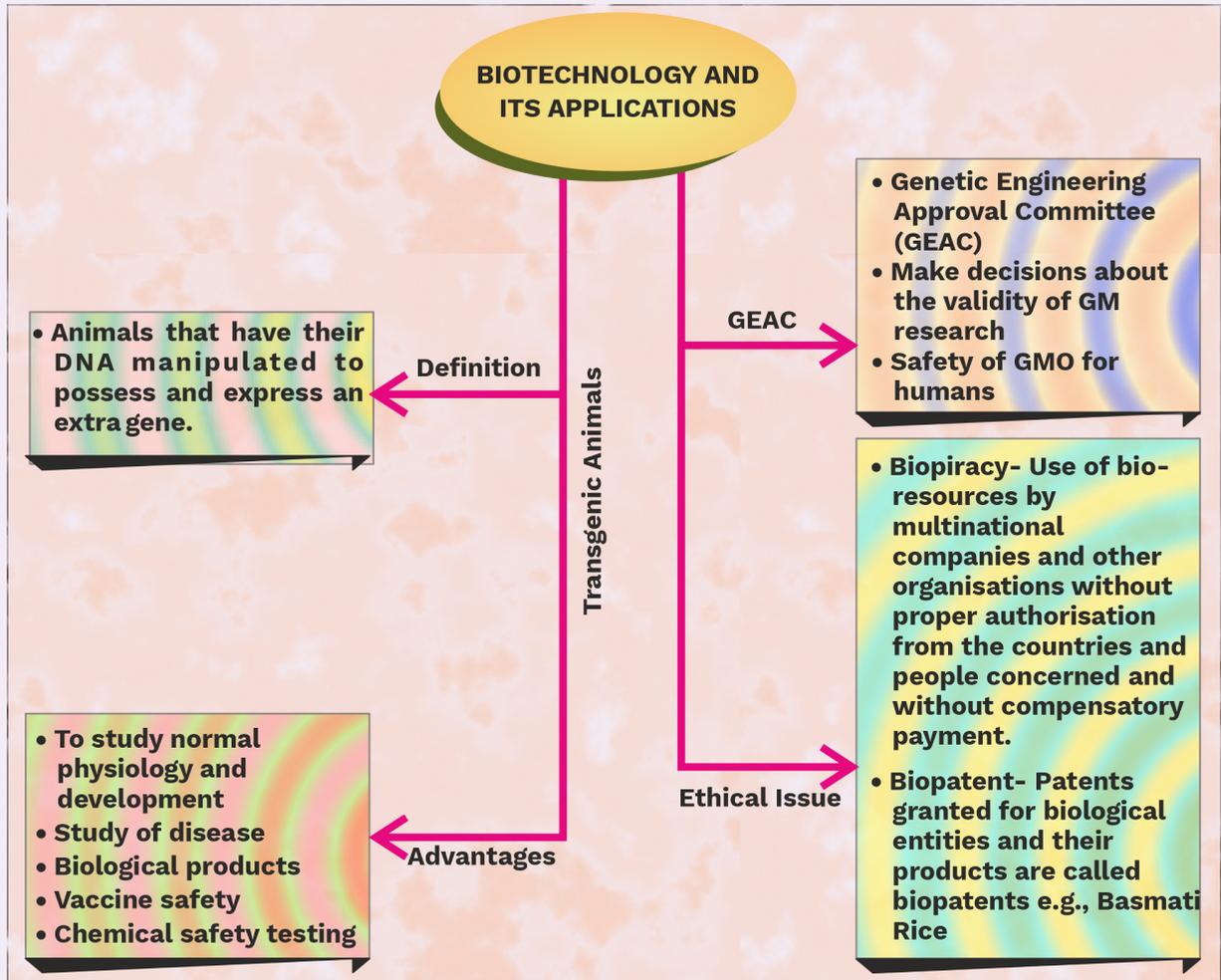


Chapter Summary





Chapter Summary





SOLVED EXERCISE

- Q1** The genetically-modified (GM) cotton has been developed for
- | | |
|-------------------------------|--------------------------|
| (1) insect-resistance | (2) enhancing shelf life |
| (3) enhancing mineral content | (4) drought-resistance |

A1 (1)
It is protected from cotton bollworm.

- Q2** Genetic engineering has been successfully used for producing
- | |
|----------------------------------------------------------------------------------|
| (1) transgenic mice for testing the safety of polio vaccine before use in humans |
| (2) transgenic models for studying new treatments for certain cardiac diseases |
| (3) transgenic cow-Rosie which produces high-fat milk for making ghee |
| (4) animals like bulls for farm work as they have super power |

A2 (1)
Transgenic animals has been used to study vaccine safety.

- Q3** Some of the characteristics of Bt cotton are
- | |
|-----------------------------------------------------------------------------------|
| (1) long fibre and resistance to aphids |
| (2) medium yield, long fibre and resistance to beetle pests |
| (3) high yield and production of toxic protein crystals which kill dipteran pests |
| (4) high yield and resistance to bollworms |

A3 (4)
Bt cotton is resistance to bollworms.

- Q4** A transgenic food crop which may help in solving the problem of night blindness in developing countries is
- | | |
|-------------------------|-----------------|
| (1) Bt soybean | (2) Golden rice |
| (3) Flavr Savr tomatoes | (4) Maize |

A4 (2)
Golden rice has a high content of vitamin A, thus protecting from night blindness.



- Q5** An improved variety of transgenic basmati rice
- (1) does not require chemical fertilizers and growth hormones
 - (2) gives high yield and is rich in vitamin A
 - (3) is completely resistant to all insect pests and diseases of paddy
 - (4) gives high yield but has no characteristic aroma

A5 (2)
Improved variety of transgenic basmati rice that is rich in vitamin A.

- Q6** Which one of the following is now being commercially produced by biotechnological procedures?
- (1) Nicotine (2) Morphine (3) Quinine (4) Insulin

A6 (4)
Insulin is being produced by recombinant DNA technology.

- Q7** What is true about Bt toxin?
- (1) Bt protein exists as active toxin in the *Bacillus*.
 - (2) The activated toxin enters the ovaries of the pest to sterilise it and thus prevent its multiplication.
 - (3) The concerned *Bacillus* has antitoxins.
 - (4) The inactive protoxin gets converted into active form in the insect gut.

A7 (4)
The prototoxin is in an inactive form in the bacteria and gets activated when it comes in contact with the alkaline pH of the gut of the insect.



-
- Q8** Transgenic plants are the ones
- (1) generated by introducing foreign DNA into a cell and regenerating a plant from that cell
 - (2) produced after protoplast fusion in artificial medium
 - (3) grown in artificial medium after hybridization in the field
 - (4) produced by a somatic embryo in artificial medium

A8 (1)
Transgenic plants are generated by recombinant DNA technology.

- Q9** What is antisense technology?
- (1) When a piece of RNA that is complementary in sequence is used to stop expression of a specific gene
 - (2) RNA polymerase producing DNA
 - (3) A cell displaying a foreign antigen used for synthesis of antigens
 - (4) Production of somaclonal variants in tissue cultures

A9 (1)
It is used for silencing mRNA.

- Q10** CryI endotoxins obtained from *Bacillus thuringiensis* are effective against
- (1) nematodes
 - (2) bollworms
 - (3) mosquitoes
 - (4) flies

A10 (2)
The toxin produced by cryI gene is effective against bollworms.