

Course- B.Sc. (Botany Honours), Part -3

Paper-VI (Group-B), Molecular Biology

Topic- Genetic Engineering.

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Genetic Engineering

Plant genetic engineering is defined as the isolation, introduction and expression of foreign DNA in the plant. In other words, it refers to direct introduction of foreign gene (DNA) into a plant's system by micromanipulation at the cellular level.

Gene transfer can be achieved in four ways, viz:

- (i) Plasmid,
- (ii) Particle bombardment,
- (iii) By micro-injection, and
- (iv) Through direct uptake.

Genetic engineering has several useful practical applications in crop improvement, medicines and industries.

Terms used in Genetic Engineering:

Before discussing various applications of genetic engineering, it is essential to define various terms which are often used in this connection.

i. Recombinant DNA:

The DNA which contains genes from different sources and can combine with DNA of any organism is called recombinant DNA. Since genetic engineering utilizes recombinant DNA, it is also known as recombinant DNA technology. Recombinant DNA is obtained by special techniques.

ii. Gene Cloning:

It is a technique of genetic engineering by which a gene sequence with many identical copies is replicated. Identical gene sequence is isolated by using restriction endonuclease enzyme. This can also be obtained by making a complementary DNA from mRNA template using reverse transcriptase.

It is then inserted into a cloning vector, i.e., a plasmid or bacteriophage. The hybrid is used to infect a cell (plant or bacteria) and replicated within the cell. Gene cloning is used for identification of molecular structure of genes.

iii. Gene Sequencing:

Gene sequencing refers to the determination of the order of bases of a DNA molecule making up a gene. The DNA is purified and then broken at a specific point using restriction endonuclease enzyme. Thus all strands have one identical end.

These strands are then broken at a random distance from this end so that there are strands ending on every base present. These strands are then separated, their end base identified and put in order of fragment size to determine the entire sequence.

iv. Gene Splicing:

In genetic engineering, the enzyme catalysed joining of DNA fragments is referred to as gene splicing. In genetics, the joining of exons after the introns sequences have been removed to produce functional messenger RNA is called gene splicing. This occurs in the cell nucleus and is catalysed by splicing enzymes such as ligase.

v. DNA Probes:

The small segments of DNA with known base sequences, origin and function are called DNA probes. DNA probes can be obtained either through DNA template or can be produced by gene cloning technique. Now microprocessor based sophisticated equipment's are available which can produce DNA probes.

These microprocessor based equipment's are known as gene synthesizing machines. DNA probes are very much useful in determining the nucleotide sequence in living as well as non-living systems.

Applications of Genetic Engineering in Crop Improvement:

Genetic engineering has several potential applications in crop improvement.

The main applications include:

- (i) Interspecific and inter-generic gene transfer (distant hybridization),
- (ii) Development of transgenic plants,
- (iii) Development of nodules in cereals,
- (iv) Development of C4 plants, etc.

These are briefly discussed below:

i. Distant Hybridization:

With the advancement of genetic engineering, it is now possible to transfer genes between distantly related species. The barriers of gene transfer between species or even genera have been overcome. The desirable genes can be transferred even from lower organisms through recombinant DNA technology.

ii. Development of Transgenic Plants:

Plants which contain foreign genetic material are known as transgenic plants. Resistance to diseases and insects can be achieved through genetic transformation and development of transgenic plants. In case of cotton, a gene has been transferred from prokaryote, viz., *Bacillus thuringiensis* which confers resistance against bollworms.

This work has been done by Monsanto Company in USA. The transgenic plants secrete a toxin when the larvae injure the buds or bolls. This toxin inactivates the larvae and ultimately leads to death of the insect. This inbuilt system of insect resistance will help in saving huge amount of money towards the use of insecticides in cotton crop.

iii. Development of Nodules in Cereals:

Leguminous plants have root nodules which contain nitrogen fixing bacteria called *Rhizobium*. These bacteria convert the free nitrogen available in the atmosphere into nitrates in the root nodules. These nitrogen fixing genes may be transferred to cereal crops like wheat, rice, maize, barley etc. through the techniques of genetic engineering. Development of nodules in cereals seems to be impossible.

iv. Development of C4 Plants:

Improvement in yield can be achieved by improving the photosynthetic efficiency of crop plants. The photosynthetic rate can be increased by conversion of C3 plants into C4 plants, which may be achieved either through protoplast fusion or recombinant DNA technology. C4 plants have higher potential rate of biomass production than C3 plants.

Most C4 crop plants are grown in tropical and subtropical zones (sorghum, sugarcane, maize, some grasses). Maize is also grown in temperate zones but as forage crop, because below 15°C temperature grain yield is significantly reduced. The chances of conversion of C3 plants into C4 plants seem to be still very remote.

Applications of Genetic Engineering in Medicine:

Biotechnology especially genetic engineering plays an important role in the production of antibiotics, hormones, vaccines and interferon in the field of medicine.

These are briefly described below:

i. Production of Antibiotics:

Penicillium and streptomyces fungi are used for mass production of antibiotics penicillin and streptomycin. Genetically efficient strains of these fungi have been developed to greatly increase the yield of above antibiotics.

ii. Production of Hormone:

Insulin, a hormone, is usually extracted from the pancreas of cows and pigs. This insulin is slightly different in structure from human insulin. As a result, it leads to allergic reactions in about 5% of the patients.

Human gene for insulin production has been incorporated into bacterial DNA and such genetically engineered bacteria are used for large scale production of this hormone. Since this is produced using human gene, it does not have allergic reactions.

iii. Production of Vaccines:

Vaccines are produced by multiplication of disease producing organisms on large scale, which is a dangerous process. Recombinant DNA technique permits production of vaccines by incorporation of specific gene into bacteria. In other words, vaccines are produced by transfer of antigen coding genes to bacteria. Such antibodies provide protection against infection by the same virus.

iv. Production of Interferon:

Interferons are virus induced proteins produced by virus infected cells. Interferons are anti-viral in action and act as first line of defence against viruses. Their response is much quicker than influenza. It also appears to be effective against cancer of breast and lymphatic system.

Natural interferon is produced in very small quantity from human blood cells. Now it is possible to produce interferon by recombinant DNA techniques at much cheaper rate.

v. Production of Enzymes:

Some useful enzymes can also be produced by recombinant DNA technique. The enzyme urokinase, which is used to dissolve blood clots, has been produced by genetically engineered microorganisms.

Industrial Applications:

In industries, recombinant DNA technique will help in the production of chemical compounds of commercial importance, improvement of existing fermentation processes and production of proteins from waste.

This can be achieved by developing more efficient strains of micro-organisms. Specially developed micro-organisms may be used even to clean up the pollutants. Thus, biotechnology has several useful applications in crop improvement, medicine and industry.

Dangers of Genetic Engineering:

Several dangers are associated with recombinant DNA technology.

The main dangers include:

- (i) Spread of new diseases,
- (ii) Effect on evolution, and
- (iii) Biological warfare.

These are briefly presented below:

i. Spread of New Diseases:

New dangerous forms of micro-organisms can be developed through recombinant DNA technique either accidentally or deliberately. Escape of such micro-organisms from the research laboratory through drainage, laboratory glassware, laboratory personnel etc., may lead to the spread and origin of new type of diseases, which may pose a serious problem.

ii. Effect on Evolution:

Nature has provided several barriers for exchange of DNA between prokaryotes and eukaryotes. Recombinant DNA technology permits exchange of DNA between these two classes of organisms and thus interferes with the natural process of evolution.

iii. Biological Warfare:

There is a fear that genetic engineering techniques will be used for biological warfare. In such warfare, disease carrying microorganisms can be used against the enemy. This will lead to disaster. Thus, genetic engineering has several demerits. Many of the results achieved through genetic engineering, can be achieved through other less dangerous techniques.

Safety Measures:

Dangers of recombinant DNA technique can be minimized:

- (i) With increasing experience and knowledge,
- (ii) By adopting safety measures to check the escape of new micro-organisms from laboratory,
- (iii) By putting international ban on the work involving tumour viruses, and
- (iv) By putting international ban on the use of such techniques in the warfare's.