Chapter 12

APPLICATION OF BIOTECHNOLOGY IN VEGETABLE PRODUCTION

OBJECTIVES

After studying this unit, you will be able to understand:

- Different techniques of biotechnology and their applications in vegetable production
- Applications of different tissue culture technologies in vegetable production
- Advantages of different techniques of tissue culture
- Genetic engineering and its role in vegetable production

INTRODUCTION

As we know that biotechnology is playing a significant role for the welfare of mankind. However, you might have certain questions in your mind. What is biotechnology? How can we apply biotechnology in vegetable production? What are the techniques of biotechnology? How these techniques can be useful in vegetable production? Where a particular technique can be applied and for what purpose? What is genetic engineering? What are the aims of genetic engineering? Where can we apply it and for what purposes? Many questions of this kind may arise in your mind. There are several techniques of biotechnology used for different purposes in vegetable production. This chapter will definitely clarify many of your doubts in the field of biotechnology and its applications for the welfare of mankind.

What is biotechnology?

The term Biotechnology was coined by Karl Ereky in 1919. Biotechnology is any technique that uses living things or their components for generating products or services for the welfare of mankind. Biotechnology deals with the manipulation of an organism at cellular or molecular level for changing and improving its characteristics efficiently for specific uses. The origin of biotechnology can be traced back to prehistoric times when microorganisms were used for processes like fermentation, making yoghurt and cheese from milk, vinegar from molasses and production of antibiotics from penicillin from *Penicillium notatum etc*. Biotechnology includes gradients of technologies ranging from traditional biotechnology

(food fermentation, biological control, *etc*.) to modern biotechnology like tissue culture and recombinant DNA technology or genetic engineering. There are two basic techniques used in vegetable biotechnology are here under:

A. **Plant tissue culture**

B. **Genetic engineering**

A. Plant tissue culture: Plant tissue culture is the cultivation of plants, seeds, plant parts, tissues, organs, embryos, single cells *etc*. on an artificial nutrient media under aseptic conditions. Plant tissue culture is also called as "i*n-vitro* technique" meaning thereby, any process carried out in sterile cultures. Plant tissue culture includes-

a) Meristem culture
b) Anther/Pollen culture
c) Embryo culture
d) Somaclonal variation
e) Germplasm conservation and cryopreservation
f) Somatic embryogenesis

B. Genetic engineering: Genetic engineering is a direct manipulation of genome of an organism for making that organism better in one or other trait. The organism thus developed is called as Genetically Modified Organism (GMO).

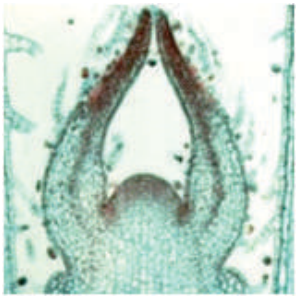
Differences between genetic engineering and plant cross breeding (*Lack et al., 2002)*

Genetic engineering	Plant cross breeding
Transfer of single gene	Transfer of hundreds of genes
Gene sequence is known	Gene sequences are not known
Small change in protein expression is there	Large changes in protein expression are there
Between species	With in species

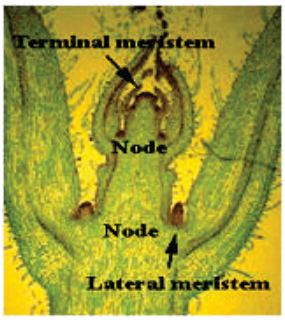
DIFFERENT TISSUE CULTURE TECHNIQUES

a) Meristem culture: Meristem culture is *in vitro* culture of excised meristems (0.2-0.5mm) on a suitable nutrient media under aseptic conditions.

Meristem is a localized group of actively dividing cells. Shoot apical meristem is also a group of undifferentiated plant cells located within the shoot tip, generally appearing as a dome like structure distal to the youngest leaf primordium. If bits of meristematic tissue are isolated and cultured on a sterile nutrient medium, the resulting plantlets are free of virus infections.



(a)
Shoot apical meristem



(b)
Meristem

Application of shoot-tip or meristem culture

1. For virus elimination

Plants developed from meristem culture are virus free. Meristematic culture is being used to produce healthy propagation stocks of vegetatively propagated vegetables like potato and ginger *etc.*

2. Micro-propagation

Micro-propagation allows the rapid propagation of large number of plantlets in a short period of time. It involves the production of plants from very small (1mm) plant part through tissue culture. Shoot tip or meristem culture of many plant species can successfully be used for micro propagation. Micropropagation of selected ornamentals, field, fruit and forest plant species is one of the best and most successful examples of commercial application of tissue culture technology. This technique is applied for

i) Production and multiplication of elite planting material (seed) of vegetatively propagated species *e.g.* micropropagation of potato through minituber production

ii) Quick popularization of new varieties of vegetatively propagated species.

iii) Rejuvenation of old varieties of vegetatively propagated species.

3. Maintenance and long term storage of genetic resources

The meristems of highly heterozygous or recalcitrant plant species can be conserved *in vitro* at minus 196^{0}C for the long term provided aseptic conditions are maintained and the material is transferred to the fresh media at appropriate intervals. Thus, the seed stock of any plant material shall be available for

propagation and export all the year round and more over the cost incurred for raising and maintenance of the crop can also be avoided.

4. Wide hybridization

In many plant species the hybrids derived after natural or artificial crossing are not viable due to one or other pre or post fertilization barriers such hybrids can be maintained through meristem or shoot-tip culture.

5. Propagation of haploid plants

Haploid plants derived from anther or pollen culture always remain sterile unless and until they are made homozygous diploid. Propagation of such haploid plants can be successfully done through meristem or shoot-tip culture.

6. Germplasm exchange:

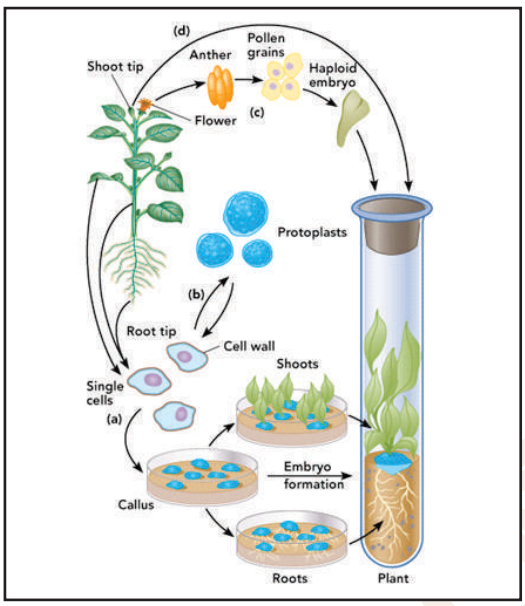
Pathogen free status of *in vitro* cultures greatly facilitates international germplasm exchange as plantlets derived from shoot-tip or meristem cultures are easily accepted by the quarantine authority for international exchange without any checking. Therefore, using this technique, crop plants can be easily exchanged in crop improvement programmes.

b) Anther culture: A plant culturing technique in which immature pollen is made to divide and grow into tissue (either callus or embryonic tissue) in either a liquid medium or on solid media. Pollen-containing anthers are removed from a flower and put in a culture medium, some micro spores survive and develop into tissue. Therefore, anther culture is the process of using anthers to culture haploid plantlets.

Applications of anther culture

1. The most important use of haploids is their use in the production of instant homozygous lines, which may be directly used as cultivars or may be used in breeding programmes. A doubled haploid, 'Marglobe tomato' has been used commercially.

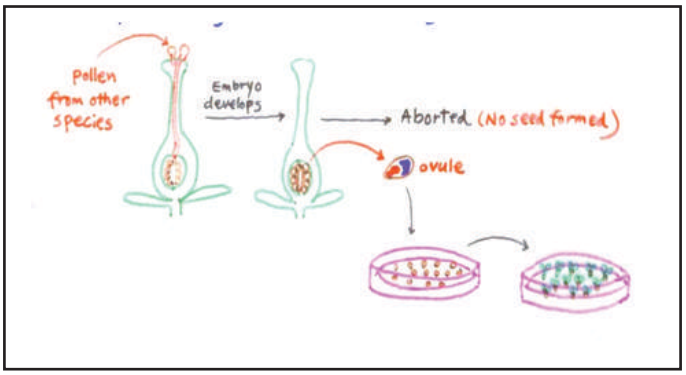
2. Production of haploids/double haploids through anther culture from F_1 plants results in true breeding plants in less than one year, which are otherwise obtained after 7-8 generations through conventional breeding methods (inbreeding or back cross method).



Advantages:

- The technique is fairly simple
- It is easy to induce cell division in the immature pollen cell
- A large proportion of the anthers used in culture respond (induction frequency is high)
- Haploids can be produced very quickly in large numbers.

c) Embryo Culture: It is the technique of *in vitro* raising of polarized egg (ovule), zygote, pro-embryo or a mature embryo.



Applications of embryo culture

1. Production of haploid plants by culturing un-fertilised ovules *e.g.* cabbage and cauliflower
2. Embryo culture is the practical approach to obtain interspecific and intergeneric hybrids among otherwise hard to cross parents.
3. It is useful where embryo fails to develop due to degeneration of embryonic tissues.
4. To overcome seed dormancy and judging the seed viability.

d) **Somaclonal Variation:** The variations seen in plants that have been produced by plant tissue culture are called as somaclonal variations. Chromosomal rearrangements are an important source of this variation. The variation among the callus derived plants is an effective aspect for broadening the genetic base. This results in obtaining incremental improvement in the commercial cultivars, more particularly, in the vegetatively propagated species. Using the technique of in vitro selection many million cells /protoplasts (cell without cell wall) can be screened against various biotic and abiotic stress factors in a single petri-dish which is more efficient as compared to the screening of similar number of plants in the field which require more time and space as well.

e) Germplasm storage and cryopreservation: Cryopreservation is a process where cells or whole tissues are preserved by cooling to sub-zero temperature with the help of chemical like liquid nitrogen. The aim of germplasm conservation is to ensure the availability of useful germplasm at any time. In seed propagated crops, seed is extensively used for conservation of germplasm using conventional methods. However, it is not possible to store germplasm in vegetatively propagated species for longer durations using conventional storage techniques.

Advantages of cryopreservation:

1. *In vitro* culture enables plant species that are in danger of being extinct to be conserved
2. Storage of vegetatively propagated plants can result in great savings in storage, space and time
3. Sterile plants that cannot be reproduced generatively can be maintained *in vitro.*
4. This method is now being practically used at several national and international germplasm banks.
5. Reduced risk of microbial contamination and cross contamination with other cell lines
6. Reduced risk of genetic drift and morphological changes

f) Somatic embryogenesis: Somatic embryogenesis is a process where a plant or embryo is derived from a single somatic cell or group of somatic cells. Somatic embryos are formed from plant cells that are not normally involved in the development of embryos, *i.e.* ordinary plant tissue. No endosperm or seed coat is formed around a somatic embryo.

Applications of somatic embryogenesis

1. Clonal propagation of genetically uniform plant material
2. Elimination of viruses
3. Synthesis of metabolite.
4. It provides source tissue in genetic transformation
5. Synthesis of artificial seeds.
6. Generation of whole plants from single cells called protoplasts

Disadvantages

1 High chance of mutations
2 Difficult method
3 Loss of regenerative ability
4 High percentage of albino shoots during regeneration

B) Genetic engineering/transformation: The science and technology of isolating gene(s) from the genome of one organism and inserting the same into the genome of another organism is termed as genetic engineering. Genes derived from unrelated

species and even other kingdoms, such as bacteria, fungi, plants, animals, that would otherwise be inaccessible to an organism, can be combined in the laboratory using genetic transformation techniques. Such insertion is done randomly and plants and seeds created thus are called Genetically Engineered Plants or Seeds. Such gene insertion would not normally happen within nature. Exchange of genes happens only between sexually compatible and closely-related species. The modern techniques of the genetic engineering facilitate the removal of an individual group of genes from one species and insertion into another, without there being a need for sexual compatibility. Genetic Engineering normally does not involve only one gene but a gene of interest accompanied by any necessary additional pieces of DNA which provide the insertion and control/regulatory sequences.

Genetic engineering can therefore be defined as the process of manipulating the pattern of protein in an organism by altering genes. New genes are added or existing genes are changed so that they are made at different times and/or different quantities. The transfer process involves cutting the desired gene out of a chromosome of a particular plant, animal or bacteria and putting that gene into a cell; the genetically modified cell is then regenerated to produce a 'transgenic' or genetically modified organism or GMO. The modified organism passes the new gene onto its progeny.

Common terms associated with genetic engineering

Recombinant DNA: Connecting fragments of DNA from different sources.
Restriction Enzymes: Bacterial proteins that have the ability to cut both strands of the DNA molecule at a specific nucleotide sequence.
Cloning: Obtaining a homogenous population of cells by repeated single colony isolation.
Anti sense RNA: RNA that is complimentary to a specific mRNA and which can interfere with translation.
DNA ligase: Enzyme for joining DNA strands by formation of phosphodiester bond.
Quantitative Trait Loci (QTL): It is a region of DNA that is associated with a particular phenotypic trait - these QTLs are often found on different chromosomes. Use of QTLs is to identify candidate genes underlying a trait.
Gene Silencing: The term gene silencing is generally used to describe the "switching off" of a gene by a mechanism other than genetic modification. That is, a gene which would be expressed (turned on) under normal circumstances is switched off by machinery in the cell.

Aims of genetic engineering

- Resistance to insect-pests and diseases.
- Resistance to herbicides.
- Tolerance to abiotic stresses.
- Enhancing nutritional attributes.
- Introduction of male sterility system for hybrid seed production.
- Prolonging the storage or shelf life of vegetables.
- Reduction of photorespiration in C_3 plants.
- Transfer of *nif* gene for atmospheric nitrogen fixation in crop plants.

Transgenic products

1. Golden tomato: Tomato with enhanced Vitamin A and lycopene
2. Golden potato: Potato with enhanced vitamin A

Common Plant Transformation Methods

1. *Agrobacterium* mediated genetic transformation- transfer of DNA from bacteria to plants.
2. Biolistics - rapidly propelled tungsten or gold microprojectiles coated with DNA are blasted into cells.
3. Electroporation - electrical impulses are used to increase membrane and cell wall permeability to DNA contained in the surrounding solution.
4. Microinjection - injection of DNA directly into the cell nucleus using an ultrafine needle.
5. Poly-ethelyne-glycol - plant cell protoplasts treated with PEG are momentarily permeable, allowing uptake of DNA from the surrounding solution

What are the basic steps for producing a genetically modified plant product?

The actual procedures for producing a genetically engineered product are very complex. However, most genetically engineered plant products are produced using the basic steps described below:

- **Trait identification**: Traits of organisms are identified.
- **Gene discovery**: Genes for the desired traits are identified.
- **Gene cloning**: The desired gene is inserted into a bacterial cell and, as bacteria reproduce, the desired gene is also reproduced.

- **Gene verification**: Researchers study the copies of the gene using molecular techniques to verify that the replicated gene is the desired one.
- **Gene implantation**: Using a bacterium or other procedure, the desired DNA (gene) is transferred into the chromosomes of the host plant cells.
- **Cell regeneration**: Select the plant cells that contain the new gene and regenerate whole plants from the selected plant cells.
- **New plant testing**: Laboratory and field testing is conducted to verify the function and safety of the new plants.
- **Seed production**: Seeds with the desired traits are produced using standards set for specific crop production.

Achievements through the application of biotechnology

1. **Improvement in nutritional quality:** Vegetables are rich in nutrient and minerals and can be further enriched with nutrients, minerals, better flavour and minimal anti-nutritional factors. Attempts have been done in this line for *e.g*. genetically modified lettuce has been developed with tocopherol and resveratol composition which prevent coronary disease. GM potato containing ***AmA1 gene*** (extracted from grain amaranth) has been developed with 35-60% more protein.

2. **Enhanced shelf life:** The vegetable crops are highly succulent as water content in them is to the tune of 90 % and as a result they are vulnerable to post harvest losses. So, we need to have vegetable crops which have long shelf life. Tomato plant was engineered for reduced synthesis of polygalacturonase (PG) which cause softening of cell wall during ripening. It was observed that an ***antisense gene*** inhibits expression of tomato *Pgu* gene during ripening, which increases shelf life. It was marketed as ***Flavr-savr*** in 1994. Another engineered variety in tomato is ***Endless Summer*** but unfortunately it was not accepted by farmers and consumers.

3. **Food safety:** Biotechnology is playing a key role in development of rapid and sensitive diagnostic tools for food borne pathogens, microbial toxins and other contaminants. Recently ***Amylase inhibitor*** was developed in kidney bean which prevent action of enzyme that break glycosidic bond of starch and work is in progress to minimise toxic substance like trypsin inhibitor, sinigrin, *etc.* from vegetable to make it safe for human consumption.

4. **Biofertilizers**: Biofertilizers are the microorganism or minute plants or their products which can absorb gaseous nitrogen and phosphorous directly from atmosphere and make it available to plants. These organisms are identified, multiplied in laboratory condition and introduced into root zone of the crop plants to supply nitrogen and phosphorous. Some bio-fertilisers are *Rhizobium, Azotobactor, Azospirillium,* BGA, *etc*. which are widely used in vegetable cultivation. In addition to nutrient availability, *Azotobactor* also produce hormones like IAA, gibberellins which are beneficial for the growth and development of the plants.

5. **Resistance to abiotic stress:** Nearly all plants are exposed to stress condition during their life cycle and stress is unpredictable and generally not controlled. In India, most of the aerable lands are affected by salt and drought. In *Cucumis melo*,a number of plants have been engineered for salt and drought tolerance. A yeast salt tolerant gene encoding a water soluble protein ***HAL1*** has been transformed *via A. tumefaciens*. An early tomato variety has been developed as it contain antifreeze gene, ***afa 3*** with increased tolerance to frost.

6. **Resistance to biotic stress:** A number of transgenics have been developed in vegetable crops. In tomato, brinjal and cauliflower, ***Cry 1Ab gene*** has been identified to generate plants resistant to lepidopteron pests and diamond back moth, respectively. In potato, a viral coat protein has been used to develop resistance against potato virus X and Y. In India, no transgenic has been accepted or released for commercial cultivation because of safety measures.

7. **Herbicide tolerance:** Due to increasing concern about contamination of environment by herbicides, new herbicides are being developed that are safer and biodegradable. This necessitates the development of resistance in crop plant against herbicides. Certain herbicides are non selective to the crop. Some approaches in detoxifying enzymes have been identified in plant which degrades herbicides like glutathione-S-transferase (GST) which detoxifies herbicide Atrazine and Nitrilase (coded by gene bxn) which detoxifies bromoxynil. In tomato, bxn gene and bar gene were found to be herbicide resistant and in *Solanum* species Paraquat tolerant mutant has also been identified.

8. **Development of parthenocarpic plants:** Parthenocarpic plants have ability to develop fruits in absence of fertilization. Parthenocarpy has been induced in brinjal and tomato using genes, ***Rol B gene*** and ***Iaah gene,*** respectively. In general the transgenic plants have higher yield than non transgenic plants.

9. Hybrid seed production: Barnase-Barstar system (***Bar gene***) of hybrid seed production is now universally applicable to all the vegetable crops where the genetic mechanism, male sterility is not present like in okra. In this system, Barnase gene induces male sterility and barstar gene induces male fertility. So, the hybrids can be produced in large quantities without emasculation. Another method includes "***arg E gene***" which destruct specific plant tissue (anther/tapetum) which cause sterility. These systems have proven successful in *Brassica*, tomato and corn.

10. Synthetic seed: Synthetic seed refers to encapsulated somatic embryo which functionally mimic seed and can develop into seedling under suitable conditions. Synthetic seeds have been developed in celery and alfalfa. This system has been well characterised in carrot, lettuce, brinjal and horseradish. Synthetic seeds reduce pest damage, time and cost also.

11. Achieving distant crosses and development of new species: Due to self incompatability and sterility barriers, it is difficult to obtain successful hybrids under ordinary conditions. First attempt of developing intergeneric hybrid was done by Karpenchenko (1927) between radish and cabbage. But the results obtained were not satisfactory *i.e.* the neo plant species generated had roots of cabbage and leaves of radish. In Japan embryo culture technique has been successfully utilized to develop a hybrid, Hakuran *(Brassica carinata)* between cabbage (*Brassica oleracae)* and Chinese cabbage *(Brassica nigra)*.

References:

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Check your progress

Fill in the blanks

1. _____ is also called as "i*n-vitro* technique".
2. The term Biotechnology was coined by _____ in 1919.
3. The organism that developed through _____ is called as Genetically Modified Organism (GMO).
4. Plants developed from _____ culture are virus free.
5. _____ technique is applied for the production and multiplication of elite planting material (seed) of vegetatively propagated species.
6. _____ is the process of using anthers to culture haploid plantlets.
7. Application of _____ is to overcome seed dormancy and judging the seed viability.
8. _____ is the process of manipulating the pattern of protein in an organism by altering genes.
9. _____ and _____ are the transgenic products developed in vegetables.
10. In Barnase and Barstar system, Barnase gene induces _____ and barstar gene induces _____.

Match the followings:

1	Meristem and bud culture	**A**	Gene transfer
2	Anther and microspore culture	**B**	*In vitro* selection, somaclonal variation, somatic embryogenesis and artificial seeds.
3	Cell and tissue culture	**C**	Micropropagation for commercial purposes, genetic conservation and exchange of material
4	Genetic engineering	**D**	Interspecific crosses
5	Zygotic embryo culture	**E**	Haploid production
6	Cryopreservation	**F**	Long term storage under sub zero temperature

Define the followings:

a) Tissue culture b) Genetic engineering c) Anther culture

d) Explant e) Micro-propagation f) Embryo culture

g) Gene cloning h) Gene silencing i) Recombinant DNA

j) Quantitative trait loci

Short answers:

1. What is meristem culture? Enlist its applications in agriculture.
2. Write two applications of anther culture and its advantages.
3. Where do we apply embryo culture?
4. List two advantages of cryopreservation.
5. List four aims of genetic engineering

Long answers

1. What is biotechnology? What are applications of biotechnology in vegetable science/breeding?
2. What do you understand by tissue culture? Discuss its application in vegetable production.
3. Define genetic engineering. Discuss its aims and applications in vegetable production.