Program	:	*B. Pharmacy*
Semester	:	4th
Subject /Course	:	Pharmacognosy & Phytochemistry-1
Subject/Course ID	:	BP405T
Module No.	:	03
Module Title	:	Plant Tissue Culture and Edible Vaccines
Maximum Hours to Complete	:	07
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Learning Outcome of Module 03

LO	Learning Outcome	Course outcome
LO1.	Student will learn about basics of Plant Tissue culture and historical development.	BP405.6
LO2.	Student will know about different methods of culturing and their nutritional requirements.	BP405.6
LO3.	Student will learn about requirements for tissue culture laboratory.	BP405.6
LO4.	Student will learn about various applications of Plant tissue culture.	BP405.6
LO5.	Student will learn about concept of Edible vaccine along with advantages, disadvantages and techniques.	BP405.6

Content Table

Topic
• Introduction to Plant Tissue Culture • Historical development • Laboratory requirements • Types of culture • Nutritional requirements • Maintenance of callus and suspension culture • Applications • Edible vaccines

Plant Tissue Culture- Introduction

This technique affords alternative solution to problems arising due to current rate of extinction and decimation of flora and ecosystem.

'Tissue culture is in vitro cultivation of plant cell or tissue under aseptic and controlled environmental conditions, in liquid or on semisolid well-defined nutrient medium for the production of primary and secondary metabolites or to regenerate plant.

This technique affords alternative solution to problems arising due to current rate of extinction and decimation of flora and ecosystem.

Advantages of Tissue Culture Technique over the Conventional Cultivation Techniques.

1. Availability of raw material Some plants are difficult to cultivate and are also not available in abundance and tissue culture technique is considered a better source for regular and uniform supply of raw material for medicinal plant industry for production of phytopharmaceuticals.
2. Fluctuation in supplies and quality The method of production of crude drugs is variable in quality due to changes in climate, crop diseases and seasons. All these problems can be overcome by tissue culture.
3. New methods for isolation It is possible to obtain new methods for isolation and newer compounds from plant by this technique and for which Patent rights can be obtained.
4. Biotransformation (Process through which the functional group of organic compound are modified by living cells) reactions are feasible using plant-cell cultures.
5. Disease free and desired propagule Large scale production of plant with disease free and desired propagule could be stored and maintained without any damage during transportation for subsequent plantation.
6. Biosynthetic pathway Tissue culture can be used for tracing the biosynthetic pathways of secondary metabolites using labelled precursor in the culture medium.
7. Immobilization of cells Tissue culture can be used for plants preservation by immobilization (entrapment)of cell further facilitating transportation and biotransformation.
8. Continuous, uniform biomass is obtained.
9. Medicinally important compound can be synthesized, which can’t be synthesized chemically.
10. Useful natural compounds can be produced, independent of soil condition & change in climatic conditions.
11. Improvement of medicinal plant species.

12. Propogation of plant without seeds in defined and controlled condition.

Disadvantages of tissue culture

1. High level of expertise is required.
2. A small error may lead to complete collapse of product/plant.
3. Lots of chemicals are required for plant tissue culture which must contain high purity.
4. There is no chance for evaluation of mutation.
5. Culture on artificial medium may lead to the depression of unusual metabolic pathways, which may not be beneficial to biotechnologist.
6. In majority cases amount of secondary metabolites produced is negligible.
7. The protocols for individual plants differ very widely and Change in the medium constitution & environmental parameters affect the rate of cell growth & accumulation of secondary metabolites.
8. To maximize on the cell mass produced the cell suspension culture eventually becomes very dense and these presents problems of even aeration.
9. Instability, Slow growth, Expensive process, Aseptic conditions are to be maintained throughout the growth of plant.

Historical development of Plant Tissue Culture-

The principles of tissue culture were involved 1838-1839 in cell theory advanced by Schleiden and Schwann. The important historical events of PTC are following-

1902	The idea of the totipotency of plant cell was given by Haberlandt
1937	White first time established successful root culture of tomato
1941	Vanoverbeek used coconut milk for growth and development of young Datura embryos
1957	Skoog and Miller demonstrated the role of auxin and cytokinin on root and shoot formation in tobacco – tissue
1962	Murashige and Skoog introduced the medium for tobacco culture
1987	Isolation of Bt. gene form bacterium *Bacillus thuringiensis*

Basic requirements of Plant Tissue Culture: •Plant material •Equipments and Glasswares •Aseptic Condition •Washing and storage facilities •Media preparation room •Sterilization room

•Nutrient medium •Transfer room •Culture room or incubators •Proper and optimum aeration •Well equipped observation or recording area

Plant material- The plant material should be disease free and should not be to old. •Also the particular species/variety/genotype which are used should be the right one. Generally in-vitro germinated seedlings are frequently chosen as seed is often also much more readily sterilized than softer plant tissues. When plants are healthy and at the desired stage for use, it is often the case that only a specific part of these plants will give the best explants. E.g. A particular internode,the youngest fully expanded leaf etc.

Equipments and Glasswares-

•Incubating chamber or laminar airflow cabinet with UV light fitting for aseptic transfer

•Incubator with temperature control ± 0.5ºC generally temperature recommended for most tissue culture studies is 36ºC.

•Autoclave-for sterilization of glassware, media etc.

•Refrigerators and freezers-For storage of reagents, tissue culture stock solutions, chemicals etc.

•Hot air oven-for dry sterilization of glassware, media etc.

•Microscope-Simple and special microscope with a provision to take camera are required. The stage of this microscope should be large enough to accommodate large roller bottles in specific cases.

•pH meter- for adjusting the pH of the medium

•A spirit burner or gas micro burner for flame sterilization of instruments

•Washing up equipments- Washing facilities for glassware, pipette etc. in deep soaking baths or washing sinks of stainless steel or polypropylene are suitable for manual washing and rinsing of almost all types of glassware except pipettes. Standard siphon type pipette washers are suitable for washing the pipettes soaked in detergent for overnight. The washed pipettes should be rinsed with deionised water and dried in a stainless steel pippette dryer.

•Water purifier- Pure water is required at most of the plant tissue culture study.

•Centrifuge- To increase the concentration of cell suspension culture

•Shakers- To maintain cell suspension culture

•Balance- To weigh various nutrients of the preparation of the medium

•Shelves- Build from rigid wire mesh to allow maximum air movement and minimum shading should be used in the culture room.

•Scissors, scalpels and forceps- For explant preparation from excies plant parts are for their transfer •Culture vessels- Usually borosilicate glass vessels are preferred, it includes test tubes, conical flasks, bottles, special flat tubes etc. Now, the common vessels are 100 ml conical flasks or large test tubes of 25 × 150 mm size.

•Glasswares- Like measuring cylinders, beakers, funnels, petri dishes, graduated pipette, conical flask etc. are required for preparation of nutrient media.

•Miscellaneous-Non absorbent cotton plug, screw cap or polyurethane foam is required to close the mouth of the culture vessel. Aluminium foil is required to cover the exposed part of plug from becoming wet when autoclaved. Labels, marking pencils, hand lens, plastic disposables like syringes, plastic bottles, hot plate, stirrer etc.

•Microwave- not essential but it melts the solidified media for pouring in culture vessels like petri dishes etc.

Aseptic Condition

The plant materials (tissues), equipments, culture media and the room should be free from microorganisms. Usually dry heat, wet heat, ultrafiltration and chemicals are used for the sterilisation process. Surface sterilisation of plant materials such as seed, fruit, stem, leaf etc. by agents like 9-10% calcium hypochlorite for 5-30 minutes 2% sodium hypochlorite solution for 5-30 minutes. The materials need to be washed thoroughly in double-distilled water, after sterilising in these solutions. 10-12% of hydrogen peroxide solution for 5-15 minutes. 1-2% bromine water, for 2-10 minutes 1% solution of chlorine water, mercuric chloride, silver nitrate or antibiotics etc. can also be used. Absolute alcohol is used for hard tissues

•Dry heat method is used for sterilisation of equipments in hot air oven.

•Sterilisation of equipment with chromic acid-sulphuric acid mixture, hydrochloric acid, nitric acid strong detergent solution, alcohol, incubator or autoclaves etc. are use for this purpose.

•Wet heat method is used for sterilisation of glassware, culture media in autoclave at 121°C and 15 lb pressure for 15 minutes. •Ultrafiltration is used for sterilisation of liquid media which are unstable at high temperature.

•Antibiotics are added to medium to prevent the growth of the microorganisms e.g. Potassium benzyl penicillin, strptomycin sulphate, gentamycin etc.

•Chemicals like alcohol are used for sterilisation of working area and the instruments. •Sterilisation of the environment is done by fumigation method, the inoculation chamber is generally laminar airflow cabinet is widely used these days.

Washing and storage facilities- Fresh water supply and disposal of waste water facility should be available. Space for distillation unit for the supply of distilled and double distilled water and de-ionized water should be available. Working table, sink or wash basin for apparatus/equipment washing should be acid and alkali resistant. Sufficient space is required for lacing hot air oven, washing machine, pipette washers etc. For storage of dried glassware separate dust proof cupboards or cabined should be provided. Media preparation room It should be spacious to accommodate lab ware, culture vessels, equipments, chemicals etc. The preparation room should also be well equipped with refrigerator, freezer etc. for storage of media and stock solutions.

Sterilization room- In the tissue culture lab it is desirable to have separate sterilization room for sterilization of culture media, glassware, metallic equipments like scissors, scalp etc. Generally sterilisation is done in autoclave or hot air oven.

Nutrient medium

Media is composed of –

Inorganic nutrients which includes macronutrients like nitrogen, phosphorous, potassium, calcium etc. and micronutrients like boron, copper, iron, manganese, zinc etc.

Organic nutrients includes Vitamins like Vitamin B1, B6, B3, B5 etc. Amino acids like L-arginine, L-asparagine, L-cysteine HCL, L-glutamine etc,

Carbon source like glucose or maltose

Growth hormones/regulators like auxin, cytokinins and gibberellins, ethylene, abscisic acid.

Others media substances like protein hydrolysates, yeast extaracts, fruit (e.g. banana) extracts, coconut milk,

Solidifying agents like agar, alginate, gelatin etc., Iron source e.g.EDTA, Antibiotics.

pH of the medium should be in a range of 5.6-6.0 before autoclaving the culture medium

Transfer room

It is provided with the laminar flow hood where most of the work of culture initiation and subsequent sub culturing is performed. Culture re-plantation, transfer or re-initiation in a clean media, harvesting of ‘ripe’ cultures is also performed in this area.

Culture room or incubators- Cultures are incubated on shelves or in incubators under specific condition of temperature, humidity, air circulation and light. Incubation chamber or area should have both light and temperature controlled devices managed for 24 hours period. Generally high output, cool, white fluorescent light is preferred for a photo-period duration (specified period for total darkness as well as for higher intenesity light) with a temperature range of 25 ± 2°C (range 18-25°C).

The rooms are required to be maintined at a relative humidity upto 70-75% (range of 20-90% controllable to ±3%) and uniform forced air circulation.

Proper and optimum aeration- Adequate aeration is required for cell to grow. Tissues which are cultured on semisolid media do not require any special method for aeration, but tissues which are grown in suspension cultures, require special devices for aeration. Aeration for submerged cultures can be provided by following methods: Placing the culture vessel with the liquid medium on an automatic shaker. The two ends of the filter paper are dipped in a medium and the middle horizontal portion on which the tissue is placed remains above the level of the medium this method is called as filter paper bridge method. Passing sterilised air through the medium and by stirring the medium. The culture vessels are closed with non-adsorbent cotton covered in cheese cloth. This process allows proper aeration but prevents the entry of microorganisms.

Well equipped observation or recording area- At regular intervals growth and maintenance of the tissue culture in the incubators should be recorded. All observations should be done in aseptic environment. For microscopic examination separate dust free space should be marked for microscopic work.

The general technique used in the isolation and growth of culture is described as follows:

1. Preparation of suitable nutrient medium: As per the selection of plant medium is autoclaved.
2. Selection of explant: Any excised part of health plant to be used e.g. Bud, leaf, root, seed etc.
3. Sterilisation of explants: by sodium hypochlorite, mercuric chloride etc. and washed asceptically for 6-10 times with sterilised water.
4. Inoculation (Transfer): The sterile explant is inoculated on solidified nutrient medium under asceptic condition.
5. Incubation: Cultures are incubated at of 25±2°C and at a relative humidity upto 50-70% from 16 hrs of photo period.

6. Regeneration: Plantlets regenerated after transferring a portion of callus into another medium and induction of roots and shoots or directly from explants.

7. Hardening: Is the gradual exposure of plantlets for acclimatisation to environment condition.

8. Plantlet transfer: Plantlet are transferred to green house or field conditions.

Types of Cultures

1. Depending upon the type of medium

2. Depending on the part used for culture

1. Depending upon the type of medium- Two types

a. Callus culture (Static culture)

b. Suspension culture- three types-

i. Batch culture

ii. Semi-continuous culture

iii. Continuous culture- Two type- Open and Closed. Open system is of two types- Turbidostat and Chemostat

2. Depending on the part used for culture

a) **Organ Culture-** i. Root tip culture (Meristem - root tip culture) ii. Shoot tip culture (Meristem - shoot tip culture) iii. Leaves or leaf primordia culture iv. Flower culture (Meristem - floral culture) v. Anther and pollens culture vi. Ovule and embryo culture vii. Ovaries culture viii. Nucellus culture ix. Seed culture x. Cotyledon culture xi. Endosperm culture xii. Fruit culture xiii. Plant cell culture

b) Hairy Root Culture

c) Protoplast Culture and Somatic Hybridization

STATIC CULTURE (CALLUS CULTURE)

Callus – a mass of undifferentiated plant cells grown on solid media from plant part/explant. It may initiate from explants of any multi-cellular plant. The organs such as root, stem tips, leaves, flowers and fruit are grown on solid media.

The cell groups are initiated from: -

-Explant/Segments of root, stem or leaf either from the mature or embryogenic plant

- Explant/Excised fragments of parenchyma or mixed tissue containing cambium or endosperm

The longer the tissue explant the more complex the range of cell types & greater the possibilities of initiating a culture of mixed cells. Callus can be induced to undergo organogenesis and/or

embryogenesis and eventually whole plant by providing suitable nutrient medium. It is useful for the production of secondary metabolites. To study the biosynthetic pathway of various metabolic processes by using tracer elements in callus culture.

SUSPENSION CULTURE- It involves active proliferation of callus as undivided unit suspended or submerged in a liquid medium. The nutrient medium in this case is in constant agitation so as to prevent the cells from settling or aggregating in to clumps. Suspension cultures are normally initiated by transferring pieces of undifferentiated callus to a liquid medium which is agitated during incubation.

Batch Suspension Culture - It means 'Culture in a fixed volume of culture medium'. In general, a nutrient medium and cellular inoculum are mixed, aerated and allowed to grow. In Batch cultures, as the cells grow, the medium is depleted of nutrients and metabolic byproducts from the cells accumulate. The system is "closed" with respect to additions or removal of culture, except for circulation of air. Batch cultures are characterized by - continuous changes in the medium - continuous internal changes in cellular composition - accumulation of metabolic products.

Semicontinuous Suspension Culture - Here, the system is 'open'. There is periodic removal of culture and the addition of fresh medium, due to which growth of the culture is continuously maintained.

Continuous Suspension Culture-

The system is 'open' here also. In this system, volume of culture remains constant and fresh medium is added continuously to a fixed volume of growing culture and withdrawn.

Two types of Continuous Suspension Culture

1. **Open type-** Regulated new medium and balancing harvest of equal volume of culture.

Chemostat- Continuous new medium input is set at a predetermined rate and determine the nature of the resulting equilibrium. It is useful to study the steady states. Desired rate of growth is maintained by adjusting the level of concentration of nutrient by constant inflow of medium. This system allows - Establishment of steady states of growth and metabolism. - Study of the changes which occur in transitions from one steady state to another. - Identification of the controlling factors. Cell proliferation takes place under constant condition.

Turbidostat –

Cell density is set at a predetermined level (as monitored by the optical properties of the culture) and new medium is added to maintain cell density within limits. Particularly valuable for work at low cell densities. It offers a simplified system for study of effects of physical factors and growth regulating substance in growth rate and associated metabolism.

2.**Closed type-** The used medium is replaced with fresh medium, hence, the cells from used medium are mechanically separated and added back to the culture and thus, the cell biomass keeps increasing.

HAIRY / TRANSFORMED ROOTS (TRANSINFECTION)

Certain soil bacteria of the genus Agrobacterium (Gram negative bacteria) infects a wide range of plant species and causes the infection in plant termed as “Hairy root” disease. The disease is transformed bye their genome t-DNA from a bacterial plasmid to plant hairy root cells. A large number of small fine hairy roots covered with root hairs originated directly from the explant in response to *Agrobacterium rhizogenes* or *Agrobacterium tumefaciens*. The hairy roots are produced by inoculating the host plant when grown in a hormone-free medium give rise to copious roots referred to as 'transformed roots' or 'hairy roots'. These are fast growing, highly branched adventitious roots at the site of infection. They are genetically stable and affect a wide range of dicotyledonous plants and have same metabolic features.

Advantages of Hairy Root Culture

1. Reproducible & predictable levels of product synthesis.
2. Plant regeneration, plant improvement and genetic manipulation in plant can be done.
3. A diversified range of plant species has been transformed using various bacterial strains.
4. Many plant cell culture systems, which did not produce adequate amount of desired compounds is being reinvestigated using hairy root culture methods.
5. Ease of culture in vitro using simple media lacking phytohormones.
6. Genetic and growth kinetic stability over prolonged period of growth in vitro.
7. More accumulation of secondary metabolites Eg. Levels of steroidal alkaloid solasodine is significantly higher in hairy root cultures than callus or suspension cultures.
8. Capability to synthesize (novel) secondary metabolites specific to that plant species from which they have developed in equal or even higher amount compared to field grown plants.

Protoplast culture-

Protoplast are plant cells with a plasma membrane but without cell wall, because of this the protoplast provide the starting point for many of the technique of genetic manipulator of plants, in particular the induction of somaclonal variation, somatic hybridization and genetic transfer. They are cultivated in liquid as well as on solid media. Isolation of Protoplasts is by two methods. Protoplasts can be isolated from almost all plant parts i.e., roots, leaves, fruits, tubers, root nodules, endosperm, pollen cells, and cells of callus tissue. 1. Mechanical method 2. Enzymatic method

Mechanical method-

The cells were kept in a suitable plasmolyticum (lysis of plasma membrane) and cut with a fine knife. Cells were cut only through the cell wall, releasing intact protoplast. This mechanical procedure gave low yield of protoplasts and could be utilized for only highly vacuolated and non meristematic cells. The method is laborious and tedious.

Enzymatic method-

Commercial preparations of purified cell wall degrading enzymes such as macroezyme, cellulase and hemicellulose became available that gave further progress to enzymatic isolation of protoplasts. By this method very large number of protoplast are obtained compared to mechanical method. Osmotic shrinkage of protoplast is much less. Cells are not damaged or broken. Enzymatic method of protoplast isolation can be classified into two groups. A) Sequential enzymatic- This involves two steps where first macerated plant tissues are incubated with pectinase to get single cells followed by cellulase treatment to get protoplast. B) Mixed enzymatic This involves simultaneous separation of cells and degradation of their walls to convert protoplast by immersing plant tissues in mixture of pectinases and cellulases.

PROTOPLAST CULTURE- Isolated protoplasts are usually cultured in either liquid or semisolid agar media plates. They require somatic protection in culture medium until they generate a strong cell wall.

Applications of protoplast culture

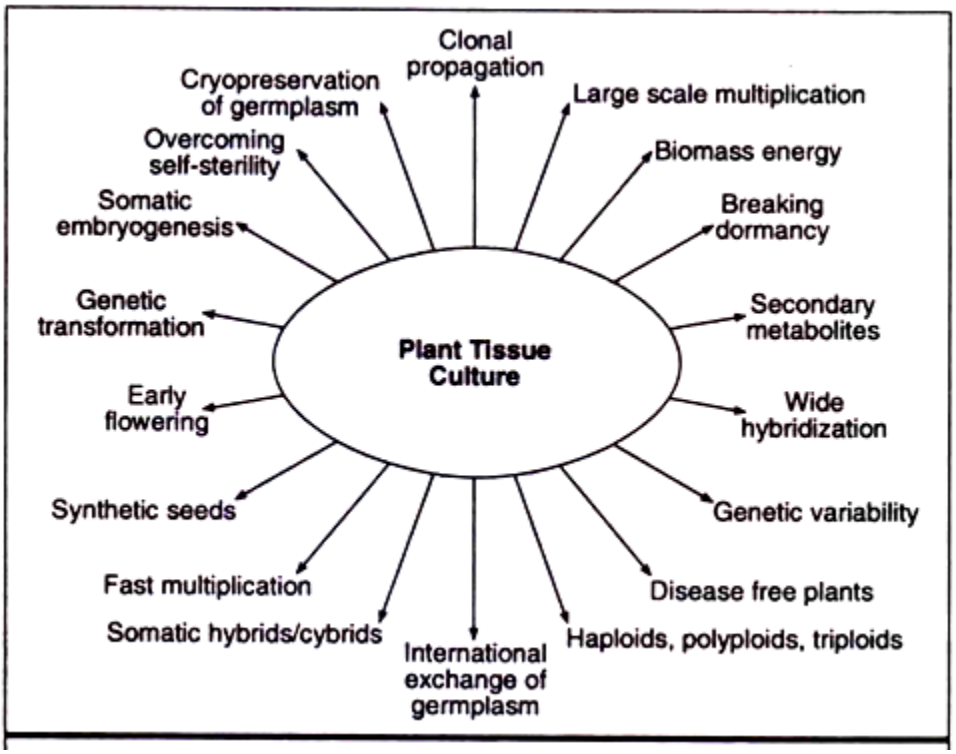
1. To develop Novel hybrid plant through protoplast fusion.
2. In single cell derived colony, isolation of mutants through mutagens is easier.
3. Single cell cloning can be easily performed with protoplasts.
4. Regeneration of entire plant or plant improvement through protoplast culture.

5. Genetic transformation through DNA uptake can be achieved.

6. Reproducible protoplast to plant systems are now available for many plants of agronomic value.

Applications of Plant Tissue Culture

The following points highlight the top ten applications of plant cell and tissue culture. The applications are: 1. Clonal Propagation and Micro-Propagation 2. Biomass Energy 3. Secondary Metabolites 4. Genetic Variability 5. Somatic Embryogenesis and Synthetic Seed 6. Breaking Dormancy 7. Haploid Plants 8. Somatic Hybrids 9. Transgenic Plants 10. Germplasm Conservation.



Application-1. Clonal Propagation and Micro-Propagation:

Plant population derived from a single donor plant is called a clone and the multiplication of genetically identical copies of that cultivar is called clonal propagation which may be an useful tool to get a large population of plant species having desirable traits. Micro-propagation is achieved through multiplication of shoot tips or axillary buds cultured in vitro.

This technique is very much used in horticulture and silviculture—in the plants which have long seed dormancy, tree species, orchids and many fruit plants. This micro-propagation technique is

also helpful for supplying the plant material throughout the year involving large scale multiplication i.e., grower and breeder gets a large number plant stocks irrespective of seasonal variation.

In tissue culture from a callus mass large numbers of shoot meristems can be regenerated within a very short time and space. As a result a large number of plantlets can be produced from such callus tissue. The most obvious advantage of this technique is the large scale production of plants of same genetic stock.

Application- 2. Biomass Energy:

In recent years, the interest has aroused in commercializing the in vitro propagation of forest trees. Micro-propagation has been successfully done in many trees of economic importance like Acacia nilotica, Albizia lebbeck, Azadirachta indica, Butea monospermous, Dendrocalamus strictus, Shorea robusta, Tectona grandis and Cedrus deodara, Cryptomeria japonia, Picea smithiana, Pinus sylvestris.

All these plant species are useful in forestry for biomass energy production. Development of automated procedure, plant delivery systems using somatic embryos and artificial seeds are also in progress.

Application-3. Secondary Metabolites:

Production of many useful compounds like alkaloids (Codeine, Vincristine, Quinine, etc.), Steroids (Diosgenin), Glycosidic compounds (Digoxin) and many other essential oils (Jasmine), flavouring and colouring agents (saffron) can be done by plant cell culture. This aim can be achieved by selection of specific cells producing high amount of desired compounds and development of a suitable medium.

In general, secondary metabolites produced by plant cell cultures are rather small in amount but by clonal selection the particular high yielding clone of cells can be isolated. Sometimes the plant cell culture may provide the helpful way for more production of secondary metabolite by feeding the culture with inexpensive product precursors (biotransformation) or by manipulating their biosynthetic control mechanisms.

Application- 4. Genetic Variability:

The variability generated by the use of a tissue culture cycle has been termed as somaclonal variation by Larkin and Scowcroft. This genetic variability is due to cells of various ploidy levels

and genetic constitution of the initial explant or also may be developed due to different cultural conditions.

The chromosomal instability in the cultured cells play an important role in polyploidization of cells and genetically variable plants can be raised.

Such kind of variations may show some useful characters such as resistance to a particular disease, herbicide resistance, stress tolerance, etc. and also some agronomical traits like tiller number, panicle size, flowering time, plant height, lodging resistance, yield, nutrient content and different kinds of morphological variations in leaf.

Application-5. Somatic Embryogenesis and Synthetic Seed:

Direct or indirect somatic embryogenesis may be achieved from pro-embryonic cell of the direct explant or the embryoids developed within the callus tissue from induced embryogenic cells. The potential application of this technique is the mass production of adventitious embryos which ultimately develop into complete plantlet in maturing media.

These somatic embryos can be encapsulated with suitable nutrient containing alginate medium which are called artificial seeds or synthetic seeds. As the somatic embryos are derived from a single cell, this method is very much useful for production of disease free propagule. This artificial seed production is also desirable in case of asexually propagating plants.

Application-6. Breaking Dormancy:

Using embryo (zygotic) culture technique the seed dormancy period can be reduced or eliminated and the breeding cycle can be shortened in many of the plants like Malus sp, Ilex sp. and Telia americana etc. The life cycle of Iris was reduced from 2-3 years to less than one year. It was possible to obtain two generations of flowering in Rosa sp.

Embryo abortion in unsuccessful crosses may be recovered by culture of immature embryo of different hybrids.

Application-7. Haploid Plants:

Haploid plants can be obtained through anther or pollen culture (androgenesis) or through ovaries or ovule culture (gynogenesis). The anther culture and haploid plant production has been attempted in many of the crop plants, where these haploids are of immense importance for production of homozygous diploid or polyploid lines by colchicine treatment within a very short period specially in case of fruit trees.

These androgenic haploids can also be used for production of different kinds of aneuploids like monosomic, nullisomic, trisomic, etc. and also for the induction of mutagenesis and doubling of those mutated lines. Many of the recessive traits can be made expressed in double haploids such as low glucosinolate content in Brassica, salt tolerance and disease resistance in rice, etc.

Generation of exclusively Y chromosome containing plant is possible also through haploid production as in case of Asparagus. The triploid or polyploid can also be produced by using protoplast fusion technique of this kind of androgenic haploids which may be used for different breeding programmes.

Application-8. Somatic Hybrids:

Isolation and regeneration of plant from the protoplasts in vitro has opened up a new avenue in various fields of plant breeding and in plant biotechnology.

Somatic hybridisation, i.e., the asexual hybridisation using isolated somatic protoplasts is a new tool to make the wide hybridisation successful. Products of fusion between two protoplasts (heterokaryon) could be cultured to regenerate a new somatic hybrid plant of desired genotype.

This technique has been mainly used for introgressing many useful criteria from the wild genotype to cultivated crop variety. Success has been achieved obtaining somatic hybrid plants between sexually compatible and incompatible plants.

Production of cybrid, i.e., the fusion between two protoplasts—one partner with nucleus and another partner with cytoplasm, is also of immense importance in the plant breeding programme, mainly for production of male sterile line with the help of extra-nuclear genome.

Application-9. Transgenic Plants:

The genetically modified (GM) plants, in which a functional foreign gene has been incorporated by biotechnological method, are called transgenic plants. A number of transgenic plants have been produced carrying genes for different traits like insect resistance, herbicide tolerance, delayed ripening, increased amino acid and vitamin content, improved oil quality, etc.

The different methods of introduction of foreign genes, direct (electroporation, microinjection or particle bombardment) or indirect (Agrobocterium mediated), have been applied either in plant tissue culture method such as embryogenic or organogenic plant development from different plant parts or in protoplast culture system.

The direct DNA uptake through protoplast is the most ideal method for production of transgenic plants. Any gene of interest that may be of eukaryotic or prokaryotic origin can be used for this purpose but should be expressed.

Application-10. Germplasm Conservation:

Many of the important crop species produce recalcitrant seeds with early embryo degeneration. Also many of the plants are vulnerable to insects, pathogens and various climatic hazards. Maintenance of these plants are very difficult. Mainly the plant species which are endangered, rare and threatened with extinction are needed to be conserved by ex-situ method of germplasm conservation.

Plant tissue culture may be applied for this purpose. In vitro germplasm storage collection provides a cost effective alternative to growing plants under field conditions, nurseries or greenhouses.

Furthermore, the cryopreservation of cells and tissue, revival of these tissue and regeneration of plants from tissue through tissue culture technique really effective in conservation biotechnology. Cryopreservation involves storage of cells, tissues, etc. at a very low temperature using liquid nitrogen.

EDIBLE VACCINES

The phrase **edible vaccines** was first used by Charles Arntzen in 1990 and refers to any foods; typically plants, that produce vitamins, proteins or other nourishment that act as a vaccine against a certain disease. Once the plant, fruit, or plant derived product is ingested orally, it stimulates the immune system. Specifically, it stimulates both the mucosal and humoral immune systems.

Edible vaccines are genetically modified crops that contain added “immunity” for specific diseases. Edible vaccines offer many benefits over traditional vaccines, due to their lower manufacturing cost and a lack of negative side effects. However, there are limitations as edible vaccines are still new and developing. Further research will need to be done before they are ready for widespread human consumption. Edible vaccines are currently being developed for measles, cholera, foot and mouth disease, Hepatitis B and Hepatitis C.

Benefits Edible vaccines differ from traditional vaccines in many ways and overcome many of their limitations.

1. Traditional vaccines can be too expensive or restricted to manufacture and develop in certain countries. In contrast, edible vaccines are easy to produce, purify, sterilize, and distribute. Since they do not require expensive manufacturing equipment, only rich soil, the cost to grow the vaccines is significantly lowered.
2. In addition, edible vaccines do not require sterilized production facilities or the biosafety standards required to cultivate certain pathogenic agents for traditional vaccines which are expensive to implement and maintain.
3. They are also easier and less expensive to store since they do not require strict refrigerated storage. This necessity for cold chain storage creates many issues in third world countries.
4. The seeds from an edible vaccine plant can also be easily dehydrated and preserved for cheap and quick distribution which makes them easily accessible in times of need.
5. Edible vaccines also offer a profuse amount of potential health benefits over traditional vaccines. Eating a vaccine is a simpler means of administration compared to injection, making them extremely economical. This reduces the need for medical personnel and sterile injection conditions that are not always achievable in developing countries.
6. Edible vaccines are considered a “pharmafood” which is a food source that increases health while also fighting diseases. The benefit of using plants is that plants are efficient vectors for vaccine production.
7. Many traditional vaccines that are developed from cultured mammalian cells can lead to contamination with animal viruses. However, edible vaccines eliminate this issue because plant viruses cannot impact humans.
8. Moreover, as a result of numerous antigens being integrated, the M-cells are randomly stimulated; leading to the possibility of second-generation vaccines.
9. Edible vaccines do not require subsidiary elements to stimulate an immune response like traditional vaccines. Some major concerns with traditional vaccines are potential side effects, for example, allergic reactions. Since edible vaccines lack certain toxic compounds and only contain therapeutic proteins, which are free of pathogens and toxins, the risk of potential side effects and allergic reactions are greatly reduced.

Limitations

Edible vaccines also have multiple disadvantages compared to traditional vaccines. Since edible vaccines are still in their infancy, there are still many unknowns left to discover.

1. The adequate dosage amount and how long it lasts is still undetermined. The dosage varies due to many factors including: the plant generation, the individual plant, the protein content, the ripeness of the fruit and how much of it is eaten.[3] The dosage also varies due to the difficulty in standardizing the concentration of the antigen in the plant tissue; it can be tedious to produce both consistently and large scale.
2. The antigen concentration can also vary significantly between individual fruits on a plant, individual plants, and between plant generations. Low doses result in the consumption of less antibodies but a high dose results in establishing an oral and immune tolerance to the vaccine proteins. The logistics of controlling dosage, quality, and consistency still need to be determined and verified.
3. Additionally, the effects and risk of using pesticides on the plants could be negative towards both the plant vaccine and the consumer.
4. There is also the risk of transgenic escape into the surrounding environment; however, this could be reduced by regulating growing practices and locations. Many plants are not eaten raw and the cooking could weaken or destroy the proteins in the vaccine. In a study, it was found that after boiling a potato for 5 minutes, half of the vaccine survived, thus showing that not all edible vaccines have to be ingested raw if dosages account for cooking times and temperatures.
5. There is also a concern that the gastric enzymes and the acidic environment of the stomach will break down the vaccine before it can activate an immune response.

Moreover, concerns have arisen regarding the vaccine behavior being different due to the differing glycosylation pattern of plants and humans.

Production

Edible vaccines are subunit vaccines; they contain the antigen proteins for a pathogen but lack the genes for the full pathogen to form. The first steps in making an edible vaccine is the identification, isolation, and characterization of a pathogenic antigen. In order to be effective, the antigen needs to elicit a strong and specific immune response. Once the antigen is identified and

isolated, the gene is cloned into a transfer vector. One of the most common transfer vectors for DNA being used for edible vaccines is *Agrobacterium tumefaciens*. The pathogen sequence is inserted into the transfer DNA (T-DNA) to produce the antigenic protein. It is then inserted into the genome, expressed, and inherited in a mendelian fashion, which results in the antigen being expressed in the fruit or plant. From that point forward, traditional vegetative methods and techniques are used to grow the plants and propagate the genetic line.

Techniques

The entire gene is inserted into a plant transformation vector to allow transcription or the epitope within the antigen is identified and the DNA fragment can be used to construct genes by fusion with a coat protein gene from a plant virus. Then, the recombinant virus can infect other plants. The epitope is first identified, and then, DNA fragment encoding is used to construct genes by fusion with a coat protein gene from plant virus (TMV or CMV). The transgene can be expressed either through a stable transformation system or through transient transformation system based on where the transgene has been inserted into the cell.

Stable transformation

A stable transformation involves a nuclear or plasmid integration in which permanent changes occur in the recipient cells' genes and the targeted transgene is integrated into the genome of host plant cells.

Transient transformation

A transient transformation involves a plasmid/vector system using *Agrobacterium tumefaciens* which integrates the exogenous genes into the T-DNA, then infects the vegetable tissue. Agrobacterium is the common technique used currently because it's a soil pathogenic bacterium that naturally infects plants and transfers their genes (T-DNA) to the nucleus of the plant. *A. tumefaciens* is the most preferred strain because it carries tumour-inducing plasmids. The genes will be made into a neutralized Ti-plasmid and the heterologous gene is inserted to form a recombinant plasmid vector. The vector is then turned into the desired strain with the help of the virulence genes of the bacterium. It is then transferred and integrated into the genomic DNA of the host plant by non-homologous recombination at random sites. This method has a

low yield and is a slow process, and it is the most effective when used with dicotyledonous plants such as, tomato, potato, and tobacco.

Bombardment method

Another technique is the microprojectile bombardment method where selected DNA sequences are processed and penetrated into the chloroplast genome. The gene containing DNA coated metal particles are fired at the plant cells using a gene gun. The plants take up the DNA, grow into new plants, then are cloned to produce large numbers of genetically identical crops. The gene transfer is independent, and it can express antigens through nuclear and chloroplast transformation.

Vaccines in development

Presently, there are edible vaccines for measles, cholera, foot and mouth disease, and hepatitis B, C, & E. However, even though there are edible vaccines, they are predominately tested in the animal testing and in development phases, with some human clinical trials being conducted. As mentioned above, the human trials have revolved around potatoes. In one cholera study, adults were given transgenic potatoes with various LT-B amounts in order to see how their IgA anti-LT and IgA anti LT amounts changed. Furthermore, they are in phase II on a potato vaccine booster for hepatitis B. Hepatitis B surface antigens were expressed in the potatoes and were given to already vaccinated patients. It was then observed if an immune response occurred. 95% of the volunteers had some form of an immune response, and 62.5% showed an increase in anti-HBsAg titers. From these studies, the National Institute of Allergy and Infectious Disease has supported that edible vaccines can safely trigger an immune response, however, it is also known that they are far from being able to begin large scale human testing for autoimmunity and infectious diseases.

Availability

While the public awareness regarding edible vaccines is increasing, they are still not available for consumer use. Currently, they have only developed and started testing edible vaccines for some diseases. During three of the recent disease outbreaks around the world, edible vaccines have been developed for testing on animals but have not reached human trials. Also, it has been found that a biotechnology company has been started to develop a patent and are working on beginning clinical trials for a transmissible gastroenteritis virus.

Important questions

For 2 marks-

1. Define Plant tissue culture.
2. Define Callus culture.
3. Define suspension culture.
4. Define inorganic nutrients with example.
5. Why Agar is used in plant tissue culture?
6. What are the working conditions for autoclave?
7. Define sterilization and which apparatus is used for it?
8. Define Edible vaccine with one example.
9. What do you mean by Biotransformation?
10. Define Micropropagation.
11. Differentiate callus and suspension culture.
12. What do you mean by Protoplast culture?
13. How and why a Hairy root culture is developed?
14. Why Auxin and Cytokinin are used in plant tissue culture?

For 5 marks-

1. Write a brief note on development of callus culture.
2. How Edible vaccines are prepared?
3. Explain various nutritional requirements for tissue culture.
4. Write a short note on Hairy root culture.
5. Explain various types of plant tissue culture
6. Write a note on establishment and maintenance of suspension culture.
7. Write the basic concept of edible vaccine with advantages and limitations.

For 10 marks-

1. Write a descriptive note on Applications of Plant tissue culture.
2. Write a detailed note on edible vaccine.