Unit-IV (BOP-474)

History of Plant Tissue Culture:

Plant tissue culture, or the aseptic culture of cells, tissues, organs, and their components under defined physical and chemical conditions in vitro, is an important tool in both basic and applied studies as well as in commercial application. It owes its origin to the ideas of the German scientist, Haberlandt, at the beginning of the 20th century.

It was Gottlieb Haberland (1902) who in the first decade of this century pioneered the field of plant tissue culture. His idea was to achieve continued cell division in explanted tissue grown on nutrient medium. Following the discovery and use of auxins, the work of Gautherel, Nobecourt and White ushered in the second phase of plant tissue culture over 30 years ago. These and other workers determined the nutritional and hormonal requirements of the cultured plant tissues. It was observed that the whole plant could be successfully regenerated from undifferentiated tissues or even single cells in culture.

Papid advances in diverse aspect of plant culture have been made during the last few years and plant tissue culture techniques have been extensively applied to agriculture and industry.

Condensed Cronology of Important Development in the Plant Tissue Culture:

Year	Worker	Contribution
1902	**C.Haberlant**	First attempt to culture isolated plant cells in vitro on artificial medium
1922	**WJ Robbins and W. Kotte**	Culture of isolated roots (for short periods) (organ culture)
1934	**P R White**	Demonstration of indefinite culture of tomato roots (long period)
1939	**R J Gautheret and P Nobecourt**	First long term plant tissue culture of callus, involving explants of cambail tissues isolated from carrot.
1939	**P R White**	Callus culture of tobacco tumor tissues from intersepcific hybird of Nicotina glaucum X N.longsdorffi

1941 J Van Overbeek Discovery of nutritional value of liquid endosperm of coconut for culture of isolated carrot embryo.

1942 P R White and A C Braun Experiments on crownn-gall and tumor formation in plants, growth of bacteria free crown-gall tissues.

1948 A Caplan and F C Stewart Use of coconut milk plus 2, 4-D fro proliferation of cultured carrot and potato tissues

1950 G Morel Culture of monocot tissues using coconut milk.

1953 W H Muir Inoculation of callus pieces in liquid medium can give a suspension of single cells amenable tosubculture. Development of technique for culture of single isolated cells.

1953 W Tulecke Haploid culture from pollen of gymnosperm (Ginkgo)

1955 C O Miller, F Skeog and others Discovery of cytokinins. E.g. Kinetin, or potent cell division factor.

1955 E ball Culture of gymnosperm tissues (Sequoia)

1957 F Skoog and C O Miller Hypotheses that shoot and root initiation in cultured callus is regulated by the proportion of auxins and cytokinins in the culture medium.

1960 E C Cocking Enzymatic isolation and culture of protoplast.

1960 G Morel Development of shoot apex culture technique.

1964 G Morel Use of modified shoot apex technique for orchid proportion.

1966 S G Guha and S C Maheshwari Cultured anthers and pollen and produce haploid embryos.

1974 J P Nitsch Culture of microspores of Datura and Nicotina, to double the chromosome number and to harvest seed from homozygous diploid plants just within five months.

1978 G Melchers Production of somatic hybrids from attached to plasmid vectors into naked plant protoplast.

1983 K A Barton , W J Brill and J H Dodds Bengochea Insertion of foreign genes attached to plasmid vectors into naked plant protoplast.

1983 M D Chilton Production of transformed tobacco plants following single cell transformation or gene insertion.

Formulation of Tissue cultur medium:

Murashige and Skoog medium (or MSO or MS0 (MS-zero)) is a plant growth medium used in the laboratories for cultivation of plant cell culture. MSO was invented by plant scientists Toshio Murashige and Folke K. Skoog in 1962 during Murashige's search for a new plant growth regulator. A number behind the letters MS is used to indicate the sucrose concentration of the medium. For example, MS0 contains no sucrose and MS20 contains 20 g/l sucrose. Along with its modifications, it is the most commonly used medium in plant tissue culture experiments in laboratorium.

As Skoog's doctoral student, Murashige originally set out to find an as-yet undiscovered growth hormone present in tobacco juice. No such component was discovered; instead, analysis of juiced tobacco and ashed tobacco revealed higher concentrations of specific minerals in plant tissues than were previously known. A series of experiments demonstrated that varying the levels of these nutrients enhanced growth substantially over existing formulations. It was determined that nitrogen in particular enhanced growth of tobacco in tissue culture.

Major salts (macronutrients)

- Ammonium nitrate (NH_4NO_3) 1,650 mg/l
- Calcium chloride ($CaCl_2 \cdot 2H_2O$) 440 mg/l
- Magnesium sulphate ($MgSO_4 \cdot 7H_2O$) 370 mg/l
- Potassium phosphate (KH_2PO_4) 170 mg/l
- Potassium nitrate (KNO_3) 1,900 mg/l

Minor salts (micronutrients)

- Boric acid (H3BO3) 6.2 mg/l
- Cobalt chloride (CoCl2 • 6H2O) 0.025 mg/l
- Cupric sulphate (CuSO4 • 5H2O) 0.025 mg/l
- Ferrous sulphate (FeSO4 • 7H2O) 27.8 mg/l
- Manganese sulphate (MnSO4 • 4H2O) 22.3 mg/l
- Potassium iodide (KI) 0.83 mg/l
- Sodium molybdate (Na2MoO4 • 2H2O) 0.25 mg/l
- Zinc sulphate (ZnSO4•7H2O) 8.6 mg/l
- Ethylenediaminetetraacetic acid ferric sodium (NaFe-EDTA) constituting 5 mL/L of a stock solution containing 5.57 g FeSO4.7H2O and 7.45 g Na2-EDTA per litre of water.

Vitamins and organics

- Myo-Inositol 100 mg/l
- Nicotinic Acid 0.5 mg/l
- Pyridoxine • HCl 0.5 mg/l
- Thiamine • HCl 0.1 mg/l
- Glycine 2 mg/l
- Lactalbumin Hydrolysate (Edamin) (optional) 1 g/l
- Indole Acetic Acid 1-30 mg/l
- Kinetin 0.04-10 mg/l

Plant Tissue Culture owes its origin to the ideas of the German Scientist, Haberlandt, in the beginning of the 20th century. This was just the beginning of the tissue culture; thereafter in 70's began the commercialization of the technology. Currently the world is revolving around it due to predicated future grain shortage; green house effect and total environmental disbalance.

Keeping all these factors in mind and to support mankind Titan Media had taken steps and introduced an extensive range of Ready to use PTC Medium; PTC Medium Ingredients like lant Growth Regulators or Phytoharmones (Auxins; Cytokinins; Gibberellins; Abscisic and & Others), Macro & Micro Nutrients (Nitrogen; Potasssium; Phosphorus; Sulphur; Magnesium; Calcium; Boron; Zinc; Iron; Iodine; Vitamins; Amino acids; Carbohydrates & Others), Gelling Agents (Gelrite; Agar Agar & Others); Adsorbing Agents; Buffering Agents; Pure Antibiotics and range of products used in the process.

Plant Tissue Culture Media Preparation is based on the unique property of the cell-totipotency. The cell-totipotency is the ability of the plant cell to regenerate into whole plant. In this process the excised bud is transferred into a tube containing a sterile nutrient medium. The success of plant tissue culture media depends very much on the stage of explant selected, the sterilization period and the type of culture media used; different types of plants require different sets of culture media. Plant tissues are grown in vitro on artificial media, which supply the nutrients necessary for growth. The success of plant culture as a means of plant propagation is greatly influenced by the nature of the culture medium used. The rich plant tissue culture media provides a good nutrient source for bacteria and fungi, therefore precautions against microbial contamination must be taken in all in vitro procedures.

Plant Tissue Culture Techniques:

Plant Tissue Culture is the process of growing an isolated plant cells or organs in an artificial nutrient media outside the parent organism.

In other words, it is an in vitro culture of plant cells or tissues on artificial nutrient media under aseptic conditions, in glass containers.

This is a technique by which new plants can be raised on artificial nutrient media by use of plant parts or cells. These small parts can be pollen, leaves, seed, root tip, embryo etc.

Since all the above organs or cells contain the same genetic material as that of parent plant, a new plant can be grown.

This capacity of plant parts or cell to grow into a full plant is termed as “toti potency”.

Media for tissue culture: Nutrient media plays an important role in tissue culture. It is very vital for proper and timely growth of cells and their multiplication.

Since nutrient media is the only source of nutrition, it should supply all the basic requirements. These include carbohydrates, amino-acids, minerals, hormones and salts etc at proper proportions. It should be sterile and be non-toxic to the tissue or cell under culture.

All the ingredient of the media are to be sterile hence one can use autoclave or membrane filters based on their thermal resistance power. Tissue culture media preparation should be done in aseptic rooms and conditions.

Also one can include activated charcoal to adsorbs impurities from media. pH of 5.2 to 5.6 is best with temp of 25o c.

Tissue culture equipment like Complete air conditioned lab, laminar air flow, autoclave, BID incubators, Shakers are also needed..

Steps in tissue culture:

1. Tissue or cell of an interesting plant is selected and sterilized (disinfected) by mercuric chloride or alcohol.

Sterilization of cells: The cells taken for tissue culture are to be surface sterilized. This helps the cell walland tissue surfaces to be free from any bacterial or fungal infections. Care should be taken during their handling, transfer etc. to keep them free from infection.

2. Then tissue is placed in media and incubated with proper oxygen supply and right temperature.

Oxygen supply: Since tissue has no direct mechanism to take up oxygen, oxygen supply has to be provided. The gas should be free from contamination and also aseptic. The rate and pressure of flow of gas into the chamber of tissue culture should be optimal.

3. The tissue or cell multiplies and then forms plant-lets.

4. This can be transplanted to green house. Tissue culture plants are highly sensitive to tolerate natural environment conditions. They have to be slowly adopted to normal atmosphere. So first they are to be grown in green houses.

Tissue growth curve:

When a cell or tissue is incubated in nutrient media, it shows phase different in growth. There are four phases of tissue growth and when a graph is plotted with growth versus curve, we obtain tissue growth curve. This growth curve has

a) Lag phase: The phase of adjustment to new environment. Here the cells just grow in size but don't.

b) Exponential phase (log phase): Here the cells multiply profusely and grow in numbers. This is a useful phase to produce bi-products in large quantities.

c) Decline phase: Here the multiplication of cells slows down. Nutrients are exhausted.

d) Stagnant phase: The cells here remain in fewer numbers without further multiplication. This is due to lack of nutrients, accumulation of toxins etc.

There are mainly two major techniques in plant tissue culture:

a) Static culture (Solid-agar Medium): It can also be called as callus plant tissue culture. In this procedure, the plant-tissue is grown on solid agar medium and always gives rise to tissue mass called a callus. This callus culture technique is easier as it is easier and even convenient for initial maintenance of cell-lines, and also for carrying out the investigation studies related to organogenesis i.e organ formation.

b) Suspension cultures (Liquid media): Here the cell aggregates, or even single cells are grown in liquid culture. The cells are kept suspended by using agitators/shakers/ impellers. The actual

growth rate of the liquid-suspension cultures are much higher in comparison to those grown solid-agar medium.Besides, this technique provide much superior control over the growth of biomass as the cells are always surrounded by the nutrient medium completely.

Types of suspension culture:

1) Batch suspension cell culture: Here the cells or tissues are grown in a fixed volume of nutrient medium. Once the cells reach exponential phase, the entire culture is replaced with new one. It is closed type of culture.

2) Continuous suspension culture: Here there is continuous addition of nutrition media. The dilution rate is such that an equivalent volume of media is removed out proportional to the in flow from top. The cells are always kept in exponential growth phase.

I) Open type: Here the system is kept continuous with constant addition and removal of cultured cells.

II) Closed type: Here cell proliferate till completion of exponential phase. Then there is fresh addition of nutrient media & culture media.

-*Several different culture types most commonly used in plant transformation studies will now be examined in more detail.*

1. Callus

-Explants, when cultured on the appropriate medium, usually with both an auxin and a cytokinin, can give rise to an unorganised, growing and dividing mass of cells.

-It is thought that any plant tissue can be used as an explant, if the correct conditions are found.

-In culture, this proliferation can be maintained more or less indefinitely, provided that the callus is subcultured on to fresh medium periodically.

-During callus formation there is some degree of dedifferentiation (i.e. the changes that occur during development and specialization are, to some extent, reversed), both in morphology (callus is usually composed of unspecialised parenchyma cells) and metabolism.

-One major consequence of this dedifferentiation is that most plant cultures lose the ability to photosynthesise.

-This has important consequences for the culture of callus tissue, as the metabolic profile will probably not match that of the donor plant. -This necessitates the addition of other components—such as vitamins and, most importantly, a carbon source—to the culture medium, in addition to the usual mineral nutrients.

-Callus culture is often performed in the dark (the lack of photosynthetic capability being no drawback) as light can encourage differentiation of the callus.

-During long-term culture, the culture may lose the requirement for auxin and/or cytokinin.

-This process, known as 'habituation', is common in callus cultures from some plant species (such as sugar beet).

-Callus cultures are extremely important in plant biotechnology. Manipulation of the auxin to cytokinin ratio in the medium can lead to the development of shoots, roots or somatic embryos from which whole plants can subsequently be produced.

-Callus cultures can also be used to initiate cell suspensions, which are used in a variety of ways in plant transformation studies.

2. Cell-suspension cultures:

-Callus cultures, broadly speaking, fall into one of two categories: compact or friable.

-In compact callus the cells are densely aggregated, whereas in friable callus the cells are only loosely associated with each other and the callus becomes soft and breaks apart easily.

-Friable callus provides the inoculum to form cell-suspension cultures. Explants from some plant species or particular cell types tend not to form friable callus, making cell-suspension initiation a difficult task.

-The friability of callus can sometimes be improved by manipulating the medium components or by repeated sub-culturing.

-The friability of the callus can also sometimes be improved by culturing it on 'semi-solid' medium (medium with a low concentration of gelling agent).

-When friable callus is placed into a liquid medium (usually the same composition as the solid medium used for the callus culture) and then agitated, single cells and/or small clumps of cells are released into the medium.

-Under the correct conditions, these released cells continue to grow and divide, eventually producing a cell-suspension culture.A relatively -large inoculum should be used when initiating cell suspensions so that the released cell numbers build up quickly.

-The inoculum should not be too large though, as toxic products released from damaged or stressed cells can build up to lethal levels.

-Large cell clumps can be removed during subculture of the cell suspension.

-Cell suspensions can be maintained relatively simply as batch cultures in conical flasks.

-They are continually cultured by repeated subculturing into fresh medium.

-This results in dilution of the suspension and the initiation of another batch growth cycle.

-The degree of dilution during subculture should be determined empirically for each culture.

-Too great a degree of dilution will result in a greatly extended lag period or, in extreme cases, death of the transferred cells.

-After subculture, the cells divide and the biomass of the culture increases in a characteristic fashion, until nutrients in the medium are exhausted and/or toxic by-products build up to inhibitory levels—this is called the 'stationary phase'.

-If cells are left in the stationary phase for too long, they will die and the culture will be lost.

-Therefore, cells should be transferred as they enter the stationary phase.

-It is therefore important that the batch growth-cycle parameters are determined for each cell-suspension culture.

3. Protoplasts:

-Protoplasts are plant cells with the cell wall removed.

-Protoplasts are most commonly isolated from either leaf mesophyll cells or cell suspensions, although other sources can be used to advantage.

-Two general approaches to removing the cell wall (a difficult task without damaging the protoplast) can be taken—mechanical or enzymatic isolation.

-Mechanical isolation, although possible, often results in low yields, poor quality and poor performance in culture due to substances released from damaged cells.

-Enzymatic isolation is usually carried out in a simple salt solution with a high osmoticum, plus the cell wall degrading enzymes.

-Protoplasts are fragile and easily damaged, and therefore must be cultured carefully.

-Liquid medium is not agitated and a high osmotic potential is maintained, at least in the initial stages.

-The liquid medium must be shallow enough to allow aeration in the absence of agitation.

-Protoplasts can be plated out on to solid medium and callus produced.

-Whole plants can be regenerated by organogenesis or somatic embryogenesis from this callus.

-Protoplasts are ideal targets for transformation by a variety of means.

4. Root cultures:

-Root cultures can be established in vitro from explants of the root tip of either primary or lateral roots and can be cultured on fairly simple media.

-The growth of roots in vitro is potentially unlimited, as roots are indeterminate organs.

-Although the establishment of root cultures was one of the first achievements of modern plant tissue culture, they are not widely used in plant transformation studies.

5. Shoot tip and meristem culture:

-The tips of shoots (which contain the shoot apical meristem) can be cultured in vitro, producing clumps of shoots from either axillary or adventitious buds.

-This method can be used for clonal propagation.

-Shoot meristem cultures are potential alternatives to the more commonly used methods for cereal regeneration (see the Case study below) as they are less genotype-dependent and more efficient (seedlings can be used as donor material).

6. Embryo culture:

-Embryos can be used as explants to generate callus cultures or somatic embryos.

-Both immature and mature embryos can be used as explants. Immature, embryo-derived embryogenic callus is the most popular method of monocot plant regeneration.

7. Microspore culture:

-Haploid tissue can be cultured in vitro by using pollen or anthers as an explant.

-Pollen contains the male gametophyte, which is termed the ‘microspore’.

-Both callus and embryos can be produced from pollen.

-Two main approaches can be taken to produce in vitro cultures from haploid tissue.

-The first method depends on using the anther as the explant. Anthers (somatic tissue that surrounds and contains the pollen) can be cultured on solid medium (agar should not be used to solidify the medium as it contains Culture types 45 inhibitory substances).

-Pollen-derived embryos are subsequently produced via dehiscence of the mature anthers.

-The dehiscence of the anther depends both on its isolation at the correct stage and on the correct culture conditions.

8. Anther culture:

-Obtain two buds at the appropriate stage. This occurs in tobacco when the sepals and the petals in the bud are the same length.

-Holding the bud by the pedicel between the thumb and first finger, dip the entire bud in 95% ethanol for 15 seconds Remove bud and allow excess alcohol to drip off.

-With a pair of sterile forceps, remove the outer layer of tissue, the sepals.

-Next, remove the inner layer of tissue, the petals, exposing the anthers.

-Open the petri dish containing the medium for the induction of haploids.

-Remove each anther from the bud and drop it onto the medium.

-Do not damage the anther or include any filament tissue.

-Repeat for another bud. When finished, seal the plates and place in incubator (25°C).

-In 2–3 weeks examine for somatic embryo initiation. Embryoid-forming cells are characterizedby dense cytoplasmic contents, large starch grains and a relatively large nucleus.

-Embryoids appear opaque among translucent cells.

-Embryoids also exhibit high dehydrogenase activity and can be detected by tetrazolium staining

Factors Influencing Plant Tissue Culture:

The following points highlight the thirteen factors influencing organogenesis in plant tissue culture.

The thirteen factors are: (1) Size of Explant (2) Source of Explant (3) Age of the Explant (4) Seasonal Variation (5) Oxygen Gradient (6) Quality and Intensity of Light (7) Temperature (8) Plant Hormones (9) Culture Medium (10) Agar-Agar (11) pH of the Medium (12) Ploidy Level and (13) Age of Culture.

Factor # 1. Size of Explant:

Organogenesis is generally dependent upon the size of the explant. The large explant consisting of parenchyma, vascular tissue and cambium have greater regenerative ability than the smaller explant. Small groups of homogeneous tissue taken from the epidermal and subepidermal layer could directly give rise to complex organs such as flower or buds or roots. A remarkable capacity to regenerate shoot buds in vitro is displayed by certain ferns such as Davallia Platycerium. The tissue pieces obtained by aseptically homogenizing the plants in a blender produce numerous new plants.

Factor # 2. Source of Explant:

The source of explant cultured is important in determining the potential of organogenesis. The most suitable part of the plant for starting culture will depend on the species. Leaves and leaf fragments of many plant species like Begonia, Solanum, Nicotiana, Crepisetc. have shown the capacity to regenerate shoot buds. Many of the monocotyledonous species with specialized storage organs possess a profound capacity to produce buds.

Factor # 3. Age of the Explant:

The physiological age of the explant is another factor which often plays an important role in organogenetic phenomenon. Regeneration of adventitious shoot bud is only noted in case of Nicotiana sp. if the leaf explants are collected from the vegetative phase i.e. prior to flowering. Leaf explants of Echeveria sp. that are collected from young leaves produce only root, whereas older leaf initiates only shoot buds and leaves of medium age produce both shoots and roots.

Factor # 4. Seasonal Variation:

The effect of seasonal variation on plant is another factor which exercises an influence on or¬gan formation. Bulb scales of Lihum speciosum regenerates bulblets freely in vitro when the ex-plant is taken during spring and autumn period of growth. But the same explant collected from summer or winter season does not produce any bulblet.

Factor # 5. Oxygen Gradient:

Oxygen gradient in a tissue culture often exercises an influence on organ formation. In some cultures, shoot bud formation takes place when the gradient of available oxygen inside the culture vessel is reduced. But rooting requires a high oxygen gradient.

Factor # 6. Quality and Intensity of Light:

The quality and intensity of incident light on culture may play an effective role in the promotion of organogenesis. Studies on spectral light on organogenesis reveals that the blue region of the spectrum promotes shoot formation and red light induces rooting.

The treatment of blue light followed the treatment of red light also stimulates the organogenetic phenomenon. Hence the nature of organogenesis can be regulated by exposure to light of different wave length. This sort of action of light on organogenesis will help us in understanding the action of auxin and cytokinin on organogenesis. In some culture, artificial fluorescent light favours rooting and inhibits in others. In case of Pisum sativum, shoot bud initiation takes place in dark followed by the sudden treatment of light.

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Factor # 7. Temperature:

Most tissue cultures are grown successfully at temperatures around 25° C, but the usual environmental temperatures of the species concerned should be taken into account. In a number of bulbous species, the optimum temperature may be much lower— 15°C in case of Galanthus and 18°C for some cultivars of Narcissus and Allium. Tropical species require higher temperatures, the optimum for date palm being 27°C and for Monstera deliciosa 30°C.

Factor # 8. Plant Hormones:

Of the many factors that influence Organogenesis in vitro, the most important single factor seems to be the phytohormones. In their classi¬cal experiments with cultured stem pith tissue of tobacco, Skoog and Miller (1957) demonstrated that different types of organogenesis can be achieved by varying the concentrations of auxins and cytokinin in the culture mediumwhen the

concentrations of cytokinin are high relative to auxin, shoots are induced; when the concentrations of cytokinin are low relative to auxins, roots are reduced; and at intermediate concentrations the tissues grow as unorganized callus .

This basic concept has been used to regenerate a wide variety of dicotyledonous plants. In general monocotyledonous plants do not show a pronounced response to cytokinins and need high concentration of auxins such as 2, 4-D to obtain changes in the development of cultured tissue. Other plant hormones, particularly abscisic acid and gibberellins have some dramatic action on in vitro organogenesis.

Factor # 9. Culture Medium:

The essential components of plant cell cul¬ture medium are the macro or major salts and micro or minor salts. Besides these, vitamins, amino-acids, carbohydrates etc. are also requir¬ed for in vitro growth and development of plant cells. Inorganic nitrogen's most important role in the plant cells is its presence in the structure of the protein molecule. In addition, nitrogen is found in such important molecules as purines, pyrimidine's and coenzymes.

Factor # 10. Agar-Agar:

Agar-agar is not an essential component of the culture medium. In plant tissue culture, the culture medium is gelled with agar. The quantity of agar is a factor that may have a determining role in organogenesis. Commercially available agar contains impurities.

Factor # 11. pH of the Medium:

The pH of the culture medium is generally adjusted between 5.6 to 5.8 before sterilization. The pH is another factor that may have a determining role in organogenesis.

Factor # 12. Ploidy Level:

Variation in chromosome number i.e. aneuploidy, polyploidy etc. of plant cell in culture has been well-documented. It is generally observed that with the increase of chromosome instability there is a gradual decline in morphogenetic potentiality of the callus tissue. So the most important

factor in maintaining organogenic potential of the callus tissue is the maintenance of chromosome stability. It has been suggested that the frequency of subculture can affect the chro¬mosome stability of cell cultures. So, in order to maintain chromosome stability, cultures are sub-cultured frequently and regularly.

Factor # 13. Age of Culture:

Age of culture often exercises an influence on organ formation. A young culture frequently produces organs. But the organogenic poten¬tial may decrease and ultimately disappear in old culture. In certain cultures of some plants, the plant regeneration capacity may retain indefinitely for many years.

Application of Plant Tissue Culture :

1. Micro Propagation:

The regeneration of whole plant through tissue culture is popularly called “micro-propagation”. This is a technique where a callus mass has been initiated from a single explant taken from any living part of a donor plant and within very short time and space, a large number of plantlets can be produced from such callus tissue. Again, it is possible to make a large number of callus pieces from the original stock culture during sub-culturing.

Then it is possible to produce hundreds of plantlets that develop on each of these callus pieces. Therefore, the most obvious advantage of micro-propagation is the numerical one. Suspension cultures can also be used to exploit this numerical advantage as they produce numerous cell aggregates relatively rapidly, generally growing faster than callus tissues.

The numbers of plantlet production depends upon the number of shoot primordia that can be induced to form within these cell aggregates. Alternatively, if the cell suspension culture happens to be embryogenic, then this propagation potential depends upon the rate at which embryoids are formed by the cell aggregates and the rate at which new embryo genic aggregates are formed in culture.

2. Clonal Propagation:

In vitro clonal propagation is a type of micropropagation. The cultured plants raised from tissue culture are derived asexually and also multiply within culture vessel by asexual means. A sexual reproduction, on the other-hand, gives rise to plants which are genetically identical to the parent plant.

Multiplication of genetically identical copies of a cultivar by asexual reproduction is called clonal propagation and a plant population derived from a single donor plant in tissue culture constitutes a clone. So, the variability that can arise from sexual reproduction and seed formation in a crop plant is omitted.

More specifically, a single plant with desirable characters can be selected from a breeding programme and propagated so that further trials and selections can be carried out as quickly as possible. The plants with long seed dormancy can be raised faster by in vitro clonal propagation than in vivo seed propagation.

3. Production of Genetically Variable Plants:

In some callus culture, there is a major tendency of the callus tissue towards the numerical variation of chromosomes in the cells that occurs after a number of serial subcultures. Such chromosomal variations in culture may arise because of two factors. First, the cells of various ploidy and genetic constitution of the initial ex- plant may be induced to divide and secondly, culture condition may contribute new irregularities.

The chromosomal instability in the cultured cells play an important role in polyploidization of cells and genetically variable plants can be raised from such polyploidized cells by subsequent micro-propagation. Thus, tissue culture is proving to be rich and novel sources of variability with a great potential in crop improve¬ment without resorting to mutation or hybridization.

Variants selected through tissue culture has been variously termed to as calliclones (from callus culture) or protoclones (from protoplast culture). Larkin and Scowcroft have pr oposed a general term “somaclones” for plant variants achieved from tissue cultures, irrespective of their origin.

Such variant plants may show some useful characters such as resistance to a particu¬lar disease, herbicide resistance, stress tolerance etc. Such changes are valuable for crops which are normally propagated by vegetative methods. Moreover, plant breeders can exploit such vari¬ants for their breeding programme.

4. Plant Pathology and Plant Tissue Culture:

There have been many valuable contributions of plant tissue culture to problems concerning plant pathology. One outstanding success is the virus eradication by apical meristem culture and the second success of tissue culture in plant pathology is the result of its application to the problems of plant tumors, especially crown gall.

Virus Eradication: In virus infected plants, the distribution of viruses in plant body is uneven. It is well known that the apical meristems are generally either free or carry a very low concentration of viruses. The apical meristem culture is the only way to obtain a clone of virus free plant which can be multiplied vegetatively under control conditions that would protect them from the chance of reinfection. The elimination procedure of virus can usually be improved by combining it with heat therapy of the host plant or the culture.

Virus eradication by apical meristem culture has enormous horticultural and agricultural value e.g. in the production of plants for the cut flower industry when stock plants of registered line must be maintained in as near-perfect condition as possible. Any infection by virus that affects growth rate or physical characteristics of size and shape is obviously very serious if it afflicts these nuclear stock, for they are the basis of all propagation and breeding.

In the agricultural world, the production or yield of a crop can fall dramatically as a result of viral infection and render that particular variety no longer saleable or commercially viable. Tissue culture techniques could be of value in restoring the original properties of the variety, by removing the infection and so bringing it bank into the commercial market. These virus tested stocks could provide ideal material for the national and international distribution of plants, either for further propagation or use as breeding material. It is hoped that these selected virus free cultures would be acceptable to quarantine authorities.

5. Plant Breeding, Plant Improvement and Plant Tissue Culture:

The conventional breeding methods are the most widely used for crop improvement. But in certain situations, these methods have to be supplemented with plant tissue culture techniques either to increase their efficiency or to be able to achieve the objective which is not possible through the conventional methods.

Embryo culture is now routinely used in recovery of hybrid plants from distant crosses. Some examples are recovery of hybrids from Hordeum vulgare x Secale cereale, Triticum aestivum x Agropyron repens, H. vulgare x Triticum species. In case of Triticale, a rare hybrid between Triticum and Secale develop viable seeds.

But most of the tetraploid and hexaploid wheat carry two dominant genes Kr1 and Kr2 which prevent seed development in crosses with Secale. The hybrid seeds are minute, poorly developed and show very poor germination. By embryo culture, 50-70% hybrid seedlings has been obtained. Hybrid seedlings from T. aestivum x H. vulgare are not obtained. But it has been achieved by embryo culture.

When H. vulgare or T. aesiivum (used as male) is crossed with H. bulbosum (used as female) the chromosome complement of H. bulbosum is eliminated from the developing embryo. Most of the seedling obtained from such crosses are haploid, having only one set of chromosomes either from H. vulgare or T. aestivum parent.

Embryo culture is also useful for propagation of orchids; shortening the breeding cycle and overcoming seed dormancy. In meristem culture, shoot apical meristem along some surrounding tissue is grown in vitro. It is used for clonal propagation and recovery of virus free plants and is potentially useful in germplasm exchange and long-term storage of germplasm through freeze preservation.

Anther and pollen culture has a potential application in plant breeding and plant improvement programme for the production of haploid as well as homozygous diploid plant. All-year-round rapid clonal propagation us¬ing plant tissue culture techniques has highlighted possibilities for new plant improvement tech¬niques.

Protoplast culture and somatic hybridization is a promising line for plant breeding and plant improvement techniques. But, at present, techniques for selection and multiplication of so¬matic hybrid and regeneration of hybrid plants is very limited to a few classical plant species. So it is expected that in near future it would be possible to use this technique for a wide variety of plant species.

Another most important approach is the mutation of tissue culture cells to produce a mu¬tant line from which plants can be raised. Production of mutant line is highly desirable for plant breeding. Callus cells, produced either from vegetative cell or reproductive tissues, can be subjected to a range of mutagenic chemicals e.g. N-nitroso-N-methyl urea or ionizing radiations e.g. gamma rays.

The hope is that permanent changes in the DNA pattern of some of the cells would be achieved by such treatment. Plants could be raised from the treated cultures and any mutant whole plants selected from the population either by physical differences or by metabolic/biochemical differences. Biochemical mutants could be selected for disease resistance, resistance to phytotoxin, improvement of nutritional quality, adaptation of plants to stress conditions e.g. saline soils and to increase the biosynthesis of plant products used for medicinal or industrial purposes.

6. Production of Useful Bio-chemicals:

Man depends on plants for many compounds other than food such as medicines, pigments, vitamins, hormones, flavoring agents, latex and tanins. If most plant somatic cells are totipotent, it should be possible to take a culture of cells from a plant that naturally produces a certain biochemical and cause the culture to produce that chemical under in vitro conditions. The main difficulty is that we do not yet understand the regulation mechanisms that control the production of most biochemical substance and so cannot manipulate them.

Even so, a surprising number of cell cultures have been found that do produce specialized bio-chemicals found in the intact parent, including alkaloid such as nicotine, atropine, ephedrine, caffeine and codeine and their precursors and derivatives. Production of cardiac glycosides and other steroids, benzoquinones, latex, phenolics, anthocyanin’s, organic acids, anti-tumor agents, antimicrobials and various flavors and odours have also been reported.

7. Preservation of Plant Genetic Resources or Gene Conservation Banks:

The need for a programme for the conservation of plant genetic resources arises from the rapid changes that are occurring in modern agriculture practice. The primitive cultivars and wild relatives of crop plants constitute a pool of genetic diversity which is invaluable for future breeding programmes. But these have already led to the replacement by new cultivars which encompass a much narrow range of genetic diversity.

As a result, there is a very real danger of future breeding being impeded by the shrinking genetic bases of some crops. Therefore, storage of this sort of irreplaceable breeding material or germplasm (gene combinations available for breeding) and establishment of a centralised gene bank are the practical ways to solve these problems.

Conventionally, germplasms are stored in the form of seeds because they occupy a relatively small space and can be stored for many years. But there are a number of important species, particularly root and tuber crops, which are normally propagated vegetatively.

In addition, the possibility of using liquid nitrogen freeze-storage techniques for the preservation of cell, tissue and apical meristem is being studied. The advantages of this technique sire that cell division and normal cellular reactions are totally arrested at the very low temperature of liquid nitrogen (-196°C), which means that there should be a high level of genetic stability and that the chemical reaction responsible for cellular damage will not occur.

The plant materials can be stored in liquid nitrogen for desirable period. This technique could be particularly valuable for storage of any germplasm which needs to be maintained in a clonal form. This technique is known as cryopreservation or freeze preservation of tissue or cell..

8. Importance of Tissue Culture in Biotechnology:

In 1981, the European Federation of Biotechnology defined “biotechnology as the integrated use of biochemistry, microbiology and chemical engineering in order to achieve the technological application of the capacities of microbes and cultured tissue and cells”. Some people equate it with the new field of genetic engineering, while others take a broader viewpoint defining it as the evaluation and use of biological agents and materials in the production of goods and service for industry, trade and commerce.

Cell suspension culture in liquid medium is a relatively young field of biotechnology. This technology involves the large-scale culture of isolated plant cell under condition which induces them to synthesize the natural secondary metabolites characteristic of parent plants from which they were obtained.

In recent years, the technique of callus culture and cell suspension culture has also been viewed, particularly from the view-point for the study of biosynthesis and metabolism of steroids and cardiac glycosides. Biotechnologists are also trying to increase the synthesis of natural compounds or new compounds by higher plant cells culture as a result of mixing or feeding transformable precursors in the culture medium.

The metabolic process of transformation or conversion of such added precursors into the natural or new compound within the cell is known as biotransformation. Biotechnologists are also trying to augment the synthesis of medicinally important alkaloids in culture by means of fungal elicitor.

This means that cells are cultured in a liquid medium by adding required quantity of the bacterial filter sterilized extract of certain fungi. It has been observed that the fungal extract in certain cases helps to increase the synthesis of a desirable compound by the higher plant cell.

Biotechnologists are also trying to modify the genetics of the cultured cells by three ways such as:

(i) Mutagenesis and selection of cell lines in cell suspension culture,

(ii) Transplantation of foreign genetic material in protoplasts by means of genetic engineering and

(iii) Somatic hybridization by the fusion of distantly related plant proplast just to widen the genetic diversity of hybrids.