Biyani's Think Tank

A CONCEPT BASED EXCLUSIVE MATERIAL

PLANT TISSUE CULTURE

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Preface

I am glad to present this book, especially designed to serve the needs of the students. The book has been written keeping in mind the general weakness in understanding the fundamental concepts of the topics. The book is self-explanatory and adopts the "Teach Yourself" style. It is based on question-answer pattern. The language of book is quite easy and understandable based on scientific approach.

Any further improvement in the contents of the book by making corrections, omission and inclusion is keen to be achieved based on suggestions from the readers for which the author shall be obliged.

I acknowledge special thanks to Mr. Rajeev Biyani, *Chairman* & Dr. Sanjay Biyani, *Director* (*Acad.*) Biyani Group of Colleges, who are the backbones and main concept provider and also have been constant source of motivation throughout this Endeavour. They played an active role in coordinating the various stages of this Endeavour and spearheaded the publishing work.

I look forward to receiving valuable suggestions from professors of various educational institutions, other faculty members and students for improvement of the quality of the book. The reader may feel free to send in their comments and suggestions to the under mentioned address.

Note: A feedback form is enclosed along with think tank. Kindly fill the feedback form and submit it at the time of submitting to books of library, else NOC from Library will not be given.

Ms. Meesha Srivastava

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Syllabus

PLANT TISSUE CULTURE AND BIOTECHNOLOGY

BT - 803
Section -A

- Historical background and terminology used in cell & tissue culture.
- Basic techniques of cell and tissue culture, surface sterilization, aseptic tissue transfer, concept of totipotency.
- Nutritional requirement of cell in vitro, various types of nutrient media.

Section -B

- Someatic embryogenesis and organogenesis in plant .
- Variability in tissue cultures, somaclonal and other variations.
- Isolation of cells, single cell cultures and cloning.

Section -C

- Micropropagation and cloning of plants, applications of micropropagation in agriculture, horticulture & forestry.
- Haploid production. various techniques, applications.

Section -D

- Production of disease free plants by tissue culture methods.
- Protoplast isolation and culture, fusion of protoplast .
- Somatic hybrids, selection methods. gene expression in somatic hybrid .

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History of Plant Tissue Culture

Q.1. **Explain in brief the history of plant tissue culture.**

Ans. The history of plant tissue culture begins with the concept of cell theory given by chleiden & chwann, that established cell as a functional unit. This concept was experimentally tested by Haberlandt who gave the idea of culturing plant cells.

The significant contributions of selected scientist are as:-

(a) G Haberlandt

- Gave the idea of culturing isolated plant cells in the nutrient solution.
- He isolated mesophyll cells with knop's nutrient solution.
- Haberlandt described the cultivation of mesophyl cells of *Lamium in purpureum* and *Eichhornia crassipes.*
- Using pieces of mature potato tubers he observed that cell division almost without exception when the explants contained a vascular strand.
- He is also known as Father of Plant Biotechnology.

(b) P. Nobecourt

- He is French plant pathologist.
- He gave the possibility of cultivating. Plant tissues for unlimited period.

(c) P.R. Gautheret

- He used piece of cambium cut form tree, attempts were made on liquid medium
- Which failed to grow but on soild medium the very healthy callus was grown .

(d) Philip R. white

- He reported for first time success full continues cultures of tomato root tips and obtained indefinite growth of roots.
- Knop's salt solution later replaced by vitamins pyrodoxine, thiamine and nicotinic acid.

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(e) F.C. Steward

He is Known as of the pioners of plant tissue culture & contributed by giving the requirment of plant tissue culture & developing techniques for the different application.

- ➢ Used coconut water for the first time and obtained good result from it.
- ➢ Gave the somatic embryogenesis concept form cell suspension of carrot cells.

(f) **J. Reinert**

- ➢ Gave the concept of totipotency of cells.
- ➢ Conducted experiment on parenchymat our cells of carrot root in complex medium.
- ➢ Worked on embryogenesis on carrot cells.

(g) **Folke Skoog**

- ➢ Done pioneering work with auxin, a plant growth hormones.
- ➢ Also work with cytokinin, he also should that number of cytokinins occur naturally.
- ➢ He was also pioneer in investigating on how to control formation of roots, stem and leaves.

(h) **Toshio Murashige**

- ➢ He worked on nutrition of plant cells using tobacco pith cells.
- ➢ He formulated the whites medium which was known as Murashige & Skoog medium.
- ➢ Developed the micro-propogation technique.
- ➢ Worked on somatic embryo. formation using carrot and citrus plants.

(i) **Guha & Maheshwari**

- ➢ First time development of haploids through anther and pollen culture.With the development of the technique plant tissue culture and nutritional requirement of plant cell, it was possible to develop news technologies by culturing plant organs such as

- Anther
- Ovary
- Ovule
- Petal

- Leaf
- Meristem
- Leading to establishment of new research lines as:-
- Haploids
- Virus free Plants
- In-Vitro fertilization
- Embryo rescue etc.

SomaticEmbryogenesis

Q.1 **What is somatic embryogenesis? Give its application.**

Ans. Plant cells are totipotent and can produce whole new plants under favourable conditions of nutritional and plant growth regulators. These somatic embryos were similar to zygotic embryos in development and structure. The origin of somatic embryos morphologically developed through the 3 stages:-

(a) Globular

(b) Heart

(c) Torpedo

Embryogenesis is a two step process:-

(1) Induction of embryogenesis

(2) Development of embryo, ultimately leading to germination.

It is of two types:-

(a) Direct Embryogenesis

(b) Indirect embryogenesis.

Indirect Embryogenesis.:-When explants produce callus and the callus forms then its known as indirect embryogenesis.

Direct Embryogenesis.:-

- When embryogenesis. Occur directly on the explants without the production of callus it is known as direct embryogenesis.
- The exogenously supplied auxin is required in appropriate concentration for the induction of somatic embryogenesis from callus or explants.
- In direct embryogenesis cells of explanted tissues are already determined for embryonic development and termed as pre-embryogenic determined cells(PEDC's).

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- In indirect embryogenesis cells require redetermination through a period in culture and termed as induced embryogenesis determined cells(IEDC's).
- Embryogenesis occurs from a single cells or from a group of cells.
- Embryogenesis cells are small, is diametric in shape, filled with dense cytoplasm and have a conspicuous nucleus.
- When somatic embryos, (early stage or developed), are transferred on induction medium they give rise to somatic embryos. This method of obtaining embryos recurrently is known as repetitive or cyclic embryogenesis. This method is useful for continuously obtaining embryos in large no for example *Atropa belledona, Ranunculus*.
- During somatic embryogenesis in cell suspension embryos of different sizes are produced, this can be achieved by sieving or fractionation of suspension with appropriate sieve size. Such cultures may be fully synchronized for their growth.

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Factor's Affecting Somatic Embryogenesis

(a) **Composition of Medium** - levels of sucrose and nitrogen is to be monitored Reduced nitrogen is not require for the induction and oxoid nitrogen alone in high amount is sufficient for induction of somatic embryogenesis However, reduced nitrogen in the embryo development medium supports embryo development. Increased osmotic conc. by sucrose affect the embryo development.

(b) **Auxins** - 2,4D appear to be required for embryo induction but adveresely affect embryo development.

(c) **Cytokinins**-

Except zeatin other cytokinins suppress embryogenesis.

(d) **Ethylene**-

Suppresses embryogenesis.

(e) **Abscisic acid**-

Suppress abnormal development of embryos.- Imparts dormancy and help in the formation of cotyledonary stage somatic embryo.

APPLICATIONS:-

(i) It provides potential in the form of somatic buds. It can be used for the production of synthetic seeds.

(ii) Somatic embryos provides organized culture system, such cultures produce organ specific or differentiation related compounds in higher amounts compared to cell culture of that species.

ADVANTAGES OF SOMATIC EMBRYOGENESIS :-

1) Rapid multiplication through cell culture and use of bioreactors.

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2) Presence of bipolar structure avoids the rooting steps required in organogenesis.

3) Possible to induce dormancy and store the culture for long duration.

4) Possibilities of encapsulation and other methods of packing and direct delivery system can be employed.

5) Provides an important resource for analysis of molecular and biochemical events that occur during induction and maturation of embryo.

6) Isolation of specific storage protein is possible.

7) It shortens the breeding cycle of deciduous trees and increases the germination of hybrid embryos where delayed germination of seed is a significant handicap in rooting of the plants of horticultural intrest.

Q.1 **What is micro propagation?**

Ans. **Introduction:-**

The technique of culturing plant became a wide subject embracing morphology,physiology, biochemistry, molecular biology and genetic engineering multiplication
of plant through plant tissue culture can be achieved by any of the following methods depending on the objectives. The basic concept is to achieve rapid multiple without creating un wanted somaclonal variation.

- Micro propagation is defined as production of miniature planting material in large number by vegetative multiplication through regeneration.
- Axillary budding- It is the development from pre-existing meristems on nodal regions to ensure genetic stability of the regenerants.
- Adventitious budding- De novo formation of adventitious buds (not from pre-existing meristems) may occur directly from the tissues of the explant.
- The technique of micro propagation is as:-

It is divided into four stages:-

Stage I-

Selection and establishment of Aseptic cultures.

i. In this, selection of typical, healthy, disease free mother plants.

ii. Selection of plant is followed by preparation of explants, surface sterilization and transfer to appropriate media.

iii. Sterilization is carried out through soaking in a calcium hypo chlorite.

iv. Main aim is to attain an aseptic culture of the plant.

Stage- II

Multiplication of Propagate

i. In this rapid multiplication of the regenerative system for obtaining large number of shoots.

ii. For this medium and tissue factors are optimized empirically.

Stage - III

Plantlet Regeneration

i. Plantlets are produced through rooting of isolated shoots or germination of somatic embryos.

ii. Shoots of appropriate length or age are required, which depends on the medium Composition.

iii. High auxin concentration composition is used for the shoot development.

iv. Low salt strength of rooting medium facilitates the rooting.

v. *In- vitro* produced shoots are treated with auxins and transferred directly to pot mixture.

Stage - IV

Preparation and transfer to field.

i. It is concerned with transfer of plantlets in pots their hardening and establishment in soil.

ii. This stage is to prepare the propogule for these successful transfer to soil.

iii. Hardening of plants imparts some tolerance to moisture stress and plants become autotrophic from heterotrophic condition.

iv. Stage organs are formed on plantlets their establishment in soil becomes easier.

v. These tuberous organs may require chilling treatment to germinate.

vi. When plantlets are taken out from the vessels adhering with running tap water

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and plantlets are transferred in a soil.

vii. Plantlets are exposed to decreasing humidity by slowly exposing the plant or reducing the mist period in the glass house.

viii. Hardened plants are then transferred to glass or poly houses with normal environmental conditions.

ix. Plants are irrigitated frequently and their growth and variation are monitored regularly.

Advantages of Micro-Propagation:-

1) Shoot multiplication can be achieved in small space so became miniature plantlets can be produced.

2) Propagation is carried out under sterile condition. No damage is caused due to insects and diseases and plantlets are produced from microbes(pathogens).

3) Virus free material is used (even through virus elimination by meristem culture) a large number of virus free plants can be obtained.

4) Plant tissue culture is carried out under defined conditions of environmental, nutritional and tissue system, therefore, it is a highly reproducible system under the defined set of reproducible system under the defined set of conditions(controlled conditions, reproducibility).

5) This production is unaffected by seasonal variations as uniform conditions are maintained (no seasonal effect).

6) No care is required between two subculture as compared to conventional vegetative propagation system like watering, weeding(less care).

7) Small glass house space is required because of miniature size of plant lets

8) Mother plant or genotype of stock plant can be stood and maintained in vitro without damage to environmental factor and stock plants.

9) Being sterile transport across countries is permissible without difficulties (transport across countries does not require phytosanitory regulation).

10) Miniature storage organs(tubers, corns, tuberous, roots) can be produced for genotype storage and subsequent plantation which is also called as Germplasm storage.

11) It is possible to mechanize whole process of vegetative propogation for large scale plantations.

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12) The plants which are difficult to propagate vegetatively by conventional method can be propagated by this method.

Disadvantages of micro-propagation:-

1) Micropropagation method involve expensive material like autoclave, laminar air flow, contaolled culture room.
2) It is a skilled work so a decision making and technique knowledge are required in the personnel.
3) Contamination cause severe damage to material and add to the cost of production, affects time schedule delivery of the material.
4) Genetic stability is not confirmed in certain methods.
5) Explants taken are delicate so it takes longer.
6) Specific conditions for micro-propogation may be required. Therefore, each material requires separate research method.

Q. **What are haploids? Give a briefs description of anther and pollen (n) in this culture.**

Ans. Haploids are plants which has gametic chromosome

- Hapliods may be grouped into two broad categories:-

(a) Monoploids

- Which possess half the number of chromosomes from a diploid species.

(b) Polyhaploids

- It possess half the number of chromosomes from a polyploid species.

Haploid production through anther culture has been referred to as and rogensis while gynogenesis is the production of haploid plants from ovary to ovule culture where the female gamete or gametophyte is triggered to sporophytic development.

Androgenic Methods - It is a method of haploid production which is done from the male gametophyte of an angiosperm plant i.e. microspore(immature pollen).

The underlying principle is to stop the development of pollen cell whose fate is normally to induce a gamete (Sexual cell) and to force its development directly into a plant. -Haploid can be obtained by the culture of excised anthers and pollen.

Anther Culture

Young flower buds with immature anthers which have the microspores are surface

sterilized and rinsed with sterile water.

One of the anthers is crushed in acetocarmine to know the stage of pollen development.

Anthers at appropriate stage are inoculated in the nutrient media.

The anthers in later stage gradually turn brown and within 3-8 weeks they burst open due to the pressure exerted by the growing pollen callus or pollen plants.

They attains a height of about 3-5 cm, the individual plantlets or shoots emerging from the callus are separated and transferred to a medium that would support further development.

Microspore culture

- Haploid plants can be produced through in vitro culture of male gametophyte cells ie microspores or immature pollen.
- General procedure of culture is : -

Anthers are collected from sterilized flower buds in a small beaker containing basal media.

Microspores are then squeezed out of the anthers by pressing them against the side of beaker with a glass rod.

Anther tissue debris is removed by filtering the suspension through a nylon sieve having a pore deameter of 40 inch.

This suspension is then centrifuged at low speed.

The supernatant containing fine debris is discarded and the pollen of pellet is resuspended, in fresh media.

The microspores obtained are then mixed with an appropriate culture medium.

Final suspension is then pipetled into small Petri dishes. (For deation, thin layer of liquid is made).

Each dish is then sealed with parafilm to avoid dehydration and is incubated.

The various factors affecting the androgenesis are :-

1. **Genotype**: - For successful culture, the genotype of anther is predominant.
2. **Physiological status of the donor plant**:-The physiological status of the plant at the time of excision of anther influences the sporophytic efficiency of microspores.
3. **Stage of pollen-** Selection of anthers at an :-Appropriate stage of pollen development. Anthers with microspores ranging from tetrad to the binuecleate stage are responsive.
4. **Pretreatment of anthers** :- As the androgenesis is the deviation from the normal development so for the induction certain treatments are given:

(a) **Cold treatment**: -It is given between 3^0 to 6^0C for 3 to 15 days. As a result weak and nonviable anthers and microspore are killed and the material gets enriched.

- This treatment retards aging of the anther wall.

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(b) **Hot Treatment** :- Explants are subjected to 3^0C for 24 hrs or 4^0C for 1 hr. stimulates embryogenesis.

(c) **Chemical Treatment:** Chemicals induce parthenogenesis example- Chloroethylphosphonic acid.

5. **Culture Media** :- Presence of sucrose, nitrate, ammonium salts and amino acids are essential components to be present in a culture medium. Activated charcoal also enhances the percentage of androgenic anthers in some.

Pollen embryogenesis can be induced on an mineral sucrose medium.

Process of androgenesis - Haploid plantlets are formed in two ways:-

(a) **Direct embryogenesis**: Embryos originate directly from the microspores of anthers without callusing.

(b) **Indirect embryogenesis**: It is also known as organogenic pathway, in this microspores undergo proliferation to form callus which can be induced to differentiate into plants.

- **Process of androgenesis** Shows microspores undergo divisions, which continues until it a undergo various stages of development, stimulation those of normal zygotic embryo formation. However when the microspore take organogenetic pathway, then these all increase in size, exerting pressure and the contents are released in the form at callus.
- These calluses differentiate into plantlets. The plants with developed shoots and roots are then transferred to pots.
- The physical environmental conditions in which the cultures are to placed can enhance differentiation. These are:-

(a) Incubation at 24-28^0C

(b) Light intensity of 500 Lux

(c) After induction kept at 14 hr day light at 2000-4000 Lux.

- For obtaining homozygous lines, the plants derived form their anther culture are analysis for this physiology status. Some of these methods are :-

1. **Counting of plastids in the stomata** :- Count the number of plastids in the stomato of a leaf.

2. **Chromosome number**:- It can be counted from pollen mother cells of buds

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which can be collected from regenerated plants. Acetocarmine is used for staining of cells.

3. **Number of nucleoli**:- Haploids contain one nucleoli whereas diploids have 2 nucleoli

4. **Flow cytometric analysis**:- Nuclear DNA content reflects the ploidy state of the donor which is determined by flow cytometery.

Depolarization

Haploids can be diploidized to produce homozygous plants by following method :-

1. Colchicine Treatment
2. Endomitosis(Chromosome duplication without nuclear division)

Significance and uses of Haploids

a) Development of pure homozygous lines.
b) Hybrid development.
c) Induction of mutation.
d) Induction of genetic variability.
e) Generation of exclusively male plants.
f) Cytogenetic Research.
g) Significance in the early release of varieties.
h) Hybrid sorting in plant breeding.
) Disease resistance.
j) Insect resistance.
k) Salt tolerance.

Gynogenic Haploids

- Recent advances has lead to the induction of haploid from ovary and ovule culture.
- The Megaspores or female gametophytes of angiosperms can be triggered in vitro saprophytic development.
- In vitro culture of unplanted ovaries and ovules represent and alternative for species

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- Ovaries can be cultured as pollinated and unpollinated.

Procedure

Ovaries are removed and surface sterilized.

Before culturing the tip of distal part of the pedicel is cut off and the ovary is implanted with the cut end inserted in the nutrient media.

When liquid medium is to be employed, the ovaries can be placed on a fitter paper and inserted into the medium.

Factors affecting gynogenesis

1) Genotype
2) Growth condition of the donor plant
3) Stage of harvest of ovule
4) Embryo sac stage
5) Culture conditions
6) Seasonal effects
7) Physical factors.

Q.1 **What is somaclonal variation?**

Ans. The growth into whole plants is an asexual process, involving only mitotic division of the cell. As expected that the process will produce genetically uniform plants orclonal multiplication is possible through callus regeneration.

This provided a basis of genetic manuplation in plants using callus. The origin of the genetic variation is as we know the plant cells are totipotent it is possible to have regeneration from single cells or protoplast, but in this process, a cell divides and redivides several hundred times to produce callus and subsequently organs.

Earlier terms such as "calliclones" and "protoclones" were coined to indicate variation arising in regenerated plants from stem and protoplast derived callus respectively. A

general term somaclones has been given for plants derived from any form of somatic cell culture and somaclonal variation is the variation displayed amongst such plants.

- Gametoclonal variation has been introduced for variation observed in gametic cells.
- The two methods of obtaining somaclonal variation are:-

(a) Without **in vitro** selection An explant is cultured on a suitable medium.

The basal medium is supplemented. With growth regulators which support the differentiation of callus.

These cultures are subcultured and then transferred to shoot induction medium for plant regeneration.

The plants so regenerated are transferred to pots grown to maturity and analyzed for variants.

Somaclonal variants for various characters are not selected with directed approach as both dominant and homozygous recessive traits can be directly selected.

(b) With **in vitro** selection

In vitro culture of higher plants can be used for selection of mutants.

Selection for resistance is the most common method for mutant selection, resistant cells in a large population can be selected by their ability to grow in the presence of an inhibitor.

The dedifferentiated culture(callus) is subjected to selection against inhibitors like antibiotics, amino acid analogs etc.

These compounds are put in the medium at a concentration such that some cell population survives and can be further grown on a selective medium.

If plants are resistant to the inhibitor, then stable transmission of that character is analayzed in subsequent generations.

In this approach, variants for a particular character are selected rather than the general variation obtained in first case where selection is done at the plant level.

➢ Various factors influencing the somaclonal variation are:-

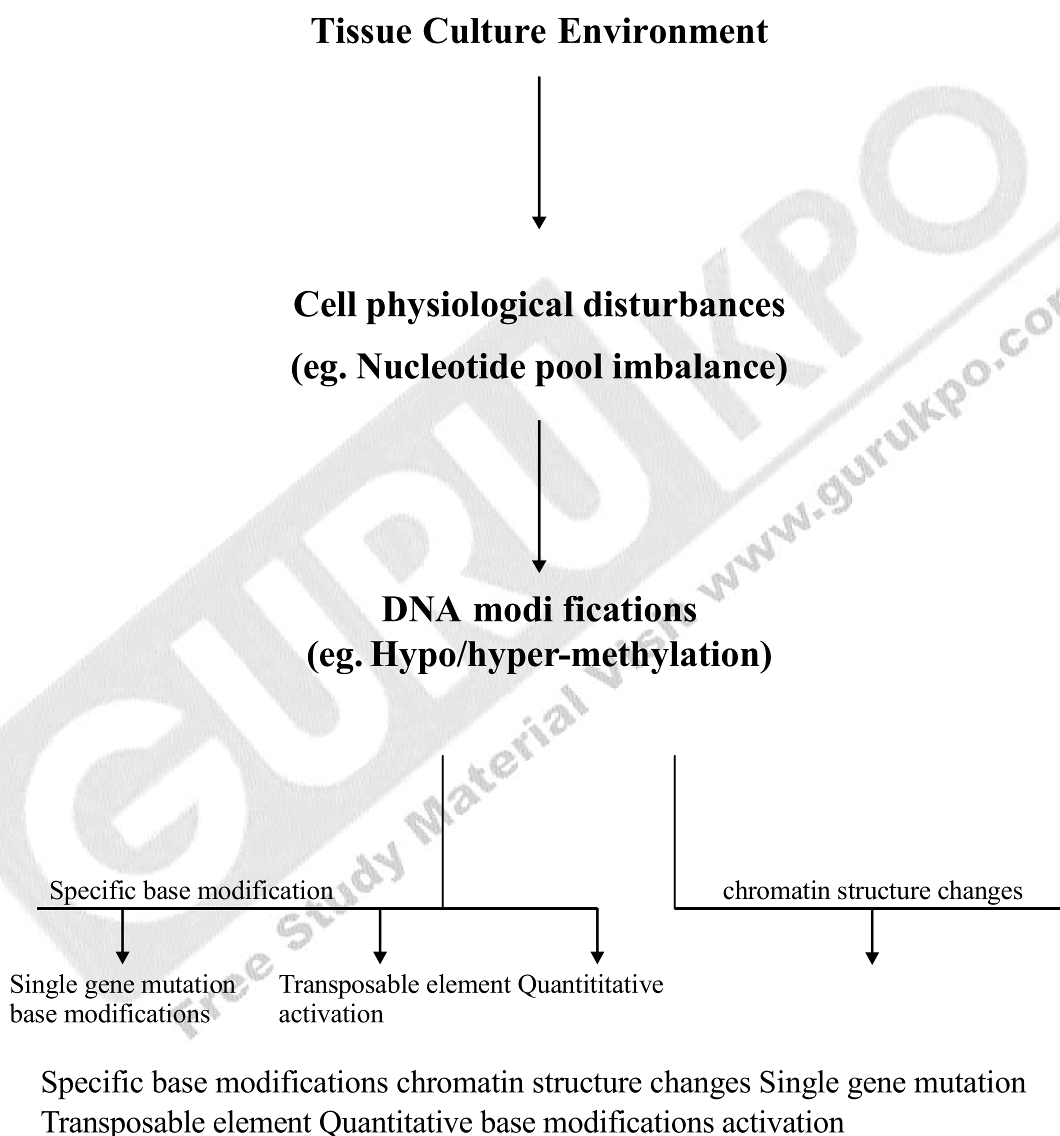
a) Genotype - It can influence both frequency of regeneration and the frequency of somaclones.

b) Explant source - It is a critical variable.

c) Duration of cell culture - Most long term cultures are chromosomally variable. Thus variation increases with increasing duration of culture.

d) Culture conditions - Growth regulators can influence the frequency of karyotypic alteration in cultured cells.

Hypothesis related DNA modification to various mutational events leading to somoclanal variation.



Specific base modifications chromatin structure changes Single gene mutation Transposable element Quantitative base modifications activation

(a) Insertion tariff variant or substitution.

(b) Excisions (c) Chromosome breakage.

Late replication- induced chromosome breakage.

1. Rearrangements dependent on heterochromatin, distribution.

2. Chromosome type, break fusion, bridge cycle.

Disadvantages of somaclonal variation:-

a) Variation is cultivar dependent.

b) Frequencies of change vary.

c) Many changes are desirable.

d) Some changes are unstable.

e) Many changes are not novel.

f) Characters of interest may not change.

Advantages of somaclonal variation:

i. A rapid source of variation is available.

ii. Some changes occur at higher frequency.

iii. Agronomic tariff can change.

iv. Novel variants can rise.

v. Improved plants through somaclones.

Various methods of assessment are:-

A. Phenotypic parameters

Quantitative eg. Leap size, plant height etc.

Qualitative eg. Branching pattern, flower colour.

B. Physiological parameters

- Protein patterns by electrophoresis for an enzyme, or total content.
- Secondary products formation eg alkaloid and steroid etc.

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C. Genetic parameters

- Chromosome number and structure.
- Giemsa/C-Banding pattern of chromosomes
- RFLP, RAPD analysis for alteration in DNA segments.

Q.1 **What is organogenesis ?**

Ans. **Introduction**- Potentiality of a plant cell to regenerate the entire organism (Plant) is termed as totipotency. This potentiality has been used for culturing of protoplast, cells, tissues and organs in vitro.

Organogenesis :-

This is a process by which cells and tissues are forced to undergo changes which lead to the production of a unipolar structure, namely a shoot or root primordium, whose vascular system is often connected to a parent tissue.

(i) **History -** controlled experiments of organogenesis by white 1939, he obtained shoots on callus of a tobacco, these finding were extended by skoog 1944. Who showed that auxin could stimulate rooting and inhibit shoot formation. Further studies of skoog and co-workers conclusively established a balanced combination of auxin and cytokinin controls the root and shoot formation he was also associated with discovery of cytokinin.

There are several advantages of plantlet regeneration through plant biotechnological method using organogenesis or embryogenesis the advantages are:-

a) Efficiency of process (formation of plantlet in four steps).

b) Potential for production of higher nos. of plantlet and the morphological and cytological uniformity of the plantlets.

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Q.1 **What are basic tools and techniques and various sterilization methods of plant tissue culture?**

Ans. Various tools and techniques used are:-

1) ph meter
2) Autoclave -works on the principle of pressure cooker
3) Plant growth chamber.
4) Laminer Air Flow- works on the principle of HEPA.
5) Microscopy.
6) Colorimeter.
7) Centrifugation.
8) Chromatograply

1. Paper
2. Thinlayer
3. Two dimensional

9) Thermometer
10) Hygrometer

The methods of sterilization are:-

1. **<u>Laboratory - cleanliness:-</u>**

1) Minimize the air current in the working area as much as possible.
2) Stare pro
3) Separate area for cleaning.
4) Laboratory, three types of sterilization is used

a) a dry heat

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b) Wet heat

c) Filter sterilization

2. **Sterilization of tools**

Disinfectants are used for the sterlization of tools. Some of them are:-

(i) Ag (ii) Chlorine

3. **Explants – Sterilization**

A suitable sized explant can be sterilized by any one of the following:-

1) 1-4% saturated solution of calcium hypochlorite.
2) 1% solution of bromine water
3) 70% ethyl alcohol
4) 0.1 - 0.2% mercuric chloride
5) 7% of sodium hypochlorite
6) 10% hydrogen peroxide solution
7) 1% silver nitrate solution

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B.Sc./M.Sc. (Part III) EXAMINATION, 2011

(Faculty of Science)

(Common to Three and Five Year Integrated Course)

BIOTECHNOLOGY

PAPER BT- 803

(Plant Tissue Culture and Biotechnology)

TIME ALLOWED: THREE HOURS

Maximum Marks-50

1) No supplementary answer -book will be given to any candidate. Hence the candidates should write the answer precisely in the main answer-book only.

2) All the parts of one question should be answered at one place in the answer-book. One complete question should not be answered at different places in the answer book.

Attempt FIVE questions in all, including Question No. 1 which is compulsory selecting one question from each Unit.

1. Fill in the blanks:-

i. Main role of growth hormone in cultural materials is …………. .

ii. Somatic embryogenesis was first induced in ………, ……..and……of…..by……. .

iii. Somaclonal variation has been proved as an alternative tool to ……. For generating……. .

iv. Fusion of nucleated or enucleated cells produces ………… .

v. Name the scientist who first isolated the protoplasts of plant tissue by using cell wall degrading enzymes. ……… year …….. .

vi. Who first indicated that organogenesis could be chemically controlled? ……….. .

vii. Gelling agent / Soldifying agent is obtained from ……….. .

viii Haploid plants are useful in :-

a) ……….

b) ……….

c) ……….

ix. Plant materials are surface sterilized by ………… ,………. .

x. Somatic embryos are also called ………. . They are similar to ………., except that they originate from ………. and ………. In size. 1x10=10

UNIT I

2. Describe the histological background of Tissue Culture Technique in detail.

Or

3. Write notes on the following:-

a) Totipotency

b) Various types of Nutrient media.

UNIT II

4. Define somatic embryogenesis and organogenesis in plants. Explain their applications.

Or

5. Define somaclonal variation. What is its importance? Explain with suitable examples.

UNIT III

6. Describe application of micropropagation in agriculture, horticulture and forestry.

Or

7. What are haploids? How do you produce through tissue culture technique? Mention their applications.

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UNIT IV

8. Explain in detail:-
   a) Role of tissue culture in producing disease free plants.
   b) Define somatic hybrid. Narrate the selection method and gene expression in somatic hybrid.

Or

9. Describe the whole technique of isolation of protoplast, culture them and fusion of protoplast. Mention its application also.

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