2.	Haberlandt	Suggest totipotency of cell First attempt of plant tissue culture (Father of Plant Tissue culture)
3.	Hannig	First attempt to culture embryo of selected crucifers
4.	Knudson	Asymbiotic germination of orchid seeds in vitro
5.	Robbins	In vitro culture of root tips
6.	Laibach	Use of embryo culture technique in interspecific crosses of linseed (linum)
7.	Gautheret	In vitro culture of the cambial tissue of a few trees and shrubs, although failed to sustain cell division
8.	P.R. White	Successful culture of tomato roots
9.	Kogl et al	Identified 1st plant growth regulator
10.	Gautheret, Nobecourt and White	Successful establishment of continuously growing callus cultures
11.	Gautheret	In vitro culture of cambial tissues of Ulmus to study adventitious shoot formation
12.	Van Overbeek	Use of coconut milk containing a cell division factor for the first time to culture Datura embryos
13.	Braun	In vitro culture of crown gall tissues
14.	Skoog	In vitro adventitious shoot formation in tobacco
15.	E. Ball	Raising of whole plants of *Lupinus* and *Tropaeolum* by shoot tip culture
16.	Morel and Martin	Use of Meristem culture to obtain virus-free Dahlias
17.	Tulecke	Production of haploid callus of the gymnosperm *Ginkgo biloba* from pollen
18.	Muir et al	First plant regenerated from a single cell
19.	Miller et al	Discovery of kinetin, a cell division hormone
20.	A. Kornberg et al	In vitro synthesis of DNA
21.	Skoog and Miller	Discovery of the regulation of organ formation by
22.	Maheshwari and Rangaswamy	Regeneration of somatic embryos in vitro from the nucellus of Citrus ovules
23.	Reinert and Steward	Regeneration of embryos from callus clumps and cell suspensions of carrot (Daucus carota)
24.	Gautheret	Publication of first handbook on "Plant Tissue Culture"
25.	Kanta	First successful test tube fertilization in Papaver rhoeas
26.	E. Cocking	Enzymatic degradation of cell walls to obtain large number of protoplasts
27.	Bergmann	Filtration of cell suspensions and isolation of single cells by plating
28.	Murashige and Skoog	Development of Murashige and Skoog nutrition Medium
29.	Guha and Maheshwari	Production of first haploid plants from pollen grains of Datura (Anther culture)
30.	Morel	Produced the protocorns from orchids under invitro condition
31.	H.G. Khorana H.G. Khorana et al.	Awarded Nobel prize for deciphering of genetic code Deduced the structure of a gene for yeast alanyl tRNA
32.	Bourgin & Nitsch	Obtained haploid plants from pollen grains of tobacco
33.	Meselson and Yuan	Coined the term "Restriction endonuclease" to describe a class of enzymes involved in cleaving DNA
34.	Carlson	Selection of biochemical mutants in vitro by the use of tissue culture derived variation
35.	Power et al.	First achievement of protoplast fusion
36.	H. Temin and D. Baltimore	Discovered the presence of reverse transcriptase (a RNA directed DNA polymerase which has the ability to synthesize cDNA using mRNA as a template
37.	Smith	Discovery of first restriction endonuclease from Haemophillus influenzae Rd. It was later purified and named Hind 11

Q. ... about the branches, the scope & importance of biotechnology in promoting human welfare. (09-10)(11-12)(13-14)

Ans:- Biotechnology is the controlled use of biological [illegible] such as microorganisms or cellular components of cells for welfare of human being.

Branches of Biotechnology

1. Medical biotechnology
2. Industrial biotechnology
3. Environmental biotechnology
4. Plant biotechnology

Scope of biotechnology

1. Medical Biotechnology helps to convert various plants as well as animal disease by highly valuable drugs or by gene therapy for the genetic disease. E.g. Insulin, antibodies etc
2. Industrial Biotechnology :- useful for the large scale production of a variety of biochemical's ranging from alcohol to antibiotics & in processing of foods & feeds
3. Agricultural Biotechnology:- Rapid & economic clonal multiplication of the fruits & forest trees, production of virus free stock of the clonal crops, transfer of the valuable genes by genetic engineering to improve their crop production & protection from the diseases & pests e.g. B.T. cotton.
4. Environmental Biotechnology:- Bacteria ,algae, etc are being utilized for detoxification of the industrial effluents , for the treatment of sewage & for the biogas production
5. Animal biotechnology :- Genetic engineering is used to develop the transgenic animals resistant to certain diseases, capable of faster growth rates & more efficient feed conversion with a capacity to produce high milk production from which various biochemicals are isolated
6. Enzyme Technology: : Various microorganisms are used for commercial production of the various restriction enzymes which are used in genetic engineering.
7. Microbial Mining:- Microorganisms are used for the extraction of metals.
8. In vitro fertilization & embryo transfer techniques (Test Tube Babies technique have permitted the childless couples, suffering from one or other kind of sterility, to have their own babies

Importance of Biotechnology

1. Tissue Culture Technique in Biotechnology: - An important aspect of all biotechnology processes is the culture of either the microorganisms or plant or animal cells (protoplast in case of plants) or tissues and organs in artificial media. While microbes in culture are used in recombinant DNA technology and in variety of industrial processes
2. Gene Technology as a tool for Biotechnology :- Most biotechnology companies make use of gene technology or genetic engineering, which involves recombinant DNA & gene cloning
3. Protein Engineering:- protein engineering will lead to production of superior enzymes & storage proteins. Biotechnology has also provide us with remarkable in the form of immobilized enzymes system which allow the production of proteins
4. Metabolic Engineering:- one of the major objective of biotechnology research is the use of living systems for production of metabolic at the industrial scale
5. Biotechnology in Medicine: - In the field of medicine, insulin and interferon synthesized by bacteria have already been released for use. A large number of vaccines for immunization against deadly diseases, DNA probes and monoclonal antibodies are discovered
6. Biotechnology in Industries:- useful for the large scale production of a variety of biochemical's ranging from alcohol to antibiotics & in processing of foods & feeds
7. Biotechnology in Environment:- Bacteria ,algae, etc are being utilized for detoxification of the industrial effluents , for the treatment of sewage & for the biogas production.
8. Biotechnology in Agriculture:- it deals with production of transgenic plants such as BT cotton , tomato for efficient production of agricultural crops

A. Callus culture

B. Cell suspension culture

a) Batch culture

I. Slowly rotating

II. Shake culture

III. spinning culture

IV. stirred culture

b) Continuous culture

I. Chemostat

II. Turbidostat

- A. Callus culture :- callus culture may be derived from a wide variety of plant organs roots, shoots, leaves (or) specific cell types Eg:- Endosperm, pollen. Thus when any tissue (or) cell cultured in an agar gel medium forms an unorganized growing and dividing mass of cells called callus culture. In culture, the proliferation can be maintained more (or) less indefinitely by sub culturing at every 4-6 weeks, in view of cell growth, nutrient depletion and medium drying.

Callus cultures are easy to maintain and most widely used in Biotechnology. Manipulation of auxin to cytokinin ratio in medium can lead to development of shoots or somatic embryos from which whole plants can be produced subsequently Callus culture can be used to initiate cell suspensions which are used in a variety of ways in plant transformation studies.

Callus cultures broadly speaking fall into one of the two categories.1) compact 2) friable callus

B. Suspension Culture:- When friable callus is placed into a liquid medium (usually the same composition as the solid medium used for callus culture) and then agitated single cells and / or small clumps of few to many cells are produced in the medium is called suspension culture Liquid cultures may be constantly agitated generally by a gyratory shaker of 100-250 rpm to facilitate aeration and dissociation of cell clumps into small pieces. Suspension cultures grow much faster than callus cultures, need to be sub-cultured at every week, allow a more accurate determination of the nutritional requirement of cells and even somatic embryos.

The suspension culture broadly grouped as 1) Batch culture 2) Continuous culture

a) Batch culture

These cultures are maintained continuously by propagating a small aliquot of inoculum in the moving liquid medium and transferring it to fresh medium (5x dilution) at regular intervals, Generally cell suspensions are grown in flasks (100-250 ml) containing 25-75 ml of the culture medium. Batch suspension cultures are most commonly maintained in conical flasks incubated on orbital platform shakers at the speed of 90-120 rpm. The biomass growth in batch culture follows the fixed pattern, When the cell number in suspension cultures is plotted against the time of incubation, a growth curve is obtained. The cells in culture exhibit the following five phases of a growth cycle.

i. lag phase where cells prepare to divide

ii. Exponential phase, where the rate of cell division is highest.

iii. linear phase, where cell division slows but the rate of cells expansion increase

iv. Decelerating phase, where the rates of cell division and elongation decreases.

v. Stationary phase, where the number and size of cells remain constant.

1. Slowly rotating

Single cells and cell aggregates are grown in a specially designed flasks, the nipple flask. Each nipple flask possesses eight nipple like projections. The capacity of each flask is 250 ml. Ten flasks are loaded in a circular manner on a large flat disc of a vertical Shaker, When the flat disc rotates at the speed of 1-2 rpm the cell within each nipple of the flask are alternatively bathed in a culture medium and exposed to air.

2. Shake culture

It is very simple and effective system of suspension culture. In this method, single cells and cell aggregates in fixed volume of liquid medium are placed in conical flask, Conical flask are mounted with the help of clip on a horizontal large square plate of an orbital platform shaker, The squire plate moves by a circular motion at 60-180 rpm

3. Spinning culture

Large volume of cell suspension may be cultured in 10 L bottles which are rotated in a cultured spinner at 120 rpm at an angle of 45°

4. Stirred culture

This system is also used for large scale batch culture (1.5 to 10.0 litre). In this method, the large culture vessel is not rotated but the cell suspension inside the vessel is kept dispersed continuously by bubbling sterile air through culture medium. The use of an internal magnetic stirrer is the most convenient way to agitate the culture medium safely. Magnetic stirrer revolves at 200-600 rpm. The culture vessel is a 5-10 litres round bottom flask

b) Continuous culture system.

In this system, the old liquid medium is continuously replaced by the fresh liquid medium to stabilize the physiological stage of the growing cells. Normally the liquid medium is not changed until the depletion of some nutrients in the medium and the cells are kept in the same medium for a certain period. As a result, the active growth phase of the cell declines the depletion of nutrient. The cells passing through out flowing medium are separated mechanically and reintroduce ill the culture

1. Chemostats

In this system culture vessels are generally cylindrical or circular in shape and possess inlet and outlet pores for aeration and for introduction of and removal of cells and medium. The liquid medium containing the cell is stirred by a magnetic-stirrer. The introduction of fresh sterile medium which is pumped in at a constant rate into the vessel is balanced by the displacement of an equal volume of spent or old medium and cells. Such a system can be maintained in a steady state so that new cells are by division at a rate which compensate the number lost in outflow of spent medium

2. Turbidostat

In this system the input of medium is intermittent as it is mainly required to control the rise in turbidity due to cell growth. The turbidity of suspension culture medium changes rapid when cells increases in no due to their steady state growth. The changes in turbidity of the culture medium can be measured by the change of optical density of medium. In Turbidostat an automatic monitoring machine is connected with the culture vessels & such units adjust the medium flow in such a way as to maintain the optical density or pH at chosen, present level

Q.): Define micropropagation. Enlist stages of micropropagation & explain in detail the application of micropropagation (09-10) (13-14)

Ans> Clonal propagation through tissue culture is called micropropagation

Stages Of Micropropagation

1. Stage 0 preparation & pre-treatment of Explant
2. Stage 1 initiation of Explant
3. Stage 2 Multiplication of tissue
4. Stage 3 regeneration of whole plant
5. Stage 4 hardening for subsequent field evaluation

Application Of Micropropogation

1. Micropropagation of hybrid has the greatest multiplication advantage since it can result in large no. of elite plants from a very tissue clump take from the hybrid plants
2. Maintenance of inbreed line for producing F1 hybrid

3. Maintenance of male sterile genotype of wheat & onion are useful in hybridization
4. Selective propagation of dioecious plants Eg: female plants of papaya, male plants of asparagus
5. Multiplication of particular heterozygous superior genotype with increased productivity Eg; oil-palm
6. Shoot culture of some spp are maintained as slow, growth culture of germplasm conservation
7. Rapid production of disease free material
8. Tissue culture can be used to minimize the growing space in commercial for maintenance of shoot plant

Q4: Give the advantages & disadvantages of Micropropagation?

Ans:- Advantages:-

1. To get genetically uniform plants in large number
2. Only a small Explant is enough to get millions of plants with extremely high multiplication rate
3. Rapid multiplication of rare and elite genotypes
4. This technique is possible alternative in plants species which do not respond to conventional bulk propagation technique
5. In plants with long seed dormancy micro propagation is faster than seed propagation
6. Useful to obtain virus free stocks
7. In dioecious species plants of one sex is more desirable than those of other sex Eg:- Male asparagus and Female papaya, In such cases plants of desired sex can be selectively multiplied by this technique
8. This technique is carried out throughout the year independent of seasons
9. Undesirable juvenile phase associated with seed raised plants does not appear in micropropagation plants of some species

Limitations

1. This technique has limited application because of high production cost
2. At each stage the technique has to be standardized
3. Suitable techniques of micro propagation are not available for many crop species
4. Somaclonal variation may arise during *in vitro* culture especially when a callus phase is involved eg- banana
5. Vitrification may be problem in some species
6. Browning of medium is a problem in woody (Adult trees) perennials
7. Requires highly advanced skills
8. Requires a transitional period before the plants are capable of independent growth
9. The plants obtained are photosynthetically not self sufficient
10. The plantlets are susceptible to water losses in external environment and they have to be hardened to the external atmosphere

Q5: Explain the term somaclonal variation. Describe in brief its application, advantages & limitations [09-10]

Ans:- Somaclonal variation:- It is the genetic variability which is generated during tissue culture.

Application of Somaclonal Variation with Achievements:-

1. Novel Variants:- Any implication of Somaclonal variation Inbreeding is that novel variants can arise and these can agronomically used. A number of breeding lines have been developed by Somaclonal variation. e.g. An improved scented Geranium variety named 'Velvet Rose' has been developed.
2. Disease resistance:- Development of disease resistant genotype in various crop species can contributed by Somaclonal variation. Ex- Sugarcane With resistance to Fiji disease, Tomato variety ONAP-17 is a somaclonal variant with monogenic Fusarium wilt resistance.
3. Abiotic stress resistance:- Somaclonal variation has resulted in several interesting biochemical mutants. In vitro selection has also used to obtain plants with increased acid soil, salt, aluminium and herbicide resistance. Ex Salt tolerance rice, maize and tobacco. Aluminium tolerance tomato, carrot sorghum.
4. Herbicide Resistance:- Through in vitro selection several cell lines resistance to herbicides have been isolated and a few have been regenerated into complete plants. Ex-

Crop	Resistance to Herbicide
Tobacco	Glyphosate
Maize	Glyphosate

5. Insect Resistance: - Zetmetra et al. Used in vitro selection technique for generation of Somaclonal variants for Russian wheat aphid (*Diuraphis noxios*) in wheat
6. Seed Quality: - Recently a variety Bio L 212 of Lathyrus(*Lathyrus sativa*) has been identified which is low in ODAP(neurotoxin)which is Developed by Somaclonal variation that improves seed quality
7. Aline gene Introgression: - Which can help widen the crop germplasm base, particularly by culturing immature embryos of wide crosses where crop and alien chromosomes cannot replicate through meiosis,
8. Production of male sterile lines
9. Production of antibiotic resistance lines

Advantages of Somaclonal Variation: -

1. The major benefit of somaclonal variation is improvement in plant.
2. Somaclonal variation leads to the creation of additional genetic variability.
3. In vitro culture in somaclonal variation includes resistance to disease pathotoxins, herbicides and tolerance to environmental or chemical stress.
4. Micropropagation can be carried out throughout the year independent of the Seasons.
5. A very effective selection can be practiced at the cell level for several traits e.g. disease resistance etc
6. This is the only approach for the isolation of biochemical mutants, especially auxotrophic mutants, in plants

Disadvantages/ limitations: -

1. Uncontrollable and unpredictable nature of variation and most of the variations are of no apparent value
2. The variation is depends on cultivar.
3. The variation obtain is not always stable and heritable.
4. The changes occur at variable frequencies.
5. All the changes obtained are not novel.
6. In majority of the cases, Improved variants have not been selected for breeding purposes

Q6: Describe in brief the procedure used for obtaining somaclonal variation along with achievements & mechanism of somaclonal variation? (10-11)(11-12)

Ans:- Procedure of Somaclonal Variation with in vitro:

1. Isolation of the callus and suspension cultures.
   Isolation is an important task. Since several changes are involved in producing somaclonal variation in different plant species, it is very difficult to sort out the somaclonal variants using a single selection system. A number of selection systems are now being used to select the variants.

   A. Selection without selection pressure
   Unorganized callus and cells grown in cultures for various periods on a medium that contains no selective agents (toxic or inhibitory substance), are induced to differentiate whole plants. The regenerated plants are ultimately transferred to the field and screened for variation. Somaclonal variants Of various crop like sugarcane, potato, tomato, geranium, cereals & grasses and Lucern have been Isolated for various desirable traits.

   B. Selection with selection pressure
   In this method variant cell lines are screened from cultures by their ability to survive in the presence of a substance in medium that may be toxic/inhibitory or under conditions of environmental stress. e.g. amino-acid analogue and amino-acids resistance ,disease resistance, herbicide resistance environmental stress tolerance, auxotrophic lines, antibiotic resistance etc.

2. Determination of the cell number:- Take the suspension and filter off the culture through a wire mesh. Volume of filtrate containing single cells and small clumps and place the drop of this suspension to haemocytometer to determine the number of cells by the equation

sr	Crops	Achievements
1	Rice	Seed weight, seed protein percentage, tiller no, panicle length, time of flowering. At IRRI were achieved
2	Wheat	Gliadin protein in seed, grain colour, plant height, heading, date & yield were manifested
3	Maize	Plant regenerate resistance to both T-toxin & infection by *Drechslera maydis*, causing southern leaf blight
4	potato	Resistance against *phytophtora infestans* & *Alternaria solani*
5	Tomato	Resistance to Fusarium oxysporium Jointless pedicel, tangerine virescent leaf, flower & fruit colour
6	sugarcane	Resistance to smut disease caused by *Ustilago scitamini*, downy mildew, eye spot disease.
7	geranium	Development of Velvet Rose

Mechanism of somaclonal variation:

1. The pre-existing genetic variation in the explants tissue;
2. Spontaneous mutation during many division cycles of the Explant
3. Numerical and structural changes in chromosome during in vitro growth
4. Intracellular mutagenic agents produced during in vitro growth
5. Activation of jumping genes are genetic entities which have the locus at which they get integrated is matured

Q.7 Define the term transgenic plant & describe in brief various applications of transgenic plant in crop improvement (09-10) (13-14)

Ans:- **Transgenic Plant:** - A plant in which a gene has been transferred through genetic engineering is called transgenic plant

Various applications of transgenic plant in crop improvement

1. **Herbicide Resistance:** - Biodegradable herbicides are most desirable but they are non selective. Therefore the development of crop plants resistance to these herbicides is necessary. This can be obtained by three ways (1) Overproduction or insensitivity of the enzyme of herbicide (2) Degradation or inactivation of enzymes (3) transgenic Petunia plant resistance to Glyphosate were developed by transferring a gene which over produce enzyme ESPS.
2. **Insect Resistance:** - Cry gene/protein (crystal protein) from a bacterium Bacillus thuringensis (Bt) effective in protecting crop plants from destructive insect attacks e.g. Hellcoverpa armigera. Bt produces a protein (delta endotoxin) during sporulation. Cry proteins are active against larvae of the target insects like lipidopterans, dipterans. Their use is limited because of their high cost & instability under field condition.
3. **Virus Resistance:** - Various approaches have been used for resistance as (1) Coat protein approach (2) cDNA of satellite RNA, (3) Defective viral genome, (4) antisense RNA approach, etc. Out of these of coat protein approach has been most successful. The effectiveness of coat protein gene in conferring virus resistance can be affected by both the amount of coat protein produced in transgenic plant and the by the conc. Of virus inoculum. The first transgenic plant of this type was tobacco by the coat protein gene tobacco Mosaic Virus (TMV)
4. **Disease Resistance:** - Disease resistance is transferred in plants by following transgenes: (1) Genes specifying toxin inactivation, 2) genes encoding insensitive target enzymes. (3. Expression of antibacterial peptides (4) Expression bacterial lysosomes, etc. Disease resistance is inserted in plant by the transfer of resistance gene in the genome of the plant

5. Drought Resistance: - Number of genes are isolated, cloned and expressed in plants which are potential source of abiotic stress in crop plant .The genes responsible for drought resistance are isolated from the other plant & transferred in the target plant which shows drought resistance.
6. Seed protein quality: - Genes for seed storage proteins are transferred into tobacco from cereals and pulses to increase the protein quality. Rice does not contain the provitamin-A i.e. β-carotene. Three transgenes were inserted in rice by Agrobacterium mediated method and a new variety is developed knows as Golden Rice
7. Biochemical production: - Many valuable biochemicals are produced by using microbes but it can be made easier cheaper by using plants. The biochemical's can be produced in plants by transferring gene, encoding valuable proteins/enzymes. The gene encoding Hirudin (antithrombin protein) has been transferred In B. napus it is expressed in seed and Hirudin accumulates in oil bodies.

Q8c. Enlist various methods of genetic transformation & explain in detail Agrobacterium mediated transformation with suitable example diagram (09-10), (10-11) (11-12)

Ans. Various Methods Of Genetic Transformation -

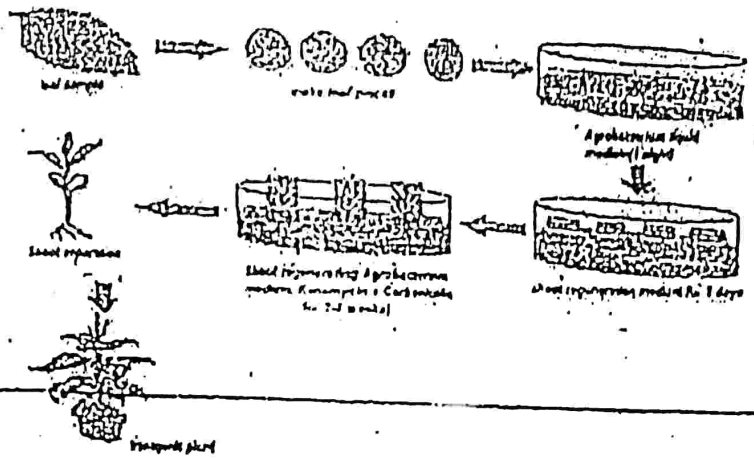
1. Agrobacterium mediated (indirect method)
2. DNA mediated/ Direct mediated
   - Chemical method ---------- 1. PEG mediated
     2. Calcium Phosphate co-ppt
     3. DMSO mediated
     4. DEAE mediated
   - Electroporation
   - Microprojectile
   - Lipofection
   - Microinjection
   - Macroinjection
   - Pollen transfer
   - DNA transfer via pollen tube
   - Ultra sound/ laser induced
   - Silicon carbide
   - Fiber mediated gene transfer

Agrobacterium mediated gene transfer:- it is achieved in following two ways -

A. Co-culture with tissue Explant
B. *In planta* transformation

A. Co-culture with tissue Explant:- the appropriate gene construction is inserted within the T-region of a disarmed Ti plasmid, either a co-integrate or a binary vector is used. The recombinant DNA is placed in Agrobacterium which is then co-cultured with the plant cell or tissue to be formed about 2 days. In case of many plant species, small leaf discs are excised from surface sterilized leaves & used for co-cultivation. E g tomato, tobacco, petunia etc

B. *In planta* transformation:- surface sterilized leaf disc of plants are inoculated with Agrobacterium tumefaciens strains having a modified tumour inducing plasmid & cultured for 2 days. The leaf disc that develop infection are transferred to a selection medium enriched only with kanamycin. The regeneration of transformed plant within 2-3 weeks of the culture



Q9: Define Somatic embryogenesis. Describe Brief the developmental pattern of somatic embryos Differentiate between somatic embryos & zygotic embryos. (16-11)

Ans:- Defn – the process by which the somatic cell or tissue develop into differentiated embryos and each fully developed embryo is capable of developing into a plantlet.

Development Pattern of Somatic Embryo

1. Somatic embryos generally originate from single cells which divide to from a group of meristematic cells.
2. This multicellular group becomes isolated by breaking cytoplasmic connections with the other cells around it and subsequently by cutinisation of the outer walls of the cell mass.
3. The cell of meristematic mass continues to divide to give rise to globular, heart shaped, torpedo and cotyledonary stages.
4. The feature after the globular stage is comparable to zygotic embryos.
5. Somatic embryos formed are bipolar structures having radical and plumule.
6. The radicular end is always oriented towards the centre of the callus or cell mass while the plumular end always sticks out from the cell mass.
7. A shoot bud is monopolar as it does not have a radicular end.
8. As the somatic embryo has both radical and plumule develops into root and shoot respectively in the same media by the Process by Deamination.
9. Sometimes in somatic embryos, radical is suppressed so that they do not produce roots, in such cases roots have to regenerate from the shoots produced by germinating somatic embryos.
10. This process of germinating plantlet from somatic embryo is called as somatic conversation
11. The somatic embryo obtained from Explants or callus are termed as primary somatic embryo. In some cases where somatic embryos regenerate from the tissue of other somatic embryos called as Secondary Somatic embryos.

Sr no	Somatic embryo	Zygotic embryo
1	A embryo derived from somatic cell	A embryo develop from zygote(union of gametes)
2	Suspensors are less developed	Suspensors is well developed
3	Do not undergo desiccation & dormancy	Undergo dormancy
4	Has no seed coat & hence direct contact with medium	Has seed coat & hence not direct contact with medium
5	Cells are sometime recalcitrant	Cells are always totipotent
6	Cohesent stage is absent	Cohesent stage is present

Q10: Define Tissue culture? Discuss the application and scope of tissue culture in agriculture? (10+3)

Ans:- Tissue Culture: - Plant tissue culture is defined as culture of cell, tissue, organ or "whole plant in a medium under aseptic and controlled conditions.

Application of invitro culture:

1. Micropropagation: - Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods. Micropropagation is used to multiply novel plants, such as those that have been genetically modified or bred through conventional plant breeding methods. It is also used to provide a sufficient number of plantlets for planting from a stock plant which does not produce seeds, or does not respond well to vegetative reproduction.

2. Clonal Propagation: - Multiplication of genetically identical copies of a cultivar by asexual reproduction is called as clonal propagation. The variability arises from sexual reproduction and seed formation in crop plants is restricted. Clonal multiplication of cultivar is very important in horticulture and silviculture.

3. Production of genetically variable plants: - Tissue culture also produce the variation in the chromosome number of cells that Occurs after a number of serial subcultures.

4. Plant Pathology and Plant tissue Culture: -Eradication of viruses is practically achieved by the apical Meristem culture because the apical Meristem 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 controlled condition

5. Plant breeding, Plant Improvement and Plant tissue culture:- The conventional breeding methods are the 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 achieve the objective which is not possible through conventional methods. Embryo culture is mainly used in the recovery of the hybrid plants from distant crosses

6. Production of useful biochemical: -Various plant cell cultures are used to obtain the various types of biochemical under In Vitro conditions. Ex.-nicotine, atropine, caffeine, etc.

7. Preservation of Plant Genetic Resources or gene conservation banks:- conservation of the plant genetic diversity or germplasm which are replaced by new plant cultivars is the need of recent agriculture. Centralize gene banks are the practical ways to solve these problems Conventionally these germplasm are stored in the form of seed but it is not applicable for the vegetatively propagated plants.

8. Somaclonal variation:- The genetic variation obtained through the tissue culture. It is most important for the breeding purpose. The variation may be beneficial or harmful but the variation is essential for the broad genetic base from which we can improve the recent cultivars with the help of Conventional or other breeding methods.

Q11: Define anther culture? Discuss the factors affecting anther culture & limitation of anther culture technique? (11+2) / (10+3)

Ans:- Anther culture – culturing anthers of precise & critical stage which is to be isolated from unopened flower buds & cultured on artificial nutrient medium.

Factors Affecting Anther Culture

1. Genotype of the donor plant:- anther culture is majorly affected by the genotype of the donar plant. some the characters exhibit by the mother plant are somewhat different from the required characters & this difference is due to the genotype of the parent plant

2. Anther wall factor:- during anther culture, the walls of anther is ruptured in order to release the pollens which are present in the anther thus new plantlets are developed from the [illegible]n material, thus if anther walls are not properly ruptured they may cause disturbance in the process of anther culture
3. Culture medium:-different types of culture media support differently resulting into the Organoids if proper culture media is not used. Generally MS media is commonly used
4. Development stage of anther:- fully matured/over developed anthers are [illegible] suited for the anther culture as they lose their viability. Proper immature, Intermediatory stage [illegible] are chosen for culture
5. Physiology of donor plant):- the donor or the parent plant should be well [illegible]oped & highly prolific which should be selected for Anther culture. It should posses health physiology
6. Temperature:- temperature is the thermal gradient for which affect the process of anther culture. Suitable temperature for anther culture is 24-28°C

Limitation Of Anther Culture Techniques

1. The frequency of haploid production is Very low.
2. Development of haploids by tissue culture technique requires high level of knowledge and management.
3. Besides haploids, different Ploidy levels are produced. Ex- triploid, tetraploid.
4. The callus derived from anther culture is usually harmful to haploid production.
5. Isolation of haploids from cultures is often difficult.
6. Haploids with harmful traits frequently develop in cultures.
7. The doubling of haploids may not always lead to formation of homozygous plant.
8. It is not profitable due to vary low success rate

Q12:- Explain in detail the Various Applications of embryo culture? (11-12)

Ans:-

1. Production of rare hybrids from Intergeneric and interspecific crosses.
   The hybrid embryo failed to develop due to poor (or) abnormal development of endosperm. But in such cases, the embryo may be potentially capable of normal growth and differention. The hybrid plants can be raised by culturing these hybrid embryos before the onset of abortion.

2. Development of disease resistant plants
   Embryo culture has been useful in evolving disease resistant plants. Eg:- Tomato resistance to virus, fungi and nematode *Lycopersicon esculentum* x *L peruvianum*, Potato - resistance to potato leaf roll virus *Solanum tuberosum* x *S. tuberosum*

3. Production of haploids
   This technique represents a considerable advancement in the production of barley haploids and it has a number of advantages over anther culture. The frequency of haploid formation is quite high by this method. In addition, no aneuploids are obtained by this method. But this technique has a disadvantage of being highly laborious because it involves crossing, embryo excision and then regeneration

4. Overcoming seed dormancy:-
   Embryo culture technique is applied to break seed dormancy which can be caused by numerous factors including endogenous Inhibitors, specific light requirements, low temperature, dry storage requirements and embryo immaturity. These factors can be circumvented by embryo excision and culture. In some fruit trees embryos required a dormancy period after ripening before germination

5. Shortening of Breeding cycle
There are many species that exhibit seed dormancy often localized in seed coat and (or) endosperm. By removing these inhibitors seeds germinate immediately. Seeds sometimes take up water and O2 very slowly (or) not at all to the seed coat and so germinate slowly if at all Eg:- Brussels sprouts, Rose, Apple, oil palm, Iris

6. Propagation of rare plants:-
This technique is used for production of seedlings from seeds of naturally vegetatively propagated plants such as banana, *colocasia esculentum* whose seeds do not germinate in nature.

7. Propagation of orchids:-
Orchids are difficult to propagate as their seeds lack any stored food (Endosperm) and embryos are virtually naked. In many orchids embryo development is incomplete at the time the seeds mature. Either young (or) mature embryos are excised from the seeds and cultured on nutrient medium to get viable seedlings plants.

8. Prevention of embryo abortion with early ripening stone fruits
Some species produce sterile seeds that will not germinate under appropriate conditions and eventually decay in soil. Eg:- Early ripening varieties, of peach, cherry, apple, plum etc.

9. Clonal Micro propagation
The regenerative potential is an essential prerequisite in non-conventional methods of plant genetic manipulations. Because of their juvenile nature, embryos have high potential for regeneration and hence may be used for in vitro clonal propagation. This is especially true of Conifers and Graminaceous members. Germination of seeds of obligatory parasites without the host is impossible in vivo but is achievable in embryo culture

10. Rapid seed viability testing:-
Germination of excised embryo is regarded as more reliable test for determining seed viability than usual staining methods. A good correlation has been shown between the growth of excised embryos of unripened seeds and germination of ripened seed of peach. Peach seeds take several months to germinate under normal conditions.

Q13. Describe the techniques of embryo culture? (13,14)

Ans:- Techniques Of Embryo Culture

1. Surface sterilization
2. Excision of embryo
3. Embryo-endosperm transplant
4. Nutritional requirement
5. Role of embryo in suspension culture

1. Surface sterilization

An embryo of seed plants normally develops inside the ovule which in turn are covered by ovaries. Since they already exist in a sterile environment, disinfection of the embryo surface is unnecessary unless the seed coats are injured or systemic infection is present. Instead, mature seeds, entire ovule or fruits are surface sterilized. Surface sterilization is carried out by immersing the material in hypochlorite containing commercial bleach for 5 to 10 min.

A small amount (0.01-0.1 %) of a surfactant (Teepol) may be added to disinfection solution. In case of infected seeds, the excised embryos may be immersed in 70% alcohol plus 5-10 min exposure to 2.6% sodium hypochlorite solution

2. Excision of embryo

Embryo excision operation is carried out aseptically in a laminar airflow hood. A stereomicroscope (90 X) equipped with cool-ray fluorescent lamp is required for excision of small embryo. The commonly used dissecting tools are forceps, dissecting needles, scalpels, razor blades and Pasteur pipettes. Mature embryo can be isolated with relative ease by splitting open the seeds. Soaking a hard-coat seeds for few hours to a few days before sterilization makes its dissection easier. In case of embryos embedded in liquid endosperm, the incision is made at micro polar end of young ovule and pressure: applied at opposite end to force the embryo out through the incision

3. Embryo-endosperm transplant

It is very difficult to culture embryo in vitro which aborts at very early stages of development because of lack of knowledge of nutritional requirements. The chances of development of immature or abortive embryos increases if they are surrounded by endosperm tissues excised from another seed of same species. Generally an endosperm older than the embryo by 5 days was more efficient as a nurse tissue than one of the same age as the embryo

4. Nutritional requirement

The Nutritional requirements of an embryo during its development in vivo constitute two phases: (a)heterotrophic phase an early phase wherein an embryo is dependent and draws upon the endosperm and material tissues and b) The autotrophic phase in which the embryo is metabolically capable of synthesizing substances required for its growth, thus becoming fairly independent for nutrition

5. Role of embryo in suspension culture

Suspensor is actively involved in embryo development. The suspensor is an ephemeral structure found at the radicular end of the proembryo and attains maximum development by the time embryo reaches globular stage. In cultures the presence of a suspensor is critical particularly for the survival at young embryos. The requirement of the suspensor may be substituted by the addition of GA or ABA to the culture medium

Q19. What is media? Explain in brief the basic constituents of media/ Nutritional requirement of PTC (5,5,2,2) (13, 14)

Ans:- Media:- it is a substrate used for plant growth such as soil, sand, agar-agar

1. Inorganic salt: Divided into two groups a) major b) minor salts
    a) Major salts: The salts of K, N, Ca, Mg P and S constitute the major salts. Nitrogen is generally used as nitrate or ammonium salts, sulphur as sulphates and phosphorous as phosphates.
    b) Minor salts: The salts of Fe, Zn, Mn, Boron, Cu, Cobalt, Mb, iodine, etc. make up the minor salts. These salts are essential for the growth of tissues and are required in trace quantities.
2. Iron Source

    Out of all the trace elements Iron is the most critical. It has been observed that iron tartarate and citrate precipitate in the medium and pose difficulty in its utilization. Therefore, Iron is used in a chelated form of EDTA i.e. Ethylene Diamine Tetra Acetic Acid. In this state, it is gradually released into the culture medium as it is utilized by the living cells.

3. Vitamins

Vitamins are organic compounds synthesized naturally in the plants. Therefore, these are supplemented in the culture media. Most commonly used are nicotinic acid, thiamine, pyridoxine, biotin, ascorbic acid, riboflavin etc.

4. Carbon Source

Carbon is supplied as a sugar, usually sucrose. It gets converted into glucose and fructose which are readily utilized by the tissue. Thus, sucrose is followed by the use of glucose and fructose. But autoclaved glucose and fructose do not prove much beneficial.

5. Plant Growth Regulators

Plant growth regulators can be categorized as:

a. Auxins:- Auxins possess the property of inducing cell division, elongation of internodes, apical dominance and rooting. There are various Auxins which are supplemented exogenously in the nutrient medium like IAA, IBA, 2,4-D, NAA, 2,4,5-T, pCPA, MCPA, Picloram
b. Cytokinins:- These adenine derivatives induce and enhance cell division, shoot differentiation. Commonly used Cytokinins are BAP, 2ip, Kinetin, Zeatin etc
c. Gibberellins:- Some plant species require gibberellins for growth, GA3, the most commonly use gibberellin, enhances cell growth and proliferation, and also helps in elongation of cells
d. Abscisic acid:- The effect of Abscisic acid is highly specific varying from species to species. It is known to inhibit and enhance growth of cells

6. Organic Supplements

Besides vitamins, organic compounds are also supplemented in the form of amino acids like Glutamine, Casein Hydrolysate, Asparagine, Proline etc. Addition of adenine sulphate also enhances shoot production. Moreover, organic extracts of unidentified nature are also added to the culture media. These are chiefly of natural origin, like coconut milk, potato extract or fruit extracts.

7. Gelling Agent

To provide a substrate for the growing cultured tissue, liquid media is solidified using Agar, Gelatin, Alginate or Phytagel. However, agar-agar is preferred the most, as it does not react with the constituents and is not digested by the plant enzymes.

8. pH

pH affects the growth of plant tissues and, therefore, it needs to be optimized. The pH affects uptake of ions for most of media formulation. Optimum pH ranges between 5.0 and 6.0.

9. Activated Charcoal

It is known to stimulate growth and differentiation in certain plant species. It also helps in reducing toxic effect of the harmful secretions of the cultures

10. Antibiotics

Although the addition of antibiotics is undesirable, in Explants suffering from systemic infection it is used to overcome contamination of the resultant cultures.

Ans:- Somatic hybridization:- production of hybrid plants through the fusion of two different plant spp is called somatic hybridization

Steps involved in somatic hybridization

1. Isolation of protoplast
2. Fusion of protoplast of desired spp
3. Selection of somatic hybrid cell
4. Culture of hybrid cell

Methods For Protoplast Fusion

1. Spontaneous Fusion

Sometimes fusion occurs without any control during the isolation and purification of protoplasts. This results in the production of homokaryons rather than desirec heterokaryons. These homokaryons contain multinuclear bodies which are mainly formed due to the expansion of plasmodesmata of adjacent cells during enzymatic digestion. This process is known as "spontaneous fusion". Sometimes these multinuclear bodies contain 2-40 nuclei. A different type of spontaneous fusion was reported in two liliaceous species (Ito and Maeda, 1973). Protoplasts of these species obtained from meiotic pollen mother cells fused just upon physical contact without any external inducer.

2. Mechanical Fusion

This type of fusion does not require chemical fusogen. Mechanical fusion of protoplasts is accomplished by pushing together two protoplasts as in case of *Acetabularia*. Moreover protoplasts could be fused by careful tapping on a depression slide readily after its release in enzymatic solution. However the main drawback of this method is that protoplasts have a great chance of injury.

3. Immunological Fusion

This type of fusion was employed by Hartmann et al. (1973) for the agglutination of protoplasts from suspension culture of *Bromus, Glycine* and *Vicia*. Immune sera is prepared against protoplasts in a rabbit which causes the agglutination of protoplasts. Antibody of *Glycine* and *Bromus* cross reacted with agglutinated protoplasts of *Vicia*. These protoplasts showed division after antibody treatment.

4. Chemical Fusion

Chemicals employed in this method are polyethylene Glycol, Polyvinylglycol, Polydelysine, Sodium Nitrate ($NaNO_3$), Dextrosulphate, etc. Other requirements are Ca ++, high pH, etc.

a) Sodium nitrate ($NaNO_3$)
b) Combination of high pH & Ca ++
c) Polyethylene glycol (PEG)

Fusion of isolated plant protoplasts is possible under certain physical conditions. These chemicals are coupled with physical conditions to alter the property of membrane which facilitated the formation of bridge between two protoplasts. The association of protoplasts has been described as "fusion like soap bubbles" as "plasmosyndnesis" and as "agglutination". If cells are 50^0 A or $>50^0$ A apart from each other fusion would not occur. For fusion removal of negative surface charges of isolatee protoplasts is a must. Nagata and Melchers (1978) demonstrated the presence of negative charges, designated as S-potentials with the help of electrophoresis. These potentials are between the range of -10 m V to -35 mV, depending upon the cell type used and its ploidy level. Ca++ helps in removal of negative charges at a concentration of 100 mM and facilitates agglutination and fusion. Any chemical compound which induces the protoplast fusion is known as fusogen or fusion inducing chemicals. Polyethylene glycol (PEG) has been found to be an efficient fusion inducing agent.

5. Electrofusion

This method of protoplast fusion in an electric field was given by Senda et al(1979) and Zimmerman and Scheurich (1981). Somatic hybridization of two auxotrophic tobacco lines by Electrofusion had been established (Kohn and Schieder, 1984).

Electrofusion acts as an alternative means to chemically induced fusion. Chemical methods have many disadvantages like: they are toxic at high concentration (40%), their removal is a must from protoplast after fusion and they also cause the production of multinucleated undesirable fusion products

Q16: What are molecular markers? Write down different molecular markers along with their applications (09-10)(10-11)(13-19)

Ans:- Molecular Markers:- A molecular marker may be defined as a DNA sequence used for a chromosome mapping as it can be located at a specific site in a chromosome

Types of marker

1. Morphological markers
2. Biochemical markers
3. Molecular markers
   - A. Non PCR-based
     1. Restriction Fragment Length Polymorphism RFLP
   - B. PCR based
     1. Random Amplified Polymorphic DNA (RAPD)
     2. Amplified Fragment Length Polymorphism (AFLP)
     3. Variable number of Tandem Repeat DNA (VNTR)
     4. DNA amplification Fingerprinting (DAF)
     5. Simple Sequence Fingerprinting (SSF)
        - i. Minisatellite
        - ii. Microsatellite

A.1.Restriction Fragment Length Polymorphism RFLP

It refers to variations found within the species in the length of DNA fragments generated by specific endonuclease. RFLPs are the first type of DNA markers developed to distinguish individuals at the DNA level

Advantages

1. It is simple & cheaper technique of DNA sequencing
2. It does not require special instrumentation
3. Majority of RFLP markers are co-dominating & high locus specific
4. RFLP are powerful tools for comparative & synteny mapping
5. Numerous sample can be simultaneously screened

Disadvantage

1. Developing sets of RFLP probes & markers is labour intensive
2. This technique requires high quality of DNA
3. The multiplex ratio is low
4. RFLP finger prints for multigene families are often complete & difficult to score
5. The genotyping throughout is low

B.1.Random Amplified Polymorphic DNA (RAPD)

It refers to polymorphism found within a species in the randomly amplified fragments of DNA generated by restriction endonuclease enzyme. RAPD are PCR based molecular markers this technique was proposed by Williams et al 1990. The RAPD markers are dominating

- Advantages

1. This technique is simple & quick
2. RAPD primers are readily available
3. It can be employed with any species using universal primers
4. It provides more polymorphism than RFLPs

Disadvantages

1. The detection of polymorphism is limited as incase of RFLPs
2. This technique only detects dominant markers.

B.2.Amplified Fragment Length Polymorphism (AFLP)

AFLPs are difference in restriction fragment length caused by SNPs(single nucle[illegible]polymorphism) or INDELs that creates or abolish restriction nuclease recognisation sites. AFLP assay are perf[illegible]d by selectively amplifying a pool of restriction fragments using PCR

Advantages

1. This technique proyides very high multiplex ratio & genotyping throu[illegible]t
2. This technique can be applied to virtual any[illegible]sm
3. Result developed are highly reproducible
4. No special instrumentation is required
5. Start up cost for AFLP is moderately low

Disadvantages

1. High qualit- DNA is needed to ensure complete restriction
2. The homology of restriction fragment cannot be unequivocally asce[illegible]ed across genotypes
3. It is difficult to develop locus specific markers
4. It generally involved radioactive methods through non radioactive methods are available but they are rarely used
5. The maximum polymorphic information content for any bi-allelle marker is 0.5

B.3.Minisatellite or variable number of tandem repeats (VNTRs): The most wide spread among the polygenic markers are the variable number of tandem repeats (VNTRs). The VNTR are highly polymorphic and less amenable to PCR analysis because they have large sequence motifs (about 1000 bp). VNTR sequence are made up of a variable number end to end duplications of identical of almost identical sequences of 2-80 cases. 5-50 bases are common in mammals

B.4.Microsatellites or simple sequence repeats (SSRs): SSRs me randomly repeated mono di, tri, tetra, penta, and hexa nucleotide motifs. The SSR length polymorphisms are caused by differences in the number of repeats. The SSR loci are individually amplified by PCR using pairs of oligonucleotide primers specific to unique DNA sequences flaking the SSR sequence.

Advantages:

1. The SSR markers tend to be highly polymorphic.
2. Most SSR markers are co-dominant and locus specific
3. This is a simple PCR based techniqui.

Disadvantage

1. The development of SSRs is labour intensive.

The cost of developing SSR markers is very high.

Q.5 Define totipotency ? Mention the importance of totipotency in plant science. 3+3=6

Ans:- Totipotency:- genetic potential of a plant cell to produce the entire plant is called as totipotency.

Importance of Totipotency

- This is a capacity which is retained even after a cell has undergone final differentiation in the plant body. In plants, even highly mature and differentiated cells retain the ability to regenerate to a meristematic state as long as they have an intact membrane system and a viable nucleus
- This is contradicting to animals, where differentiation is generally irreversible. For a differentiated cell, to express its totipotency, it first undergoes dedifferentiation followed by redifferentiation. The phenomenon of a mature cell reverting to the meristematic state and forming undifferentiated callus tissue is termed 'dedifferentiation'.

➢ The phenomenon of conversion of component cells of callus tissue to whole plant or plant organs is called 'redifferentiation'.

Q1 B. Write the protocol for following culture

1. Protocol for Organogenesis in Tobacco

1. Select upper part of stem 3-4' tall, cut 2cm long internode.
2. Surface sterilize the internode 70% ethanol for 30sec
3. Incubate it with NaOCl (sodium hypochlorite) for 15min
4. Wash it 2-3 times with distilled water
5. Take sterilized Explant in petridish, cut it longitudinally into 2 pieces
6. To initiate organogenesis use ms medium + 2mg/lit IAA + 0.2 mg/lit kinetin
7. Culture is incubated at 25° c, 100 LUX, 16 hrs photoperiod
8. Callus formation take place in 3weeks by using -ms + 0.02 mg/lit IAA+ 1 mg/lit kinetin
9. Shoot formation takes place in 2-3 weeks by using- ms + 0.2 mg/lit IAA + 0.02mg/lit Kinetin
10. After 6 weeks roots can be induced using-ms + 0.02 mg/lit IAA+ 0.02mg/lit kinetin
11. The plantlets are carefully transplanted in the polythene bags and taken to greenhouse for hardening purpose

2. Protocol for Callus Culture of Tap Root

1. A fresh tap root of carrot is taken and washed thoroughly under running tap water to remove all surface dirts
2. The tap root is then dipped into 5% "Teepol" for 10 minutes and then the root is washed
3. The tap root is surface sterilized by immersing in 70% v/v ethanol for 60 seconds followed by 20-25 minutes in sodium hypochlorite 0.8%
4. The root is washed 3 times with sterilized distilled water to remove NaOCl.
5. The carrot is then transferred to a sterilized petridish containing a filter paper. A series of transverse slice 1 mm in thickness is cut from the tap root using a sharp scalpel
6. Each piece is transfer to another sterile petridish. Each piece contains a whitish circular ring of cambium around the pith. An area of 4 mm^2 across the cambium is cut from each piece so that each piece contains part of phloem. Cambium and xylem size and thickness of Explant should be uniform
7. Always the lid of petridish is replaced after each manipulation
8. The closure (cotton plug) from a culture tube is removed and flamed the uppermost 20mm of the open end. While holding the tube at an angle of 45°, an Explant is transferred using forceps onto surface of the agarified nutrient medium. Nutrient medium is Gamborg's B_5 or MS medium supplemented with 0.5 mg/l 2, 4-D
9. The closure is immediately placed on the open mouth of each tube. Date medium and name of the plant are written on the culture tube by a glass marking pen or pencil
10. Cultures are incubated in dark at 25°C in culture room.
11. After 4 weeks in culture the whole callus mass is taken out aseptically on a sterile petridish and should be divided into two or three pieces
12. Each piece of callus tissue is transferred to a tube containing same fresh medium
13. Prolonged culture of carrot tissue produces large calluses.

3. Protocol for Suspension Culture

1. Take 150/250 ml conical flask containing autoclaved 40/60 ml liquid medium
2. Transfer 3-4 pieces of pre-established callus tissue (approx. 1 g each) from culture tube using the spoon headed spatula to conical flask.

3. Flame the [illegible] conical flask; close the mouth of conical flask [illegible] piece of Aluminium foil or a cotton plug. Cover [illegible] ure with piece of brown paper.
4. Place the [illegible] in the clamps of a rotary shaker moving at the [illegible] rpm.
5. After seve[illegible]; our the content of each flask through the st[illegible] sieve (pore diameter 60-100 µ) and collect the filtrate in a big sterilized container. The nitrate [illegible] only free cells and cell aggregates.
6. Allow the [illegible] settle for 10-15 minute or centrifuge the [illegible] 500 to 1000 rpm and finally pour off the su[illegible].
7. Resuspe[illegible] cells in a requisite volume of fresh liq[illegible] and dispense the cell suspension equally in [illegible] sterilized flasks (150/250 ml). Place the flask[illegible] and allow the free cells and cell aggregates to grow.
8. At the next subculture, repeat the previous steps but take only one-fifth of the residual cells as the inoculum and dispense equally in flasks and again place them on shaker.
9. After 3-4 subcultures, transfer-10 ml of cell suspension from each flask into new flask containing 30 ml fresh liquid medium

2. Protocol for inducing somatic embryogenesis in carrot culture

- The plant material used is *Daucus carota*

1. Select leaf petiole (0.5 to 1cm) or root segment from 7days old seedling (1cm) or cambium tissue (0.5 cm^3).
2. Transfer it to semi solid ms medium + 0.1 mg/l 2,4-d + 3% sucrose
3. Incubate culture in dark for 4 weeks...it forms the callus
4. After 4 weeks cell suspension culture is initiated by transferring 0.2gm of callus + 20-25 ml liquid media in 250 ml Erlenmeyer flask
5. Place flask on horizontal shaker @ 120-160 rpm, temp 25°c
6. Sub culture the medium after 4 weeks by transferring 5ml cell suspension + 65 ml of fresh liquid medium
7. For uniform embryo development from cell suspension is to be passed through a series of stainless steel mesh sieve ,sieve dia=74mm
8. Sieved cell suspension is cultured on liquid semisolid ms medium free from 2,4-d
9. Add ABA 0.1 µm used to inhibit precocious germination especially root elongation
10. Incubate in dark for 3-4 weeks, culture would contains different developmental stages
11. Somatic embryos can be placed on a agar medium devoid of 2,4-d for plantlet development
12. Plantlets are finally transferred to pots or vermiculite for subsequent development

3. Protocol for Meristem culture

1. Remove the young twigs from the healthy plant. Cut the tip (1 cm) portion of the twig.
2. Surface sterilized the shoot apices by incubation in a sodium hypochlorite solution 15 for 10 min, The Explants are thoroughly rinsed 4 times in sterile distilled water.
3. Transfer each Explant to a sterilize petridish.
4. Remove the outer leaves from each shoot apices with a pair of Jeweller's forceps. This lessens the possibility of cutting into the softer underlying tissue.
5. After the removal of all the outer leaves, the apex is exposed. Cut off the ultimate apex with the help of scalpel and transfer only those less than 1 mm in length to the surface of agar medium or to the surface of filter paper bridge. Flame the neck of culture tube before and after the transfer of excised tips. Binocular dissecting microscope can be used for cutting the true Meristem or shoot-tip perfectly
6. Inoculate the culture under 16hrs light at 25°C.
7. As soon as the growing single leafy shoot or multiple shoots obtained from single shoot tip or Meristem transfer them to hormone free medium to develop roots.
8. The plants form by this way are later transferred to pots containing compost and kept under green house condition for hardening

3. Protocol for pollen culture

Method-1

- This method is described for the culture of isolated pollen protoplasts. This technique can be considered as basic protocol for pollen culture & involves the following steps

1. Transfer the selected unopened flower bud to the lab & surface sterilize the flower buds with 70% ethanol solution for 10sec followed immediately by 20 min in 2% sodium hypochlorite, then washed three times with distilled water
2. Remove the anthers by sterile sharp scalpel & about 50 anthers are placed in small sterile beaker containing 20ml liquid basal MS medium
3. Anthers are then pressed against the side of beaker with the sterile glass piston of a syringe to squeeze out pollens
4. The homogenized anthers are than filtered through a nylon sieve (40-50um dia)
5. The filtrate is centrifuged at low speed 500-800rpm for 5min, supernatant with fine debris is discarded & pillet of pollen is suspended in fresh liquid medium & washed twice by repeated centrifugation & resuspension in fresh liquid medium
6. Pollen are mixed finally with measured volume of liquid basal MS medium at density of 10^3-10^4 pollens /ml
7. A 2.5 ml pollen suspension is pipette out & spread in 5cm petridish
8. Petri dish incubated at 27-30 C under low intensity of light 500LUX, 16hrs photoperiod
9. Young embryoids can be observed after 30 days which ultimately give rise to haploid plantlets
10. Haploid plantlets are then incubated at 27-30 C in a 16hrs day light 2000LUX. Plantlets at maturity are transferred to soil as described in anther culture

Method-2

- This method is known as nurse culture technique. Sharp et al(1972) first introduced this method.

1. Selection of flower bud, sterilization, excision of anther, isolation of suitable pollen are the same as described previously
2. The intact anthers are placed horizontally on the top of solid or semisolid basal medium within a conical flask
3. A small filter paper disc is placed over the intact anther & about 10 pollen grains in the suspension are then placed on the filter paper disc. Here the intact anthers are considered as the nurse tissue.
4. A control set is also prepared in exactly the same way except that the pollens grains on the filter paper are directly kept on solid medium. Sometimes, callus tissue derived from any part of the plant is used as nurse tissue
5. With this method pollen grains in the control set did not grown at all. The pollen grains kept on nurse tissue grow and form a culture of green parenchymatous issue within 2 weeks such tissue ultimately form the haploid callus tissue

4. Protocol for ovule culture

1. Collect the open flower (unfertilized ovule). If fertilized ovule are desired collect the open flowers where anthers are dehisced and pollination has taken place. To ensure the Fertilization, collect the flower after 48 hrs of anther dehiscence.
2. Remove sepals, petals, androecium etc from the ovaries containing either fertilized or unfertilized ovules.
3. Soak the ovaries in 6% NaOCl solution.
4. Rinse the ovaries 3-4 times with sterile distilled water.

5. Using sterile technique ovules are gently prodded with the help of spoon shaped spatula by breaking the funicules at its junction placental tissue.
6. The spatula with ovules is gently lowered into the sterile solid or liquid medium as the culture vial is slanted about 45°C.
7. Damaged or unorganized ovule are rejected when possible during transfer
8. Incubate the ovule culture in either dark or Light(16 hrs, 3000 LUX) at 25°C

8. Protocol for Culture of Protoplast

1. The protoplasts in liquid NT (Nagata and Takebe) medium are counted with the help of haemocytometer. The protoplast density is adjusted to 1×10^5 to 2×10^5 protoplast /ml.
2. Agar solidified (1.6% 'Difco' agar) NT medium is melted.
3. The tight lid of falcon plastic petridish (35 mm diameter 5 mm thickness is opened and 1.5 ml of protoplast suspension is taker. To this equal aliquot of melted agar medium is added when it cools down at 37°e to 40°C
4. The lid is quickly replaced tightly and whole dish is swirled gently to disperse the protoplast-agar medium mixture uniformly throughout the dish.
5. The medium is allowed to solidify. The petridish is then inverted
6. The culture is incubated at 25°C with 500 lux illumination (16 hrs light) initially.
7. The cultures are sub cultured periodically in the same solid medium (0.8% agar) gradually reducing mannitol

9. Protocol for Embryo Culture

1. The capsules in the desired stages of development are surface sterilized for 5-10 minutes in 0.1 % $HgCl_2$ in a laminar air flow.
2. Wash repeatedly in sterile water.
3. Further operations are carried out under a specially design dissecting microscope at a magnification of about 90X. The capsules are kept in a depression slide containing few drops of liquid medium
4. The outer wall of capsule is removed by a cut in the region of the placenta; the halves are push apart with forceps to expose the ovules.
5. A small incision in the ovule followed by slight pressure with a blunt needle is enough to free the embryos.
6. The excised embryos are transferred by micropipette or small spoon headed spatula to standard 10 cm petridishes containing 25 ml of solidified standard medium. Usually 6-8 embryos are cultured in petridish.
7. The petridishes are sealed with cellotape to prevent desiccation of the culture.
8. The cultures are kept in culture room at 25± 1°C and given 16 hrs illuminations by cool white fluorescent tube.
9. Subcultures into fresh medium are made at approximately four weeks interval

Q 19. Explain the factors affecting organogenesis?

Ans:

1. **Size of Explant**

Organogenesis is generally dependent upon size of Explant. The large Explant consisting of parenchyma, vascular tissue & cambium trace greater regeneration ability than the smaller Explant. Small group of homogenous tissue taken from epidermal or sub epidermal layer could directly give rise to complex organs like flower or bud or root

2. Source of Explant

The most suitable part of the plant for starting culture will depend on species. Leaves & leaf fragments of many plant spp like *Begonia, Solanum, Nicotiana, Creps* etc have shown capacity to regenerate shoot buds. Bulb scales of *Hillum* spp regenerate adventitious bulblets. Flowers stem Explant of *Tullpa* spp regenerate shoot buds.

3. Age of the Explant

Physiological age of Explant is important for Invitro organogenesis. In Nicotiana spp regeneration of adventitious shoot is only more if the leaf Explant is collected from vegetative stage i.e. before flowering. Leaf Explants of *Echeveria* spp. That are collected from young leaves only produce roots, whereas older leaves initiate only shoots buds & leaves of medium age produce both shoots & roots.

4. Seasonal variation

Bulb scales of *Lilium speciosum* regenerates bulbs freely Invitro when Explant is taken during spring & autumn period of growth but same Explant collected from summer or winter season does not produce any bulblets.

5. Oxygen gradient

In some culture shoot bud formation takes place when the gradient of available, oxyger inside the culture vessel is reduce. But rooting requires a high oxygen gradient

6. Quality & Intensity of light

The blue region of spectrum promotes root formation & red lights induce rooting. The treatment of blue light followed by treatment of red light also stimulates the organogenetic phenomenon In some cultures artificial fluorescent light favours rooting & inhibits in other. Normally organogenesis in culture takes place with an illumination of 2000-3000 lux, 16 hrs of light.

7. Temperature

Most tissue cultures are grown successfully at temperature around 25°C. In number of bulbous spp optimum temperature may be much lower of about 15-18°C. Increase in temperature upto 33°C may be associated with rise in growth of tobacco callus but for shoot bud initiation a lower temp of about 18°C may be optimum.

8. Culture medium

Medium solidified with agar favours bud formation although there are some reports about the development of leaf shoot buds on culture grown in a solid medium.

9. pH of the medium

The pH of the culture medium is generally adjusted between 5.6-5.8, before sterilization. The pH may have a determining role in organogenesis

10. Ploidy level

Variation In chromosome number i.e. aneuploidy, polyploidy etc of plant cell in culture has been well documented. With the increase in chromosome instability there is a general decline in morphogenetic potentiality of callus tissue. So the most important factor in maintaining organogenic potential of callus culture is the maintenance of chromosome stability. Frequency of subculture can affect the chromosome stability of cell culture. So in order to maintain chromosome stability, cultures are subcultured frequently & regularly

11. Age of culture

A young culture frequently produces organs, but the organogenic potential may decrease & ultimately disappears in old culture. In certain cultures of some plants, the plant regeneration capacity may retain indefinitely for many years

Q20. Explain in detail the different stages of micropropagation [15]

Ans:-

1) Stage 0
2) Stage I
3) Stage II
4) Stage III
5) Stage IV

1. Stage 0

This is a initial step of micropropag[illegible]n which stock plant[illegible]sed for culture initiation are g[illegible]wn at least for 3 months under carefully monitored [illegible]tion. Stock plants [illegible]grown at a relatively low humidity and watered either with irrigation tubes or by ca[illegible]y sand beds or ma[illegible]s stock plant preconditioning stage also includes measures to be accepted for reduction f surface & systemic [illegible]robial contamination

2. Stage I

Murashige.(1974) defined this stage as [illegible]ntiation [illegible]tablishment of aseptic cultures. The main steps involved are preparation of the Explan[illegible]owed by [illegible]blishment on a suitable culture medium. Cultures are initiated from explants of several or[illegible]s but shoot [illegible]uxillary buds are most often used for commercial micropropagation. Procedure to su[illegible]e sterilize the Ex[illegible] growth in the culture medium defined for each species may be devised. It may also be advisable to conti[illegible]obial contamination within Explant tissues in case such efforts at Stage [illegible]not successful. Star[illegible]months to 2 years and require at least four passages of the subculture.

3. Stage II

a. This stage takes up the bulk of [illegible]ropagation a[illegible]ed culture medium that stimulates maximum proliferation of reg[illegible]l shoots. V[illegible]owed for micropropagation include

b. Multiplication through the growth and proliferation of meristems excised from apical and axillary shoots of the parent plant.

c. Induction and multiplication of adventitious meristems through a process of Organogenesis or somatic embryo-genesis directly on explants.

Multiplication of calli derived from organs, tissues, cells or protoplasts and their subsequent expression of either organogenesis or somatic embryogenesis in serial subcultures. Shoots obtained from these calli can be further multiplied following procedures (a) and (b).

A passage or harvest cycle generally requires 4 weeks. Shoots are harvested from the multiplying culture to either be sold as a Stage II product of carried onto Stage III. Generally stage II last to 10-36 months with large number of subcultures of similar age

4. Stage III

Shoots proliferated during Stage II are transferred to a rooting medium (storage). Sometimes shoots are directly established in the soil as microcuttings to develop roots. Since such a possibility depends on the particular species and at present a large no of species, cannot be handled in this manner, the shoots are generally rooted in vitro. When the shoots or plantlets are prepared for soil, it may be necessary to evaluate survival factors such as (i) dividing the shoots and rooting individually (ii) hardening the shoots to increase their resistance to moisture stress and diseases (iii) rendering the plants capable of autotrophic development in contrast to the heterotrophic state induced by culture and (iv) fulfilling requirement of breaking dormancy especially of bulb crops. Stage III required 1-6 weeks

5. Stage IV

Steps taken to ensure successful transfer of the plantlets of stage III from the aseptic environment of the laboratory to the environment of green house comprise stage IV. Unrooted stage II shoots are also acclimatized in suitable compost mixture or sol in pots under controlled conditions of light, temperature, humidity inside the

green house. In such cases stage III is s... ...plying bottom heat aids to pot with plantlets ... ... maintenance of dense fine-particle fo... ...tinin the green house enhance the rooting proces... ...plete plants can be established in the artificia... ...edia such as soilless mixes, Rockwood plugs, ...sponges. It takes 4 – 16 weeks for the finished prod... ready for sale or shipment.

Q.2.5 Explain in detail the steps involved in ... genic technology/Genetic engineering? (12)

Ans:-

1. Identification of useful Genes
   The desirable genes may be located in wild species, unrelated plant species, unrelated, organisms i. e. micro-organisms (bacteria, viruses or fungi) and animals. This work is carried out, in the research laboratory.

2. Designing Gene for Insertion
   The gene of interest is isolated from the donor source and cloned in the laboratory. The cloning is done generally using plasmids.

3. Insertion of Gene Into Target Plant
   The cloned gene i.e. multiple copies of the gene of interest are inserted into the host plant or the recipient plant. Two methods, viz. Agrobacterium mediated and gene gun or particle bombardment methods are used for gene transfer. This is done in the research laboratory (Protoplast culture for plasmid method and meristematic tissue or embryogenic callus are used for gene gun method.

4. Identification of Transgenic Cells
   Transformed cells are identified using selectable marker (Kanamycin) and are regenerated into whole plant in nutrient medium. The regenerated plant is compared with parent variety. It should look like parent variety except gene of interest. This work is done in the laboratory and glass house.

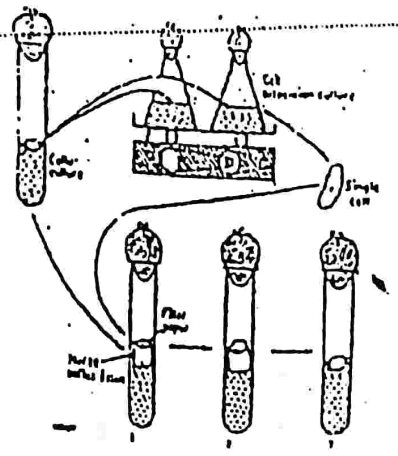
5. Small scale Field trials
   The transgenic plants are evaluated for their performance in small scale field trials. The seed of the transgenic plants such as cotton, soybean, rapeseed, etc. is tested in laboratory for biosafety i.e. allergenicity and toxicity. These tests are conducted with animals such as rats rabbits, poultry, goats etc.

6. Larger Field Trials
   Transgenic plants which are passed by regulatory authority are evaluated in multilocation trials for their performance for the gene of interest. Superior performing genotypes are released after testing and stable performance for three years

Q.2.6 Explain in detail the different methods of single cell culture?

Ans:

1. The filter paper raft nurse technique
2. The petridish plating technique
3. The micro-chamber technique
4. The nurse callus technique
5. The microdroplet technique



1. The filter paper raft nurse technique
   i. Single cell are isolated from suspension culture or a friable callus with the help of a micropipette
   ii. Sterile 8X8 mm sq of filter paper are placed aseptically on the surface of actively growing callus tissue
   iii. The filter paper gets wetted by soaking water & nutrients

from the callus tissue

iv. The isolated single cell is placed aseptically on the wet filter paper raft.

v. Whole system is incubated under 16hrs cool light, 3000 LUX, 25 °C

vi. Single cells divides & redivides & ultimately form a small cell colony. When cell colony reaches to a suitable size, it is transferred to another fresh medium

The callus tissue on which the single cell is growing is called the nurse tissue

2. The petridish plating technique

This technique was developed by Bergmann 7 is most popular of plating of single cells

i. A suspension of purely single cell is prepared aseptically from the stock cell suspension culture.

ii. The solid medium (1.6 % Difco Agar added) is melted in water bath

iii. In laminar air flow light lid of falcon plastic petridish is opened. With the help of pipette 1.5ml of single cell suspension is put on melted agar medium, when it is cooled down at 35°C, it is added in the single cell suspension

iv. The lid is quickly replaced & whole dish is swirled gently to disappear the cell & medium mixture uniformly. A thin layer of 1mm should be formed at the bottom of petridish by eventually disturbing the medium & cell suspension culture

v. The medium is allowed to solidify & petridish is kept inverted position

vi. The culture is incubated at 16 hrs light period 3000 Lux at 25°C

vii. When the cells start to divide, a grid is drawn on the under surface of petridish to facilitate the counting of the no of dividing cells

viii. The dividing cells ultimately form pin head shape colonies within 21 days of incubation

ix. The plating efficiency can be calculated from the counting of cell colonies by the formula

$$PE = \frac{\text{Number of colonies per plate}}{\text{Number of total cells per plate}} \times 100$$

x. Pin head colonies when they reach of suitable size they are transfer to the fresh medium for further growth

3. The micro-chamber technique

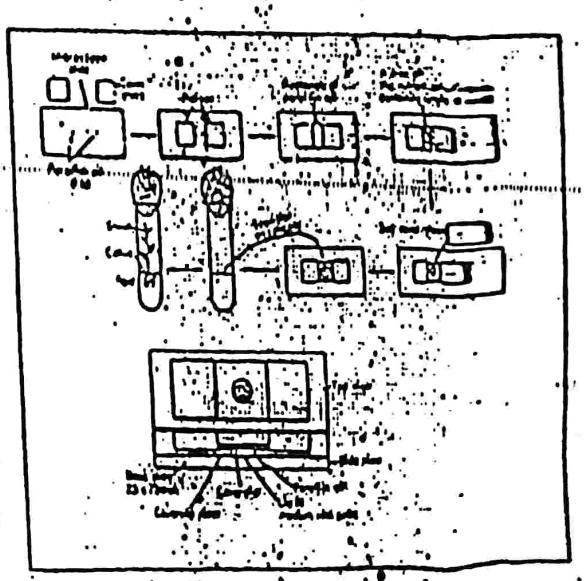
i. A drop of liquid nutrient medium containing single cell is isolated aseptically from stock suspension culture with the help of long fine Pasteur pipette

ii. The culture drop is placed on the center of a sterile microscopic slide (25x75 mm) and ringed with sterile paraffin oil

iii. A drop of paraffin oil is placed on either side of the culture drop and a cover glass (called raiser) is placed on each oil drop.

iv. A third cover glass is then placed on the culture drop bridging the two raiser cover glasses and forming a micro-chamber to enclose the single cell aseptically within the paraffin oil. The oil prevents the Water loss from the culture drop but permit gaseous exchange

v. The whole micro-chamber slide is placed in a petridish and is incubated under 16 hrs white cool illumination 3000 lux at 25 °C.

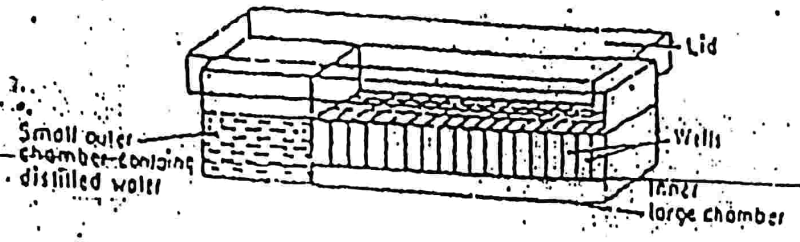


vi. Cell colony derived from single cell ... 10 single cell clone
vii. When the Cell colony become ... large the cover glass is removed and tissue ... transferred to fresh solid or semisolid medium

4. The nurse callus technique

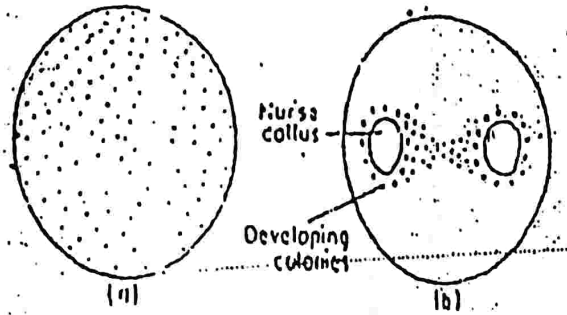
This method s actually a modification of petri ... plating method & paper raft nurse culture method

i. In this method, single cell are plated on to a agar medium in a petri dish. Two to three callus masses derived from the same plant tissue are also embedded directly along with the single cell in the same medium.
ii. Here the paper barrier between single cell & the nurse tissue is removed
iii. Cells first began to divide in the regions near the nurse callus indicated that the single cells closer to nurse callus in the solid medium gets the essential growth factor that are liberated from the callus mass.
iv. The developing colonies growing near to nurse callus also stimulates the division & colony formation of the other cells.



5. The microdroplet technique

i. In this method single cells are cultured in a special cunrak dishes which have two chamber-a outer smaller chamber & a large inner chamber.
The large inner chamber carles numerous numbered wells each with a capacity of 0.25 – 25 µl of nutrient medium
ii. Each well of Inner chamber is filled with a micro droplet of liquid medium containing isolated single cell.
The outer chamber is filled with sterile distilled water to maintain the humidity inside the dish
iii. After covering the dish with lid, the dish is sealed with paraffin
iv. The dish is incubated under 16 hrs white cool light 3000 LUX at 25°C
v. The cell colony derived from the single cell is transferred on to a fresh semi-solid medium in culture tube for further growth



Q2: Write short note on

A. Gene cloning (09,10)

Gene cloning:- refers to the process of isolating a gene from one organism & then inserting it into another organism resulting into the formation of GMO. It both the development of recombinant DNA's as well as their cloning in suitable host. Gene cloning is the basic strategy that must be used to obtain the first ever preparation of all the genes. Gene cloning produces large no. of copies of the gene/ DNA being cloned.

Steps In gene cloning

1. Selection of suitable vector
2. Generating DNA fragment
3. Insertion of target DNA into vector
4. Introduction of recombinant DNA into host cell
5. Screening & selection of transformed cell
6. Analysis of clone
7. Expression of inserted foreign gene

B: Anther culture (09-10) (11-12)

Anther culture is the technique By which the developing anthers of precise and critical stage are excised aseptically from unopened flower bud and are cultured on a nutrient medium where the microspores within the cultured anther develop into callus tissue that give rise to haploid plantlet either through organogenesis or embryogenesis.

Procedure/Protocol for anther culture

1. Collect unopened flower bud
2. Transfer to laminar air flow
3. Surface sterilize with 70% ethanol(10 sec)
4. Remove 5 anthers with scalpel
5. Transfer to another petridish containing Agar medium.
6. Incubate in dark 3-4weeks @ 24-28°C for 14 hrs
7. 50 mm tall plantlets free from agar by gentle washing
8. Transfer to small autoclave pots
9. Fihally shift to play house or green house

C: Marker Assisted Selection (MAS) (09-10)

Marker assisted selection refers to indirect selection for a desired plant phenotype based on the pattern of linked molecular (DNA) markers. It is also known as marker aided selection. Marker assisted selection is based on the concept that it is possible to infer the presence of a gene from the presence of a marker that is tightly link to the gene. A tight linkage between marker and gene of interest and high heritability of the gene of interest are prerequisite for marker assisted seleetion.

Merits

1. It permits early screening of traits that are expressed late in the life of plant.
2. It permits screening of traits that are extremely difficult, expensive or time consuming to score phenotypically.
3. It helps in distinguishing the homozygous verses heterozygous condition of many loci in a single generation without the need of progeny testing because of molecular markers are co-dominant.
4. The accuracy of marker assisted selection (MAS) is very high, molecular marker is not affected by the environmental condition.

Demerits

1. It requires a sophisticated and well equipped laboratory
2. It is very expensive
3. it requires well trained man power.

D: Mapping population (10-11) (11-12)

In plant breeding and genetics various types of plant material which are used for gene mapping or gene tagging or for construction of genetic linkage maps are known as mapping populations.

The most commonly used populations for gene mapping include- recombinant inbred lines, near isogenic lines, F2 population, Back cross population, bulk segregant analysis,& double haploid.

- Types of mapping population
  1. Recombinant Inbred L[illegible] which are developed from a cross between 2 pure lines or inbred lines are re[illegible]
  2. Near Isogenic Lines:- [illegible] single locus difference are referred to as NILs
  3. F2 Population:- the p[illegible] which is obtained by self pollination of F1 plants referred as F2 P.
  4. Back Cross Population [illegible] to crossing of F1 with either of its parents
  5. Bulk Segregant Analysis:- this technique compares bulks of DNA of individuals having the same genotype
  6. Double Haploid:- these are individuals or plants which are produced from haploids through chromosome doubling.

F:- Southern blotting (10-11) (11-12)

- Southern blotting is a method which is used to detect presence of specific DNA sequence.
- This method is named after its inventor, the British biologist Edwin Southern.
- Steps in southern Blotting
  1. Isolation & purification of DNA
  2. Cutting of DNA into Fragments
  3. Separation of Fragments by size
  4. Denaturing of DNA
  5. Transfer into membrane
- Advantages
  1. The technique is very simple
  2. It has very high degree of accuracy
  3. It can detect single gene from the entire genome
  4. It can analyse DNA from specific DNA clones
  5. It can use CDNA genomic sequence, pCR products
  6. It can use both DNA & RNA probes
- Disadvantages
  1. It is time consuming method
  2. It is an expensive method

F:- Restriction enzymes (10-11)

- The restriction enzymes are also called as molecular scissors
- These act as foundation of recombinant DNA technology
- These enzymes are present in bacteria & provide a type of defense mechanism
- It was discovered when that phage λ infects E. coli
- Bacterium protects itself through
  1. Restriction Mechanism
  2. Modification System
- A recombinant DNA molecule is a produce of joining together two or DNA segments usually originate from different organisms

G:- Test tube fertilization (10-11)

- Test tube fertilization:- Transfer of the pollen from anther to stigma of ovaries cultured invitro is called as in vitro pollination or test tube fertilization or invitro fertilization

Procedure of test tube fertilization or In vitro pollination:-

1. Flower buds which to be used as female parent and are emasculated before anthesis and bagged in order to prevent undesired pollination.
2. The buds are brought in laboratory for pollination & the ovaries alone are sterilized by 70% alcohol and wash with distilled water.
3. Thus ovaries collected from emasculated flowers 1-2 days after anthesis are cultured to expose placenta. Wetting of ovules and stigma should be avoided for the better pollen tube germination.
4. Collect pollen from the anther in the aseptic condition and kept in sterile petriplates containing a presterilized filter paper until their dehiscence.
5. The pollen is then aseptically deposited on the cultured ovules or stigma depending on the nature of experiment

H. RFLP (1...

- It refer to variations found within a species in length of DNA fragments generated by specific endonuclease.
- RFLPs are useful as molecular markers
- RFLP's are the first type of DNA markers developed to distinguish individuals at the DNA level.

Advantages

1. It is simple & cheaper technique of DNA sequencing
2. It does not require special instrumentation
3. Majority of RFLP markers are co-dominant & high locus specific
4. RFLP are powerful tools for comparative synteny mapping
5. Numerous sample can be simultaneously screened

Disadvantage

1. Developing sets of RFLP probes & markers is labour intensive
2. This technique requires high quality of DNA
3. The multiplex ratio is low
4. RFLP finger prints for multigene families are often complete & difficult to score
5. The genotyping throughout is low.

I. Cryopreservation (13-14)

The principle underlying this technique basically involves bringing plant cells and tissue culture to a non-dividing of zero metabolism stage subjecting them to super low temperature in the presence of cryoprotectants

In this technique the plant material is frozen and maintained at the temperature of liquid nitrogen which is around -196 °C. Following are the different techniques

1. Slow freezing method
2. Rapid freezing method
3. Stepwise freezing method
4. Dry- freezing method
5. Cold storage
6. Low pressure and low oxygen storage

J. Synthetic seed (11-12) (13-14)

Synthetic seeds:- are the living seeds like structures which are made experimentally by a technique where somatic embryoids are derived from PTC are encapsulated by a hydrogel & such encapsuletd embryoids behave like a true seeds if grown in soil & can be used as a substitute for natural seeds

Several steps are followed for making artificial seeds as follows:

1. establishment of callus culture
2. induction of somatic embryogenesis in callus culture
3. maturation of somatic embryos

4. Encapsulation of somatic embryos

After encapsulation, the artificial seeds are tested by

1. Test for embryoid to plant conversion
2. Green-house and field planting

K. Methods for Protoplast Isolation (1+2)

1. Mechanical Method

In this method by Klercker (1892), first the tissue is plasmolysed which increases the gap between plasmalemma and the cell wall; at the same time improving the chances of getting a higher yield of protoplasts. This method is based on cutting of the plasmolysed tissue by a razor blade or sharp edged knife. However, this method has only theoretical importance rather than practical one. Thus, a better approach having practical importance has emerged in the form of an enzymatic method.

2. Enzymatic Method

The idea of enzymatic isolation of protoplasts was conceived by Cocking in 1960. It basically implies the use of cell wall degrading enzymes like pectinase and cellulase for isolation of viable protoplasts. This method can be applied in two ways:

(a) Sequentially: Initially, macerated plant tissue is treated with pectinase to dissolve middle lamella, followed by treatment with cellulase to dissolve the cell wall in order to isolate protoplast.

(b) Simultaneously:

Both the enzymes (pectinase and cellulase) are employed in a single step to isolate protoplasts in the same media composition. Now-a-days, this method is extensively used because it is time saving as well as it decreases the chances of contamination by micro-organisms.

The enzymes employed for isolation of protoplasts are extracted from wood-eating fungi. These fungi grow on roots and secrete an enzyme "cellulase" and also contain lipase pectinase, pepsinase, etc

L. Importance & limitation of artificial seeds

Importance /uses of artificial seeds

1. Artificial seeds can be produced within a month
2. Production of true seeds in not season bounded
3. Reduce the life cycle of plants
4. A.S can be produced in any desired crop
5. They can be used for sole as well as mixed cropping
6. It gives protection to meiotically unstable elite genotypes
7. A.S coating used for delivering beneficial adjuvants like Rhizobacteria , plant nutrients
8. A.S. help to study the role of endosperm & seed coat formation

Limitation of artificial seeds

1. Large scale production is costly
2. Poor germination of artificial seeds
3. Chances of occurrence of somaclonal variation
4. Special skills are required to handle

M. Application/Advantages of anther or polen culture

1. Development of homozygous line: - The chromosome number of haploid plants is doubled by using colchicines, the plants such obtain are called as double haploids (DH). They are completely homozygous and produced in two years where as by conventional breeding method it takes 6 years, Thus DH lines save 4 years for obtaining homozygous lines

2. Hybrid Sorting: - The het... gene combinations present in the hybrids ar... homozygous combination. ...mozygous strain may be released as a new va... it performs well or used as a parent in hybrid ... ...gramme.

3. Induction of mutation:- ... ...vide a convenient system for the induct... ... and selection of mutants with desired t... ... than diploid cells

4. Analytic breeding:- Extraction of the dihaploid from the tetraploid species and then chromosome doubling of selected dihaploid lines to obtain tetraploid varieties is called as analytic breeding.

5. Use in gene transfer:- pollen embryos are highly regenerative therefore they can be used for gene transfer by Agrobacterium or by a technique like particle gun method.

6. Production of exclusively male plants:- by the process of androgenesis it is possible to produce androgenic haploids followed by chromosome doubling to obtain exclusively male plants
7. Used in evolutionary male plants:- used for the comparison of dihaploids with diploid wild plant species.
8. The steps of androgenesis can be observed starting from a single cell

Q23: Application of DNA fingerprinting (2.5)

1. In medical science
   - i. Diagnosis of genetic disorders
   - ii. Developing cure for genetic disorders
   - iii. Identification of paternity & maternity
   - iv. Confirming legal nationality
   - v. Identification of exchanged child
   - vi. Identification of bodies of soldiers killed in war

2. Forensic science
   DNA testing is very effective in detecting crimina cases such as murder, rape, robbery, assault, kidnapping, car accidents , extortion & blackma...

3. Genetics & plant breeding
   - i. In gene mapping
   - ii. Markers assisted selection
   - iii. Identification of transgenes
   - iv. Protection of legal rights

Q24: Define the following

1. Biotechnology :- Biotechnology is the controlled use of biological agents such as microorganisms or cellular components of cells for welfare of human being
2. Explant:- any plant material which is used to initiate the tissue culture is called Explant
3. Transformation:- the process by which a part of foreign DNA integrate in the Chromosome of recipient cell by a process of recombination is called transformation
4. Southern blotting:- is a method which is used to detect presence of specific DNA sequence
5. Plasmids:- circular DNA other than bacterial chromosome capable of independent replication & transmission is called plasmid
6. Totipotency:- genetic potential of a plant cell to produce the entire plant is called as totipotency
7. Morphogenesis:- formation of multiple shoot from Explant invitro is called Morphogenesis

8. Vector:- vectors are the carrier of foreign [illegible] which are used to transfer the recombinant DNA
9. Callus:- unorganized mass of proliferating [illegible] is called as callus
10. Quantitative trait loci:- genes governing quantitative traits are called as Quantitative trait loci.
11. Synthetic seed: -are the living seeds like structures which are made experimentally by a technique where somatic embryoids are derived from PTC are encapsulated by a hydrogel & such encapsulated embryoids behave like a true seeds if grown in soil & can be used as a substitute for natural seeds
12. Organ culture: - Culture of isolated plant [illegible] such as root tips, shoot tips, leaf primordial, immature parts of flower etc.
13. Ovule culture:- culture in which ovule are aseptically detached from the ovary & are grown on chemically defined nutrient under controlled condition
14. Batch culture:- cultured which are maintained continuously by propagating a small aliquot of inoculum in a fresh moving liquid medium & transferring into fresh medium at regular intervals
15. Asymmetric hybrid:-
16. DNA probes:- are very short pieces of DNA used to find specific sequence of letters in a very long pieces of DNA from a chromosome
17. Double haploids:-
18. Differentiation:- process of biochemical & structural changes by which the unorganized callus become specialized in form & function
19. Dedifferentiation: The resumption of meristematic activity by more or less mature cells through a reversal of the process of cell of tissue differentiation
20. Cybrid :- it is a cell containing nucleus from one species but cytoplasm from both the species involved in fusion
21. Sub culture :- transfer of Explant/callus from old medium to new medium
22. Somaclonal variation:- it is the genetic variability which is generated during tissue culture
23. Organoids:- in some tissue culture an error occur in development programming or organogenesis & an anomalous structure is formed called as Organoids
24. Aseptic:- it means free from all micro organisms
25. Invitro:- culturing Explant under aseptic condition literally in glass medium
26. Continuous culture : A suspension culture continuously supplied with nutrients by continuous flow of fresh medium. The volume of culture medium is normally constant
27. Passage time : The time interval between two successive sub cultures
28. Meristem : A group of actively dividing cells from which permanent tissue systems such as root, shoot, leaf, flower etc are derived
29. Amplification: Creation of many copies of a segment of DNA by PCR / Duplication of genes within a chromosomal segment.
30. Heterokaryon: A cell in which two or more nuclei of unlike genetic makeup are present
31. Homokaryon: A cell with two or more nuclei of similar genetic make up
32. Synkaryon: Hybrid cell produced by fusion of nuclei in Heterokaryon
33. Hetroplast : Cell containing foreign organelles
34. Plant tissue culture:-is the aseptic method of growing cells and organs such as meristems, leaves, roots etc. either in solid or liquid medium under controlled condition
35. Plant biotechnology:- is a branch of biotechnology which deals with all such activities that aim at either improving genetic makeup, phenotypic performance or multiplication rate of economic plants or plant cell
36. Micropropagation:- Clonal propagation through tissue culture is called micropropagation
37. Transgenic Plant:- A plant in which a gene has been transferred through genetic engineering is called transgenic plant
38. Somatic Embryogenesis:- the process by which the somatic cell or tissue develop into differentiated embryos and each fully developed embryo is capable of developing into a plantlet.
39. Anther culture – culturing anthers of precise & critical stage which is to be isolated from unopened flower buds & cultured on artificial nutrient medium
40. media:- it is a substrate used for plant growth such as soil, sand, agar-agar
41. somatic hybridization:- production of hybrid plants through the fusion of two different plant spp is called as somatic hybridization

42. Molecular Markers:- A molecular marker may be defined as a DNA sequence used for chromosome mapping as it can be located at a specific site in a chromosome
43. Organogenesis:- the development of adventitious organs or primordial from undifferentiated cell mass in tissue culture by the process of differentiation is called organogenesis
44. Caulogensis:- development of adventitious shoot buds in callus.
45. Rhizogenesis:- development of adventitious roots in callus.
46. Suspension culture:- it is a type of culture in which single cell or mass of aggregates of cell multiple while suspended in agitated liquid medium.
47. Single cell culture:- it is a method of growing isolated single cell aseptically on a nutrient medium under controlled condition.
48. Embryoids:- these are small, well organized structure comparable to sexual embryo, which is produced in tissue culture.
49. Marker assisted selection refers to indirect selection for a desired plant phenotype based on the pattern of linked molecular (DNA) markers
50. Mapping population:- In plant breeding and genetics various types of plant material which are used for gene mapping or gene tagging or for construction of genetic linkage maps are known as mapping populations
51. Southern blotting:- is a method which is used to detect presence of specific DNA sequence.
52. Northern blotting technique:- is a method which is used to determine the presence of a specific mRNA from a mixture of RNA.
53. Western blotting technique:- is a method which is used to detect the presence of a specific protein sample by using antibody specific to that protein.
54. Test tube fertilization:- Transfer of the pollen from anther to stigma of ovaries cultured invitro is called as in-vitro pollination or test tube fertilization or invitro fertilization
55. Synthetic seeds/ Artificial seeds:- are the living seeds like structures which are made experimentally by a technique where somatic embryoids are derived from PTC are encapsulated by a hydrogel & such encapsulated embryoids behave like a true seeds if grown in soil & can be used as a substitute for natural seeds
56. Clonal propagation:- multiplication of genetically identical copies of a cultivar by asexual reproduction is called C.P
57. Cybridisation:- the process of protoplast fusion resulting in the development of Cybrids.
58. Gene cloning:- refers to the process of isolating a gene from one organism & then inserting it into another organism resulting into the formation of GMO
59. DNA finger printing:- Is an analysis of nitrogen base sequence in the DNA of an individual.

29. Contribution of following scientist (asked in now date in exams)

1. Kary mullis:- Developed polymerase chain reaction technique in 1985
2. Alexander Flemming:- produced the antibiotic penicillin
3. G. morali:- developed shoot tip / Meristem culture technique first time to obtain virus free plants
4. F. Laibach:- used embryo culture technique to raise viable plants from unsuccessful crosses
5. L. Bergmann :- grew the first single cells first using Petri dish plating technique
6. E. M. Southern:- Invented DNA-DNA hybridization technique
7. Alec Jaffrey:- discovered the DNA finger printing technique
8. Hargobind Khorana:- he developed procedure for artificial gene DNA synthesis
9. Guha & Maheswari :- obtained haploid(n) Dhatura plants by culturing anther & pollens
10. Prof. Stanford:- discovered Biolistic gene transfer method
11. Murashige & skoog:- discovered MS medium for tissue culture — Micropropagation by Ereky
12. Brown :- Invitro culture of crown gall tissue
13. Karl Ereky :- The term Biotechnology was coined by karl Ereky
14. P. R. White:- he maintain long term culture of tomato roots
15. Edward Cocking:- worked on enzymatic isolation & culture of protoplast
16. W.H. Muir :- inoculate the callus through callus technique
17. G. Haberlaud :- made 1st attempt to culture plant

Q26: Answer in one sentence

1. Thermoliable compounds are sterilized by filter sterilization. Why ?
   Ans :- Thermoliable compounds are sterilized by filter sterilization because they unstable at high temperature
2. Why virus free plants are produced from shoot tip culture
   Ans :- virus free plants are produced from shoot tip culture because of fast mitotic activity of meristematic tissue present in shoot tip.
3. Sub-culturing is essential for in vitro culture. Give reasons
   Ans:- Sub-culturing is essential for in vitro culture to ensure undisturbed supply of nutrients to growing culture
4. What is a shuttle vector?
   Ans – the vector designed to replicate in cells of two different species is called as vector shuttle
5. Why RAPD marker is not reproducible & consistent
   Ans:- RAPD marker is not reproducible & consistent because it is more sensitive to experimental conditions
6. Mannitol is used for isolation of cells. Why?
   Ans:- Manitol is used for isolation of cells because manitol sugar provide energy to cell & maintain osmotic pressure
7. Ph of tissue culture medium is adjusted to 5.8 before autoclaving
   Ans:- Ph of tissue culture medium is adjusted to 5.8 before autoclaving as it helps in better nutrient uptake, the salts remain in dissolved condition
8. What is disarming of Ti-plasmid ?
   Ans: the deletion of genes governing auxin & cytokinin production from T/DNA of Ti-plasmid is known as disarming of Ti-plasmid
9. Why suspension cultures are constantly shaken .
   Ans:- suspension cultures are constantly shaken to keep cells in suspension & to make the culture homogenous
10. Browning of Explant occurs during microprogation. Why?
    Ans:- Browning of Explant occurs during microprogation because of oxidation of phenolic compounds

Q27: Fill ups

1. The process of organogenesis resulting in the formation of shoot is known as Caulogensis
2. Friable callus is suitable for suspension culture
3. The plating culture for culturing cells or protoplasts is developed by Bergmann
4. Commercially exploited technique of tissue culture is Micropropogation
5. Disease free or virus free plants are obtained by shoot tip culture
6. Embryos isolated before Globular stage are most suitable for embryo culture
7. Somatic hybrids plants which retain the full or nearly full somatic complements of the two parental species are called Cybrids
8. Primer is a short sequence that pairs with one strand of DNA & provides free 3' off end at which DNA polymerase starts synthesis of a deoxyribonucleotide chain.
9. Leaf Mesophylls are commonly used as Explants for the protoplast culture

Q28: Give the full form along with headquarters of following Research Central Organization for Biotechnology

1. IARI ——— Indian Agricultural Research Institute, New Delhi
2. JNU ——— Jawaharlal Nehru University, New Delhi
3. IVRI : ——— Indian Veterinary Research Institute, Izatnagar
4. CFTRI : ——— Central Food Technology Research Institute, Mysore
5. NDRI ——— National Dairy Research Institute - Karnal - Haryana
6. MRC ——— Malaria Research Center – New Delhi
7. RRL : ——— Regional Research Laboratory – Jammu
8. CDRI : ——— Central Drug Research Institute – Lucknow
9. CIMAP : ——— Central Institute of Medicine and Aromatic plants - Lucknow and Hyderabad

10. IIT :----------Indian Institute of Technology – Kanpur, New Delhi
11. IISC :----------Indian Institute of sciences – Bangalore
12. IMTECH :---------Institute of Microbial Technology – Chandigarh
13. NIM/NII :---------National Institute of Immunology – New Delhi
14. NCL :----------National Chemical Laboratory – Pune
15. CCMB :----------Center for Cellular and Molecular Biology – Hyderabad
16. CDFD :----------Center for DNA Finger Printing and Diagnostics – Hyderabad
17. CPMB :---------Center for Plant Molecular Biology – 7 centers
18. BARC :---------Baba Atomic Research Center – Mumbai
19. UNEP :---------United Nations Environment Programme
20. ICRO :---------International Cell Research organization
21. IIB :----------International Institute of Biotechnology – Canterbury Kent in UK

29. Give the full forms of following abbreviations

1. PAGE :---------Poly Acrylamide Gel Electrophoresis
2. RFLP :---------Restriction Fragment Length polymorphism
3. RAPD :---------Randomly Amplified polymorphic DNA
4. cDNA :---------Complementary DNA
5. mt DNA :---------Mitochondrial DNA
6. PCR :---------Polymerase Chain Reaction
7. HPLC :---------Higrowth hormone Performance Liquid Chromatography
8. PEG :---------Poly Ethylene Glycol
9. HFCS :---------Higrowth Hormone Fructose Corn Syrup
10. HEPA :---------Higrowth Hormone Efficiency Particulate Air
11. GMO :---------Genetically Modified Organisms
12. MAS :---------Marker Assisted Aided Selection
13. ELISA :---------Enzyme Linked Immuno Sorbent Assay
14. NAA :---------Naphthalene Acetic Acid
15. IAA :---------Indole – 3 - Acetic acid
16. IBA :---------Indole – 3 – Butyric acid
17. BAP :---------Benzyl Amino Purine
18. BA :---------Benzyl Adenine
19. HGH :---------Human Growth Hormone
20. SSRs :---------Simple Sequence Repeats
21. QTL :---------Quantitative Trait loci
22. VNTRS :---------Variable Number of Tandem Repeats.
23. GEAC :---------Genetic Engineering Approval Committee
24. GEM :---------Genetically Engineered Micro Organism
25. CMV :---------Cauliflower Mosaic Virus
26. TMV :---------Tobacco Mosaic Virus
27. STS :---------Sequence Tagged Sites
28. EDTA :---------Ethylene Diamine Tetra Acetic acid.
29. Pg :---------Picograms
30. Ppm :---------Parts Per Million
31. MOET :---------Multiple Ovule and Embryo transfer

[illegible] Important Contributors in Plant Tissue Culture

Sr	Scientist	Contribution
1.	Scheilden & Schwann	Hypothesis of cell theory, suggest totipotency of cell
2.	Haberlandt	First attempt of plant tissue culture (Father of Plant Tissue culture)
3.	Hannig	First attempt to culture embryo of selected crucifers
4.	Knudsen	Asymbiotic germination of orchid seeds in vitro
5.	Robbins	In vitro culture of root tips
6.	Laibach	Use of embryo culture technique in interspecific crosses of linseed (Linum)
7.	Gautheret	In vitro culture of the cambial tissue of a few trees and shrubs, although failed to sustain cell division
8.	P.R. White	Successful culture of tomato roots
9.	Kogl et al	Identified 1st plant growth regulator
10.	Gautheret, Nobecourt and White	Successful establishment of continuously growing callus cultures
11.	Gautheret	In vitro culture of cambial tissues of Ulmus to study adventitious shoot formation
12.	Van Overbeek	Use of coconut milk containing a cell division factor for the first time to culture Datura embryos
13.	Braun	In vitro culture of crown gall tissues
14.	Skoog	In vitro adventitious shoot formation in tobacco
15.	E. Ball	Raising of whole plants of *Lupinus* and *Tropaeolum* by shoot tip culture
16.	Morel and Martin	Use of Meristem culture to obtain virus-free Dahlias
17.	Tulecke	Production of haploid callus of the gymnosperm *Ginkgo biloba* from pollen
18.	Muir et al	First plant regenerated from a single cell
19.	Miller et al	Discovery of kinetin, a cell division hormone
20.	A. Kornberg et al	In vitro synthesis of DNA
21.	Skoog and Miller	Discovery of the regulation of organ formation by
22.	Maheshwari and Rangaswamy	Regeneration of somatic embryos in vitro from the nucellus of Citrus ovules
23.	Reinert and Steward	Regeneration of embryos from callus clumps and cell suspensions of carrot (Daucus carota)
24.	Gautheret	Publication of first handbook on "Plant Tissue Culture"
25.	Kanta	First successful test tube fertilization in Papaver rhoeas
26.	E. Cocking	Enzymatic degradation of cell walls to obtain large number of protoplasts
27.	Bergmann	Filtration of cell suspensions and isolation of single cells by plating
28.	Murashige and Skoog	Development of Murashige and Skoog nutrition Medium
29.	Guha and Maheshwari	Production of first haploid plants from pollen grains of Datura (Anther culture)
30.	Morel	Produced the protocorms from orchids under invitro condition
31.	H.G. Khorana H.G. Khorana et al.	Awarded Nobel prize for deciphering of genetic code Deduced the structure of a gene for yeast alanyl tRNA
32.	Bourgin & Ritsch	Obtained haploid plants from pollen grains of tobacco
33.	Meselson and Yuan	Coined the term "Restriction endonuclease" to describe a class of enzymes involved in cleaving DNA
34.	Carlson	Selection of biochemical mutants in vitro by the use of tissue culture derived variation First achievement of protoplast fusion
35.	Power et al.	Discovered the presence of reverse transcriptase (a RNA directed DNA polymerase which
36.	H. Temin and D. Baltimore	has the ability to synthesize cDNA using mRNA as a template
37.	Smith	Discovery of first restriction endonuclease from Haemophilus influenzae Rd. It was later purified and named Hind II