SEMESTER V

17BTU503A PLANT BIOTECHNOLOGY 4H - 4C

Total hours/week: L:4 T:0 P:0 Marks: Internal: 40 External: 60 Total: 100

Scope: This will enable for learning the techniques to save endangered species which will be useful for mankind.

Objective: This paper aims at introducing students to the basic and applied aspects of plant biotechnology.

UNIT-I

Introduction: Cryo and organogenic differentiation, Types of culture: Seed, Embryo, Callus, Organ, Cell and Protoplast culture. Micropopagation Axillary bud proliferation, Meristem and shoot tip culture and culture, organogenesis, embryogenesis, advantages and disadvantages of micropropagation

UNIT-II

***In vitro* culture:** haploid production Androgenic methods: Anther culture, Microspore culture and ogenesis. Significance and use of haploids, Ploidy level and chromosome doubling, diplodization, Gynogenic haploids, factors effecting gynogenesis, chromosome elimination techniques for production of haploids in cereals.

UNIT-III

Hybridization: Protoplast Isolation and fusion Methods of protoplast isolation, Protoplast development, Somatic hybridization, identification and selection of hybrid cells, Cybrids, Potential of somatic hybridization limitations. Somaclonal variation Nomenclature, methods, applications basis and disadvantages. Plant Growth Promoting bacteria. Nitrogen fixation, Nitrogenase, Hydrogenase, Nodulation, Bio control of pathogens, Growth promotion by free-living bacteria.

UNIT-IV

Structure and organization of plant genome: regulation of plant genome expression, transcriptional, translational and post transcriptional regulation of plant genome. Transfer of nucleic acid to plant cells - Direct transformation by electroporation and particle gun bombardment. - *Agrobacterium*, Ti plasmid vector Theory and techniques for the development of new genetic traits.

UNIT-V

Transgenic plants: herbicides and pest resistant plants, Drought, Salinity and cold tolerant plants; Molecular farming / pharming: carbohydrates, lipids, theraputic proteins, edible vaccines, purification strategies; Oleosin partition technology.

References

1. Gardner, E.J., Simmonns, M.J., & Snustad, D.P. (2008). (8th ed.). *Principles of Genetics*. India: Wiley.
2. Bhojwani, S.S., & Razdan, (2004). *Plant Tissue Culture and Practice*.

3. Brown, T.A., (2006). *Gene Cloning and DNA Analysis* (5th ed.). Oxford: UK, Blackwell Publishing.
4. Raven, P.H., Johnson, G.B., Losos, J.B., & Singer, S.R. (2005). *Biology*. Tata MC Graw Hill.
5. Reinert, J., & Bajaj, Y.P.S. (1997). *Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture*. Narosa Publishing House.
6. Russell, P.J. (2009). *Genetics – A Molecular Approach* (3rd ed.). Benjamin Co.

7. Sambrook, & Russel. (2012). *Molecular Cloning: A laboratory manual* (4th ed.). Cold Spring Harbor Laboratory Press.
8. Slater, A., Scott, N.W., & Fowler, M.R. (2008). *Plant Biotechnology: The Genetic Manipulation of Plants*. Oxford University Press.

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LECTURE PLAN
DEPARTMENT OF BIOTECHNOLOGY

STAFF NAME: Dr.A.SANGILIMUTHU
SUBJECT NAME: PLANT BIOTECHNOLOGY SUB.CODE:17BTU503A
SEMESTER: V CLASS: III B.Sc (A Sec)

S.No.,	Topic to be covered	Duration Hours	Total hours	Ref. Book/ Author
	Unit I			
1.	Introduction and Principles of plant breeding	1	10	T1 378-379 T2 20-24
2.	Conventional method of plant breeding Self pollinated crops Cross pollinated crops Vegetative propagated crop	1		T2 25-29 T2 29-32
3.	Non conventional method of plant breeding Polyploidy Genetic variability	1 1 1		T2 413-426
4.	Importance of plant breeding methods	1		T2 10-12
5.	Genome organization of plant Mitochondrial genome Chloroplast genome	1 1		R1 1-17
6.	Cytoplasmic male sterility	1		T1 488 - 490
7.	Recapitulation of Unit I	1		
	Unit II			
8.	*In vitro* culture/ Plant tissue culture Tissue culture media Composition and preparation	1 1	10	T1 332-334 T1 337- 339
9.	Callus and suspension culture Somaclonal variation, Micropropagation Organogenesis	1 1		T1339-344 T1 389-393 T1 345 -346
10.	Somatic embryogenesis	1		T1 391 - 392
11.	Embryo culture and embryo	1		T1 368 - 369

	rescue- haploid			
12.	Protoplast fusion and somatic hybridization, Cybrid, anther, pollen culture	1		T1 361 - 366 T1 367 - 368
13.	Ovary culture for production of haploid plant and homozygous line	1		T1 355-356 T1 403- 405
14.	Plant hardening transfer to soil, Green house technology	1		R2 243- 244
15.	Recapitulation of Unit II	1		
Unit III				
16.	Plant genome organization Chloroplast genome organization Mitochondrial genome organization, Nuclear genome organization	1 1	09	R1 1-2 R 3-7
17.	Production of pharmaceutical compounds	1		
18.	Mass cultivation of plant cell Cell suspension culture and stages	1 1		T1 378-382
19.	Secondary metabolites production from cell suspension culture, Bioreactor – photo bioreactor	1 1		T382-3881
20.	Production and Stages of secondary metabolite production in plants	1		T1 490 - 495
21.	Use of plant tissue culture techniques	1		T1 496- 499
Unit IV				
22.	Plant genetic engineering	1	09	T1 458 – 460
23.	Methodology : Physical method and chemical methods	1		T1 426 – 430 T1 449 - 450
24.	Plant transformation with Ti plasmid of *Agrobacterium tumifaciens*	1		T1 447 - 449
25.	Mechanism and interaction Gene expression in plants	1		T1 453 - 455
26.	Ti plasmid derived vector system and Ri plasmid	1		T1 435 – 440
27.	Physical method of transferring gene to plants Microprojectile bombardment, Electroporation	1 1		T1 449 - 451
28.	Production of marker free	1		T1 459 – 460

	transgenic plants			
29.	Recapitulation of Unit IV	1		
Unit V				
30.	Application of genetic transformation productivity and performance	1	10	**T1 426 – 427**
31.	Herbicide resistance crop Insect resistance crop	1		**T1 459 – 460** **T1 461-466**
32.	Virus resistance crop, Fungal resistance crop, Nematode resistance crop and Delay in fruit ripening	1 1		**T1 467 – 470** **T1 470 -472**
33.	Induction of abiotic stress and cold stress resistance plant	1		**T1 473**
34.	Terminator and seed technology and Plant bodies	1		**T1 503 - 504**
35.	Edible vaccines primary and secondary metabolite modification	1		**T1 495 - 499**
36.	Biopolymer, Plant based enzyme engineering	1		**T1 637 - 639**
	ESE question paper discussion	1		
	ESE question paper discussion	1		

T1. Singh B.D., 2010, Biotechnology, Kalyani Publishers
T2. Principles of Plant Breeding , G.S.Chahal & S.S.Gosal (2007), Narosa Publishing House, New Delhi

R1. Adrian Slater, Nigel W. Scott, 2008. Plant Biotechnology, Oxford University Press, UK
R2. Introduction to plant tissue culture, M.K.Razdan (2008), Oxford & IBH Publishing House, New Delhi.

UNIT-I

SYLLABUS

Introduction: Cryo and organogenic differentiation, Types of culture: Seed, Embryo, Callus, Organ, Cell and Protoplast culture. Micropopagation Axillary bud proliferation, Meristem and shoot tip culture and culture, organogenesis, embryogenesis, advantages and disadvantages of micropropagation.

Organogenic differentiation

- ✓ Growing any part of the plant (explants) like, cells, tissues and organs, in an artificial medium under controlled conditions (aseptic conditions) for obtaining large scale plant propagation is called micropropagation.
- ✓ The basic concept of micropropagation is the plasticity, totipotency, differentiation, dedifferentiation and redifferentiation, which provide the better understanding of the plant cell culture and regeneration. Plants, due to their long life span, have the ability to withhold the extremes of conditions unlike animals.
- ✓ The plasticity allows plants to alter their metabolism, growth and development to best suit their environment.
- ✓ When plant cells and tissues are cultured *in vitro*, they generally exhibit a very high degree of **plasticity**, which allows one type of tissue or organ to be initiated from another type.
- ✓ Hence, whole plants can be subsequently regenerated and this regenerated whole plant has the capability to express the total genetic potential of the parent plant.
- ✓ This is unique feature of plant cells and is not seen in animals. Unlike animals, where differentiation is generally irreversible, in plants even highly mature and differentiated cells retain the ability to regress to a meristematic state as long as they have an intact membrane system and a viable nucleus.
- ✓ However, sieve tube elements and xylem elements do not divide any more where the nuclei have started to disintegrate, According to Gautheret (1966) the degree of regression a cell can undergo would depend on the cytological and physiological state of the cell.
- ✓ The meristematic tissues are differentiated into simple or complex tissues called **differentiation**. Reversion of mature tissues into meristematic state leading to the formation of callus is called **dedifferentiation**.
- ✓ The ability of callus to develop into shoots or roots or embryoid is called **redifferentiation**.

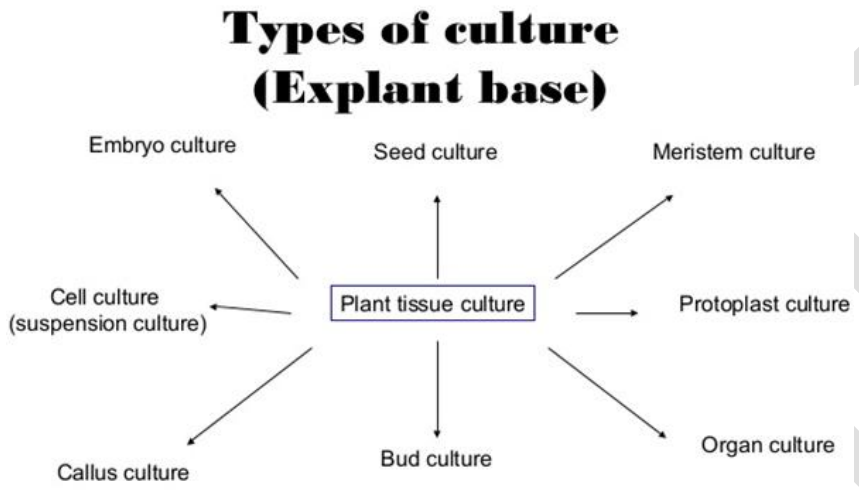
- ✓ The inherent potentiality of a plant cell to give rise to entire plant and its capacity is often retained even after the cell has undergone final differentiation in the plant system is described as cellular **totipotency**.
- ✓ During *in-vitro* and *in vivo* cytodifferentiation (cell differentiation), the main emphasis has been on vascular differentiation, especially tracheary elements (TEs).
- ✓ These can be easily observed by staining and can be scored in macerated preparations of the tissues. Tissue differentiation goes on in a fixed manner and is the characteristic of the species and the organs.

Factors affecting vascular tissue differentiation

- Vascular differentiation is majorly affected qualitatively and quantitatively by two factors, auxin and sucrose.
- Cytokinins and gibberellins also play an important role in the process of xylogenesis.
- Depending upon the characteristics of different species, concentration of phytohormones, sucrose and other salt level varies and accordingly it leads to the vascular tissue differentiation.
- The vegetative propagation has been conventionally used to raise genetically uniform large scale plants for thousands of years
- However, this technique is applicable to only limited number of species. In contrast to this, micropropagation has several advantages.

Types of culture: Seed, Embryo, Callus, Organ, Cell and Protoplast culture.

Organogenesis : generation of specific organs of the plants under *in vitro* conditions by using artificial media are called organogenesis.



Embryogenesis

generation of embryo of the plant under *in vitro* conditions by using artificial media are called embryogenesis.

Seed Culture

Seed culture is the type of tissue culture that is primarily used for plants such as orchids. For this method, explants (tissue from the plant) are obtained from an in-vitro derived plant and introduced in to an artificial environment, where they get to proliferate. In the event that a plant material is used directly for this process, then it has to be sterilized to prevent tissue damage and ensure optimum regeneration.

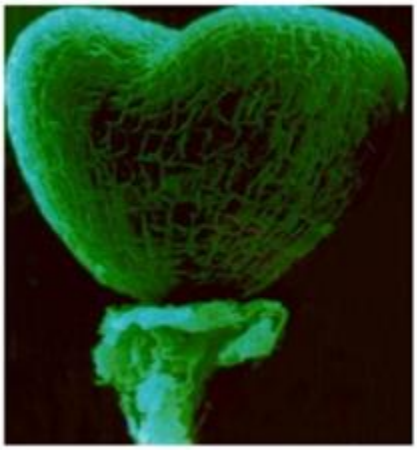
- Growing seed aseptically *in vitro* on artificial media.
- Increasing efficiency of germination of seeds that are difficult to germinate in vivo
- It is possible to independent on asymbiotic germination. Production of clean seedlings for explants or meristem culture.

Embryo culture is the type of tissue culture that involves the isolation of an embryo from a given organism for *in vitro* growth.

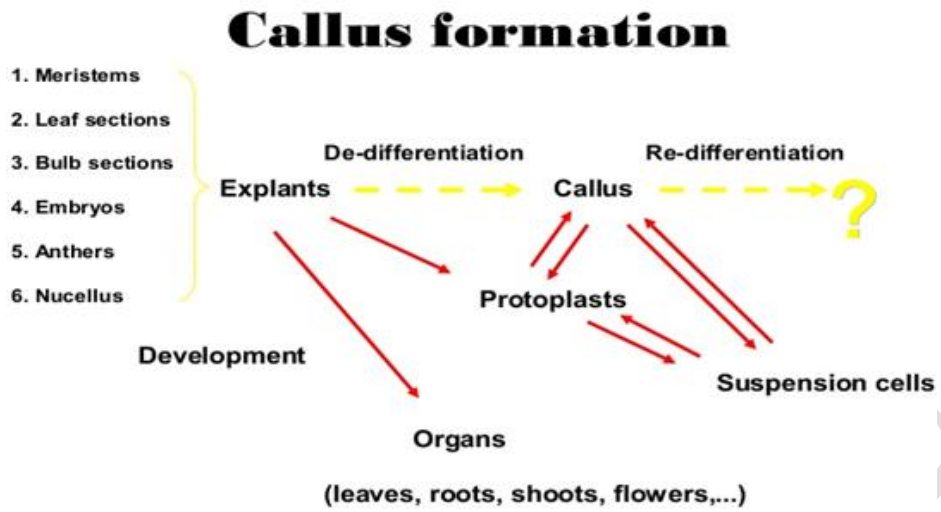
Growing embryo aseptically *in vitro* on artificial nutrient media

Overcoming seed dormancy and self –sterility of seeds

Study embryo development



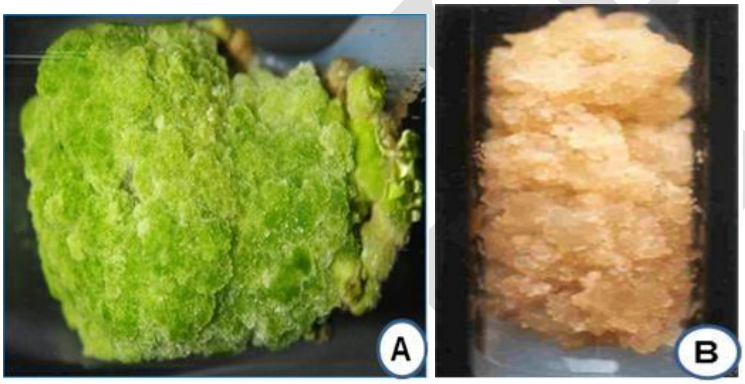
Callus culture



Callus cultures

- When an organ of a plant is damaged a wound repair response is induced to bring about the repair of the damaged portion.
- This response is associated with the induction of division in the undamaged cells adjacent to the lesion, thus sealing of the wound. If, however, wounding is followed by the aseptic culture of the damaged region on a defined medium, the initial cell division response can be stimulated and induced to continue indefinitely through the exogenous influence of the chemical constitution of the culture medium.
- The result is a continually-dividing mass of cells without any significant differentiation and organization and this proliferated mass of cell aggregate is called callus.
- The first step to establish cell suspension cultures is to raise callus from any explants of the plant.
- To maximize the production of a particular compound, it is desirable to initiate the callus from the plant part that is known to be high producer.
- Calli are generally grown on medium solidified with gelling agents like, agar, gelrite, agarose, in Petri-dishes, glass test-tubes or extra-wide necked Erlenmeyer flasks.
- In morphological terms it can vary extensively, ranging from being very hard/compact and green or light green in color, where the cells have extensive and strong cell to cell contact, to being ‘friable' where the callus consists of small, disintegrating aggregates of poorly-associated cells and has brownish or creamy appearance .

- Friable callus is most demanded since it shows fast and uniform growth of cells and is highly suitable to initiate cell suspension cultures.
- Callus morphology is explants and species dependent but can be altered by the modification of plant growth regulators in the medium.
- The callus cultures shows inherent degree of heterogeneity and this may be due to their size and nature, unidirectional supply of nutrient medium (below the callus) and gases and light (predominantly from above).
- The heterogeneity may be disadvantageous in uniform production of cell biomass but may be useful in the developmental responses of the callus like, shoot regeneration.



Callus cultures- **A.** Hard and compact callus; **B.** Friable and brown callus

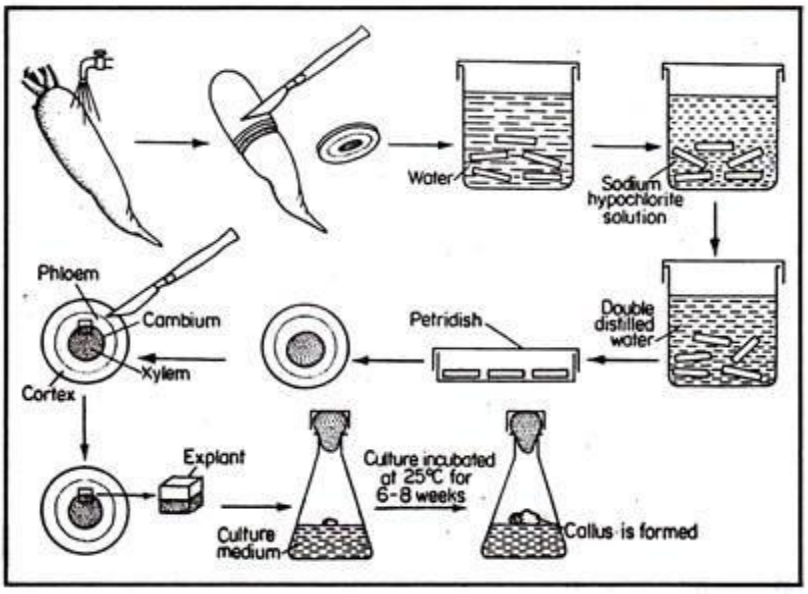


Fig 3.1

Procedure for the callus culture from carrot root

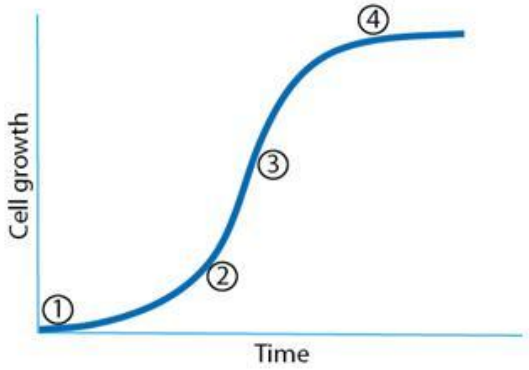
Cell suspension culture

- A suspension culture is developed by transferring the relatively friable portion of a callus into liquid medium and is maintained under suitable conditions of aeration, agitation, light, temperature and other physical parameters .
- The increased cell dissociation means increased culture uniformity.
- Plant cells are significantly larger and slower growing cells than most microbial organisms.
- They mostly resemble to parenchymatous cells in having relatively large vacuoles, a thin layer of cytoplasm and thin, rounded cell walls. The species/genotypes and medium composition used can influence *in vitro* cell morphology and different cell types with different morphological/physiological properties can co-exist within a single culture.

Cells in liquid medium showing fine suspension of cells

Cell growth: The most commonly used cell suspensions are of the closed (or batch) type where the cells are grown in fixed volume of liquid medium and which are routinely maintained through the transfer of a portion (ca 10%) of a fully-grown culture to fresh medium at regular intervals.

- The growth curve of a cell suspension culture has a characteristic shape consisting of four essential stages- an initial lag phase, an exponential phase, stationary phase and death phase.
- The duration of each phase is dependent on the species or genotype selected, explant used, culture medium and subculture regime.
- The lag phase is shortened when relatively large inocula are used although paradoxically, growth terminates earlier and overall biomass production is reduced.



Growth curve for plant cell suspension grown in closed system. The four different growth phases are labeled: (1) Lag phase, (2) Exponential phase, (3) Linear phase, (4) Stationary phase.

Aggregation:

- Due to larger size of a plant cell, it is capable of withstanding tensile strain but is sensitive to shear stress.
- Aggregation is common, largely due to failure of the cells to separate after division. The secretion of extracellular polysaccharides, particularly in the later stages of growth, may further contribute to increased adhesion.
- This tendency of plant cells to grow in clumps results in sedimentation, insufficient mixing and diffusion-limited biochemical reaction. Even the fine suspension culture consists of micro-to sub-macroscopic colonies made up of around 5-200 cells and such degree of cell aggregation is acceptable.
- Cultures consisting of larger aggregates like, 0.5-1.0 mm in diameter, are more readily attainable, grow perfectly well and depending on the aim of the research are often sufficient to meet all requirements.
- This so called cell-cell contact is desirable for the biosynthesis of many secondary metabolites by the plant cells in suspension cultures.
- Therefore, controlled aggregation of plant cells may be of interest from process engineering point of view.

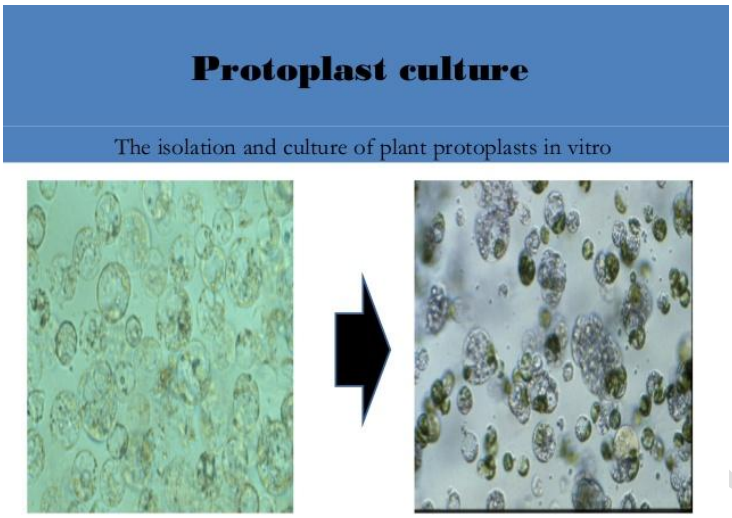
Oxygen and aeration effect:

- Oxygen requirements of plant cells are comparatively lower than that of microbial cells due to their low growth rates.
- In some cases high oxygen concentration is even toxic to the cell's metabolic activities and may strip nutrients such as carbon dioxide from the culture broth.
- Carbon dioxide is often considered as essential nutrient in the culture of plant cells and has a positive effect on cell growth. Moderate shaking speed like, 90-120 rpm is ideal for standard aeration.
- As the plant cells are shear sensitive and the immediate effects of high agitation are the cell damage, reduction in cell viability, release of intracellular compounds while low agitation (<90rpm) results in cell aggregation.

Protoplast culture

The protoplasm of a living plant cell whose cell wall has been removed or cell without cell wall.

- Variants obtained using callus cultures are referred as "**Calliclones**" (Skirvin, 1978) while variants obtained using protoplast cultures are known as "**Protoclones"**
- Protoplast can be isolated either directly from the different parts of whole plant or indi-rectly from in vitro cultured tissue.
- Convenient and suitable materials are leaf, mesophyll and cells from liquid suspension cul-tures. Protoplast yield and viability are pro-foundly influenced by the growing conditions of plants serving leaf mesophyll sources.



Micropopagation Axillary bud proliferation, Meristem and shoot tip culture and culture, organogenesis, embryogenesis, advantages and disadvantages of micropropagation.

Organ culture

Culture separate organs of the plants like shoot, stem, leaf and root.

Organ culture

Any plant organ can serve as an explant to initiate cultures

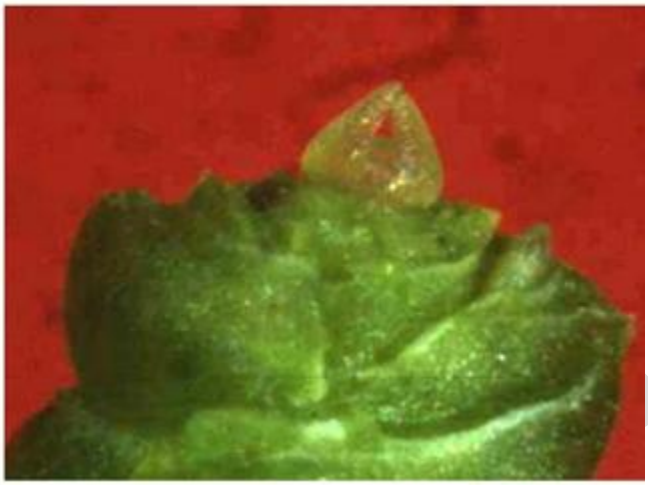
No.	Organ	Culture types
1.	Shoot	Shoot tip culture
2.	Root	Root culture
3.	Leaf	Leaf culture
4.	Flower	Anther/ovary culture

Production separate organs under *in vitro* condition using the hormones specific growth.

Shoot apical meristem culture

- Production of virus free germplasm
- Mass production of desirable genotypes
- Facilitation of exchange between locations (production of clean material)

- Cryopreservation (cold storage) or in vitro conservation of germplasm



Physico-chemical conditions for propagation of plant cells and tissues

PHYSICO CHEMICAL CONDITION (NUTRIENT MEDIA)

Both the media listed in the below tables can be prepared from stock solutions of:

i. Macronutrients: As its name suggests, in plant tissue culture media these components provide the elements which are required in large amounts (concentrations greater than 0.5 mmole l^{-1}) by cultured plant cells.

Macronutrients are usually considered to be carbon, nitrogen, phosphorous, magnesium, potassium, calcium and sulphur.

ii. Micronutrients: It provides the elements that are required in trace amounts (concentrations less than 0.5 mmole l^{-1}) for plant growth and development.

These include, manganese, copper, cobalt, boron, iron, molybdenum, zinc and iodine.

iii. Iron source: It is considered the most important constituent and required for the formation of several chlorophyll precursors and is a component of ferredoxins (proteins containing iron) which are important oxidation : reduction reagents.

iv. Organic supplements (**vitamins**): Like animals, in plants too vitamins provide nutrition for healthy growth and development.

Although plants synthesize many vitamins under natural conditions and, therefore, under in vitro conditions they are supplied from outside to maintain biosynthetic capacity of plant cells in vitro.

There are no firm rules as to what vitamins are essential for plant tissues and cell cultures. The only two vitamins that are considered to be essential are myo-inositol and thiamine.

Myo-inositol is considered to be vitamin B and has many diverse roles in cellular metabolism and physiology. It is also involved in the biosynthesis of vitamin C.

v. Carbon source: This is supplied in the form of carbohydrate.

Plant cells and tissues in the culture medium are heterotrophic and are dependent on external source of carbon.

Sucrose is the preferred carbon source as it is economical, readily available, relatively stable to autoclaving and readily assimilated by plant cells.

During sterilization (by autoclaving) of medium, sucrose gets hydrolyzed to glucose and fructose. Plant cells in culture first utilize glucose and then fructose. Besides sucrose, other carbohydrates such as, lactose, maltose, galactose are also used in culture media but with a very limited success.

$$\textbf{Dilutions :}\ \frac{\text{Required concentration} \times \text{medium volume}}{\text{Concentration of stock solution}} = \text{Volume of stock required}$$

The media elements and their functions

S.No.,	Elements	Functions
1	Oxygen	Common cell components, electron acceptor
2	Carbon	Common cellular components, forms basic backbone of most biochemicals
3	Nitrogen	Part of proteins, vitamins, amino acids and coenzymes
4	Sulphur	Part of some amino acids and some coenzymes
5	Potassium	Principal inorganic actions
6	Magnesium	Important coenzyme factor and part of chlorophyll molecule
7	Manganese	Important cofactor
8	Calcium	Important constituents of cell wall and enzyme cofactor
9	Iron	Part of cytochromes
10	Cobalt	Part of some vitamins
11	Copper	Enzyme cofactor
12	Zinc	Enzyme cofactor
13	Molybdenum	Enzyme cofactor

The composition of Gamborg's B_5 Medium (1968)

Component	Concentration in stock (mg l^{-1})	Concentration in medium (mg l^{-1})	Volume of stock per litre of medium (ml)
Macronutrients			
KNO_3	50000	2500	50
$CaCl_2.2H_2O$	3000	150	
$(NH_4)_2SO_4$	2680	134	
$MgSO_4.7H_2O$	5000	250	
$NaH_2PO_4.H_2O$	3000	150	
Micronutrients			
KI	30	0.75	25
H_3BO_3	120	3	
$MnSO_4.4H_2O$	400	10	
$ZnSO_4.7H_2O$	80	2	
$Na_2MoO_4.2H_2O$	10	0.25	
$CuSO_4.5H_2O$	1	0.025	
$CoCl_2.6H_2O$	1	0.025	
Iron Source			
FeNaEDTA	3670	36.7	10
Vitamins			
Myo-inositol	Add freshly to the medium	100	1
Pyridoxine-HCl	1000	1	
Thiamine-HCl	10000	10	
Nicotinic acid	1000	1	
Carbon Source			
Sucrose	Add freshly to the medium	30g l^{-1}	
Adjust pH to 5.5 before autoclaving			

• Stock concentration of macronutrients is for 20 litres of medium, while micronutrients stock is for 40 litres of medium, iron for 100 litres of medium and vitamins stock is for 1000 litres of medium.

• Myoinositol and sucrose are added freshly to the medium.

The composition of Murashige and Skoog (MS) Medium (1962)

Component	Concentration in stock (mg l^{-1})	Concentration in medium (mg l^{-1})	Volume of stock per litre of medium (ml)
Macronutrients			
NH_4NO_3	33000	1650	50
KNO_3	38000	1900	
$CaCl_2.2H_2O$	8800	440	
$MgSO_4.7H_2O$	7400	370	
KH_2PO_4	3400	170	
Micronutrients			
KI	166	0.83	5
H_3BO_3	1240	6.2	
$MnSO_4.4H_2O$	4460	22.3	
$ZnSO_4.7H_2O$	1720	8.6	
$Na_2MoO_4.2H_2O$	50	0.25	
$CuSO_4.5H_2O$	5	0.025	
$CoCl_2.6H_2O$	5	0.025	
Iron Source			
$FeSO_4.7H_2O$	5560	27.8	5
$Na_2EDTA.2H_2O$	7460	37.3	
Vitamins			
Myo-inositol	Add freshly to the medium	100	
Nicotinic acid	100	0.5	5
Pyridoxine-HCl	100	0.5	
Thiamine-HCl	100	0.5	
Glycine	400	2	
Carbon Source			
Sucrose	Add freshly to the medium	30g l^{-1}	
Adjust pH to 5.7-5.8 before autoclaving			

• Stock concentration of macronutrients is for 20 litres of medium, while micronutrients, iron and vitamins stock concentrations are prepared for 200 litres of medium.

• Myoinositol and sucrose are added freshly to the medium.

• Dissolve 5.56 g of $FeSO_4 .7H_2O$ in 350 ml of water. Apply heat if needed. Dissolve 7.46 g of Na_2EDTA in 350 ml of water. Apply heat if needed. When both solutions are dissolved, combine

and bring to 1 litre final volume. The chelation reaction is forced to completion by autoclaving. The final stock solution should be deep golden yellow in color.

The steps involved for the preparing nutrient medium

- ✓ Add appropriate quantities of various stock solutions, including growth regulators and other special supplements. Make up the final volume of the medium with distilled water.
- ✓ Add and dissolve sucrose.
- ✓ After mixing well, adjust the pH of the medium in the range of 5.5-5.8, using 0.1 N NaOH or 0.1 N HCl (above 6.0 pH gives a fairly hard medium and pH below 5.0 does not allow satisfactory gelling of the agar).
- ✓ Add agar, stir and heat to dissolve. Alternatively, heat in the autoclave at low pressure, or in a microwave oven.
- ✓ Once the agar is dissolved, pour the medium into culture vessels, cap and autoclave at 121°C for 15 to 20 min at 15 pounds per square inch (psi). If using pre-sterilized, non-autoclavable plastic culture vessels, the medium may be autoclaved in flasks or media bottles. After autoclaving, allow the medium to cool to around 60°C before pouring under aseptic conditions.
- ✓ Allow the medium to cool to room temperature. Store in dust-free areas or refrigerate at 7°C (temperature lower than 7°C alter the gel structure of the agar).

Gelling agents

The media listed above are only for liquids, often in plant cell culture a ‘semi-solid' medium is used. To make a semi-solid medium, a gelling agent is added to the liquid medium before autoclaving. Gelling agents are usually polymers that set on cooling after autoclaving.

i. Agar: Agar is obtained from red algae- *Gelidium amansii* . It is a mixture of polysaccharides. It is used as a gelling agent due to the reasons: (a) It does not react with

the media constituents (b) It is not digested by plant enzymes and is stable at culture temperature.

ii. Agarose: It is obtained by purifying agar to remove the agaropectins. This is required where high gel strength is needed, such as in single cell or protoplast cultures.

iii. Gelrite: It is produced by bacterium *Pseudomonas elodea* . It can be readily prepared in cold solution at room temperature. It sets as a clear gel which assists easy observation of cultures and their possible contamination. Unlike agar, the gel strength of gelrite is unaffected over a wide range of pH. However, few plants show hyperhydricity on gelrite due to freely available water.

iv. Gelatin: It is used at a high concentration (10%) with a limited success. This is mainly because gelatin melts at low temperature (25°C) and as a result the gelling property is lost.

Plant growth regulators

- In addition to nutrients, four broad classes of growth regulators, such as, auxins, cytokinins, gibberellins and abscisic acid are important in tissue culture.
- In contrast with animal hormones, the synthesis of a plant growth regulator is often not localized in a specific tissue but may occur in many different tissues.
- They may be transported and act in distant tissues and often have their action at the site of synthesis.
- Another property of plant growth regulators is their lack of specificity- each of them influences a wide range of processes.
- The growth, differentiation, organogenesis and embryogenesis of tissues become feasible only on the addition of one or more of these classes of growth regulators to a medium.

- In tissue culture, two classes of plant growth regulators, cytokinins and auxins, are of major importance. Others, in particular, gibberellins, ethylene and abscisic acid have been used occasionally.

- Auxins are found to influence cell elongation, cell division, induction of primary vascular tissue, adventitious root formation, callus formation and fruit growth.

- The cytokinins promote cell division and axillary shoot proliferation while auxins inhibit the outgrowth of axillary buds.

- The auxin favours DNA duplication and cytokinins enable the separation of chromosome. Besides, cytokinin in tissue culture media, promote adventitious shoot formation in callus cultures or directly from the explants and, occasionally, inhibition of excessive root formation and are, therefore, left out from rooting media.

- The ratio of plant growth regulators required for root or shoot induction varies considerably with the tissue and is directly related to the amount of growth regulators present at endogenous levels within the explants. In general, shoots are formed at high cytokinin and low auxin concentrations in the medium, roots at low cytokinin and high auxin concentrations and callus at intermediate concentrations of both plant growth regulators.

- Commonly used plant growth regulators are listed in below.

Stock solutions of growth regulators

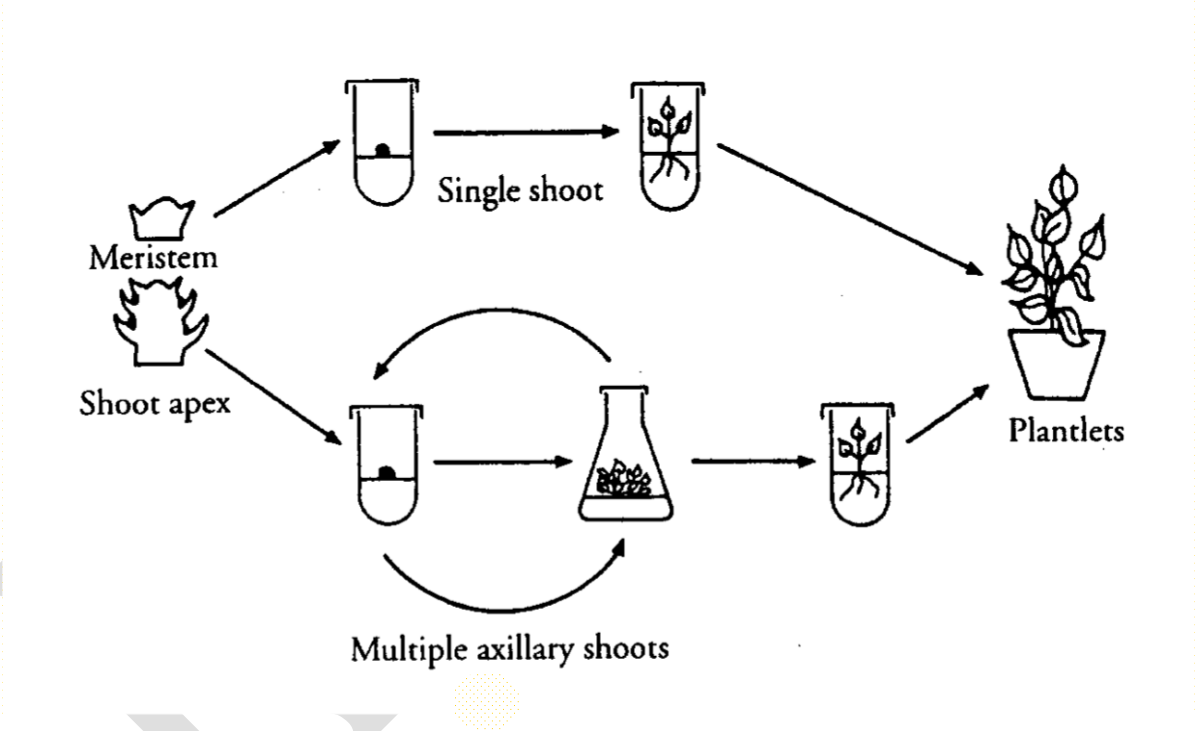
Compound	Abbreviations	mg/50 ml (1 mM or 10^{-3} Molar)
CYTOKININS		
6-Benzyladenine	BA	11.25
N^6-(2-isopentenyl) adenine	2-iP	10.15
6-Furfurylaminopurine	Kinetin	10.75
Zeatin	ZEA	10.95
Thidiazuron	TDZ	11.00
Note: Dissolve cytokinins in few drops of 1 N NaOH; stir; heat gently and make to volume. TDZ is dissolved in 95% ethanol.		
AUXINS		
Indole-3-acetic acid	IAA	8.76
Indole-3-butyric acid	IBA	10.16
α-Naphthaleneacetic acid	NAA	9.31
2,4-Dichlorophenoxyacetic acid	2,4-D	11.05
2,4,5-Trichlorophenoxyacetic acid	2,4,5-T	12.78
p-Chlorophenoxyacetic acid	4-CPA	9.33
Picloram	PIC	12.06
Note: Dissolve auxins in 95% ethanol or 1N NaOH; stir, heat gently; gradually add water to volume. Dissolve picloram in DMSO.		
OTHERS		
Silver Nitrate	$AgNO_3$	9.00
Gibberellic acid	GA_3	17.32
Abscisic acid	ABA	13.20
Note: Dissolve in 95% ethanol or 1N NaOH; stir, heat gently; gradually add water to volume.		

1 molar = the molecular weight in g/l; 1 mM = the molecular weight in mg/l ;ppm = parts per million = mg/l

Micropropagation

- Plant tissue culture has become popular among horticulturists, plant breeders and pharmaceutical industries because of its variety of practical applications.
- The earliest application of plant tissue culture was to rescue hybrid embryos, and the technique became a routine aid with plant breeders to raise rare hybrids, which normally failed due to post-zygotic sexual incompatibility.
- Currently, the most popular commercial application of plant tissue culture is in clonal propagation of disease-free plants, salt tolerance, stress tolerance plants. *In vitro* clonal propagation, popularly called micropropagation, offers many advantages over the conventional methods of vegetative propagation.
- The enhanced rate of multiplication can considerably reduce the period between the selection of trees and raising enough planting material for field trials.
- In tissue culture, propagation occurs under pathogen and pest-free conditions. An important contribution made through tissue culture is the revelation of the unique property of plant cells, called "cellular totipotency".
- Due to this character the plant tissue culture techniques have greatly evolved.
- The technique has developed around the concept that a cell has the capacity and ability to develop into a whole organism irrespective of their nature of differentiation and ploidy level.
- Therefore, it forms the backbone of the modern approach to crop improvement by genetic engineering.
- The principles involved in plant tissue culture are very simple and primarily an attempt, whereby an explant can be to some extent freed from inter-organ, inter-tissue and inter-cellular interactions and subjected to direct experimental control.
- Regeneration of plants from cultured cells has many other applications. Plant regeneration from cultured cells is proving to be a rich source of genetic variability, called "somaclonal variation".
- Several somaclones have been processed into new cultivars.
- Regeneration of plants from microspore/pollen provides the most reliable and rapid method to produce haploids, which are extremely valuable in plant breeding and genetics.

- In haploids, homozygosity can be achieved in a single step, cutting down the breeding period to almost half.
- This is particularly important for highly heterozygous, long-generation tree species. Pollen raised plants also provide a unique opportunity to screen gametic variation at sporophytic level.
- This approach has enabled selection of several gametoclones, which could be developed into new cultivars.
- Even the triploid cells of endosperm are totipotent, which provides a direct and easy approach to regenerate triploid plants difficult to raise *in vivo.*



The entire plant tissue culture techniques can be largely divided into two categories based on to establish a particular objective in the plant species:

1. Quantitative Improvement (Micropropagation)

- Adventitious shoot proliferation (leaves, roots, bulbs, corm, seedling- explants etc.)
- Nodal segment culture
- Meristem/Shoot-tip culture
- Somatic embryogenesis

- Callus culture

2. Qualitative improvement

- Anther/ Microspore culture
- Ovary/ Ovule culture
- Endosperm culture
- Cell culture
- Protoplast culture

1. Quantitative Improvement (Micropropagation)

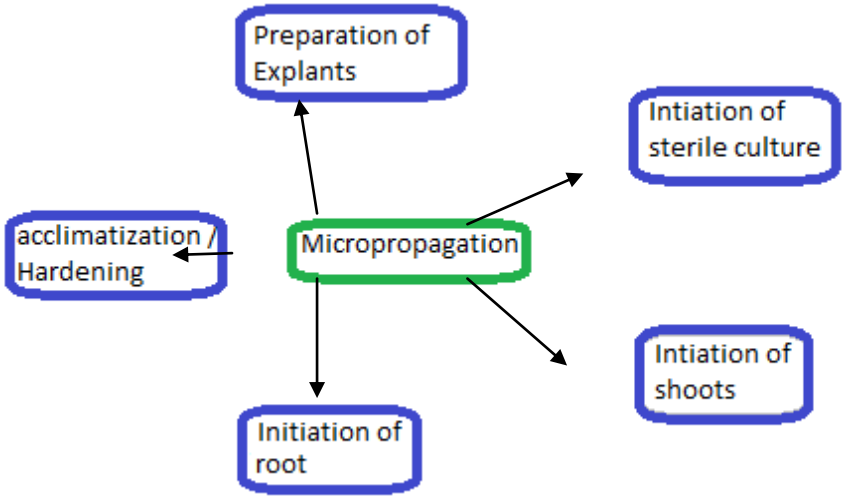
- ✓ Growing any part of the plant (explants) like, cells, tissues and organs, in an artificial medium under controlled conditions (aseptic conditions) for obtaining large scale plant propagation is called micropropagation.
- ✓ The basic concept of micropropagation is the plasticity, totipotency, differentiation, dedifferentiation and redifferentiation, which provide the better understanding of the plant cell culture and regeneration. Plants, due to their long life span, have the ability to withhold the extremes of conditions unlike animals.
- ✓ The plasticity allows plants to alter their metabolism, growth and development to best suit their environment.
- ✓ When plant cells and tissues are cultured *in vitro*, they generally exhibit a very high degree of **plasticity**, which allows one type of tissue or organ to be initiated from another type.
- ✓ Hence, whole plants can be subsequently regenerated and this regenerated whole plant has the capability to express the total genetic potential of the parent plant.
- ✓ This is unique feature of plant cells and is not seen in animals. Unlike animals, where differentiation is generally irreversible, in plants even highly mature and differentiated cells retain the ability to regress to a meristematic state as long as they have an intact membrane system and a viable nucleus.
- ✓ However, sieve tube elements and xylem elements do not divide any more where the nuclei have started to disintegrate, According to Gautheret (1966) the degree

of regression a cell can undergo would depend on the cytological and physiological state of the cell.

- ✓ The meristematic tissues are differentiated into simple or complex tissues called **differentiation**. Reversion of mature tissues into meristematic state leading to the formation of callus is called **dedifferentiation**.
- ✓ The ability of callus to develop into shoots or roots or embryoid is called **redifferentiation**.
- ✓ The inherent potentiality of a plant cell to give rise to entire plant and its capacity is often retained even after the cell has undergone final differentiation in the plant system is described as cellular **totipotency**.
- ✓ During *in-vitro* and *in vivo* cytodifferentiation (cell differentiation), the main emphasis has been on vascular differentiation, especially tracheary elements (TEs).
- ✓ These can be easily observed by staining and can be scored in macerated preparations of the tissues. Tissue differentiation goes on in a fixed manner and is the characteristic of the species and the organs.

Stages of micropropagation

- Selection of mother plant and preparation of explant.
- Initiation of a sterile culture of the explant in a particular enriched medium
- Initiation of multiplication of shoots
- Development of roots on the shoots to produce plantlets.
- Acclimatization: it is a process of plantlets from invitro condition to soil under green-house condition after acclimatization to transfer to the field (hardening). Produce the self-sufficient plants



Micropropagation stages

Preparation of explants

- Chose the opte pièce of plants to grow under invitro conditions after surface sterilization using surface sterilization methods.

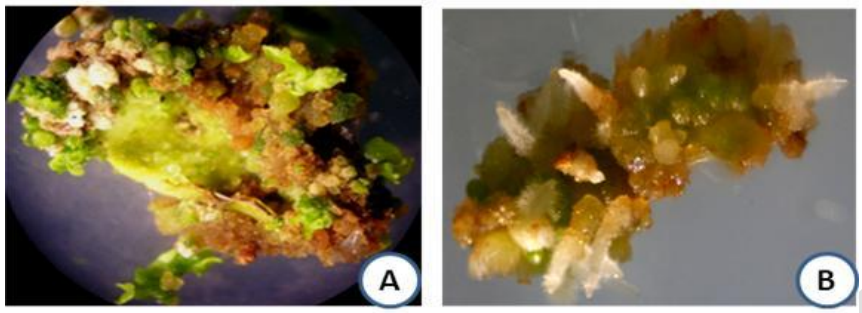
Initiation of sterile culture

- After inoculating the explants to the artificial media to maintain the sterility in the invitro culture.

Initiation of shoots and roots

- Adventitious shoot proliferation in plant cell and tissue culture, in response to hormonal manipulation of the culture medium, require *de novo* differentiation of meristematic region, randomly, all over the tissue other than the pre-existing meristem. It is a multistep process and a series of intracellular events, collectively called induction that occurs before the appearance of morphologically recognizable organs
- Micropropagation via adventitious shoot regeneration may occur directly or indirectly via an intervening callus phase.

- Indirect regeneration often results in somaclonal variations, making this strategy less desirable for large-scale clonal multiplication.
- Therefore, regeneration of shoots directly from the explants is regarded as the most reliable method for clonal propagation.
- Various explants like leaf, cotyledon, embryo and root have been tried with different media combinations by the scientists to obtain adventitious shoot proliferation.

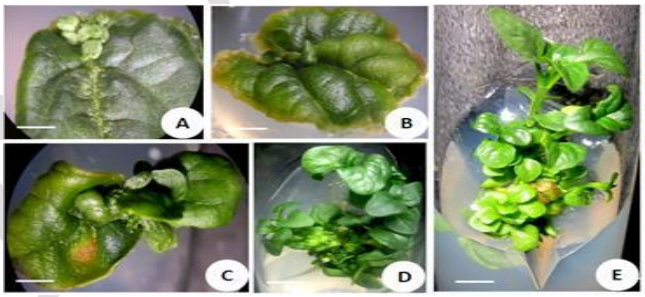


. Organogenesis from leaf explants indirectly via callusing

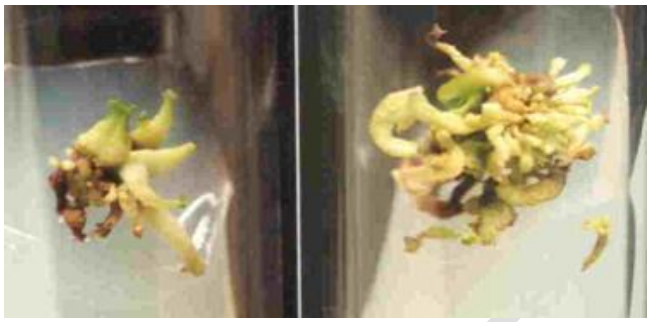
A. Shoot differentiation **B.** Root differentiation

Organogenic differentiation

Regeneration of plant from the cultured explant may occur either through differentiation of shoot-buds or somatic embryogenesis.

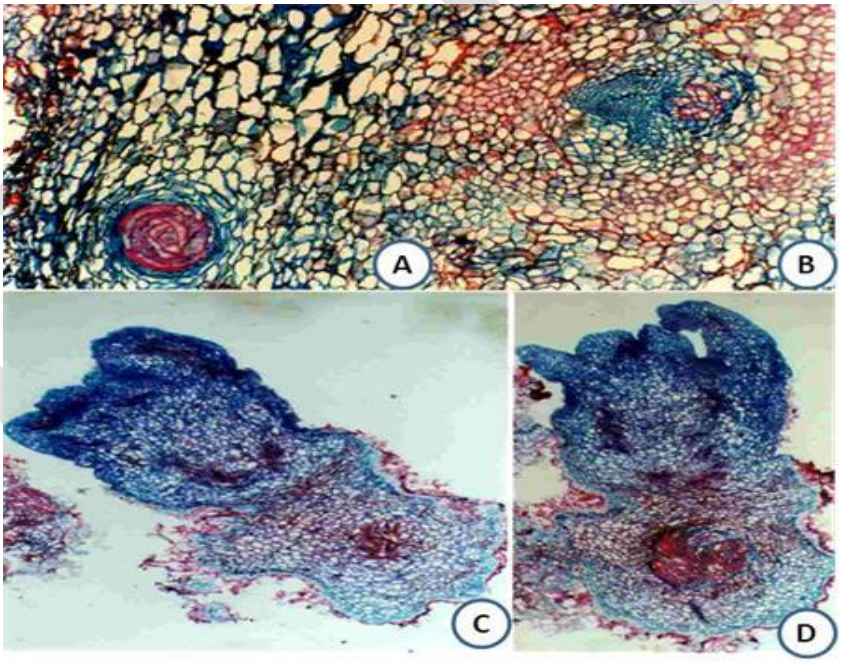


Direct shoot proliferation from leaf-disc culture



Direct differentiation of somatic embryos from hypocotyl explants

- The shoot-bud and embryo formation can be distinguished by the distinct morphological features.
- The shoot-bud is a monopolar structure.
- It develops from the procambial strands which establish a connection with the pre-existing vascular tissue dispersed within the callus or the cultured explants



Shoot differentiation from callus tissue. **A**-**B**, Development of vascular nodules randomly in the callus, note a small shoot-bud originated from vascular tissue in **figure B**. **C**-**D,** Shoot-buds establish a connection with pre-existing vascular

tissue developed from the callus

- Plant regeneration from isolated cells, protoplasts or unorganized mass of cells (callus) is generally more difficult than that obtained from the intact explants such as, cotyledons, hypocotyl segments and immature embryos
- The regeneration obtained through de novo differentiation of shoot buds or somatic embryogenesis directly from explants may also exhibit genetic variability.

Acclimatization process

Self sufficient plantlets transfer to soil under green house condition and train the plant to natural soil. The plantlet acclimatize to natural soil is called hardening process.

Factors affecting vascular tissue differentiation

- Vascular differentiation is majorly affected qualitatively and quantitatively by two factors, auxin and sucrose.
- Cytokinins and gibberellins also play an important role in the process of xylogenesis.
- Depending upon the characteristics of different species, concentration of phytohormones, sucrose and other salt level varies and accordingly it leads to the vascular tissue differentiation.
- The vegetative propagation has been conventionally used to raise genetically uniform large scale plants for thousands of years
- However, this technique is applicable to only limited number of species. In contrast to this, micropropagation has several advantages.

Advantages of micropropagation

i. The rapid multiplication of species difficult to multiply by conventional vegetative means. The technique permits the production of elite clones of selected plants.
ii. The technique is independent of seasonal and geographical constraints.

iii. It enable large numbers of plants to be brought to the market place in lesser time which results in faster return on the investment that went into the breeding work.

iv. To generate disease-free (particularly virus-free) parental plant stock.

v. To raise pure breeding lines by *in vitro* haploid and triploid plant development in lesser time.

vi. It can be utilized to raise new varieties and preservation of germplasm.

vii. It offers constant production of secondary medicinal metabolites.

Possible Questions
Short questions

1. What is embryogenesis?
2. What is micropropagation.
3. Write short note on seed culture.
4. Give short note on Embryo rescue.
5. What is Totipotency?
6. What is meant by precursor?
7. Define redifferentiation.
8. Write short note on cell suspension culture.
9. What is hairy root culture?
10. What are pollen culture?
11. Define somatic embryogenesis.
12. What is somatic hybridization?
13. What is callus?
14. How will you check the product bioavailability?
15. Define dedifferentiation.

Essay type questions

1. Discuss the various types of plant tissue culture.
2. Write short notes on i. Shoot culture & ii. Hairy root culture.
3. Describe advantage and disadvantage of micropropagation.
4. Describe major steps involved in micropropagation.
5. Write short notes on i) Organogenic differentiation ii) Protoplast culture iii). Meristem culture.

UNIT-II

SYLLABUS

***In vitro* culture:** haploid production Androgenic methods: Anther culture, Microspore culture and ogenesis. Significance and use of haploids, Ploidy level and chromosome doubling, diplodization, Gynogenic haploids, factors effecting gynogenesis, chromosome elimination techniques for production of haploids in cereals.

Anrogenic methods

Haploid production occurs through anther or pollen culture, and they are referred to as androgenic haploids.

In androgenesis, the male gametophyte (microspore or immature pollen) produces haploid plant. The basic principle is to stop the development of pollen cell into a gamete (sex cell) and force it to develop into a haploid plant.

Haploid plants are characterized by possessing only a single set of chromosomes (gametophytic number of chromosomes i.e. n) in the sporophyte.

This is in contrast to diploids which contain two sets (2n) of chromosomes. Haploid plants are of great significance for the production of homozygous lines (homozygous plants) and for the improvement of plants in plant breeding programmes.

Androgenesis:

In androgenesis, the male gametophyte (microspore or immature pollen) produces haploid plant. The basic principle is to stop the development of pollen cell into a gamete (sex cell) and force it to develop into a haploid plant. There are two approaches in androgenesis— anther culture and pollen (microspore) culture. Young plants, grown under optimal conditions of light, temperature and humidity, are suitable for androgenesis.

Anther Culture:

The selected flower buds of young plants are surface-sterilized and anthers removed along with their filaments. The anthers are excised under aseptic conditions, and crushed in 1% acetocarmine to test the stage of pollen development.

If they are at the correct stage, each anther is gently separated (from the filament) and the intact anthers are inoculated on a nutrient medium. Injured anthers should not be used in cultures as they result in callusing of anther wall tissue.

The anther cultures are maintained in alternating periods of light (12-18 hr.) and darkness (6-12 hrs.) at 28°C. As the anthers proliferate, they produce callus which later forms an embryo and then a haploid plant.

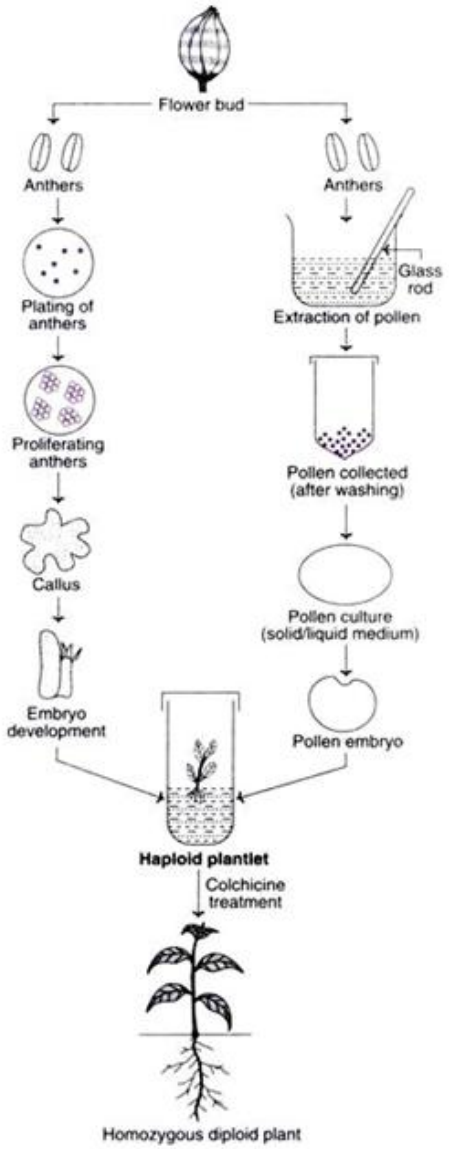


Figure: Anther and pollen culture for the production of haploids

Pollen (Microspore) Culture:

Haploid plants can be produced from immature pollen or microspores (male gametophytic cells). The pollen can be extracted by pressing and squeezing the anthers with a glass rod against the sides of a beaker. The pollen suspension is filtered to remove anther tissue debris.

Viable and large pollen (smaller pollen do not regenerate) are concentrated by filtration, washed and collected. These pollen are cultured on a solid or liquid medium. The callus/embryo formed is transferred to a suitable medium to finally produce a haploid plant, and then a diploid plant (on colchicine treatment).

Comparison between anther and pollen cultures:

Anther culture is easy, quick and practicable. Anther walls act as conditioning factors and promote culture growth. Thus, anther cultures are reasonably efficient for haploid production. The major limitation is that the plants not only originate from pollen but also from other parts of anther. This results in the population of plants at different ploidy levels (diploids, aneuploids). The disadvantages associated with anther culture can be overcome by pollen culture.

Many workers prefer pollen culture, even though the degree of success is low, as it offers the following advantages:

i. Undesirable effects of anther wall and associated tissues can be avoided.

ii. Androgenesis, starting from a single cell, can be better regulated.

iii. Isolated microspores (pollen) are ideal for various genetic manipulations (transformation, mutagenesis).

iv. The yield of haploid plants is relatively higher.

Development of Androgenic Haploids:

The process of in vitro androgenesis for the ultimate production of haploid plants is depicted in the above figure.

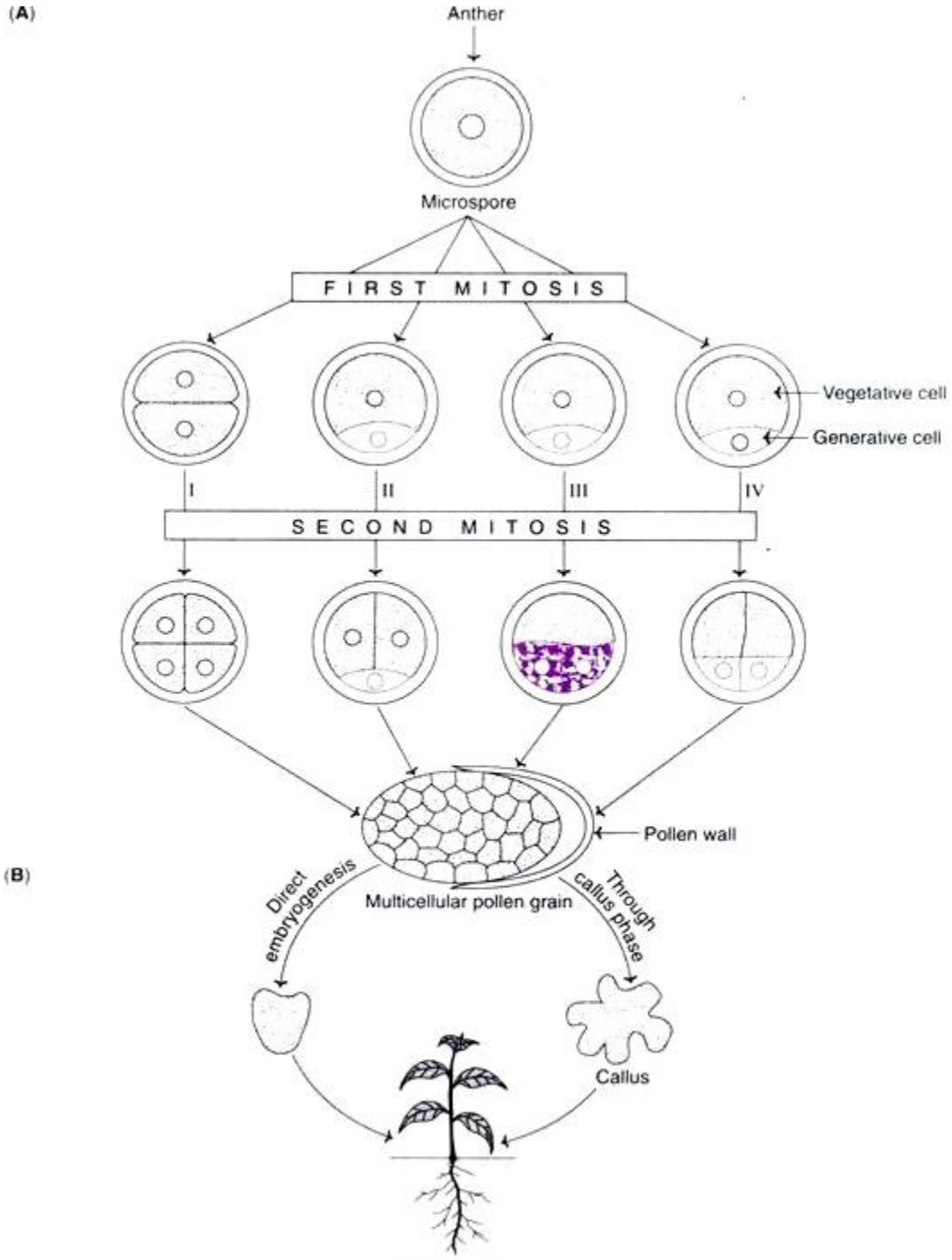


Figure. Formation of multucellular pollen grains A. formation of haploid sporophytes B). I, II, III and IV indicative pathways.

The cultured microspores mainly follow four distinct pathways during the initial stages of in vitro androgenesis.

Pathway I:

The uninucleate microspore undergoes equal division to form two daughter cells of equal size e.g. *Datura innoxia.*

Pathway II:

In certain plants, the microspore divides unequally to give bigger vegetative cell and a smaller generative cell. It is the vegetative cell that undergoes further divisions to form callus or embryo. The generative cell, on the other hand, degenerates after one or two divisions—e.g., *Nicotiana tabacum, Capsicum annuum.*

Pathway III:

In this case, the microspore undergoes unequal division. The embryos are formed from the generative cell while the vegetative cell does not divide at all or undergoes limited number of divisions e.g. *HyoScyamus niger.*

Pathway IV:

The microspore divides unequally as in pathways I and II. However, in this case, both vegetative and generative cells can further divide and contribute to the development of haploid plant e.g. *Datura metel, Atropa belladonna.*

At the initial stages, the microspore may follow any one of the four pathways described above. As the cells divide, the pollen grain becomes multicellular and burst open. This multicellular mass may form a callus which later differentiates into a plant (through callus phase). Alternately, the multicellular mass may produce the plant through direct embryogenesis.

Factors Affecting Androgenesis:

A good knowledge of the various factors that influence androgenesis will help to improve the production of androgenic haploids. Some of these factors are briefly described.

Genotype of donar plants:

The success of anther or pollen culture largely depends on the genotype of the donor plant. It is therefore important to select only highly responsive genotypes. Some workers choose a breeding approach for improvement of genotype before they are used in androgenesis.

Stage of microspore or pollen:

The selection of anthers at an ideal stage of microspore development is very critical for haploid production. In general, microspores ranging from tetrad to bi-nucleate stages are more responsive. Anthers at a very young stage (with microspore mother cells or tetrads) and late stage (with bi-nucleate microspores) are usually not suitable for androgenesis. However, for maximum production of androgenic haploids, the suitable stage of microspore development is dependent on the plant species, and has to be carefully selected.

Physiological status of a donar plant:

The plants grown under best natural environmental conditions (light, temperature, nutrition, CO_2 etc.) with good anthers and healthy microspores are most suitable as donor plants. Flowers obtained from young plants, at the beginning of the flowering season are highly responsive. The use of pesticides should be avoided at least 3-4 weeks preceding sampling.

Pretreatment of anthers:

The basic principle of native androgenesis is to stop the conversion of pollen cell into a gamete, and force its development into a plant. This is in fact an abnormal pathway induced to achieve in vitro androgenesis. Appropriate treatment of anthers is required for good success of haploid production.

Treatment methods are variable and largely depend on the donor plant species:

1. Chemical treatment:

Certain chemicals are known to induce parthenogenesis e.g. 2-chloroethylphosphonic acid (ethrel). When plants are treated with ethreal, multinucleated pollens are produced. These pollens when cultured may form embryos.

2. Temperature influence:

In general, when the buds are treated with cold temperatures (3-6°C) for about 3 days, induction occurs to yield pollen embryos in some plants e.g. Datura, Nicotiana. Further, induction of androgenesis is better if anthers are stored at low temperature, prior to culture e.g. maize, rye. There are also reports that pretreatment of anthers of certain plants at higher temperatures (35°C) stimulates androgenesis e.g. some species of Brassica and Capsicum.

Effect of light:

In general, the production of haploids is better in light. There are however, certain plants which can grow well in both light and dark. Isolated pollen (not the anther) appears to be sensitive to light. Thus, low intensity of light promotes development of embryos in pollen cultures e.g. tobacco.

Effect of culture medium:

The success of another culture and androgenesis is also dependent on the composition of the medium. There is, however, no single medium suitable for anther cultures of all plant species. The commonly used media for anther cultures are MS, White's, Nitsch and Nitsch, N6 and B5. These media in fact are the same as used in plant cell and tissue cultures. In recent years, some workers have developed specially designed media for anther cultures of cereals.

Sucrose, nitrate, ammonium salts, amino acids and minerals are essential for androgenesis. In some species, growth regulators — auxin and/or cytokinin are required for optimal growth. In certain plant species, addition of glutathione and ascorbic acid promotes androgenesis. When the anther culture medium is supplemented with activated charcoal, enhanced androgenesis is observed. It is believed that the activated charcoal removes the inhibitors from the medium and facilitates haploid formation.

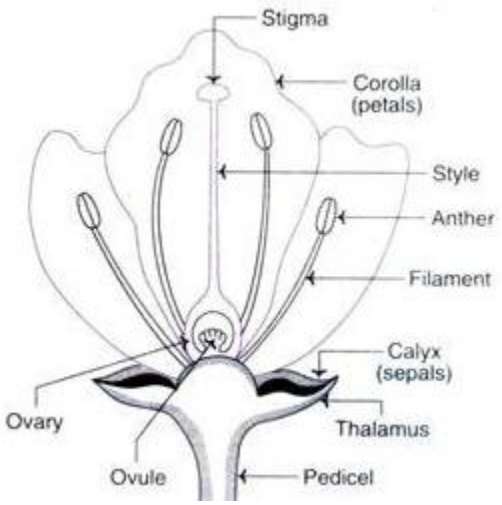
Gynogenesis:

Haploid plants can be developed from ovary or ovule cultures. It is possible to trigger female gametophytes (megaspores) of angiosperms to develop into a sporophyte. The plants so produced are referred to as gynogenic haploids.

Gynogenic haploids were first developed by San Noem (1976) from the ovary cultures of Hordeum vulgare. This technique was later applied for raising haploid plants of rice, wheat, maize, sunflower, sugar beet and tobacco.

In vitro culture of un-pollinated ovaries (or ovules) is usually employed when the anther cultures give .unsatisfactory results for the production of haploid plants. The procedure for gynogenic haploid production is briefly described.

The flower buds are excised 24-48 hr. prior to anthesis from un-pollinated ovaries. After removal of calyx, corolla and stamens, the ovaries are subjected to surface sterilization. The ovary, with a cut end at the distal part of pedicel, is inserted in the solid culture medium.



Parts of the flower

Whenever a liquid medium is used, the ovaries are placed on a filter paper or allowed to float over the medium with pedicel inserted through filter paper. The commonly used media are MS, White's, N6 and Nitsch, supplemented growth factors. Production of gynogenic haploids is particularly useful in plants with male sterile genotype. For such plant species, this technique is superior to another culture technique.

Limitations of Gynogenesis:

In practice, production of haploid plants by ovary/ ovule cultures is not used as frequently as anther/ pollen cultures in crop improvement programmes.

Polyploidy:

- Polyploid cells and organisms are those containing more than two paired (homologous) sets of chromosomes.
- Most species whose cells have nuclei (Eukaryotes) are diploid, meaning they have two sets of chromosomes one set inherited from each parent.

Classification of Polyploids : Polyploids may be classified based on their chromosomal composition into either euploids or aneuploids. Euploids constitute the majority of polyploids.

Euploidy

- Euploids are polyploids with multiples of the complete set of chromosomes specific to a species. Depending on the composition of the genome, euploids can be further classified into either autopolyploids or allopolyploids. Tetraploidy is the most common class of euploids

Autopolyploidy

- Autopolyploids are also referred to as autoploids.
- They contain multiple copies of the basic set (x) of chromosomes of the same genome (Acquaah, 2007; Chen, 2010).
- Autoploids occur in nature through union of unreduced gametes and at times can be artificially induced (Chen, 2010).
- Natural autoploids include tetraploid crops such as alfafa, peanut, potato and coffee and triploid bananas.
- They occur spontaneously through the process of chromosome doubling. Chromosome doubling in autoploids has varying effect based on the species.
- Spontaneous chromosome doubling in ornamentals and forage grasses has led to increased vigour.
- Due to the observed advantages in nature, breeders have harnessed the process of chromosome doubling *in vitro* through induced polyploidy to produce superior crops.
- For example, induced autotetraploids in the watermelon crop are used for the production of seedless triploid hybrids fruits.
- Such polyploids are induced through the treatment of diploids with mitotic inhibitors such as dinitroaniles and colchicine .
- To determine the ploidy status of induced polyploids, several approaches may be used. These include, chloroplast count in guard cells, morphological features such as leaf, flower or pollen size (gigas effect) and flow cytometry .

Allopolyploidy

- Allopolyploids are also called alloploids.
- They are a combination of genomes from different species
- They result from hybridization of two or more genomes followed by chromosome doubling or by the fusion of unreduced gametes between species.

- This process is key in the process of speciation for angiosperms and ferns and occurs often in nature.
- Economically important natural alloploid crops include strawberry, wheat, oat, upland cotton, oilseed rape, blueberry and mustard.
- To differentiate between the sources of the genomes in an alloploid, each genome is designated by a different letter.

Aneuploidy

- Aneuploids are polyploids that contain either an addition or subtraction of one or more specific chromosome(s) to the total number of chromosomes that usually make up the ploidy of a species.
- Aneuploids result from the formation of univalents and multivalents during meiosis of euploids
- For example, several studies have found that 30-40% of progeny derived from autotetraploid maize are aneuploids
- With no mechanism of dividing univalents equally among daughter cells during anaphase I, some cells inherit more genetic material than others.
- Smilarly, multivalents such as homologous chromosomes may fail to separate during meiosis leading to unequal migration of chromosomes to opposite poles. This mechanism is called non-disjunction
- These meiotic aberrances result in plants with reduced vigor. Aneuploids are classified according to the number of chromosomes gained or lost .

Classification of aneuploids

Term	Chromosome number
Monosomy	2n-1
Nullisomy	2n-2
Trisomy	2n+2
Tetrasomy	2n+2
Pentasomy	2n+3

- Another major route for polyploid formation is through somatic doubling of chromosomes during mitosis.
- In nature, the formation of polyploids as a result of mitotic aberrations has been reported in the meristematic tissue of several plant species including tomato and in non-meristematic tissues of plants such as bean.
- Artificial inducement of polyploids through the inhibition of mitosis is routine in plant breeding. High temperatures above 40°C have been used to induce tetraploid and octoploid corn seedlings albeit with low success of 1.8% and 0.8% respectively.

A doubled haploid (DH) is a genotype formed when haploid cells undergo chromosome doubling. Artificial production of doubled haploids is important in plant breeding.

Diploidization is the process of converting a polyploid genome back into a diploid one. Polyploidy is a product of whole genome duplication (WGD) and is followed by diploidization as a result of genome shock.

Possible questions

Short question

1. What is haploid plants?
2. What is Androgenic haploid?
3. Write short note on Anther culture.
4. Define Microspore culture
5. Define ogenesis.
6. What is Ploidy?
7. What is meant by chromosome doubling?
8. What is diplodization?
9. What is Gynogenic haploids?
10. List factors effecting gynogenesis.

Essay questions

1. Explain haploid production through Androgenic methods.
2. Discuss Anther culture and Microspore culture.
3. Significance and use of haploids-explain.
4. Explain Ploidy level and chromosome doubling.
5. Elaborate Gynogenic haploids.
6. Explain factors effecting gynogenesis.

UNIT-III

SYLLABUS

Hybridization: Protoplast Isolation and fusion Methods of protoplast isolation, Protoplast development, Somatic hybridization, identification and selection of hybrid cells, Cybrids, Potential of somatic hybridization limitations. Somaclonal variation Nomenclature, methods, applications basis and disadvantages. Plant Growth Promoting bacteria. Nitrogen fixation, Nitrogenase, Hydrogenase, Nodulation, Bio control of pathogens, Growth promotion by free-living bacteria.

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Protoplast Isolation and fusion Methods of protoplast isolation

Protoplast fusion and somatic hybridization

Protoplast fusion

- Protoplast fusion could be spontaneous during isolation of protoplast or it can be induced by mechanical, chemical and physical means. During spontaneous process, the adjacent protoplasts fuse together as a result of enzymatic degradation of cell walls forming homokaryons or homokaryocytes, each with two to several nuclei.
- The occurrence of multinucleate fusion bodies is more frequent when the protoplasts are prepared from actively dividing callus cells or suspension cultures.
- Since the somatic hybridization or cybridization require fusion of protoplasts of different origin, the spontaneous fusion has no value.
- To achieve induced fusion, a suitable chemical agent (fusogen) like, $NaNO_3$, high Ca^{2+}, polyethylene glycol (PEG), or electric stimulus is needed.

Fusion by means of $NaNO_3$:

- It was first demonstrated by Kuster in 1909 that the hypotonic solution of $NaNO_3$ induces fusion of isolated protoplast forming heterokaryon (hybrid).
- This method was fully described by Evans and Cocking (1975), however this method has a limitation of generating few no of hybrids, especially when highly vacuolated mesophyll protoplasts are involved.

High pH and Ca^{++} treatment:

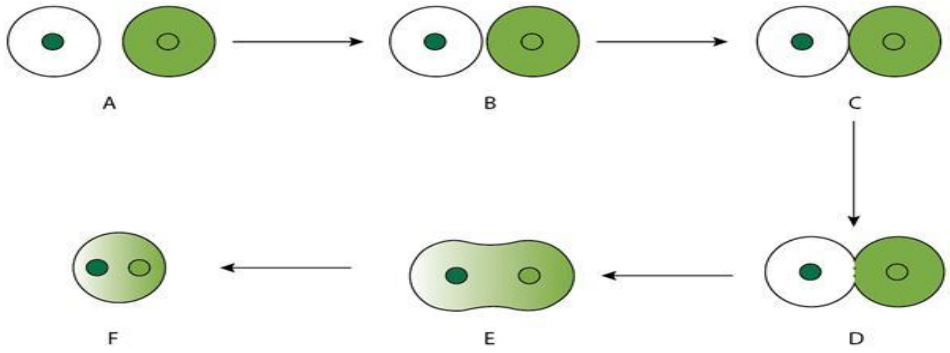
- This technique lead to the development of intra- and interspecific hybrids. It was demonstrated by Keller and Melcher in 1973.
- The isolated protoplasts from two plant species are incubated in 0.4 M mannitol solution containing high Ca^{++}(50 mM $CaCl_2.2H_2O$) with highly alkaline pH of 10.5 at 37°C for about 30 min.
- Aggregation of protoplasts takes place at once and fusion occurs within 10 min.

Polyethylene glycol treatment:

- Polyethylene glycol (PEG) is the most popularly known fusogen due to ability of forming high frequency, binucleate heterokaryons with low cytotoxicity. With PEG the

aggregation occurred mostly between two to three protoplasts unlike Ca^{++} induced fusion which involves large clump formation.

- The freshly isolated protoplasts from two selected parents are mixed in appropriate proportions and treated with 15-45% PEG (1500-6000MW) solution for 15-30 min followed by gradual washing of the protoplasts to remove PEG.
- Protoplast fusion occurs during washing. The washing medium may be alkaline (pH 9-10) and contain a high Ca^{++} ion concentration (50 mM).
- This combined approach of PEG and Ca^{++} is much more efficient than the either of the treatment alone. PEG is negatively charged and may bind to cation like Ca^{++}, which in turn, may bind to the negatively charged molecules present in plasma lemma, they can also bind to cationic molecules of plasma membrane.
- During the washing process, PEG molecules may pull out the plasma lemma components bound to them. This would disturb plamalemma organization and may lead to the fusion of protoplasts located close to each other
- The technique is nonselective thus, induce fusion between any two or more protoplasts.



Sequential stages in protoplast fusion. (**A**) two separate protoplasts, (**B**) agglutination of two protoplasts, (**C** and **D**) Membrane fusion at localized site, and (**E** and **F**) development of spherical heterokaryon.

Electrofusion:

- The chemical fusion of plant protoplast has many disadvantages –
- (1) The fusogen are toxic to some cell systems,
- (2) it produces random, multiple cell aggregates, and

- (3) must be removed before culture. Compare to this, electrofusion is rapid, simple, synchronous and more easily controlled. Moreover, the somatic hybrids produced by this method show much higher fertility than those produced by PEG-induced fusion.

Selection of fusion products

- ✓ The somatic hybridization by electrofusion of protoplasts allow one-to-one fusion of desired pairs of protoplasts and, therefore, it is easy to know the fate of fusion products.
- ✓ However, protoplast suspension recovered after chemical treatments (fusogen) consists of the following cell types:

i. unfused protoplasts of the two species/strains

ii. products of fusion between two or more protoplasts of the same species (homokaryons), and

iii. 'hybrid' protoplasts produced by fusion between one (or more) protoplasts of each of the two species (heterokaryons)

Morpho-physiological basis:

- The whole mixture of the protoplasts are cultured after fusion treatment and the resulting calli or regenerants are screened for their hybrid characteristics.
- Occasionally the hybrid calli outgrow the parental cell colonies and are identified by their intermediate morphology, i.e. green with purple coloured cells. However, the process is labour intensive and requires glasshouse facilities.
- It is limited to certain combinations showing differences in their regeneration potential under specific culture conditions.

Complementation:

- In this case complementation or genetic or metabolic deficiencies of the two fusion partners are utilized to select the hybrid component.
- When protoplasts of two parents, (one parent bearing cytoplasmic albino trait and the other parent bearing green trait) each parent carrying a non-allelic genetic or metabolic defect are fused, it reconstitutes a viable hybrid cell, of wild type in which both defects are mutually abolished by complementation, and the hybrid cells are able to grow on minimal medium non-permissive to the growth of the parental cells bearing green trait.

- Later, the calli of hybrid nature could be easily distinguished from the parental type tissue (albino trait) by their green color. The complementation selection can also be applied to dominant characters, such as dominant resistance to antibiotics, herbicides or amino acid analogues.

Isolation of heterokaryons or hybrid cells:

- The manual or electronic isolation of heterokaryons or hybrid cells is the most reliable method. Manual isolation requires that the two parental type protoplasts have distinct morphological markers and are easily distinguishable.
- For example, green vacuolated, mesophyll protoplasts from one parent and richly cytoplasmic, non green protoplasts from cultured cells of another parent.
- The dual fluorescence method also helps easy identification of fusion products. In this case, the protoplast labeled green by treatment with fluorescein diacetate (FDA, 1-20 mgl^{-1}) are fused with protoplasts emitting a red fluorescence, either from chlorophyll autofluorescence or from exogenously applied rhodamine isothiocyanate (10-20 mgl^{-1}).
- The labeling can be achieved by adding the compound into the enzyme mixture. This can be applied even for morphologically indistinguishable protoplasts from two parents.

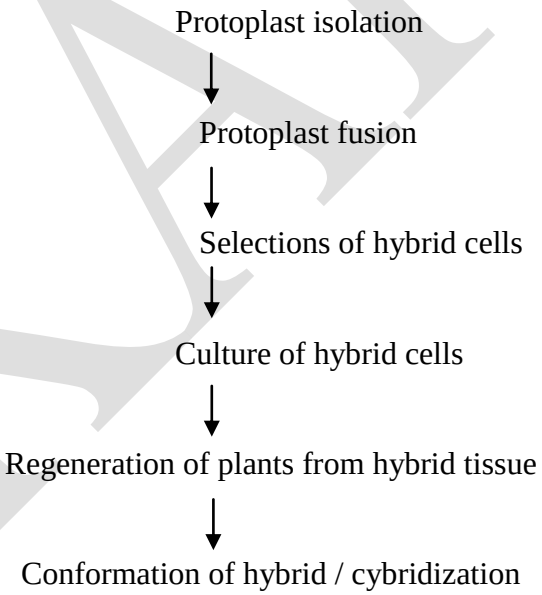
Somatic hybridization

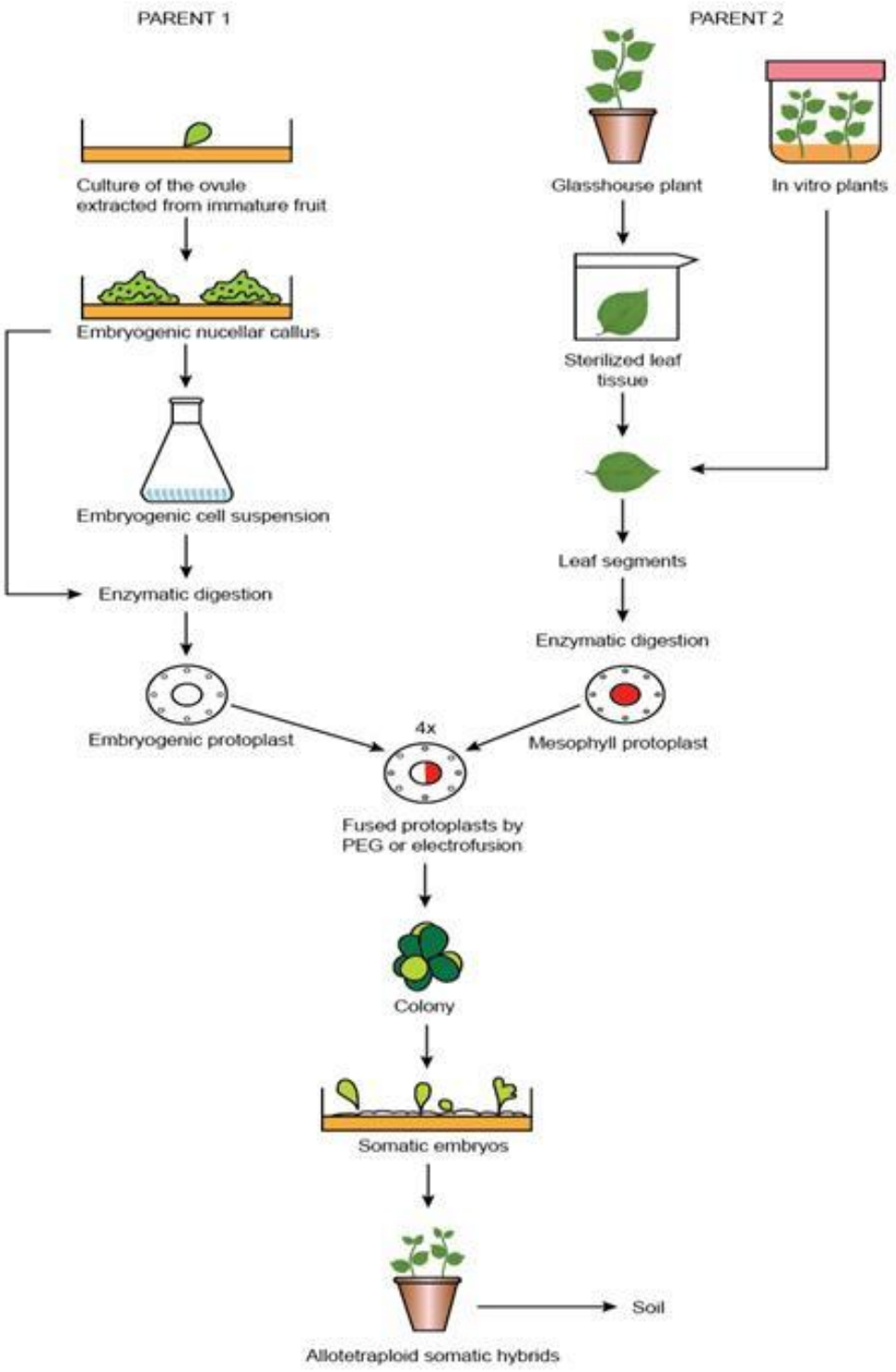
- Sexual hybridization since time immemorial has been used as a method for crop improvement but it has its own limitations as it can only be used within members of same species or closely related wild species.
- Thus, this limits the use of sexual hybridization as a means of producing better varieties. Development of viable cell hybrids by somatic hybridization, therefore, has been considered as an alternative approach for the production of superior hybrids overcoming the species barrier.
- The technique can facilitate breeding and gene transfer, bypassing problems associated with conventional sexual crossing such as, interspecific, intergeneric incompatibility.
- This technique of hybrid production via protoplast fusion allows combining somatic cells (whole or partial) from different cultivars, species or genera resulting in novel genetic

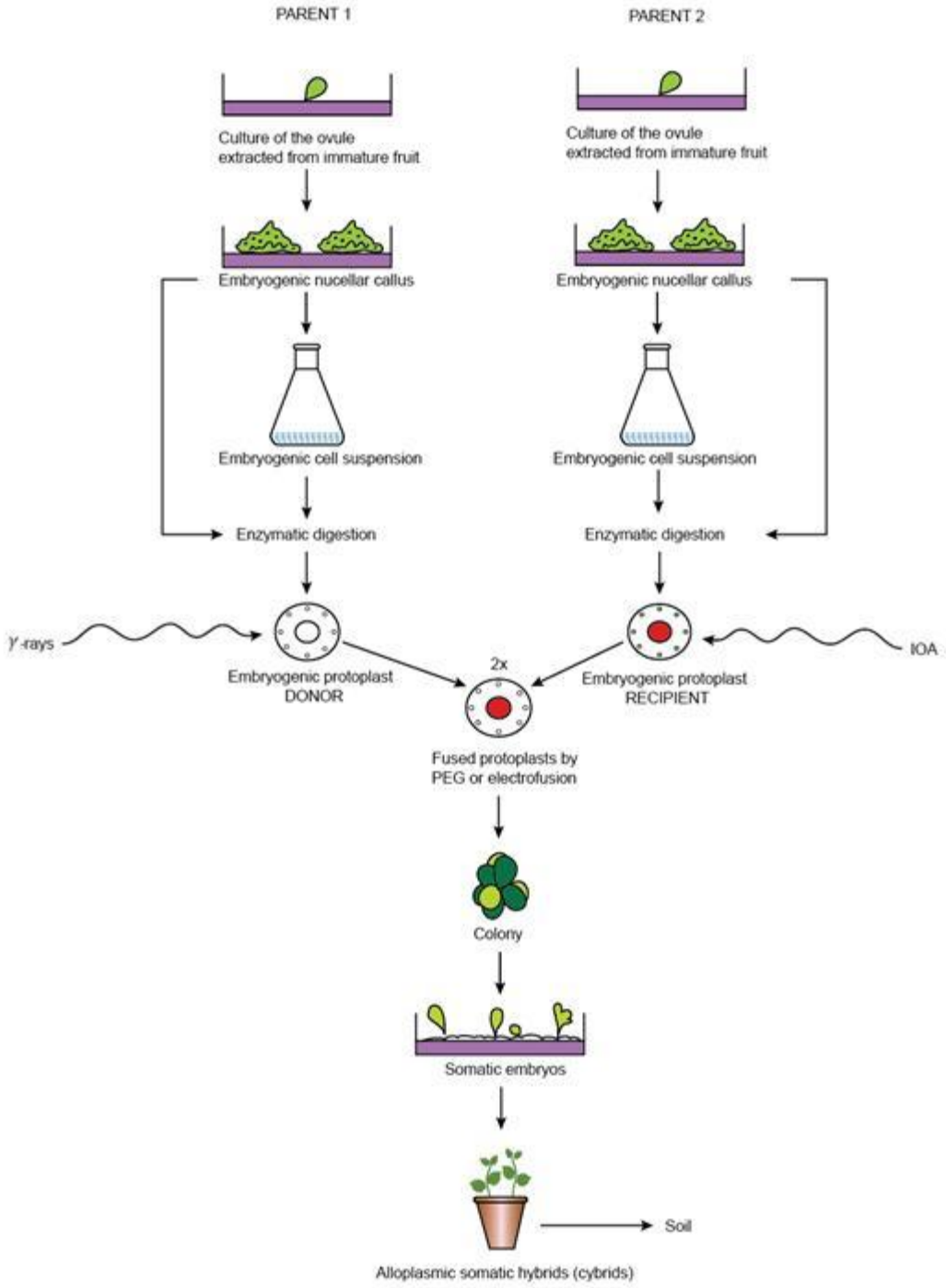
combinations including symmetric somatic hybrids, asymmetric somatic hybrids or somatic cybrids.

- The most common target using somatic hybridization is the gene of symmetric hybrids that contain the complete nuclear genomes along with cytoplasmic organelles of both parents.
- This is unlike sexual reproduction in which organelle genomes are generally contributed by the maternal parent.
- On the other hand, somatic cybridization is the process of combining the nuclear genome of one parent with the mitochondrial and/or chloroplast genome of a second parent.
- Cybrids can be produced by donor-recipient method or by cytoplast-protoplast fusion.
- Incomplete asymmetric somatic hybridization also provides opportunities for transfer of fragments of the nuclear genome, including one or more intact chromosomes from one parent (donor) into the intact genome of a second parent (recipient).

Steps involved in somatic hybridization




PARENT 1
PARENT 2
Culture of the ovule extracted from immature fruit
Glasshouse plant
In vitro plants
Embryogenic nucellar callus
Sterilized leaf tissue
Embryogenic cell suspension
Leaf segments
Enzymatic digestion
Enzymatic digestion
Embryogenic protoplast
4x
Mesophyll protoplast
Fused protoplasts by PEG or electrofusion
Colony
Somatic embryos
Soil
Allotetraploid somatic hybrids



Plant hardening and green house technology

- In order to give plants a chance to grow from seed to mature, fruit-bearing plant, gardeners need to start plants indoors during the cold late winter, and transplant them outside once the temperatures are warm enough to support proper plant growth.
- "Hardening off" is the process of moving plants outdoors for a portion of the day to gradually introduce them to the direct sunlight, dry air, and cold nights.
- Harden off gradually, so that seedlings become accustomed to strong sunlight, cool nights and less-frequent watering over a 7-10 day period.
- On a mild day, start with 2-3 hours of sun in a sheltered location.
- Protect seedlings from strong sun, wind, hard rain and cool temperatures.
- Increase exposure to sunlight a few additional hours at a time and gradually reduce frequency of watering, but do not allow seedlings to wilt. Avoid fertilizing.
- Keep an eye on the weather and listen to the low temperature prediction. If temperatures below the crop's minimum are forecast, bring the plants indoors or close the cold frame and cover it with a blanket or other insulation.
- Know the relative hardiness of various crops. Onions and brassicas are hardy and can take temperatures in the 40's. After they are well hardened off, light frosts won't hurt them. Warm-season crops such as eggplants, melons and cucumbers prefer warm nights, at least 60° F. They can't stand below-freezing temperatures, even after hardening off.
- Gradually increase exposure to cold.
- After transplanting to the garden, use a weak fertilizer solution to get transplants growing again and to help avoid transplant shock. Be sure to water plants after hardening them off.

Recommended Minimum Temperatures		
Hardy	40° F.	Broccoli, Brussels sprouts, kohlrabi, cabbage, onions, leeks, parsley
Half-Hardy	45° F.	Celery, Chinese cabbage, lettuce, endive
Tender	50° F.	Squash, pumpkin, sweet corn
	60° F.	Cucumber, muskmelon
	65° F.	Basil, tomatoes, peppers

Somaclonal variation

- Plants generally exhibit cytogenetic and genetic variations which help the plant breeders in crop improvement.
- When such variants arise through the cell and tissue culture process using any plant portion as an explant material, variations arising are termed as **somaclonal variations**.
- Variants obtained using callus cultures are referred as "**Calliclones**" (Skirvin, 1978) while variants obtained using protoplast cultures are known as "**Protoclones"**
- Larkin and Scowcroft (1981) proposed a general term 'Somaclonal variation' to describe genetic variation in plants regenerated from any form of cell cultures. Accordingly, the plants derived from cell and tissue cultures are termed as '**somaclones**', and the plants displaying variation as 'somaclonal variants'.
- Another term suggested by Evans et al. (1984) as '**gametoclonal variation**' for those variations arising in cell cultures of gametic origin like, in pollen and microspores cultures, to distinguish them from somatic cell derived regenerants.
- However, generally the term somaclonal variation is used for genetic variability present among all kinds of cell/plants obtained from cell cultures *in vitro*.
- Plants regenerated from tissue and cell cultures show heritable variation for both qualitative and quantitative traits. Several useful somaclonal variants have been obtained in large number of plant species such as, potato, sugarcane, banana, tomato etc.
- Chaleff (1981) labeled plants regenerated from tissue cultures as R_0 generation and their successive sexual generations as R_1, R_2 and so on.
- The basic cause of these variations may be attributed to changes in karyotype (chromosome number and structure), chromosome rearrangements, somatic crossing over, sister chromatid exchange, DNA amplification and deletion, transposable elements and DNA methylation. Somaclonal variation can be characterized based on morphological, biochemical (isozymes) and DNA markers such as, Random Amplified Polymorphic DNA (RAPDs), Restriction Fragment Length Polymorphism (RFLPs) and Inter-Simple Sequence Repeats (ISSR).
- The variations could also arise in tissue culture due to physiological changes induced by the culture conditions.

- Such variations are temporary and are caused by **epigenetic changes**. These are non-heritable variations and disappear when the culture conditions are removed.
- There are different approaches (steps) to create somaclonal variations, which include:

i. Growth of callus or cell suspension cultures for several cycles.
ii. Regeneration of a large number of plants from such long term cultures.
iii. Screening for desirable traits in the regenerated plants and their progenies.
For example, *invitro* selection to select agronomically desirable somaclones for tolerance to various biotic and abiotic stresses, herbicides, high salt concentration and extremes of temperature.
iv. Testing of selected variants in subsequent generations for desirable traits.
v. Multiplication of stable variants to develop new breeding lines.

- To be of commercial use, a somaclonal variant must fulfill certain basic requirements:

i. It must involve useful characters.
ii. It should be superior to the parents in the character(s) in which improvement is sought.
iii. The improved character(s) must be combined with all other desirable characters of the parent, and
iv. The variations must be inherited stably through successive generations by chosen means of propagation.

Origin of Somaclonal variation

- The somaclonal variations observed in plants regenerated from cultured cells are derived from two sources:
- (i) some of the variations could be revelation of the inherent cellular heterogeneity of the explant, and
- (ii) culture conditions may bring about new genetic changes.

Pre-existing variability

- ✓ Plant development in general involves change in nuclear DNA, such as change in

chromosome number, structure (Bennici and D'Amato, 1990).

- ✓ Cells of plant apical meristems like, root-tips and shoot-tips are uniformly diploid in their genome due to DNA synthesis immediately followed by karyokinesis and cytokinesis (normal cell cycle).
- ✓ However, the derivatives of these meristematic cells do not divide by normal mitosis but may undergo DNA duplication and endoreduplication.
- ✓ The varying degree of endoreduplication results in somatic cells with 4C, 8C or higher DNA content or may result in polysomaty.
- ✓ Usually these genetic changes are not noticed as these cells do not divide. However, under culture conditions these cells may divide and undergo redifferentiation and express this change in their genome content as an inheritable character within the whole plant.
- ✓ Another type of pre-existing chromosomal variability which is rarely observed in hybrid plants is **aneusomaty**.
- ✓ In such plants the apical meristems and, consequently, the mature tissues comprise a mosaic of cells with varying number of aneuploid chromosome numbers.
- ✓ This condition is transferred or enhanced in callus cultures derived from such tissues.

***In vitro* induced variability**

- ✓ Under the stressful culture conditions, the plant cells undergo genetic and epigenetic changes. This could happen even in the explants from non-polysomatic species.
- ✓ Generally less variations are found in plants than the callus because in mixed population of cells with different ploidy, euploid cells tend to be more regenerative than aneuploid cells.
- ✓ Several factors affect the type and frequency of somaclonal variations, explant source, genotype, culture conditions and age of the culture.

i. Culture medium

- ➢ Culture media constituents, particularly certain growth regulators, BAP, NAA, 2,4-D, induce mutations in the cultured cells.
- ➢ Sunderland (1977) reported that *Haplopappus* cells in 2,4-D containing medium is converted from entirely diploid state to a entirely tetraploid state within few months.
- ➢ Torrey (1965) observed that in the cultures of pea root segments on a medium with 2,4-D

as the sole hormone, only diploid cells divide but when the medium contained Kinetin and yeast extract in addition to 2,4-D, the tetraploid cells were selectively induced to divide.

- Most of the literature suggests that growth regulators influence somaclonal variation during the culture phase by affecting cell division, degree of disorganized growth and selective proliferation of specific cell types.

ii. Growth pattern and regeneration mode

- In vitro growth may occur from meristem cultures, which may form callus (undifferentiated mass of cells) or direct shoot formation.
- Callus is further differentiated into organized structures by organogenesis or somatic embryogenesis. The departure from organized growth is a key element in somaclonal variation.
- In general, longer the duration of callus and cell suspension in culture phase, the greater the chances of generating somaclonal variation.
- These somaclonal variation can also occur in embryogenic cultures, if they are kept for a long time in cultures, depending upon the plant species.

Analysis of Somaclonal variants

- Most useful somaclones are those which carry almost all of the good parental characters as well as incorporate within it certain desirable characters which were lacking in its parents.
- It becomes extremely important to select variants as early as possible, with minimal exposure of cells to tissue culture environment.
- With prolonged culture gross abnormalities may appear. The variants are generally assessed at the phenotypic level, and in over 50% cases it is based on R_0 plants.
- However, this approach of screening R_0 plants would the screening of only homozygous or dominant traits.
- The recessive mutations in heterozygous regenerants can be recognized only in the segregating R_1 and R_2 progenies.

- It is, therefore, important that the variants should be assessed in the sexual progenies of the in vitro regenerated plants so that their heritability is established.
- The degree of variation of a plant can be determined by estimating the standard deviation for a particular quantitative trait.
- It is usually determined as the percentage of plants showing aberrations for one or more defined characteristics, such as plant height, time of flowering, fertility, flower and fruit color.
- The effect of environment on the phenotype of plant can also be detected using biochemical characterization mostly involving protein electrophoresis.
- These above mentioned methods can be very well used for the assessment of phenotypic variations but the variation or change at genome level cannot be monitored.
- In order to detect the variation at DNA level, use of certain molecular markers is encouraged.
- RFLP appears to be a better technique as it helps in identifying slight changes and also in studying plants grown in different environments.

Applications of Somaclonal Variations

i. Variability generated at the genetic level proves to be a source of crop improvement which can be greatly beneficial to plant breeders.

ii. Distinctive mutations may sometimes give rise to elite characters in the regenerants which cannot be achieved by conventional methods of breeding.

iii. Disease resistant genotypes of various plants can be attained. Resistance was first reported in sugarcane for eye spot disease (*Heliminthosporium sacchari*) and Fiji virus disease by regenerating plants from callus of susceptible clones.

iv. Plants with characteristic resistance to abiotic stress (cold, draught, acidic or alkaline soil) can be obtained as somaclones.

v. Somatic genome exchange may give rise to regenerants where a part of alien genome can be introgressed thereby leading to germplasm widening.

Limitations of Somaclonal variations

i. Poor plant regeneration from long-term cultures of various cell lines.

ii. Regeneration being limited to specific genotypes which may not be of much interest to breeders.

iii. Some somaclones have undesirable features, such as aneuploidy, sterility etc.

iv. Unpredictable variations that are often generated are of no use.

v. Variations attained may not always be stably integrated.

vi. Variants attained may not always be novel. In majority of cases improved variants are not even selected for breeding programs.

Nitrogen fixation

A relatively small amount of ammonia is produced by lightning. Some ammonia also is produced industrially by the Haber-Bosch process, using an iron-based catalyst, very high pressures and fairly high temperature. But the major conversion of N_2 into ammonia, and thence into proteins, is achieved by microorganisms in the process called nitrogen fixation (or dinitrogen fixation).

The nitrogen-fixing organisms

All the nitrogen-fixing organisms are prokaryotes (bacteria). Some of them live independently of other organisms - the so-called free-living nitrogen-fixing bacteria. Others live in intimate symbiotic associations with plants or with other organisms (e.g. protozoa).

The following free living bacteria fix the nitrogen in the plants through non-symbiotic association

Aerobic free living bacteria

- *Azotobacter*
- *Beijerinckia*
- *Klebsiella*
- *Cyanobacteria*

Anaerobic free living bacteria

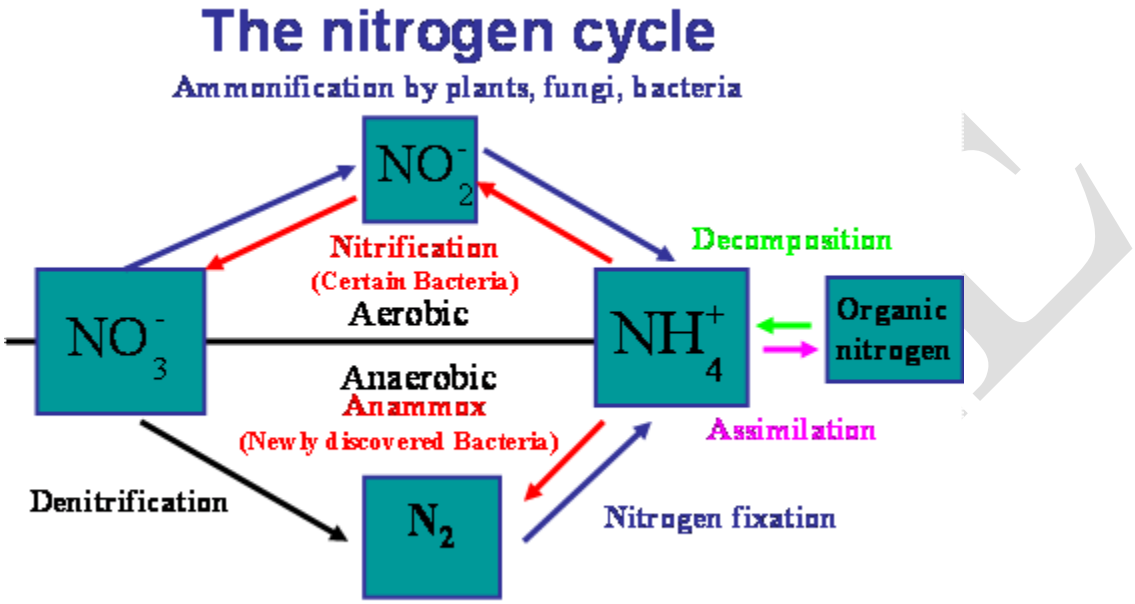
Clostridium

Desulfovibrio

Symbiotic nitrogen fixation

Rhizobium mainly play a vital role to fix the nitrogen in the plants through symbiotic action.

The most familiar examples of nitrogen-fixing symbioses are the root nodules of legumes (peas, beans, clover, etc.).



Nitrogenase (Nase) is an enzyme that fixes atmospheric nitrogen (N2) into ammonia. Though abundantly present in the atmosphere, most organisms cannot utilize N2 directly, and must instead take it in through other forms, like ammonia or nitrate.

Hyrdogenase an enzyme which catalyses the reduction of a particular substance by hydrogen.

Nodulation. Nodulation involves the production of a special organ, the nodule, and also what has been called a novel organelle, the symbiosome, consisting of nitrogen-fixing bacteroids enclosed in a primarily host-derived peribacteroid membrane.

Bio-control pathogens

Plant diseases need to be controlled to maintain the quality and abundance of food, feed, and fiber produced by growers around the world. Different approaches may be used to prevent, mitigate or control plant diseases. Beyond good agronomic and horticultural practices, growers often rely heavily on chemical fertilizers and pesticides. Such inputs to agriculture have contributed significantly to the spectacular improvements in crop productivity and quality over the past 100 years.

Mechanisms of biological control

Because biological control can result from many different types of interactions between organisms, researchers have focused on characterizing the mechanisms operating in different experimental situations. In all cases, pathogens are antagonized by the presence and activities of other organisms that they encounter. Here, we assert that the different mechanisms of antagonism occur across a spectrum of directionality related to the amount of interspecies contact and specificity of the interactions. Direct antagonism results from physical contact and/or a high-degree of selectivity for the pathogen by the mechanism(s) expressed by the BCA(s). In such a scheme, hyperparasitism by obligate parasites of a plant pathogen would be considered the most direct type of antagonism because the activities of no other organism would be required to exert a suppressive effect. In contrast, indirect antagonisms result from activities that do not involve sensing or targeting a pathogen by the BCA(s). Stimulation of plant host defense pathways by non-pathogenic BCAs is the most indirect form of antagonism. However, in the context of the natural environment, most described mechanisms of pathogen suppression will be modulated by the relative occurrence of other organisms in addition to the pathogen. While many investigations have attempted to establish the importance of specific mechanisms of biocontrol to particular pathosystems, all of the mechanisms described below are likely to be operating to some extent in all natural and managed ecosystems. And, the most effective BCAs studied to date appear to antagonize pathogens using multiple mechanisms. For instance, pseudomonads known to produce the antibiotic 2,4-diacetylphloroglucinol (DAPG) may also induce host defenses (Iavicoli et al. 2003). Additionally, DAPG-producers can aggressively colonize roots, a trait that might further contribute to their ability to suppress pathogen activity in the rhizosphere of wheat through competition for organic nutrients (Raaijmakers and Weller 2001).

Table Types of interspecies antagonisms leading to biological control of plant pathogens.

Type	Mechanism	Examples
Direct antagonism	Hyperparasitism/predation	Lytic/some nonlytic mycoviruses *Ampelomyces quisqualis* *Lysobacter enzymogenes* *Pasteuria penetrans* *Trichoderma virens*
Mixed-path antagonism	Antibiotics	2,4-diacetylphloroglucinol Phenazines Cyclic lipopeptides
	Lytic enzymes	Chitinases Glucanases Proteases
	Unregulated waste products	Ammonia Carbon dioxide Hydrogen cyanide
	Physical/chemical interference	Blockage of soil pores Germination signals consumption Molecular cross-talk confused
Indirect antagonism	Competition	Exudates/leachates consumption Siderophore scavenging Physical niche occupation
	Induction of host resistance	Contact with fungal cell walls Detection of pathogen-associated, molecular patterns Phytohormone-mediated induction

Possible Questions

Short question

1. What is protoplast?
2. What is fusogen?
3. How will you isolate the protoplast?
4. Define somatic hybridization.
5. What is cybrids?
6. Define somaclonal variation.
7. What is meat by nodulation?
8. Role of nirogenase and hydrogenase.
9. What is nitrogen fixation?
10. List out the nitrogen fixing bacteria.
11. List out the plant growth promoting bacteria.

Essay type questions.

12. Discuss about the isolation and fusion of protoplast.
13. Explain protoplast hybridization.
14. Explain Somatic hybridization
15. Discuss in detail about the Somaclonal variation
16. Explain in detail about the N2 fixation in plants.
17. Write short notes on i.Nitrogenase, ii.Hydrogenase, iii. Nodulation.
18. Write short notes on i. Bio control of pathogens, ii. Growth promotion by free-living bacteria.

UNIT-I

SYLLABUS

Structure and organization of plant genome: regulation of plant genome expression, transcriptional, translational and post transcriptional regulation of plant genome. Transfer of nucleic acid to plant cells - Direct transformation by electroporation and particle gun bombardment. - *Agrobacterium*, Ti plasmid vector Theory and techniques for the development of new genetic traits

Transformation of plant cells

- Genetic transformation involves the integration of gene into genome by means other than fusion of gametes or somatic cells.
- The foreign gene (termed the "transgene") is incorporated into the host plant genome and stably inherited through future generations.
- This plant transformation approach is being used to generate plant processing trails, unachievable by conventional plant breeding, especially in case where there is no source of the desired trait in the gene pool.
- In the gene of interest, the correct regulatory sequences are incorporated i.e. promoters and terminators, and then the DNA is transferred to the plant cell or tissue using a suitable vector.
- The gene of interest is attached to a selectable marker which allows selection for the presence of the transgene. Confirmation for the presence of inserted genes is generally tested by resistance to a specific antibiotic present in the medium.
- Once the plant tissue has been transformed, the cells containing the transgene are selected and regeneration back into whole plants is carried out.
- This is possible as plant cells are totipotent, which means that they contain all the genetic sequence to control the development of that cell into a normal plant.
- Therefore, the gene of interest is present in every single plant cell; however, where its expression is controlled by the promoter.
- Plant transformation can be carried out by various ways depending on the species of the plant.
- A major method of DNA transfer in plants is *Agrobacterium* mediated transformation.
- *Agrobacterium is* a natural living soil bacteria and is capable of infecting a wide range of plant species, causing crown gall diseases. It has natural transformation abilities. When *A. tumefaciens* infects a plant cell, it transfers a copy of its T-DNA, which is a small section of DNA carried on its Ti (Tumour inducing) plasmid.
- This T-DNA is flanked by two (imperfect) 25 base pair repeats. Any DNA contained within these borders will be transferred to the host cell when used as transformation vector.

Different types of plant transformation vectors

- Plant transformation vectors comprises of plasmids that have been purposely designed to facilitate the generation of genetically modified plants.
- The most commonly applicable plant transformation vectors are binary vectors which have the ability to replicate in *E. coli*, a common lab bacterium, as well as in *Agrobacterium tumefaciens*, bacterium used to insert the recombinant (customized) DNA into plants. Plant transformation vectors contain three essential elements:

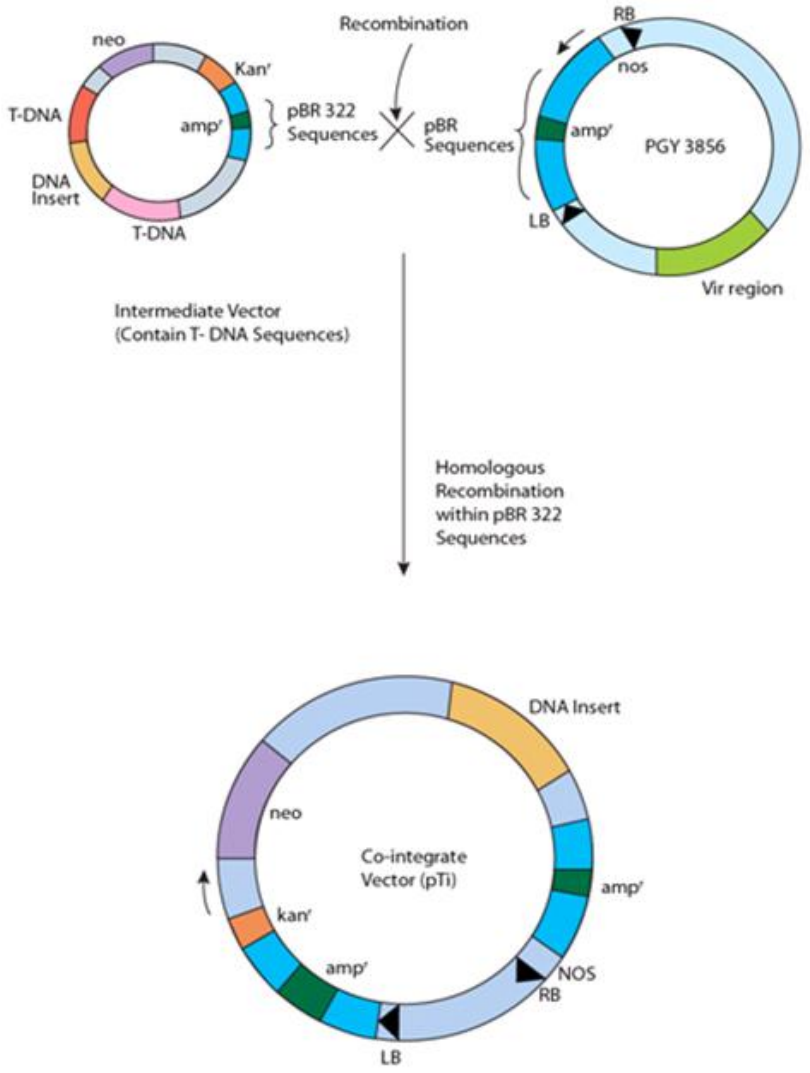
• Plasmids selection (creating a custom circular strand of DNA)

• Plasmids replication (so that it can be easily worked with T-DNA)

• T-DNA region (inserting the DNA into the *Agrobacterium*)

Co-integrate pTi vector

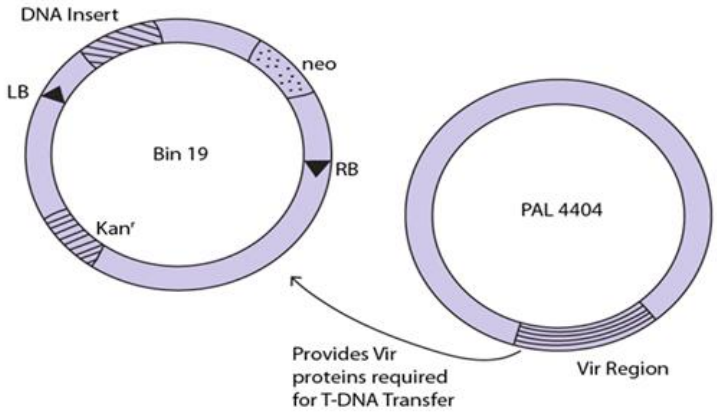
- The discovery that the vir genes do not need to be in the same plasmid with a T-DNA region to lead its transfer and insertion into the plant genome led to the construction of a system for plant transformation where the T-DNA region and the vir region are on separate plasmids.
- A co-integrative vector produced by integration of recombinant intermediate vector (IV containing the DNA inserts) in to a disarmed pTi.
- Transformed gene is initially cloned in *E. coli* for easy in cloning procedure. A suitably modified *E. coli* plasmid is used to initiate cloning of gene
- The subsequent gene transfer in to plants is obtained by co-integrative vectors. Co-integration of the two plasmids is achieved with in *Agrobacterium* by homologous recombination.



Diagrammatic representation of homologous recombination between disarmed pTi and recombinant IV (intermediate vector) containing the desired DNA insert to produce a cointegrative vector. (LB & RB – left and right borders of T-DNA; neo- neomycin phosphotransferase; kan r - Kanamycin resistance; ampr- ampicillin resistance).

Binary vector

- A binary vector consists of a pair of plasmids of which one contain *vir* region and other contains disarmed T-DNA sequence with right and left border sequences. The plasmid contain disarmed T-DNA are called micro-Ti or mini-Ti for e.g. Bin 19



Binary vectors Bin19 and PAL 4404 of pTi

Plant virus vector

• Viruses have following features as a vector

• Infect cells of adult plant (dicotyledonous and monocotyledonous both)

• They produce large number of copies per cell which facilitate gene amplification and produce large quantities of recombinant protein.

• Some are systemic that they can spread throughout the plant.

- Mostly plant viruses have RNA genome; two such viruses have great potential for vectors are brome mosaic virus (BMV) and tobacco mosaic virus (TMV).
- But maximum processes have been made with two DNA genome containing viruses as a vector, *viz* ., Caulimoviruses and Gemini viruses.

Cauliflower mosaic virus (**CaMV**)

- The Cauliflower Mosaic Virus (CaMV) is a double-stranded DNA virus which infects a wide range of crucifers, especially Brassicas, such as cabbage, cauliflower, oilseed rape or mustard.
- In order to get itself and its DNA replicated (multiplied) within a plant cell, the virus must trick the plant's own molecular ‘machinery' to do this task.

- For this purpose the virus has two promoters (35S and 19S) in front of its genes, which the plant cell believes to be its own. Furthermore, these promoters override the plant's own regulatory system, as they are constitutive, i.e. they are constantly switched on and can't be regulated or switched off by the plant.
- The CaMV 35S well known promoter is being used in almost all GM crops currently grown or tested, especially GM maize.
- It is the promoter of selection for plant genetic engineering, as it is a strong and constitutive promoter.
- Failure to distinguish or to ignore its capacity to be universally active in almost any organism is irresponsible and careless and shows a serious lack of scientific rigor and commitment to safety.

Gemini viruses

- Gemini viruses are small circular DNA viruses that replicate in plant nuclei. The Gemini virus vectors lack a coat protein gene, they are not transmissible by insect vectors, which are required for plant-to-plant spread and, thus, use of the disarmed vectors does not require a permit.
- Viruses from the Gemini virus family normally infects a wide range of crop plants, including maize, cotton, wheat, bean and cassava and are, therefore, an ideal system of choice for VIGS-based gene function analyses in a broad range of crop plants.
- Now vectors have been developed for use in cotton, and work is also ongoing for suitable vectors for roses. Using these new VIGS vectors, recombinant virus bearing a partial sequence of a host gene is used to infect the plant.
- As the virus spreads, the endogenous gene transcripts, which are homologous to the insert in the viral vector, are degraded by post-transcriptional gene silencing.
- These VIGS virus vectors have been used in a range of studies to silence single or multiple genes, including the meristematic gene, Proliferating Cell Nuclear Antigen (PCNA).

Tobacco mosaic virus (TMV)

- TMV have single-stranded RNA genome which also serves as mRNA. It encodes at least four proteins in three open reading frames.

- Its genome contains 4 genes, of these the coat protein (cp) gene seems to be nonessential and can be site of integration of transgene. Viral RNA promoters are successfully manipulated for the synthesis of recombinant messenger RNAs in whole plants.
- This vector consist of two steps, first, is the use of cDNA copy of viral genome for cloning in *E. coli* and, second, is *in vitro* transcription of the recombinant viral genome cDNA to produce infectious RNA copies to be used for plant infection.

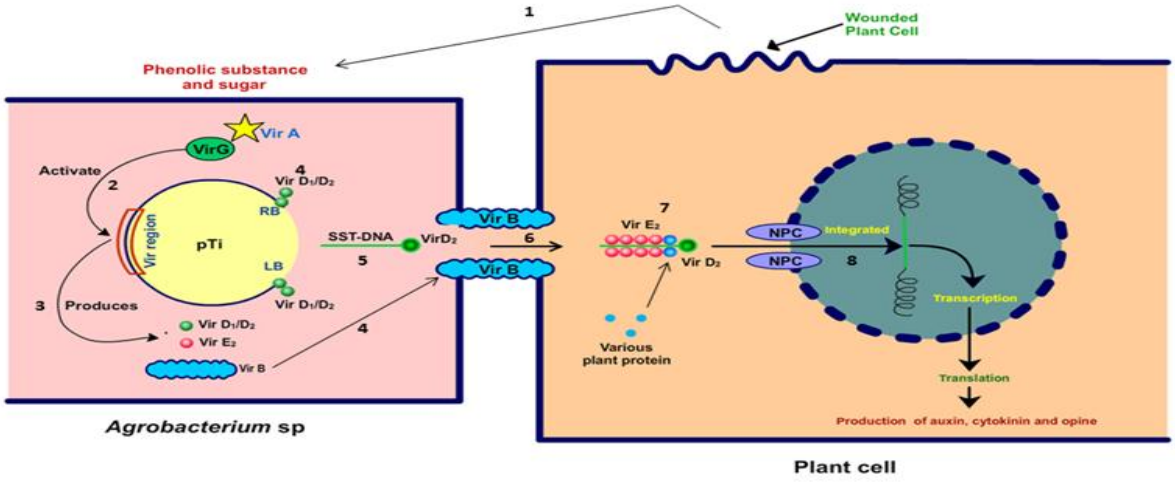
Brome mosaic virus (BMV)

- Brome mosaic virus (BMV) belongs to the family *Bromoviridae* of plant RNA viruses. BMV is a eukaryotic RNA virus, and its replication is entirely cytoplasmic. BMV genome is divided among three RNAs (1, 2 and 3) each packed into separate particle.
- Viral replication is dependent on well-organized interaction between nonstructural proteins 1a and 2a, encoded, respectively, by genomic RNA1 (gB1) and RNA2 (gB2). Genomic RNA3 (gB3) is dicistronic.
- Another nonstructural movement protein (MP) which promotes cell-to-cell spread encoded by 5′ half, while the capsid protein gene (CP) encoded in the 3′ half is translationally silent but is expressed from a subgenomic RNA (sgB4) that is synthesized from progeny minus-strand gB3 by internal initiation mechanisms.
- It was found in the absence of a functional replicase, assembled virions contained non-replicating viral RNAs (RNA1 or RNA2 or RNA3 or RNA1 + RNA3 or RNA2 + RNA3) as well as cellular RNAs.
- This indicates that placing a transgene downstream to the regulatory sequences of the *cp* gene of BMV will give high yields of the protein encoded by it.
- Different systems are now available for gene transfer and successive regeneration of transgenic plants and the most common being *Agrobacterium* -mediated transformation. However, the preferred host of *Agrobacterium* is the dicot plants and its efficiency to transfer genes in monocots is still unsatisfactory.
- The alternative to this, is the introduction of DNA into plants cells without the involvement of a biological agent like, *Agrobacterium* , and leading to stable transformation is known as direct gene transfer.

- T he most often applied direct methods are microprojectile bombardment or protoplast transformation.

T-DNA transfer and integration

The steps involved in T-DNA transfer and integration in to the plant genome



T-DNA transfer and integration

Wounded plant cell releases phenolics substances and sugars (1); which are sensed by *vir* A, *vir* A activates *vir* G, *vir* G induces expression of *vir* gene of Ti-plasmid (2); *vir* gene produces all the *vir* -protein (3); *vir* D_1 and *vir* D_2 are involve in ssT-DNA production from Ti-plasmid and its export (4) and (5); the ssT-DNA (with associated *vir* D_1 and *vir* D_2) with *vir* E_2 are exported through transfer apparatus *vir* B (6); in plant cell, T-DNA coated with *vir* E_2 (7); various plant proteins influence the transfer of T-DNA + *vir* D_1 + *vir* D_2 + *vir* E_2 complex and integration of T-DNA to plant nuclear DNA(8). (LB= left border; RB= Right border; pTi = Ti plasmid, NPC = nuclear pore complex)

Signal recognition by *Agrobacterium* spp.

- The wounded plant cells release certain chemicals, such as phenolics and sugars. These chemicals are recognized by *Agrobacterium* as signals.

- This in turn results in a sequence of biochemical events in*Agrobacterium* that helps in transfer of T-DNA of Ti plasmid.

Attachment to plant cell

- Attachment of this bacterium to plant cells is a two step process. It involves an initial attachment via a polysaccharides (the product of *att* R locus).
- Subsequently, a mesh of cellulose fibres is produced by*Agrobacterium*. Several chromosomal virulence genes (*chv* genes) are involved in attachment of bacterial cells to the plant cells.

Induction of virulence gene

- *vir* A (a membrane-linked sensor kinase) senses phenolics (such as acetosyringone) and autophosphorylates, subsequently phosphorylating and, thereby, activating *vir* G.
- This activated *vir* G induces expression of virulence gene of Ti plasmid to produce the corresponding virulence proteins (D, D2, E2, B).
- It has been also identified that certain sugars (e.g. glucose, galactose, xylose etc.) also induce virulence gene.

Table : *Agrobacterium* virulence protein function

Virulence protein	Function in *Agrobacterium* spp.	Function in plant
*vir*A	• Phenolic sensor • Part of two component system with VirG; phosphorylation and activates VirG	-
*vir*G	• Transcriptional factor • Responsible for *vir* gene expression	-
*vir*B1-B11	Components of membrane structure for T-DNA transfer	-
*vir*D1	• In T-DNA processing • Modulate *vir*D2 activity	-
*vir*D2	• Nick the T-DNA • Directs the T-DNA through *vir*B transfer apparatus	-
*vir*E2		• Single stranded DNA-binding protein • Prevents T-DNA degradation by nucleases • Involved in nuclear targeting and helps in passage through nuclear pore complex (NPC).

Production of T-DNA strand

- The right and left border sequence of T-DNA are identified by *vir* D1/ *vir* D2 protein complex and *vir*D2 produces single stranded DNA (ss-T-DNA).
- After nicking, *vir* D2 becomes covalently attached to the 5'end of ss-T- DNA strand and protect and export the ss-T-DNA to plant cells.

Transfer of T-DNA out the bacterial cell

The ss-T-DNA – *vir* D2 complex in association with *vir* E2 is exported from bacterial cell by a 'T-pilus' (a membrane channel secretary system).

Transfer T-DNA into plant cell and integration

- The single stranded T-DNA–*vir* D2 complex and other *vir* proteins cross the plant plasma membrane. In the plant cells, T-DNA gets covered with *vir* E2.
- This covering of *Vir* E2 helps in protection of ss-T-DNA from degradation by nucleases. *vir* D2 and *vir* E2 interact with variety of plant proteins which influence the T-DNA transport and integration.

- The T-DNA – *Vir* D2 – *Vir* E2 – plant proteins complex enters the nucleus through nuclear pore complex (NPC).
- In the nucleus, T-DNA gets integrated into the plant genome by a process referred to as 'illegitimate recombination'. This process is unlike homologous recombination as it does not depend on extensive region of sequence similarity.
- *Agrobacterium* species harboring tumor-inducing (Ti) or hairy root-inducing (Ri) plasmids cause crown gall or hairy root diseases, respectively in plants. *Agrobacterium tumefaciens* is a plant pathogen that induces tumor on a wide variety of dicotyledonous plants and the disease is caused by tumor-inducing plasmid (pTi).
- Similarly *Agrobacterium rhizogenes* is a plant pathogen that induces hairy roots on a wide variety of dicotyledonous plants and the disease is caused by root-inducing plasmid (pRi). Virulence *(vir)* genes of Ri as well as of Ti plasmids are essential for the T-DNA (Figure 26.1 & 26.2) transfer into plant chromosomes .
- These natural plasmids provide the basis for vectors to make transgenic plants. The plasmids are approximately 200 kbp in size.
- Both pTi and pRi are unique in two respects: **(i)** they contain some genes, located within their T-DNA, which have regulatory sequences recognized by plant cells, while their remaining genes have prokaryotic regulatory sequences, **(ii)**both plasmids naturally transfer a part of their DNA, the T-DNA, into the host genome, which makes*Agrobacterium* a natural genetic engineer.

The Ti plasmid

- The Ti plasmid contains all the genes which required for tumor formation. Virulence genes (*vir*-genes) are also located on the Ti plasmid.
- The vir genes encode a set of proteins responsible for the excision, transfer and integration of the T-DNA into the plant nuclear genome.

The basic elements of the vectors designed for *Agrobacterium*-mediated transformation that were taken from the native Ti-plasmid

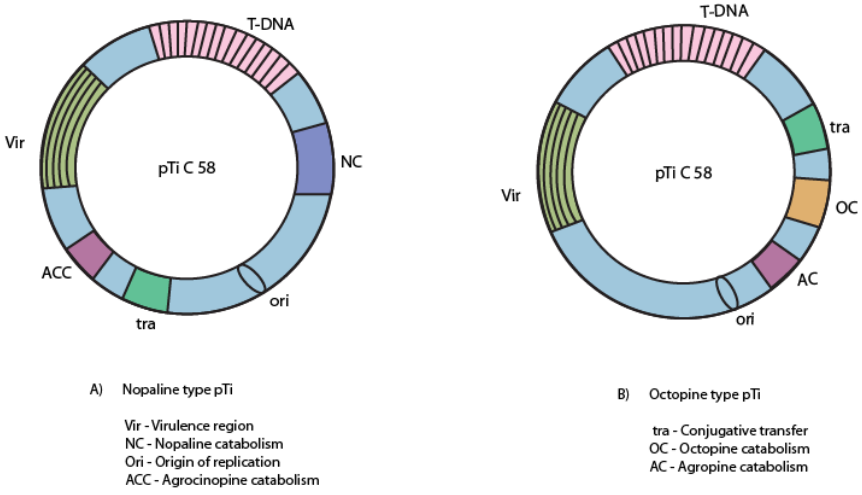
• The **T-DNA border sequences**, at least the right border, which initiates the integration of the T-DNA region into the plant genome

• The ***vir* genes** , which are required for transfer of the T-DNA region to the plant, and

- A **modified T-DNA region** of the Ti plasmid, in which the genes responsible for tumor formation are removed by genetic engineering and replaced by foreign genes of diverse origin, e.g., from plants, bacteria, virus.
- When these genes are removed, transformed plant tissues or cells regenerate into normal-appearing plants and, in most cases, fertile plants.The T-DNA region genes are responsible for the tumorigenic process. Some of them control the production of plant growth hormones that cause proliferation of the transformed plant cells.
- The T-DNA region is flanked at both ends by 24 base pairs (bp) direct repeat border sequence called T-DNA borders.
- The T-DNA left border is not essential, but the right border is indispensable for T-DNA transfer. Ti plasmid is grouped into two general categories:

i) Nopaline type pTi

ii) Octopine type pTi



Ti plasmid: **(A)** nopaline type pTi; **(B)** Octopine type pTi

Ri plasmid

- *Agrobacterium rhizogenes* is a soil born gram negative bacterium. It causes hairy root disease of many dicotyledonous plants.
- The ability of *A. rhizogenes* to incite hairy root disease is confirmed by a virulence plasmid, which is similar to that found in *Agrobacterium tumefaciens* which causes Crown gall tumors of plants.
- The virulence plasmid of *A. rhizogenes* is commonly known as the Ri-plasmid (pRi).
- The pRi have extensive functional homology with the pTi. The pRi contains distinct segment(s) of DNA, which is transferred to plant genome during infection.
- The transfer T-DNA to the plant genome is mediated by another segment on the plasmid known as the virulence (vir) region.
- All strains of *A. rhizogenes* are known to produce agrocinopine.

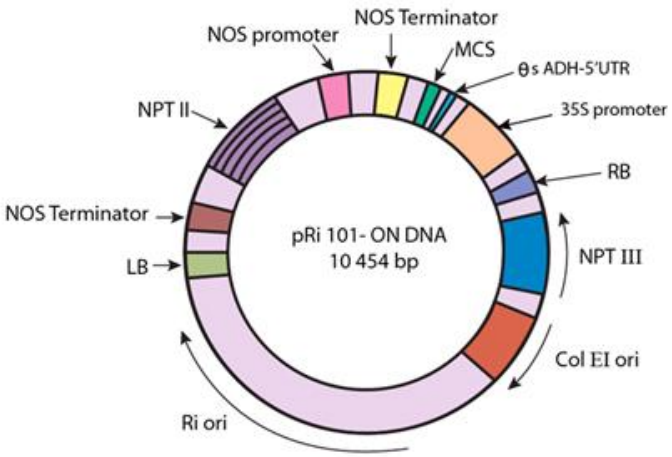


Figure : Ri plasmid

Selection and screening of transformation

- Genetic selection of transformed cells is a significant step of any plant transformation.
- Screening of transformed cells or plants for gene integration and expression in transformed cells or plants is a process that involves several techniques, including DNA and RNA blot hybridization analysis, PCR, ELISA analysis. In the absence of a correct selection system one would face with the option of screening every shoot that regenerates in a transformation experiment.

- In cases where transformation frequency is high this may be possible but for plant species that transform with low frequencies this would be a laborious, if not impossible, task.

Table : Selectable marker genes used in plant transformation

Gene	Enzyme encoded	Selective agent(s)
Antibiotics		
ble	Enzymic activity not known	Bleomycin
dhfr	Dihydrofolate reductase	Methotrexate Trimethoprim
hpt	Hygromycin phosphotransferase	Hygromycin B
npt II	Neomycin phosphotransferase	G418 Kanamycin
Herbicides		
als	Mutant forms of acetolactate synthase	Chlorsulfuron Imidazolinones
Aro A	5-Enolpyruvylshikimate 3-phosphate synthase	Glyphosate (Roundup)
bar	Phosphinothricin acetyltransferase	Phosphinothricin (Bialaphos)

- A screening can also be possible by screening or scorable or reporter gene, incorporated into the transformation vectors, which allows for the detection of transformed cells, tissues or plants The essential features of an ideal reporter gene are:

i. An efficient and easy detection with high sensitivity
ii. Lack of endogenous activity in plant cells
iii. A relatively rapid degradation of the enzyme

The screening markers presently used are mostly derived from bacterial genes coding for an enzyme that is readily detected by the use of chromogenic, fluorigenic, photon emitting or radioactive substrates.

Table : Screenable marker genes used in plant transformation

Gene	Enzyme encoded	Substrate(s) and assays
CAT	Chloramphenicol acetyl transferase	[^{14}C]chloramphenicol and acetyl CoA; TLC separation of acetylated [^{14}C]chloramphenicol - detection by autoradiography
lac Z	β-galactosidase	As β-glucuronidase; problems with background activity in some species
GUS	β-glucuronidase	Range of substrates depending on assay; colourimetric, fluorometric, and histochemical techniques available
lux	Luciferase: bacterial insect	Decanal and $FMNH_2$ ATP and O_2 and luciferin Bioluminescent assays: quantitative tests on extracts or in situ tissue assays with activity detected by exposure of X-ray film
npt-II	Neomycin phosphoryltransferase	Kanamycin and [^{32}P]ATP In situ assay on enzyme fractionated by non-denaturing PAGE; enzyme detected by autoradiography Quantitative dot-binding assay on reaction products

Direct gene transfer methods

Mode of gene delivery in plants

The direct DNA transfer methods have been subdivided into three categories:

1. Physical gene transfer method
2. Chemical gene transfer method
3. DNA imbibitions by cell, tissue and organ

Physical gene transfer method

Particle Bombardment

- The Particle bombardment device, well known as the gene gun, was developed to enable penetration of the cell wall so that genetic material containing a gene of interest can be transferred into the cell.
- This physical direct gene transfer method, gene gun (Figure 24.1) is used for genetic transformation of several organisms to introduce a diverse range of desirable traits.

- Plant transformation using particle bombardment follows the same steps as in *Agrobacterium* mediated transformation method:

i. Isolation of desired genes from the source organism

ii. To develop a functional transgenic construct including the selected gene of interest; promoters to drive expression; modification of codon, if needed, to increase successful protein production; and marker genes to facilitate tracking of the introduced genes in the host plant

iii. Insertion of transgenic construct into a useful plasmid

iv. Introduce the transgenes into plant cells

v. Regenerate the plants cells, and

vi. Test the performance of traits or gene expression under *in vitro*, greenhouse and field conditions.

Figure : A gene gun apparatus

- In particle bombardment method, 1-2 µm tungsten or gold particles (called micro-projectiles) coated with genetically engineered DNA are accelerated with air pressure at high velocities and shot into plant tissues on a Petri-plate.
- . This is the second most widely used method, after*Agrobacterium* mediated transformation, for plant genetic transformation.

- The device accelerates particles in one of the two ways: (1) by means of pressurized helium gas or (2) by the electrostatic energy released by a droplet of water exposed to high voltage.
- The earlier devices used blank cartridges in a modified firing mechanism to provide the energy for particle acceleration, and thus, the name particle gun. It is also called Biolistics, Ballistics or Bioblaster).
- The microcarriers (or microprojectiles), the tungsten or gold particles coated with DNA, are carried by macrocarriers (macro projectiles) which are then inserted into the apparatus and pushed downward at high velocities.
- The Macro-projectile is stopped by a perforated plate, while allowing the microprojectiles to propelled at a high speed into the plant cells on the other side. As the micro-projectiles enter the plant cells, the transgenes are free from the particle surface and may inserted into the chromosomal DNA of the plant cells.
- Selectable markers help in identifying those cells that take up the transgene or are transformed. The transformed plant cells are then regenerated and developed into whole plants by using tissue culture technique.

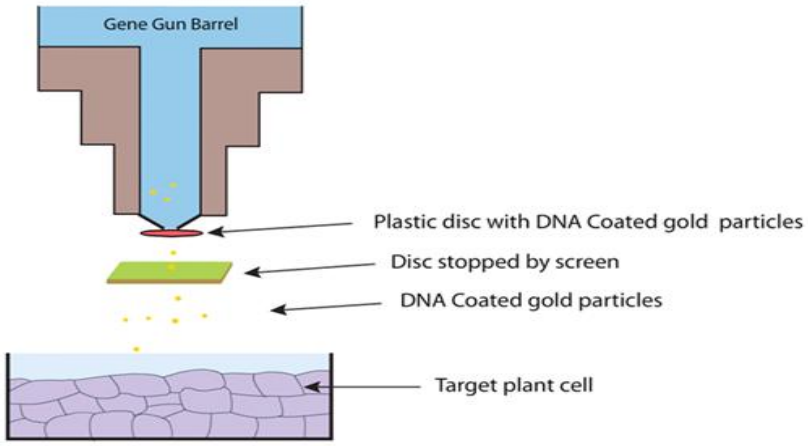


Figure : Diagrammatic illustration of gene transfer using Gene Gun method

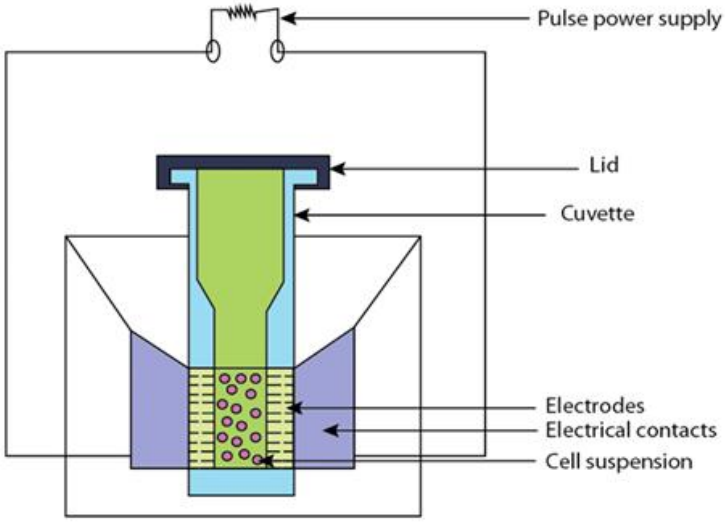
- The technique has many advantages and can be used to deliver DNA into virtually all the tissues, like immature and mature embryos, shoot-apical meristem, leaves, roots etc.

- Particle bombardment methods are also useful in the transformation of organelles, such as chloroplasts, which enables engineering of organelle-encoded herbicide or pesticide resistance in crop plants and to study photosynthetic processes.
- Limitations to the particle bombardment method, compared to *Agrobacterium*-mediated transformation, include frequent incorporation of multiple copies of the transgene at a single insertion site, rearrangement of the inserted genes, and insertion of the transgene at multiple insertion sites.
- These multiple copies can be associated with silencing of the transgene in subsequent progeny. The target tissue may often get damaged due to lack of control of bombardment velocity.

Electroporation

- Electroporation is another popular physical method for introducing new genes directly into the protoplasts.
- In this method, electric field is playing important role. Due to the electric field protoplast get temporarily permeable to DNA.
- In electroporation, plant cell protoplasts are kept in an ionic solution containing the vector DNA in a small chamber that has electrodes at opposite ends.
- A pulse of high voltage is applied to the electrode which makes the transient pores (ca. 30 nm) in the plasma membrane, allowing the DNA to diffuse into the cell.
- Immediately, the membrane reseals. If appropriately treated, the cells can regenerate cell wall, divide to form callus and, finally, regenerate complete plants in suitable medium.
- The critical part of the procedure is to determine conditions which produce pores that are sufficiently large and remain open long enough to allow for DNA diffusion.
- At the same time, the conditions should make pores that are temporary. With a 1 cm gap between the electrodes and protoplasts of 40-44µm diameter, 1-1.5 $kVcm^{-2}$ of field strength for 10µs is required for efficient introduction of DNA. It was seen that presence of 13% PEG (added after DNA) during electroporation significantly raised the transformation frequency.

- The other factors which may improve the transformation frequency by electroporation are linearizing of plasmid, use of carrier DNA, and heat shock (45 ~ for 5 min) prior to addition of vector, and placing on ice after pulsing. Under optimal conditions transformation frequencies of up to 2% have been reported. Stably transformed cell lines and full plants of a number of cereals have been produced through electroporation.



Electroporation

There are some parameters that can be considered when performing *in vitro* electroporation:

Cell size

- Cell size is inversely correlated to the size of the external field needed to generate permeabilization. Consequently, optimization for each cell type is essential. Likewise, cell orientation matters for cells that are not spherical.

Temperature

- It has been observed that plant membrane resealing is effectively temperature dependent and shows slow closure at low temperatures.
- For DNA transfer, it has been found that cooling at the time of permeabilization and subsequent heating in incubator increases transfer efficacy and cell viability.

Post-pulse manipulation

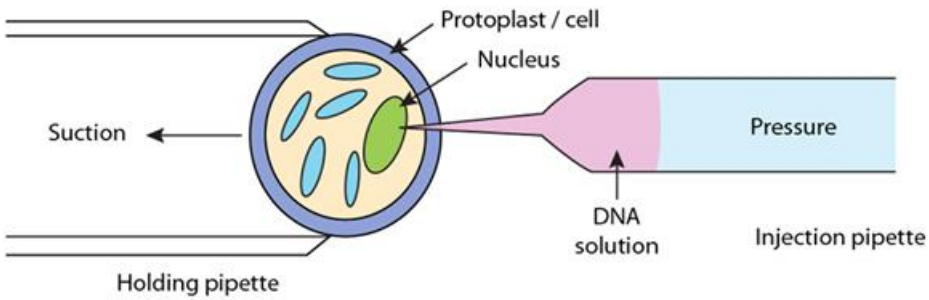
- Cells are susceptible when in the permeabilized state, and it has been shown that waiting for 15min after electroporation in order to allow resealing before pipetting cells, increases cell viability.

Composition of electrodes and pulsing medium

- For short pulses is needed for release of metal from the standard aluminium electrodes used in standard disposable cuvettes. Some authors advocate the use of low conductivity or more resistance media for DNA transfer in order to increase viability and increase stransfection efficacy.

Microinjection

- The microinjection technique is a direct physical approach to inject DNA directly into the plant protoplasts or cells (specifically into the nucleus or cytoplasm) using fine tipped (0.5-1.0 µm diameter) capillary glass needle or micropipettes.
- Through microinjection technique, the desired gene introduce into large cells, such as oocytes, eggs, and the cells of early embryo.



Microinjection

Liposome-mediated transformation

- The idea of a method of direct plant transformation elaborated in the middle eighties was to introduce DNA into the cell by means of liposomes. Liposomes are microscopic spherical vesicles that form when phospholipids are hydrated. Liposomes are circular lipid molecules with an aqueous interior that can carry nucleic acids.
- Liposomes encapsulate the DNA fragments and then adhere to the cell membranes and fuse with them to transfer DNA fragments.

- Thus, the DNA enters the cell and then to the nucleus. Lipofection is a very efficient technique used to transfer genes in bacterial, animal and plant cells.
- They can be loaded with a great variety of molecules, including DNA.
- In the case of protoplasts, the transfection (lipofection) occurs through the membrane fusion and endocytosis. When pollen grains are transformed, liposomes are delivered inside through pores.
- The efficiency of bioactive-beads-mediated plant transformation was improved using DNA-lipofection complex as the entrapped genetic material instead of naked DNA used in the conventional method.
- Liposome-mediated transformation is far from routine, in spite of the low expense and equipment requirement.
- A probable reason is its laboriousness and low efficiency.
- Only few reports on the integration of genes introduced by means of liposomes followed by transgenic plant regeneration for tobacco and wheat have been published thus far.

Silicon carbide fiber mediated transformation (SCMT)

- SCMT is one of the least complicated methods of plant transformation. Silicon carbide fibers are simply added to a suspension containing plant tissue (cell clusters, immature embryos, callus) and plasmid DNA, and then mixed in a vortex, or in other laboratory apparatus such as, commercial shakers, blenders etc. DNA-coated fibers penetrate the cell wall in the presence of small holes created in collisions between the plant cells and fibers.
- The most often used fibers in this procedure are single crystals of silica organic minerals like, siliconcarbide, which have an elongated shape, a length of 10–80 mm, and a diameter of 0.6 mm, and which show a high resistance to expandability.
- Fiber size, the parameters of vortexing, the shape of the vessels used, the plant material and the characteristics of the plant cells, especially the thickness of the cell wall are the factors depending on the efficiency of SCMT.

- There are several known examples of deriving transgenic forms, cell colonies or plants in maize, rice, Wheat, tobacco, *Lolium multiflorum* , *Lolium perenne, Festuca arundinacea* , *and Agrostis stolonifera* by SCMT.
- SCMT is an easy, fast and inexpensive procedure. Therefore, it could be an attractive alternative method of plant transformation in particular situations, e.g. when a gene gun is not available and*Agrobacterium* -mediated transformation is difficult or not possible (as in the case of numerous monocots).
- The other advantages of the SCF-mediated method over other procedures include the ability to transform walled cells, thus, avoiding protoplast isolation .
- The main disadvantages of this method are low transformation efficiency, damage to cells, thus, negatively influencing their further regeneration capability.
- Another disadvantage is that silicon fibers have similar properties to asbestos fibers and care must be taken when working with them as breathing the fibers can lead to serious sicknesses. Silicon carbide has some carcinogenic properties as well.

The pollen-tube pathway method

- The transformation method via pollen-tube pathway has great function in agriculture molecular breeding. Foreign DNA can be applied to cut styles shortly after pollination. The DNA reaches the ovule by flowing down the pollen-tube.
- This procedure, the so-called pollen-tube pathway (PTP), was applied first time for the transformation of rice.
- The authors obtained transgenic plants at remarkably high frequency. Afterward PTP was used for other species e.g. wheat, soybean, *Petunia hybrida* and watermelon.
- A bacterial inoculum or plasmid DNA can also be injected into inflorescence with pollen mother cells in the pre- meiotic stage without removing the stigma. In that case, it is expected that foreign DNA will be integrated with the gamete genome.
- Such an approach has been employed for rye. Pollen collected from inflorescences injected with a suspension of genetically engineered *A. tumefaciens* strain was predestined for the pollination of the emasculated spikes of the maternal plant.
- But the transformation efficiency was about 10-fold lower than that approximately reached for this species via microprojectile bombardment.

Chemical gene transfer method

- This involves plasma membrane destabilizing and/or precipitating agents. Protoplasts are mainly used which are incubated with DNA in buffers containing PEG, poly L-ornithine, polyvinyl alcohol or divalent ions.
- The chemical transformation techniques work for a broadspectrum of plants.

Polybrene–Spermidine Treatment

- The combination polybrene–spermidine treatment greatly enhanced the uptake and expression of DNA and, hence, the recovery of nonchimeric germline transgenic cotton plants.
- The major advantages of using the polybrene–spermidine treatment for plant genetic transformation are that polybrene is less toxic than the other polycations; spermidine protects DNA from shearing because of its condensation effect; and because no carrier DNA is used, and the integration of plasmid DNA into the host genome should enable direct analysis of the sequences surrounding the site of integration.
- To deliver plasmid DNA into cotton suspension culture obtained from cotyledon-induced callus, polybrene and/or spermidine treatments were used.
- The transforming plasmid (pBI221.23) contained the selectable hpt gene for hygromycin resistance and the screenable gus gene.
- Primary transformant cotton plants were regenerated and analyzed by DNA hybridization and b-glucuronidase assay.

PEG mediated gene transfer

- In this method protoplasts are isolated and a particular concentration of protoplast suspension is taken in a tube followed by addition of plasmid DNA (donor or carrier).
- To this 40% PEG4000(w/v) dissolved in mannitol and calcium nitrate solution is slowly added because of high viscosity, and this mixture is incubated for few minutes (ca 5 min.). As per the requirements of the experiments, transient or stable transformation studies are conducted.

- Among the most important parameters that affect the efficiency of PEG-mediated gene transfer are the concentration of calcium and magnesium ions in the incubation mixture, and the presence of carrier DNA.
- The linearized dsDNA are more efficiently expressed and integrated in the genome than the supercoiled forms.
- The advantage of the method is that the form of DNA applied to the protoplast is controlled entirely by the experimenter and not by intermediate biological vector. Main disadvantage is that the system requires a protoplast.

Calcium-Phosphate co-precipitation

- DNA when mixed with calcium chloride solution isotonic phosphate buffer DNA-CaPO 4 precipitate.
- The precipitate is allowed to react with actively dividing cells for several hours, washed and then incubated in the fresh medium.
- Giving them a physiological shock with DMSO can increase the efficiency of transformation to a certain extent.
- Relative success depends on high DNA concentration and its apparent protection in the precipitate.

Regulation of Plant genome expression

Transcription

- Transcription is the first step of gene expression, in which a particular segment of DNA is copied into RNA by the enzyme RNA polymerase.
- Both RNA and DNA are nucleic acids, which use base pairs of nucleotides as a complementary language.
- The two can be converted back and forth from DNA to RNA by the action of the correct enzymes.
- During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand called a primary transcript.

Transcription proceeds in the following general steps:

- One or more sigma factor protein binds to the RNA polymerase holoenzyme, allowing it to bind to promoter DNA.
- RNA polymerase creates a transcription bubble, which separates the two strands of the DNA helix. This is done by breaking the hydrogen bonds between complementary DNA nucleotides.
- RNA polymerase adds matching RNA nucleotides to the complementary nucleotides of one DNA strand. RNA sugar-phosphate backbone forms with assistance from RNA polymerase to form an RNA strand.
- Hydrogen bonds of the untwisted RNA-DNA helix break, freeing the newly synthesized RNA strand.
- If the cell has a nucleus, the RNA may be further processed. This may include polyadenylation, capping, and splicing. The RNA may remain in the nucleus or exit to the cytoplasm through the nuclear pore complex..

Transcription is divided into pre-initiation, initiation, promoter clearance, elongation and termination.

Pre-initiation

- In eukaryotes, RNA polymerase, and therefore the initiation of transcription, requires the presence of a core promoter sequence in the DNA.
- Promoters are regions of DNA that promote transcription and, in eukaryotes, are found at -30, -75, and -90 base pairs upstream from the transcription start site (abbreviated to TSS). Transcription factors are proteins that bind to these promoter sequences and facilitate the binding of RNA Polymerase.
- The most characterized type of core promoter in eukaryotes is a short DNA sequence known as a TATA box, found 25-30 base pairs upstream from the TSS.
- The TATA box, as a core promoter, is the binding site for a transcription factor known as TATA-binding protein (TBP), which is itself a subunit of another transcription factor, called Transcription Factor II D (TFIID).

- After TFIID binds to the TATA box via the TBP, five more transcription factors and RNA polymerase combine around the TATA box in a series of stages to form a preinitiation complex.
- One transcription factor, Transcription factor II H, has two components with helicase activity and so is involved in the separating of opposing strands of double-stranded DNA to form the initial transcription bubble.
- However, only a low, or basal, rate of transcription is driven by the preinitiation complex alone.
- Other proteins known as activators and repressors, along with any associated coactivators or corepressors, are responsible for modulating transcription rate.

Thus, preinitiation complex contains

Core Promoter Sequence

Transcription Factors

RNA Polymerase

Activators and Repressors.

- The transcription preinitiation in archaea is, in essence, homologous to that of eukaryotes, but is much less complex.
- The archaeal preinitiation complex assembles at a TATA-box binding site; however, in archaea, this complex is composed of only RNA polymerase II, TBP, and TFB (the archaeal homologue of eukaryotic transcription factor II B (TFIIB)).

Initiation

- Simple diagram of transcription initiation. RNAP = RNA polymerase
- In bacteria, transcription begins with the binding of RNA polymerase to the promoter in DNA.
- RNA polymerase is a core enzyme consisting of five subunits: 2 α subunits, 1 β subunit, 1 β' subunit, and 1 ω subunit.

- At the start of initiation, the core enzyme is associated with a sigma factor that aids in finding the appropriate -35 and -10 base pairs downstream of promoter sequences.
- When the sigma factor and RNA polymerase combine, they form a holoenzyme.
- Transcription initiation is more complex in eukaryotes. Eukaryotic RNA polymerase does not directly recognize the core promoter sequences.
- Instead, a collection of proteins called transcript
- tion factors mediate the binding of RNA polymerase and the initiation of transcription. Only after certain transcription factors are attached to the promoter does the RNA polymerase bind to it.
- The completed assembly of transcription factors and RNA polymerase bind to the promoter, forming a transcription initiation complex.
- Transcription in the archaea domain is similar to transcription in eukaryotes.

Promoter clearance

- After the first bond is synthesized, the RNA polymerase must clear the promoter. During this time there is a tendency to release the RNA transcript and produce truncated transcripts.
- This is called abortive initiation and is common for both eukaryotes and prokaryotes.
- In prokaryotes, abortive initiation continues to occur until an RNA product of a threshold length of approximately 10 nucleotides is synthesized, at which point promoter escape occurs and a transcription elongation complex is formed. The σ factor is released according to a stochastic model.
- Mechanistically, promoter escape occurs through a scrunching mechanism, where the energy built up by DNA scrunching provides the energy needed to break interactions between RNA polymerase holoenzyme and the promoter.
- In eukaryotes, after several rounds of 10nt abortive initiation, promoter clearance coincides with the TFIIH's phosphorylation of serine 5 on the carboxy terminal domain of RNAP II, leading to the recruitment of capping enzyme (CE). The exact mechanism of how CE induces promoter clearance in eukaryotes is not yet known.

Elongation

- One strand of the DNA, the template strand (or noncoding strand), is used as a template for RNA synthesis.
- As transcription proceeds, RNA polymerase traverses the template strand and uses base pairing complementarity with the DNA template to create an RNA copy.
- Although RNA polymerase traverses the template strand from 3' → 5', the coding (non-template) strand and newly formed RNA can also be used as reference points, so transcription can be described as occurring 5' → 3'.
- This produces an RNA molecule from 5' → 3', an exact copy of the coding strand (except that thymines are replaced with uracils, and the nucleotides are composed of a ribose (5-carbon) sugar where DNA has deoxyribose (one fewer oxygen atom) in its sugar-phosphate backbone).
- mRNA transcription can involve multiple RNA polymerases on a single DNA template and multiple rounds of transcription (amplification of particular mRNA), so many mRNA molecules can be rapidly produced from a single copy of a gene.
- Elongation also involves a proofreading mechanism that can replace incorrectly incorporated bases.
- In eukaryotes, this may correspond with short pauses during transcription that allow appropriate RNA editing factors to bind.
- These pauses may be intrinsic to the RNA polymerase or due to chromatin structure.

Termination

- Bacteria use two different strategies for transcription termination - Rho-independent termination and Rho-dependent termination. In Rho-independent transcription termination, also called intrinsic termination, RNA transcription stops when the newly synthesized RNA molecule forms a G-C-rich hairpin loop followed by a run of Us. When the hairpin forms, the mechanical stress breaks the weak rU-dA bonds, now filling the DNA-RNA hybrid.

- This pulls the poly-U transcript out of the active site of the RNA polymerase, in effect, terminating transcription. In the "Rho-dependent" type of termination, a protein factor called "Rho" destabilizes the interaction between the template and the mRNA, thus releasing the newly synthesized mRNA from the elongation complex.
- Transcription termination in eukaryotes is less understood but involves cleavage of the new transcript followed by template-independent addition of adenines at its new 3' end, in a process called polyadenylation.

Translation

- In molecular biology and genetics, **translation** is the process in which cellular ribosomes create proteins.
- In translation, messenger RNA (mRNA)—produced by transcription fromDNA—is decoded by a ribosome to produce a specific amino acid chain, orpolypeptide.
- The polypeptide later folds into an active protein and performs its functions in the cell. The ribosome facilitates decoding by inducing the binding of complementary tRNA anticodon sequences to mRNA codons.
- The tRNAs carry specific amino acids that are chained together into a polypeptide as the mRNA passes through and is "read" by the ribosome.
- The entire process is a part of gene expression.

In brief, translation proceeds in three phases:

1. **Initiation**: The ribosome assembles around the target mRNA. The first tRNA is attached at the start codon.
2. **Elongation**: The tRNA transfers an amino acid to the tRNA corresponding to the next codon. The ribosome then moves (*translocates)* to the next mRNA codon to continue the process, creating an amino acid chain.
3. **Termination**: When a stop codon is reached, the ribosome releases the polypeptide.

Post-transcriptional regulation

Post-transcriptional regulation is the control of gene expression at the RNA level, therefore between the transcription and the translation of the gene.

Mechanism

- After being produced, the stability and distribution of the different transcripts is regulated (post-transcriptional regulation) by means of RNA binding protein (RBP) that control the various steps and rates of the transcripts: events such as alternative splicing, nuclear degradation (exosome), processing, nuclear export (three alternative pathways), sequestration in P-bodies for storage or degradation and ultimately translation.
- These proteins achieve these events thanks to a RNA recognition motif (RRM) that binds a specific sequence or secondary structure of the transcripts, typically at the 5' and 3' UTR of the transcript.

Capping

- changes the five prime end of the mRNA to a three prime end by 5'-5' linkage, which protects the mRNA from 5' exonuclease, which degrades foreign RNA. The cap also helps in ribosomal binding.

Splicing removes

- the introns, noncoding regions that are transcribed into RNA, in order to make the mRNA able to create proteins.
- Cells do this by spliceosomes binding on either side of an intron, looping the intron into a circle and then cleaving it off. The two ends of the exons are then joined together.

Addition of poly(A) tail

- otherwise known as polyadenylation. That is, a stretch of RNA that is made solely of adenine bases is added to the 3' end, and acts as a buffer to the 3' exonuclease in order to increase the half life of mRNA. In addition, a long poly(A) tail can increase translation. Poly(A)-binding protein (PABP) binds to a long poly(A) tail and mediates the interaction between EIF4E and EIF4G which encourages the initiation of translation.

RNA editing

- is a process which results in sequence variation in the RNA molecule, and is catalyzed by enzymes.
- These enzymes include the Adenosine Deaminase Acting on RNA (ADAR) enzymes, which convert specific adenosine residues to inosine in an mRNA molecule by hydrolytic deamination.
- Three ADAR enzymes have been cloned, ADAR1, ADAR2 and ADAR3, although only the first two subtypes have been shown to have RNA editing activity.
- Many mRNAs are vulnerable to the effects of RNA editing, including the glutamate receptor subunits GluR2, GluR3, GluR4, GluR5 and GluR6 (which are components of the AMPA and kainate receptors), the serotonin2C receptor, the GABA-alpha3 receptor subunit, the tryptophan hydroxlase enzyme TPH2, the hepatitis delta virus and more than 16% of microRNAs.

mRNA Stability

- can be manipulated in order to control its half-life, and the poly(A) tail has some effect on this stability, as previously stated.
- Stable mRNA can have a half life of up to a day or more which allows for the production of more protein product; unstable mRNA is used in regulation that must occur quickly.

Possible questions

Short questions

1. What is electroporation?
2. Draw the Ti plasmid.
3. What is crown gall diseases?
4. Short note on Particle bombardment.
5. Write a short note on gene gun.
6. Give short note on Ri plasmid.
7. What is meant by translation?
8. Draw the typical structure of plant genome.
9. What is capping?
10. What is post transcriptional regulations?

Essay type questions

1. Describe in detail transcription regulation in plants.
2. Explain in detail about structure and organization of plant genome.
3. Write short notes on i. Gene gun & ii. Electroporation
4. Discuss in detail about regulations of plant genome expression.
5. Explain in detail about gene transformation using *Agrobacterium*.
6. Write short notes on i. Particle bombardment & ii. Electroporation
7. Describe i. Translational ii. Transcriptional regulation of gene expression
8. Explain in detail about structure and organization of plant genome.
9. Describe i. Ti plasmid and ii. Ri plasmid
10. Illustarte the physical gene transformation methods.

UNIT-V

SYLLABUS

Transgenic plants: herbicides and pest resistant plants, Drought, Salinity and cold tolerant plants; Molecular farming / pharming: carbohydrates, lipids, theraputic proteins, edible vaccines, purification strategies; Oleosin partition technology

Application of genetic engineering

Introduction

The knowledge of the molecular basis of diseases caused by various pathogens has allowed testing different strategies to produce disease resistant transgenic plants. Genetic engineering has also been successful in producing herbicide resistance plants. Some other applications are to develop high degree of tolerance or resistance to pests (insects, nematodes, etc.) and diseases. Below are some examples of genetic engineering applications in agriculture

- **Virus resistance**
- **Insect resistance**
- **Golden rice**
- **Long lasting tomatoes**

Virus resistance

- Plant viruses can cause severe damage to crops by substantially reducing vigor, yield, and product quality.
- Viruses cause more than 1400 plant diseases and thus, decreasing the agricultural productivity.
- Unfortunately, there is no viracidal compound to control these diseases. Some diseases, such as rice tungro disease, are caused by two or more distinct viruses and attempts to incorporate genes for resistance against them have not met with success.
- Virus resistance is achieved usually through the antiviral pathways of RNA silencing, a natural defense mechanism of plants against viruses.
- The experimental approach consists of isolating a segment of the viral genome itself and transferring it into the genome of a susceptible plant. Integrating a viral gene fragment into a host genome does not cause disease (the entire viral genome is needed to cause disease).
- Instead, the plant's natural antiviral mechanism that acts against a virus by degrading its genetic material in a nucleotide sequence specific manner via a cascade of events involving numerous proteins, including ribonucleases (enzymes that cleave RNA), is activated. This targeted degradation of the genome of an invader virus protects plants from virus infection.

- Three hypothesis have been investigated to engineer development of virus resistance plants

• Expression of the virus coat protein (CP) gene

• Expression of satellite RNAs and

• Use of antisense viral RNA

Expression of the virus coat protein (CP) gene

- This technique is most common. In this CP-mediated resistance (CP-MR) is developed, based on the well known process of cross protection.
- It is protected against super infection by a severe strain of related virus. This method has been commonly used in agriculture to confer protection against severe virus infection.
- However, the technique has following disadvantages:

• Due to synergistic interaction, infection of cross protected plants with a second unrelated virus may cause a severe disease,

• The suspicious virus strain might mutate to a more severe form, leading to extensive crop losses,

• Protecting virus strain may cause a small but significant decrease in yields, and

• In cross protection, the protecting virus must be applied each growing season.

- Most of these problems can be offset by genetic engineering of CP-MR in plants. CP-MR produced a c-DNA encoding the capsid protein (CP) sequences of TMV, ligated it to a strong transcriptional promoter (CaMV 35S promoter) and transport sequences to provide constitutive expression of the gene throughout the transgenic plant, and flanked on the 3' end by poly A signal from the nopaline synthase gene.
- This chimeric gene was introduced into a disarmed plasmid of *A. tumefaciens* and the modified bacterium was used.

Expression of satellite RNAs

- Some viruses have specific feature to contain, in addition to their genomic RNA, a small RNA molecule known as satellite RNA (S-RNA).

- The S-RNAs require the company of a specific 'helper' virus (closely related virus) for their replication. S-RNA does not have sequence to encode CP.
- They are encapsulated in the coat protein of their helper virus or satellite vi-ruses which encode their own coat protein.
- Due to ability to modify disease symptoms S-RNAs now have point of attention in genetic engineering.
- Most of the S-RNAs decrease the severity of viral infection, presumably through interference with viral replication. By this method, tomato, a number of pepper varieties, cucumber, eggplant, cabbage and tobacco plants against CMV have been protected.
- The first time S-RNA induced attenuation of viral symptoms involved the introduction of cDNA copies of CMV S-RNA into the genome of tobacco plants.

Use of antisense viral RNA

- Here using the antisense RNA, which is a single stranded RNA molecule complementary to the mRNA (sense RNA), transcribed by a given gene, is another approach suggested for introducing viral resistance in plants.
- The sense RNA carries codons to translate to a specified sequence of amino acids. The antisense RNA, on the other hand, does not contain the functional protein sequences.
- When both sense and antisense RNA are present together in cytoplasm they anneal to form a duplex RNA molecule which cannot be translated. Using this methodology, transgenic plants expressing 3' region of antisense RNA, including CP gene of TMV or CMV.
- RNAs were produced which have property to protect against infection with respective viruses or viral RNA.

Insect resistance

- Insects cause serious losses in agricultural products in the field at the time of cultivation and during storage.
- Insects belonging to the orders, Coleoptera, Lepidoptera and Diptera, are the most serious plant pests which cause agricultural damages. Use of insecticides, bio-pesticides has

several harmful side effects. *Bacillus thuringiensis* (Bt), a free-living, Gram-positive soil bacterium, has been employed as insecticide specificity towards lepidopteran pests.

- It is environmentally safe and thus, is high in demand. On the other hand, the major problems in using Bt sprays for controlling the insect attack on plants

• The high cost of production of Bt insecticide and

• The instability of the protoxin crystal proteins under field conditions, necessitating multiple applications.

- To avoid these problems transgenic plants expressing Bt toxin genes have been engineered. Insect resistant transgenic plants have also been created by introducing trypsin inhibitor gene.

Bt Cotton

- Two Bt proteins have been recognized as being of particular use for the control of the major pests of cotton and the genes encoding for these proteins have been incorporated into cotton plants by Monsanto.
- In the 1980's a lot work was undertaken by Monsanto to identify and extract the Bt genes and during this decade the gene encoding for the Bt protein Cry1Ac was successfully inserted into a cotton plant.
- Nowadays, several plant genes are transformed and used as insect resistant plants.

Golden rice

- Golden rice is genetically modified rice which contains a large amount of A-vitamins. Or more correctly, the rice contains the constituent beta-carotene which is converted in the body into Vitamin-A. So when you eat golden rice, so can get more amount of vitamin of A.
- Beta-carotene is orange colour so genetically modified rice is golden color. For the making of golden for synthesis of beta-carotene three new genes are implanted: two from daffodils and the third from a bacterium.

Advantages:

- The rice can be considered for poor people in underdeveloped countries. They eat only an extremely limited diet lacking in the essential bodily vitamins.

Disadvantage

- Critics terror that poor people in underdeveloped countries are becoming too dependent on the rich western world. Generally, genetically modified plants are developed by the large private companies in the West.
- The customers who buy patented transgenic seeds from the company may need to sign a contract not to save or sell the seeds from their harvest, which raises concerns that this technology might lead to dependence for small farmers.

Long-lasting tomatoes

- Long-lasting, genetically modified tomatoes now came in to the market.
- This is the first genetically modified food available to consumers.
- The genetically modified tomato produces less of the substance that causes tomatoes to rot, so remains firm and fresh for a long time.

Transgenic Plants

- Progress is being made on several fronts to introduce new traits into plants using recombinant DNA technology.
- The genetic manipulation of plants has been going on since the dawn of agriculture, but until recently this has required the slow and tedious process of cross-breeding varieties.
- Genetic engineering promises to speed the process and broaden the scope of what can be done.

Making transgenic plants

There are several methods for introducing genes into plants, including

- infecting plant cells with plasmids as vectors carrying the desired gene;
- shooting microscopic pellets containing the gene directly into the cell.

In contrast to animals, there is no real distinction between somatic cells and germline cells. Somatic tissues of plants, e.g., root cells grown in culture,

- can be transformed in the laboratory with the desired gene;
- grown into mature plants with flowers.

If all goes well, the transgene will be incorporated into the pollen and eggs and passed on to the next generation.

In this respect, it is easier to produce transgenic plants than transgenic animals.

Improved Nutritional Quality

- Milled rice is the staple food for a large fraction of the world's human population. Milling rice removes the husk and any beta-carotene it contained.
- Beta-carotene is a precursor to vitamin A, so it is not surprising that vitamin A deficiency is widespread, especially in the countries of Southeast Asia.
- The synthesis of beta-carotene requires a number of enzyme-catalyzed steps.
- In January 2000, a group of European researchers reported that they had succeeded in incorporating three transgenes into rice that enabled the plants to manufacture beta-carotene in their endosperm.

Insect Resistance.

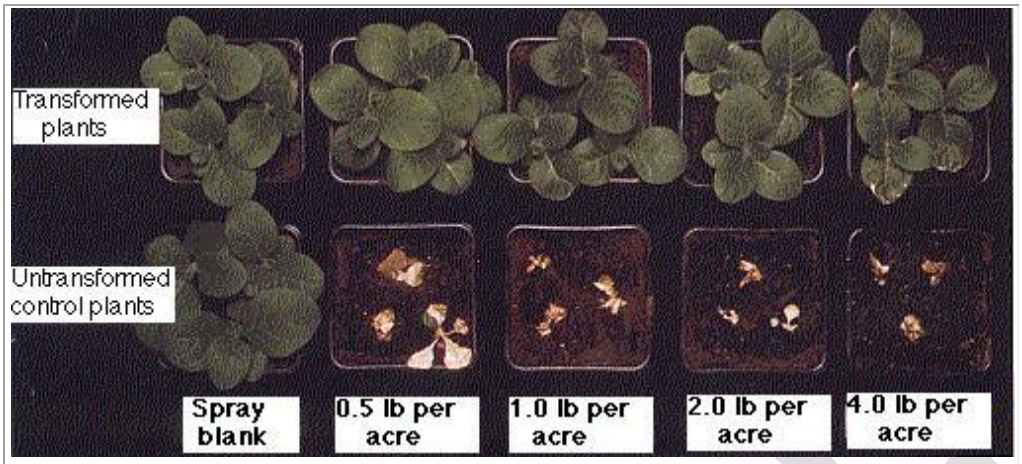
- *Bacillus thuringiensis* is a bacterium that is pathogenic for a number of insect pests. Its lethal effect is mediated by a protein toxin it produces.
- Through recombinant DNA methods, the toxin gene can be introduced directly into the genome of the plant where it is expressed and provides protection against insect pests of the plant.

Disease Resistance.

- Genes that provide resistance against plant viruses have been successfully introduced into such crop plants as tobacco, tomatoes, and potatoes.
- Tomato plants infected with tobacco mosaic virus (which attacks tomato plants as well as tobacco).
- The plants in the back row carry an introduced gene conferring resistance to the virus.
- The resistant plants produced three times as much fruit as the sensitive plants (front row) and the same as control plants. (Courtesy Monsanto Company.)

Herbicide Resistance.

- Alternatives are available, but they may damage the crop as well as the weeds growing in it. However, genes for resistance to some of the newer herbicides have been introduced into some crop plants and enable them to thrive even when exposed to the weed killer.



Effect of the herbicide bromoxynil on tobacco plants transformed with a bacterial gene whose product breaks down bromoxynil (top row) and control plants (bottom row). "Spray blank" plants were treated with the same spray mixture as the others except the bromoxynil was left out. (Courtesy of Calgene, Davis, CA.)

Salt Tolerance

- A large fraction of the world's irrigated crop land is so laden with salt that it cannot be used to grow most important crops.
- However, researchers at the University of California Davis campus have created transgenic tomatoes that grow well in saline soils.
- The transgene was a highly-expressed sodium/proton antiport pump that sequestered excess sodium in the vacuole of leaf cells.
- There was no sodium buildup in the fruit.

Terminator Genes

- This term is used (by opponents of the practice) for transgenes introduced into crop plants to make them produce sterile seeds (and thus force the farmer to buy fresh seeds for the following season rather than saving seeds from the current crop).

The process involves introducing three transgenes into the plant:

- A gene encoding a toxin which is lethal to developing seeds but not to mature seeds or the plant. This gene is normally inactive because of a stretch of DNA inserted between it and its promoter.
- A gene encoding a recombinase — an enzyme that can remove the spacer in the toxin gene thus allowing to be expressed.
- A repressor gene whose protein product binds to the promoter of the recombinase thus keeping it inactive.

How they work

When the seeds are soaked (before their sale) in a solution of tetracycline

- Synthesis of the repressor is blocked.
- The recombinase gene becomes active.
- The spacer is removed from the toxin gene and it can now be turned on.

Because the toxin does not harm the growing plant — only its developing seeds — the crop can be grown normally except that its seeds are sterile.

The use of terminator genes has created much controversy:

- Farmers — especially those in developing countries — want to be able to save some seed from their crop to plant the next season.
- Seed companies want to be able to keep selling seed.

Production of edible vaccines

- Transgenic plants provide an alternative system for the production of recombinant vaccines.
- The major advantage of vaccine production in plants is the direct use of edible plants tissue for oral administration.
- By the use of edible vaccines the problems associated with the purification of vaccines can be avoided.
- The stable or transient expression system can be used to produce vaccines in plants. Transgenic plants have been developed for expressing antigens derived from animal viruses.

- The need for use of edible vaccines comes from the fact that larger numbers of people are the victims of enteric diseases.

Table : Examples of plant edible subunit vaccines

Recombinant protein (vaccine)	Transgenic plant	Protection against
Rabies glycoprotein	Tomato	Rabies virus
Foot and mouth virus (VPI)	Arabidopsis	Foot and mouth virus
Herpes virus B surface antigen	Tobacco	Herpes simplex virus
Cholera toxin B subunit	Potato	*Vibrio cholerae*
Human cytomegalovirus glycoprotein B	Tobacco	Human cytomegalovirus

Choice of plants for edible vaccines

- Most of the vaccines production was carried out in tobacco plant that is not edible.
- These vaccines are now being produced in edible plants such as banana, tomato and potato. For use in animals the common fodder crops are used.
- Banana is an ideal system for the production of edible vaccine since it is grown in most part of the world and eaten raw.

Edible vaccine production and use

- The bacterium, *Agrobacterium tumefaciens* is commonly used to deliver the DNA for bacterial or viral antigens.
- A plasmid carrying the antigen gene and an antibiotic resistance gene are incorporated into the bacterial cells.
- The cut pieces of potato leaves are exposed to an antibiotic to kill the cells that lack the new genes.
- The surviving cells (gene altered ones) can multiply and form a callus. This callus will sprout and form shoots and roots, which are grown in soil to form plants.

- After 3 weeks the plant produces potatoes containing antigen vaccines
- The first clinical trials in humans involved the ingestion of transgenic potatoes with a toxin of *E.coli* causing diarrhoea.

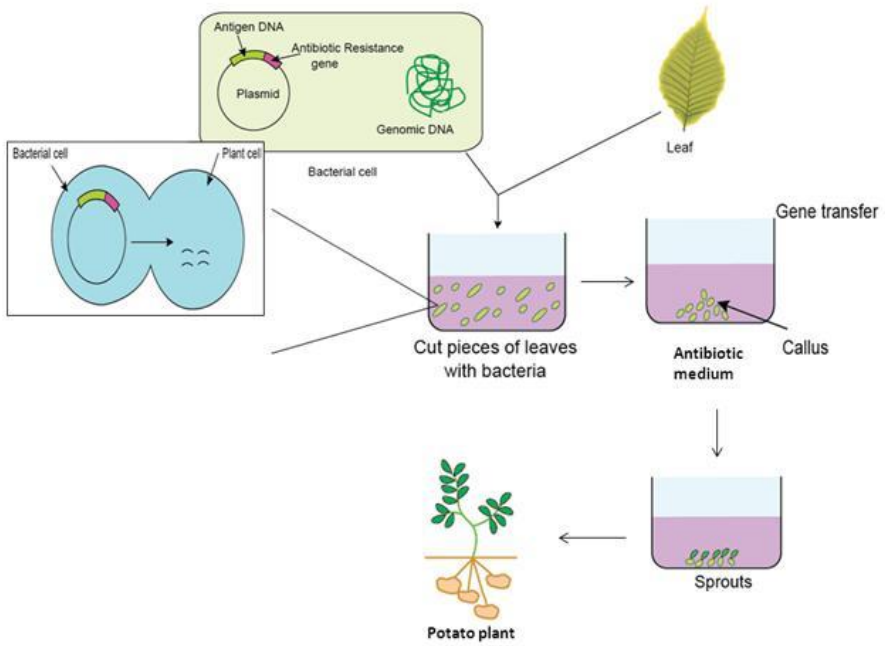


Figure : Schematic representation of production of edible vaccine

Delivery of vaccine to the gut

- Vaccines, being protein are likely to be degraded in the stomach. But in the case of edible vaccine it has been found that orally administered plant material can induce immune response.
- There is a difficulty of dose adjustment when edible vaccines are consumed as a part of food stuff.
- Instead of the direct use of plant material, a food based tablet containing a known dose of vaccine has been produced.
- This approach is being applied to vaccines produced in tomatoes.

Limitations of edible vaccines

Direct consumption of transgenic fruit or vegetable or food based tablets have some problems.

- The risk of loss of vaccines by the action of enzymes in stomach and intestine.
- The possibility of allergic reactions as they enter circulation.

Fungus Resistant Transgenic Plant Production:

i. On the basis of known defense mechanisms, overall transgenic approaches can be grouped into seven categories .

1. Over-expression of genes that produce compounds e.g.pathogenesis-related proteins (PR proteins) and phytoalexins, which are directly toxic to pathogens or reduce their growth.

2. Expression of genes, e.g.polygalacturonase, oxalic acid and lipases that destroy or neutralize the components of pathogen arsenal.

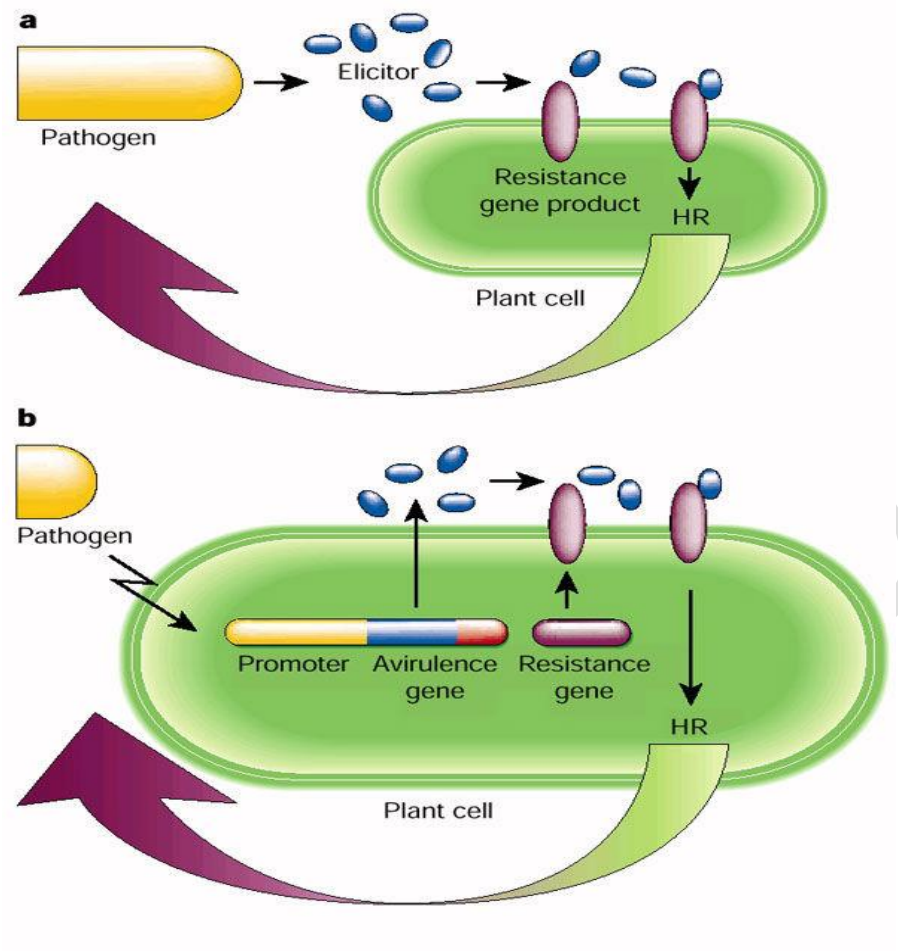
3. Expression of gene products e.g.peroxidase and lignin, that can potentially enhance structural defense in the plants.

4. Expression of genes, e.g. elicitor, hydrogen peroxidase (H2O2), salicylic acid (SA) and ethylene (C2H4), that regulate signals to control plant defenses.

5. Expression of the resistance gene (R) products involved in hypersensitive response (HR) for their interaction with avirulence (Avr) gene.

6. Binding or inactivation of fungal toxins thus stopping invasion of fungus by expression of Rgene.

7. Other strategies are production of RNAi, RNase and lysozyme . In such cases genesisolated from sources apart fromplantsareexploited.Availablereportsincludeintroduction of doublestranded RNA from viruses found in fungi genes of lysozymes cloned from human tissues and genes islated frm fungi and microbes.

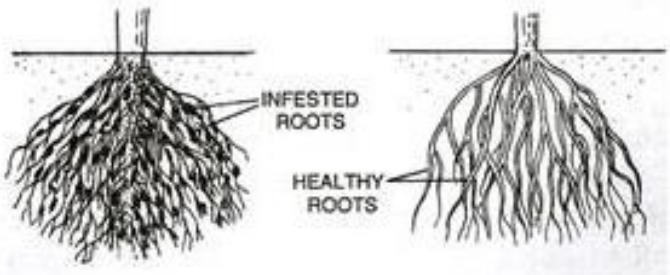


Resistance plant production

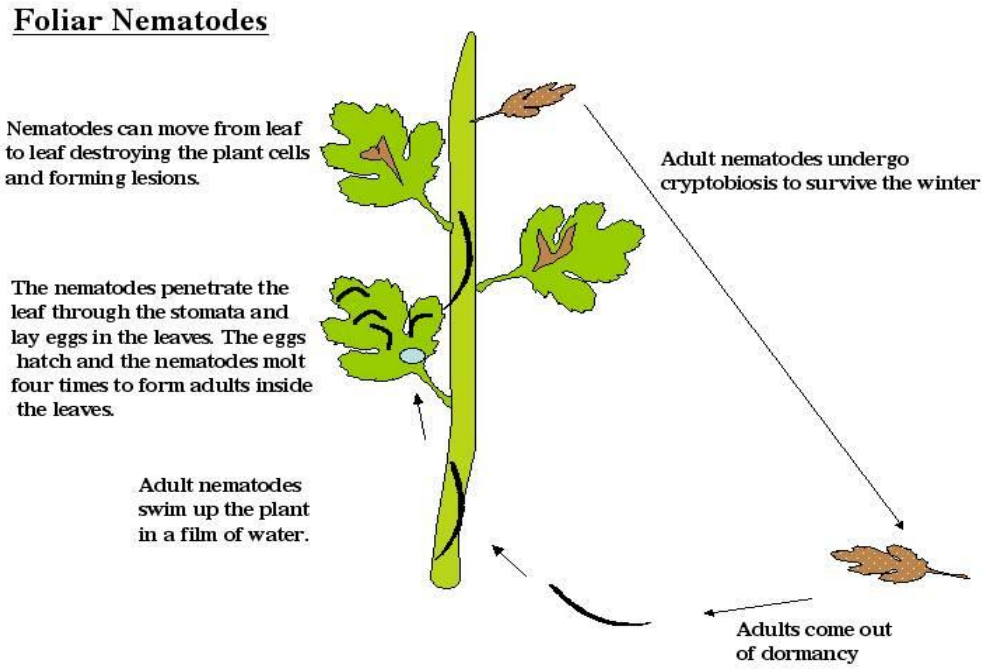
Nematode resistance

ii. Resistant cultivars can be very effective for nematode control but few have been developed so far

- Examples : Stem nematode in alfalfa
- Soyabean cyst nematode in soyabean
- Susceptible varieties of woody plants can often be grafted onto resistant rootstock



Development of nematode resistance through RNAi. A, Roots of infested plant. B, Roots of a transgenic plant even after deliberate infection of nematode.



Abiotic stress and cold stress

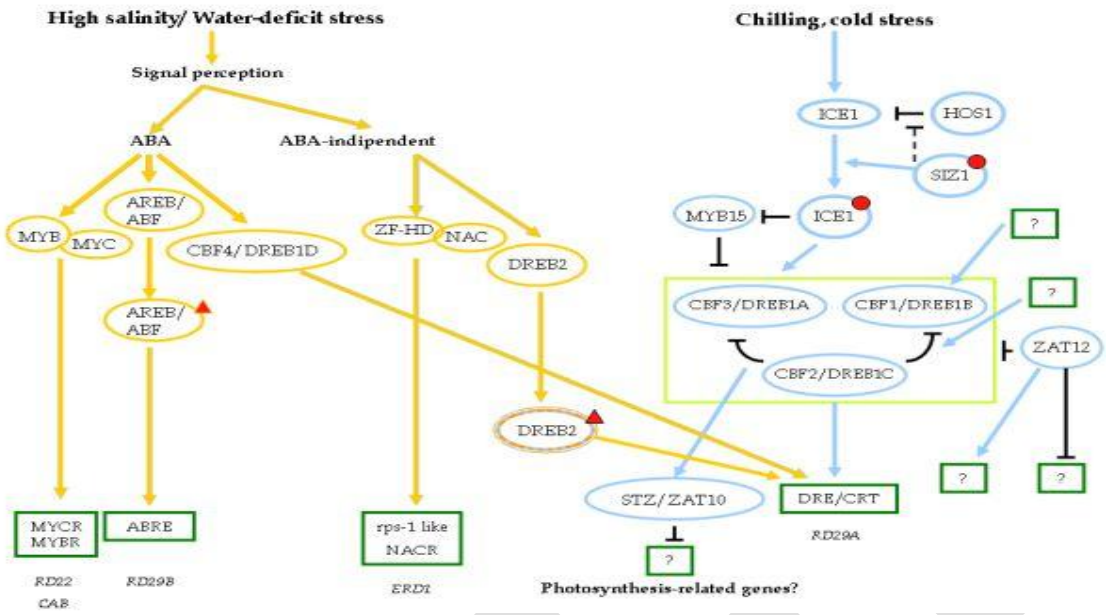
- Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50%.
- Plants as sessile organisms are constantly exposed to changes in environmental conditions. When these changes are rapid and extreme, plants generally perceive them as stresses.

- However stresses are not necessarily a problem for plants because they have evolved effective mechanisms to avoid or reduce the possible damages.
- A number of abiotic stresses such as extreme temperatures, high light intensity, osmotic stresses, heavy metals and a number of herbicides and toxins lead to over production of reactive oxygen species (ROS) including H_2O_2 causing extensive cellular damage and inhibition of photosynthesis

Stress	Consequences	Plant Responses
Heat stress	High temperature lead to high evaporation and water deficit. The consequent increased turnover of enzymes leads to plant death.	Efficient protein repair systems and general protein stability support survival, temperature can lead to acclimation.
Chilling and cold stress	Biochemical reactions proceed at slower rate, photosyntesis proceeds, carbon dioxide fixation lags, leading to oxigen radical damage. Indeed, freezing lead to ice crystal formation that can distrupt cells membranes.	Cessation of growth in adaptable species may be overcome by changes in metabolism. Ice crystal formation can be prevent by osmolyte accumulation and synthesis of hydrophilic proteins.
Drought	Inability to water transport to leaves leads to photosyntesis declines.	Leaf rolling and other morphological adaptations. Stoma closure reduces evaporative transpiration induced by ABA. Accumulation of metabolities, consequently lower internal water potential and water attracting.
Flooding and submergence	Generates anoxic or microaerobic conditions interfering with mitochondrial respiration.	Development of cavities mostly in the roots that facilitate the exchange of oxigen and ethylene between shoot and root (aerenchyma).

Heavy metal accumulation and metal stress	In excess, detoxification reactions may be insufficient or storage capacity may exceeded.	Excess of metal ions may be countered by export or vacuolar deposition but metal ions may also generate oxygen radicals.
High light stress	Excess light can lead to increased production of highly reactive intermediates and by-products that can potentially cause photo-oxidative damage and inhibit photosynthesis.	Exposure of a plant to light exceeding what is utilized in photochemistry leads to inactivation of photosynthetic functions and the production of reactive oxygen species (ROS). The effects of these ROS can be the oxidation of lipids, proteins, and enzymes necessary for the proper functioning of the chloroplast and the cell as a whole.

- Also, accumulation of abscisic acid (ABA) plays an important role in abiotic stress signalling and transduction pathways, mediating many responses
- It is well known that abiotic stresses in general, through regulation of both gene expression and protein turnover, alter the abundance of many transcripts and proteins indicating that transcriptional and post-transcriptional regulation play an essential role in the adaptation of cellular functions to the environmental changes.
- Environmental stress-inducible genes can be mainly divided into two groups in terms of their protein products:
- one type of genes, whose coding products directly confer to plant cells the resistence to environmental stress such as late embryogenesis abundant (LEA) protein, anti-freezing protein, osmotic regulatory protein, enzymes for synthesizing betaine, proline and other osmoregulators; the other groups of genes, whose coding products play an important role in regulating gene expression and signal transduction such as the transcriptional elements
- . At least four different regulons can be identified, two ABA independent (1 and 2) and two ABA dependent (3 and 4): (1) the CBF/DREB regulon; (2) the NAC (NAM, ATAF and CUC) and ZF-HD (zinc-finger homeodomain) regulon; (3) the AREB/ABF (ABA-responsive element-binding protein/ ABA-binding factor) regulon; and (4) the MYC (myelocytomatosis oncogene)/MYB (myeloblastosis oncogene) regulon.



Biopharmaceuticals

The genes for proteins to be used in human (and animal) medicine can be inserted into plants and expressed by them.

Advantages:

- **Glycoproteins can be made (bacteria like E. coli cannot do this).**
- **Virtually unlimited amounts can be grown in the field rather than in expensive fermentation tanks.**
- **It avoids the danger from using mammalian cells and tissue culture medium that might be contaminated with infectious agents.**
- **Purification is often easier.**

Corn is the most popular plant for these purposes, but tobacco, tomatoes, potatoes, rice and carrot cells grown in tissue culture are also being used.

Some of the proteins that have been produced by transgenic crop plants:

- **human growth hormone with the gene inserted into the chloroplast DNA of tobacco plants**
- **humanized antibodies against such infectious agents as**

- HIV
- respiratory syncytial virus (RSV)
- sperm (a possible contraceptive)
- herpes simplex virus, HSV, the cause of "cold sores"
- Ebola virus, the cause of the often-fatal Ebola hemorrhagic fever

- **protein antigens to be used in vaccines**
  - **An example: patient-specific antilymphoma (a cancer) vaccines. B-cell lymphomas are clones of malignant B cells expressing on their surface a unique antibody molecule. Making tobacco plants transgenic for the RNA of the variable (unique) regions of this antibody enables them to produce the corresponding protein. This can then be incorporated into a vaccine in the hopes (early trials look promising) of boosting the patient's immune system — especially the cell-mediated branch — to combat the cancer.**
- **other useful proteins like lysozyme and trypsin**
- **However, as of April 2012, the only protein to receive approval for human use is glucocerebrosidase, an enzyme lacking in Gaucher's disease. It is synthesized by transgenic carrot cells grown in tissue culture.**

Controversies

- **The introduction of transgenic plants into agriculture has been vigorously opposed by some.**
- **There are a number of issues that worry the opponents. One of them is the potential risk of transgenes in commercial crops endangering native or nontarget species.**

Examples:

- **A gene for herbicide resistance in, e.g. maize (corn), escaping into a weed species could make control of the weed far more difficult.**
- **The gene for Bt toxin expressed in pollen might endanger pollinators like honeybees.**
- **resistance to the herbicide glyphosate ("Roundup Ready®") thus making it practical to spray the crop with glyphosate to kill weeds without harming the crop;**
- **resistance to insect attack (by expressing the toxin of Bacillus thuringiensis).**

Biopolymers

- **Biopolymers are polymers produced by living organisms; in other words, they are polymeric biomolecules.**
- **Since they are polymers biopolymers contain monomeric units that are covalently bonded to form larger structures.**
- **There are three main classes of biopolymers, classified according to the monomeric units used and the structure of the biopolymer formed: polynucleotides (RNA and DNA), which are long polymers composed of 13 or more nucleotide monomers; polypeptides,which are short polymers of amino acids; and polysaccharides, which are often linear bonded polymeric carbohydrate structures.**
- **Other examples of biopolymers include rubber, suberin, melanin and lignin.**
- **Cellulose is the most common organic compound and biopolymer on Earth. About 33 percent of all plant matter is cellulose. The cellulose content of cotton is 90 percent, for wood it is 50 percent.**

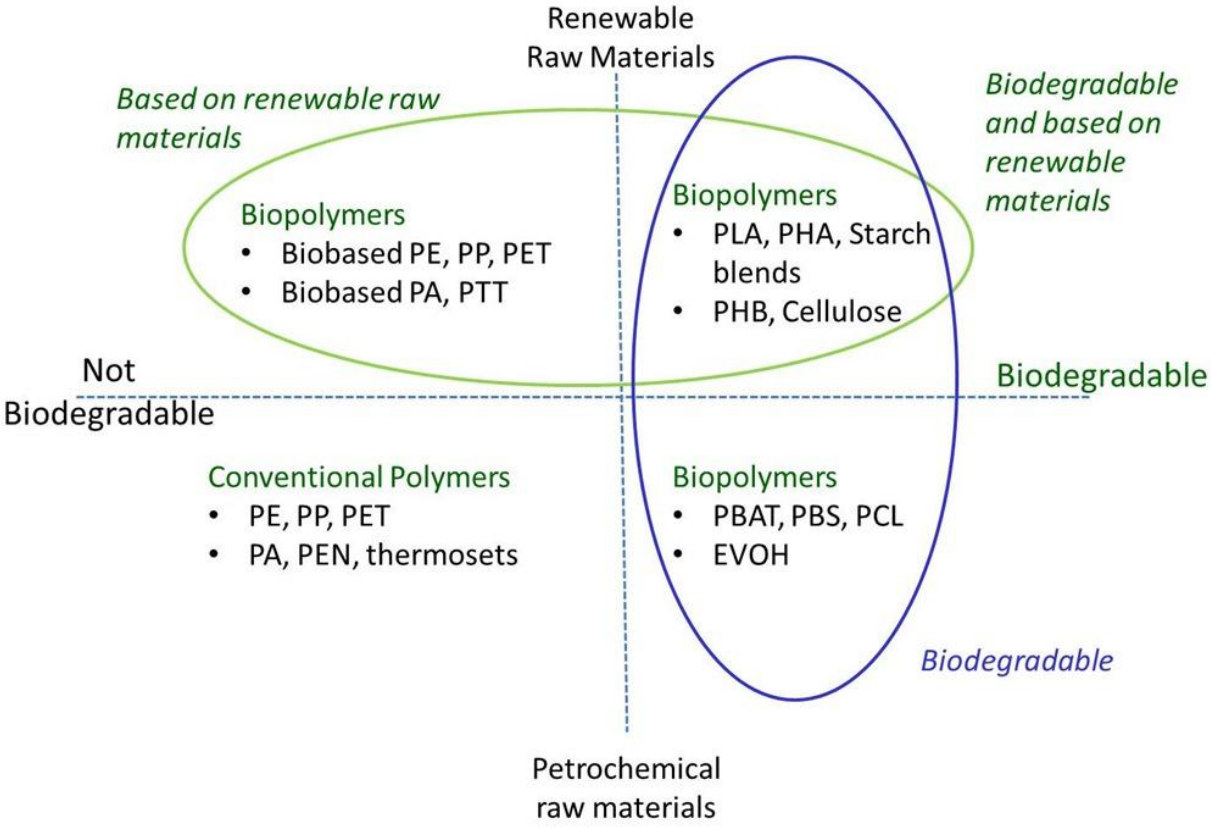
Biopolymers as materials

- **Some biopolymers- such as PLA, naturally occurring zein, and poly-3-hydroxybutyratecan be used as plastics, replacing the need for polystyrene or polyethylened based plastics.**
- **Some plastics are now referred to as being 'degradable', 'oxy-degradable' or 'UV-degradable'.**
- **This means that they break down when exposed to light or air, but these plastics are still primarily (as much as 98 per cent) oil-based and are not currently certified as 'biodegradable' under the European Union directive on Packaging and Packaging Waste**
- **Biopolymers will break down, and some are suitable for domestic composting.**
- **Biopolymers (also called renewable polymers) are produced from biomass for use in the packaging industry.**
- **Biomass comes from crops such as sugar beet, potatoes or wheat: when used to produce biopolymers, these are classified as non food crops.**
- **These can be converted in the following pathways:**

Sugar beet > Glyconic acid > Polyglyconic acid

Starch > (fermentation) > Lactic acid > Polylactic acid(PLA)

Biomass> (fermentation) > Bioethanol > Ethene > Polyethylene



plantibody

- **A plantibody is an antibody that is produced by plants that have been genetically engineered with animal DNA.**
- **An antibody (also known as an immunoglobulin) is a complex protein within the body that recognizes antigens on viruses and other dangerous compounds in order to alert the immune system that there are pathogens within the body.**

- **The transgenic plants become transformed with the DNA and produce antibodies that are similar to those inserted.**
- **The term plantibody and the concept are trademarked by the company Biolex.**
- **A plantibody is produced by insertion of antibodies into a transgenic plant**
- **The plantibodies are then modified by intrinsic plant mechanisms (N-glycosylation).[**
- **Plantibodies are purified through processes such as filtration, immunofluorescence chromatography, and diafiltration**
- **It is more cost effective to produce antibodies in transgenic plants than in transgenic animals.**

Advantages

- **Transgenic plants offer an attractive method for large-scale production of antibodies for immunotherapy.**
- **Antibodies produced in plants have many advantage that are beneficial to humans, plants, and the economy as well.**
- **They can be purified cheaply and in large numbers.**
- **The many seeds of plants allow for ample storage, and they have no risk of transmitting diseases to humans because the antibodies are produced without the need of the antigen or infectious microorganisms.**
- **Plants could be engineered to produce antibodies which fight off their own plant diseases and pests, for example, nematodes, and eliminate the need for toxic pesticides.**

Applications

- **Antibodies generated by plants are cheaper, easier to manage, and safer to use than those obtained from animals**
- **The applications are increasing because recombinant DNA is very useful in creating proteins that are identical when exposed into a plant's.**
- **A recombinant DNA is an artificial DNA that is created by combining two or more sequences that would not normally come together.**

- **In this way, DNA injected into a plant is turned into recombinant DNA and manipulated. The favorable properties of plants are likely to make the plant systems a useful alternative for small, medium and large scale production throughout the development of new antibody-based pharmaceuticals**

Possible questions

Short questions

1. What is plant bodies?
2. What is meant by biopolymer ?
3. Write short note on Cry proteins.
4. Give short note on molecular pharming.
5. Write short note on Oliesin partition technology.
6. What is edible vaccines?
7. List out any two edible vaccines.
8. List any two genetically modified crop.
9. Short note on Bt cotton.
10. Write short note on satellite RNAs.

Essay type questions

1. Discuss in detail about Oleosin partitioning technology.
2. Discuss in detail about virus resistance engineered in plants.
3. Explain in detail about production of edible vaccines.
4. Describe the metabolic engineering for production of fatty acids.
5. Discuss: oleosin partition technology
6. Write an account on pest resistant plants.
7. Explain in detail about Bt crops.
8. Explain in detail about golden rice.

Reg. No……

[17BTU501A]

Karpagam Academy of Higher Education, Coimbatore
First Internal Examinations, July, 2019
(For Candidates admitted from 2017 and onwards)
Fifth Semester
Department of Biotechnology

PLANT BIOTECHNOLOGY

Date : **Class : III B.Sc., BT**
Time : 2 hours **Maximum : 50 marks**

Part –A
Answer All Questions **20 X 1 = 20 marks**

1. The most common solidifying agent used in micropropagation is----
   a) Agar b) dextran c) Mannose d) carbohydrate
2. Root formation in
   a) Intermediate raio of auxin to cytokinin b) Low auxin to cytokinin ratio
   c) High auxin to cytokinin ratio d) Low auxin to gibberellin ratio
3. Alteration of chemical atmosphere for plant regeneration under *in vitro* condition are called
   a)Totipotency b) Plasticity c) Regeneration d) cybrids
4. ……male sex organ of the plants
   a) egg b) ovules c) ovary d) Stamen
5. Artificial seeds are
   a) seeds produced in laboratory condition b) seeds encapsulated in a gel
   c) somatic embryos encapsulated in a gel d) zygotic embryos encapsulated
6. Shoot formation occurs when
   a) Intermediate ratio of auxin to cytokinin b)Low auxin to cytokinin ratio
   c) High auxin to cytokinin ratio d) Low auxin to gibberellins ratio
7. …… are used as encapsulating agent for synthetic seed preparation.
   a) sodium chloride b) sodium alginate
   c) sodium hydroxide d) sodium hypo chloride
8. Explants are ……
   a) tissue taken for *In vitro* culture b) Dried plant tissue for culture
   c) synthetic seed d) callus
9. Which nutrient media is widely used in tissue culture techniques?
   a) MS media b) Whites media c) B5 media d) Salt
10. Mass of undifferentiated cell is……..
    a) shoot b) root c) callus d) plant
11. Microsporogenesis are also know as
    a) adrogenesis b) gynogenesis c) somatic embryogenesis d) callus
12. Somatic embryogenesis are
    a) embryos from egg cell b) embryo from pollen cell
    c) embryo from body cell d) embro from both egg and pollen cells
13. Induction of callus ….. hormone will be used
    a) BAP b) BA c) 2,4-D d) Gibberellins
14. A cell containing two complete sets of chromosomes one from each parent are
    a) Diploid b) haploid c) polyploidy d) triploid
15. Who discovered plant tissue culture?
    a) Gottlieb Haberlandt b) Murashige and Skoog c) Gamborg d) White's
16. Encapsulated somatic embryos are known as
    a) natural seed b) synthetic seed c)callus d)organogenesis
17. Microspores in plants are
    a) male sex cell b) Female sex cell c) somatic cells d) egg cells
18. Induction of multiple shoot …. hormone will be used
    a) IBA b)NAA c)BAP d)2,4-D
19. What is the role of agar in media?
    a) chelating agent b) solidifying agent c) nutrient d) mineral salts
20. What is the role of calcium elements in media for plant regeneration?
    a) cell wall synthesis b) enzyme co-factor
    c) protein synthesis d) carbohydrate synthesis

Part –B **3 X 2 = 6 marks**
Answer All the Questions

21. What is totipotency?
22. Write short note on Organogenic differentiation.
23. Define haploid.

Part – C **3 X 8 = 24 marks**
Answer all questions choosing either a or b. All questions carry equal marks

24. a. Explain in detail about the micropropagation.
    (OR)
    b. Write in detail about the advantage and disadvantage of micropropagation.

25. a. Write short notes on i. embryogenesis, ii. Embryo culture iii. Callus culture.
    (OR)
    b. Illustrate the haploid production – Androgenic method.

26. a. Give in detail on MS media composition for plant regeneration.
    (OR)
    b. Discuss the different types of plant hormones and its role in organogenesis.

Reg. No……

[17BTU501A]

Karpagam Academy of Higher Education, Coimbatore
Second Internal Examinations, August, 2019
(For Candidates admitted from 2017 and onwards)
Fifth Semester
Department of Biotechnology

PLANT BIOTECHNOLOGY

Date : **Class : III B.Sc., BT**
Time : 2 hours **Maximum : 50 marks**

Part –A
Answer All Questions **20 X 1 = 20 marks**

1. Transplastomics
   a) Targets genes in the chloroplast b) Provides low protein yield
   c)Produces genes that are released in pollen d) Provide high protein yield
2. Protoplast are
   a) Cell without cell wall b) callus
   c) Cell with cell wall d) dead cell
3. Agent for using protoplast fusion are
   a)chelating b) fusogen c) fission d) cybrids
4. ……female sex cells of the plants
   a) anther b) ovules c) pollen d) Stamen
5. …….. will be used for synthetic seed
   a) calcium chloride b) sodium chloride c) sodium alginate d) sodium hydroxide
6. Friable callus are
   a) dead cells b)young cells c) mature cell d) leaves
7. Abbreviated for of PEG……
   a) Poly ethelene glycol b) Polyethanoid
   c) poly amine d) polyetheleamine
8. $HgCl_2$ will be used for
   a) surface sterilization b) fumigation
   c) callus induction d) shoot induction
9. Somatic hybridization are….
   a) hybrid from somatic cell b) hybrid from sex cell
   c) hybrid from dead cell d) hybrid from pollen cell
10. In N_2 fixation ….
    a) N_2 converted to NaCl b) N_2 converted to Na OH
    c) N_2 converted to ammonia d) N_2 converted to CaCl
11. Tetraploidy means
    a) organism having one set of chromosome
    b) organism having two set of chromosome
    c) organism having three set of chromosome
    d) organism having four set of chromosome
12. Somatic embryogenesis are
    a) embryos from egg cell b) embryo from pollen cell
    c) embryo from body cell d) embro from both egg and pollen cells
13. The protoplast hybrids are
    a) cybrid b)hybrid c) synthetic seed d) diploids
14. A cell containing multiple sets of chromosomes one from each parent are
    a) Diploid b) haploid c) polyploidy d) triploid
15. Who discovered the PEG protoplast fusion
    a) Kao and Michayluk b) Murashige and Skoog c) Gamborg d) White's
16. Hyrodolase are
    a) alkilation b) reversible oxidation c)amination d)deamination
17. Nitrite to nitrate are
    a) nitrification b) denitrification c) nodulation d) deamination
18. Azotobacter are help to
    a) fix H_2 b)fix O_2 c)Fix N_2 d)fix CO
19. Nitrogenase are …..
    a) chelating agent b) fixing nitrogen c) to help fission d) mineral salts
20. What is the role of nitrogen elements in media for plant regeneration
    a) cell wall synthesis b) enzyme co-factor
    c) protein synthesis d) carbohydrate synthesis

Part –B **3 X 2 = 6 marks**
Answer All the Questions

21. What is somatic embryogenesis?
22. Write short note on gynogenesis.
23. Define nodulation.

Part – C **3 X 8 = 24 marks**
Answer all questions choosing either a or b. All questions carry equal marks

24. a. Explain in detail about the somatic hybridization.
    (OR)
    b. Write in detail about the production of gynogenic haploids.

25. a. Write short notes on i. Somaclonal variation, ii. Plant growth promoting bacteria
    (OR)
    b. Illustrate the protoplast isolation and fusion.

26. a. Give in detail on nitrogen fixation in plants.
    (OR)
    b. Give notes on i) Cybrids ii) Advantage and disadvantage of somatic hybridization.

Reg. No……

[17BTU503A]

Karpagam Academy of Higher Education, Coimbatore
Third Internal Examinations, October, 2019
(For Candidates admitted from 2017 and onwards)
Fifth Semester
Department of Biotechnology
PLANT BIOTECHNOLOGY

Date : **Class : III B.Sc., BT**
Time : 2 hours **Maximum : 50 marks**

Part –A
Answer All Questions **20 X 1 = 20 marks**

1. ……have single-stranded RNA genome.
a) TMV b) CaMV c) TMV and CaMV d) None
2. *Agrobacterium tumefaciens* is a
a) gram (+) bacteria b) gram (-) bacteria c) a fungi d) a yeast
3. In CaMV ….. promoter commonly used for GM crop
a)16S b) 35S c) 18S d) 19S
4. Function of Vir A protein in *Agrobacterium*
a) Phenolic sensor b) T-DNA processing
) nick the T-DNA d) Transcriptional factor
5. In Ti plasmid the operon required for virulence is
a) Vir C b) Vir H c) Vir B d) Vir F
6. _____ is a major group of oncogenic Ti plasmid
a) Histipine b) Octopine c) Octopinic acid d) Lysopine
7. A metal used in particle bombardment DNA transfer is……
a) tungsten b) thorium c) silver d) iron
8. ___ is the monopartite RNA virus
a) Tobacco mosaic virus b) Tobacco ringspot virus
c) Tomato black ring virus satellite d) shoot induction
9. Agropines are….
a) Sugar derivatives b) amino acid derivatives
c) fatty acid derivatives d) purine derivatives
10. Curcuma longa used for the production of _____
a) withaferin b) tropane
c) curcumin d) whithanolides
11. The secondary metabolite piperidine alkaloids extracted from_____
a) *Withania somnifera* b) *Curcuma longa* c) *Solanum* d) *Piper nigrum*
12. The DNA of gemini virus is.....
a) double stranded b) coiled c) single stranded d) both single and double
13. The red and blue color for the flowers are due
a) anthocyanins b) carotenoids c) lignins d) terpenes
14. Which of the following gene is responsible for resistance against chilling?
a) Polygalactouranase b) ACC deaminase
c) Glycerol 1 phosphate acyl transferase d) Sucrose phosphate synthase gene
15. Nitrilase is encoded by
a) gene bxn in *Klebsiella pneumoniae* b) bar gene in Streptomyces spp
c) vir gene d) TATA box
16. Which of the following not dies from Ti plasmid infection?
a) rice b) tomato c)corn d)sorghum
17. Antisense technology
a.selectively blocks expression of a gene
b.combines genetic material from different species
c.combines organelles and cells
d.alters or transfers cells
18. Ethylene formation triggers
a) red coloration b) fruit softening c) ripening d) sweetness
19.Palmitic acid has _____number of carbon atoms
a)18 b) 16 c) 20 d) 24
20. Insect resistance in the transgenic plant has been achieved by..
a.transferring genes for other non- insecticidal secondary metabolities
b.transferring genes for non- protease inhibitors
c) transferring genes for Bt toxins
d) transferring genes for non-Bt toxins

Part –B **3 X 2 = 6 marks**
Answer All the Questions

21. What is electroporation?
22. Write short note on Transcriptional regulation.
23. List out genetically modified crop.

Part – C **3 X 8 = 24 marks**
Answer all questions choosing either a or b. All questions carry equal marks

24. a. Explain in detail about the *Agrobacterium tumefaciens* mediated gene transformation.
(OR)
b. Write in detail about the regulation of gene expression.

25. a. Write short notes on i. Gene gun, ii. PEG mediated gene transfer
(OR)
b. Illustrate the T-DNA construct and its functions
26. a. Give in detail on pest resistant crop.
(OR)
b. Give notes on i). Ri plasmid ii) Oleosin partition technology

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Plant Biotechnology - 17BTU503A
mutiple choice Questions

Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
_____ allows plants to alter their metabolism, growth and development to best suit their environment.	Totipotency	Plasticity	Regeneration	Somatic embryogenesis	Plasticity
Regeneration of whole organisms depends upon the concept that all plant cells can, given the correct stimuli, express the total genetic potential of the parent plant. This maintenance of genetic potential is called _____.	Totipotency	Plasticity	Regeneration	Somatic embryogenesis	Totipotency
Many plant cell cultures, as they are not photosynthetic, require the addition of a _____.	fixed nitrogen	fixed hydrogen	fixed carbon	fixed oxygen	fixed carbon
A(n) _____ is an excised piece of leaf or stem tissue used in micropropagation.	microshoot	medium	explant	scion	explant
Protoplasts can be produced from suspension cultures, callus tissues or intact tissues by enzymatic treatment with	cellulotyic enzymes	pectolytic enzymes	both cellulotyic and pectolytic enzymes	proteolytic enzymes	both cellulotyic and pectolytic enzymes
Which of the following is considered as the disadvantage of conventional plant tissue culture for clonal propagation?	Multiplication of sexually derived sterile hybrids	Less multiplication of disease free plants	Storage and transportation of propogates	multiplication of haploids	Storage and transportation of propogates
What is meant by 'Organ culture' ?	Maintenance alive of a whole organ, after removal from the organism by partial immersion in a nutrient fluid	Introduction of a new organ in an animal body with a view to create genetic mutation in the progenies of that animal	Cultivation of organs in a laboratory through the synthesis of tissues	The aspects of culture in community which are mainly dedicated by the need of a specified organ of the human body	Maintenance alive of a whole organ, after removal from the organism by partial immersion in a nutrient fluid
Which method of plant propagation involves the use of girdling?	Grafting	Cuttings	Layering	Micropropagation	Layering

Organogenesis is	formation of callus tissue	formation of root and shoots on callus tissue	formation of mass of cells	apical meristem	formation of root and shoots on callus tissue
Which of the following is used in the culture of regenerating protoplasts, single cells or very dilute cell suspensions?	Nurse medium	salts	acids	elements alone	Nurse medium
In a callus culture	increasing level of cytokinin to a callus induces shoot formation and increasing level of auxin promote root formation	increasing level of auxin to a callus induces shoot formation and increasing level of cytokinin promote root formation	auxins and cytokinins are not required	only auxin is required for root and shoot formation	increasing level of cytokinin to a callus induces shoot formation and increasing level of auxin promote root formation
The phenomenon of the reversion of mature cells to the meristematic state leading to the formation of callus is known as	redifferentiation	dedifferentiation	callus	shoot multiplication	dedifferentiation
Cell fusion method includes the preparation of large number of	plant cells stripped of their cell wall	single plant cell stripped of their cell wall	plant cells with cell wall	cells from different species	single plant cell stripped of their cell wall
Subculturing is similar to propagation by cuttings because	it separates multiple microshoots and places them in a medium	it uses scions to produce new microshoots	they both use in vitro growing conditions	all of the above	it separates multiple microshoots and places them in a medium
The ability of the component cells of callus to form a whole plant is known as	redifferentiation	dedifferentiation	callus	explants	redifferentiation
What is/are the benefit(s) of micropropagation or clonal propagation?	Rapid multiplication of superior clones	Multiplication of diseased plants	Multiplication of sexually derived non sterile hybrids	production of contaminated plants	Rapid multiplication of superior clones
Cellular totipotency is the property of	plants	animals	bacteria	virus	plants

Agrobacterium based gene transfer is efficient	only with dicots	only with monocots	with both monocots and dicots	with majority monocots and few dicots	only with dicots
The proteins that forms the walls of the microtubules are	actin	tubulin	pectin	hydroxyproline	tubulin
The colour of flower is due to the presence of	chlorophyll	xanthophylls	florigen	chromoplast or anthocyanin	chromoplast or anthocyanin
Which is the most common carbon source used in the plant cell culture media?	Sucrose	Glucose	Fructose	Maltose	Sucrose
Which of the following is an ethylene biosynthesis inhibitor?	Citric acid	Succinic acid	Activated charcoal	Silver thiosulphate	Silver thiosulphate
Nitrogen in the plant cell culture media is provided by either ammonia or nitrate salt. In the media	utilization of ammonium cause culture pH to drop while utilization of nitrate cause culture pH to rise	utilization of nitrate cause culture pH to drop while utilization of ammonium cause culture pH to rise	utilization of both ammonium and nitrate result in rise in pH	utilization of both ammonium and nitrate result in drop in pH	utilization of ammonium cause culture pH to drop while utilization of nitrate cause culture pH to rise
Which of the following growth regulator is added for short initiation during plant regeneration from callus?	Auxins	Cytokinins	Gibberellins	Brassinosteroids	Cytokinins
Which of the following growth regulator promote cell division?	Auxins	Cytokinins	Gibberellins	Brassinosteroids	Cytokinins
Which of the following growth regulator is used to stimulate embryo or shoot development?	Auxins	Cytokinins	Gibberellins	Brassinosteroids	Gibberellins
Which of the following growth regulator cause plant cells to grow?	Auxins	Cytokinins	Gibberellins	Brassinosteroids	Auxins
Silver thiosulphate is added to culture medium as it helps to	maintain the pH	remove toxic phenolics from plant cells	prevent the gaseous plant hormone, ethylene dioxide from accumulating to detrimental condition.	all of the above	prevent the gaseous plant hormone, ethylene dioxide from accumulating to detrimental condition.

In plant cell culture media, auxins and cytokinins are used in the range of	l-50µM	50-100µM	100-125µM	more than 125µM	l-50µM
Concentration of sucrose generally used in plant cell culture media is	10-15 g/l	20-30 g/l	40-50 g/l	60-70 g/l	20-30 g/l
Which is/are the naturally occurring plant auxins?	Indole acetic acid (IAA)	Naphthalenacetic acid (NAA)	2,4-dichlorophenoxy acetic acid	gebberellin	Naphthalenacetic acid (NAA)
Which is/are the disadvantage/(s) of using IAA in plant cell culture media?	It is stable in solution	Gets easily oxidized	non-Conjugated to inactive form by plant cells	non-oxidised	Gets easily oxidized
To maintain the pH of the culture	agar will be used	synthetic buffers will be used	synthetic any chemical	ammonium salts are used	synthetic buffers will be used
Which of the following is not a cytokinin?	2,4-dichlorophenoxyacetic acid	6 benzylaminopurine	Zeatin	Kinetin	2,4-dichlorophenoxyacetic acid
Which of the following is not an auxin?	Indole acetic acid (IAA)	Naphthalenacetic acid (NAA)	zeatin	Indole butyric acid	zeatin
Very high sugar concentration (40-100 g/l) have been used	not-adjust the osmotic potential	to adjust the osmotic potential of the media in short term treatment for regeneration	for high organ growth	for root formation	to adjust the osmotic potential of the media in short term treatment for regeneration
Common source of fixed carbon in PTC media is	sucrose	fructose	cellulose	glucose	sucrose
_____ is required in greatest amount in PTC media.	potassium	calcium	magnesium	nitrogen	nitrogen
Neutralized activated charcoal is occasionally added to young regenerating cultures to	remove toxic phenolics produced by the stressed plant cell	solidifying agent	Basal media	maintain the pH of the medium	remove toxic phenolics produced by the stressed plant cell
_____ regulates osmotic potential in PTC media.	potassium	calcium	magnesium	nitrogen	potassium
In the PTC media _____is important for cell wall synthesis, membrane function and cell signaling.	potassium	calcium	magnesium	nitrogen	calcium
_____ is a component of chlorophyll.	potassium	calcium	magnesium	nitrogen	magnesium

_____ is a component of intermediates in respiration and photosynthesis.	potassium	calcium	magnesium	phosphorus	phosphorus
Virulence trait of Agrobacterium tumefaciens is borne on	chromosomal DNA	tumour inducing plasmid DNA	both chromosomal and plasmid DNA	cryptic plasmid DNA	tumour inducing plasmid DNA
The size of the virulent plasmid of Agrobacterium tumefaciens is	40-80 kb	80-120 kb	140-235 kb	>235 kb	140-235 kb
Which of the following is not true about the helper plasmids?	These can replicate in Agrobacterium	These help in the mediating conjugation of intermediate vectors	These can't replicate in Agrobacterium	no further process	These can replicate in Agrobacterium
Direct DNA uptake by protoplasts can be stimulated by	polyethylene glycol (PEG)	decanal	luciferin	NaOH	polyethylene glycol (PEG)
The enzyme beta-glucuronidase is produced in	maize	rice	wheat	oats	maize
The enzyme, produced in plants, used for brewing is	cellulase	avidin	(1-3) (1-4) beta Glucanase	phytase	(1-3) (1-4) beta Glucanase
In the initial stage of somatic embryogenesis, _____ is used.	a high concentration of 2, 4-D	no or very low levels of 2, 4-D	low auxin to cytokinin ratio	high auxin to cytokinin ratio	a high concentration of 2, 4-D
In the second stage of somatic embryogenesis, _____ is used	a high concentration of 2, 4-D	no or very low levels of 2, 4-D	low auxin to cytokinin ratio	high auxin to cytokinin ratio	no or very low levels of 2, 4-D
Somatic embryogenesis is improved by supplying a source of ____	fixed carbon	reduced nitrogen	vitamins	potassium	reduced nitrogen
The enzymes in calvin cycle are in excess to	sustain electron transfer	sustain carbondioxide fixation	activate Rubisco	activate dark reactions	sustain carbondioxide fixation
The first commited step in the fatty acid biosynthesis is the	formation of malonyl coenzyme A	formation of acetoacetate ACP	liberation of carbondioxide	none of the above	formation of malonyl coenzyme A
What is IPR?	integrated patents regulation	intellectual property rights	intellectual property regulation	none of the above	intellectual property rights
_____ is most commonly associated with fruit ripening in climacteric fruits.	abscisic acid	ethylene	gibberellic acid	cytokinin	ethylene

Callus formation	Intermediate raio of auxin to cytokinin	Low auxin to cytokinin ratio	High auxin to cytokinin ratio	Low auxin to gibberellin ratio	Intermediate raio of auxin to cytokinin
Shoot formation	Intermediate raio of auxin to cytokinin	Low auxin to cytokinin ratio	High auxin to cytokinin ratio	Low auxin to gibberellin ratio	Low auxin to cytokinin ratio
Root formation	Intermediate raio of auxin to cytokinin	Low auxin to cytokinin ratio	High auxin to cytokinin ratio	Low auxin to gibberellin ratio	High auxin to cytokinin ratio
During callus formation, there is some degree of	differentiation	dedifferentiatio n	redifferentiation	morphogenesis	dedifferentiation
The process -culture loses the requirement for auxin and/or cytokinins during long-term culture	habituation	Totipotency	plasticity	vitrification	habituation
_____ is commonly used to initiate cell suspension cultures.	root cultues	meristem cultures	embryo culture	friable callus	friable callus
Friability of the callus can be improved by	manipulation of media components	non subculturing	culturing on solid medium	addition of BAP	manipulation of media components
Protoplasts are most commonly isolated from	leaf mesophyll cells	mature callus	friable callus	root	leaf mesophyll cells
Co-integrating transformation vectors must include a region of homology in	the vector plasmid	the Ti-plasmid	between vector plasmid and Ti-plasmid	none of these	between vector plasmid and Ti-plasmid
Unit II					
The deals with improvement and culture of agricultural crops are known as _____	Plant genetics	Plant breeding	Plant pathology	None of the above	Plant breeding
What is the name of the male sex organ of the plant?	Stigma	stamen	ovary	ovules	stamen
What is the name of the female sex organ of the plant?	Stigma	stamen	Pollen	microspore	Stigma
How many number of the nucleates are there in embryo sac?	2 cell nucleate	4 cell nucleate	8 cell nucleate	10 cell nucleate	4 cell nucleate
Plants that grow under average temperature and moisture are called	halophytes	hydrophytes	mesophytes	xerophytes	mesophytes
The most common solidifying agent used in micropropagation is	agar	dextran	Mannan	agarose	agar
The culturing of cells in liquid agitated medium is called	liquid culture	micropropagatio n	Agar culture	suspension culture	suspension culture

Batch cultures are type of suspension culture where	medium is continuously replaced	medium is loaded only at the beginning	no depletion of medium occurs	cellular wastes are continuously removed and replaced	medium is loaded only at the beginning
Immobilized cell bioreactors are based on	cells cultures in solid medium	cells cultured in liquid medium	cells entrapped in gels	non-entroped in gel	cells entrapped in gels
All are plant derived alkaloids except	menthol	nicotine	quinine	codeine	menthol
Elicitors are molecules that	induce cell divison	stimulate production secondary metabolites	stimulate hairy root formation that accumulate secondary metabolites	non-stimulate production of secondary metabolites	stimulate production secondary metabolites
All are plant derived elicitors except	chitin	pectin	cellulose	pectic acid	chitin
The modification of exogenous compounds by plant cells is called	Biotransformation	gene modification	gene trasnformation	biophytomodification	Biotransformation
Artificial seeds are	seeds produced in laboratory condition	seeds encapsulated in a a gel	somatic embryos encapsulated in a gel	zygotic embryos encapsulated in a gel	somatic embryos encapsulated in a gel
Hairy root cultures for secondary metabolite production are induced by transforming plant cells withs	*E.coli*	*Agrobacterium tumefaciens*	*Bacillus thuringiensis*	*Agrobacterium rhizogenes*	*Agrobacterium rhizogenes*
The variation in invitro culture is called as	invitro variation	mutation	somaclonal variation	cybrids	somaclonal variation
Haploid plants are produced in large numbers by	anther culture	sepal	petal	calyx	anther culture
Cybrids are	nuclear hybrids	hybrid plants derived from cross pollination	cytoplasmic hybrids	cytological hybrids	cytoplasmic hybrids
The controversy regarding the use of Bt corn is that it	is potentially harmful to monarch butterflies	is a potential non-allergen to humans	it is non toxic butterflies	can contaminate groundwater	is potentially harmful to monarch butterflies

Organism containing more than two paired (homologous) sets of chromosomes are called	homologous chromosome	ploidy	polyploidy	monoploidy	polyploidy
Polyploids with multiples of the complete set of chromosomes specific to a species known as ____	Euploidy	*Autopolyploidy*	anuploidy	diploidy	Euploidy
Polyploids contains multiple copies of the basic set (x) of chromosomes of the same genome are called _____	*Autopolyploidy*	autoploids	Auto and Alloploidy	diploidy	Auto and Alloploidy
Polyploids contains multiple copies of the basic set (x) of chromosomes of the different genome are called _____	*Autopolyploidy*	Allopolyploids	anuploidy	diploidy	Allopolyploids
Polyploids that contain either an addition or subtraction of one or more specific chromosome(s) to the total number of chromosomes are called _____	*Autopolyploidy*	Aneuploidy	Euploidy	Allopolyploids	Aneuploidy
Flower that contains sepals, petals, stamens and carpel are called ____	*complete flower*	incomplete flower	moderate flower	anther	*complete flower*
Flower that not contains any one or more of there sepals, petals, stamens and carpel are called ____	*complete flower*	incomplete flower	moderate flower	anther	incomplete flower
The female gametophyte are also called ____	8-nucleate embryo sac	4-nucleate embryo sac	2-nucleate embryo sac	3-nucleate embryo sac	8-nucleate embryo sac
In micropyler end of embryosac contains one egg cell ans two _____ cells	*synergids*	endosperm	antipodal	egg cells	*synergids*
The nuiclei present in the chalazal end are called ___	*antipodals cells*	endosperm	synergids	eggs cells	*antipodals cells*
The process of transfering pollen grains from the anther to the stigma of female reproductive organ are called ____	*Pollination*	embryosac	endosperm	synergids	*Pollination*
The pollen grains may fall on the stigma of the same flower or another flower born on the same plant are called ___	*allogamy*	autogamy	syngamy	auto and Allogamy	autogamy
The pollen grains may fall on the stigma of the same flower or another flower born on the different plant are called ___	*allogamy*	autogamy	syngamy	auto and Allogamy	*allogamy*
Pollination takes place through the wind are known as	*entomophilly*	anemophylus	zoophily	entomo and zoophily	anemophylus
Pollination takes place through the insects are known as	*entomophilly*	anemophylus	zoophily	entomo and zoophily	*entomophilly*
Pollination takes place through the animals are known as	*entomophilly*	anemophylus	zoophily	entomo and zoophily	zoophily
The transfer of pollen from the male reproductive organ of one plant to the female reproductive organ of another plant such polination process are called _____.	*self pollination*	cross pollination	labeling	non-cros pollination	cross pollination

The transfer of pollen from the male reproductive organ of one plant to the female reproductive organ of same plant such polination process are called _____.	*self pollination*	cross pollination	labeling	non-self pollination	*self pollination*
The male gametophyte are also called as	microsporocyte	megasporocyte	oospore	egg cells	microsporocyte
The female gametophyte are also called as	microsporocyte	megasporocyte	pollen	anther	megasporocyte
Production of plants through vegetative parts are known as ___	*Sexual reproduction*	a sexual reproduction	vegetative reproduction	non-vegetative reproduction	vegetative reproduction
Production of plants through pollination are known as ___	*Sexual reproduction*	a sexual reproduction	vegetative reproduction	non-sexual reproduction	*Sexual reproduction*
What is meant by complete flower?	*only sepal*	*Contains sepals, petals, stamens and carpel*	contains sepal and petal	contains only stamen	*Contains sepals, petals, stamens and carpel*
What is meant by incomplete flower?	*only sepal*	*Contains sepals, petals, stamens and carpel*	contains sepal and petal	only egg cells	a, c and d
The failure of plants to produce functional anther, pollen are called ____	*Male sterility*	female sterility	Sterility of male and female	non male sterility	male sterility
The failure of plants to produce functional stigma, ovary and egg are called ____	*Male sterility*	female sterility	male and female sterility	non-female sterility	female sterility
Male sterility cuased by extranuclear genome such as mitochondrial or plastid genomes such male sterility known as ___	*cytoplasmic male strility*	cytoplasmic female sterility	sterility	non-sterility	*cytoplasmic male strility*
Extra nuclear genome cuases _____	*genetc sterility*	cytoplasmic sterility female sterility	sterility	non-sterility	cytoplasmic sterility female sterility
Male sterility classified by ___	2	3	4	1	3
When pollen sterility is controlled by both cytoplasmic and nuclear genes is known as _____	*Male sterility*	cytoplasmic genetic male sterility	cytoplasmic sterility	genetic male sterility	cytoplasmic genetic male sterility

The pollen sterility which is controlled by cytoplasmic genes is known as _____	cytoplasmic male sterility	Male sterility	female sterility	cytoplasmic sterility	cytoplasmic male sterility
The pollen sterility, which is caused by nuclear genes are known as ___	Genetic Male Sterility	cytoplasmic male sterility	Male sterility	genetic and cyto sterility	Genetic Male Sterility
Mitochondrial DNA are also called	mDNA	mtDNA	mDNA and mtDNA	tRNA	mDNA and mtDNA
mDNA located in	chloroplast	nucleus	mitochondria	cytoplasm	mitochondria
Protoplasts are the cells devoid of	cell membrane	cell wall	cell wall and cell membrane	vacuole	cell wall
Which breeding method uses a chemical to strip the cell wall of plant cells of two sexually incompatible species?	Mass selection	Protoplast fusion	Transformation	Transpiration	Protoplast fusion
stamen is the _____	male sex organ	female sex organ	not sex organ	vegetative body	male sex organ
Stigma is the _____	male sex organ	female sex organ	not sex organ	vegetative body	female sex organ
Gynogenic haploids	haloids from megaspore	haploid from microspore	haploid from pollen	haploid from anther	haloids from megaspore
Androgenic haploids are	haloids from megaspore	haploid from microspore	haploid from egg	haploid from stigma	haploid from microspore
Unit III					
The transfer of intermediate vectors into Agrobacterium are brought about by	transformation	biparental mating	triparental mating	transduction	triparental mating
In response to the activating signal molecule, an endonuclease is produced that causes nicks in the T-DNA. It is encoded by	vir A	vir B	vir C	vir D	vir D
Microinjection involves	injection of large amount of DNA	injection of DNA into bigger cells	injection with needle having diameter greater than cell diameter	all of the above	injection with needle having diameter greater than cell diameter
Which of the following are used as selection marker for the cells transformed with Agrobacterium?	Neomycin phosphotransferase	non-Streptomycin phosphotransferase	non-Hygromycin phosphotransferase	non-Neomycin phosphotransferase	Neomycin phosphotransferase
Vir genes required for the T-DNA transfer and processing are located	on the T-DNA	outside the T-DNA region	on the plant genome	on the viral genome	outside the T-DNA region
Plant transformation vectors based on Agrobacterium can generally be divided into	two vectors	four vectors	six vectors	eight vectors	two vectors

Maximum size of foreign DNA that can be inserted into a replacement vector is	25-30 kb	18-20 kb	20-25 kb	40-50 kb	20-25 kb
Which of the following is not true about phagemid?	Contain functional origin of replication of the plasmid and λ phage	May be propagated as a plasmid or as phage in appropriate strain	Contain λ att site	Can only be propagated as phage	Can only be propagated as phage
pBR 322 has/have which of the following selection marker(s)?	Amp^r	Tet^r	Ampr and Tetr	Kan^r	Ampr and Tetr
Difference between λ gt 10 and λ gt 11 vectors is that	λ gt 11 is an expression vector	λ gt 10 is an expression vector	λ gt 10 is a replacement vector	λ gt 11 is a replacement vector	λ gt 11 is an expression vector
Select the wrong statement about plasmids?	It is extrachromosomal	It is double stranded	Its replication depends upon host cell	It is closed and circular DNA	Its replication depends upon host cell
Stuffer is	the right arm of the vector DNA	the left arm of the vector DNA	central fragment of the vector DNA	right and left arm of the vector DNA	central fragment of the vector DNA
Conjugative plasmids	exhibit antibiotic resistance	do not exhibit antibiotic resistance	carry transfer genes called the tra genes	do not carry transfer genes	carry transfer genes called the tra genes
Plasmid incompatibility is	inability of a plasmid to grow in the host	inability of two different plasmids to coexist in the same host cell in the absence of selection pressure.	ability of two different plasmids to coexist in the same host cell in the absence of selection pressure.	ability of a plasmid to grow in the host	inability of two different plasmids to coexist in the same host cell in the absence of selection pressure.
P1 cloning vector allow cloning of DNA of the length of	100 kbp	50 kbp	20 kbp	10 kbp	100 kbp
P1 cloning vector is the example of	plasmid	cosmid	bacteriophage	phagemid	bacteriophage
Cos site of the cosmids	consists of 20 bases	helps whole genome in circularization and ligation	consists of 24 bases	contains cleavage site	helps whole genome in circularization and ligation

Phagemid vectors are	combination of plasmid and phage λ	combination of phages and cosmid	phages carrying properties of plasmids	phagemid	combination of plasmid and phage λ
Single stranded vectors are useful	for sequencing of cloned DNA	for oligonucleotide non-directed mutagenesis	mutagenesis	nucleotide	for sequencing of cloned DNA
Size of the DNA that can be packaged into a λ phage is	50 kb	35-53 kb	40-50 kb	any size	35-53 kb
Cosmid vectors are used for	cloning small fragments of DNA	cloning large fragments of DNA	cloning prokaryotic DNA only	cloning eukaryotic DNA only	cloning large fragments of DNA
Plasmids which are maintained as limited number of copies per cell are known as	stringent plasmids	relaxed plasmids	cryptic plasmids	plamid	stringent plasmids
Cryptic plasmids	do not exhibit any phenotypic trait	exhibit many phenotypic traits	exhibit one phenotypic traits	exhibit antibiotic resistance	do not exhibit any phenotypic trait
Phagemid consist of	plasmid vector carrying λ phage's cos site	plasmid vector carrying λ attachment (λ att) site	plasmid vector carrying origin of replication of λ phage only	plasmid vector carrying origin of replication of plasmid only	plasmid vector carrying λ attachment (λ att) site
Maximum size of foreign DNA that can be inserted into an insertion vector is	35 kb	18 kb	50 kb	27 kb	18 kb
Plasmids which are maintained as multiple copy number per cell are known as	stringent plasmids	relaxed plasmids	cryptic plasmids	plsmids	relaxed plasmids
Cosmid vectors are	plasmids that contain fragment of λ DNA including the cos site	phages that lack cos site	plasmids that have no selection marker	cryptic plasmids	plasmids that contain fragment of λ DNA including the cos site
The length of T-DNA region can vary from	12 to 24 kb	10 to 20 kb	14 to 26 kb	9 to 19 kb	12 to 24 kb

Crown gall tumors are induced by	*E. coli.*	*Agrobacterium tumefaciens*	*Pseudomonas aeruginosa*	*Bacillus* sp.	*Agrobacterium tumefaciens*
_____, a phenolic compound, is responsible for the activation of vir genes of A. tumefaciens.	Acetosyringone	Acetylcholine	Acetic acid	glacial acitic acid	Acetosyringone
_____ genes are encoded on the Ti plasmid of *A. tumefaciens*	*nif* genes	*ras* genes	*vir* genes	*coz*	*vir* genes
In T-DNA region, the gene which encodes isopentenyl transferease is	*tmr* gene,	*tms1*	*tms2*	*nif*	*tmr* gene,
_____ permits the plasmid to be stably maintained in *A. tumefaciens*	Ti plasmid	Ori region	Vir genes	Opine catabolism region	Ori region
Tms1 or aux1 in T-DNA encodes	isopentyl transferase	indole 3-acetamide hydralase	Tryptophan –2-monoxygenase	tryptophan	Tryptophan –2-monoxygenase
Tms2 or aux2 in T-DNA encodes	isopentyl transferase,	indole 3-acetamide hydralase	Tryptophan –2-monoxygenase	tryptophan	indole 3- acetamide hydralase
Fructopine synthesis is encoded by	mas gene	ags gene	frs gene	tmr	frs gene
Mannopine synthesis is encoded by	*ma* s gene	*ags* gene	*frs* gene	*tm* r	*ma* s gene
ags gene encodes	octapine synthesis	agropine synthesis	nopaline synthesis	mannopine synthesis	agropine synthesis
In angiosperm, the endosperm is	haploid	diploid	triploid	monoploid	triploid
In a _____ protocol, bacteria with engineered abilities to detoxify pollutants are intentionally released in an area.	microcosm establishment	mibridization	bioremediation	rhizosecretion	bioremediation
Induction of callus ….. hormone will be used	BAP	BA	2,4-D	Gibberellins	2,4-D
A cell containing two complete sets of chromosomes one from each parent are	Diploid	haploid	polyploidy	triploid	Diploid
In N2 fixation ….	N_2 converted to NaCl	N_2 converted to Na OH	N_2 converted to ammonia	N_2 converted to CaCl	N_2 converted to ammonia
Who discovered the PEG protoplast fusion	Kao and Michayluk	Gamborg	Murashige and Skoog	White's	Kao and Michayluk
Hydrolase are	alkilation	reversible oxidation	amination	deamination	reversible oxidation
Nitrite to nitrate are	nitrification	deamination	denitrification	nodulation	nitrification
Azotobacter are help to	fix H_2	fix O_2	Fix N_2	fix CO	Fix N2
Nitogenase are …..	chelating agent	to help fission	fixing nitrogen	mineral salts	fixing nitrogen
…… enzyme help to fix the nitrogen.	Nitogenase	pectinase	aldolase	hydrogenase	Nitogenase

……. Organism use to fix the nitrogen	Azotobacter	E-coli	Bacillus	Aspergillus	Azotobacter
What is the role of magnesium elements in media for plant regeneration	cell wall synthesis	enzyme co-factor	Co-factor	carbohydrate synthesis	Co-factor
Unit IV					
In the liposome mediated gene transfer in plants, nucleic acids are	protected from nuclease digestion	not stable in lisosomes	protected from non-nuclease digestion	not stable in liposomes	protected from nuclease digestion
In the liposome mediated gene transfer in plants, nucleic acids are	protected from nuclease digestion	stable in liposomes	protected from non-nuclease digestion	not stable in liposomes	not stable in liposomes
On Ti-plasmid T-region or T-DNA is flanked by a direct repeat of	12 bp	20 bp	25 bp	30 bp	25 bp
Agrobacterium tumefaciens is a	gram (+) bacteria	gram (-) bacteria	a fungi	a yeast	gram (-) bacteria
Microprojectile method of gene transfer in plants involves delivery of DNA	with the help of micromanipulator	with the help of bolistics	with the help of needles	any of the above	with the help of bolistics
Which of the following genes are constitutively expressed and control the plant induced activation of other vir genes?	vir A and vir G	vir C and vir D	vir B and vir E	vir A and vir B	vir A and vir G
Integrated octopine T DNA occurs as	single segment	two segments	three segments	four segments	two segments
Liposomes mediated gene transfer in plants involves	plasmid DNA enclosed in a lipid bag	fusion of liposomes without protoplast	use of NoOH	use of ammonia	plasmid DNA enclosed in a lipid bag
Which of the following plant signal molecules regulate the expression of vir B, C, D and E in case of tobacco?	Acetosyringone	α-hydroxy syringone	Acetosyringone and α-hydroxy syringone	noropine	Acetosyringone and α-hydroxy syringone
Opines that are present in crown gall tumour include	octopine	Acetosyringone	α-hydroxy syringone	Acetosyringone and α-hydroxy syringone	octopine
Octopine synthesis is encoded by	Nos gene	Ocs gene	Frs gene	Ags gene	Ocs gene
Nopaline synthase is encoded by	Nos gene	Ocs gene	Frs gene	Ags gene	Ocs gene

Hairy root disease in higher is caused by	*A. tumefaciens*	*A. rhizogenes*	*E.coli*	*Bacillus* sp.	*A. rhizogenes*
The plasmid found in virulent strain of *A. tumefaciens* is	Ti plasmid	Pla	Ri plasmid	S	Ri plasmid
In ti plasmid the operon required for virulence is	VIR B	VIR C	VIR H	VIR F	VIR B
_____ is a major group of oncogenic Ti plasmid	Histipine	Octopinic acid	Lysopine	Octopine	Octopine
The genes that provide endonucleases to initiate the transfer process by nicking T-DNA at a specific process are	vir C1 and vir C2	vir D, vir D1 and vir D2	vir H	vir E2	vir D, vir D1 and vir D2
The proteins that contain a nuclear targeting sequence to direct the T- strand to the nucleus of the transformed cells are	vir D1	vir E2	vir H	vir D2	vir D2
The genes with overdrive sequence to stimulates the T-DNA transfer process are	vir C1 and vir C2	vir D, vir D1 and vir D2	vir H	vir E2	vir C1 and vir C2
_____ operon is essential for virulence in Agrobacterium transformation.	vir A	vir E2	vir H	vir B	vir B
_____ is the process where electrical impulses of high strength size used for DNA transfer	Microprojectiles	Particle bombardment	Electroporation	Biolistics	Electroporation
_____ used to assist the association of the DNA with membrane in Electroporation mediated DNA transfer	Polyethylene glycol	Gun powder	Silicon-Carbide	Calcium	Polyethylene glycol
A metal used in particle bombardment DNA transfer is	thorium	silver	tungsten	iron	tungsten
The gas used in microprojectile bombardment is	argon	helium	neon	nitrogen	helium
_____ DNA transfer has the advantage of avoiding protoplast isolation	liposome mediated transformation	silicon carbide fiber transformation	ultrasound mediated transformation	DNA transfer via pollen	silicon carbide fiber transformation
_____ has been used to transfer DNA via pollen as a vector to overcome the nuclease action on DNA	*E.coli*	*P. aeruginosa*	*Bacillus* sp	*A. tumeficiens*	*A. tumeficiens*
Cauliflower mosaic virus (CaMV) is potential vector cited under the group	caulimoviruses	Gemini viruses	RNA viruses	virus	caulimoviruses
Dahlia mosaic virus is a vector cited under the group	caulimoviruses	Gemini viruses	RNA viruses	vitrus	caulimoviruses
Maize streak virus vector is a member of	RNA viruses	Caulimoviruses	Gemini viruses	virus	Gemini viruses
The DNA of gemini virus is	double stranded	coiled	single stranded	both single and double	single stranded
TMV and bromo mosaic viruses are the members of	caulimoviruses	RNA viruses	Gemini viruses	virus	RNA viruses

_____ is the monopartite RNA virus	TMV	Tobacco ringspot virus	BMV	Tomato black ring virus satellite	TMV
_____ is the multipartite RNA virus	TMV	Tobacco ringspot virus	BMV	Tomato black ring virus satellite	BMV
_____ RNAs are unable to self replicate in the infected plants	Satellite RNAs	Monopartite viruses	Multipartite viruses	Subgenomic RNAs	Subgenomic RNAs
The first plant RNA viral vector is	RNA IV virus	BMV	Tobacco ringspot virus satellite	TMV vector TB2	TMV vector TB2
_____ are the critical media components in determining the developmental pathway of the plant cells.	microelements	macroelements	plant growth regulators	agar	plant growth regulators
The most frequently used amino acid in PTC media is	arginine	asparagine	aspartic acid	glycine	glycine
The most common type of gelling agent in PTC media is _____.	Agar	Agarose	Polyacrylamide	gelatin	Agar
Agropines are	amino acid derivatives	fatty acid derivatives	sugar derivatives	purine derivatives	sugar derivatives
Datura spp , used for the production of ____	quercetin	withaferin	tropane	whithanolides	tropane
Curcuma longa used for the production of _____	withaferin	tropane	curcumin	whithanolides	curcumin
The secondary metabolite piperidine alkaloids extracted from_____	*Piper nigrum*	*Withania somnifera*	*Curcuma longa*	solanum	*Piper nigrum*
The secondary metabolite withaferin A extracted from_____	*Piper nigrum*	*Withania somnifera*	*Curcuma longa*	solanum	*Withania somnifera*
Which solevent used for the extraction of fat molecules from plants?	methanol	ethanol	petroleum ether	water	petroleum ether
_____ apparatus used for thermo stable compound extraction	soxhlet	vacuum desicator	Drier	vaccum	soxhlet
_____ assay used for cell viability test of the compounds	DPPH	ABTS	MTT	FRAP	MTT
_____ assay used for rDNA expression conformation	DPPH	ABTS	GUS	FRAP	GUS
For the preparation of synthetic seed _____ solution used as immobilizing agent	silver chloride	sodium alginate	copper chloride	NaCl	sodium alginate
_____ hormone used for callus induction	2,4-dichlorophenoxyacetic acid	Naphthalenacetic acid (NAA)	BAP	NAD	2,4-dichlorophenoxyacetic acid

_____ hormone used for multiple shoot induction	2,4-dichlorophenoxyacetic acid	Naphthalenacetic acid (NAA)	BAP	NAD	BAP
_____ gas used for fruite repening	methane	nitrogen	oxygen	Hydrogen	methane
for surface sterilization of the explant _____ used for sterilizing agent	mercury chloride	sodium chloride	silver nitrate	copper sulphate	mercury chloride
_____ region used for organogenesis	nodal and inter nodal region of the stem	inter nodal region of the stem		shoot	nodal and inter nodal region of the stem
_____ part used for hairy root culture	root	leaf	apical meristem	rhizome	root
Metabolic interference is a term used to describe a method to metabolize a compound and prevent the synthesis of something that is normally produced. What compound(s) have been targeted for metabolic interference in tomato?	ACC (1-aminocyclopropane-1-carboxylic acid)	SAM (S-adenosylmethionine)	ACC (1-aminocyclopropane-1-carboxylic acid) and SAM (S-adenosylmethionine)	AOA (aminooxyacetic acid)	ACC (1-aminocyclopropane-1-carboxylic acid) and SAM (S-adenosylmethionine)
Starch content of potatoes can be increased by using a bacterial gene, known as	sucrose phosphate synthase gene	ADP glucose pyrophosphorylase gene	polygalactouranase gene	none of the above	ADP glucose pyrophosphorylase gene
Which tropical fruit crop has been successfully engineered to be protected against a lethal virus?	Passion fruit	Papaya	Mango	Lychee	Papaya
Which of the following metabolites are implicated in stress tolerance?	Proline	Betaines	Proline and betaines	Citrate	Proline and betaines
Which of the agricultural challenges below cannot be solved with transgenic techniques?	Crops are damaged by frost	Crops are killed by a virus	Public concern about safety of synthetic pesticides	Public preference for organic vegetables	Public preference for organic vegetables
Auxanometer is used for measuring	respiratory activity	photosynthetic activity	growth activity	osmotic pressure	growth activity

Crown gall tissue	can be cultivated in vitro in absence of bacteria	retains non tumorous properties when cultivated	can be cultivated in vitro in the presence of bacteria	shows tumorous properties only in presence of bacteria	can be cultivated in vitro in absence of bacteria
Integrated nopaline T-DNA occurs as	single segment	two segments	three segments	four segments	single segment
Opines are	amino acid derivatives found in tumor tissues	amino acid derivatives found in normal tissues	amino acid derivatives found in both normal as well as tumor tissues	amino acid derivatives found in non-tumor tissues	amino acid derivatives found in tumor tissues
Which of the following is true about Agrobacterium tumefaciens?	It causes crown gall disease of plants	It infects lichens also	It infects only monocot	it will not cause crown gall disease	It causes crown gall disease of plants
Unit V					
Which of the following gene is responsible for resistance against chilling?	Glycerol 1 phosphate acyl transferase	Polygalactouranase	ACC deaminase	Sucrose phosphate synthase gene	Glycerol 1 phosphate acyl transferase
Which of the following gene detoxifies herbicide phosphinothricin?	Nitrilase	Glutathione S-transferase (GST)	Phosphinothricin acetyl transferase	reductase	Phosphinothricin acetyl transferase
Plants derived sexually from the same plant are____ while those derived from somatic tissue from the same plant are____.	identical, different	different, also different	different, identical	plants cannot be derived from somatic tissue	different, identical
Transgenic plants with increased tolerance to aluminum have been produced by making plants that	secrete phytosiderophores into the soil	make more metal-binding peptides like phytochelatins	bind aluminum to the cell wall	secrete citrate into the soil	secrete citrate into the soil

Transplastomics	targets genes in the chloroplast	provides exceptionally low yields of protein products	produces genes that are released in pollen	offers little opportunity for practical use	targets genes in the chloroplast
Plants containing genes encoding cytokines and blood clotting factors are used in	nutrition improvement	pharmaceutical production	vaccine production	textile production	pharmaceutical production
The first transgenic plants expressing engineered foreign genes were tobacco plants produced by the use of	*Agrobacterium tumefaciens*	*Bacillus thuringiensis*	*Arabidopsis thaliana*	*Streptomyces hygroscopicus*	*Agrobacterium tumefaciens*
Transgenic plants	contain foreign genes in their cells	it is not useful for human	are weeds	are plants that differ in geographical locations	contain foreign genes in their cells
Low temperatures induce the expression of many cold-induced genes. Transgenic plants with improved cold tolerance have been produced by	expressing the protein that activates expression of these genes all the time in plants	cooling plants using the Peltier effect	expressing a gene for production of antifreeze (ethylene glycol) in plants	increasing evaporative cooling from leaf surfaces	expressing the protein that activates expression of these genes all the time in plants
Nitrilase is encoded by	gene bxn in Klebsiella pneumoniae	bar gene in Streptomyces spp	vir gene	TATA box	gene bxn in Klebsiella pneumoniae
If the goal were to create a plant resistant to an insecticide, which cell-based plant technology would be most effective?	Clonal propagation	Cybridization	Protoplast fusion	Mutant selection	Mutant selection
What are the various advantages of cross protection?	Possibility of mutations in inducing mild virus strain	Possibility of synergism between inducing virus and other unrelated virus	Possibility of unnecessary spread of mild virus	possibility of new breed	possibility of new breed
Which of the following gene is transferred to plants that detoxify the herbicide atrazine?	Nitrilase	Glutathione S-transferase (GST)	Phosphinothriu m acetyl transferase	reductase	Glutathione S-transferase (GST)

Which of the following self-pollinating plant/(s) tend to be homozygous?	Peas	Papaya	banana	Beet root	Peas
Which cell-based plant technology involves the combining of two cells without cell walls from different species?	Clonal propagation	Cybridization	Protoplast fusion	Mutant selection	Protoplast fusion
A naturally occurring variant, possessing characteristics of interest, is identified. This plant is selectively bred. This is an example of	traditional plant breeding	transgenic technology	mutant selection	non-traditional plant breeding	traditional plant breeding
Which of the following not dies from Ti plasmid infection?	Rice	Corn	Sorghum	tomato	tomato
Which of the following genes can be used for making resistances against viral infection?	Genes for non-capsid protein	Gene for non-nucleocapsid protein	Satellite RNA	DNA	Satellite RNA
Which of the following has been widely used to provide resistance against plant viruses?	Virus resistance genes from bacteria	Expression of virus coat protein genes in transgenic plants	Expression of anti-virus genes in vectors that transmit viruses	Expression of ribonuclease (RNase) genes in host plants	Expression of virus coat protein genes in transgenic plants
Cross protection against viruses in transgenic plants can be obtained by	inoculating the susceptible strain of a crop with a mild strain of a virus that helps in developing resistance against more virulent strain	inoculating the susceptible strain with the coat proteins of virulent strain	inoculating the susceptible strain with genes of nucleocapsid	any of the above	inoculating the susceptible strain of a crop with a mild strain of a virus that helps in developing resistance against more virulent strain
Which cell-based technology endows a cell with increased ability to harness energy?	Chlybridization	Cybridization	Mibridization	Protoplast fusion	Chlybridization
The first field tests were conducted with which of the following genetically altered organism?	bt Corn	Vaccinia virus containing a gene from the rabies virus	The flavrsavr tomato	Strawberry seedlings sprayed with ice-minus bacteria	Strawberry seedlings sprayed with ice-minus bacteria

The delayed ripening tomato was created by a biotechnologist who _____ a gene.	altered	silenced	replaced	Relocated	silenced
Antisense technology	selectively blocks expression of a gene	combines genetic material from different species	combines organelles and cells	alters or transfers cells	selectively blocks expression of a gene
Antisense transgenic plants produced fruit that softened	more slowly than the normal fruit	more rapidly than the normal fruit	as the normal fruits	cell wall	more slowly than the normal fruit
Insect resistance in the transgenic plant has been achieved by	transferring genes for Bt toxins	transferring genes for non-protease inhibitors	transferring genes for other non- insecticidal secondary metabolities	transferring genes for non-Bt toxins	transferring genes for Bt toxins
Which group of plants has the greatest diversity (i.e. the most species) living today?	Bryophyta	Lycophyta	Gymnosperms	Angiosperms	Angiosperms
The first transgenic tomato was	Flavr Savr	Flavroma	Tom	Tom2	Flavr Savr
The polygalacturonase enzyme functions in	lycopene synthesis	cellwall degradation	ethylene formation	protect cellwall	cellwall degradation
The phytoene synthase is the gene product of the gene	pTOM5	pTOM6	pTOM13	p53	pTOM5
The ACC oxidase gene product function as	lycopene synthesis	cellwall degradation	ethylene formation	methane formation	ethylene formation
Ethylene formation triggers	red coloration	fruit softening	ripening	sweetness	ripening
The genetically modified flower was marketed by	Calgene	Zeneca	Florigene	Flamogene	Florigene
Golden rice was produced by the manipulation of _____pathway	provitamin A	.provitamin B	starch	protein	provitamin A
The photosynthetic pigments of the leaves absorb in the range	400-700nm	200-400nm	700-800nm	300-350 nm	400-700nm
A protein which accumulates in transgenic potato tubers	cyclodextrin	.fructose	Patatin	agar	Patatin
The carbohydrate which is produced due osmotic stress in yeast is	Sucrose	fructose	trehalose	cyclodextrin	trehalose
.The storage oils in plant seeds are	Saturated	.triacylglycerols	.triazenes	latones	.triacylglycerols
Palmitic acid has _____number of carbon atoms	18	16	20	22	16
Coconut and palm kernel oils contain fatty acids with the carbon number as	C8-C14	C5-C10	C15-C20	C12-C23	C8-C14
A rare fatty acid which is found in Umbiliferae	lauric acid	adipic acid	petroselenic acid	linolenic acid	petroselenic acid

Name an essential fatty acid	lauric acid	adipic acid	petroselenic acid	linolenic acid	linolenic acid
Hirudin is produced transgenically in	.Arabidopsis thaliana	.Emblica officianalis	.Oscimum sanctum	Brassica napus	Brassica napus
Expand CMV	cauliflower mosaic virus	cucumber mosaic virus	curcuma mosaic virus	mosaic virus	cucumber mosaic virus
The genetic manipulation of ethylene biosynthesis is also known as	antisense strategy	genesilencing strategy	gene knock out strategy	non-antisense strategy	antisense strategy
The red and blue color for the flowers are due	.carotenoids	anthocyanins	lignins	terpenes	anthocyanins
Abscisic acid is formed from_____	Anthocyanins	.beta carotene	alpha carotene	alakaloids	beta carotene
Both root apical & shoot apical meristem is apparent at the __ stage of embryo development.	globular	heart-stage	torpedo	rectangular	torpedo
Which group of land plants is most restricted to moist environments?	Lycophyta	Sphenophyta	Bryophyta	Angiosperms	Bryophyta
What single feature is probably most responsible for the success of angiosperms?	Seeds	Fruit	Broad leaves	Flowers	Flowers