Application

1. The commercial production of plants which uses meristem & shoot culture to produce large numbers of identical individuals.

2. To conserve rare or endangered plant species.

3. A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters.

4. Large scale growth of plants in liquid culture in bioreactors for the production of valuable compounds.

5. To cross distantly related species by protoplast fusion & regeneration of the novel hybrid.

Types of In Vitro Culture (explant based)

1. Culture of intact plants (seed and seedling culture)

2. Embryo culture (immature embryo culture)

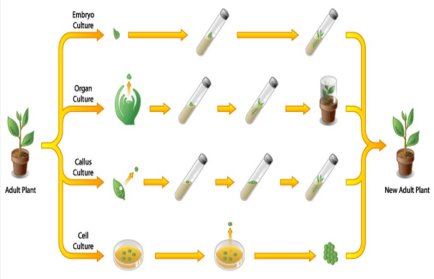
3. Organ culture (Hairy Root Culture, Shoot tip and meristem culture)

4. Callus culture

5. Cell suspension culture

6. Protoplast culture

Types of In Vitro Culture



1. Seed Culture

-growing seed aseptically in vitro on artificial media

-Use:

->Increasing efficiency of germination of seeds that are difficult to germinate in vivo

->Precocious germination by application of plant growth regulators

->Production of clean seedlings for explants or meristem culture

->In vitro selection

2. Embryo Culture

-growing embryo aseptically in vitro on artificial nutrient media

-Use:

->Rescue embryos (embryo rescue) from wide crosses where fertilization occurred, but embryo development did not occur

->Production of plants from embryos developed by non-sexual methods (haploid production)

->Overcoming embryo abortion due to incompatibility barriers

->Overcoming seed dormancy and self-sterility of seeds

->Shortening of breeding cycle

The advantages of growing an embryo isolated from the rest of the seed

-To remove the immature plant from the endosperm and/or cotyledon(s) which may in particular cases prevent or modify the development of the plant.

-As a means of propagating species which resist attempts to use standard methods of vegetative propagation.

-Rescue of weak/ aborting embryos resulting from breeding/ crossing process

3. Organ Culture

-Any plant organ can serve as an explant to initiate cultures

Organ	Culture Types
Shoot	a) Shoot tip culture
Root	b) Root culture
Leaf	c) Leaf culture
Flower	d) Anther/ovary culture

3a) Shoot Apical Meristem Culture

-Shoot tip can be cultured in vitro, producing clumps of shoots from either axillary or adventitious buds.

-This method can be used for clonal propagation

-Shoot meristem cultures are potential alternatives methods for cereal regeneration as they are less genotype-dependent and more efficient

-Use:

1. Production of virus free germplasm

3a) Shoot Apical Meristem Culture (cont)

2. Mass production of desirable genotypes

3. Facilitation of exchange between locations (production of clean material)

4. Cryopreservation (cold storage) or in vitro conservation of germplasm

3b) Root Organ Culture

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

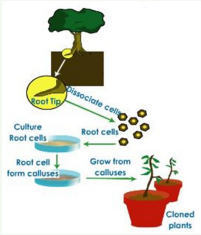
-growth of roots in vitro is potentially unlimited, as roots are indeterminate organs

-Use:

1. Production secondary metabolites

2. Study the physiology and metabolism of roots, and primary root to determinate growth patterns

Root Culture



3d) Anther/Ovary culture

-production of haploid plants

Ovary/Ovule Culture

-Use:

1. Production of haploid plants

2. A common explant for the initiation of somatic embryogenic cultures

3. Overcoming abortion of embryos of wide hybrids at very early stages of development due to incompatibility barriers

4. In vitro fertilization for the production of distant hybrids avoiding style and stigmatic incompatibility that inhibits pollen germination and pollen tube growth

Anther and Microspore Culture

3d) Anther/Ovary culture (cont)

1. Production of haploid plants

2. Production of homozygous diploid lines through chromosome doubling, thus reducing the time required to produce inbred lines

3. Uncovering mutations or recessive phenotypes

4. Callus Culture

-un-organized mass of cells

-tissue that develops in response to injury caused by physical or chemical means

-Most cells of which are differentiated although may be and are often highly unorganized within the tissue

Culturing Callus

-Production of plantlets through somatic embryogenesis or organogenesis

-Secondary metabolites production

-Any plant tissue can be used as an explant

-often performed in the dark as light can encourage differentiation of the callus

-During long-term culture, the culture may lose the requirement for auxin and/or cytokinin - ‘habituation’ - common in callus cultures

-Manipulation of the auxin to cytokinin ratio in the medium can lead to the development of shoots, roots or somatic embryos

-Callus cultures can also be used to initiate cell suspensions

Development of Callus

-The proliferation can be maintained more or less indefinitely, if the callus is subcultured on to fresh medium periodically

-Callus is usually composed of unspecialized parenchyma cells

-During callus formation there is some degree of dedifferentiation

-i.e. the changes that occur during development and specialization are, to some extent, reversed, both in morphology and metabolism

-One major consequence of dedifferentiation: most plant cultures lose the ability to photosynthesize

Two categories of callus:

a) Compact callus: the cells are densely aggregated

4. Callus Culture (cont)

b) Friable callus: the cells are only loosely associated with each other and the callus becomes soft and breaks apart easily

The friability of callus can be improved by:

a) manipulating the medium components

b) repeated subculturing

c) culturing it on 'semi-solid' medium (medium with a low concentration of gelling agent)

5. Cell Suspension Culture

-When callus pieces are agitated in a liquid medium, they tend to break up.

-Suspensions are much easier to bulk up than callus since there is no manual transfer or solid support.

-Friable callus provides the inoculum to form cell-suspension cultures

Growing of cell suspension from friable callus

->liquid medium

->agitated

->single cells and/or small clumps of cells are released

->continue to grow and divide

->cell-suspension culture

Criteria in growing cell suspension from friable callus:

-A relatively large inoculum should be used when initiating cell suspensions so that the released cell numbers build up quickly.

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

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

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

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

5. Cell Suspension Culture (cont)

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

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

-cells should be transferred as they enter the stationary phase

-important to determine batch growth-cycle parameters for each cell-suspension culture

Advantage

1. it can ultimately provide a continuous, reliable source of natural products.

2. synthesis of bioactive secondary metabolites

3. running in controlled environment

4. independently from climate and soil conditions

Importance and Application of cell suspension culture as an experimental technique

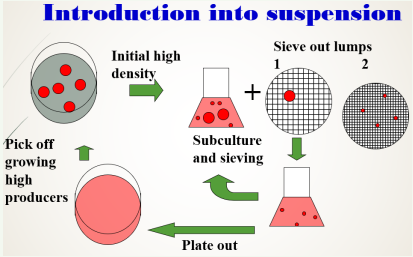
-Contribute information about cell physiology, biochemistry, metabolic events at the level of individual cells and small cell aggregates

-Develop understanding of an organ formation or embryoid formation starting from single cell or small cell aggregates

-Suspension culture derived from medicinally important plants can be studied for the production of secondary metabolites

-Mutagenesis studies may be facilitated by the use of cell suspension cultures to produce mutant cell clones from which mutant plants can be raised

Introduction into Suspension



6. Protoplast Culture

-The living material of a plant or bacterial cell, including the protoplasm and plasma membrane after the cell wall has been removed.

Somatic Hybridization

-development of hybrid plants through the fusion of somatic protoplasts of two different plant species/varieties

Process:

1. Isolation of protoplast
2. Fusion of the protoplasts of desired species/varieties
3. Identification and Selection of somatic hybrid cells
4. Culture of the hybrid cells
5. Regeneration of hybrid plants

How to make a protoplast?

-Two general approaches to removing the cell wall (without damaging the protoplast):

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

b) Enzymatic isolation: usually carried out in a simple salt solution (with a high osmoticum) + cell wall degrading enzymes.

-It is usual to use both cellulase and pectinase enzymes (must be of high quality and purity)

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

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

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

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

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

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

Uses for Protoplast Fusion:

6. Protoplast Culture (cont)

1. Combine two complete genomes

->another way to create allopolyploids

2. In vitro fertilization
3. Partial genome transfer

->Exchange single or few traits between species

->May or may not require ionizing radiation

4. Genetic engineering

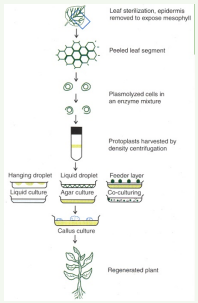
->Micro-injection, electroporation, Agrobacterium

5. Transfer of organelles

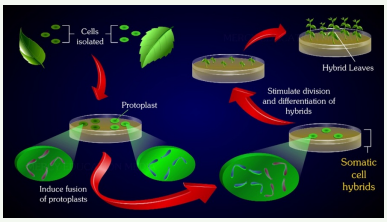
->Unique to protoplast fusion

->The transfer of mitochondria and/or chloroplasts between species

Protoplast



Protoplast Fusion



Plant Genetic Engineering

Genetic Transformation

-Introduction of foreign DNA to generate novel genetic combinations.

-Transfer of desirable genes for disease and pest resistance from related or unrelated plant species into high yielding susceptible cultivars.

-Study of structure and function of genes.

Purposes of Introducing Novel Traits

Plant Genetic Engineering (cont)

1. Biodiversity screening: To look for new traits in other or wild population

2. Management of germplasm: Propagation of elite germplasm through micropropagation

3. Interspecific or intergeneric crosses: Introduction of traits from other genus or species

4. Overcome crossing barrier: Use protoplast fusion or somatic hybridization to produce hybrids

5. Genetic engineering: Introduce genes from the same species, distantly related species or unrelated species (e.g. bacteria into plants)

6. Incorporation into plant breeding: Use selected inbreeds for gene transformation

Creation of Novel Traits

Somaclonal variation

-Variation arise in culture (especially those undergone long period of culturing) due to genetic or epigenetic mutation

Mutagenesis

-Treatment of cultures with mutagens, such as ethyl methane sulfonate (EMS), UV and radioactive radiations

-Dosage of mutagens can be vital, sub-vital or sub-lethal

-Mild mutation - opt for vital

-Heavy mutation – opt for subvital to sublethal

Purposes of Creating Novel Traits

Propagate high yielders

-Micropropagation of high yielders could increase yield

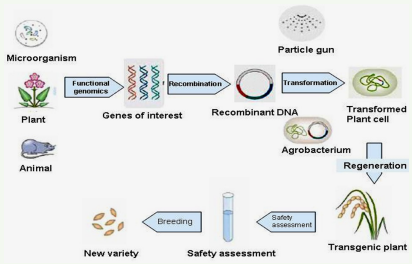
-This could be a strategy until full inbreeds are produced

Disease susceptibility

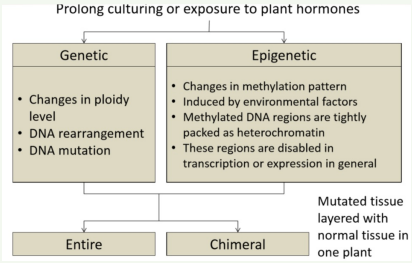
-Reliance on one clone is dangerous to food security, since disease susceptibility could decimate the entire crop

-At least a few clones of the high yielders must be grown in a field and best if they are separated by rows

Plant Genetic Engineering Process



Somaclonal Variation



Germplasm Conservation

-Germplasm is living tissue from which new plants can be grown

(google)-Germplasm is the term used to describe the seeds, plants, or plant parts useful in crop breeding, research, and conservation efforts.

An extension of micropropagation techniques through two methods:

-Slow growth techniques e.g.: ↓ Temp., ↓ Light, media supplements (growth retardants).

-Medium-term storage (1 to 4 years)

Cryopreservation

-Ultra low temperatures in liquid nitrogen at -196°C.

-Stops cell division & metabolic processes

-Very long-term (indefinite)

Pros and Cons of Plant Tissue Culture

Advantages	Disadvantages

Pros and Cons of Plant Tissue Culture (cont)

1. In plants prone to virus diseases, virus free explants (new meristem tissue is usually virus free) can be cultivated to provide virus free plants	1. It is a labor intensive & expensive process.
2. Plant “tissue banks” can be frozen, the regenerated through tissue culture	2. Not all plants can be successfully tissue cultured - it is usually because the medium of growth is not known.
3. Plant culture in approved media are easier to export than soil-grown plants, as they are pathogen free and take up little space (most current plant export is now done in this manner	
4. Tissue culture allows fast selections for crop improvement – explants are chosen from superior plants then cloned	
5. High degree of uniformity (true type plants) when compared to conventionally produced plants	