Introduction

plant cells re-differentiate

plant tissues can be regenerated from explants

Application

increase crop yield (developing countries)

produce consistent yield quality (private at-home grower)

produce exact replicas of species for profit (businesses)

Plasticity

-plant's ability to adapt and cope with changes in the environment

-plant's ability to different developmental pathways (alter their phenotype) in response to a particular stimuli/changes in the environment

-alter its metabolism, growth, and development which suit the current environment the best

Plant cells and tissues with high plasticity is needed for plant tissue culture.

EXAMPLE: Fanwort (aquatic weed)

1. feathery underwater leaves

2. floating surface leaves

-both leaf types are genetically identical cells, but the dissimilar environments cause certain genes involved in leaf formation to be expressed or unexpressed in different environments

Plant Adaptation/Response

plastic structural responses to specific environment	etc. growth of plant towards sunlight source and growth of root towards source of water
morphological adaptations in specific environment	etc. cactus's leaves are reduced to spines and a stem to reduce water lost in desert

Fundamental Abilities of Plants

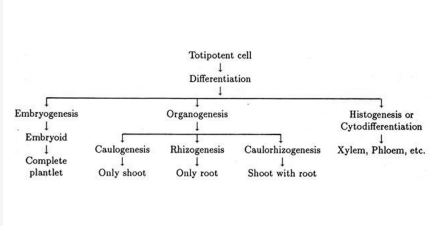
1. Totipotency	potential of a cell/group of cells to develop into an entire organism if suitably stimulated

Fundamental Abilities of Plants (cont)

2. Dedifferentiation	development of differentiated explant into a undifferentiated callus (mature cells return to meristematic condition)
3. Competency??	endogenous potential of a given cells or tissue to develop in a particular way

*Differentiation	physiological and morphological changes that occur in a cell, tissue or organ during development
*Meristematic Condition	unmatured plant which does not have specific differentiated meristematic tissues (etc. apical meristem, lateral meristem) and all meristem tissues function for the 'simple growth' of the plants
*Redifferentiation	development of undifferentiated callus into planta differentiated

Plant Regeneration Pathway Image



Plant Regeneration Pathway

1. Organogenesis

-initiation and development of tissues and organ from cells which is not meristems

a) Dedifferentiation	-starts shortly after the isolation of explant
	-rapid cell division and formation of undifferentiated cells (callus)
b) Redifferentiation (budding)	-starts after the first callus cell forms

Plant Regeneration Pathway (cont)

	-tissue named organ primordia is differentiated from callus cells
	-organ primordia will give rise to small meristems (cells densely filled with protoplasm and strikingly large nuclei)
	-different types of specialized cells will further differentiate
	-vascular system formed will connect new organs with the parent explant/callus mass

2. Somatic Embryogenesis

-dedifferentiation of plant somatic cell into totipotent embryonic stem cell then to differentiated embryos

-embryonic stem cell need to have the ability to give rise to an embryo which can further develop into a whole new plant without sexual fertilization of zygotic embryos

a) initiated directly	from explants
b) initiated indirectly	from callus

Process

1) induction of embryogenic cultures from zygotic seed, leaf or stem segment

2) further multiplication of embryos

3) mature embryos are then cultured for germination and plantlet development

4) transferred to soil

3. Histogenesis

-differentiation of undifferentiated cells and their component cell types into specific tissues and organ

*Somatic embryos - embryos form from ordinary plant cells(2n) which normally are not involved in embryo development

Plant Tissue Culture

collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition

1. Appropriate tissue

2. Sterile conditions with aseptic techniques

3. Suitable growth medium

Factors Affecting Plant Tissue Culture

1. Growth Media	-minerals, growth factors, carbon source, hormones
2. Environmental Factors	-light, temperature, photoperiod, sterility, media
3. Explant Source	-usually, younger, less differentiated explant is a better explant source
4. Genetics	-different species show difference in amenability to tissue culture
	-different genotype within a species will have variable response to tissue culture

Explant

-small pieces of plant parts or tissues that are aseptically cut from a matured plant and used to initiate a culture in a nutrient medium

-almost all parts of plant are amenable to in vitro plant regeneration provided that they are able to dedifferentiate into totipotent cells

-to grow, it require a nutrient medium consisting of mineral salts mixture, a carbon source, (usually sucrose) and vitamins

-to initiate and maintain cell division, it need phytohormones (auxins and cytokines) in the nutrient medium

-occasionally, to ensure the prolonged growth of the excised tissue to give an established callus, other organic supplements (amino acids or hexitols) is also needed

correct choice of explant material can have an important effect on the success of a tissue culture experiment

Explants used in Micropropagation	
shoot tip	leaf tip
axillary bud	shoot tip
inflorescence segment	nodal segment
lateral bud	flower stalk segment
leaf base	root tips

Plant Explant Selection	
-correct choice of explant material can have an important effect on the success of a tissue culture experiment	
1. Season in which the explant is obtained	season of the year can affect on the contamination and response in culture
2. Positi-on/part of plant	explants of various organs of a same parent plant vary in their rate of growth & regeneration
	in certain plants some organs may be more regene-rative than the others
3. Quality of the source plant	best to obtain explants from healthy plants compared to plants under nutritional or water stress or plants which are exhibiting disease symptoms

Plant Explant Selection (cont)	
4. Size of explant (commonly: 1-1.5 x 104 cells/ml)	minimum inoculation size of explant varies according to the genotype of the plant being cultured and the cultural conditions
	-large explants generally survive more frequently and grow more rapidly at the outset than very small ones
	-large explants probably contain more nutrient reserves and plant growth regulators to sustain the culture
	-smaller explant harder to culture where the medium of culture has to have additional components
	-smaller explant increase the chance of virus elimination from subsequent cultures
5. The purpose/ goal of the proposed culture	choice of explant tissue will vary depending on what type of a response is desired from the cell culture

Plant Explant Selection (cont)

	a) clonal propagation	lateral or terminal shoot or bud
	b) callus induction	cotyledon, hypocotyl, stem, leaf, or embryo
	c) protoplast isolation	leaf tissue from aseptically germinated seed
6. The kind of culture to be initiated	choice of explant material also determines if the plantlets developed via tissue culture are	
	a) haploid/diploid	
	b) cell/organ	
7. Physiological condition/age of the explant source	younger tissue is more responsive in vitro, usually the newest formed and is easier to surface disinfect and establish clean cultures	
	older tissue will not form callus that is capable of regeneration	

Micropropagation

practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods

Advantage of Plant Tissue Culture

In plants prone to virus diseases, virus free explants (new meristem tissue is usually virus free) can be cultivated to provide virus free plants

Plant “tissue banks” can be frozen, the regenerated through tissue culture

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

Tissue culture allows fast selections for crop improvement – explants are chosen from superior plants then cloned

High degree of uniformity (true type plants) when compared to conventionally produced plants

Disadvantage of Plant Tissue Culture

It is a labor intensive & expensive process.

There is a chance that the propagated plants will be less resilient to diseases due to the type of environment they are grown in.

It is imperative that, before being cultured, the material is screened; failure to pick up any abnormalities could lead to the new plants being infected.

While the success rate is high if the correct procedures are followed, success with the tissue culture is not a guarantee. There is still a chance that the process triggers a secondary metabolic chemical reaction, and the new explants or cells' growth gets stunted, or even die off