Part II
PLANT TISSUE CULTURE (OREVIEW)

Le Bui Van

University of Science

Plant Biotechnology

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2.1. Plant tissue culture techniques

Tissue culture is the culture and maintenance of plant cells or organs in sterile, nutritionally and environmentally supportive conditions (in vitro). Tissue culture produces clones, in which all product cells have the same genotype (unless affected by mutation during culture). It has applications in research and commerce. In commercial settings, tissue culture is primarily used for plant propagation and is often referred to as micropropagation.

- ✓ *The first commercial use of plant tissue culture on artificial media was in the germination and growth of orchid plants, in the 1920's*
- ✓ *In the 1950's and 60's there was a great deal of research, but it was only after the development of a reliable artificial medium (Murashige & Skoog, 1962) that plant tissue culture really 'took off' commercially.*
- ✓ *Tissue culture techniques are used for virus eradication, genetic manipulation, somatic hybridization and other procedures that benefit propagation, plant improvement and basic research.*

What conditions do plant cells need to multiply *in vitro*?

Tissue culture has several critical requirements:

- *Appropriate tissue (some tissues culture better than others)*
- *A suitable growth medium containing energy sources and inorganic salts to supply cell growth needs. This can be liquid or semisolid*
- *Aseptic (sterile) conditions, as microorganisms grow much more quickly than plant and animal tissue and can overrun a culture.*
- *Growth regulators - in plants, both auxins & cytokinins.*
- *Frequent subculturing to ensure adequate nutrition and to avoid the build-up of waste metabolites*

Appropriate tissue (Explant)

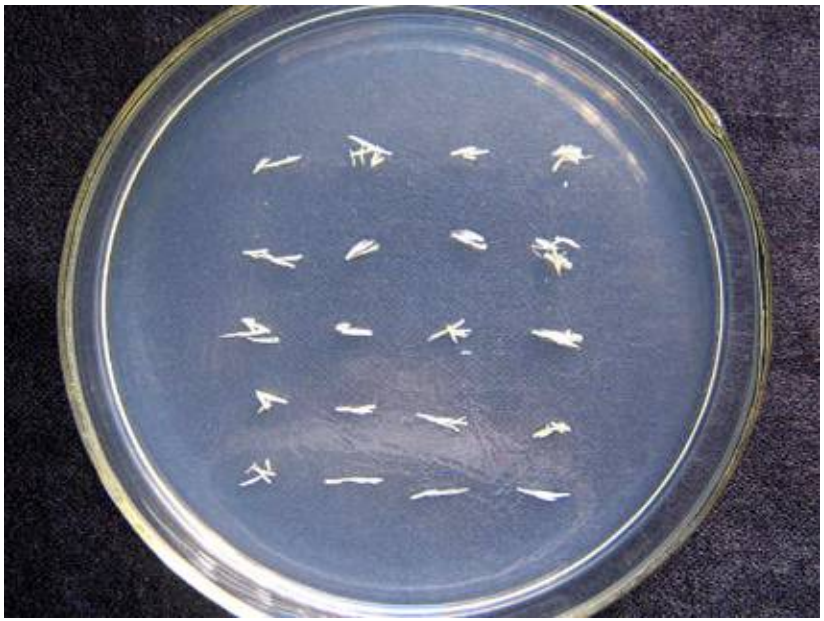
- ✓ *Explants: Cell, tissue or organ of a plant that is used to start in vitro cultures. Many different explants can be used for tissue culture, but axillary buds and meristems are most commonly used.*
- ✓ *The explants must be sterilized to remove microbial contaminants. This is usually done by chemical surface sterilization of the explants with an agent such as bleach at a concentration and for a duration that will kill or remove pathogens without injuring the plant cells beyond recovery.*

Plant source

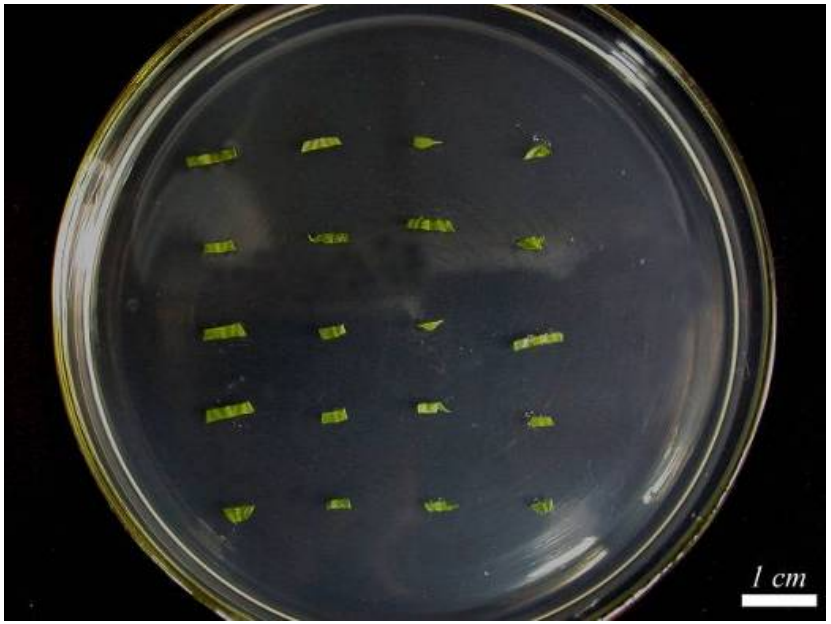
(axillary buds, meristems Leaves, stems, roots, hypocotyl…)

↓

Surface sterilization of explants



Young flower stalk of *Vertiver* sp



Leaf explants of *Stevia* sp

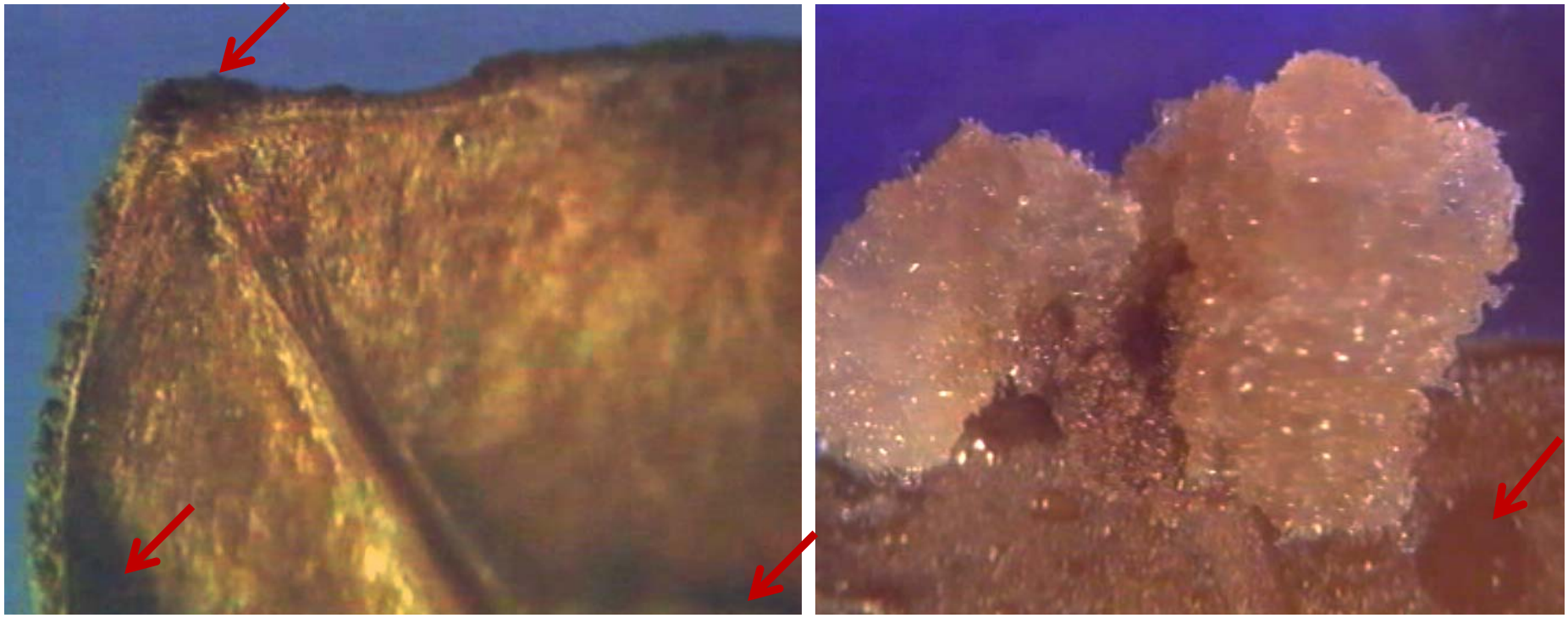
Many plants are rich in polyphenolics

- *After tissue injury during dissection, such compounds will be oxidized by polyphenol oxidases → tissue turn brown/black*
- *Phenolic products inhibit enzyme activities and may kill the explants*

Methods to overcome browning:

- *adding antioxidants [ascorbic acid, citric acid, PVP (polyvinylpyrrolidone), dithiothreitol], activated charcoal or presoaking explants in antioxidant*
- *incubating the initial period of culturing in reduced light/darkness*
- *frequently transfer into fresh medium*

The appearance of phenolic compound and death tissues



Nutrition medium

When an explant is isolated, it is no longer able to receive nutrients or hormones from the plant, and these must be provided to allow growth in vitro. The composition of the nutrient medium is for the most part similar, although the exact components and quantities will vary for different species and purpose of culture. Types and amounts of hormones vary greatly. In addition, the culture must be provided with the ability to excrete the waste products of cell metabolism. This is accomplished by culturing on or in a defined culture medium which is periodically replenished.

- *A nutrient medium is defined by its mineral salt composition, carbon source, vitamins, plant growth regulators and other organic supplements.*
- *pH determines many important aspects of the structure and activity of biological macromolecules. Optimum pH of 5.0-6.0 tends to fall during autoclaving and growth.*

Mineral salt

• **NH_4NO_3**	**Ammonium nitrate**
• **KNO_3**	**Potassium nitrate**
• **$CaCl_2 \cdot 2H_2O$**	**Calcium chloride (Anhydrous)**
• **$MgSO_4 \cdot 7H_2O$**	**Magnesium sulfide (Epsom Salts)**
• **KH_2PO_4**	**Potassium hypophosphate**
• **FeNaEDTA**	**Fe / Na ethylene-diamine-tetra acetate**
• **H_3BO_3**	**Boric Acid**
• **$MnSO_4 \cdot 4H_2O$**	**Manganese sulfate**
• **$ZnSO_4 \cdot 7H_2O$**	**Zinc sulfate**
• **KI**	**Potassium iodide**
• **$Na_2MoO_4 \cdot 2H_2O$**	**Sodium molybdate**
• **$CuSO_4 \cdot 5H_2O$**	**Cupric sulfate**
• **$CoCl_2 \cdot H_2O$**	**Cobaltous sulfide**

Mineral salt composition

- ✓ Macroelements: *The elements required in concentration > 0.5 mmol/l*
- ✓ The essential macroelements: *N, K, P, Ca, S, Mg, Cl*
- ✓ Microelements: *The elements required in conc. < 0.5 mmol/l*
- ✓ The essential microelements: *Fe, Mn, B, Cu, Zn, I, Mo, Co*
- ✓ The optimum concentration → maximum growth rate

Mineral salt composition of media

		Murashige Skoog	White	Gamborg	Schenk Hildebrandt	Nitsch& Nitsch
NO_3	mmol/l	40	3.8	25	25	18.5
NH_4	"	20	---	2	2.5	9
Total N	"	60	3.8	27	27.5	27.5
P	"	1.5	0.15	1	2.5	0.5
K	"	21.5	1.65	25	25	10
Ca	"	3	1.5	1	1.5	1.5
Mg	"	1.5	3	1	1.5	0.75
Cl	"	6	0.85	2	3	3
S	"	1.73	4.545	2.112	1.619	0.985
Fe	µmol/l	100	15	50	55	100
Na	"	202	3180	1102	111	202
B	"	100	25	50	80	150
Mn	"	100	20	60	60	100
Zn	"	30	10	7	3.5	35
Cu	"	0.1	0.04	0.1	0.8	0.1
Mo	"	1	0.007	1	0.4	1
Co	"	0.1	---	0.1	0.4	---
I	"	5	4.5	4.5	---	---

Mineral salts
Function of nutrients in plant growth

Element	Function
Nitrogen	Component of proteins, nucleic acids and some coenzymes Element required in greatest amount
Potassium	Regulates osmotic potential, principal inorganic cation
Calcium	Cell wall synthesis, membrane function, cell signalling
Magnesium	Enzyme cofactor, component of chlorophyll
Phosphorus	Component of nucleic acids, energy transfer, component of intermediates in respiration and photosynthesis
Sulphur	Component of some amino acids (methionine, cysteine) and some cofactors
Chlorine	Required for photosynthesis
Iron	Electron transfer as a component of cytochromes
Manganese	Enzyme cofactor
Cobalt	Component of some vitamins
Copper	Enzyme cofactor, electron-transfer reactions
Zinc	Enzyme cofactor, chlorophyll biosynthesis
Molybdenum	Enzyme cofactor, component of nitrate reductase

Carbon sources and vitamins

- Sucrose or glucose (sometimes fructose), concentration 2-5%

- Most media contain myo-inositol, which improves cell growth

- An absolute requirement for vitamin B1 (thiamine)

- Growth is also improved by the addition of nicotinic acid and vitamin B6 (pyridoxine)

- Some media contain pantothenic acid, biotin, folic acid, p-amino benzoic acid, choline chloride, riboflavine and ascorbic acid (C-vitamin)

Plant growth regulators
(Body building Plants)

Auxins:

- *induces cell division, cell elongation, swelling of tissues, formation of callus, formation of adventitious roots.*
- *inhibits adventitious and axillary shoot formation*

  - 2,4-D, NAA, IAA, IBA, pCPA...

Cytokinins: *- shoot induction, cell division*

- BAP, Kinetin, zeatin, 2iP...

Gibberellins: *plant regeneration, elongation of internodes*

- GA3...

Abscisic acid: *induction of embryogenesis*

- ABA

Plant growth regulators used in plant tissue culture media
Normal concentration range is 10^{-7} ~ 10^{-5}M

Class	Name	Abbreviation	MW
Auxin	p-chlorophenoxyacetic acid	pCPA	186.6
	2,4-Dichlorophenoxyacetic acid	2,4-D	221.0
	Indole-3-acetic acid	IAA	175.2
	Indole-3-butyric acid	IBA	203.2
	1-Naphthaleneacetic acid	NAA	186.2
Cytokinin	6-Benzylaminopurine	BAP	225.2
	N-Isopenteylaminopurine	2iP	203.3
	6-Furfurylaminopurine (Kinetin)	K	215.2
	Zeatin	Zea	219.2
Gibberellin	Gibberellic acid	GA_3	346.4
Abscisic acid	Abscisic acid	ABA	264

Organic supplements

- *N in the form of amino acids (glutamine, asparagine) and nucleotides (adenine)*
- *Organic acids: TCA cycle acids (citrate, malate, succinate, fumarate), pyruvate*
- *Complex substances: yeast extract, malt extract, coconut milk, protein hydrolysate*
- *Activated charcoal is used where phenol-like compounds are a problem, absorbing toxic pigments and stabilizing pH. Also, to prevent oxidation of phenols PVP (polyvinylpyrrolidone), citric acid, ascorbic acid, thiourea and L-cysteine are used.*

2.2. Cellular totipotency and plant regeneration

Unlike an animal cell, a plant cell, even one that highly maturated and differentiated, retains the ability to change a meristematic state and differentiate into a whole plant if it has retained an intact membrane system and a viable nucleus.

1902 Haberlandt raised the totipotentiality concept of plant totipotency in his Book "Kulturversuche mit isolierten Pflanzenzellen" (Theoretically all plant cells are able to give rise to a complete plant)

Totipotency or Totipotent: The capacity of a cell (or a group of cells) to give rise to an entire organism.

Cultured tissue must contain competent cells or cells capable of regaining competence (dedifferentiation). e.g. an excised piece of differentiated tissue or organ (Explant) → dedifferentiation → callus (heterogenous) → redifferentiation (whole plant) = cellular totipotency.

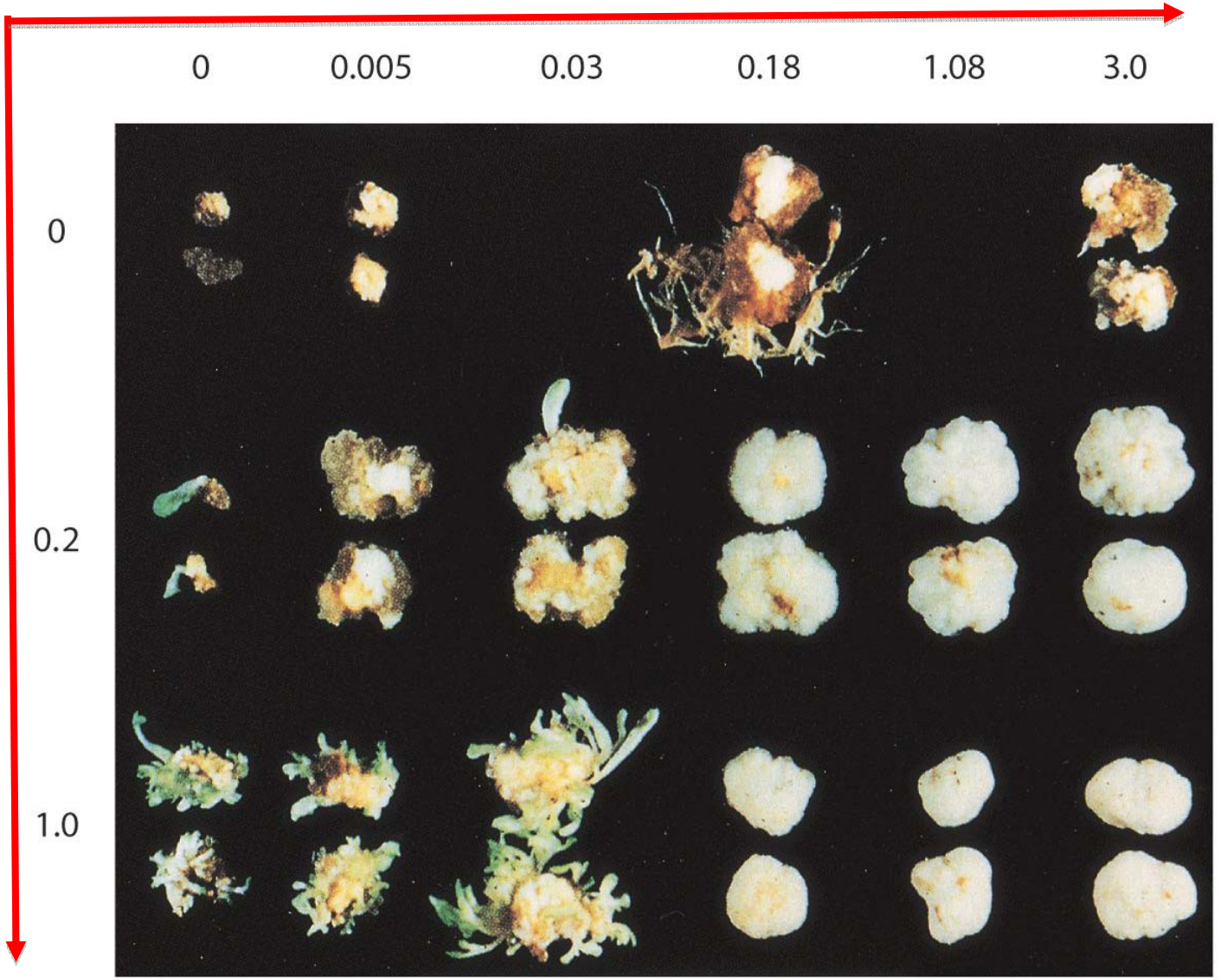
1957 Skoog and Miller demonstrated that two hormones affect explants' differentiation:

- *Auxin: Stimulates root development*
- *Cytokinin: Stimulates shoot development*

- *Generally, the ratio of these two hormones can determine plant development:*
  - *↑ Auxin ↓Cytokinin = Root development*
  - *↑ Cytokinin ↓Auxin = Shoot development*
  - *Auxin = Cytokinin = Callus development*

Skoog & Miller 1957, *Symp.Soc.Exp. Biol* 11:118-131

Increase IAA concentration (mg/l)



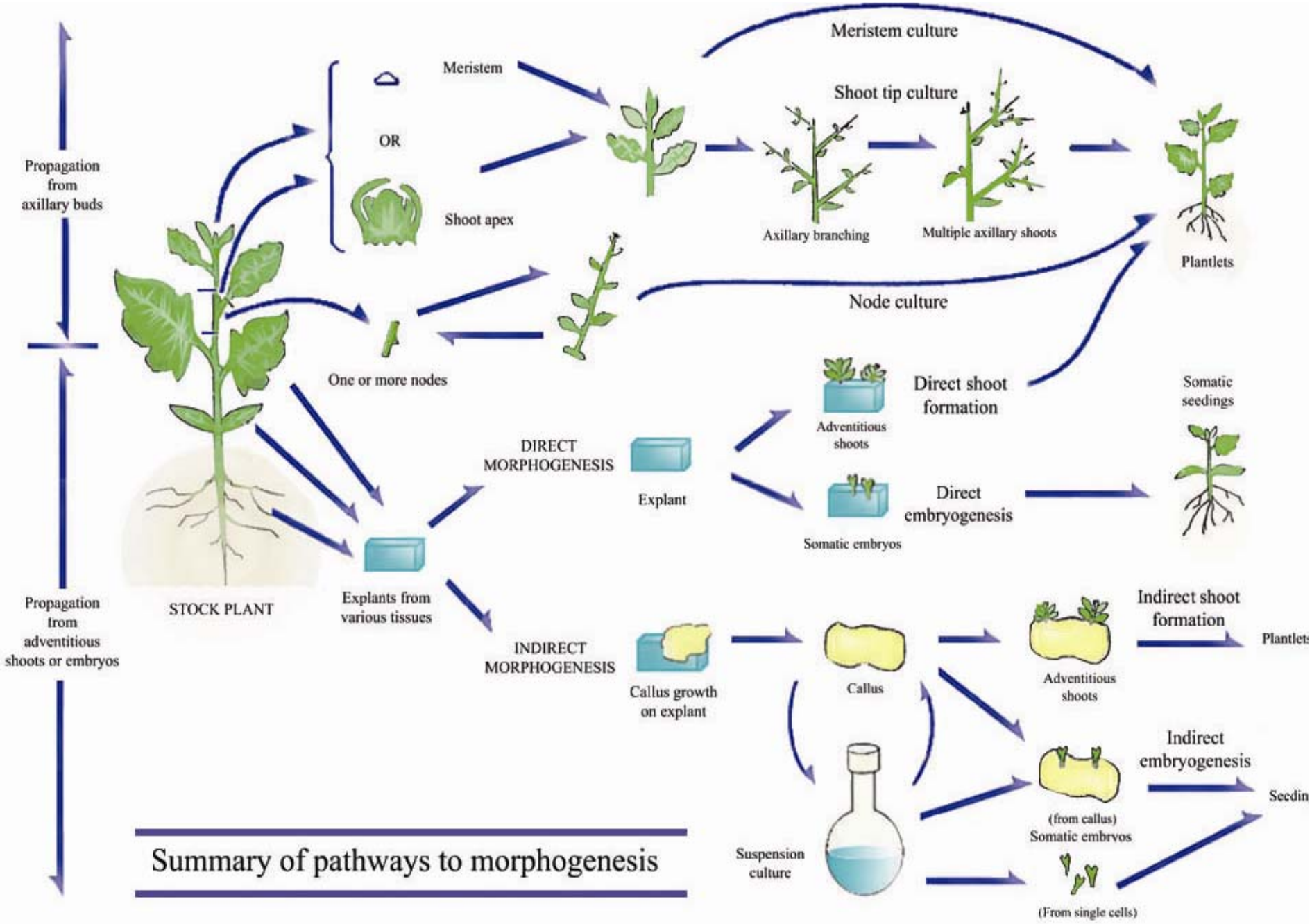
Callus of *Nicotiana* (Solanaceae family)

Morphogenetic processes that lead to plant regeneration

Can be achieved by culturing tissue sections either lacking a preformed meristem (adventitious origin) or from callus and cell cultures (de novo origin)

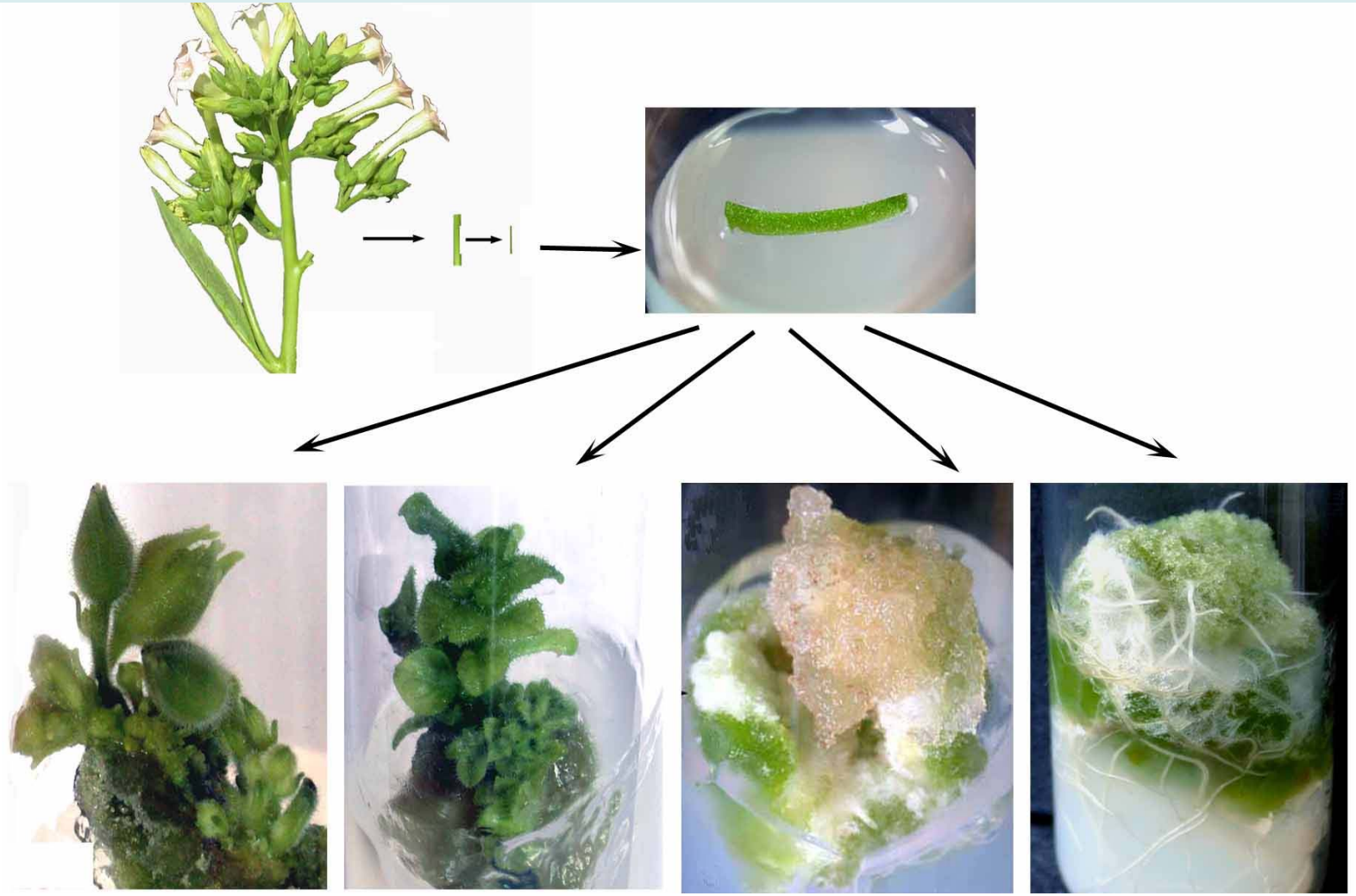
- adventitious regeneration occurs at unusual sites of a culture tissue (e.g. leaf blade, internode, petiole) where meristems do not naturally occur

- adventitious or de novo regeneration can occur by organogenesis and embryogenesis



Modified from Edwin F. Geoge. Plant propagation by tissue culture 3rd Ed. Springer publisher (2008).

Morphogenetic response of thin cell layers explants of tobacco



Flower induced Shoot cluster Callus Root induce

Callus culture

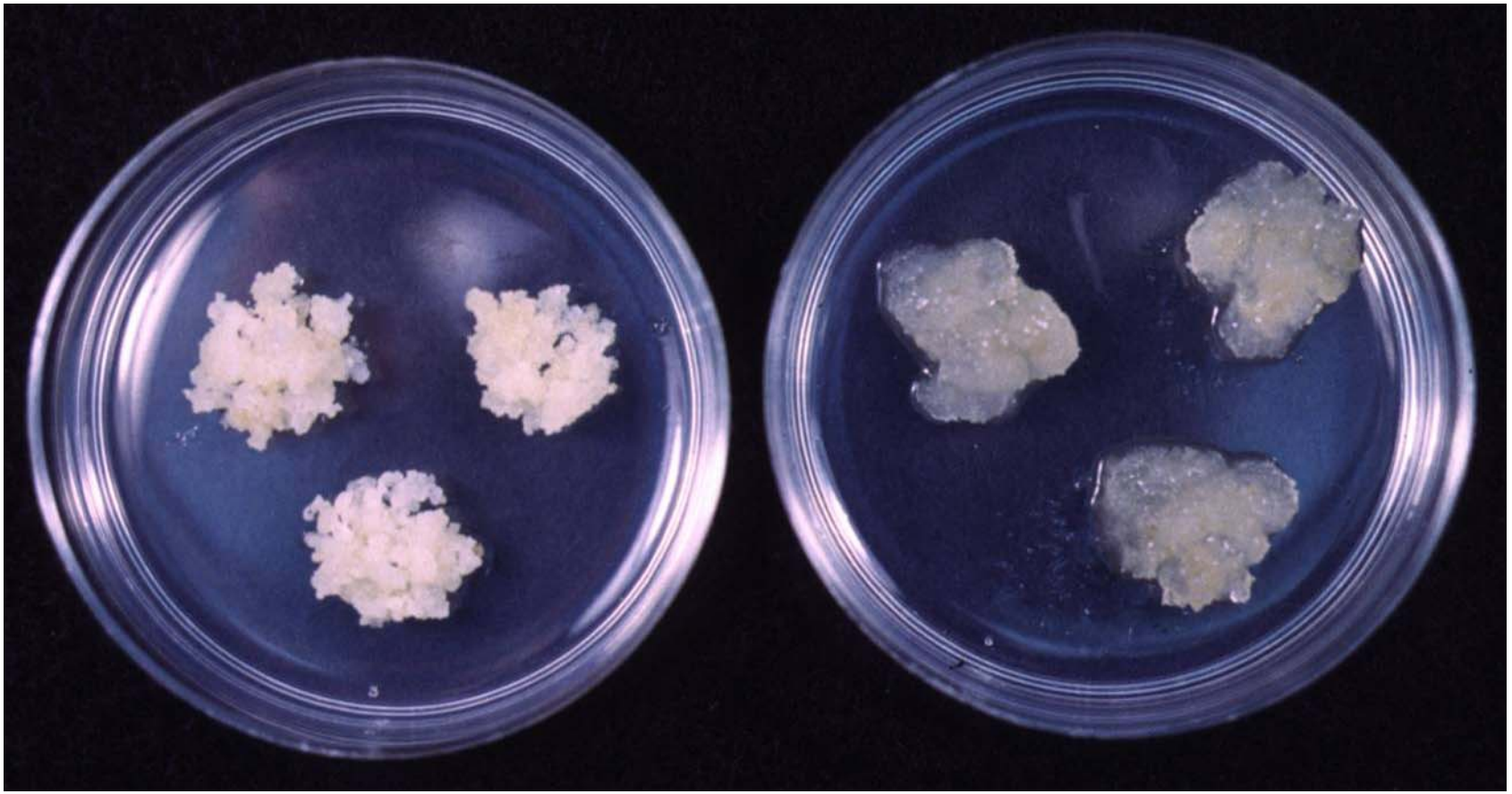
A tissue that develops in response to injury caused by physical or chemical means, most cells of which are differentiated although they may be and often are highly unorganized within the tissue. Callus differs in compactness or looseness, i.e. cells may be tightly joined and the tissue mass is one solid piece or cells are loosely joined and individual cells readily separate (friable). This can be due to the genotype or the medium composition. A friable callus is often used to initiate a liquid cell suspension culture

- Callus is formed at the peripheral surfaces as a result of wounding and hormones (auxin, high auxin/low cytokinin).
- Genotype, composition of nutrient medium, and physical growth factors are important for callus formation.
- Explants with high mitotic activity are good for callus initiation.
- Immature tissues are more plastic than mature ones.
- The size and shape of the explants is also important.
- In some instances it is necessary to go through a callus phase prior to regeneration via somatic embryogenesis or organogenesis.
- Callus is ideal material for in vitro selection of useful somaclonal variants (genetic or epigenetic)
- A friable callus is often used to initiate a liquid cell suspension culture for production of metabolites
- Friable callus is a source of protoplasts.

Genotypic Effects on Callus Morphology

Arabidopsis **Tobacco**

3.0 mg/L 2,4-D



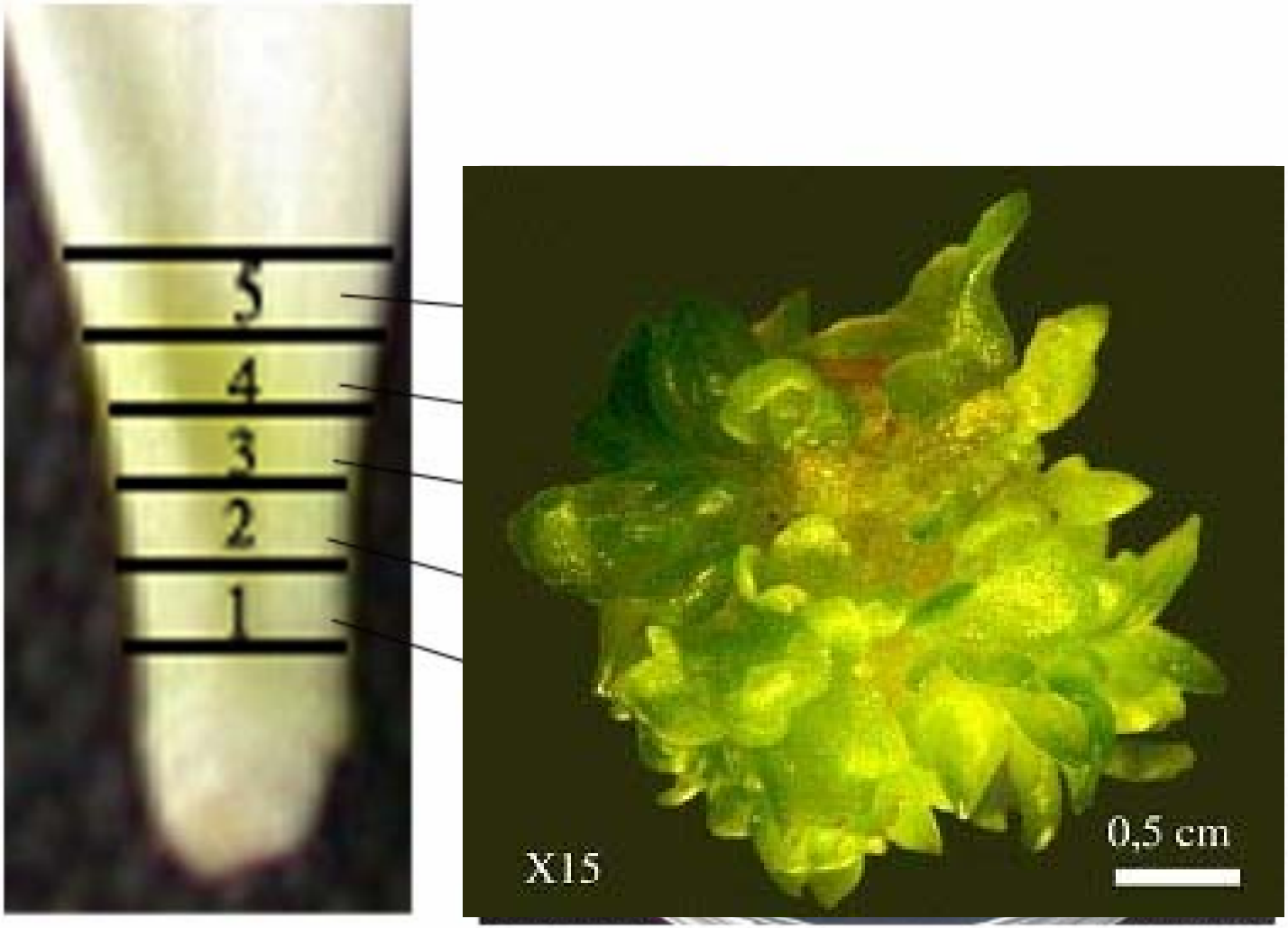
Compact Callus Friable Callus

Organogenesis

Process of differentiation by which plant organs are formed (roots, shoot, buds, stem etc.)

- ✓ *Adventitious refers here to the development of organs or embryos from unusual points of origin of an organized explants where a preformed meristem is lacking*
- ✓ *Adventitious shoots or roots are induced on tissues that normally do not produce these organs*
- ✓ *Plant development through organogenesis is the formation of organs either de novo (from callus) or adventitious (from the explants) in origin.*

Induce of *adventitious shoots on Petals explants of Chrysanthemum*



Direct adventitious organ formation

The somatic tissues of higher plants are capable, under certain conditions, of regenerating adventitious plants

The formation of adventitious organs will depend on the reactivation of genes concerned with the embryonic phase of development

Adventitious buds are those which arise directly from a plant organ or a piece thereof without an intervening callus phase

Suitable for herbaceous plants: Begonia (buds from leaves), most frequently used micropropagation system

Somatic embryogenesis

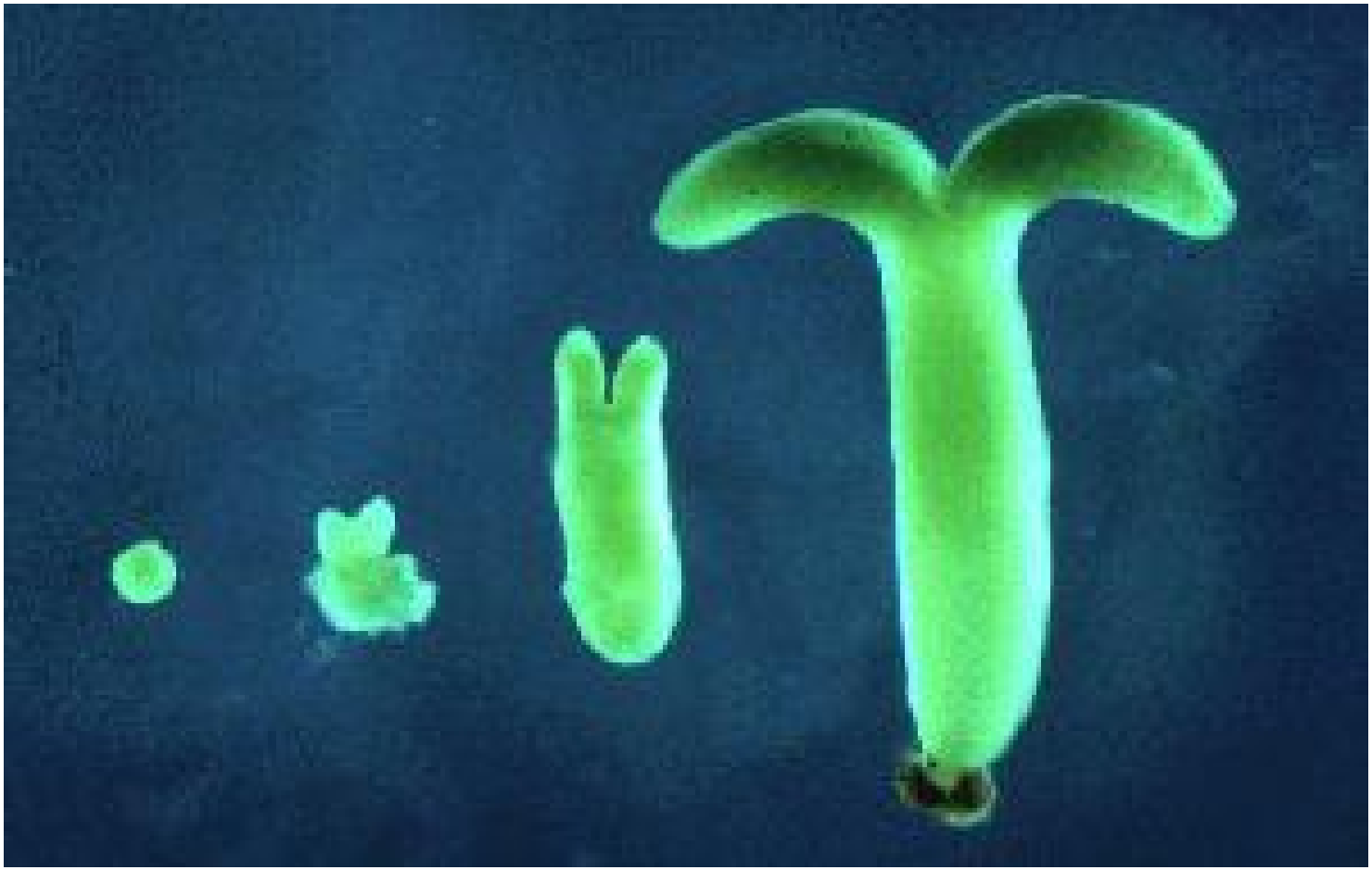
Somatic embryogenesis differs from organogenesis in the embryo, being a bipolar structure rather than monopolar.

The embryo arises from a single cell and has no vascular connections with the maternal callus tissue or the cultured explants.

For some species any part of the plant body serves as an explants for embryogenesis (e.g. carrot) whereas in some species only certain regions of the plant body may respond in culture (e.g. cereals).

Direct embryogenesis of coffee leaf

Morphological statement of embryogenesis in soybean



Floral and reproductive tissues in general have proven to be excellent source of embryogenic material.

Further, induction of somatic embryogenesis requires a single hormonal signal while in the organogenesis two different hormonal signals are needed to induce first a shoot organ, then a root organ.

The presence of auxin is always essential,

- Cytokinins, L-glutamine play an important role, enhance the process of embryogenesis in some species.
- Addition of activated charcoal to the medium is useful in lowerring phenylacetic acid and benzoic acid compounds which inhibit somatic embryogenesis.

Two routes to somatic embryogenesis

1. **Direct embryogenesis**

   The embryo initiates directly from the explant tissue through ″pre-embryogenic determined cells.″

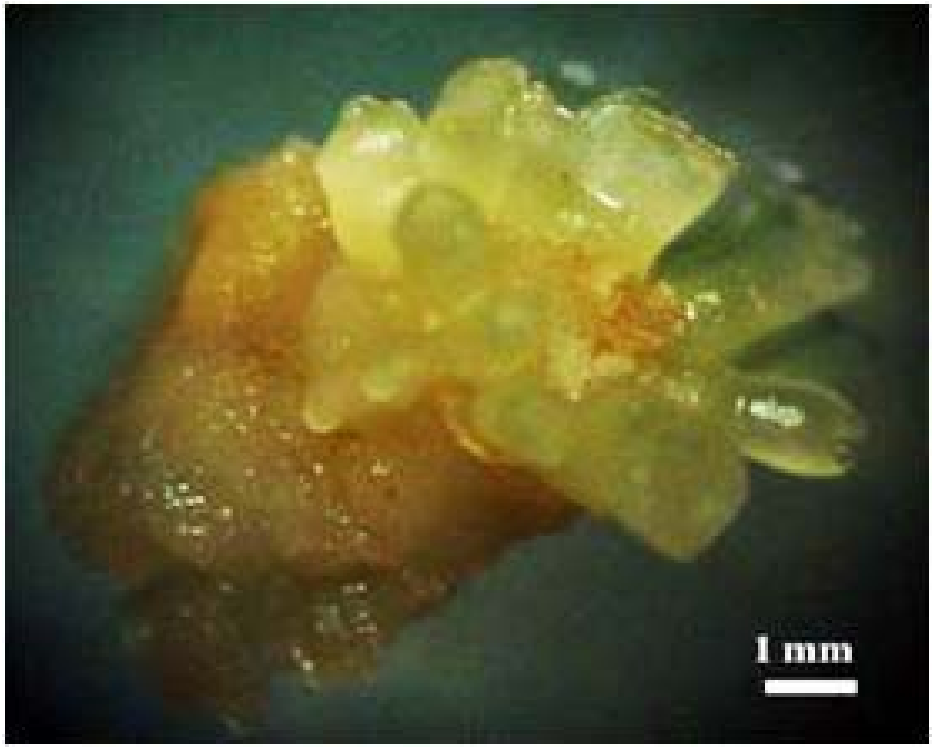
   Such cells are found in embryonic tissues (e.g. scutellum of cereals), hypocotyls and nucellus.

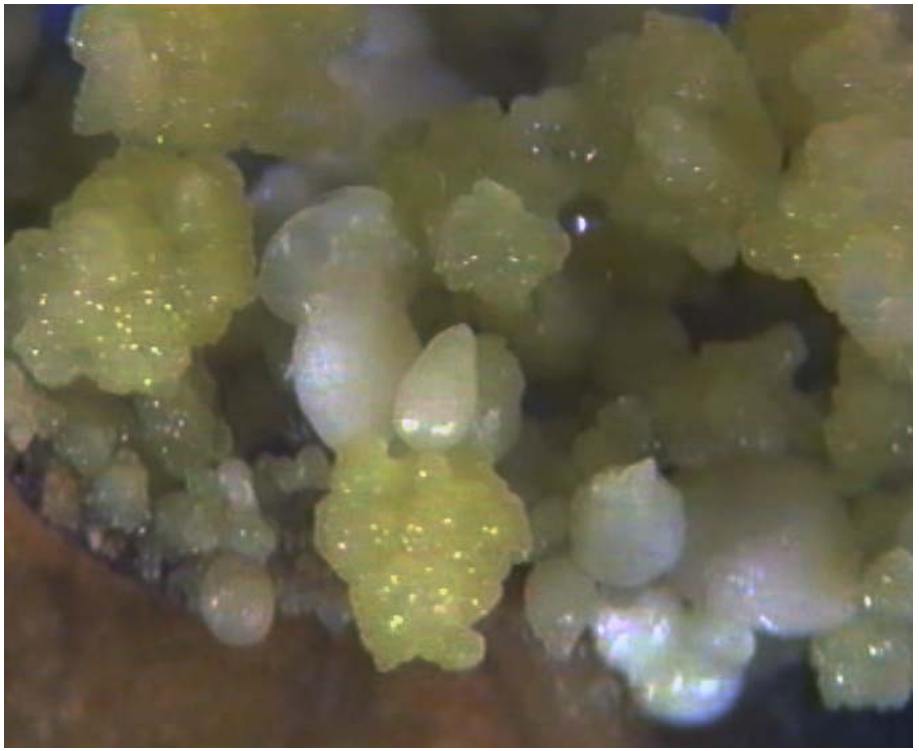
2. **Indirect embryogenesis**

   Cell proliferation,i.e. callus from explant, takes place from which embryos are developed.

   The embryo arises from ″induced embryogenic determined cells.”

e.g. Direct embryogenesis (in cassava) and indirect embryogenesis (in coffee)





Plant regeneration categories

1. *Enhanced release of axillary bud proliferation, multiplication through growth and proliferation of existing meristem.*
2. ***Organogenesis** is the formation of individual organs (shoots, roots, flower ....) either directly on the explants where a preformed meristem is lacking or de novo origin from callus and cell culture induced from the explants.*
3. ***Somatic embryogenesis** is the formation of a bipolar structure containing both shoot and root meristem either directly from the explants (adventitive origin) or de novo origin from callus and cell culture induced from the explants.*

e.g. Indirect shoot formatiom from callus of tobacco

- ✓ *Somatic embryogenesis:* Not used often in plant propagation because there is a high probability of mutations arising.
- ✓ The method is usually rather difficult.
- ✓ The chances of losing regenerative capacity become greater with repeated subcultures
- ✓ Induction of embryogenesis is often very difficult or impossible with many plant species.
- ✓ A deep dormancy often occurs.

Clonal propagation

The success of many in vitro selection and genetic maniplation techniques in higher plants depends on the success of in vitro plant regeneration.

A large number of plants can be produced (cloned) starting from a single individual:

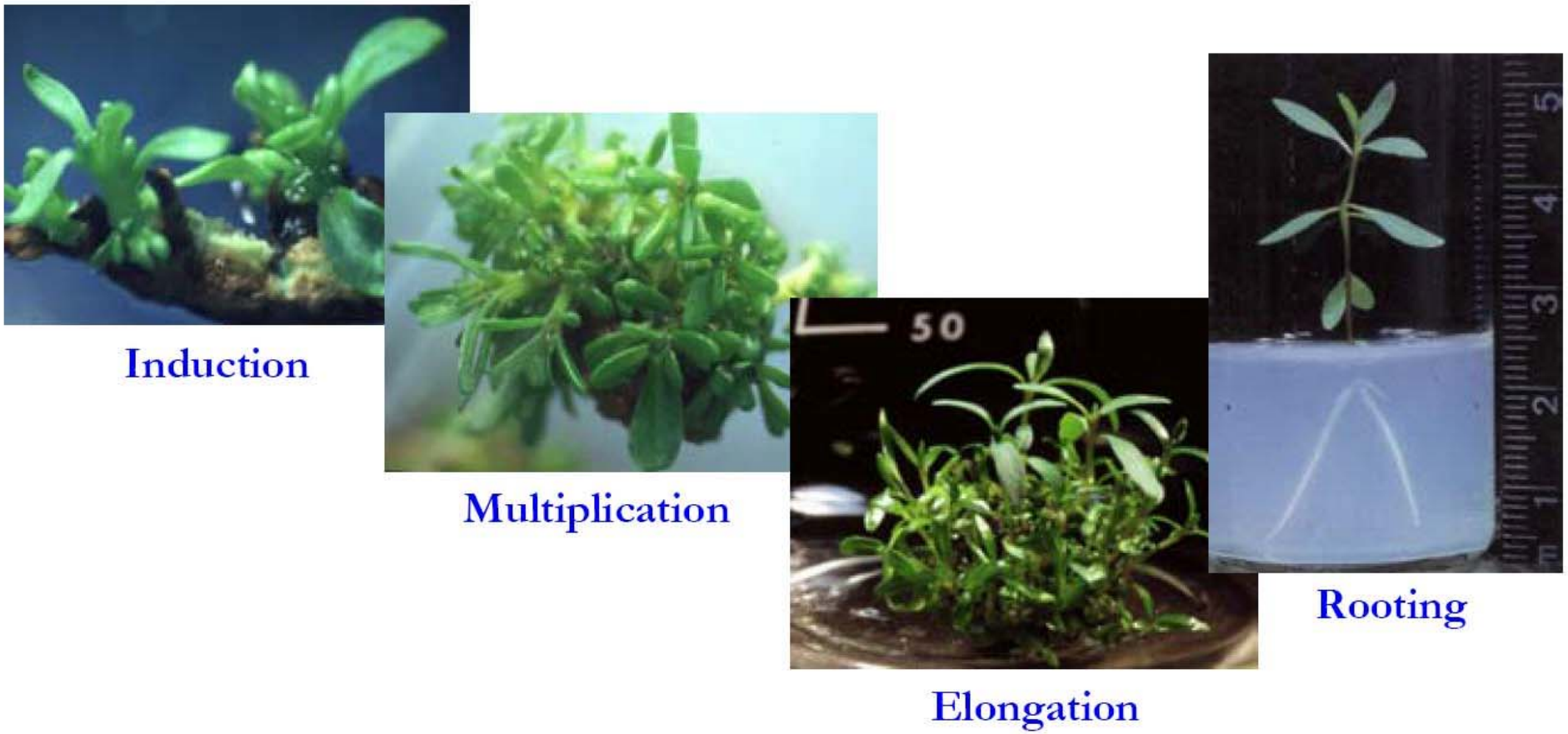
1,000,000 propagules in 6 months from a single plant

Vegetative (asexual) methods of propagation → crop improvement

Stages in micropropagation

1. *Selection of suitable explants, their sterilization, and transfer to nutrient media*
2. *Proliferation or multiplication of shoots from the explant*
3. *Transfer of shoots to a rooting medium followed later by planting into soil*

Clonal propagation in plants



Advantages of clonal propagation

- Mass clonal propagation: Rather than 1M propagules in 6 months from a single plant, which actually impossible in the natural world.

Orchids one of first crops to which propagation was applied

- Propagation of difficult to root plants
  - Woody plants - pears, cherry, hardwoods
- Introduction of new cultivars
  - Decreases time from first selection to commercial use by about half
  - Very useful in bulb crops - freesia, narcissus

Vegetative propagation of parent plants used for hybrid seed

- *Repeated selfing of parents leads to inline depression*
- *Undesirable traits emerge, loss of vigor over time*
- *Used in cabbage seed production*

Eradication of viruses, fungi, bacteria: First used by Morel in dahlia- Found to be useful in orchids. Used in a great many horticultural crops.

Without this technique there is no other way of eradicating many of the viruses, fungi, bacteria that infect plant tissues.

Storage of germplasm

- Uses considerably less space than land
  - Consider the area required for fruit trees
- May be possible to reduce mutations to zero
  - In the field there is always a chance of bud sports or other mutations developing
  - Storage in cold room still has chance of mutation because of slow growth
- The ideal germplasm storage is at temperature of liquid nitrogen
  - All cellular activity is halted

2.3. Applications in Plant tissue culture

Seed culture

Important in propagation of orchids

In nature, germination of orchid seedlings is dependent on a symbiotic relationship with a fungus.

However, in vitro it is possible to be independent of the fungus by substituting its action with a nutrient medium (= asymbiotic germination).

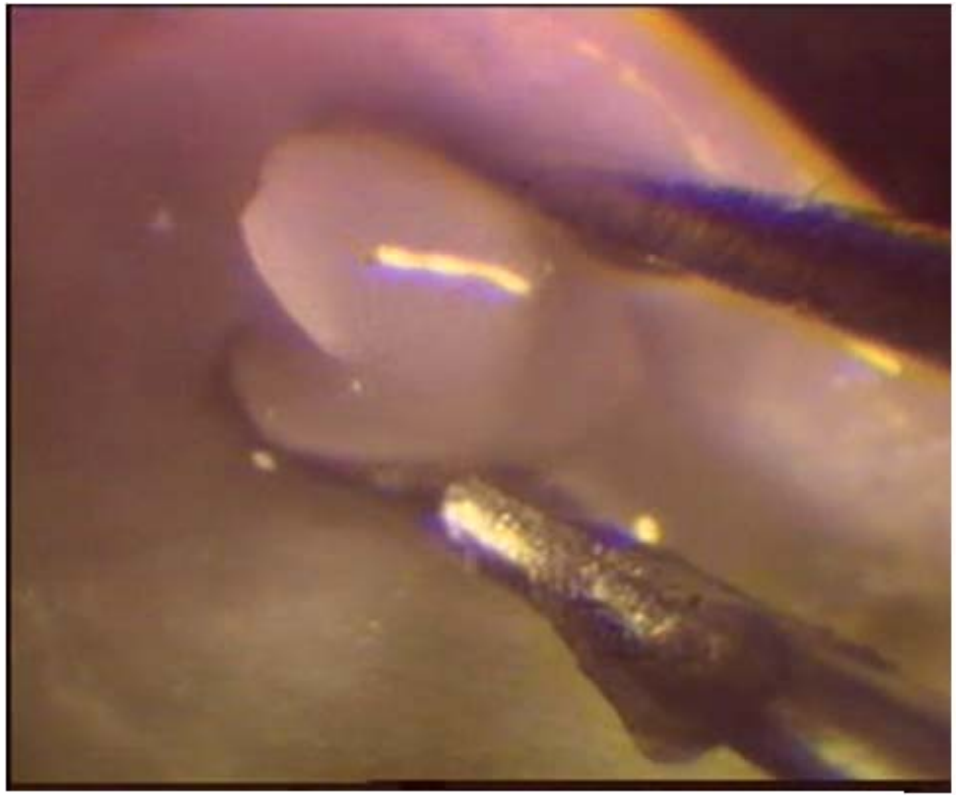
Orchid cloning in vivo is a very slow process; therefore, seed cultures are carried out on a large scale

→ germination and development much quicker in vitro (no competition with fungi or bacteria)

Embryo Culture

- ✓ *Embryo culture is a sterile isolation and growth of an immature or mature embryo in vitro, with the goal of obtaining a viable plant.*
- ✓ *Embryo abortion in wide crosses often occurs during embryogeny (e.g. endosperm degradation) and it is sometimes possible to culture these embryo and recover hybrid plants.*
- ✓ *Embryo culture may include the culture of embryos within an ovule or ovary. In these instances test-tube fertilization may overcome stigmatal or stylar, and pollen incompatibility barriers.*

e.g. embryo culture of potato



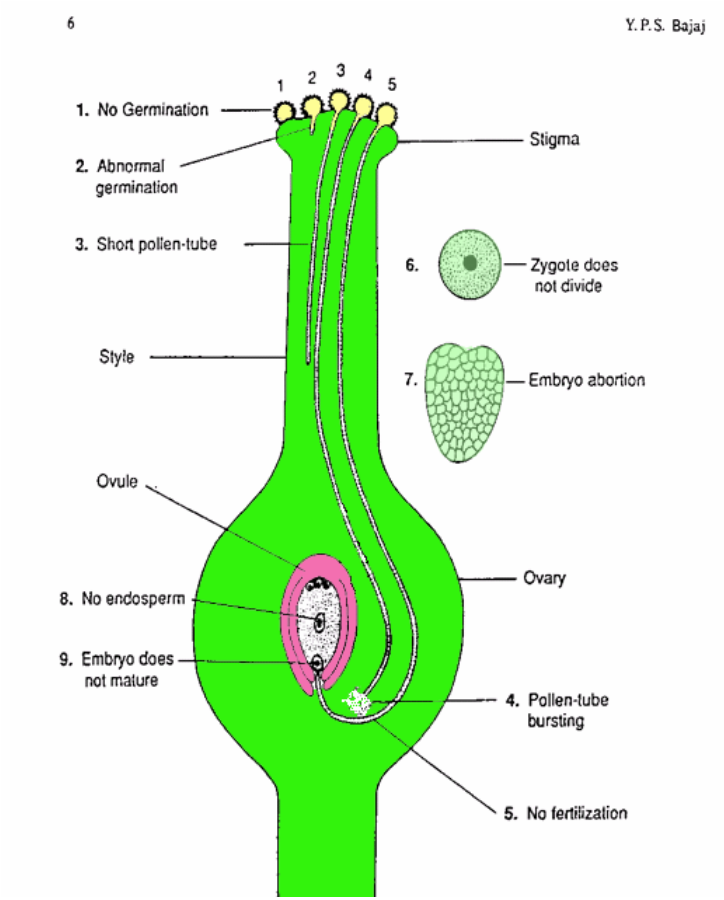
Mature embryo culture:

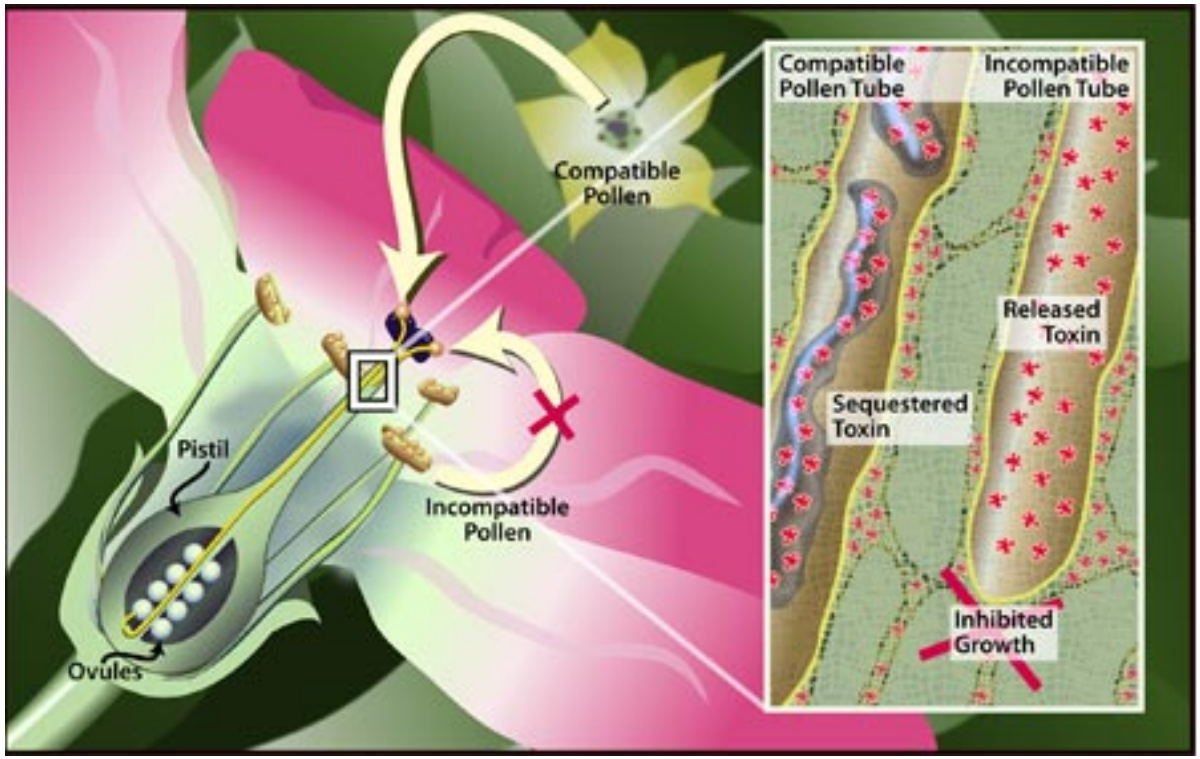
- *mature embryos derived from ripe seeds, autotrophic, grow on a simple inorganic medium*
- *seed dormancy can be avoided*

Immature embryo culture:

- *production of interspecies and intergeneric hybrids, particularly in gene transfers from wild species to cultivated ones*
- *embryo rescue = avoidance of embryo abortion due to post-fertilization barriers*

(failure of hybrid endosperm to develop properly → starvation)





Source: *www.nfs.gov*

Schematic representation showing various causes of incompatibility where in vitro technology can be applied for wide hybridization

Applications of embryo culture

1. Prevention of embryo abortion in wide crosses

- successful interspecific hybrids: cotton, tomato, rice, legume, barley

- intergeneric hybrids: wheat x barley, wheat x rye, maize x Tripsacum

2. Shortening of breeding cycle

- removing the seed coat

3. Prevention of embryo abortion with early ripening stone fruits

- avocado, peach, plum, cherry, apricot

4. *Seed dormancy is due to endogenous inhibitors, specific light requirements, low temperature, dry storage requirements and embryo immaturity.*

   A potential use of the technique is the production of seedlings from seeds naturally vegetatively propagated plants such as bananas (Musa balbisiana)

5. *Embryos are excellent materials for in vitro clonal propagation.*

   - especially in conifers, Gramineae-family

6. *Production of haploids*

Organ culture

It can be given different names depending upon the organ used as an explant:

-meristem culture,

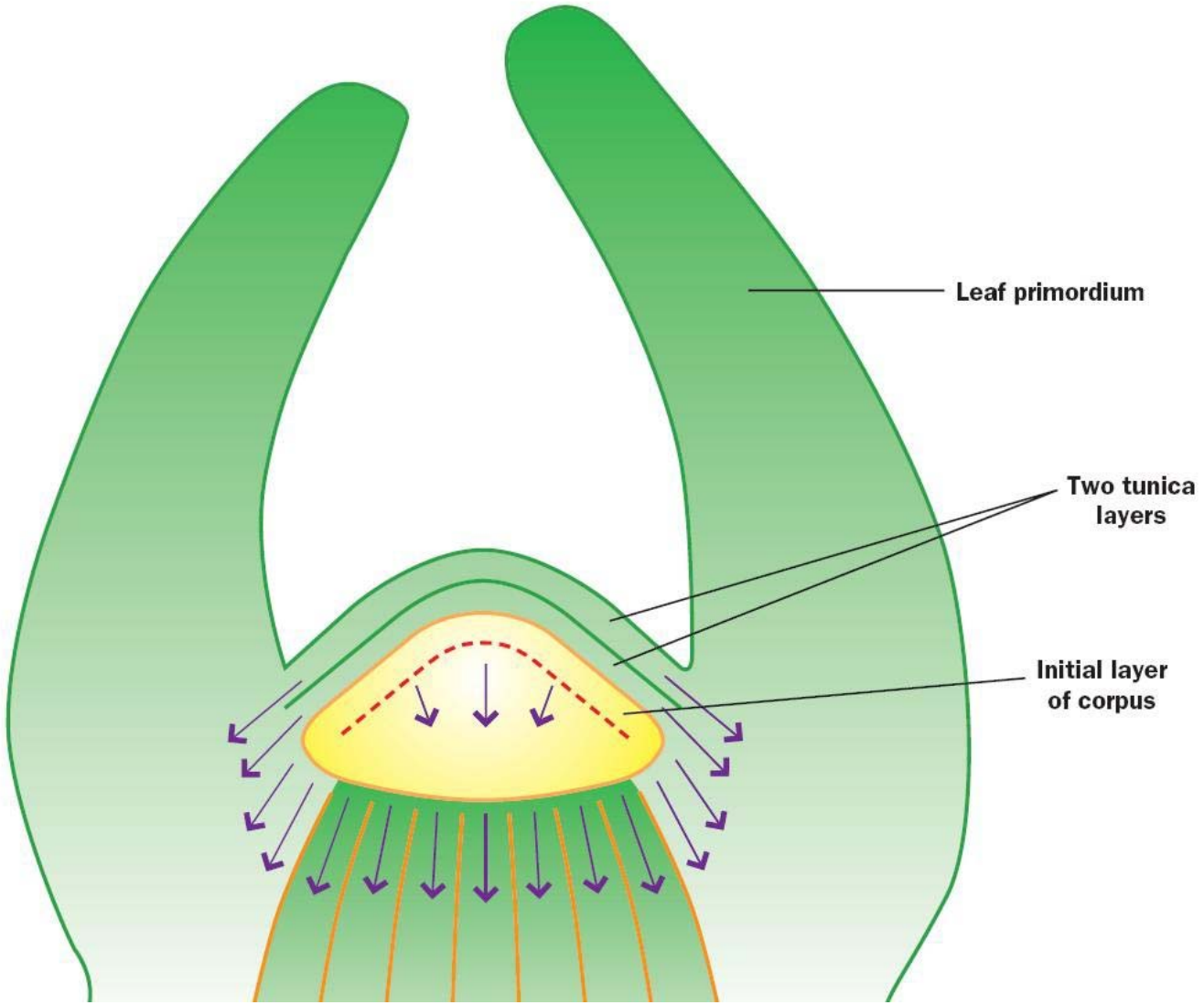
-anther culture (→ androgenic haploids),

-ovule culture (→ gynogenic haploids),

-nucellus culture,

--endosperm culture.

Meristem culture



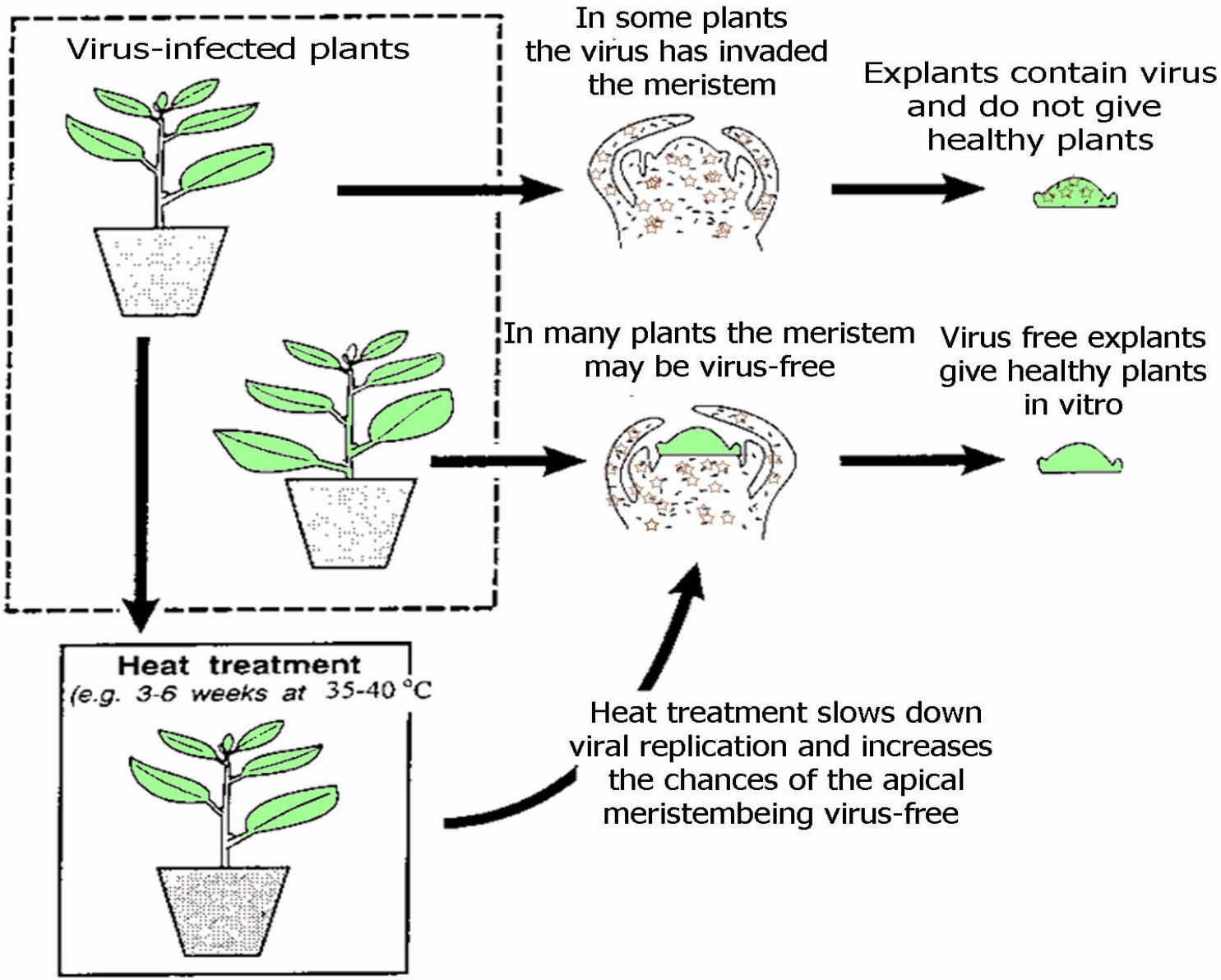
Advantages of Meristem Culture

- 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



Strategies for obtaining virus-free plants by meristem culture

Anther and microspore culture

Haploid plants *are derived from microspores (pollen) cultured individually or in anthers*

Processes Leading to Production of Haploid Plants:

- ***Androgenesis:*** *haploid plant derived from male gamete, most common method in vitro*
- ***Parthenogenesis:*** *from unfertilized egg*

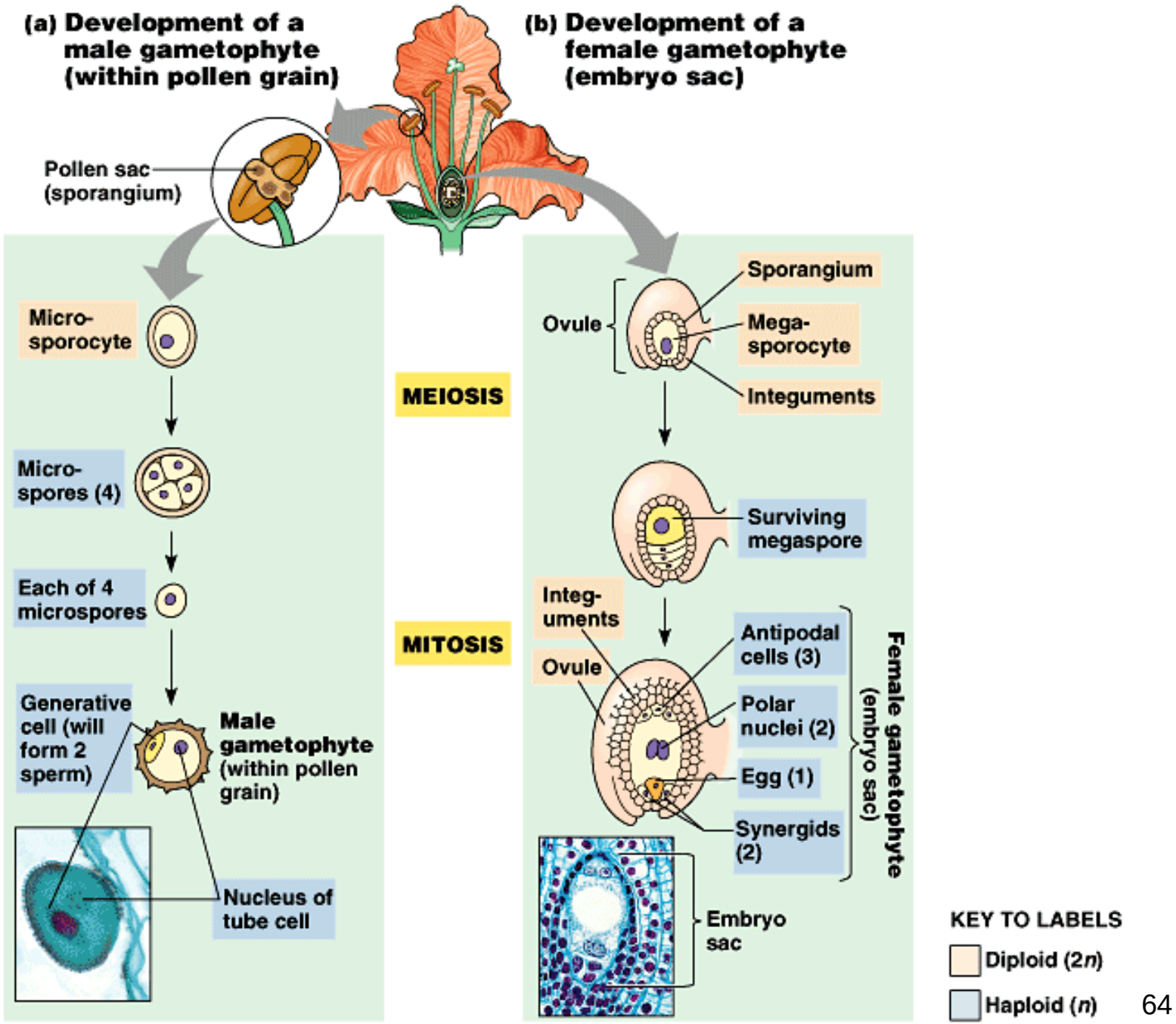
Chromosome elimination:

chromosome elimination in somatic cells, most common method used with plant breeding

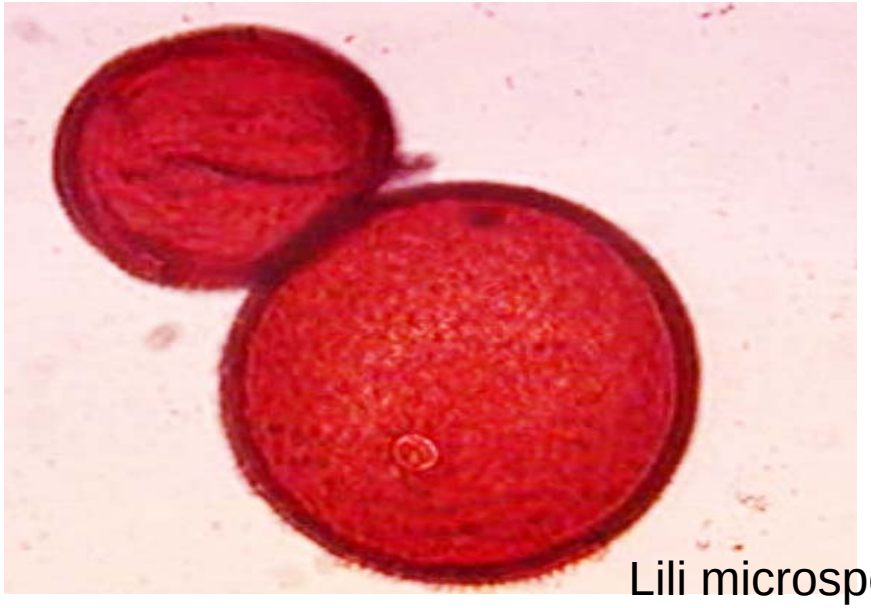
- ✓ *Haploids are very valuable in plant breeding for several reasons*
- ✓ *Since they carry only one allele of each gene, mutations and recessive characteristics are expressed in the plant.*
- ✓ *Plants with lethal genes are eliminated from the gene pool.*
- ✓ *Can produce homozygous diploid or polyploid plants - valuable in breeding*
- ✓ *Shorten the time for inbreeding for production of superior hybrids genotypes*

Production of haploid

- In vitro methods:
  - **Anther / microspores culture** (androgenesis) - production of haploid plants from microspores
    - Anther culture for production of haploids reported in about 250 species
    - Solanaceae, Cruciferae, Gramineae, Ranunculaceae most common
  - **Ovule culture** (gynogenesis) - production of haploid plants from unfertilized egg cell



Pollen sac of Lili



Lili microspores

Poppy ovule

Androgenic methods

From the male gametophyte of an angiosperm plant, i.e. microspore (immature pollen)

- The underlying principle is to stop the development of pollen cell → direct development in a plant (no gamete phasis)

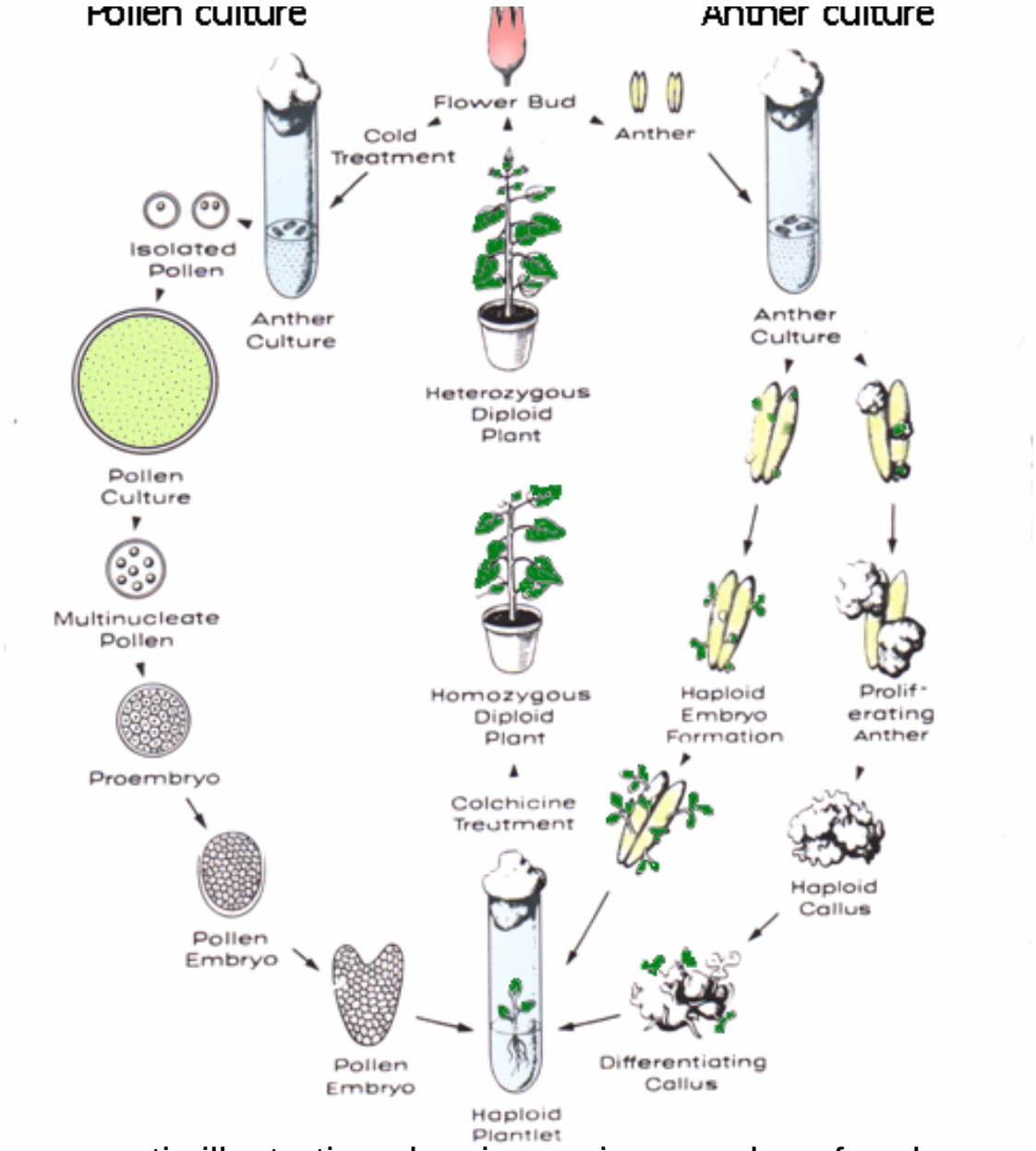
- Anther techniques are simple, quick and efficient:

- immature anthers sterilized

- acetocarmine test for pollen development

- solid media, pollen callus → shoots

- disadvantages: plants may originate from various parts of the anther → plants with various ploidy levels



Diagrammatic illustration showing various modes of androgenesis and haploid plant formation by anther and isolated pollen culture. The homozygous plants are obtained by treating haploids with colchichine. (Bajaj et al. 1983)

The composition of culture media

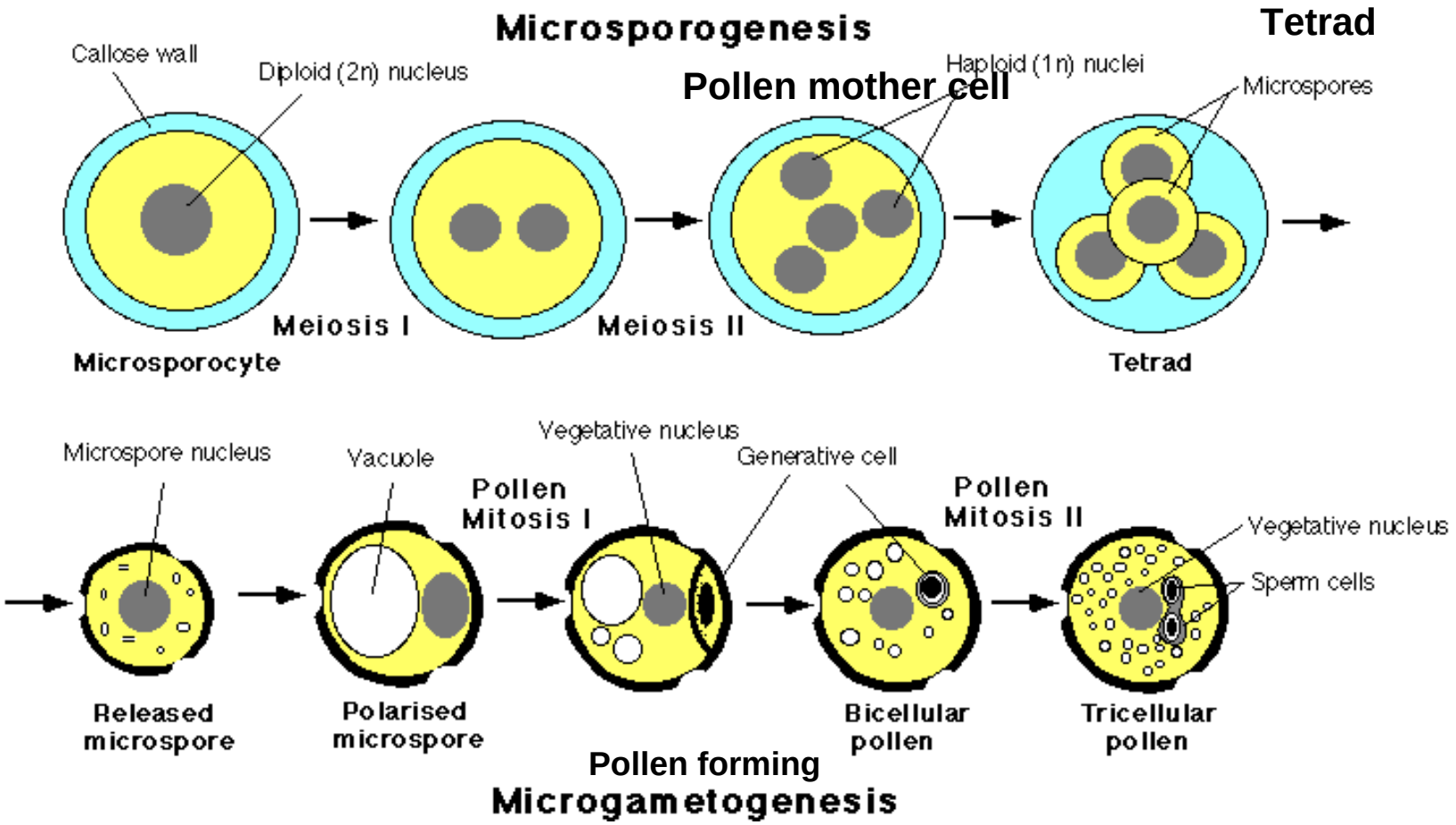
- *sucrose, N (glutamine), auxin/cytokinins*

Stage of pollen: anthers with microspores ranging from tetrad to the binucleate stage are responsive

- *optimum stage of pollen for each species*

Development of the polled can be stopped by taking the pollen away from its normal environment in the living plant and placing in other specific conditions

This induction is enhanced by giving certain treatments: cold pretreatment, hot treatment, chemical treatment (ethrel)



Source : *www.le.ac.uk*

Microspore culture

Haploid plants can also be produced through culture of male gametophytic cells i.e. microspores or immature pollen → embryo (directly) or via callus

Advantages:

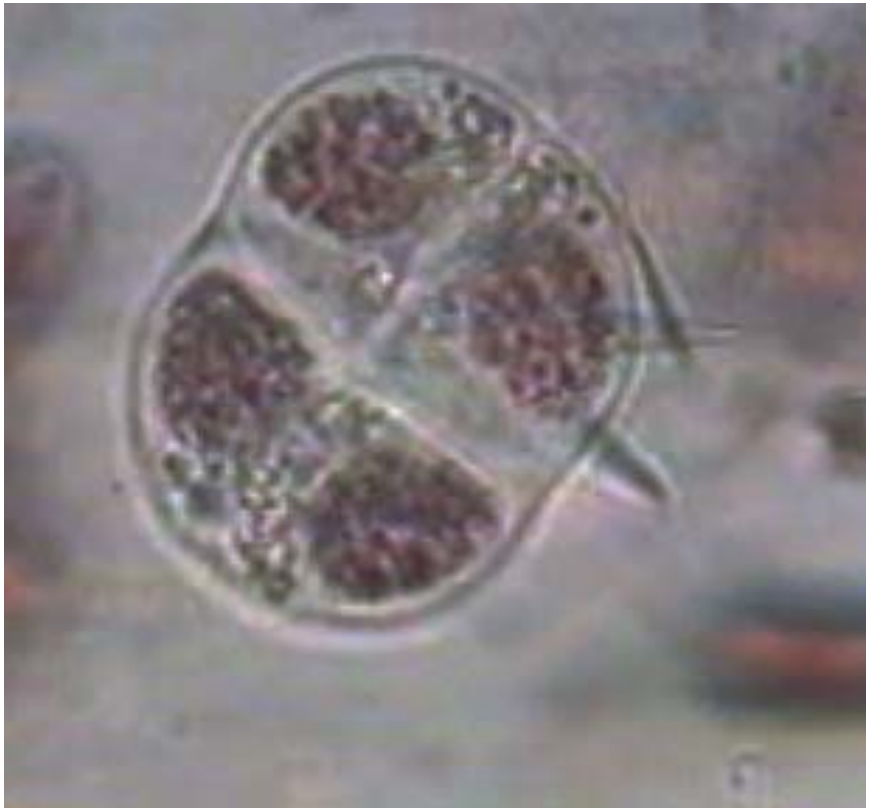
- uncontrolled effects of the anther wall and other associated tissue are eliminated

- the sequence of androgenesis can be observed starting from a single cell

- microspores are ideal for mutagenic and transformation studies

- high yield of plants per anther can be obtained

e.g. Microspore culture of rice



Tetrad stage

Embryos and callus

Success of androgenesis

Anther culture ability is genetically controlled:

- ✓ *testing various cultivars in a simple medium*
- ✓ *physiological status of the mother plant*
- ✓ *no pesticides*
- ✓ *optimal environmental conditions: light, photoperiod, temperature, nutrition, CO2 concentration*
- ✓ *outdoor plants more responsive than greenhouse material*
- ✓ *young plants better than old*

Gynogenic methods

Megaspores or female gametophytes of angiosperms can be triggered in vitro to sporophytic development.

- ✓ *Culture of unpollinated ovaries and ovules represents an alternative for the production of haploid plants in species for which anther culture has failed (e.g. albino plants).*
- ✓ *Not used as much as androgenic method.*
- ✓ *Problems in dissection of unfertilized ovules/ovaries.*
- ✓ *Promising for gymnosperms.*
- ✓ *In addition to unpollinated ovaries, pollinated can be also used in some cases.*

Nucellus culture

Nucellus culture has been utilized to study factors responsible for the formation of adventive embryos (the embryos arise adventitiously from cells of nucellus or integuments, e.g. citrus, mango)

The adventive embryos are of considerable importance to the horticulturists. Genetically uniform reproduce the characteristic of the maternal parent, they are disease-free clones retaining growth vigor and fruiting characteristics

Endosperm culture

Endosperm culture successful e.g. in maize, wheat, barley, apple

✓ *the induction of organogenesis has always been a challenging problem.*

✓ *applications: production of triploid plants (from triploid endosperm) e.g. in Citrus, banana, apple, tea, mulberry).*

✓ *endosperm culture can also be used as a nurse tissue for raising hybrid embryos.*

Cell suspension culture

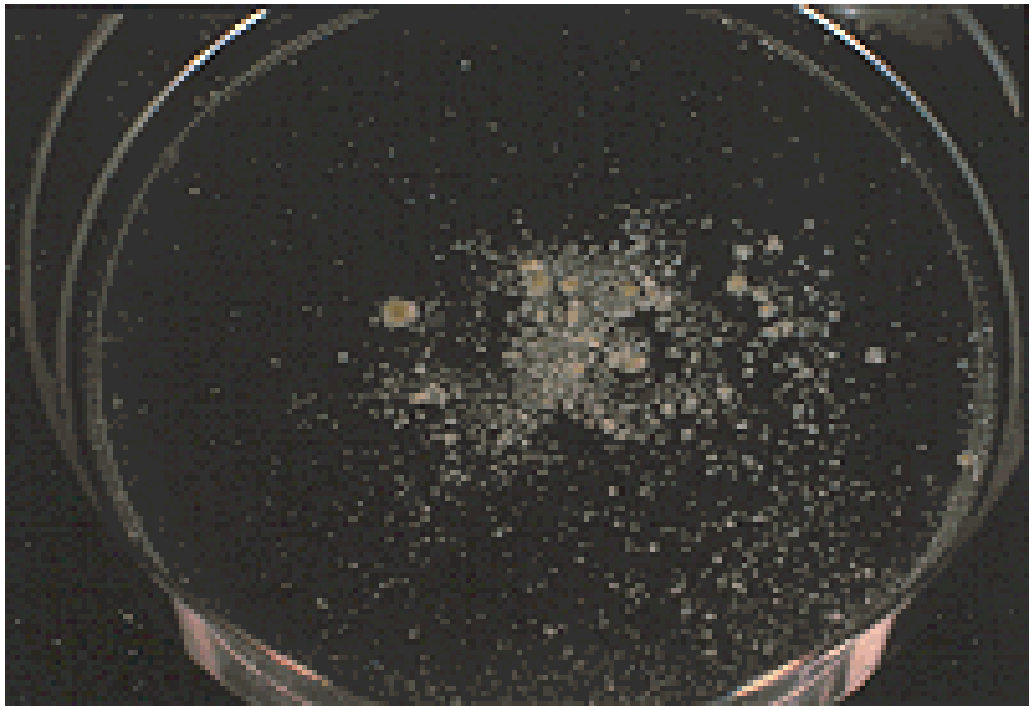
A friable callus is often used to initiate a liquid cell suspension culture .

Consists of cell aggregates dispersed and growing in moving liquid media

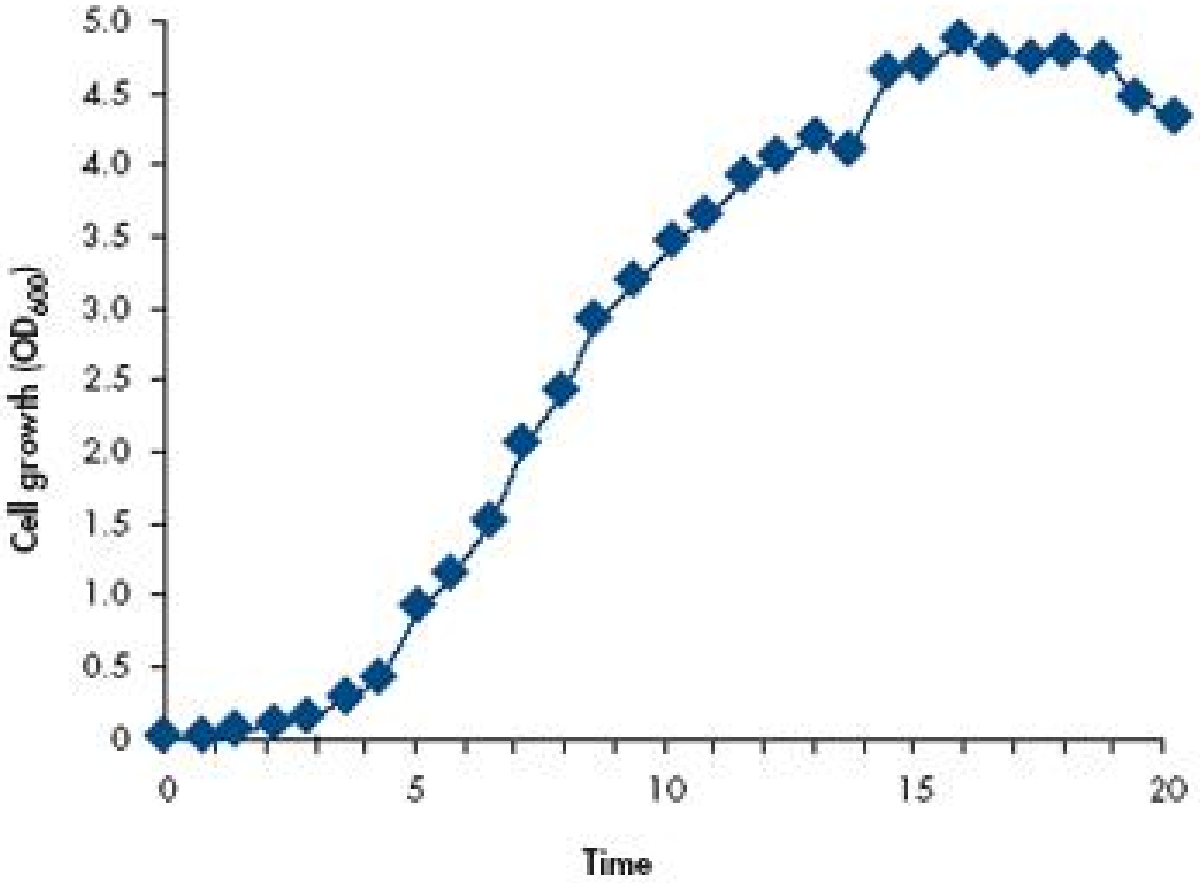
Agitation of medium in a shaker:

- *a mild pressure on cell aggregates breaking them into smaller clumps and single cells*
- *maintains uniform distribution of cell and cell clumps in the medium*
- *good gaseous exchange*

e.g. Cell suspension culture of *Helianthus* sp callus in Erlenmeyer flask.



Cells culture, five phases of growth



Source: *www.qiagen.com*

1. *Lag phase: cells prepare to divide*
2. *Exponential phase: the rate of cell division is highest*
3. *Linear phase: cell division slows but the rate of cell expansions increases*
4. *Deceleration phase: rates of cell division and elongation decreases*
5. *Stationary phase: number and size of cells remain constant*

Production of secondary metabolites

Two main routes:

1) The rapid growth of suspension cultures in large volumes

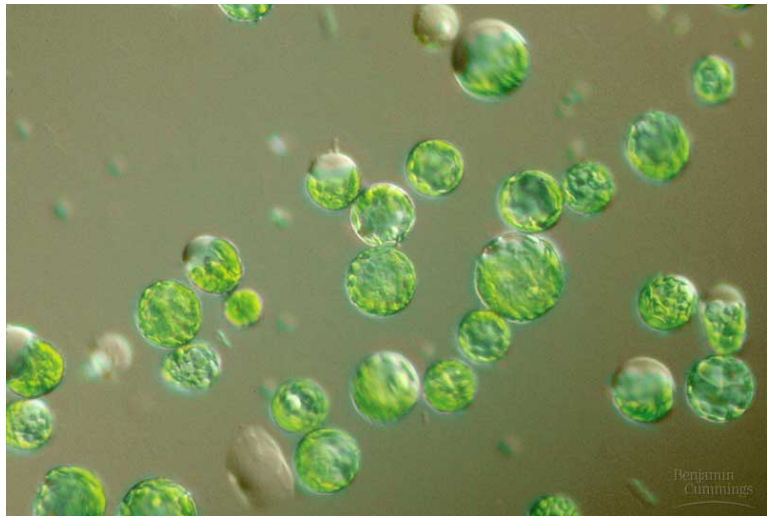
2) The growth and subsequent immobilization of cells, which are used for the production of compounds over a prolonged time

In many cases, secondary product synthesis of an intact plant cannot occur in rapidly growing undifferentiated cell cultures, but requires some degree of morphological or biochemical differentiation and slow growth.

- ✓ *Fragrances, flavours, natural sweeteners, pharmaceuticals, anti-microbial etc.*
- ✓ *Independent from various environmental factors (climate, pests, diseases)*
- ✓ *Any cell of a plant could be multiplied to yield specific metabolites*
- ✓ *A consistent product quality with the use of characterized cell lines*
- ✓ *New routes of synthesis from mutant cell lines → novel products*

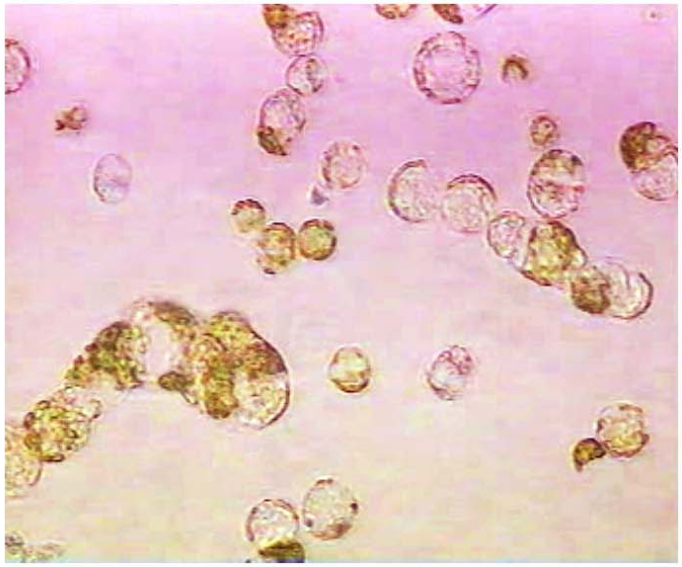
Protoplast Fusion

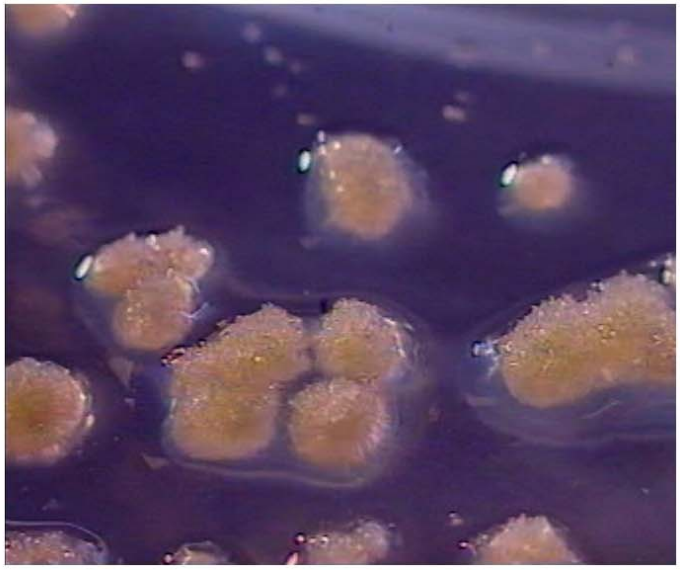
- Protoplasts are plant cells that have had their cell walls removed enzymatically by cellulases and pectinases.
- It is possible in some cases to fuse two protoplasts from different plant species that would otherwise be incompatible.
- The hybrids can regenerate a wall, be cultured, and produce a hybrid plantlet.



Source: *http://www.felix.ib.us.br*

Protoplast fusion and the regeneration of microcallus potato





Genetic transformation

- Many different explants can be used, depending on the plant species and its favored method of regeneration as well as the method of transformation
- Introduction of foreign DNA to generate novel (and typically desirable) genetic combinations
- Used to study the function of genes