Callus culture

Callus cells are not necessarily genetically homogeneous because a callus is often made from structural tissue , not individual cells

Plant callus (plural *calluses* or *calli*) is a mass of unorganized parenchyma cells derived from plant tissue (explants) for use in biological research and biotechnology. In plant biology, callus cells are those cells that cover a plant wound.

Callus formation is induced from plant tissues after surface sterilization and plating onto *in vitro* tissue culture medium. Plant growth regulators, such as auxins, cytokinins, and gibberellins, are supplemented into the medium to initiate callus formation or somatic embryogenesis.

Callus:

- **Definition:** It is an unspecialized and unorganized, growing and dividing mass of cells,.
- During callus formation there is some degree of dedifferentiation both in morphology and metabolism, resulting in the lose the ability to photosynthesis.
- Compact callus
- Friable callus

Plant callus is usually derived from somatic tissues. The tissues used to initiate callus formation depends on plant species and which tissues are available for explant culture.

The cells that give rise to callus and somatic embryos usually undergo rapid division or are partially undifferentiated such as meristematic tissue.

Plant hormones are used to initiate callus growth.

Morphology

Specific auxin to cytokinin ratios in plant tissue culture medium give rise to an unorganized growing and dividing mass of callus cells.

Callus cultures are often broadly classified as being either compact or friable. Friable calluses fall apart easily, and can be used to generate cell suspension cultures. Callus can directly undergo direct organogenesis and/or embryogenesis where the cells will form an entirely new plant.

- The explant is commonly cultured on a nutrient medium solidified in agar. Explants from most species of plants may be induced to divide in an unorganized manner on specifically formulated nutrient media
- An undifferentiated mass of cells, known as callus (plural, calli), is formed within 4 to 8 weeks.
- The callus may be divided, with clusters of cells transferred to fresh agar media to form subcultures. Repeated subculturing of the callus permits rapid multiplication of the cultured material.

- Plant regenerability may decline, and genetic stability of the plant material may be altered, with successive subculturing.
- Callus cultures are incubated under aseptic conditions, normally in dim light, with temperatures around 25°C.

Callus induction

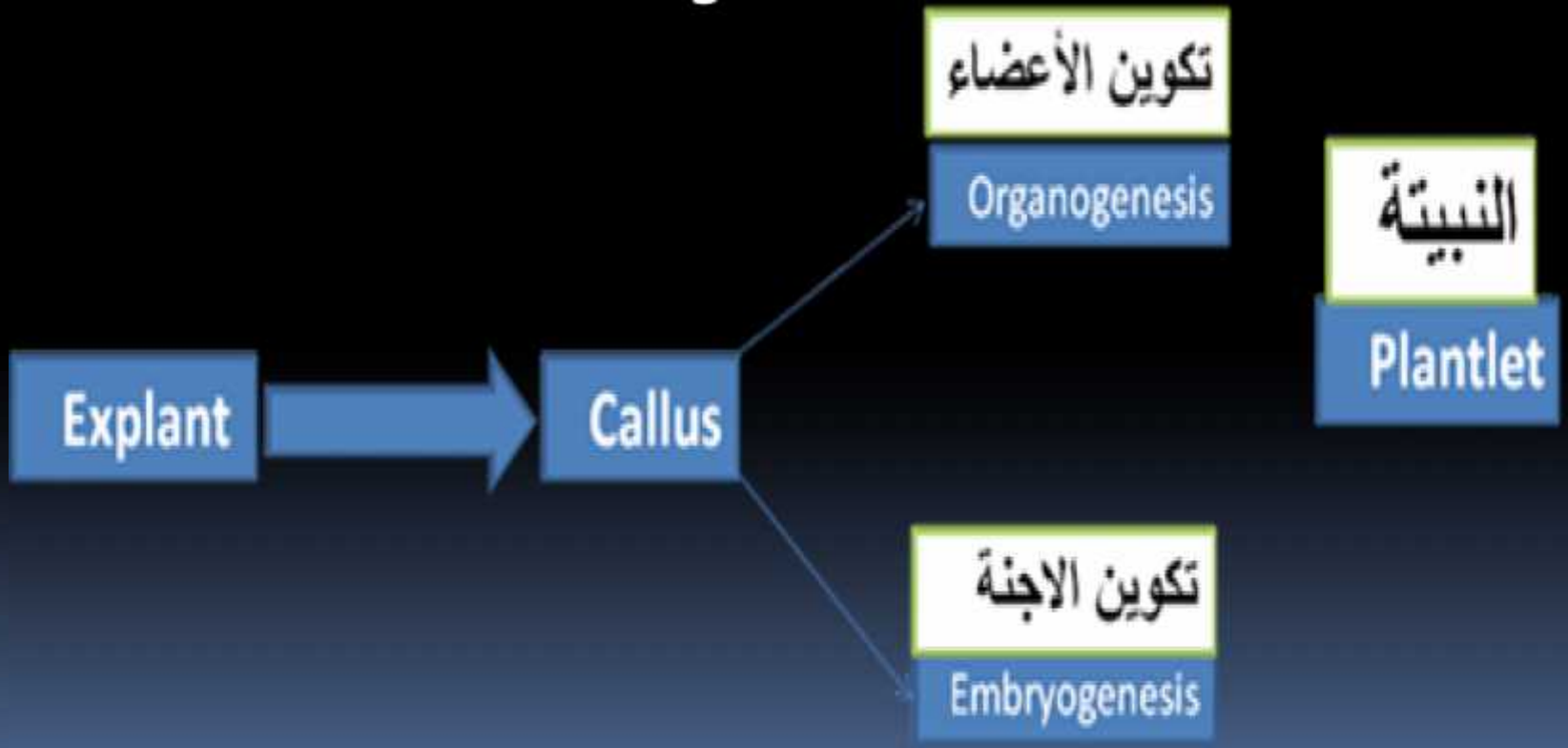
A callus cell culture is usually sustained on gel medium. Callus induction medium consists of agar and a mixture of macronutrients and micronutrients for the given cell type.

Murashige and Skoog medium,White's medium (woody plant medium).

Vitamins are also provided to enhance growth such as B5 vitamins.For plant cells, enrichment with nitrogen, phosphorus, and potassium is especially important.

Stages of initiation callus

- Induction stage
- Cell division stage
- Differentiation stage



Callus cells deaths

Callus can brown and die during culture, but the causes for callus browning are not well understood.

Browning has also been associated with oxidation and phenolic compounds in both explant tissues and explant secretions.

Uses

Nevertheless, callus cells are often considered similar enough for standard scientific analysis to be performed as if on a single subject. For example, an experiment may have half a callus undergo a treatment as the experimental group, while the other half undergoes a similar but non-active treatment as the control group

Plant calli can differentiate into a whole plant, a process called regeneration, through addition of plant hormones in culture medium. This ability is known as totipotency. Regeneration of a whole plant from a single cell allows researchers to recover whole plants that have a copy of the transgene in every cell.

Regeneration of a whole plant that has some genetically transformed cells and some untransformed cells is called a chimera. In general, chimeras are not useful for genetic research or agricultural applications.

- Genes can be inserted into callus cells using biolistic bombardment, also known as a gene gun, or *Agrobacterium tumefaciens*. Cells that receive the gene of interest can then be recovered into whole plants using a combination of plant hormones. The whole plants that are recovered can be used to experimentally determine gene function(s), or to enhance crop plant traits for modern agriculture.
- Callus is of particular use in micropropagation where it can be used to grow genetically identical copies of plants with desirable characteristics

Application of Callus Culture

1 . The whole plant can be regenerated in large number from callus tissue through manipulation of the nutrient and hormonal constituents in the culture medium which is called as organogenesis or morphogenesis. Similarly, callus can be induce to form somatic embryo which can gives rise to whole plant.

2 . Callus tissue is good source of genetic or karyotypic variability, so it may be possible to regenerate a plant from genetically variable cells of the callus tissue.

3 . Cell suspension culture in moving liquid medium can be initiated from callus culture.

4 . Callus culture is very useful to obtain commercially important secondary metabolites. If a bit tissue from a medicinally important plant is grown in vitro and produced callus culture, then secondary metabolites or drugs can be directly extracted from the callus tissues without sacrfting the whole plant.

5 . Several biochemical assays can be performed from callus culture.

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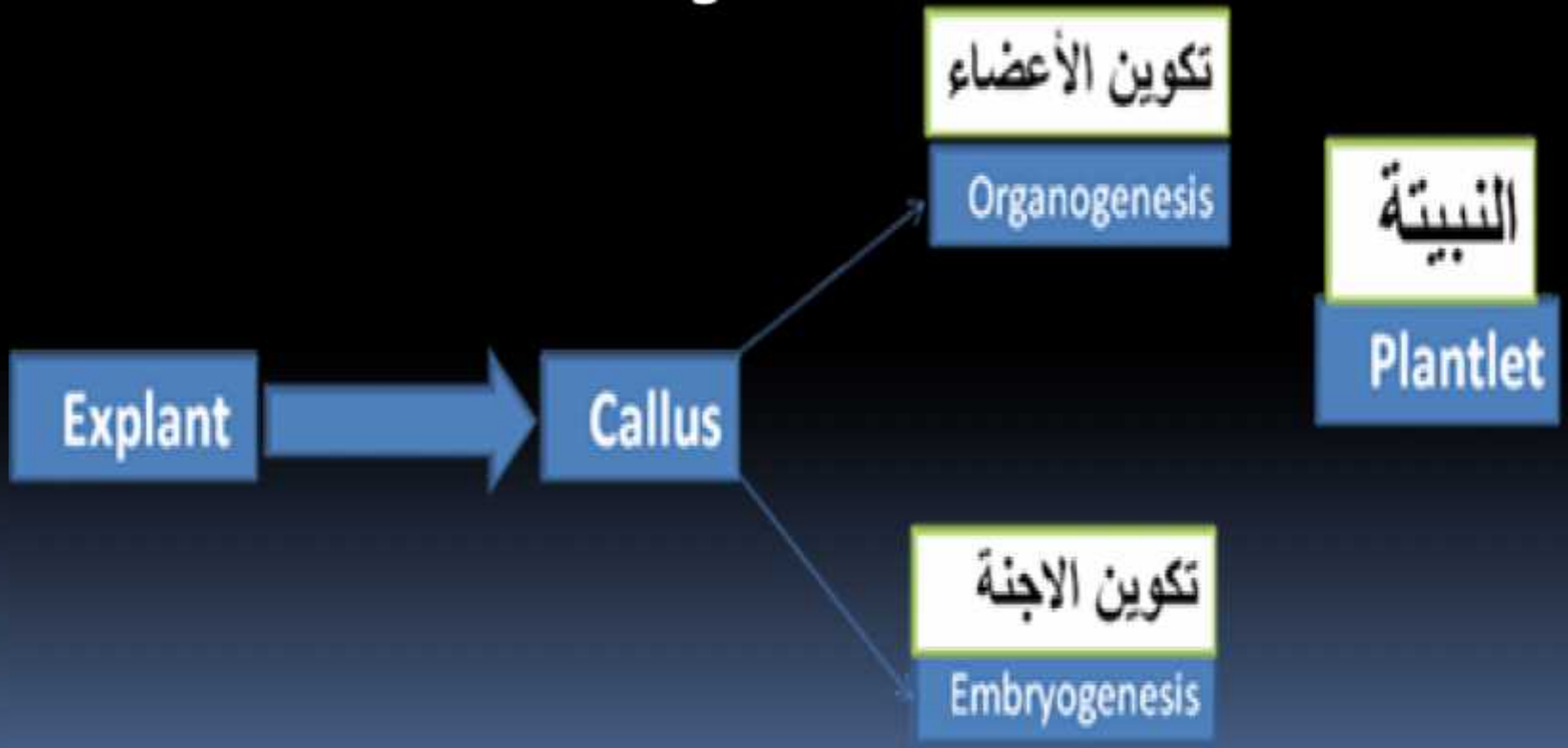

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In Vitro cell culture and Developmental Pathways

Explant

- Piece of tissue put into culture
-Tissue selection depends on purpose, species,
many factors

Explants

- Pieces of organs
  - Leaves
  - Stems
  - Roots
  - Cotyledons
  - Embryos
  - Other

Explants

- Specific cell types
  - Leaf tissue
  - Embryo
  - Pollen
  - Endosperm
  - Nucellus

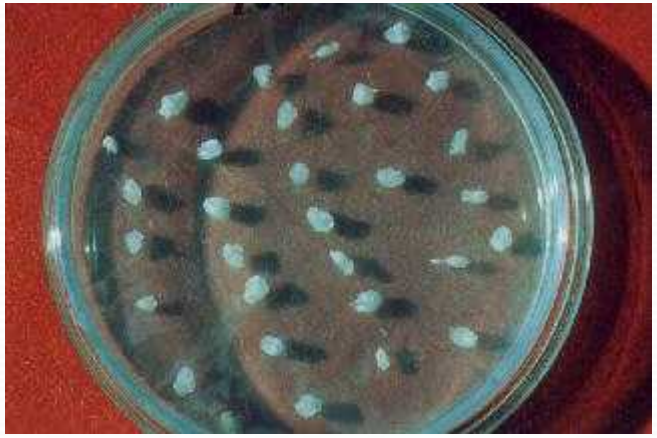
Callus

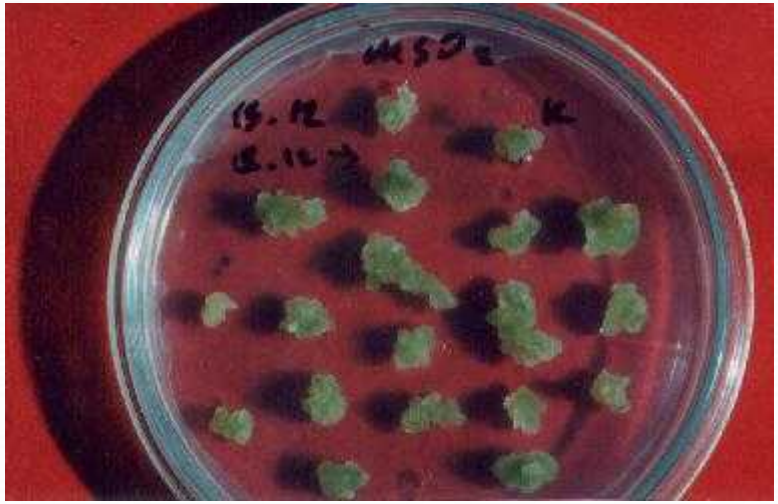
- Unorganized, growing mass of cells
- Dedifferentiation of explant
  - Loosely arranged thinned walled, outgrowths from explant
  - No predictable site of organization or differentiation
- Auxin + cytokinin
- Often can be maintained indefinitely by subculture, but may lose ability to redifferentiate
- Compact vs friable
- Habituation

Three stages of callus culture

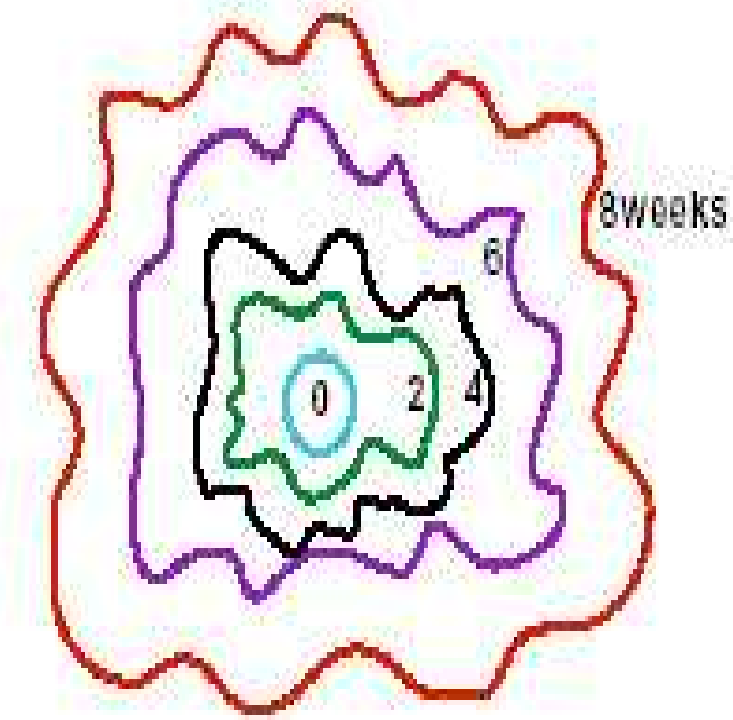
- Induction: Cells in explant dedifferentiate and begin to divide
- Proliferative Stage: Rapid cell division
- Differentiation stage (sometimes): organogenesis or embryogenesis

Induction





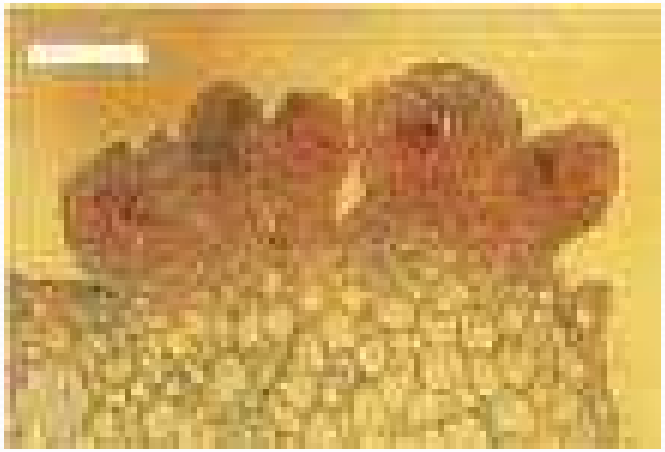
Division

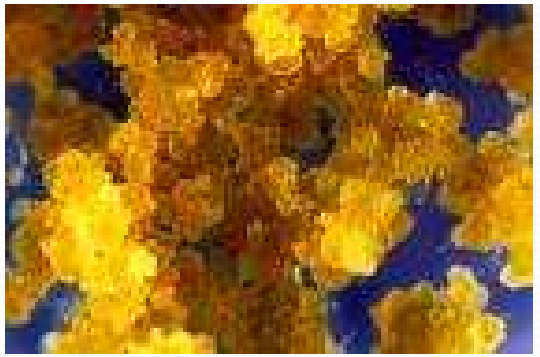


Growth of callus over time — t
8 weeks

E. Sutton, UC Davis

Callus





Differentiation

- Organogenesis
- Somatic embryogenesis

Cell and Suspension Culture

- **Cell Cultures?**
- **Suspension Cultures**

Suspension cultures

- **Can be initiated from any part of the plant.**
- **Usually initiated from friable callus already growing in culture.**
- **Transferred into liquid medium.**

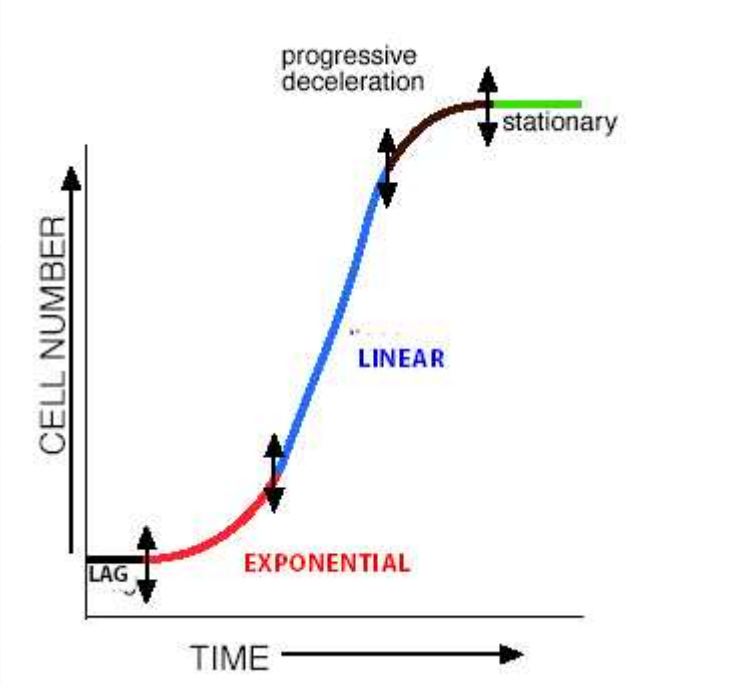
Agitation

- Breakdown of cell aggregates into smaller clumps of cells
- Maintains a uniform distribution of cells and cell clumps in the medium
- Provides gas exchange

Medium

- Same as for callus culture?
- Gamborg B5
- Conditioning

Growth Curve



E. Sutton, UC Davis

Batch Cultures

- A certain number of cells is used to inoculate the culture, in a given volume
- Erlenmeyer flask: volume should be about 20% of flask capacity for aeration.
- Roller cultures

Continuous Culture

- Bioreactors
- Closed continuous cultures: Remove some of the media and replace with fresh. Continuous removal or periodic. Terminate growth at harvest. Start over.
- Open continuous culture: Not only remove some of media, but cells too. Maintain cell density at optimal level. Can be grown for years.

Why is it possible to regenerate in vitro?

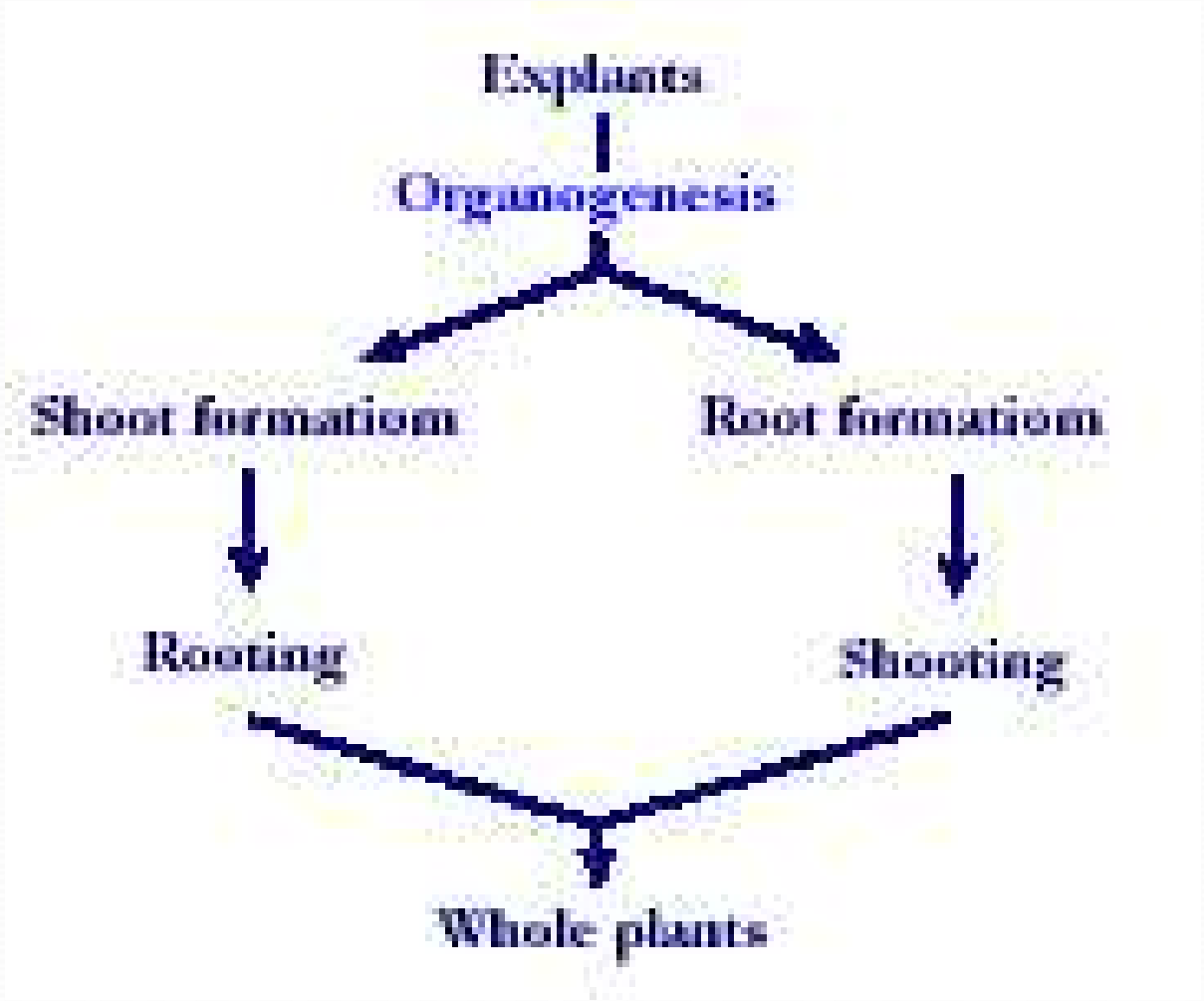
- **Totipotency**
  - **Initial state**
  - **Competence**
  - **Determination**
  - **Differentiation**

Only occurs in a few cells in culture. Why?

- Pre-determination prior to culture
- Newly formed meristems may act as sinks
- Meristematic centers might actually produce compounds that inhibit neighboring cells.

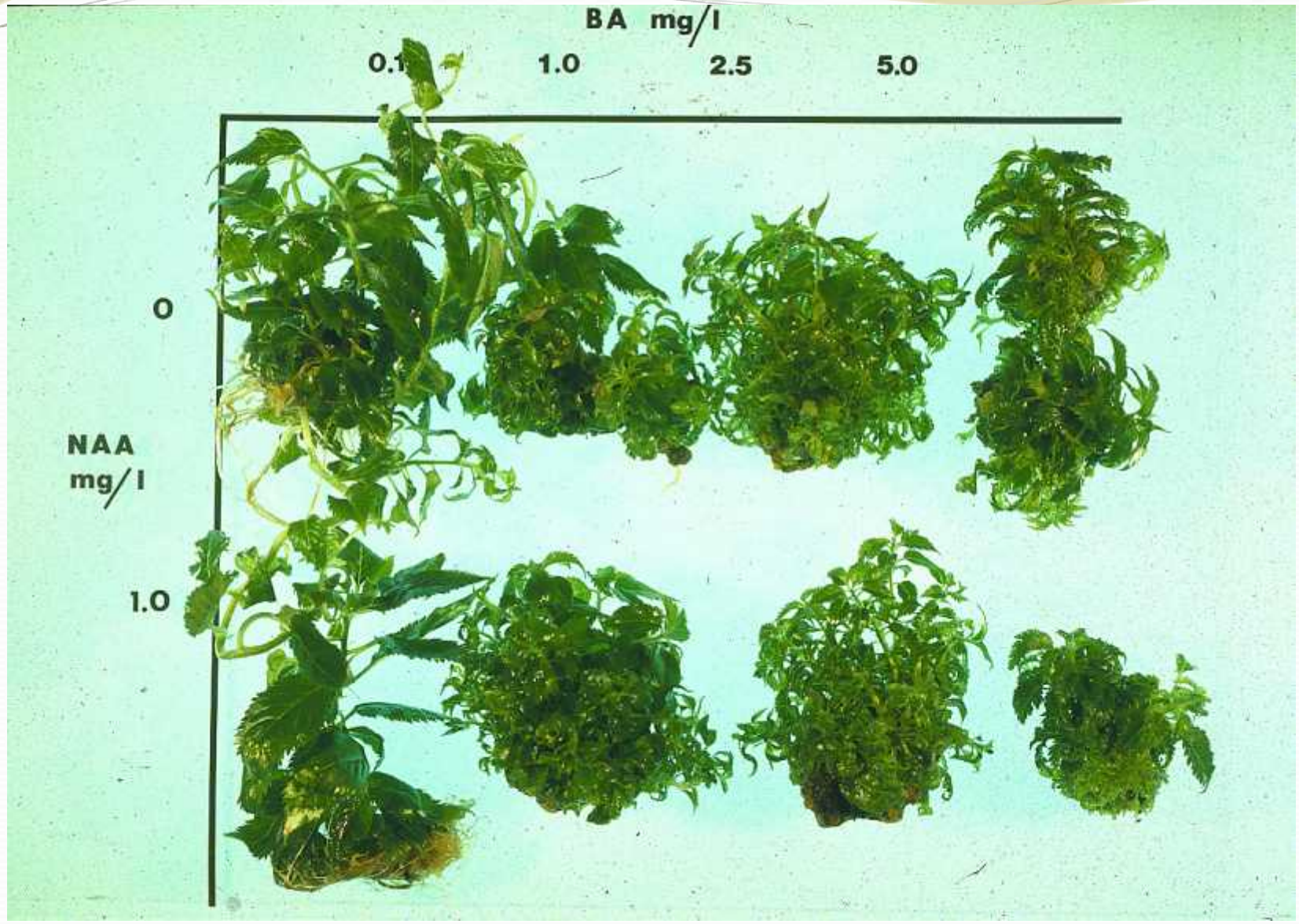
Organogenesis

The formation of organs (such as leaves, shoots, roots) on a plant organ, usually of a different kind.

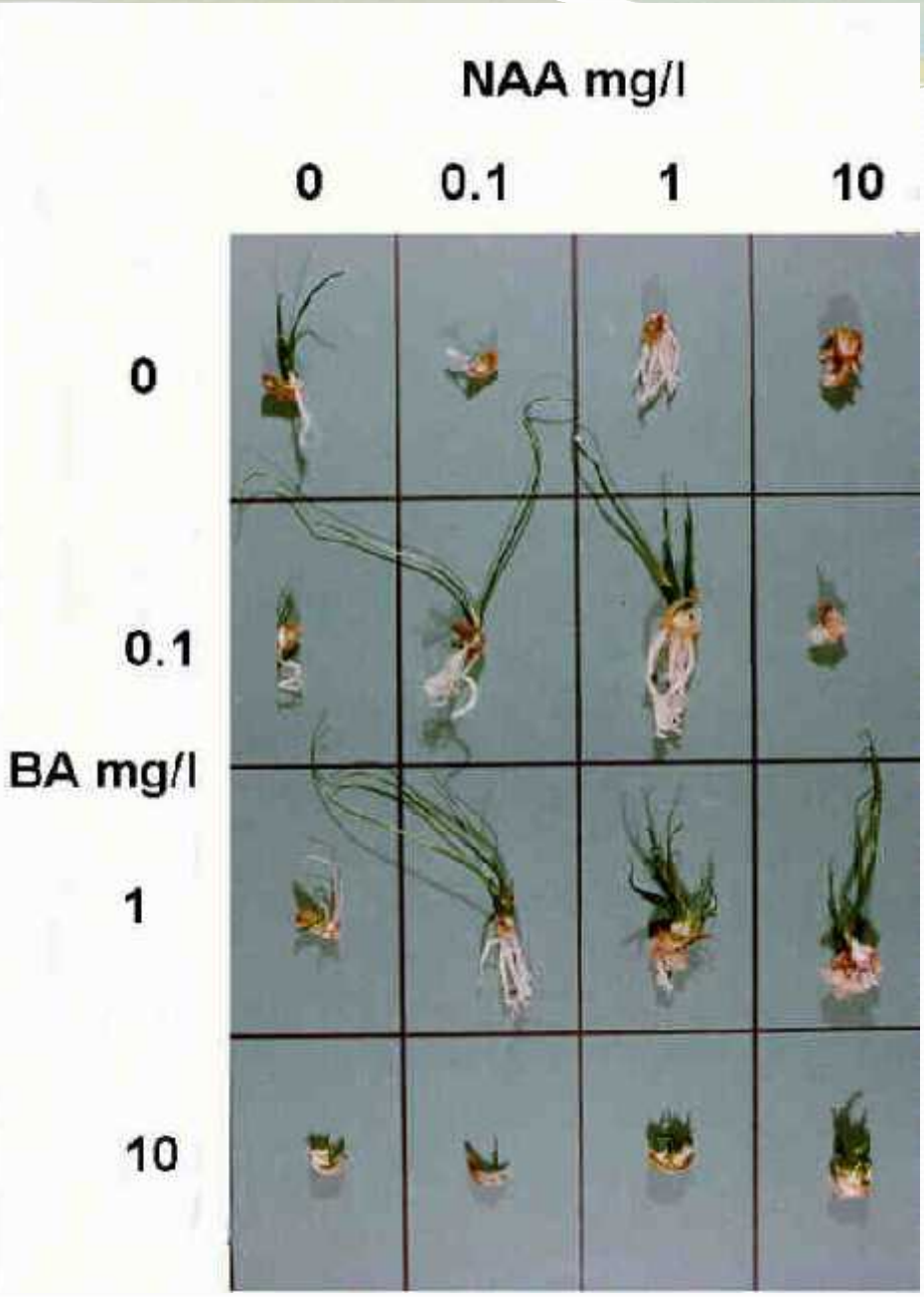

Explants
Organogenesis
Shoot formation
Root formation
Rooting
Shooting
Whole plants

Organogenesis

- Rule of thumb: Auxin/cytokinin 10:1-100:1 induces roots.
- 1:10-1:100 induces shoots
- Intermediate ratios around 1:1 favor callus growth.


BA mg/l
0.1
1.0
2.5
5.0
NAA
mg/l
0
1.0


BAP
Stücke
10^{-3}
10^{-4}
10^{-6}
0
0
10^{-7}
10^{-5}
10^{-5}
IAA

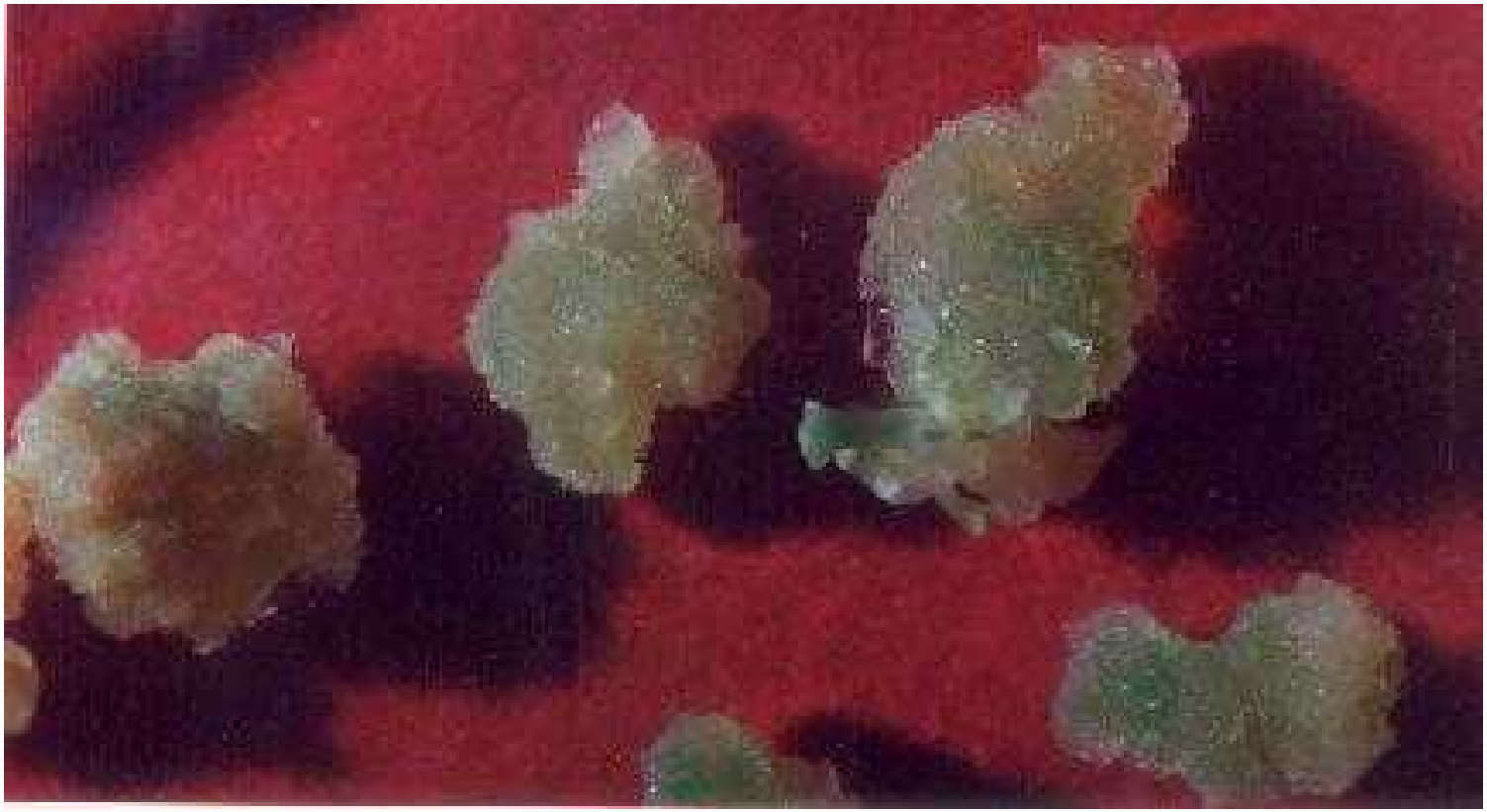

NAA mg/l
0
0.1
1
10
BA mg/l
0
0.1
1
10

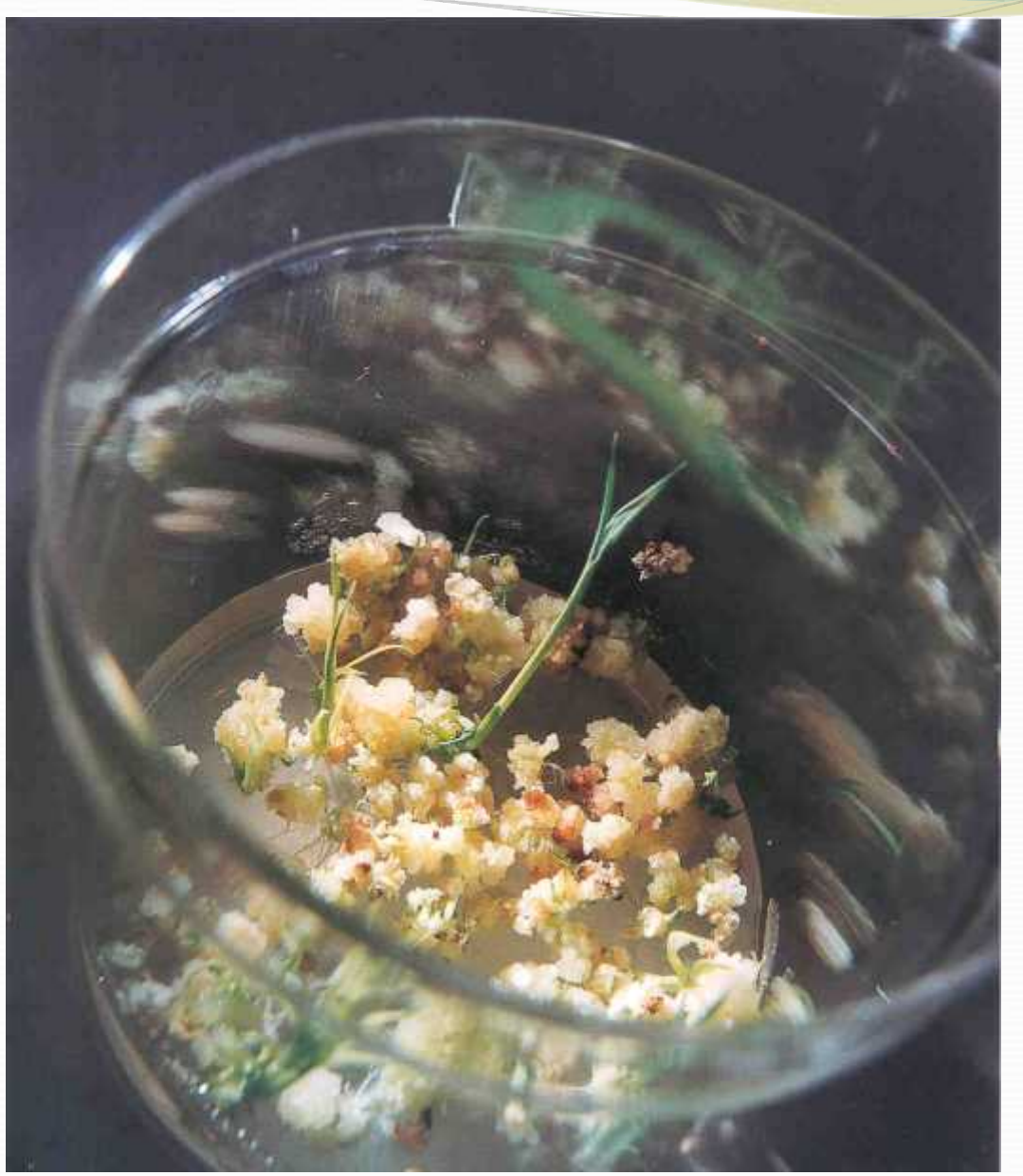
Indirect organogenesis

Explant → Callus → Meristemoid → Primordium

Indirect Organogenesis

- Dedifferentiation
  - Less committed, more plastic developmental state
- Induction
  - Cells become organogenically competent and fully determined for primordia production
  - Change in culture conditions?
- Differentiation

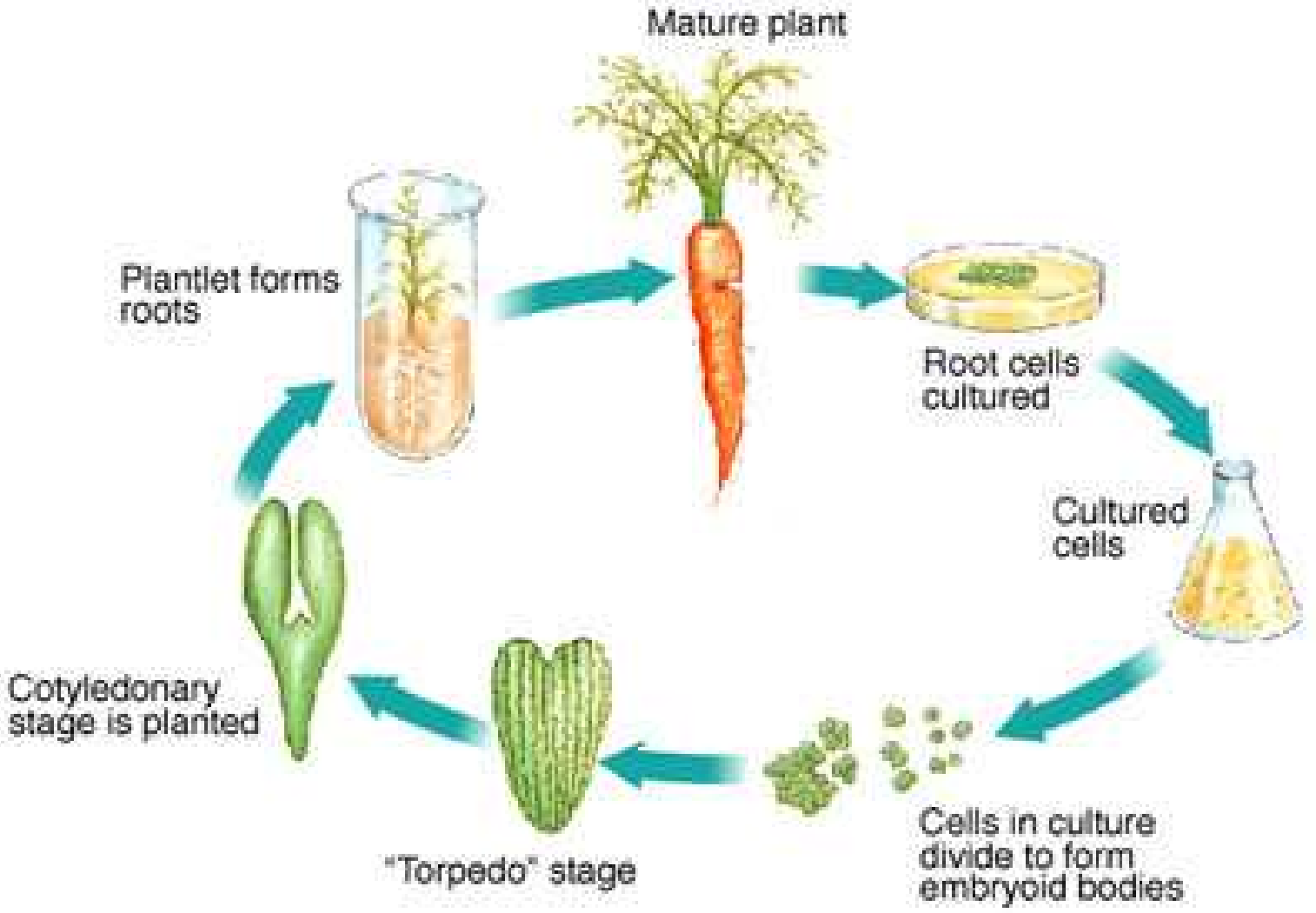


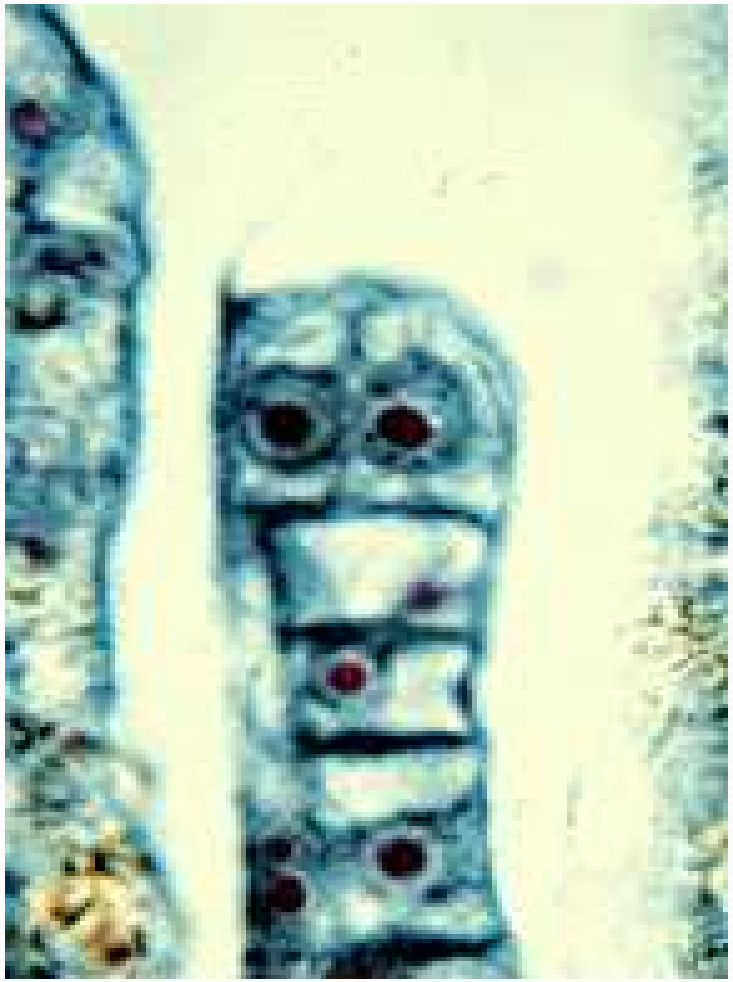


Direct Organogenesis

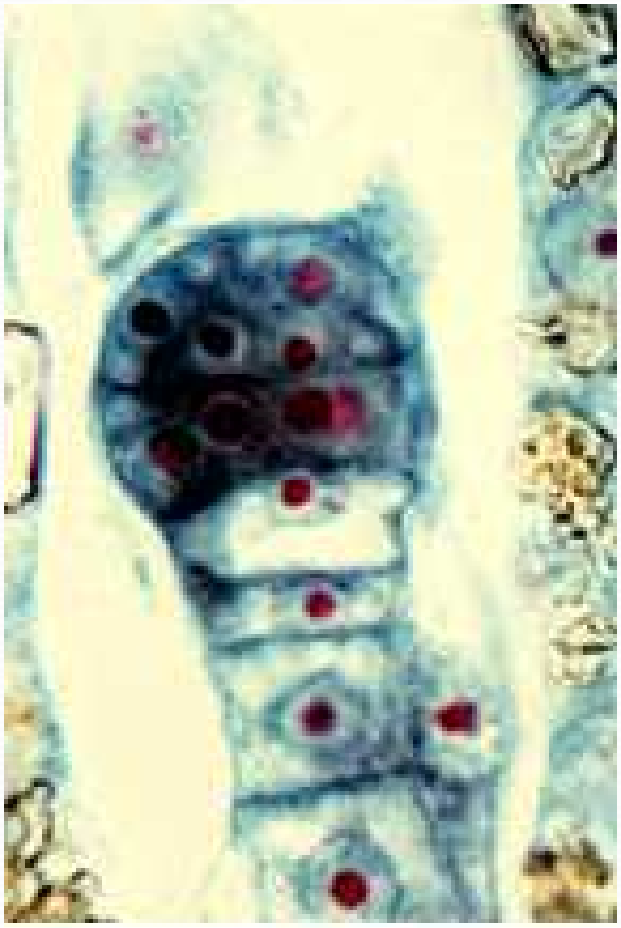
Somatic Embryogenesis

- **Parthenocarpy**
- **Apomixis**
- **In vitro somatic embryogenesis**

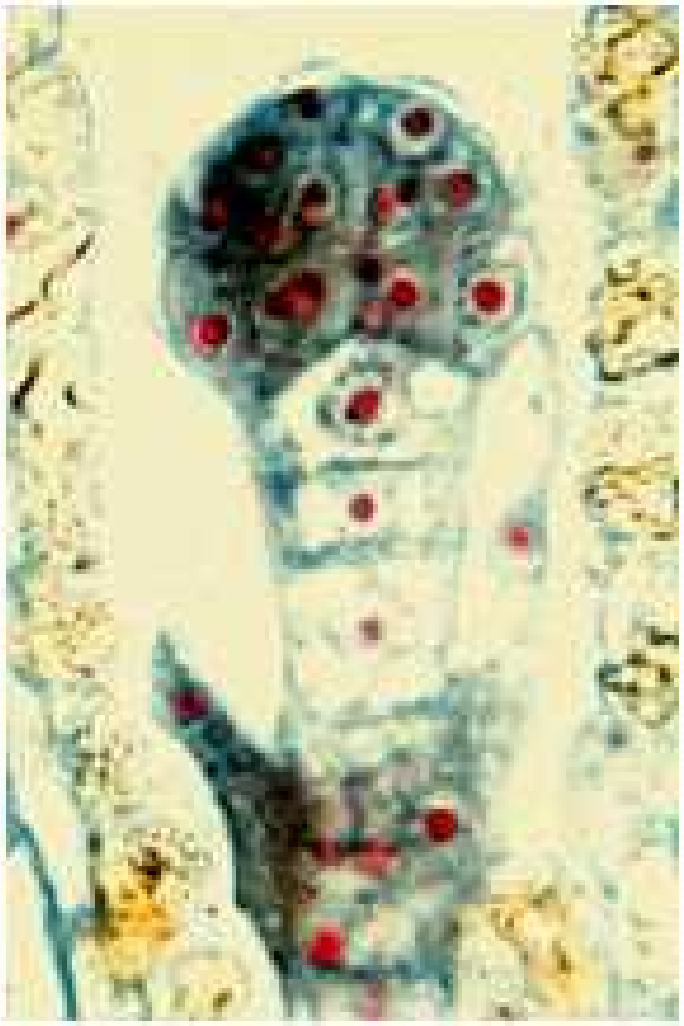

Mature plant
Plantlet forms roots
Root cells cultured
Cultured cells
Cotyledonary stage is planted
"Torpedo" stage
Cells in culture divide to form embryoid bodies



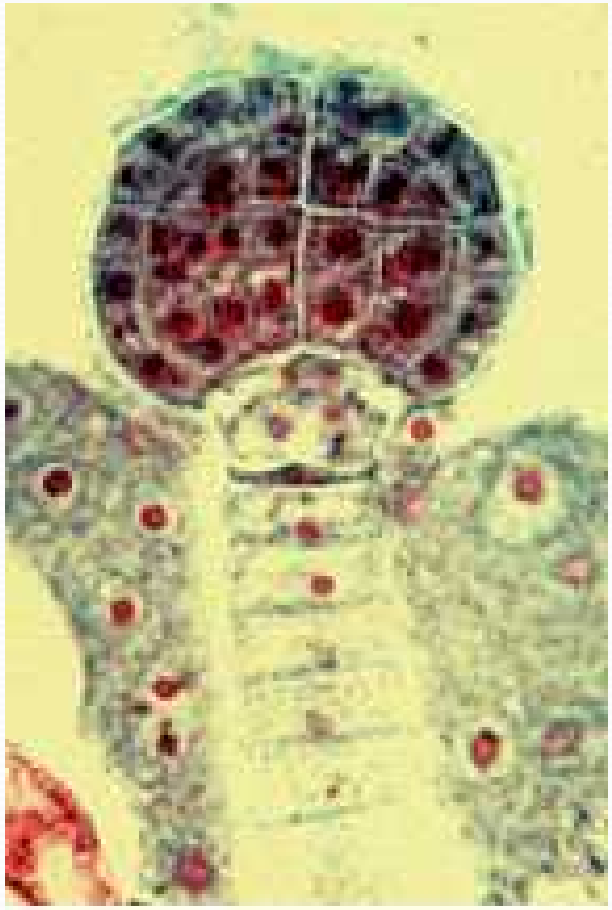
2 cell proembryo



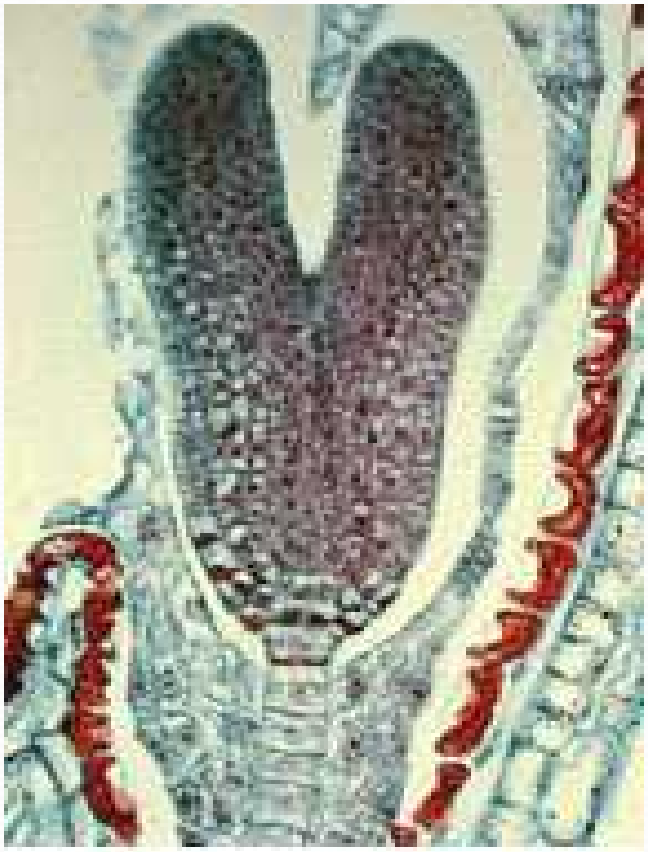
8 cell proembryo



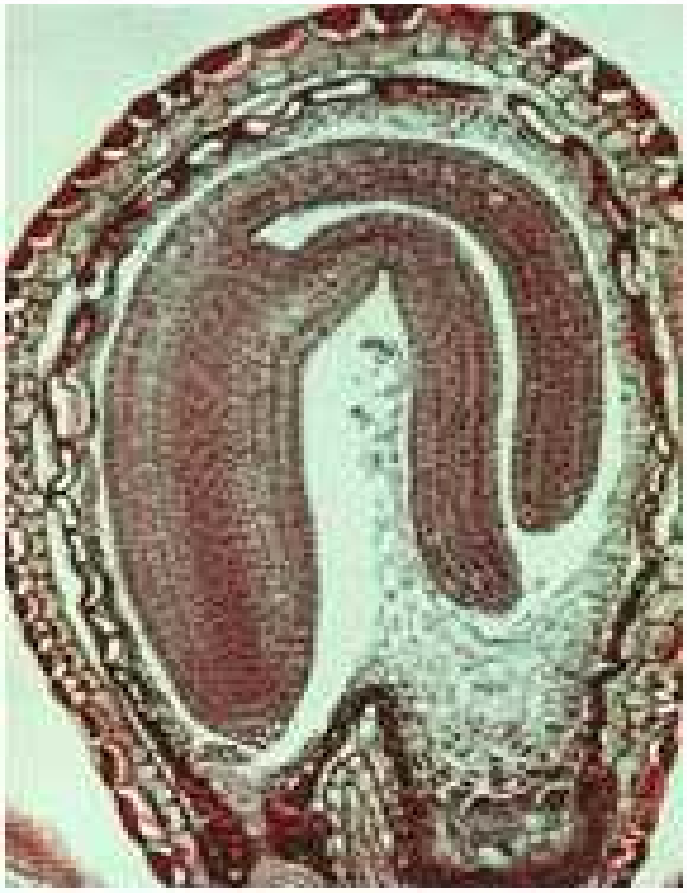
32 cell embryo



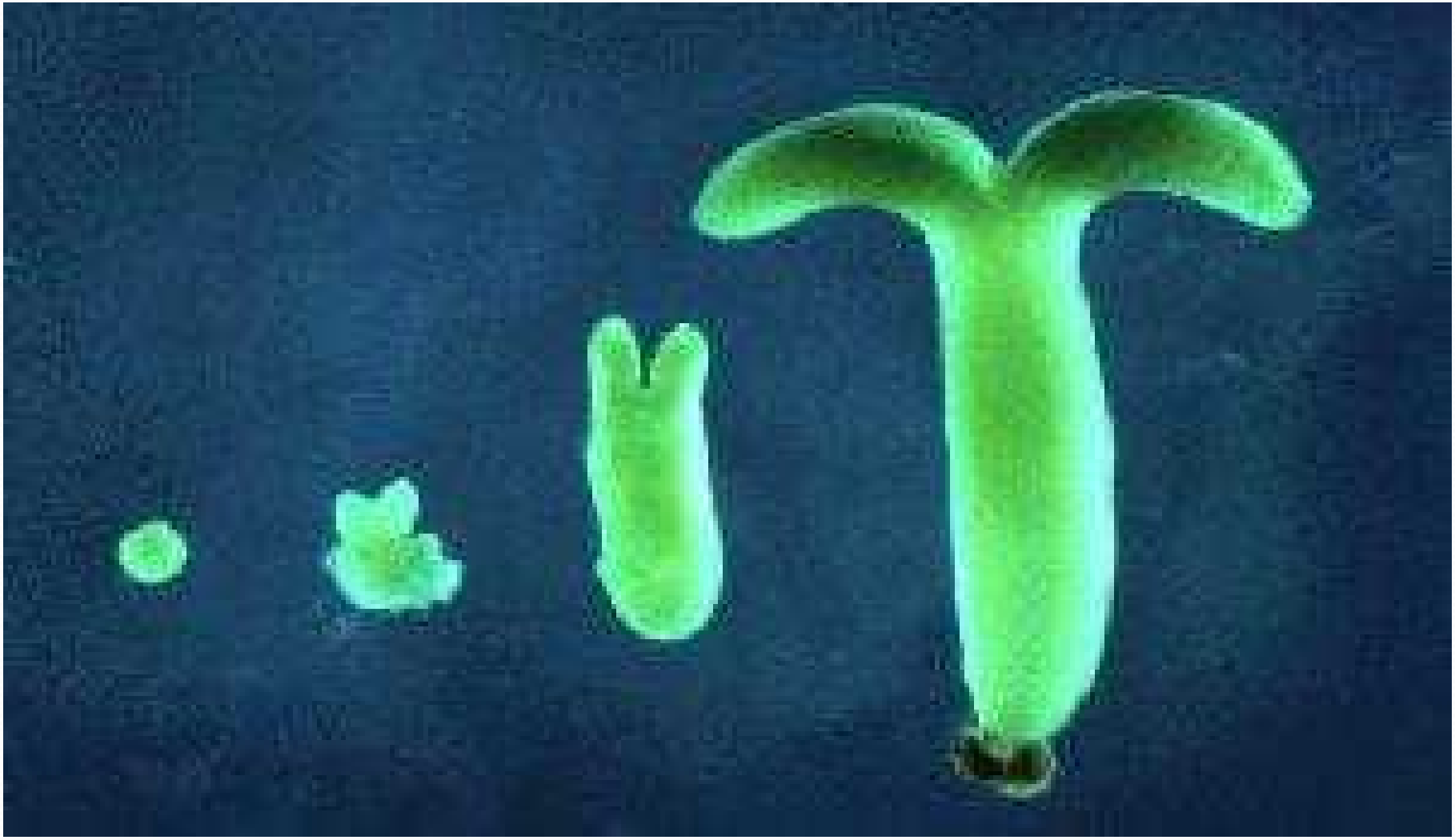
globular embryo



heart stage
embryo



curled cotyledon embryo



Soybean – Wayne Parrot, UGA

Somatic Embryos

- Bipolar
- Not connected to explant or callus cells by vascular tissue
- In most woody plants, tissue must be juvenile or reproductive

Indirect Somatic Embryogenesis

Induction

- Auxins required for induction
  - Proembryogenic masses form
  - 2,4-D most used
  - NAA, dicamba also used

Development

- Auxin must be removed for embryo development
- Continued use of auxin inhibits embryogenesis
- Stages are similar to those of zygotic embryogenesis
  - Globular
  - Heart
  - Torpedo
  - Cotyledonary
  - Germination (conversion)

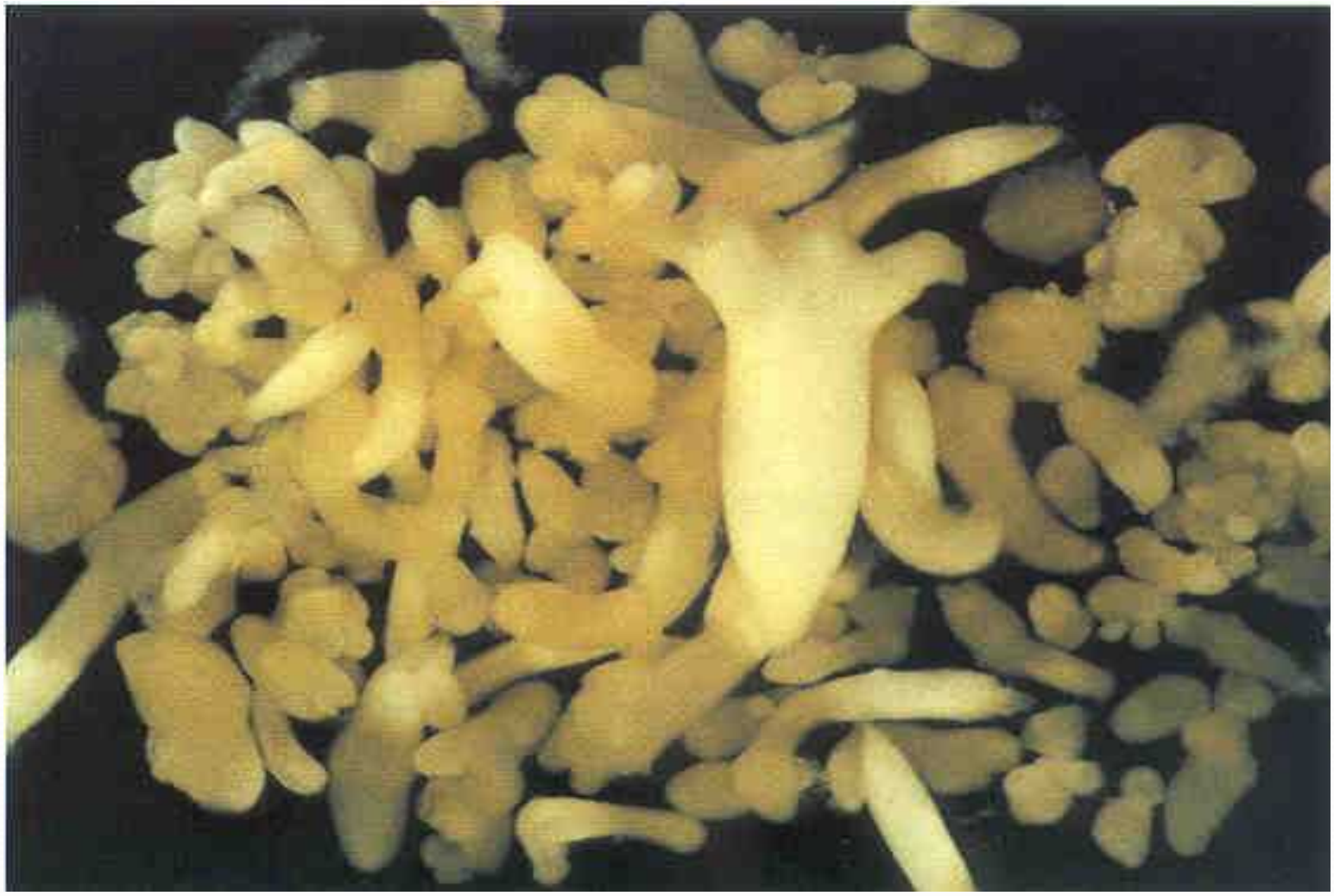
Maturation

- Require complete maturation with apical meristem, radical, and cotyledons
- Often obtain repetitive embryony
- Storage protein production necessary
- Often require ABA for complete maturation
- ABA often required for normal embryo morphology
  - Fasciation
  - Precocious germination

Germination

- May only obtain 3-5% germination
- Sucrose (10%), mannitol (4%) may be required
- Drying (desiccation)
  - ABA levels decrease
  - Woody plants
  - Final moisture content 10-40%
- Chilling
  - Decreases ABA levels
  - Woody plants

Rubber tree from somatic embryo CIRAD



Factors that Influence SE

- Genotype
- Growth regulators
- Carbon source
- Nitrogen

Maturation and Germination (Conversion)