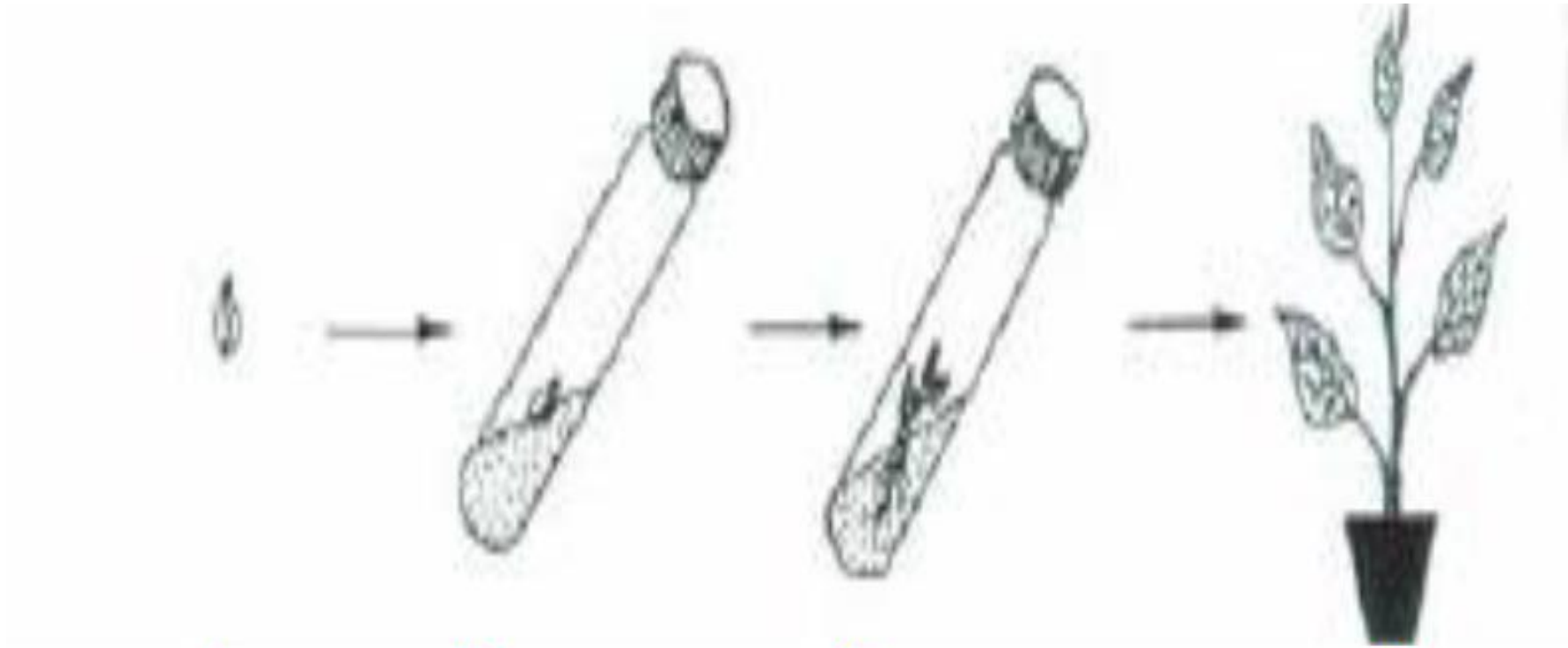
3. Embryo Culture

Embryo Culture as a Source of Genetic Variation

- **Hybridization**
  – Can transfer mutant alleles between species
  – Can introduce new genetic combinations through interspecific crosses

- **Polyploidy**
  – Can combine embryo culture with chromosome doubling to create new polyploid species (allopolyploidy)



A: Proembryo dissected 3 to 5 days after pollination

B: Proembryo cultured on solid agar medium

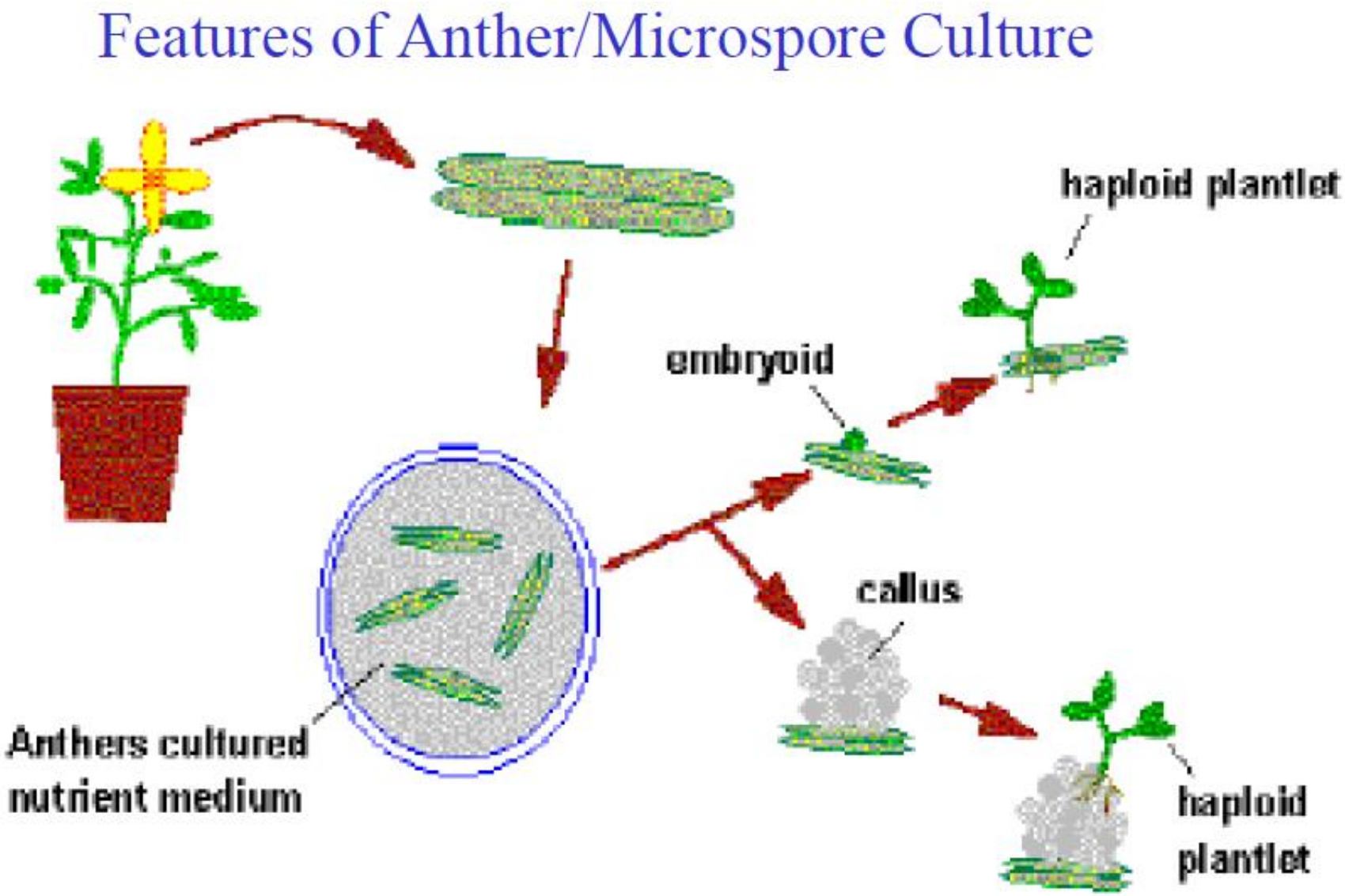
C: Plantlet developing from embryo

D: Plantlet transplanted into soil

Embryo Rescue Process

- Make cross between two species
- Dissect embryo (usually immature)
  – The younger the embryo, the more difficult to culture
- Grow on culture medium
- Many times, resulting plants - **haploid** - because of lack of pairing between the chromosomes of the different species
  – This can be overcome by doubling the chromosomes, creating allotetraploids
  – Polyploids are another source of genetic variation

4. Anther/Microspore Culture



Anther/Microspore Culture Factors

- **Genotype**
  – As with all tissue culture techniques

- **Growth of mother plant**
  – Usually requires optimum growing conditions

- **Correct stage of pollen development**
  – Need to be able to switch pollen development from gametogenesis to embryogenesis

- **Pretreatment of anthers**
  – Cold or heat have both been effective

- **Culture media**
  – Additives, Agar vs. 'Floating'

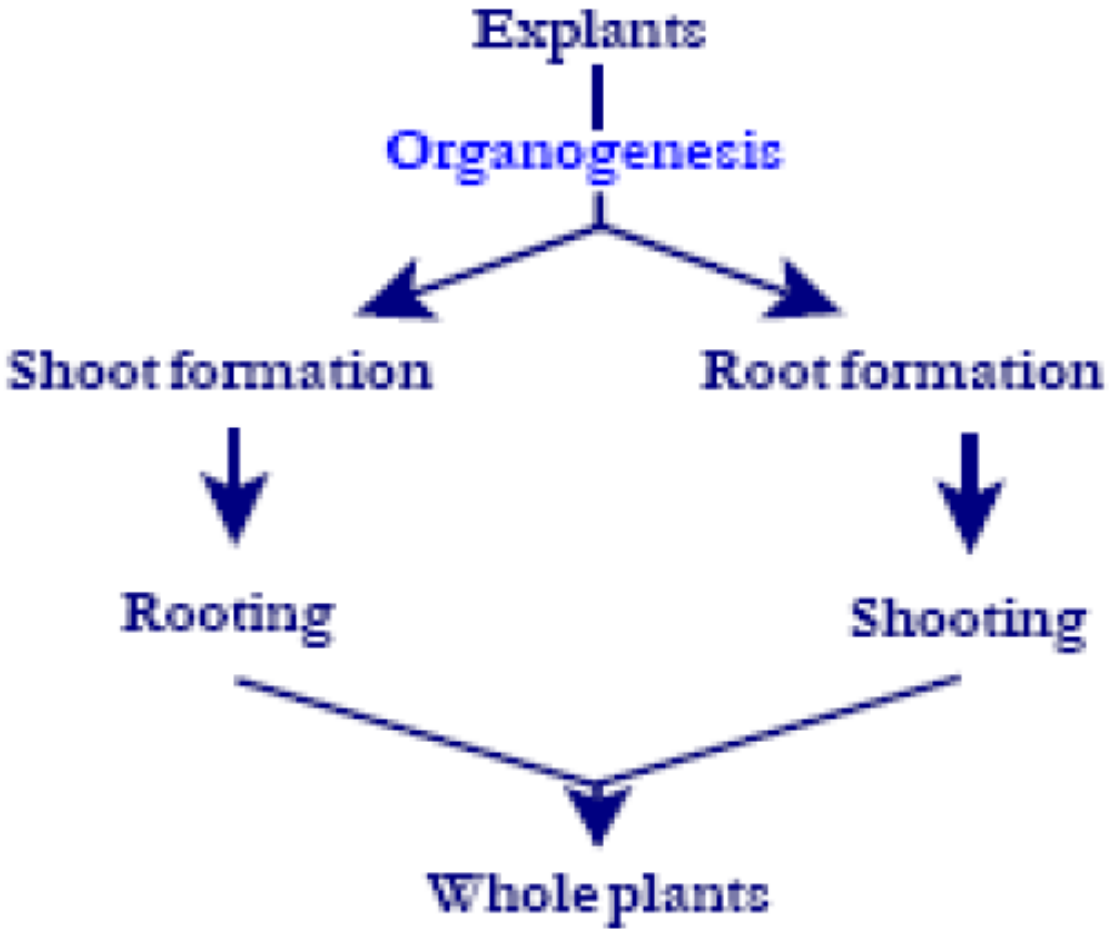
Ovule Culture for Haploid Production

- **Essentially** the same as embryo culture
  - Difference is an unfertilized ovule instead of a fertilized embryo

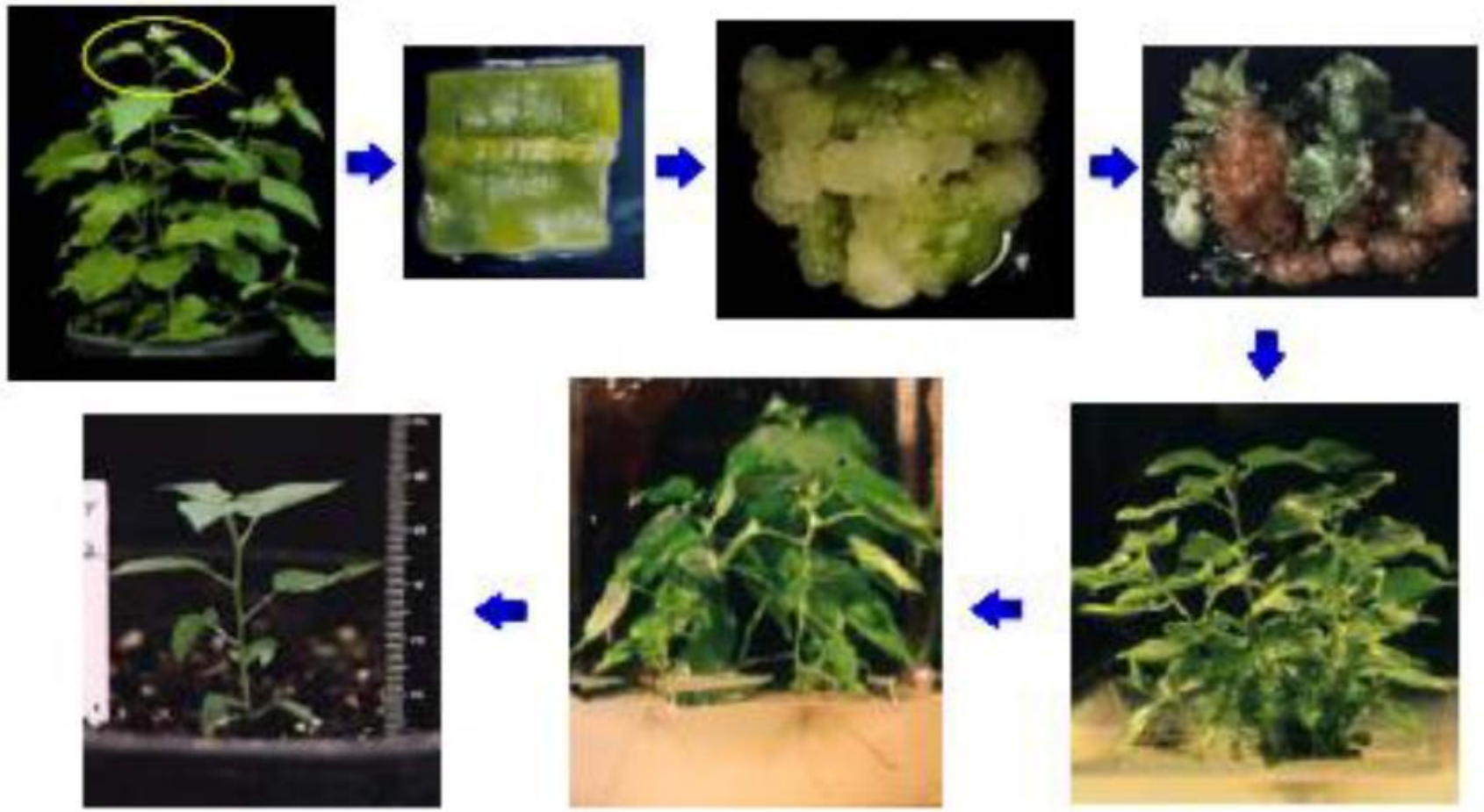
- **Effective** for crops that do not yet have an efficient microspore culture system
  - *e.g.*: melon, onion

5. Organogenesis

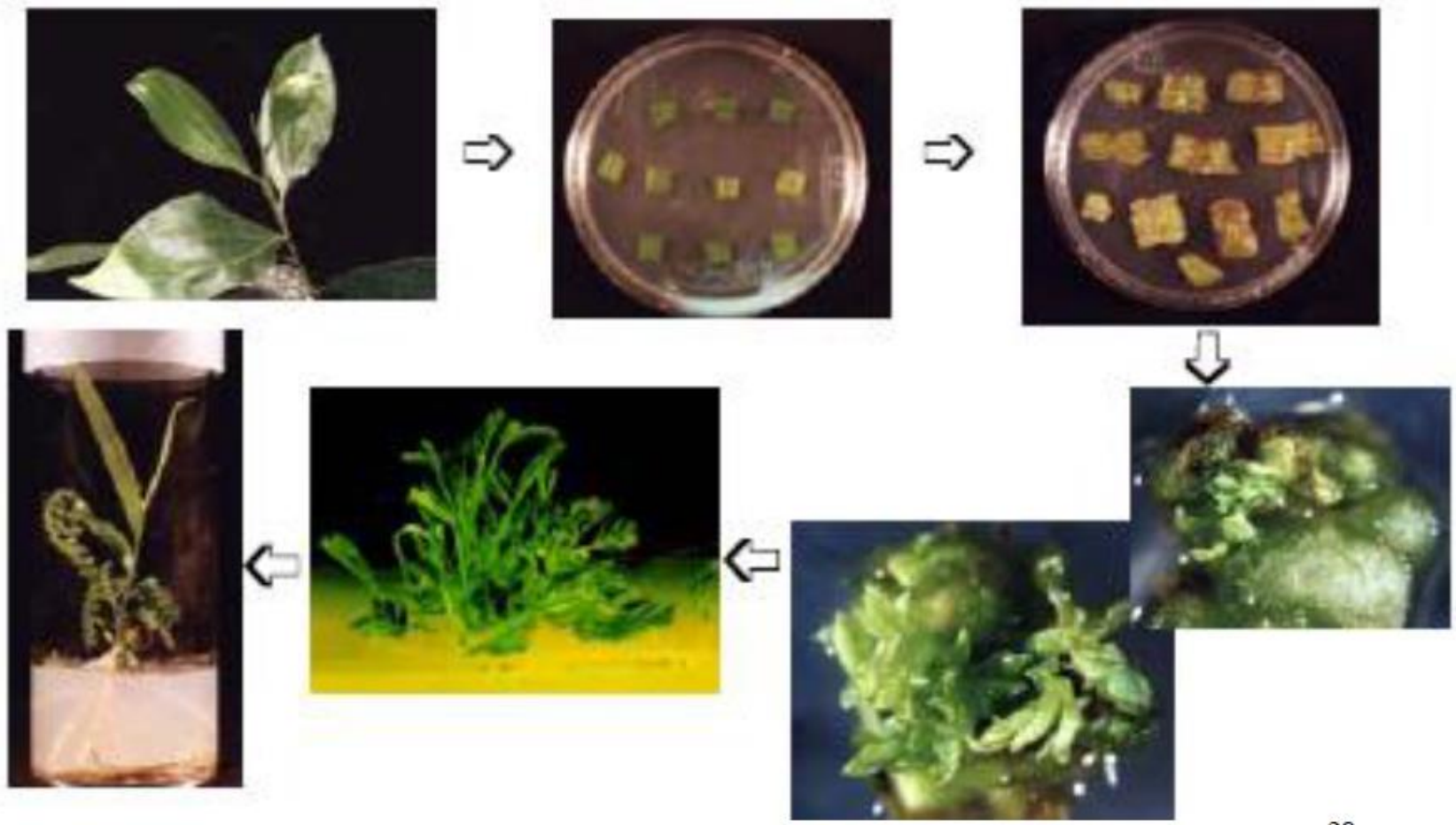
- Organ formation
- The formation of a shoot, root, flower, etc.
- This allows whole plant regeneration



Organogenesis – Aspen



Organogenesis – Acacia

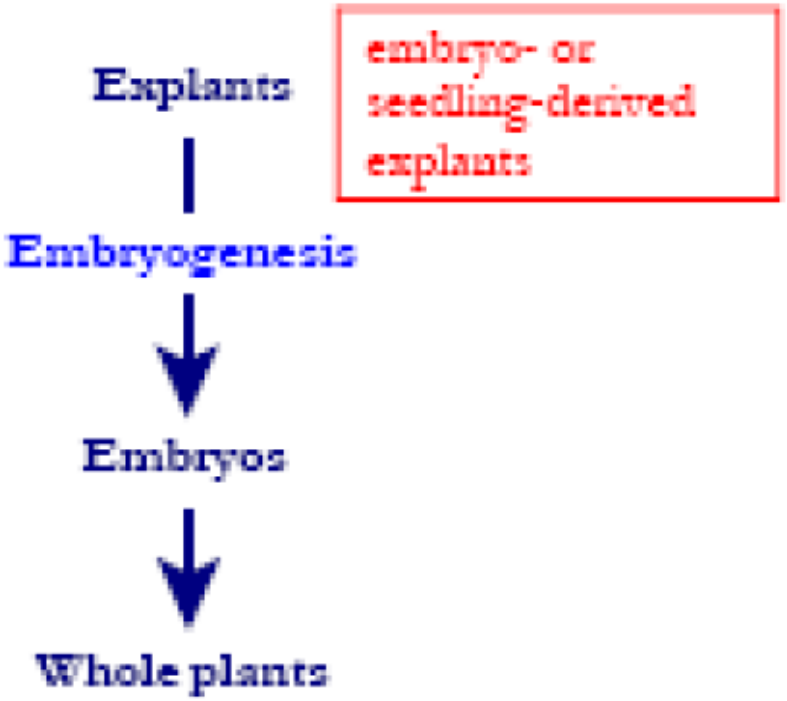


6. Embryogenesis

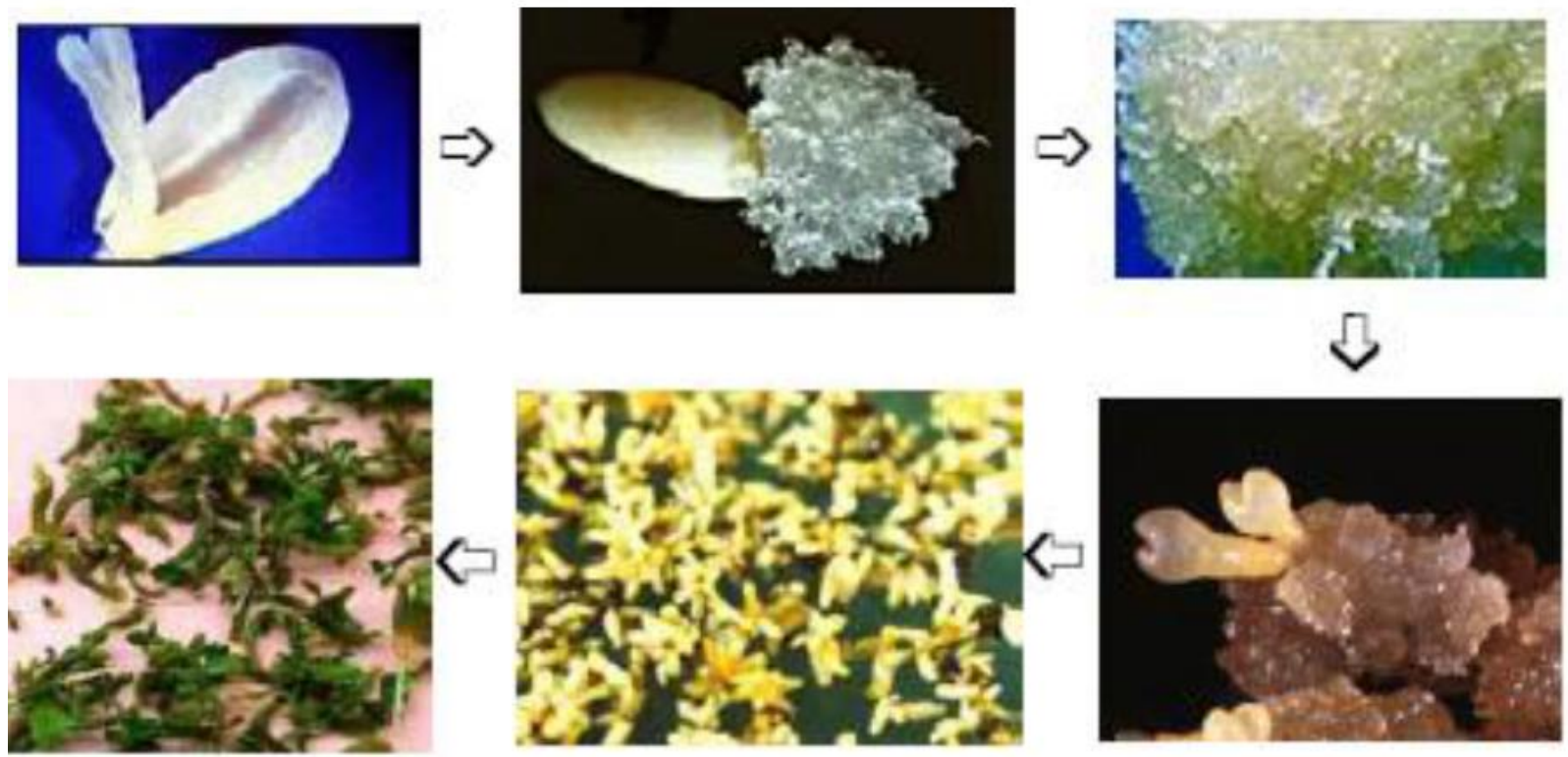
- **Embryo formation**

- Process by which an embryo develops from a **fertilized egg cell** or **asexually** from a group of **somatic cells**

- The ability to differentiate embryogenic structures is reduced with increased age of the donor tissue



Somatic Embryogenesis



Procedure for plant regeneration via somatic embryogenesis

1. **Initiation**: embryogenic callus
2. **Proliferation**: embryogenic callus, proembryogenic mass (PEM)
3. **Embryo development and maturation**: embryos
4. **Embryo germination** (Regeneration): emblings

Developmental Stages of Somatic Embryo in Dicots

1. **Proembryonic mass**
2. **Globular stage**
3. **Heart stage**
4. **Torpedo stage**
5. **Somatic embryos**

7.Protoplast isolation, culture and fusion

Protoplast - a plant cell without cell wall or a naked plant cell

Procedure:

1. **Cell wall digestion**: cellulase, hemicellulase, pectinase
2. **Cell wall regeneration**: usually within 24 hrs
3. **Cell division**: first division occur within 24-48 hrs
4. **Proliferation and differentiation**

In vitro hybridization – Protoplast Fusion

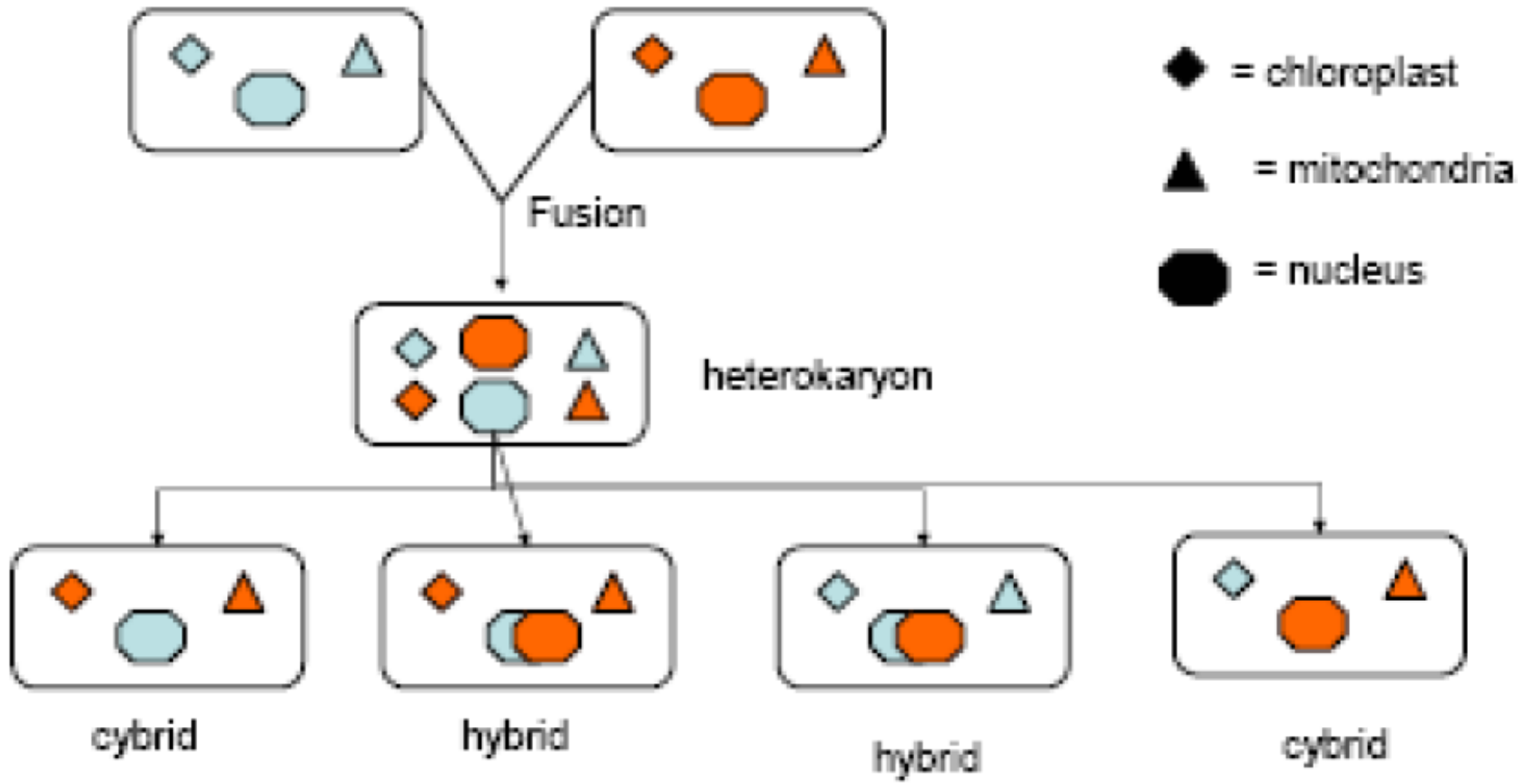
- **Created** by degrading the cell wall using enzymes
- **Very fragile**, can't pipette
- **Protoplasts can be induced** to fuse with one another:

– **Electrofusion**: A high frequency AC field is applied between 2 electrodes immersed in the suspension of protoplasts

– **Polyethylene glycol** (PEG): causes agglutination of many types of small particles, including protoplasts which fuse when centrifuged in its Presence

– **Addition of calcium ions** at high pH values

Possible Result of Fusion of Two Genetically Different Protoplasts



Identifying Desired Fusions

- **Complementation selection**
  – Can be done if each parent has a different selectable marker (e.g. **antibiotic** or **herbicide resistance**), then the fusion product should have both markers
- **Fluorescence-activated cell sorters**
  – First label cells with different **fluorescent markers**; fusion product should have both markers
- **Mechanical isolation**
  – Tedious, but often works when you start with different cell types
- **Mass culture**
  – Basically, no selection; just regenerate everything and then screen for desired traits