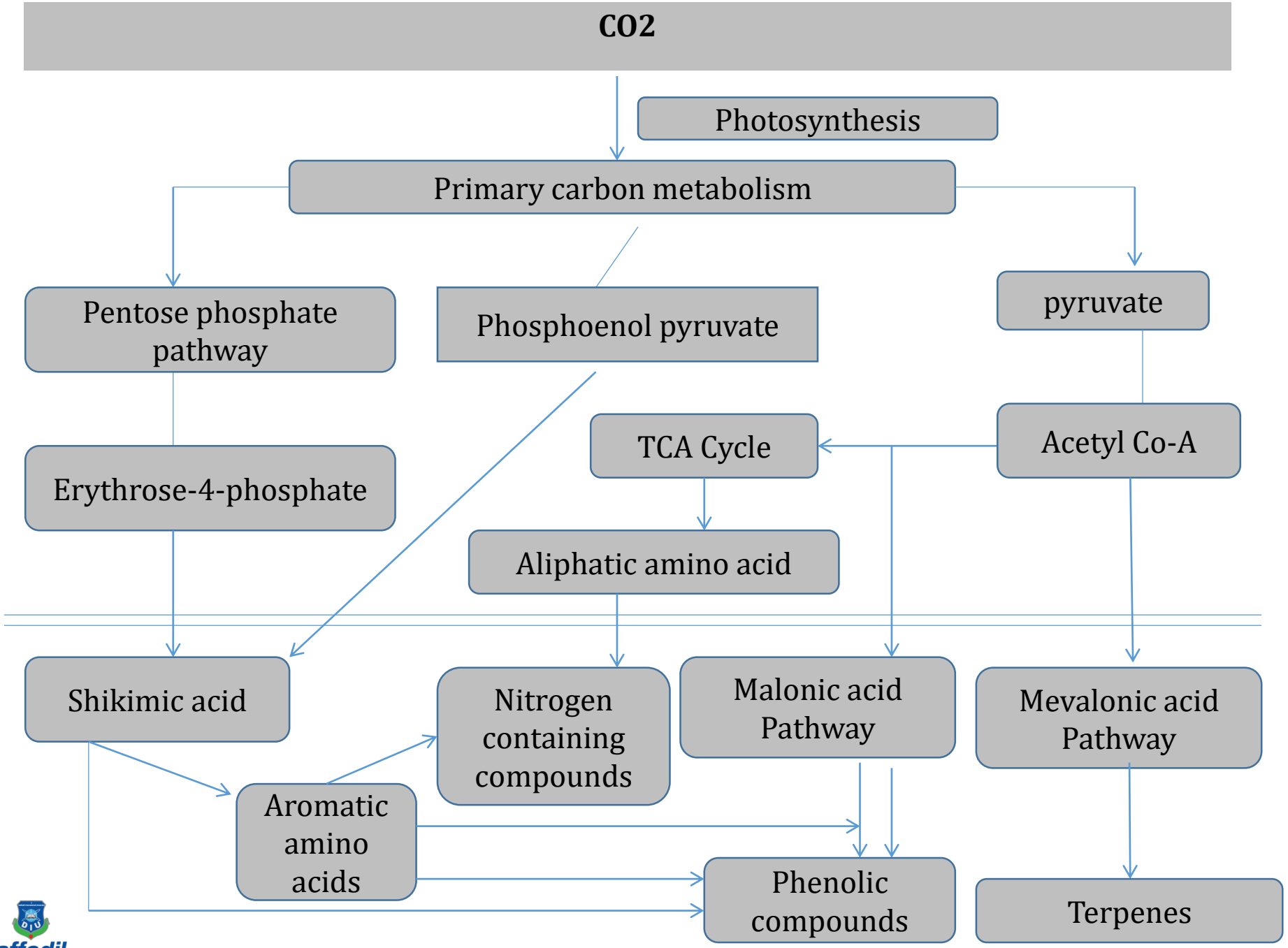
FOOD BIOTECHNOLOGY



Nitrogen containing compounds!

Alkaloids (pseudo-, True-, proto-)

- ✓ **Extremly heterogenous group**
- ✓ **alkali like**
- ✓ **have important pharmacological properties**
- ✓ **further classified in to many groups**

-Pyridine alkaloids , e.g. nicotine

- pyrrolidine alkaloids , e.g. stachydrine

- piperidine alkaloids , e.g. coniine

- tropane alkaloids , e.g. atropine

- quinoline alkaloids , e.g. quinine

- Isoquinoline alkaloids , e.g. berberine

-Quinolizidine alkaloids , e.g. lupinine

- Indol alkaloids , e.g. resrpine

- Imidazol alkaloids , e.g. pilocarpine

Plant Tissue Culture

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Definition

The culture of plant seeds, organs, tissues, cells, or protoplasts on nutrient media under sterile conditions.

Basis for Plant Tissue Culture

Two Hormones Affect Plant 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

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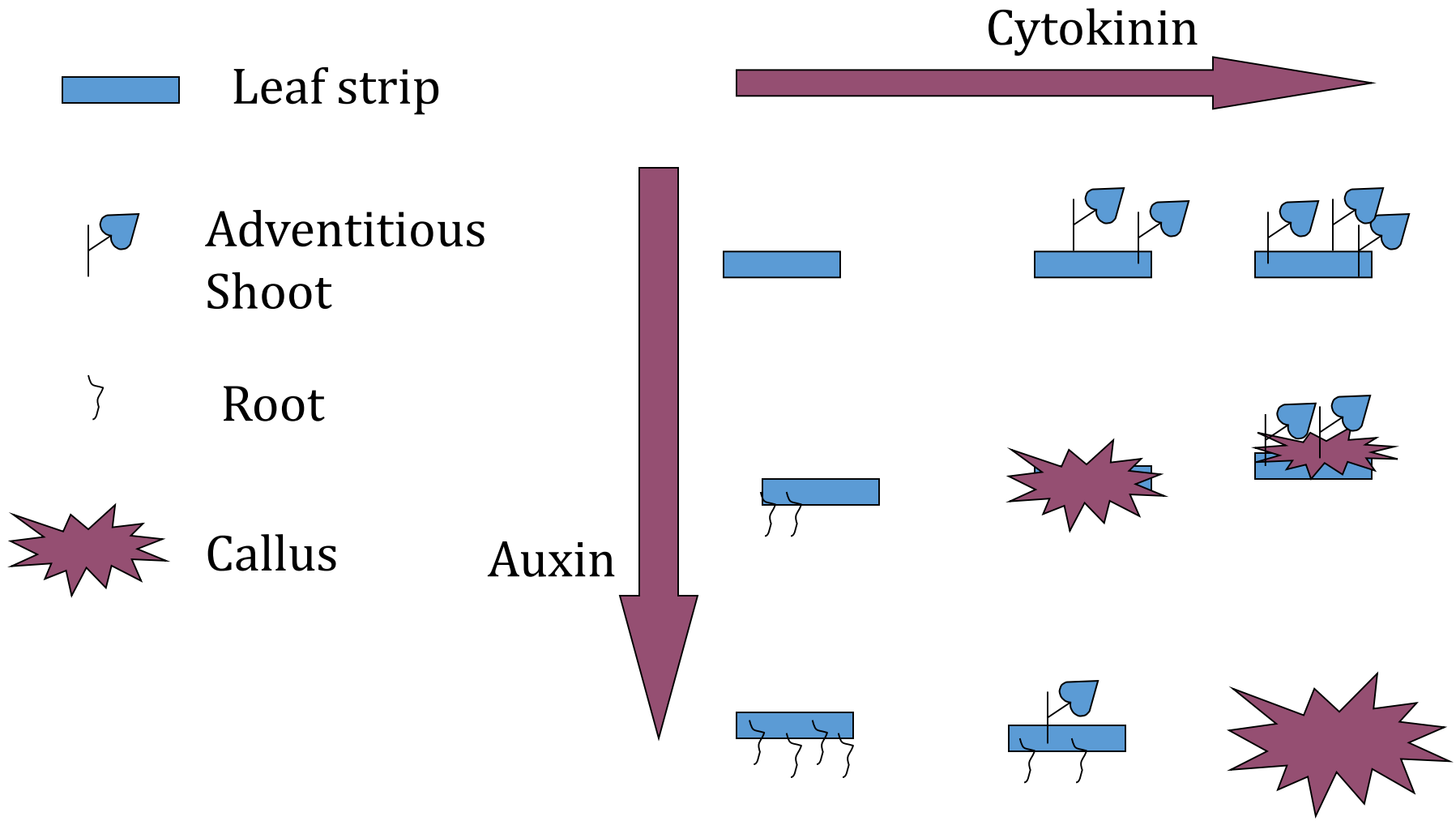
Control of in vitro culture



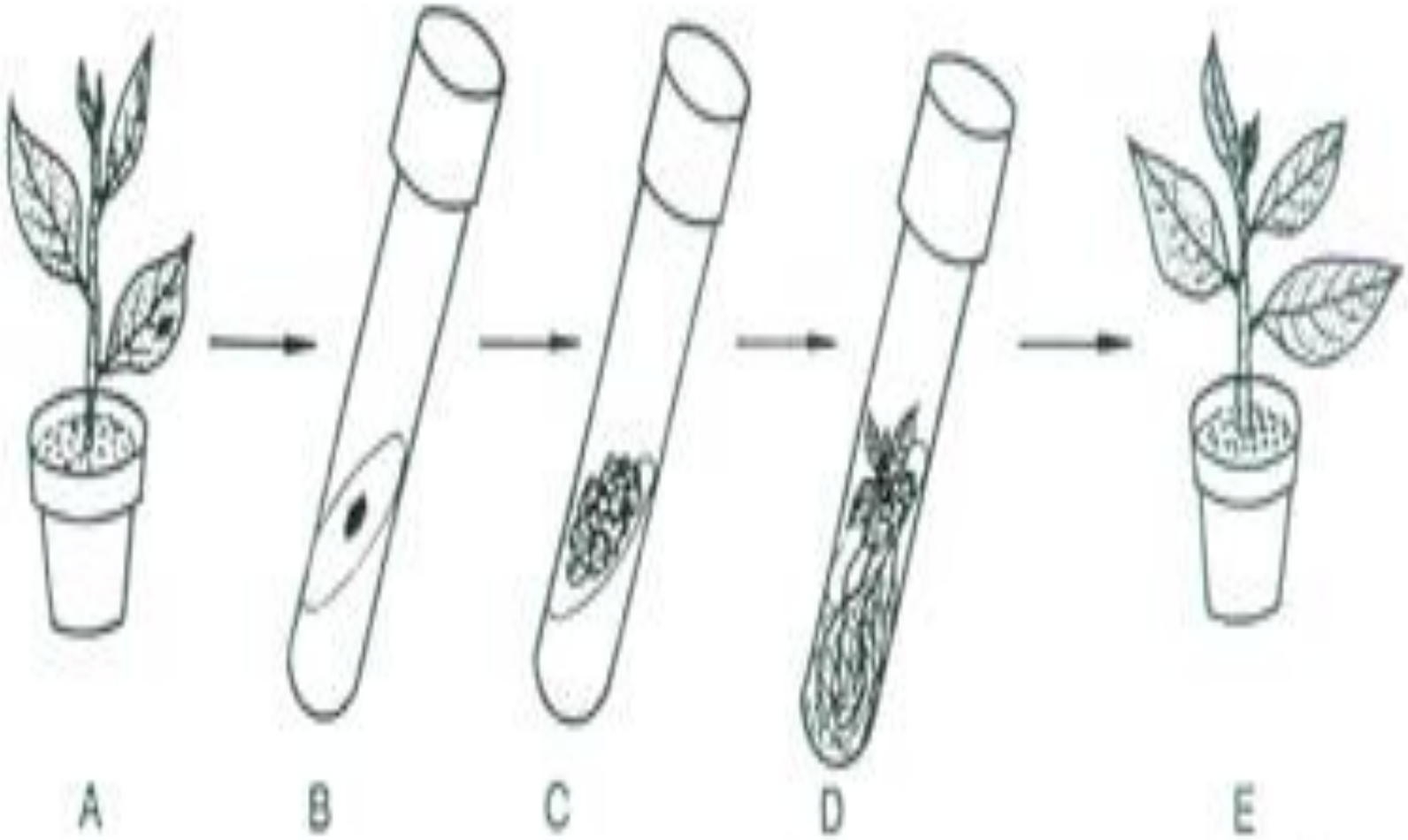
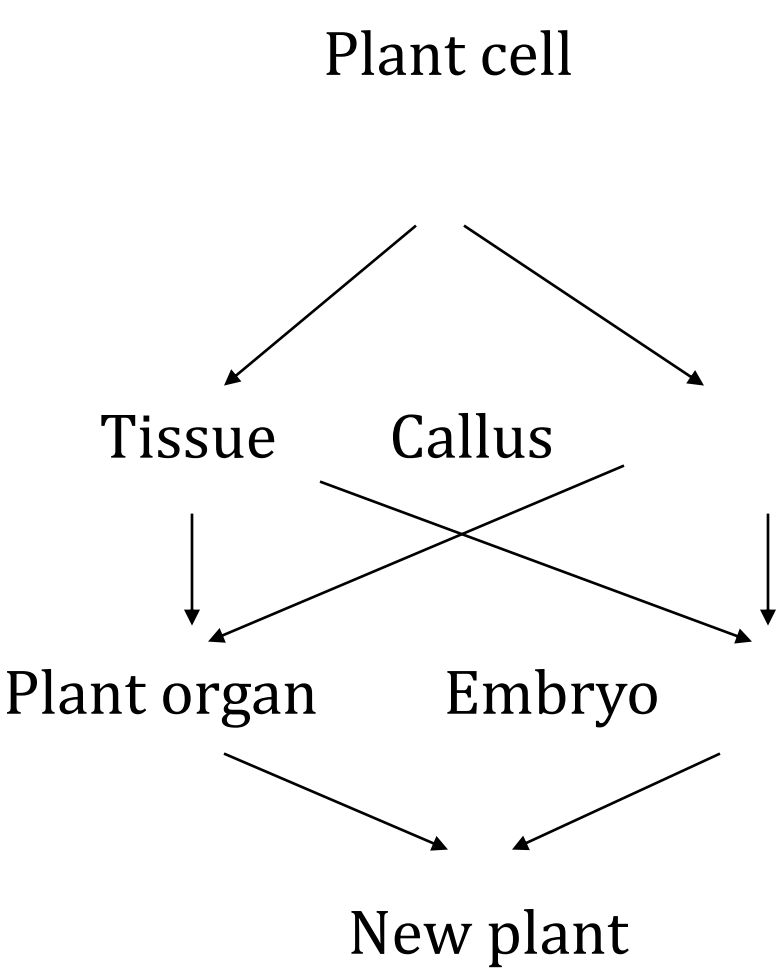


FIG. 8.1. In Vitro regeneration of plants through tissue culture. (A) Source of tissue explant. (B) Explant culture on agar medium. (C) Callus production. (D) Plantlet regenerated. (E) Plantlet transplanted into sterile soil.



Factors Affecting Plant Tissue Culture

Growth Media

Minerals, Growth factors, Carbon source, Hormones

Environmental Factors

Light, Temperature, Photoperiod, Sterility, Media

Explant Source

Usually, the younger, less differentiated the explant, the better for tissue culture

Genetics

Different species show differences in amenability to tissue culture

In many cases, different genotypes within a species will have variable responses to tissue culture; response to somatic embryogenesis has been transferred between melon cultivars through sexual hybridization

Three Fundamental Abilities of Plants

✓Totipotency

the potential or inherent capacity of a plant cell to develop into an entire plant if suitably stimulated.

It implies that all the information necessary for growth and reproduction of the organism is contained in the cell

✓Dedifferentiation

Capacity of mature cells to return to meristematic condition and development of a new growing point, follow by redifferentiation which is the ability to reorganise into new organ

✓Competency

the endogenous potential of a given cells or tissue to develop in a particular way

HISTORY OF PLANT TISSUE CULTURE

1838-39	cellular theory (Cell is autonom and totipotent)	Schleiden-Schwann
1902	First attempt of plant tissue culture	Harberlandt
1939	Continuously growing callus culture	White
1946	Whole plant developed from shoot tip	Ball
1950	Organs regenerated on callus	Ball
1954	Plant from single cell	Muir
1960	Protoplast isolation	Cocking

HISTORY OF PLANT TISSUE CULTURE

1962cam	MS media	Murashige - Skoog
1964	Clonal propagation of orchids	Morel
1964	Haploids from pollen	Guha
1970	Fusion of protoplasts	Power
1971	Plants from protoplasts	Takebe
1981	Somaclonal variation	Larkin

Types of In Vitro Culture

- ✓ Culture of intact plants (seed and seedling culture)
- ✓ Embryo culture (immature embryo culture)
- ✓Organ culture
    1. shoot tip culture
    2. root culture
    3. leaf culture
    4. anther culture
- ✓Callus culture
- ✓Cell suspension culture
- ✓Protoplast culture

Tissue Culture Applications

- ✓Micropropagation
- ✓Germplasm preservation
- ✓Somaclonal variation
- ✓dihaploid production
- ✓Protoplast fusion
- ✓Secondary metabolites production
- ✓Genetic engineering

Micropropagation

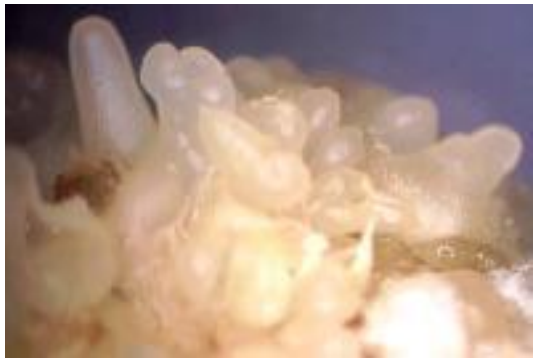
- Embryogenesis
  - Direct embryogenesis
  - Indirect embryogenesis
- Organogenesis
  - Organogenesis via callus formation
  - Direct adventitious organ formation
- Microcutting
  - Meristem and shoot tip culture
  - Bud culture

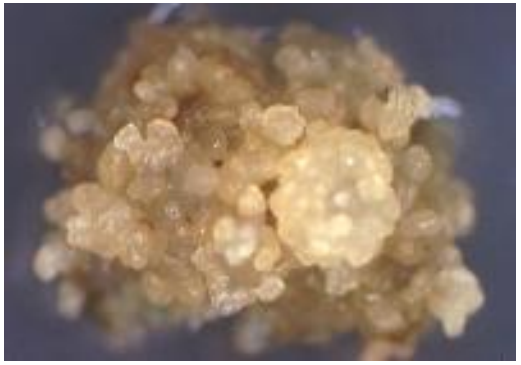
Somatic Embryogenesis

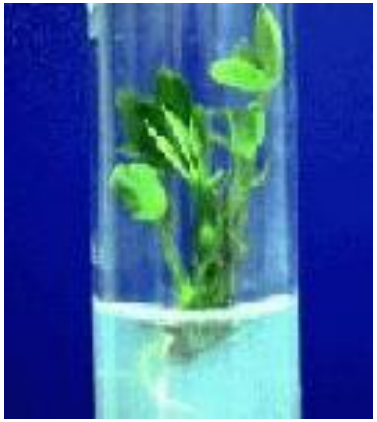
The production of embryos from somatic or “non-germ” cells.

Usually involves a callus intermediate stage which can result in variation among seedlings

Peanut somatic embryogenesis







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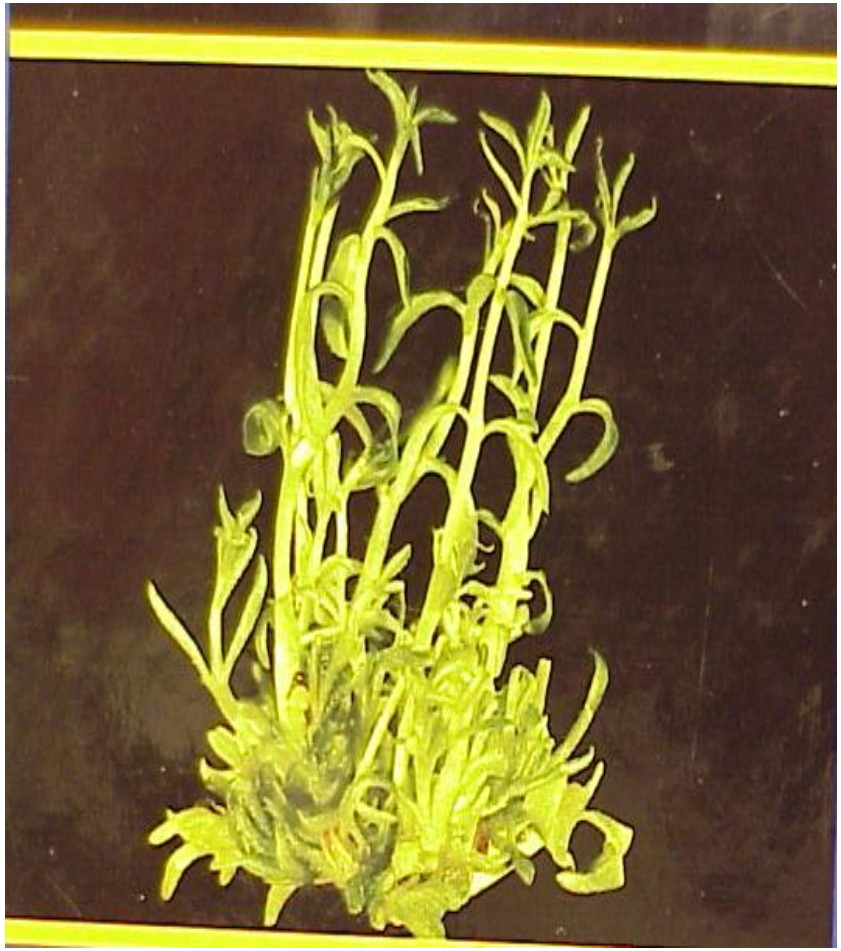
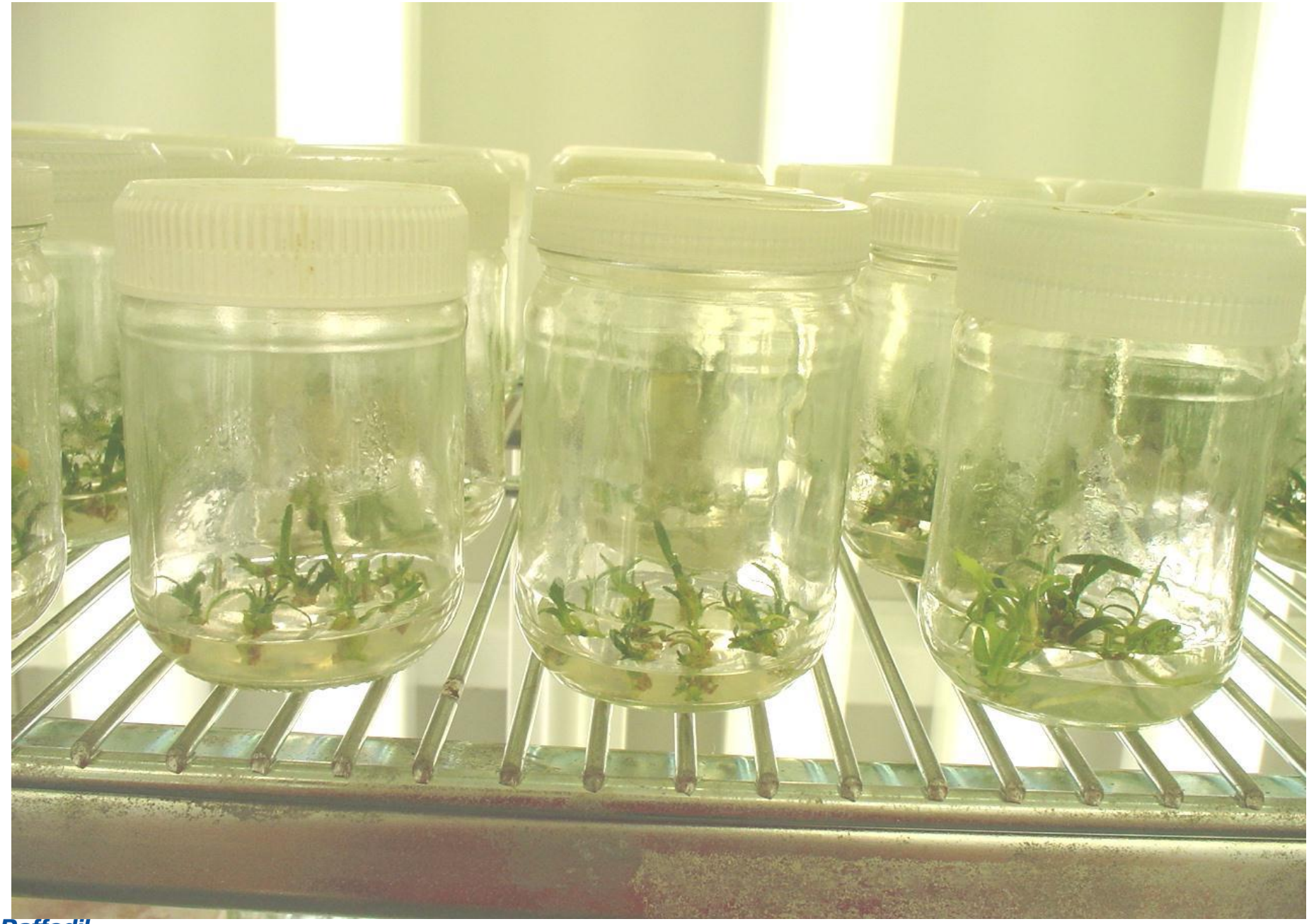
Organogenesis



- The production of roots, shoots or leaves.
- These organs may arise out of pre-existing meristems or out of differentiated cells.
- This, like embryogenesis, may involve a callus intermediate but often occurs without callus.



Somatic Embryogenesis and Organogenesis

Both of these technologies can be used as methods of micropropagation.

Not always desirable because they may not always result in populations of identical plants.

The most beneficial use of somatic embryogenesis and organogenesis is in the production of whole plants from a single cell (or a few cells).

Microcutting propagation

This is a specialized form of organogenesis

It involves the production of shoots from pre-existing meristems only.

Requires breaking apical dominance

Microcuttings can be one of three types:

- Nodal
- Shoot cultures
- Clump division

Micropropagation

The art and science of plant multiplication *in vitro*

Usually derived from meristems (or vegetative buds) without a callus stage

- Tends to reduce or eliminate somaclonal variation, resulting in true clones

Can be derived from other explant or callus (but these are often problematic)

Steps of Micropropagation

Stage 0 – Selection & preparation of the mother plant

sterilization of the plant tissue takes place

Stage I - Initiation of culture

explant placed into growth media

Stage II - Multiplication

explant transferred to shoot media; shoots can be constantly divided

Stage III - Rooting

explant transferred to root media

Stage IV - Transfer to soil

explant returned to soil; hardened off

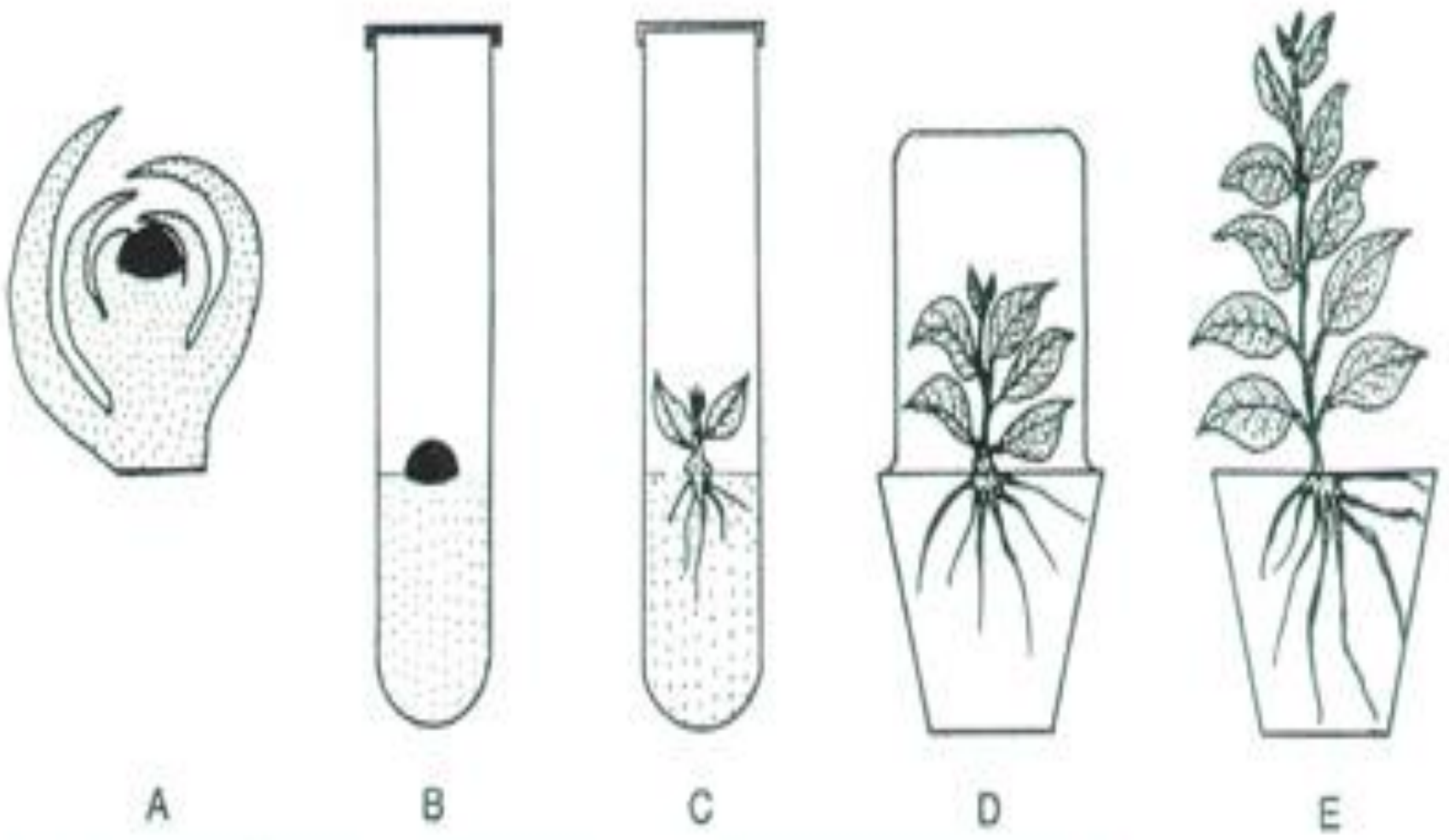


FIG. 8.3. Meristem-tip culture. (A) Apical meristem showing section to be excised. (B) Excised meristem tip cultured on agar medium. (C) Plantlet regenerated from excised meristem tip. (D) Plantlet transferred to sterile soil. (E) Virus-free plant growing in soil.

Features of Micropropagation

- Clonal reproduction
  - Way of maintaining heterozygozity
- Multiplication Stage can be recycled many times to produce an unlimited number of clones
  - Routinely used commercially for many ornamental species, some vegetatively propagated crops
- Easy to manipulate production cycles
  - Not limited by field seasons/environmental influences
- Disease-free plants can be produced
  - Has been used to eliminate viruses from donor plants

Embryo Culture

- Embryo culture developed from the need to rescue embryos (embryo rescue) from wide crosses where fertilization occurred, but embryo development did not occur
- These techniques have been further developed for the production of plants from embryos developed by non-sexual methods (haploid production discussed later)

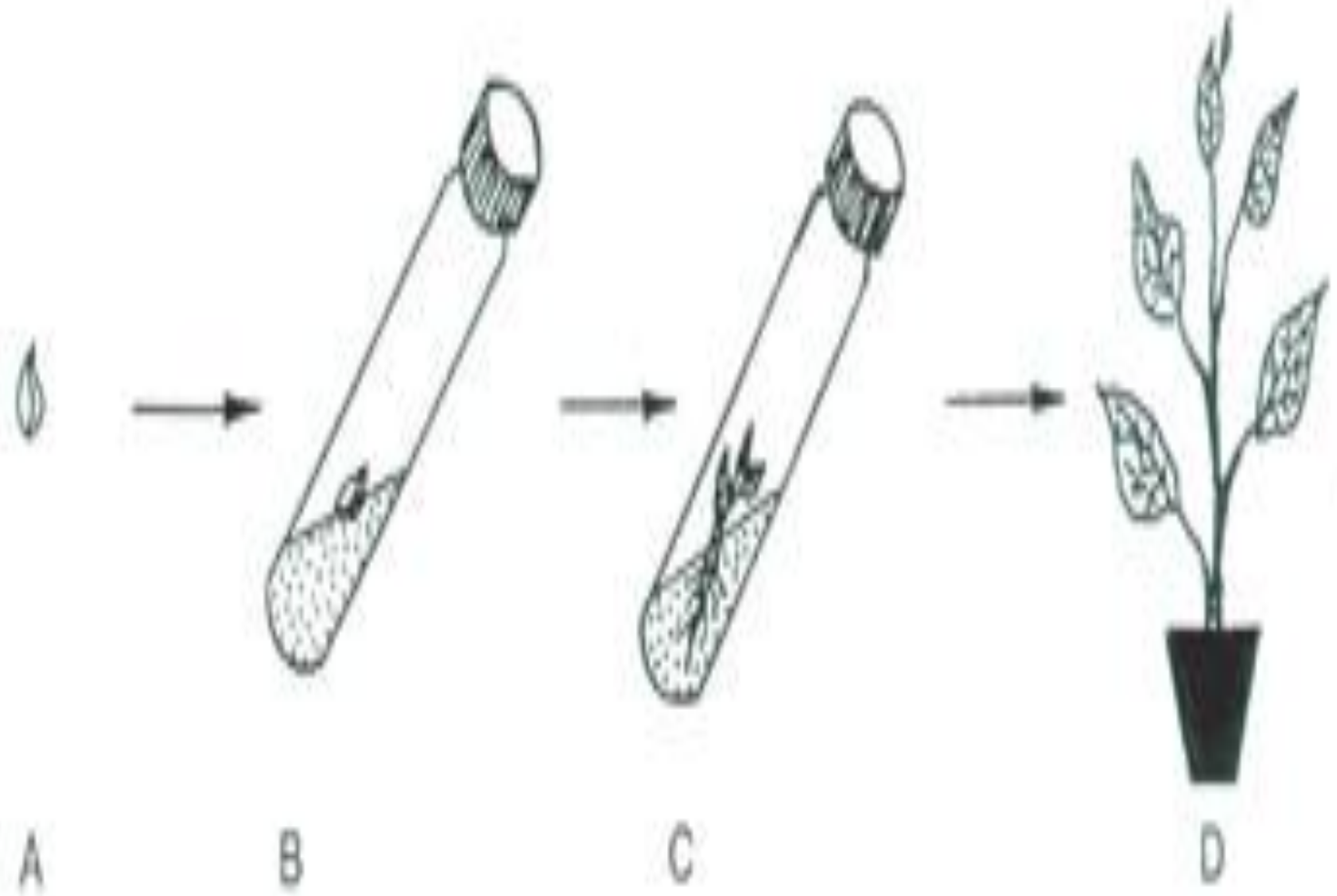
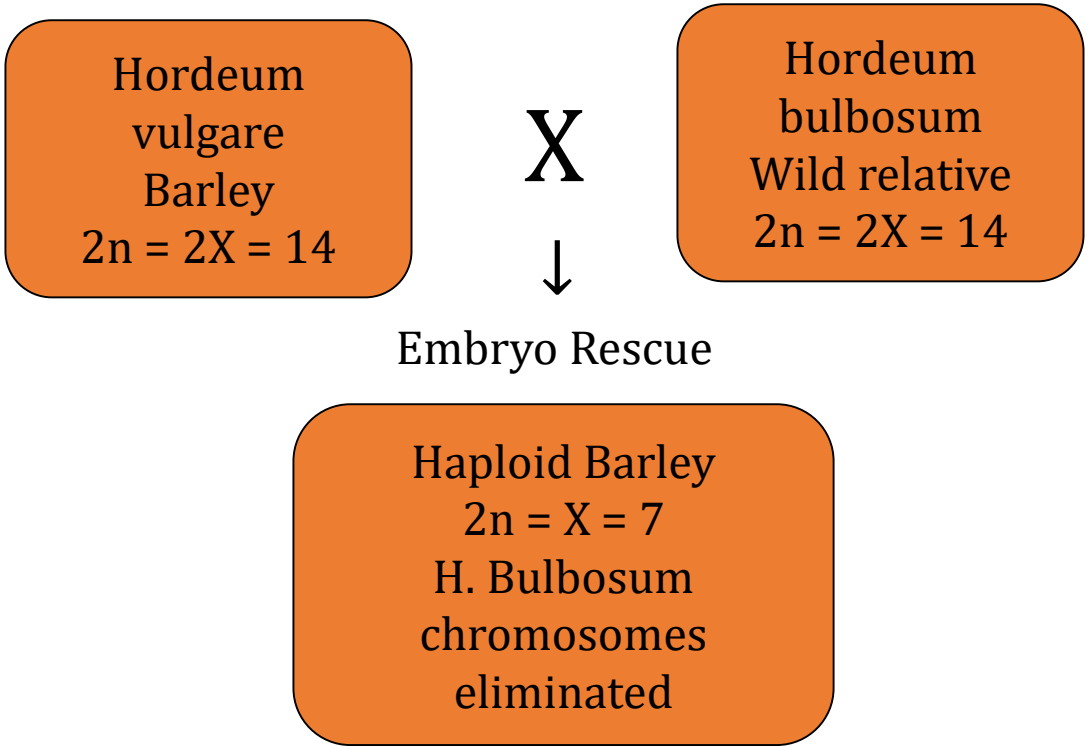


FIG. 8.4. Embryo culture. (A) Proembryo dissected 3 to 5 days after pollination. (B) Proembryo cultured on solid agar medium. (C) Plantlet developing from embryo. (D) Plantlet transplanted into soil.

Haploid Plant Production

- Embryo rescue of interspecific crosses
  - Creation of alloploids (*e.g.* triticale)
  - Bulbosum method
- Anther culture/Microspore culture
  - Culturing of Anthers or Pollen grains (microspores)
  - Derive a mature plant from a single microspore
- Ovule culture
  - Culturing of unfertilized ovules (macrospores)
  - Sometimes “trick” ovule into thinking it has been fertilized

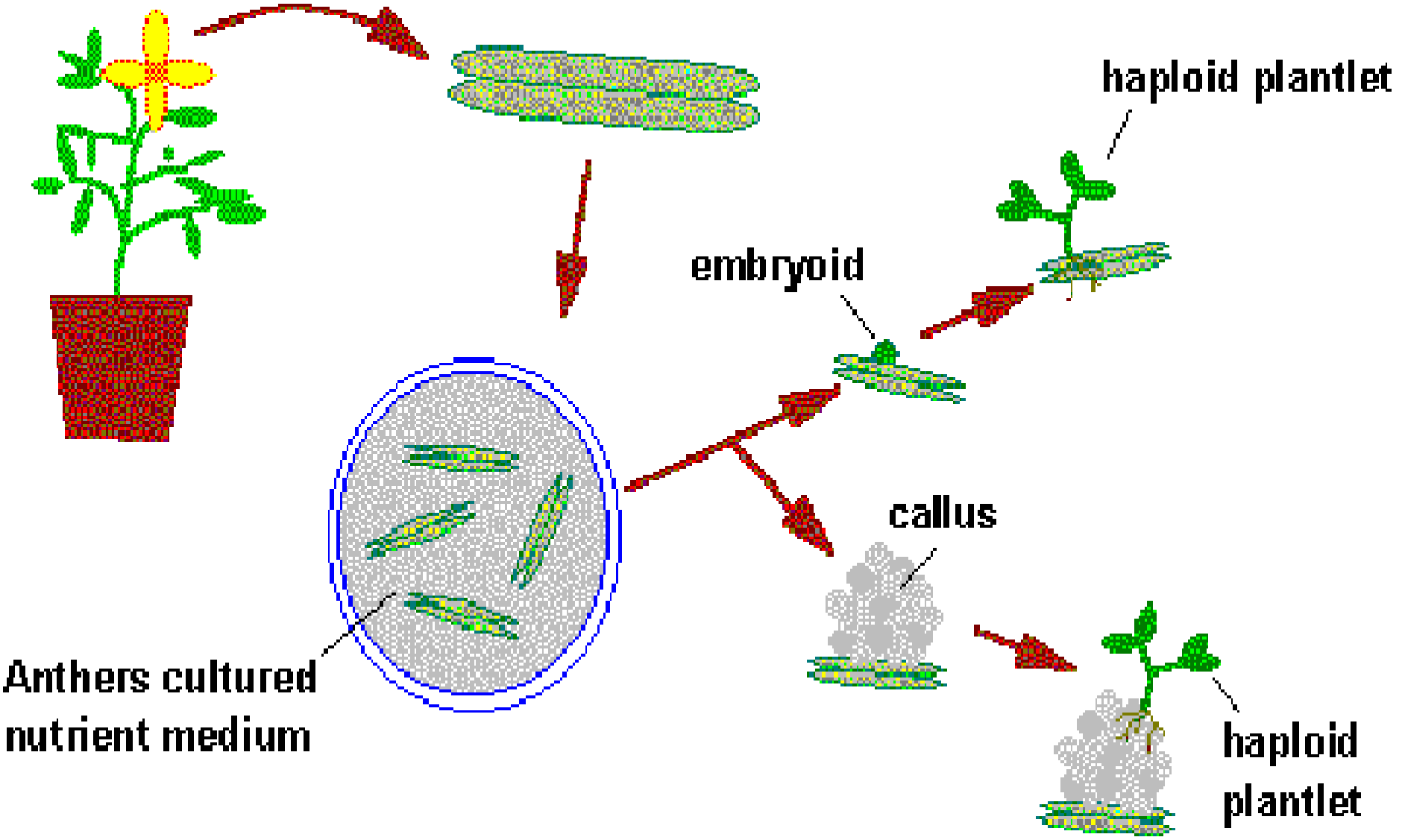
Bulbosum Method



This was once more efficient than microspore culture in creating haploid barley

Now, with an improved culture media (sucrose replaced by maltose), microspore culture is much more efficient (~2000 plants per 100 anthers)

Anther/Microspore Culture



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
  - In the case of melon, you have to "trick" the fruit into developing by using irradiated pollen, then x-ray the immature seed to find developed ovules

What do you do with the haploid?

Weak, sterile plant

Usually want to double the chromosomes, creating a dihaploid plant with normal growth & fertility

Chromosomes can be doubled by

- Colchicine treatment
- Spontaneous doubling
  - Tends to occur in all haploids at varying levels
  - Many systems rely on it, using visual observation to detect spontaneous dihaploids
  - Can be confirmed using flow cytometry

Specific Examples of DH uses

Evaluate fixed progeny from an F_1

- Can evaluate for recessive & quantitative traits
- Requires very large dihaploid population, since no prior selection
- May be effective if you can screen some qualitative traits early

For creating permanent F2 family for molecular marker development

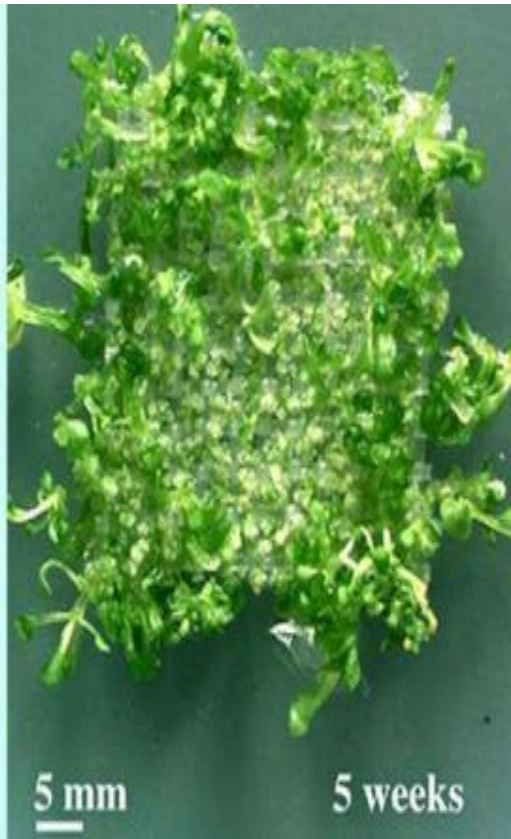
For fixing inbred lines (novel use?)

- Create a few dihaploid plants from a new inbred prior to going to Foundation Seed (allows you to uncover unseen off-types)

For eliminating inbreeding depression (theoretical)

- If you can select against deleterious genes in culture, and screen very large populations, you may be able to eliminate or reduce inbreeding depression
- e.g.: inbreeding depression has been reduced to manageable level in maize through about 50+ years of breeding; this may reduce that time to a few years for a crop like onion or alfalfa

Protoplasts Isolation and Culture



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Protoplast fusion

Protoplasts are made from two species that you want to cross

The membranes are made to fuse

osmotic shock, electrical current, virus

Regenerate the hybrid fusion product

Contain genome from both organisms

Very, very difficult

Uses for Protoplast Fusion

- Combine two complete genomes
  - Another way to create allopolyploids
- Partial genome transfer
  - Exchange single or few traits between species
  - May or may not require ionizing radiation
- Genetic engineering
  - Micro-injection, electroporation, Agrobacterium
- Transfer of organelles
  - Unique to protoplast fusion
  - The transfer of mitochondria and/or chloroplasts between species

Introduction of callus into suspension

‘Friable’ callus goes easily into suspension.

2,4-D

Low cytokinin

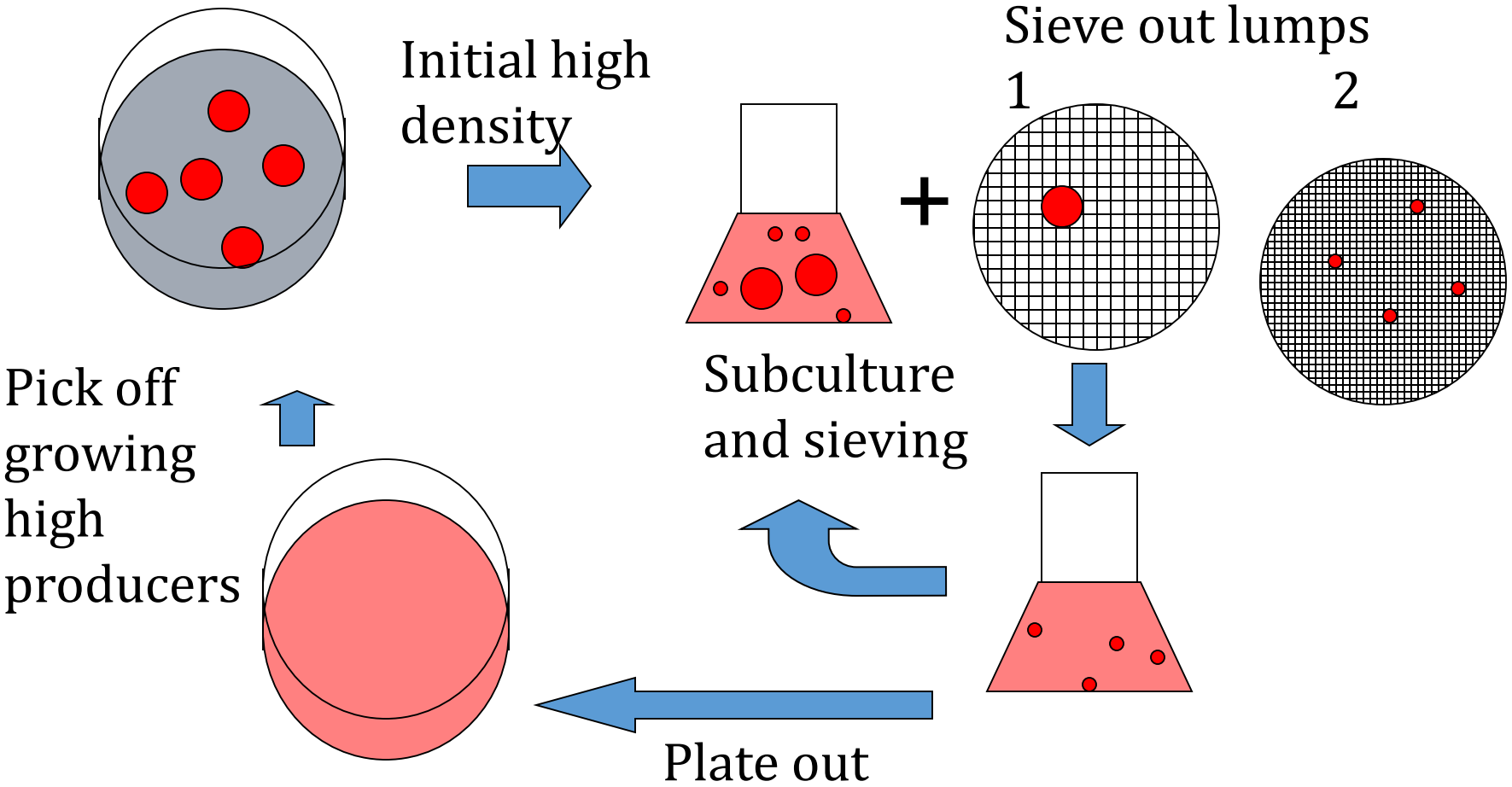
semi-solid medium

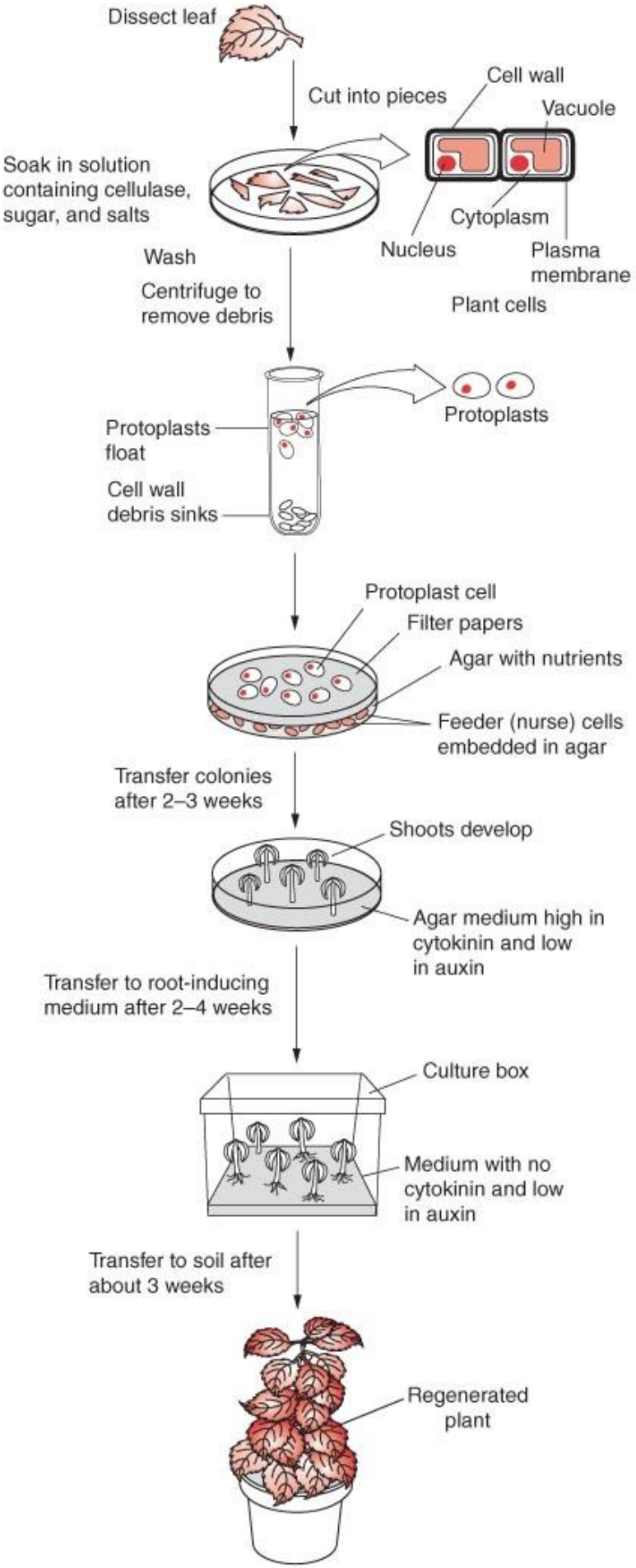
enzymic digestion with pectinase

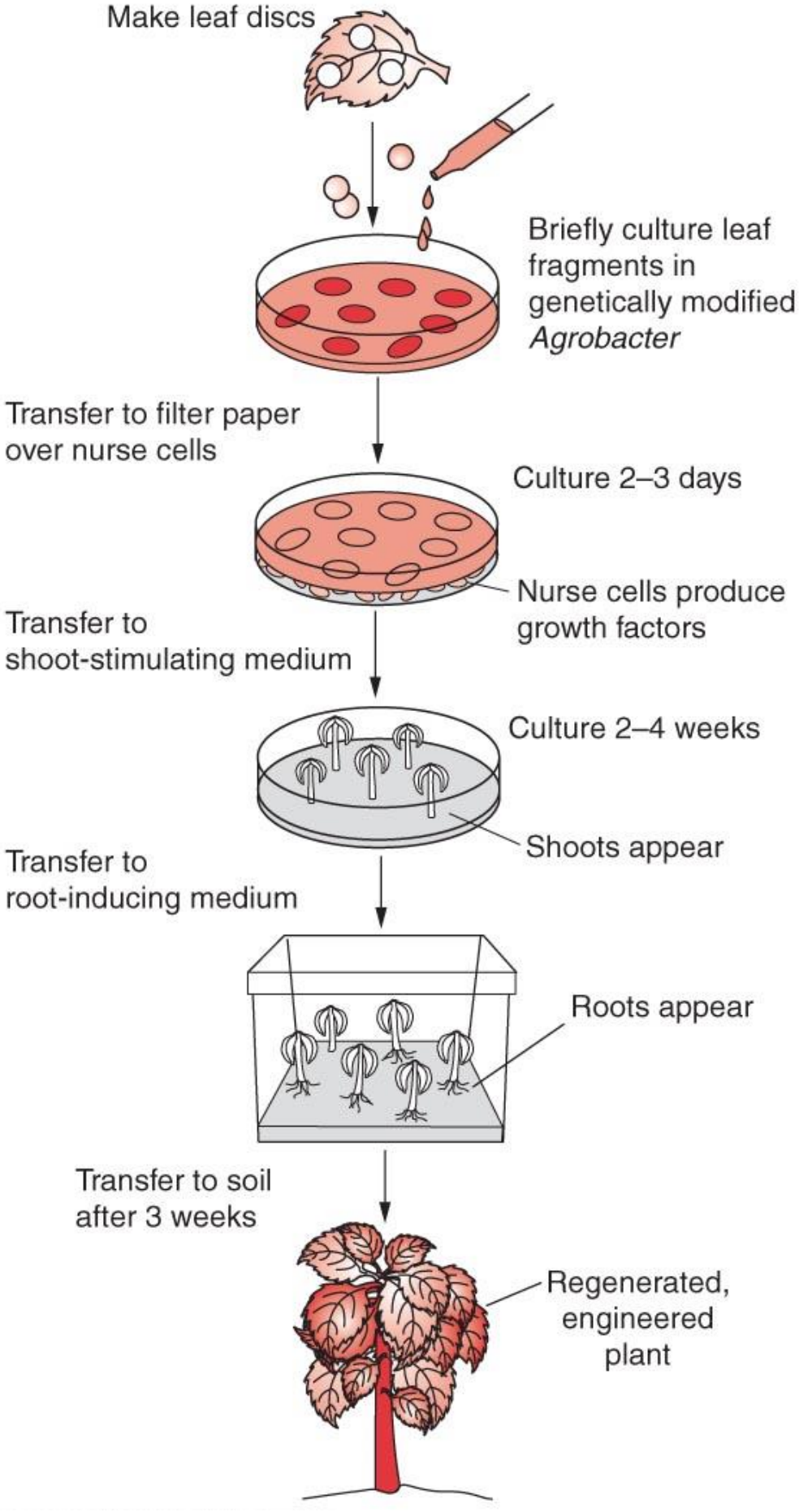
blending

- Removal of large cell aggregates by sieving.
- Plating of single cells and small cell aggregates - only viable cells will grow and can be re-introduced into suspension.

Introduction into suspension




Dissect leaf
Cut into pieces
Cell wall
Vacuole
Soak in solution containing cellulase, sugar, and salts
Cytoplasm
Nucleus
Plasma membrane
Plant cells
Wash
Centrifuge to remove debris
Protoplasts
Protoplasts float
Cell wall debris sinks
Protoplast cell
Filter papers
Agar with nutrients
Feeder (nurse) cells embedded in agar
Transfer colonies after 2–3 weeks
Shoots develop
Agar medium high in cytokinin and low in auxin
Transfer to root-inducing medium after 2–4 weeks
Culture box
Medium with no cytokinin and low in auxin
Transfer to soil after about 3 weeks
Regenerated plant


Make leaf discs
Briefly culture leaf fragments in genetically modified *Agrobacter*
Transfer to filter paper over nurse cells
Culture 2–3 days
Nurse cells produce growth factors
Transfer to shoot-stimulating medium
Culture 2–4 weeks
Shoots appear
Transfer to root-inducing medium
Roots appear
Transfer to soil after 3 weeks
Regenerated, engineered plant

Methods Used in Plant Transgenesis