

Plant Tissue Culture

What is Plant Biotechnology?

Plant biotechnology is the use of scientific techniques to **modify plants** for human benefit.
It involves:

Removing genes from an organism.

Altering them in the lab.

Transferring them into a plant to give it new traits (e.g., disease resistance, better yield).

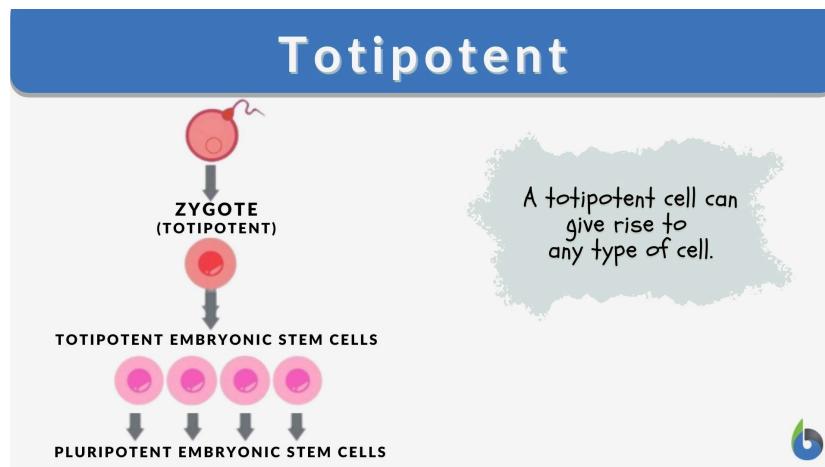
Main techniques:

Plant tissue culture

Plant genetic engineering

1. Concept of Totipotency

- Every plant cell has the ability (**totipotency**) to grow into a complete plant under the right conditions.
- Differentiation in plants (different cells forming roots, stems, leaves, etc.) is not permanent — cells can “reprogram” under special conditions.



2. Gottlieb Haberlandt – Father of Plant Tissue Culture (1902)

- First to attempt culturing isolated plant cells (from *Lamium*, *Eichhornia*, etc.).
Used Knop's solution + sucrose → cells stayed alive but didn't divide.
Predicted:
Cells could resume growth when isolated.

Presence of “growth enzymes” (now known as hormones).
Possible to grow artificial embryos from vegetative cells.
His vision laid the foundation for modern tissue culture.

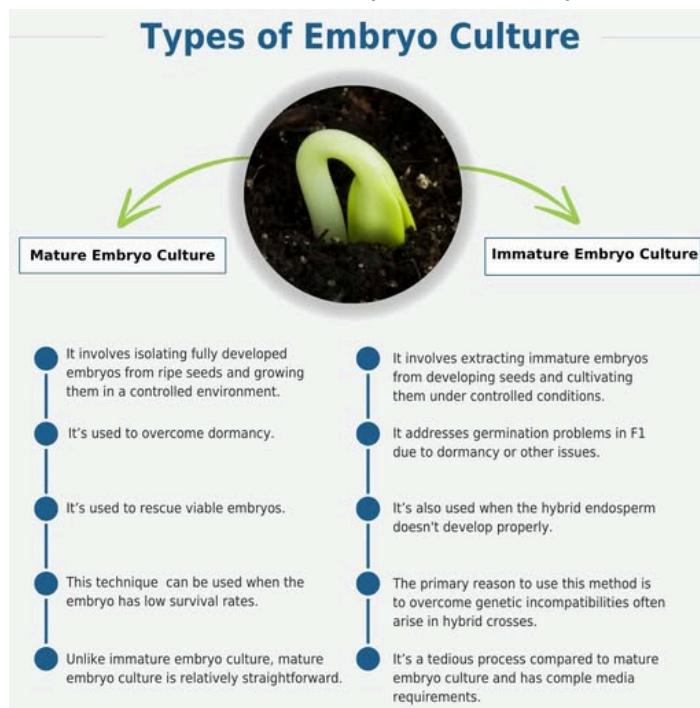


3. Early Developments (1904–1930s)

Hannig (1904): Grew excised embryos on nutrient media.

Van Overbeek (1941): Showed coconut milk (embryo sac fluid) promotes **embryo and callus** growth → led to synthetic media development.

Laibach (1925): Used embryo culture for hybrid plant breeding.



4. Root and Callus Cultures

- **White (1934):** Established continuous root cultures (tomato roots).

What Is a Callus?

A **callus** is a soft, lumpy mass of cells that forms when a plant is **injured** or when small plant parts are **grown in the lab** (in vitro).

Callus cells can later grow into **roots, shoots, or whole plants** — like a healing or growing stage for plants!



1. Callus Formation In Vitro (in the Lab)

In labs, scientists grow plant pieces (explants) on a **nutrient medium**.

When special **plant hormones** called **auxin** and **cytokinin** are added, the cells start dividing and form **callus**.

Hormone Ratios:

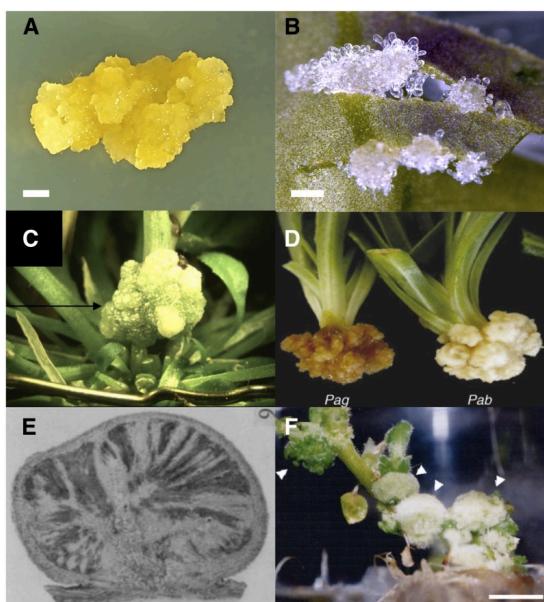
Auxin : Cytokinin Ratio What Happens

Balanced (equal) Callus forms

More auxin Roots form

More cytokinin Shoots form

- This process helps scientists **grow new plants, clone useful traits, or add new genes** (genetic engineering).
- Other hormones like **abscisic acid** and **brassinosteroids** can also help in callus formation.



 **Example – Arabidopsis (a model plant)**

- When its **roots or shoots** are placed in hormone-rich media, **callus forms** from certain inner cells.
Scientists found that these calli are **not random**, but are **organized** like early root tissues.
This means the plant's genes for **root growth** are active even in lab-made callus.
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2. Callus Formation in Nature

Plants can also form callus **naturally** — especially when they are **injured** or **infected**.

A. Wound-Induced Callus

- Happens when a plant is **cut or damaged**.
Cells near the wound start dividing to **heal the area**.
These calli may later form **new roots, stems, or leaves**.
They also help the plant **prevent infection** and **stop water loss**.
Example: When you cut a tree branch or stem, a thick tissue grows over the wound — that's a natural callus!
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B. Pathogen-Induced Callus (Tumors or Galls)

Sometimes, **bacteria or viruses** infect plants and make them grow **tumors** (big callus-like lumps).



Examples:

- Crown Gall Disease** – caused by the bacteria *Agrobacterium tumefaciens*.
The bacteria enter through a wound and insert their **DNA** into the plant.
The plant cells then grow uncontrollably, forming a **gall (tumor)**.
These gall cells can grow even without hormones and can form whole plants again
→ **they are totipotent**.
 - Galls by Other Bacteria or Viruses** –
Pantoea agglomerans bacteria cause galls on *Gypsophila*.
Wound Tumor Viruses (WTVs) cause galls in clover and other crops.
These galls disturb the plant's normal growth.
 - Other Causes** – Insects, nematodes (worms), and tiny parasites can also cause galls.
-

C. Genetic Tumors (in Hybrid Plants)

- When two **different plant species** are crossed (hybridized), sometimes their offspring (hybrids) show **uncontrolled cell growth** (callus-like lumps).

These are called **genetic tumors**.

They can grow without hormones and can become new plants.

This happens because the **balance of hormones (auxin and cytokinin)** gets disturbed.



How Does Callus Form? (Molecular Basis)

Callus formation is a **complex process** controlled by **many genes** and **signals** inside the plant.

Normal plant cells **stop dividing** when mature.

To form callus, cells must “**wake up**” and start dividing again.

Hormones like **auxin** and **cytokinin** turn on special **genes** that restart cell division.

Many **transcription factors** (like LBD, WIND, WUS, BBM, LEC) help in this process.

Chromatin modifiers (proteins that control gene activity) also play key roles.

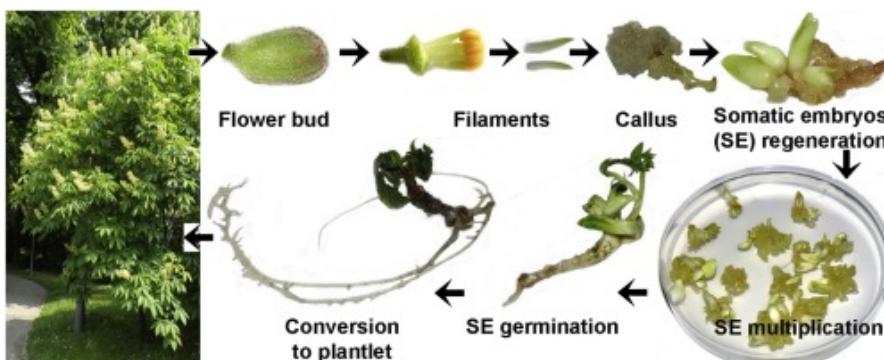
- So, callus formation is like **pressing the “reset” button** in a plant cell — making it young again and ready to grow new parts.
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Type of Callus	How It Forms	Example
In vitro callus	Formed in lab using auxin + cytokinin	Arabidopsis explants on MS medium
Wound callus	Forms when plant is cut or damaged	Tree bark wounds
Pathogen-induced callus (Gall)	Caused by bacteria or virus infection	Crown gall by <i>Agrobacterium</i>
Genetic tumor	Caused by hybridization between species	<i>Nicotiana</i> hybrids

Introduced vitamins (B₁, B₆, nicotinic acid) → essential for plant growth.

Gautheret, White, Nobecourt (1939): First continuous callus (undifferentiated tissue) cultures.

These became the basis for all later plant tissue culture work.



5. Discovery of Plant Hormones

Auxin (1930s) and **cytokinins (1950s)** discovered → key to tissue growth.

Skoog & Miller (1957): Developed the *hormonal control theory*:

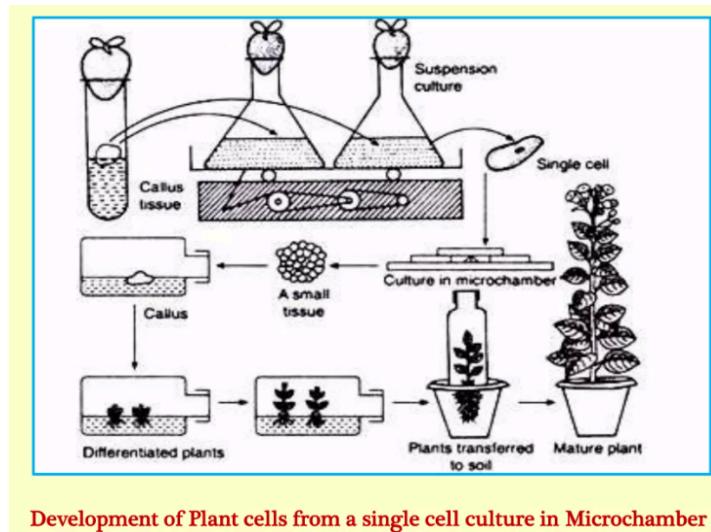
High **auxin** → roots

High **cytokinin** → shoots

Equal levels → callus (undifferentiated tissue)

6. Single Cell Culture

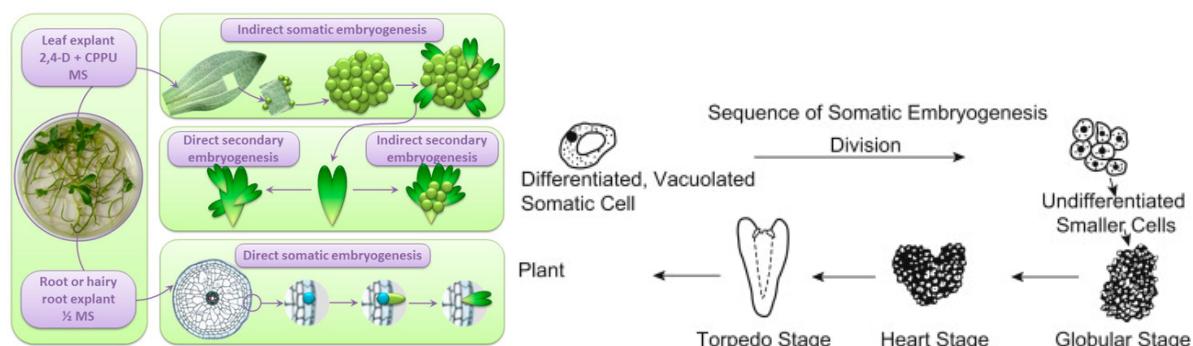
- **Muir (1953):** Developed *suspension cultures* (liquid media + shaking → single cells).
- **Bergmann (1960):** Developed *single-cell cloning* technique.
- **Vasil & Hildebrandt (1965):** Regenerated whole plants from single tobacco cells → proved totipotency.

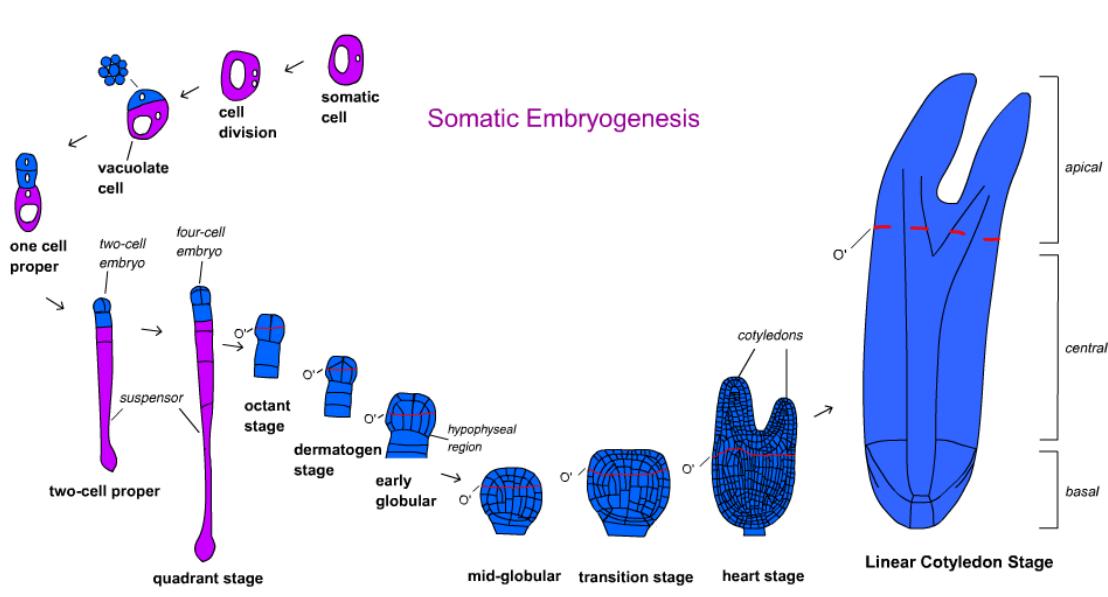


Development of Plant cells from a single cell culture in Microchamber

7. Somatic Embryogenesis (1958–1960s)

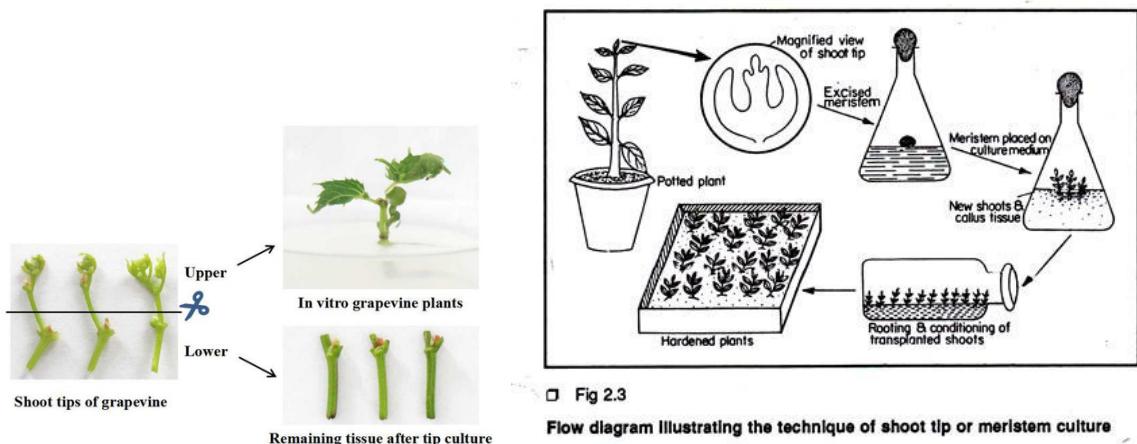
- **Reinert and Steward:** Developed *somatic embryos* from carrot callus → alternative method for plant regeneration.





8. Micropropagation (Shoot Tip Culture)

- **Morel (1952):** Produced virus-free plants using shoot tip culture (Dahlia).
- **Morel (1960):** Used shoot tips to rapidly multiply orchids → commercial propagation.
- **Murashige & Skoog (1962):** Developed the famous *MS Medium* (standard for tissue culture).



Videos that we discussed in the class:

<https://www.youtube.com/watch?v=xuwV3ywCxW8>

<https://www.youtube.com/watch?v=NXNFR4cj68U>



Category	Chemicals	Amount
Macro salts	NH ₄ NO ₃	1.65 g
	KNO ₃	1.90 g
	CaCl ₂ .2H ₂ O	0.44 g
	MgSO ₄ .7H ₂ O	0.37 g
	KH ₂ PO ₄	0.17 g
Micro salts	FeSO ₄ .7H ₂ O	27.80 mg
	Na ₂ EDTA2H ₂ O	33.60 mg
	KI	0.83 mg
	H ₃ BO ₄	6.20 mg
	MnSO ₄ .4H ₂ O	22.30 mg
	ZnSO ₄ .7H ₂ O	8.60 mg
	Na ₂ MoO ₄ .H ₂ O	0.25 mg
	CuSO ₄ .5 H ₂ O	0.025 mg
	CoCl ₂ .6 H ₂ O	0.025 mg
Organic supplements	Myoinositol	100.00 mg
	Nicotinic acid	0.05 mg
	Pyridoxine HCl	0.05 mg
	Thiamine HCl	0.05 mg
	Glycine	0.02 mg
	Sucrose	30.00 g

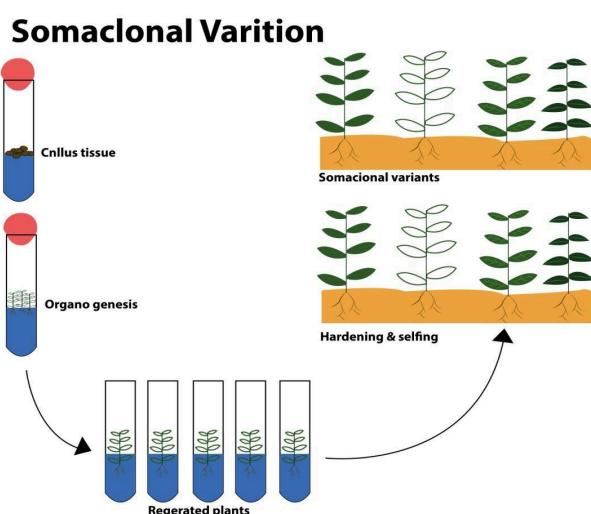
Components of MS medium

9. Somaclonal Variation

- Variations seen in plants regenerated from tissue cultures.

Heinz & Mee (1971): First reported in sugarcane.

Larkin & Scowcroft (1981): Coined the term “**somaclonal variation**” → useful in crop improvement.



Source / Cause:

Callus: A mass of unorganized parenchyma cells formed at the plant wound site.

Chromosomal rearrangements and genetic mutations during tissue culture lead to variation.

Types of Variation:

1. **Phenotypic Variation:** Can be **genetic** (permanent DNA changes) or **epigenetic** (reversible modifications).
2. **Genotypic Variation:** Changes in chromosome number (polyploidy, aneuploidy).
DNA sequence alterations.
Chromosomal structural changes: translocation, insertion, deletion, duplication.

Affected Plants:

- Somaclonal variation can occur in **outcrossing, inbreeding, vegetative, seed-propagated, cultivated, and wild plants.**

Effects on Traits:

- Both **qualitative** (e.g., flower color) and **quantitative** (e.g., yield, growth rate) traits may be affected.

Applications / Advantages:

- **Plant breeding:** Introduces new genetic variability.
- **Disease and stress resistance:** Somaclonal mutants can show resistance to diseases, herbicides, mineral toxicity, and chemical stress.
- **Crop improvement:** Enhanced survival, creation of new species, improved secondary metabolite production.

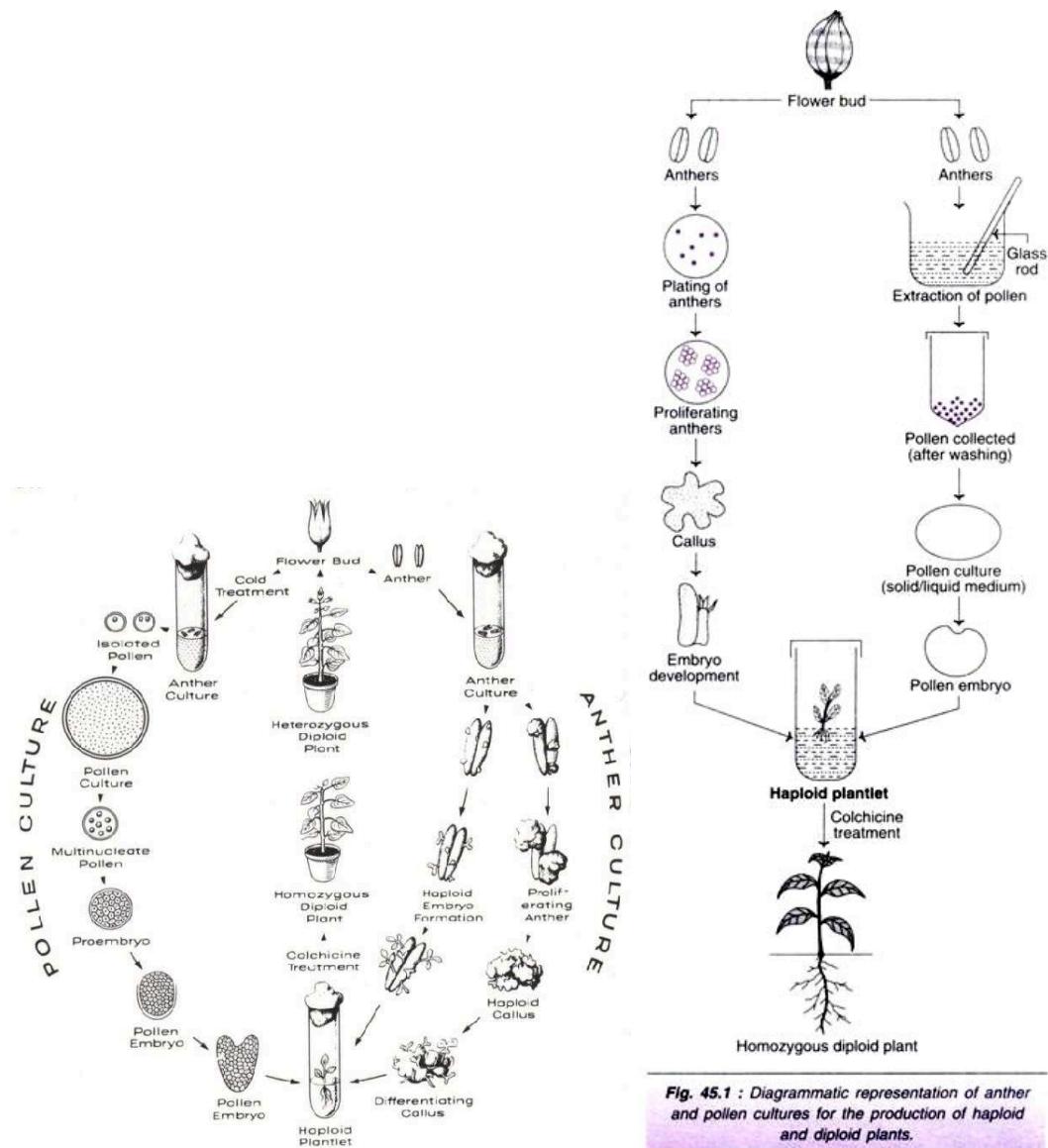
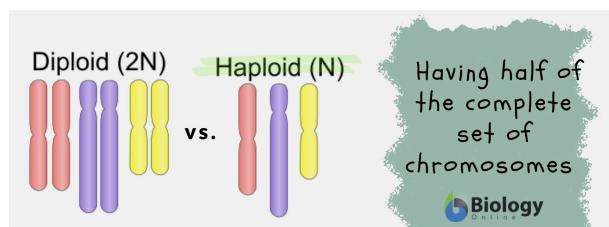
Disadvantages / Limitations:

- Variants are **random and genetically unstable.**
- Not all variations are agronomically desirable (yield, quality, uniformity).
- Requires **extended field trials** to select useful traits.
- **Clonal uniformity** may be compromised.

10. Haploid and Anther Culture

- **Guha & Maheshwari (1966):** Raised haploid plants from pollen (anther culture) in *Datura*.
- **Nitsch (1970s):** Developed isolated microspore culture → haploid production used in plant breeding.

- The production of haploid plants is important for rapidly developing pure, homozygous lines for plant breeding, significantly shortening the time needed to create improved crop varieties.



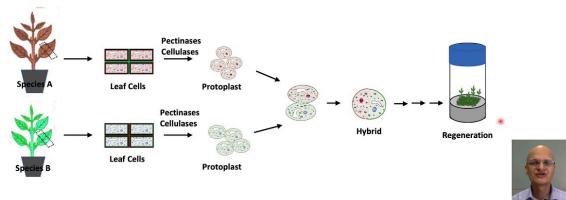
11. Protoplast Culture and Somatic Hybridization

- Cocking (1960):** Isolated protoplasts (cells without walls) using enzymes.
Nagata & Takebe (1971): Proved protoplasts are totipotent.

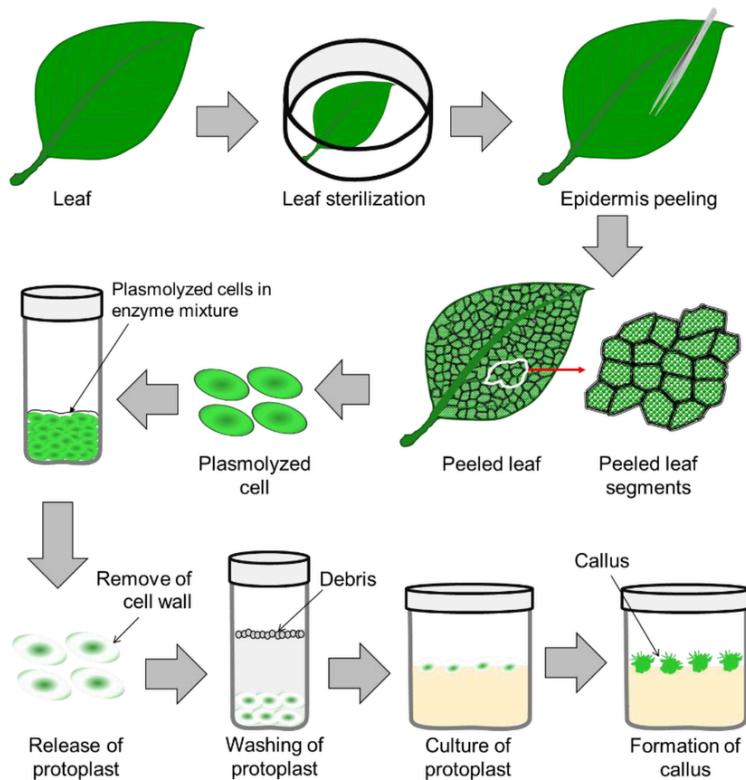
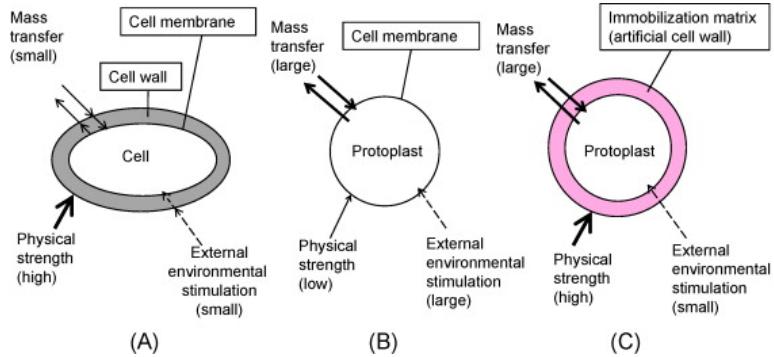
Carlson (1972): Produced first somatic hybrid (*Nicotiana* species).

Melchers (1978): Made intergeneric hybrid (potato × tomato).

The process of fusion of protoplasts isolated from somatic cells of two different plant species/cultivars to regenerate hybrid plants is called as Somatic hybridization.



Protoplast culture is a process in plant biotechnology where plants are regenerated from individual plant cells that have had their cell walls removed, called protoplasts. This technique allows for the fusion of protoplasts to create hybrids, genetic transformation, and the regeneration of whole plants from single cells, which is valuable for plant breeding and genetic research.



12. Genetic Engineering in Plants

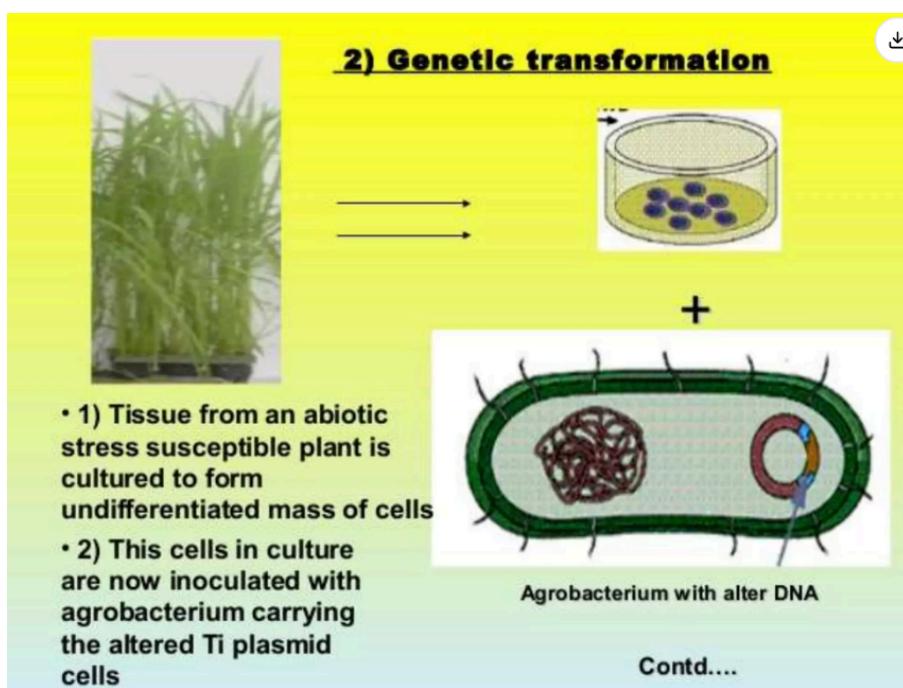
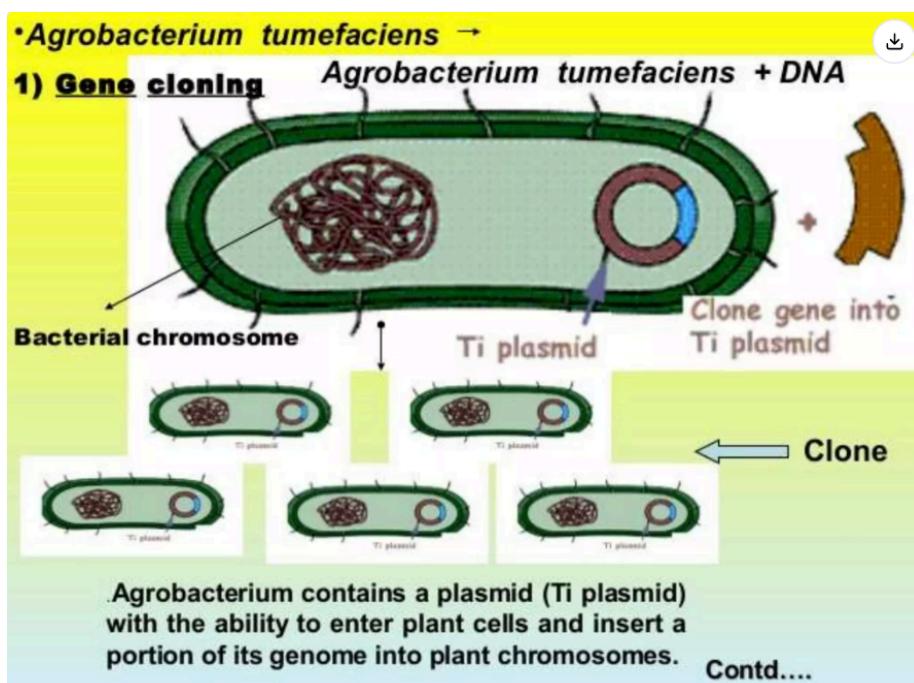
- **Agrobacterium tumefaciens** causes *crown gall disease* by transferring part of its DNA (Ti plasmid) into plants.
Chilton et al. (1977): Proved T-DNA integration into plant genome.
Horsch et al. (1984): Created first transgenic tobacco plants.
Modern techniques: **Agrobacterium vectors, gene gun, electroporation, microinjection** → used for making GM crops.

Method	How It Works	Key Points / Limitations	Applications
Using viruses	Desired gene is inserted into viral DNA; virus infects host cells, carrying the gene into the host genome.	Virus modified so it cannot replicate uncontrollably; gene replicates as cells divide.	Efficient in many organisms; used for gene therapy and research.
Microinjection	Gene is removed with a fine micropipette and injected directly into the nucleus of the host cell.	Simple, precise; limited by cell size; can damage the cell.	Common in animal cells and early embryo modification.
Electroporation	Weak electric current creates temporary pores in the cell membrane; DNA enters the cell.	Single genes can enter nucleus naturally; works for many cell types.	Widely used in bacteria, yeast, plant cells, and mammalian cells.
Chemical poration	Cells are bathed in chemicals that induce pores in the membrane for DNA entry.	Less invasive than physical methods; effectiveness varies with cell type.	Used for plants, bacteria, and mammalian cells.
Laser poration	Tiny laser beams create pores in the cell membrane; new genes are introduced.	Highly precise; requires specialized equipment.	Suitable for research and experimental gene delivery.
Gene scissors (e.g., CRISPR/Cas)	Lasers or molecular tools cut genes/chromosomes; recipient DNA is opened for insertion.	Enables targeted modification; reduces random integration.	Genome editing, functional genomics, crop improvement.
Gene guns / biolistics	Genes are coated on tiny metal particles and “fired” into cells; particles penetrate and deliver DNA.	Originally used on large plant cells; still mainly for plants.	Plant genetic engineering, especially cereals and crops.

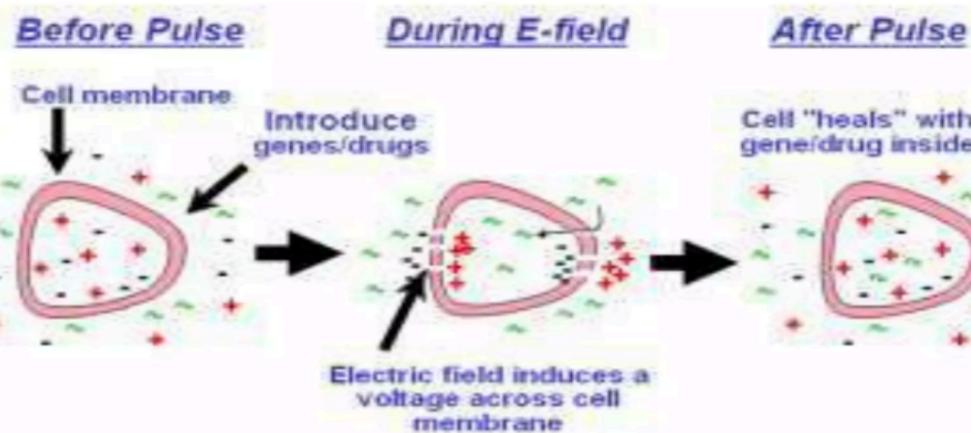
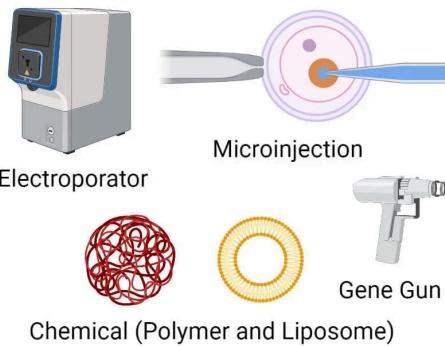
Nanoparticles Genes are carried on biodegradable polymer nanoparticles; penetrate cell and organelle membranes.

Can target mitochondria and chloroplasts; biocompatible.

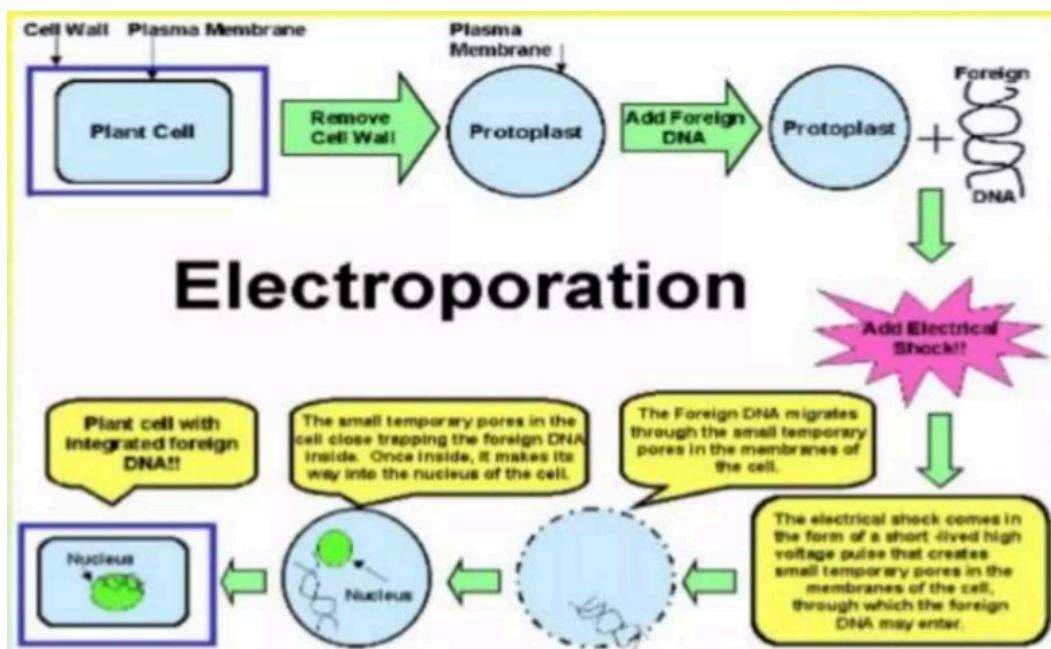
Emerging technique for precise genetic modification in plants and animals.



Gene Delivery Methods



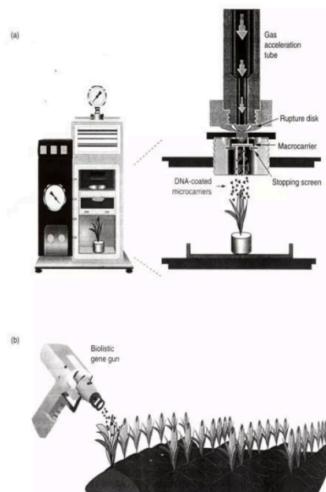
Method of Electroporation: under the influence of electric shocks, holes will create in the plasma membrane, through which foreign DNA fragment enter into the host cytoplasm and after that into the nucleus



Microprojectile bombardment or
biolistic-mediated DNA
transfection equipment

- (a) lab version
(b) portable version

When the helium pressure builds to a certain point, the plastic rupture disk bursts, and the released gas accelerates the flying disk* with the DNA-coated gold particles on its lower side. The gold particles pass the stopping screen, which holds back the flying disk, and penetrate the cells of the plant.



<https://www.slideshare.net/slideshow/gene-transfer-methods-148119209/148119209>

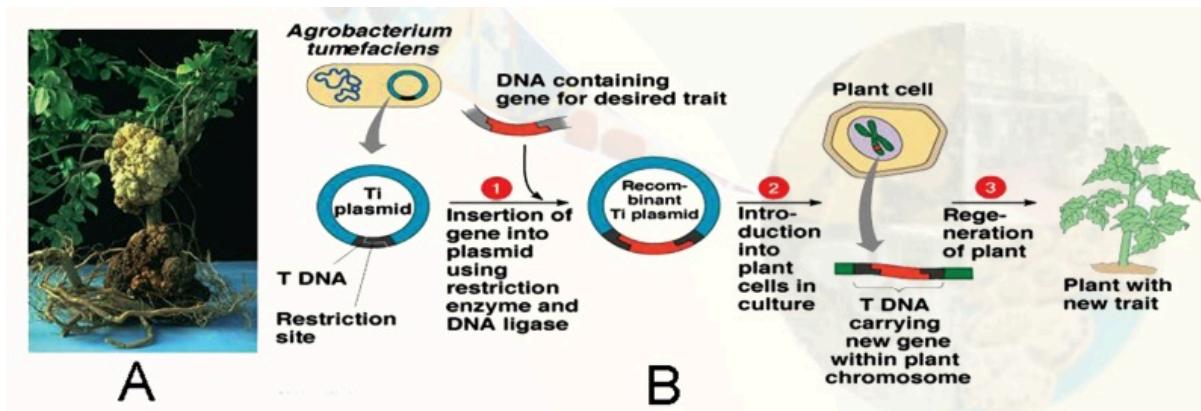
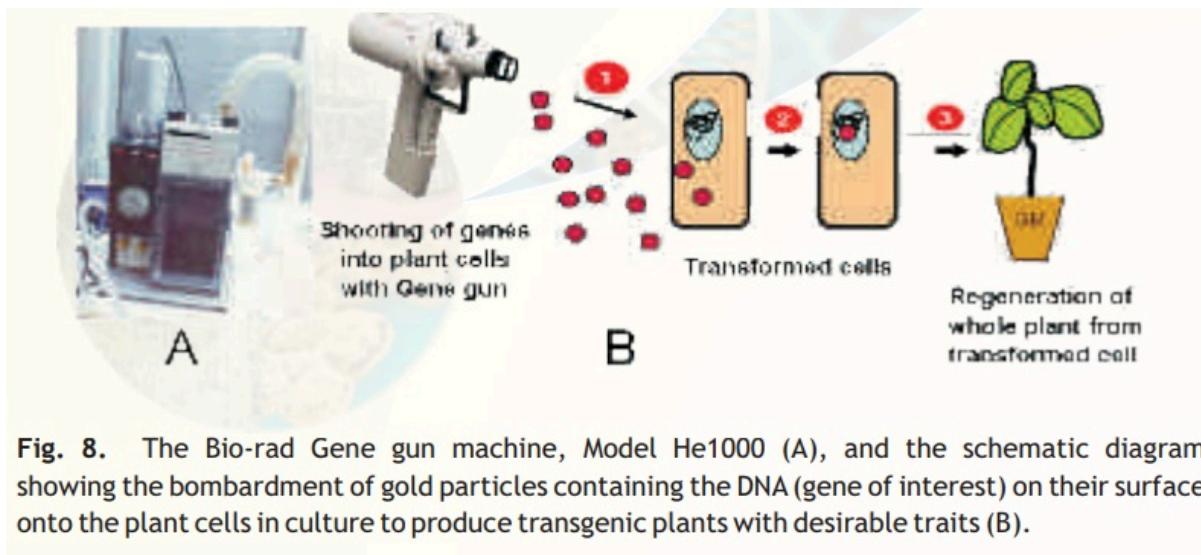
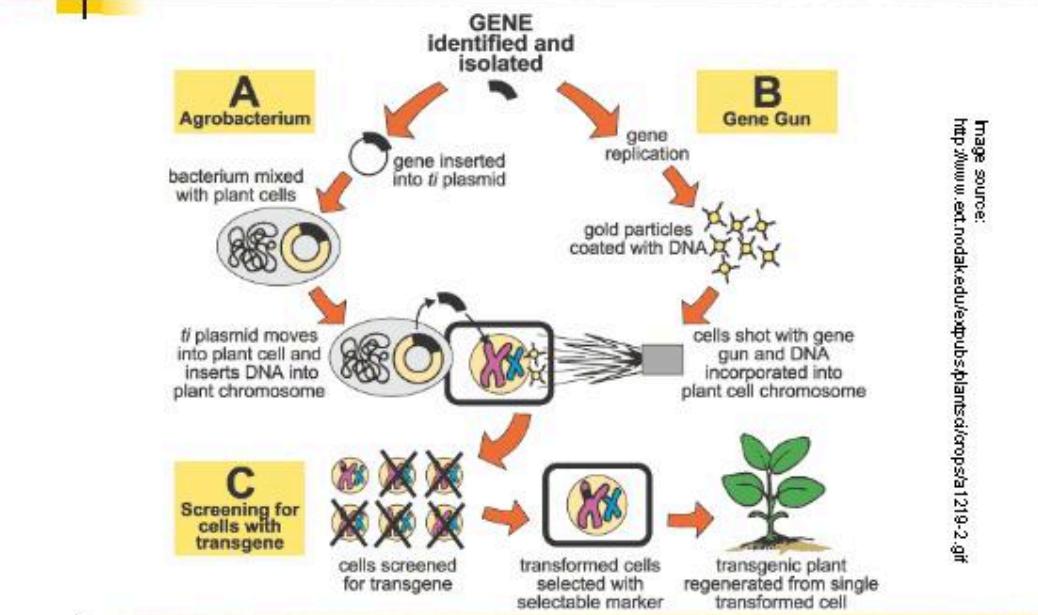


Fig. 7. Induction of crown gall on the wounded stem induced by wild-type virulent *Agrobacterium tumefaciens* (A), and the schematic diagram showing the cloning of the gene of interest in *Ti*-plasmid of *Agrobacterium* and its transfer to plant cells in culture to produce transgenic plants with desirable traits (B).

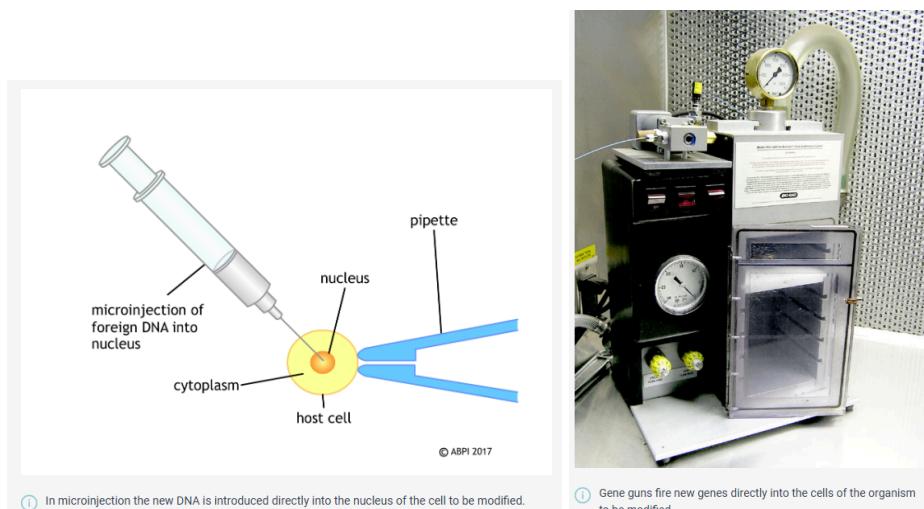


Gene transfer techniques...



13. Applications of Tissue Culture

- Rapid **clonal propagation** (micropropagation)
- Virus elimination via shoot tip culture
- Germplasm storage (cryopreservation at -196°C)
- Production of secondary metabolites in cell cultures (e.g., shikonin, ginseng)
- Somaclonal variation for breeding
- Somatic hybridization and genetic engineering



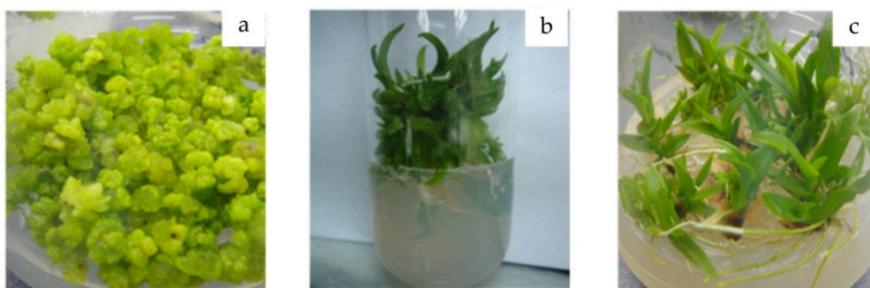
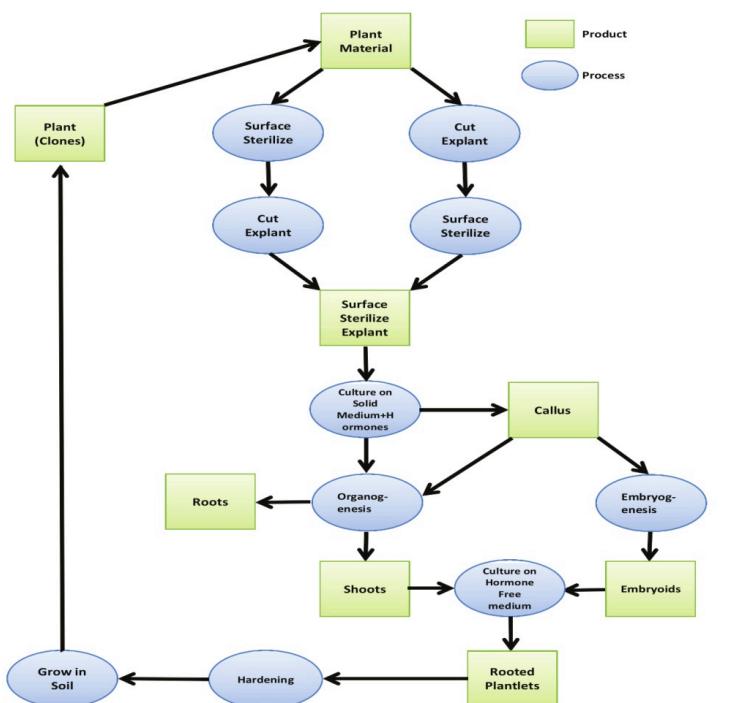


Figure 3.

Micropagation of Orchids (a) callus culture (b) shoot regeneration (c) rooted plantlets

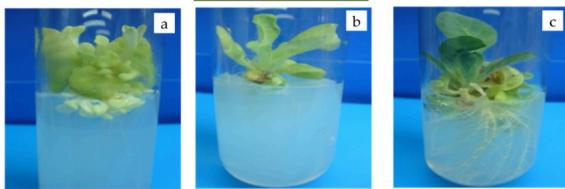
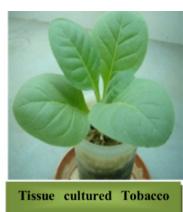


Figure 4.

Tissue culture of Nicotianatabacum (a) callus (b) shoot regeneration (c) root induction

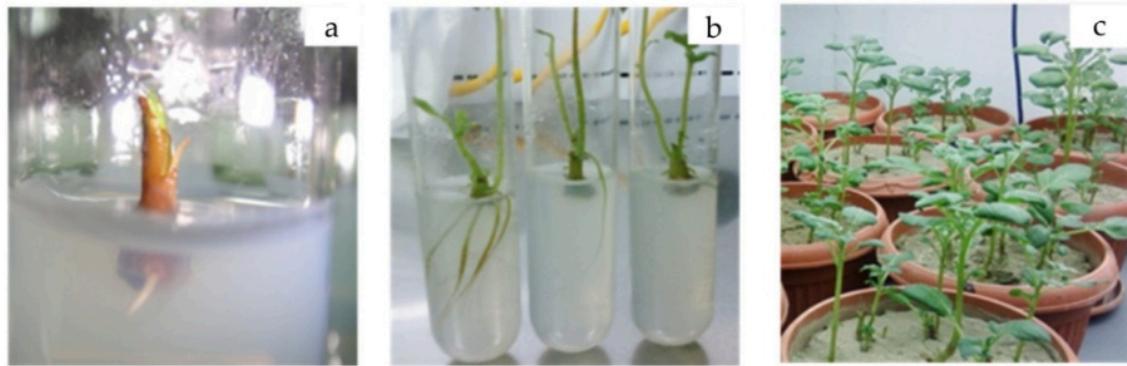


Figure 6.

Tissue culture of Potato (a) nodal segment (b) regenerated shoots and roots (c) tissue culturedpotato

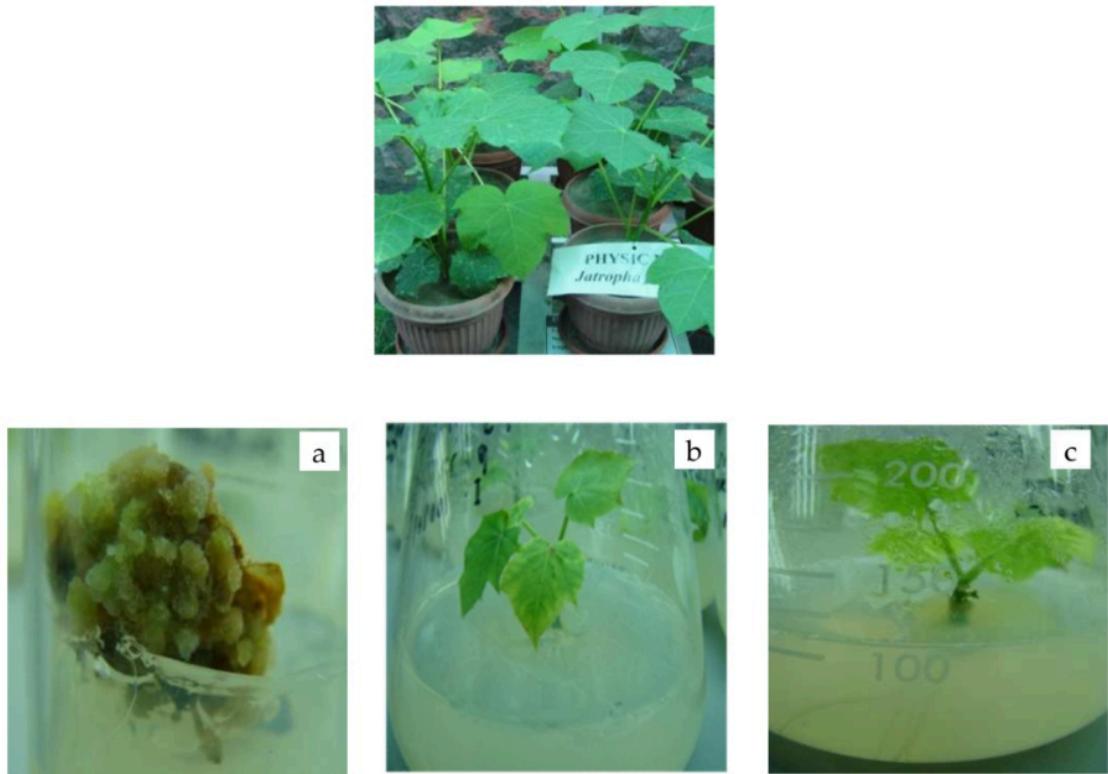


Figure 7.

Tissue culture of Jatropha curcas (a) callus of Jatropha (b) shoot regeneration (c) root induction.

Plant Tissue Culture: Overview

Aspect	Details
Definition	Aseptic in vitro culture of plant cells, tissues, organs, or whole plants under controlled nutritional and environmental conditions to produce clones.
Purpose / Applications	- Large-scale plant multiplication - Production of disease-free plants - Plant improvement and breeding - Production of secondary metabolites - Conservation of rare and endangered species
Key Advantages	- True-to-type clones - Rapid propagation irrespective of season - High multiplication coefficient - Produces virus-free plants - Enables somaclonal and gametoclonal variation for crop improvement

Historical Milestones in Plant Tissue Culture

Year	Discovery / Event
1838	Schleiden & Schwann: Cell theory; plant cells are totipotent
1902	Haberlandt: Attempted in vitro culture of single leaf cells
1926	Went: Discovered first plant growth hormone – Indole Acetic Acid
1939	Gautheret, White, Nobécourt: Callus culture proliferation
1955	Skoog & Miller: Kinetin discovered; hormonal control of organogenesis
1959	Reinert & Steward: Carrot embryos regenerated from callus
1960s	Cocking: Protoplast isolation; Murashige & Skoog: MS medium
1970s	Protoplast fusion, interspecific hybrids, somatic hybridization
1981	Larkin & Scowcroft: Term “somaclonal variation” introduced
1984–1987	Development of transgenic plants (Agrobacterium & biolistic methods)
2005	Rice genome sequenced

Basics of Plant Tissue Culture

Component	Role / Details
Medium	Provides nutrients, vitamins, carbon source, gelling agents; MS medium commonly used.

Plant Growth Regulators (PGRs)	Auxins, cytokinins, gibberellins; balance determines root/shoot formation or callus induction.
Totipotency	Single plant cell can regenerate into an entire plant under suitable conditions.
pH	Typically 5.4–5.8; affects growth and hormone activity.
Callus Formation	High auxin + cytokinin balance → undifferentiated mass of cells; source for regeneration or somaclonal variation.
Example Applications	- Stevia: 0.5 mg/L NAA → root induction - Black pepper: BA 0.5 mg/L → shoot proliferation - Phalaenopsis orchids: GA3 0.5 mg/L → shoot elongation

Applications in Agriculture and Biotechnology

Application	Details
Crop Improvement	Production of superior varieties through somaclonal variation and genetic engineering.
Disease-Free Plants	Virus-free propagation (e.g., banana, potatoes, <i>Corydalis</i>).
Mass Propagation	Rapid multiplication of high-quality plants for commercial agriculture.
Secondary Metabolites	Production of important compounds in vitro.
Stress Tolerance	Developing varieties tolerant to salinity, drought, heat, and chemical stress.
Genetic Engineering	Enables genetic transformation, creation of interspecific hybrids, and enhanced crop traits.

5. Germplasm Conservation

- **Purpose:** To conserve endangered plant genotypes and maintain genetic diversity.
- **Methods:**
 - **In vitro culture:** Preserves vegetative tissues, especially for clones, sterile plants, or plants with recalcitrant seeds.
 - **Cryopreservation:** Long-term storage of tissues in liquid nitrogen.

- Success depends on tissue survival and regeneration.
 - Genetic integrity of recovered plants must be assessed (phenotypic, cytological, molecular levels).
 - **Cryobionomics:** Emerging approach to study stability in cryopreserved material.
 - **Applications:** Maintenance of gene banks, preservation against natural disasters or stresses.
-

6. Embryo Culture

- **Definition:** Culturing embryos from seeds or ovules in nutrient medium.
 - **Purpose:**
 - Break seed dormancy
 - Test seed viability
 - Produce rare species or haploid plants
 - Shorten plant breeding cycles
 - **Examples:**
 - Jatropha intra-varietal hybrids for mass multiplication
 - Jucara Palm somatic embryogenesis for rapid cloning
 - Khaya grandifoliola for timber and medicinal plant propagation
 - **Applications:** Conservation, forestry, plant improvement.
-

7. Genetic Transformation

- **Purpose:** Introduce desirable genes into host plants for improved traits.
- **Methods:**

- **Vector-mediated (indirect):** e.g., Agrobacterium-mediated transformation
 - **Vectorless (direct):** e.g., particle bombardment
- **Applications:**
 - Disease and pest resistance (e.g., potato resistant to PVY)
 - Reduction of toxic compounds in seeds (e.g., Jatropha)
 - Production of transgenic crops with improved yield and quality
-

8. Protoplast Fusion (Somatic Hybridization)

- **Definition:** Fusion of protoplasts from different species to produce hybrid plants.
 - **Purpose:** Overcome sexual incompatibility, transfer desired traits.
 - **Examples:**
 - Rice × ditch reed hybrids for salt tolerance
 - Citrus hybrids for horticulture
 - Wheat improvement using *Haynaldia villosa* as donor
 - **Applications:** Crop improvement, horticulture, genetic diversity enhancement
-

9. Haploid Production

- **Definition:** Production of plants with a single set of chromosomes; doubled haploids are fertile homozygous plants.
- **Methods:** Protoplast, anther, or microspore culture
- **Applications:**
 - Speed up breeding cycles
 - Produce inbred lines

- Introduce desired traits at haploid stage for genetic improvement
 - **Example:** Drought-tolerant wheat via double haploids
-

10. Current and Future Status of Plant Tissue Culture

- Tissue culture enables:
 - Mass propagation
 - Introduction of genetic modifications
 - Production of proteins, vaccines, and antibodies in transgenic plants
 - Transgenic plants offer cost-effective, disease-free systems for industrial and pharmaceutical uses.
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11. Micropropagation

- **Stages:**
 1. **Preparation of donor plant:** Healthy, contamination-free mother plants
 2. **Initiation:** Surface sterilization of explants, culture in nutrient medium
 3. **Multiplication:** Increase propagule numbers via subcultures
 4. **Rooting:** Induce root formation, sometimes requiring media modification
 5. **Acclimatization:** Gradual adaptation to ex vitro conditions
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12. Somatic Embryogenesis and Organogenesis

- **Somatic Embryogenesis:**
 - Somatic cells develop into embryos → whole plants without sexual fertilization
 - Can be direct (from explant) or indirect (via callus)

- Applications: Clonal propagation, stress-resistant plants, genetic transformation
 - **Organogenesis:**
 - Formation of roots, shoots, and leaves from callus or meristem
 - Regulated by hormone ratios (cytokinin:auxin for shoots, auxin:cytokinin for roots)
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13. Tissue Culture in Pharmaceuticals

- **Cell suspension culture:**
 - Mass cultivation of plant cells for secondary metabolites
 - Controlled environment, independent of climate or soil
 - Examples: Shikonin, paclitaxel production in bioreactors
 - **Applications:** Production of alkaloids, terpenoids, steroids, flavonoids, amino acids, and recombinant proteins
 - **Table 1:** Lists plant metabolites produced via cell suspension culture
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14. Hairy Root Cultures

- **Definition:** Cultures derived from Agrobacterium rhizogenes-infected roots
- **Advantages:** High growth rate, genetic stability, hormone-free growth
- **Applications:** Production of root-specific secondary metabolites comparable to whole plants
- **Table 2:** Lists metabolites produced via hairy root cultures

Here's a structured summary and analysis of the tissue culture facilities and case studies at Qarshi Industries, highlighting key techniques, protocols, and outcomes:

Qarshi Industries Tissue Culture Lab

- **Established:** 2004
 - **Objectives:**
 - Propagate endangered medicinal plants and plants difficult to grow by traditional methods.
 - Conservation and mass propagation.
 - Commercialization of fruit and vegetable crops.
 - **Medicinal plants propagated (12 species):**
 - *Plumbago zeylanica, Nicotiana tabacum, Artemisia absinthium, Rosa damascena, Althea rosea, Stevia rebaudiana, Jatropha curcas, Phalaenopsis, Piper nigrum, Solanum tuberosum, Araucaria heterophylla, Taxus wallichiana.*
 - **Current focus:** Propagation of endangered woody plants (e.g., *Taxus wallichiana*) and commercialization of certain crops.
 - **Protocols developed for:** Moth Orchid, Tobacco, Honey Plant (Stevia), Potato, Physic Nut (Jatropha).
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Case Studies

1. Micropropagation of Phalaenopsis “Moth Orchids”

- **Challenges:** Slow vegetative propagation; seedlings often lose desired traits.
- **Methodology:**
 - Callus obtained from mature plants.
 - Callus cultured on MS medium + 3% sucrose, 0.8% agar, BAP, 2,4-D.
 - Subcultured every 30 days; optimal callus proliferation at 0.5 mg/l BAP.
 - Shoot regeneration on MS + BAP + GA3; max elongation at 1.0 mg/l GA3.
 - Rooting on medium + 2.0 mg/l IBA.

- **Outcome:** Efficient shoot and root regeneration, enabling mass propagation for commercial use.
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2. Tissue Culture of Tobacco (*Nicotiana tabacum*)

- **Objective:** Clonal propagation of low-nicotine hybrids (PGH-01, 02, 04, 09).
 - **Methodology:**
 - Leaves and meristems used as explants.
 - Callus induction: MS + 1.0 mg/l 2,4-D.
 - Shoot regeneration: MS + 0.5 mg/l BAP.
 - Rooting: MS + 2.0 mg/l IBA.
 - **Outcome:** Commercially viable tissue-cultured tobacco plants.
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3. In Vitro Propagation of Stevia (*Stevia rebaudiana*)

- **Objective:** Mass clonal propagation.
 - **Methodology:**
 - Seeds germinated on MS medium (16h light/8h dark).
 - Nodal segments (0.5 cm) used for shoot multiplication.
 - Best shoot formation: MS + 2.0 mg/l BAP.
 - Optimal shoot length: MS + 2.0 mg/l Kn + 0.25 mg/l IAA.
 - Rooting: MS + 0.5 mg/l NAA, 81% success.
 - **Outcome:** Protocol optimized for local conditions; suitable for commercial cultivation.
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4. Multiplication and Regeneration of Potato (*Solanum tuberosum*)

- **Objective:** Mass propagation of high-yield varieties (Desiree, Diamant, Cardinal).
 - **Methodology:**
 - Disease-free sprouts (5-day-old) used as explants.
Sterilization: detergent + 0.1% mercuric chloride.
Medium: Espinosa + vitamin B5 + BAP and GA3.
Shoot induction: 0.5 mg/l BAP + 0.4 mg/l GA3.
Root induction: 2.0 mg/l NAA.
 - **Outcome:** Disease-free plantlets ready for cultivation.
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5. Tissue Culture of Physic Nut (*Jatropha curcas*)

- **Objective:** Mass propagation of elite trees with high oil yield.
 - **Methodology:**
 - Explants: leaf and apical meristems from 7-day-old seedlings.
Callus induction: MS + 1.0 mg/l 2,4-D (white, friable callus).
Shoot regeneration: apical meristem used.
Rooting: MS + 2.0 mg/l IBA; secondary roots developed.
 - **Outcome:** Efficient clonal propagation; plans for somatic embryogenesis and field release.
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Key Observations Across Case Studies

1. **MS Medium Dominance:** Murashige & Skoog medium was the base for most protocols.
 2. **Use of Plant Growth Regulators (PGRs):**
Cytokinins (BAP, Kinetin) for shoot induction.
Auxins (IAA, IBA, NAA) for rooting.
GA3 used for shoot elongation.
 3. **Callus as Explant Source:** Critical for plants with limited vegetative propagation potential (e.g., orchids, *Jatropha*).
 4. **Acclimatization:** Rooted plantlets were successfully transferred to soil/greenhouse conditions for all species.
 5. **Commercial Implications:** Protocols enable large-scale, disease-free propagation of high-value crops for farmers and industry.
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