📄 Cheat Sheet 1 – Introduction to Plant Tissue Culture 🌱🧪

🌟 Definition / Concept

- **Plant Tissue Culture (PTC)** = *In vitro* aseptic culture of plant cells, tissues, organs, or whole plants on a nutrient medium under controlled conditions.
- Based on the principle of **Totipotency** – the ability of a single cell to regenerate into a complete plant.

🗝️ Basic Requirements

1. **Explant** – Small piece of plant tissue used to start culture (e.g., meristem, leaf, root, stem, anther).
2. **Culture medium** – Nutrient solution (MS medium = most common).
3. **Aseptic conditions** – Use of laminar airflow, autoclave sterilization.
4. **Controlled environment** – Light, temperature, humidity.

🕰️ Historical Background

- **1902 – Haberlandt**: Proposed totipotency, father of tissue culture.
- **1939 – White**: Cultured root tips.
- **1941 – Gautheret**: Demonstrated callus culture.
- **1958 – Steward**: Regenerated whole carrot plants from single cells.
- **1962 – Murashige & Skoog (MS)**: Developed standard medium → revolutionized plant tissue culture.

🔬 Types of Tissue Culture

- **Callus culture** – Unorganized cell mass.
- **Organ culture** – Whole organ/part (e.g., root/shoot).
- **Single-cell culture** – Individual cell isolation.
- **Meristem culture** – Virus-free plants.
- **Protoplast culture** – Fusion & hybrid plants.
- **Embryo culture** – Embryo rescue, hybridization.

🧠 Mnemonics

- **“Every Medium Always Controls”** → Explant, Medium, Asepsis, Controlled environment.
- **PTC Pioneers** → *Haberlandt (concept) → White (roots) → Gautheret (callus) → Steward (plant regen) → MS (medium).*

📊 Key Uses

- Clonal propagation (micropropagation).
- Production of disease-free plants.
- Germplasm conservation.
- Genetic engineering & transgenic plants.
- Production of secondary metabolites (medicinal compounds).

🎯 Exam Capsule

- **Totipotency** – coined by Haberlandt (1902).
- **MS medium** – Murashige & Skoog, 1962 (most used).
- **Meristem culture** – virus elimination.
- **Carrot culture by Steward (1958)** – classic example of totipotency.

📄 Cheat Sheet 2 – Plant Tissue Culture Media Composition & Types □‍

🌟 Concept

Culture medium = **artificial nutrient environment** that supports *in vitro* plant growth.
The most widely used = **MS (Murashige & Skoog, 1962)**.

🔑 Major Components of Culture Medium

1. **Inorganic Salts**
    - **Macronutrients**: N, P, K, Ca, Mg, S.
    - **Micronutrients**: Fe, Mn, Zn, Cu, B, Mo, Co, I.
2. **Carbon Source**
    - Usually **Sucrose (2–3%)** → main energy source.
3. **Vitamins**
    - Thiamine (B_1), Nicotinic acid, Pyridoxine (B_6), Glycine.

4. **Plant Growth Regulators (PGRs)** 🌿
    - **Auxins** (IAA, NAA, 2,4-D) → root induction, callus formation.
    - **Cytokinins** (BAP, Kinetin, Zeatin) → shoot induction.
    - **Gibberellins** → elongation, embryogenesis.
    - **Abscisic acid (ABA)** → maturation, dormancy.
5. **Organic Additives**
    - Amino acids, coconut milk, yeast extract, casein hydrolysate.
6. **Gelling Agent**
    - Agar (0.8–1.0%), Gelrite, Phytagel → solid support.
7. **pH**
    - Optimal: **5.6–5.8** before autoclaving.

📖 Common Media Types

- **MS Medium (1962)** → High salts, supports most plants.
- **White's Medium (1939)** → First root culture medium.
- **Gamborg's B5 Medium (1968)** → Soybean cell suspension, useful for protoplasts.
- **Nitsch & Nitsch Medium (1969)** → Anther/pollen culture.
- **SH Medium (Schenk & Hildebrandt, 1972)** → Cell suspension cultures.

📊 Comparison Table

Medium	Special Feature	Application
MS (1962)	Rich in salts & nutrients	General use, micropropagation
White (1939)	First medium	Root culture
Gamborg B5	High vitamins	Protoplast & cell suspension
Nitsch	Balanced hormones	Anther/pollen culture
SH	Rich nitrate	Cell suspensions

🧠 Mnemonics

- **"Some Vitamins Add Real Growth"** = Salts, Vitamins, Additives, Regulators, Gelling agent.
- **Media history**: *White → MS → Gamborg → Nitsch → SH.*

🎯 Exam Capsule

- **MS Medium** = standard, high nitrate & ammonium.
- **Sucrose** = common carbon source.
- **pH 5.6–5.8** optimal.
- **Gamborg B5** = protoplast culture.
- **Nitsch medium** = pollen culture.

📄 Cheat Sheet 3 – Callus Culture & Organogenesis 🌱🧫

🌟 Callus Culture (Unorganized Growth)

- **Callus** = Mass of **undifferentiated, unorganized cells** produced from explants in presence of **auxins + cytokinins**.
- First demonstrated by **Gautheret (1939)**.

🔑 Process

1. **Explant inoculation** on solid medium.
2. **Dedifferentiation** → Explant cells revert to meristematic state.
3. **Proliferation** → Cell mass forms (callus).
4. **Redifferentiation** → Under hormonal control → roots, shoots, embryos.

🌟 Organogenesis (Organ Formation in vitro)

- **Organogenesis** = Differentiation of callus or single cells into **shoots or roots**.
- **Direct organogenesis** – Explant produces organs without callus.
- **Indirect organogenesis** – Via callus phase.

Hormonal Regulation

- **High Auxin : Low Cytokinin** → Root induction.
- **High Cytokinin : Low Auxin** → Shoot induction.
- **Balanced** → Callus proliferation.

🕮 Applications

- Micropropagation (mass clonal propagation).

- Virus-free plants (from meristems).
- Secondary metabolite production.
- Genetic engineering (regeneration of transgenic plants).

📊 Quick Table

Ratio (Auxin : Cytokinin)	Result	Example
High Auxin : Low Cytokinin	Root induction	NAA → roots in tomato
Low Auxin : High Cytokinin	Shoot induction	BAP → shoots in tobacco
Balanced	Callus growth	2,4-D + Kinetin

□ Mnemonics

- **"Root at Bottom, Shoot at Top"** 🌱
  - Auxin (bottom, roots).
  - Cytokinin (top, shoots).

🎯 Exam Capsule

- **Callus = unorganized mass** (Gautheret, 1939).
- **Organogenesis = shoot/root induction** under hormonal control.
- **Direct organogenesis** = no callus stage → preserves genetic stability.
- **Indirect organogenesis** = via callus → prone to somaclonal variation.

📄 Cheat Sheet 4 – Somatic Embryogenesis & Synthetic Seeds 🌱💎

🌟 Somatic Embryogenesis (SE)

- Process where **somatic (non-reproductive) cells** form embryos that resemble zygotic embryos.
- First reported in **carrot by Steward (1958)**.
- **Totipotency at cellular level** → basis of SE.

🔑 Stages of Somatic Embryogenesis

1. **Induction** – Somatic cells dedifferentiate → embryogenic callus.

2. **Development** – Embryo passes stages: globular → heart → torpedo → cotyledonary.
3. **Maturation** – Accumulation of proteins, starch, lipids.
4. **Germination** – Somatic embryo → seedling.

🌟 Types of Somatic Embryogenesis

- **Direct SE** – Embryos form directly from explant without callus → genetically stable.
- **Indirect SE** – Via callus → prone to somaclonal variation.

🌟 Synthetic (Artificial) Seeds

- Somatic embryos encapsulated in **hydrogel (e.g., sodium alginate + $CaCl_2$)**.
- Can be sown like seeds.

Advantages

- Easy handling, storage, transport.
- Uniform clonal propagation.
- Disease-free planting material.
- Used in crops with poor seed viability or sterility.

🕮 Applications

- Mass clonal propagation.
- Germplasm conservation.
- Production of **uniform hybrids**.
- Tool in **genetic engineering & biotechnology**.

📊 Quick Table

Feature	Somatic Embryogenesis	Synthetic Seeds
Definition	Embryos from somatic cells	Encapsulation of somatic embryos
Stability	Direct SE → stable	High genetic fidelity
Storage	Short-term	Long-term possible
Application	Micropropagation, transgenics	Seed-like handling, field planting

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□ Mnemonics

- **Embryo Stages** → *"Good Healthy Tomatoes Cook"* = Globular → Heart → Torpedo → Cotyledonary.
- **Synthetic seeds** → "Alginate = Artificial."

🎯 **Exam Capsule**

- **Steward (1958)** → carrot SE.
- **Somatic embryos** mimic zygotic embryos, but lack endosperm.
- **Synthetic seeds** = somatic embryos in sodium alginate gel.
- Used in **banana, sugarcane, carrot, orchids**.

📄 **Cheat Sheet 5 – Protoplast Culture & Somatic Hybridization □🧬**

🌟 **Protoplast – Definition**

- **Protoplast** = Plant cell without a cell wall, surrounded only by plasma membrane.
- Obtained by:
  - **Enzymatic method** → Cellulase + Pectinase.
  - **Mechanical method** → Micromanipulation (less common).

🔍 **Protoplast Culture**

- Culture of isolated protoplasts in **osmotic stabilizers** (mannitol, sorbitol).
- First successful culture: **Cockings (1960)** – tobacco protoplasts.
- Protoplasts can:
  - Regenerate cell wall.
  - Divide & form callus.
  - Undergo somatic hybridization.

🌟 **Somatic Hybridization**

Fusion of two somatic protoplasts → **somatic hybrids (cytoplasmic + nuclear fusion)**.

Methods of Fusion

1. **Spontaneous fusion** – Rare, occurs naturally.

2. **Induced fusion**
    - **Chemical**: Polyethylene glycol (PEG).
    - **Electrical**: Electrofusion (electric pulses).

🌟 Types of Hybrids

- **Symmetric hybrid** – Fusion of nuclei + cytoplasm of both parents.
- **Asymmetric hybrid** – One parent contributes partial genome.
- **Cybrid (Cytoplasmic hybrid)** – Only cytoplasmic fusion; nucleus from one parent + cytoplasm from both.

🕮 Applications

- Overcoming sexual incompatibility barriers.
- Creation of novel hybrids (e.g., **Pomato** = Potato + Tomato).
- Transfer of cytoplasmic traits (disease resistance, male sterility).
- Genetic studies & crop improvement.

📊 Quick Table

Type of Fusion	Feature	Example
Symmetric hybrid	Both nuclei + cytoplasm fused	Pomato (Potato × Tomato)
Asymmetric hybrid	Partial genome from one parent	Useful for gene introgression
Cybrid	Nucleus from one, cytoplasm mix	Disease resistance, CMS

□ Mnemonics

- **Fusion types** → *"SAC"* = Symmetric, Asymmetric, Cybrid.
- **Enzymes for protoplast** → *"Cellu-Pectu"* = Cellulase + Pectinase.

🎯 Exam Capsule

- **Protoplast definition** = cell without wall.
- **First protoplast culture** → Tobacco (1960).
- **Somatic hybridization** → PEG or electrofusion.
- **Pomato** = famous somatic hybrid.

- **Cybrids** → cytoplasmic transfer of traits.

📄 Cheat Sheet 6 – Anther & Pollen Culture (Androgenesis & Haploid Plants) 🌾

🌟 Concept

- **Androgenesis** = Development of haploid plants from **male gametophyte (anther or pollen)** under *in vitro* conditions.
- Produces **haploids (n)** → doubled haploids (2n) for pure line production.

🔬 Methods

1. Anther Culture

- Introduced by **Guha & Maheshwari (1964)** in *Datura innoxia*.
- Anthers placed on nutrient medium → microspores divide → form callus or embryos → haploid plantlets.

2. Pollen Culture

- Isolated pollen grains cultured directly → develop into haploid embryos.
- Advantage: Avoids diploid somatic tissue contamination.

🌟 Pathways of Haploid Formation

1. **Direct androgenesis** – Microspores → embryos → plantlets.
2. **Indirect androgenesis** – Microspores → callus → organogenesis/embryogenesis.

🕮 Applications

- **Crop improvement**: Rapid production of homozygous pure lines.
- **Mutation breeding**: Easy detection of recessive mutations.
- **Genetic studies**: Chromosome mapping, gene expression.
- **Hybrid breeding**: Doubled haploids = stable hybrids.

📊 Quick Table

Method	Source	Outcome	Example
Anther culture	Whole anther	Haploid plants via callus or embryos	*Datura innoxia*
Pollen culture	Isolated microspores	Direct haploids	Barley, Rice
Direct pathway	Microspore → embryo	Genetically stable	Cereals
Indirect pathway	Callus → plantlets	Somaclonal variation possible	Tobacco

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□ Mnemonics

- **"A → Datura, P → Cereals"**
  - Anther culture first in *Datura*.
  - Pollen culture widely in cereals (barley, rice).
- **Haploid advantage** → *"Half genome, Whole truth"* (recessive traits visible).

🎯 Exam Capsule

- **First anther culture** → *Datura innoxia* (Guha & Maheshwari, 1964).
- **Pollen culture** → cereals (barley, rice).
- **Haploids (n)** → doubled using **colchicine** → instant pure lines.
- **Applications**: Mutation breeding, hybrid breeding, genetic studies.

📄 Cheat Sheet 7 – Ovary, Ovule & Embryo Culture (Gynogenesis & Embryo Rescue) 🌸□

🌟 Concept

- **Gynogenesis** = Development of haploid plants from **female gametophyte (ovary/ovule)**.
- **Embryo culture** = In vitro growth of isolated immature or mature embryos.
- **Embryo rescue** = Technique to save hybrid embryos that would normally abort.

🔑 Methods

1. Ovary Culture

- Whole ovary cultured.
- Useful for studying fertilization and early embryo development.

2. Ovule Culture

- Ovules isolated & cultured.
- Induces haploid development (gynogenic haploids).

3. Embryo Culture

- Introduced by **Hanning (1904)**.
- Excised embryo → placed on nutrient medium → develops into seedling.
- Types:
  - **Mature embryo culture** → normal seed germination in vitro.
  - **Immature embryo culture** → embryo rescue (before abortion).

🌟 Applications

- **Embryo rescue**: Overcomes post-zygotic hybridization barriers.
  - Wide crosses (e.g., *Triticum* × *Secale* → Triticale).
- **Gynogenesis**: Haploid plants from ovules.
- **Seedless fruit production** (embryo culture in grapes, orchids).
- **Study of embryogenesis** (nutritional & hormonal requirements).
- **Hybrid breeding** → enables production of hybrids from distant crosses.

📊 Quick Table

Culture Type	Explant	Use	Example
Ovary	Whole ovary	Fertilization, embryo study	*Petunia*
Ovule	Ovule	Haploid (gynogenesis)	Sugar beet
Embryo	Embryo	Embryo rescue, hybridization	Triticale, Grapes

□ Mnemonics

- **"Ovary → Fertilization, Ovule → Haploids, Embryo → Rescue"**.

- **Embryo rescue crops** → *"Triticale Grapes Orchid"*.

🎯 Exam Capsule

- **Embryo culture discovered by Hanning (1904)**.
- **Gynogenesis** → haploids via female gametophyte.
- **Embryo rescue** → saves wide hybrid embryos (e.g., triticale).
- Used in overcoming **interspecific & intergeneric barriers**.
- Key tool in **hybridization, crop improvement, and seedless fruit production**.

📄 Cheat Sheet 8 – Somaclonal Variation & Applications 🧬

🌟 Definition

- **Somaclonal variation** = Genetic variation observed among plants regenerated from *in vitro* culture (callus, protoplast, cell suspensions).
- Term coined by **Larkin & Scowcroft (1981)**.

🔑 Sources of Somaclonal Variation

1. **Chromosomal changes**
    - Polyploidy, aneuploidy.
    - Structural changes (deletions, duplications, inversions, translocations).
2. **Gene mutations**
    - Point mutations, altered gene expression.
3. **Epigenetic changes**
    - DNA methylation, transposons, gene silencing.
4. **Culture-induced stress**
    - Prolonged callus culture → genetic instability.

📖 Examples

- Disease resistance: *Potato, Sugarcane*.
- Stress tolerance: Salt-tolerant rice, aluminum-tolerant maize.
- Quality traits: High alkaloid content in *Datura*, high shikonin in *Lithospermum*.

🌟 Applications

- **Crop improvement**: Traits like resistance to disease, pests, drought, salinity.
- **Secondary metabolite production**: Alkaloids, steroids, pigments.
- **Germplasm enhancement**: Introducing variability in clonally propagated crops.
- **Alternative to mutation breeding**.

📊 Quick Table

Trait Improved	Crop Example
Disease resistance	Potato, Sugarcane
Salt tolerance	Rice
Aluminium tolerance	Maize
High alkaloid content	*Datura*

🧠 Mnemonics

- **"C-GECS"** = Chromosome, Gene, Epigenetic, Culture Stress.
- **Somaclonal uses** → *"R-SQ"* = Resistance, Stress tolerance, Quality.

🎯 Exam Capsule

- **Coined by Larkin & Scowcroft (1981)**.
- Major source = **prolonged callus culture**.
- **Somaclonal variants** → disease resistance (potato, sugarcane), stress tolerance (rice, maize).
- Useful for **clonal crops** where traditional breeding is limited.

📄 Cheat Sheet 9 – Cryopreservation & Germplasm Conservation 🌸🌾

🌟 Cryopreservation – Definition

- **Cryopreservation** = Long-term storage of biological material (cells, tissues, embryos, seeds) at **ultra-low temperatures (–196°C in liquid nitrogen)**.

- Metabolic activities almost stop → material remains viable indefinitely.

🔑 Methods of Cryopreservation

1. **Slow Freezing**
    - Gradual cooling → water exits cell → less ice crystal damage.
2. **Rapid Freezing** ⚡
    - Direct immersion in liquid nitrogen.
3. **Vitrification**
    - Use of cryoprotectants → water turns into **glassy state** (no crystals).

🔑 Cryoprotectants

- Substances that prevent ice damage.
- Examples: **DMSO, Glycerol, Proline, Mannitol, Sucrose, PEG**.

🌱 Germplasm Conservation

- **Germplasm** = total genetic resources of a species.
- Two types:
    1. **In situ conservation** → natural habitat (biosphere reserves, sanctuaries).
    2. **Ex situ conservation** → outside habitat (seed banks, gene banks, tissue culture).

In vitro Germplasm Conservation

- Short-term: slow growth using osmotic agents (mannitol, sorbitol).
- Long-term: cryopreservation.

🕮 Applications

- Conservation of endangered, rare, or wild species.
- Preservation of vegetatively propagated crops (potato, banana).
- Conservation of recalcitrant seeds (coconut, coffee, cocoa).
- International germplasm exchange.

📊 Quick Table

Method	Example Application
Slow growth in vitro	Potato, Sugarcane
Cryopreservation	Banana, Coffee, Coconut
Seed bank storage	Rice, Wheat, Maize

□ Mnemonics

- **Cryoprotectants** → *"Good Scientists Prefer Making Safe Plants"*
  - Glycerol, Sucrose, Proline, Mannitol, Sorbitol, PEG.
- **Conservation** → *"In = Inside nature, Ex = Exit nature."*

🎯 **Exam Capsule**

- **Cryopreservation = –196°C in LN_2** .
- **Cryoprotectants** prevent ice crystal damage.
- **Recalcitrant seeds (coconut, cocoa, coffee)** → cannot be stored in seed banks → cryopreservation essential.
- **Potato, Banana, Sugarcane** widely conserved using in vitro methods.

📄 Cheat Sheet 10 – Applications of Plant Tissue Culture 🌱🔬

🌟 Major Applications

1. Micropropagation (Clonal Propagation)

- Rapid multiplication of elite genotypes.
- Produces uniform, disease-free plants.
- Widely applied in **banana, sugarcane, potato, orchids**.

2. Production of Disease-Free Plants

- **Meristem culture** eliminates systemic viruses.
- Example: Virus-free sugarcane, potato, banana.

3. Haploid Breeding

- **Anther/pollen culture** → haploids (n) → doubled haploids (2n).
- Produces homozygous pure lines in a single generation.

4. Somaclonal Variation

- Source of novel variability → disease resistance, stress tolerance.
- Eg: Salt-tolerant rice, resistant sugarcane.

5. Synthetic Seeds

- Somatic embryos encapsulated in sodium alginate → artificial seeds.

6. Secondary Metabolite Production

- Valuable compounds from cell cultures.
- Examples:
    - Shikonin (*Lithospermum*).
    - Berberine (*Coptis*).
    - Diosgenin (*Dioscorea*).

7. Genetic Engineering & GM Crops

- Tissue culture = platform for transformation & regeneration.
- Applications: Bt cotton, Golden rice.

8. Germplasm Conservation

- In vitro storage, cryopreservation.
- For recalcitrant seed crops (coconut, coffee).

🕮 Industrial Applications

- **Pharmaceuticals** → alkaloids, steroids, pigments.
- **Food industry** → flavor compounds (vanillin).
- **Bioenergy** → in vitro biomass production.

📊 Quick Table

Application	**Example**
Micropropagation	Banana, Sugarcane
Meristem culture	Virus-free potato
Haploid breeding	Barley, Rice

Application	Example
Somaclonal variation	Salt-tolerant rice
Secondary metabolites	Shikonin, Berberine
GM crops	Bt cotton, Golden rice

□ Mnemonics

- **"M-H-S-S-G"** = Micropropagation, Haploids, Somaclonal variation, Secondary metabolites, GM crops.
- **Secondary metabolites** → *"Big Strong Drugs"* = Berberine, Shikonin, Diosgenin.

🎯 Exam Capsule

- **Micropropagation = clonal propagation.**
- **Meristem culture = virus-free crops.**
- **Anther culture (1964, Datura)** → haploids.
- **Somaclonal variation = genetic diversity source.**
- **Synthetic seeds = sodium alginate encapsulated embryos.**
- **Secondary metabolites** from cultures = pharmaceutical use.