Paper -V

SCIENCE AND TECHNOLOGY

Introduction to Genetic Engineering and Biotechnology. Basic concepts of genetic engineering. Tissue culture methods and applications. Biotechnology in agriculture- Bio-pesticides, Bio-fertilizers, Bio-fuels, Genetically modified crops.

INTRODUCTION

Biotechnology and genetic engineering represent transformative scientific domains with profound implications for agriculture, healthcare, industrial sustainability, and environmental conservation. Biotechnology harnesses biological systems, living organisms, or their derivatives to develop products and processes for specific use. Genetic engineering specifically involves the direct manipulation of an organism's genome using recombinant DNA technology and precision gene-editing tools to introduce, delete, or modify specific traits.

India has emerged as a significant global player in biotechnology, with a bioeconomy valued at approximately USD 130 billion in 2024 and projections to reach USD 150 billion by 2030. The integration of genetic engineering, tissue culture, and agricultural biotechnology has enabled India to address challenges of food security, climate resilience, sustainable farming, and rural livelihoods.

PART 1: INTRODUCTION TO GENETIC ENGINEERING AND BIOTECHNOLOGY

Definitions and Conceptual Framework

Biotechnology

- According to the United Nations Convention on Biological Diversity (1992): "Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use"
- Integrates biology with engineering, chemistry, data science, and artificial intelligence
- Four major branches:
    - Red Biotechnology: Medical and pharmaceutical applications (vaccines, gene therapy, diagnostics)
    - Green Biotechnology: Agricultural applications (GM crops, biofortification, biopesticides)

- White Biotechnology: Industrial applications (enzymes, biofuels, bioplastics)
- Blue Biotechnology: Marine and aquatic applications (algae-based products, aquaculture).

Colour Classification	Field / Area	Important Examples / Uses
Blue Biotechnology	Marine & Aquatic Biotechnology	Increase seafood production, control harmful water-borne organisms, development of new drugs.
Green Biotechnology	Agricultural Biotechnology	Transgenic/GM crops, improved nutritional quality, higher yield, eco-friendly products.
Red Biotechnology	Medical & Health Biotechnology	Production of insulin, antibiotics, vaccines, therapeutic enzymes.
White Biotechnology	Industrial Biotechnology	Use of enzymes as industrial catalysts, eco-friendly chemical production.

Genetic Engineering

- The direct manipulation of an organism's genes using biotechnology to alter, add, or delete specific genetic material
- Also known as recombinant DNA technology, gene editing, or genetic modification
- Enables transfer of desirable traits across species boundaries, development of disease-resistant varieties, production of therapeutic proteins, and potential correction of genetic disorders
- Key distinction: All genetic engineering is biotechnology, but not all biotechnology involves genetic engineering (e.g., traditional fermentation)

Historical Evolution and Key Milestones

Foundational Discoveries (Pre-1970s)

- 1865: Gregor Mendel establishes laws of inheritance through pea plant experiments
- 1944: Avery, MacLeod, and McCarty identify DNA as the genetic material
- 1953: Watson and Crick describe double-helix structure of DNA
- 1960s: Discovery of restriction enzymes that cut DNA at specific sequences

Birth of Genetic Engineering (1970s)

- 1973: First recombinant DNA organism created by Stanley Cohen and Herbert Boyer – insertion of frog DNA into bacterial plasmid

- 1975: Asilomar Conference establishes voluntary guidelines for recombinant DNA research safety
- 1976: Genentech founded – first biotechnology company; produces human insulin using E. coli
- 1978: First synthetic human insulin produced via recombinant DNA technology

Commercialization and Indian Progress (1980s-Present)

- 1982: First recombinant drug (Humulin – human insulin) approved by US FDA
- 1996: Bt cotton commercialized globally; India begins field trials
- 2002: Bt cotton approved for commercial cultivation in India – only GM crop currently grown
- 2012: CRISPR-Cas9 gene-editing system developed; revolutionizes precision editing
- 2017: Genome India Project launched to sequence 10,000 Indian genomes
- 2020-2023: Indigenous COVID-19 vaccines (Covaxin, ZyCoV-D) developed using recombinant and DNA vaccine platforms
- 2023: NexCAR19 – India's first indigenous CAR-T cell therapy approved by CDSCO
- 2024: BioE3 Policy approved to position India as global biomanufacturing hub

PART 2: BASIC CONCEPTS OF GENETIC ENGINEERING

Foundational Principles

Central Dogma of Molecular Biology

- DNA → RNA → Protein; genetic engineering intervenes at DNA or RNA level to alter protein expression and phenotype
- Understanding transcription, translation, and gene regulation is essential for designing genetic modifications

Recombinant DNA Technology

- Cutting, joining, and replicating DNA fragments from different sources to create novel genetic combinations
- Key steps: Gene isolation, vector construction, transformation, selection, and expression

Gene Expression Regulation

- Promoters, enhancers, silencers, and epigenetic modifiers control when, where, and how much a gene is expressed

- Tissue-specific and inducible promoters enable targeted expression in desired organs or under specific conditions

Selectable Markers and Reporter Genes

- Antibiotic resistance genes (nptII, hpt) or visual markers (GFP, GUS) enable identification and selection of successfully transformed cells
- Essential for screening large populations of transformed cells

Core Molecular Tools

Restriction Endonucleases

- Molecular scissors that recognize and cut specific palindromic DNA sequences
- Examples: EcoRI cuts at GAATTC, HindIII at AAGCTT
- Enable precise cutting of DNA for recombinant construction

DNA Ligases

- Enzymes that join DNA fragments by forming phosphodiester bonds between adjacent nucleotides
- Essential for sealing recombinant DNA constructs

Vectors (Gene Carriers)

- Plasmids: Circular extrachromosomal DNA (pUC19, pBR322, Ti plasmid in Agrobacterium)
- Cosmids, BACs (Bacterial Artificial Chromosomes), YACs (Yeast Artificial Chromosomes) for cloning large DNA inserts
- Viral vectors: Adenovirus, lentivirus, AAV for efficient delivery into mammalian cells

Host Systems

- Prokaryotic: E. coli for rapid cloning and protein expression
- Eukaryotic: Saccharomyces cerevisiae (yeast), Pichia pastoris for post-translational modifications
- Mammalian: CHO, HEK293 cell lines for therapeutic protein production
- Plant: Protoplasts, callus, embryogenic tissue for regeneration of transgenic plants.

KEY BIOTECHNOLOGY TECHNIQUES

Concept, Mechanism, Variants, and Applications for Mains Examination

1. POLYMERASE CHAIN REACTION (PCR)

Polymerase Chain Reaction (PCR) is a laboratory technique that **amplifies a specific DNA segment exponentially**, producing millions to billions of copies from a tiny initial sample. Invented by **Kary Mullis in 1983** (Nobel Prize in Chemistry, 1993), it is often called the **"DNA photocopier"** of molecular biology.

PCR mimics natural DNA replication but replaces cellular machinery with **controlled temperature cycles** and a **heat-stable enzyme**. It relies on the fact that DNA strands separate at high temperatures and rebind at lower temperatures, allowing targeted copying in a test tube.

In simple terms,

PCR is like a DNA photocopier. It takes a tiny amount of DNA and makes millions of exact copies in just a few hours. This allows scientists to work with enough DNA to study, diagnose, or modify.

How It Works (3-Step Cycle Repeated 30–40 Times):

PCR works by repeating a simple 3-step process 30–40 times. Each cycle doubles the amount of target DNA.

Step 1: Denaturation (94–98°C)

- Heat separates the double-stranded DNA into two single strands by breaking hydrogen bonds
- Like unzipping a zipper – now each strand is available as a template

Step 2: Annealing (50–65°C)

- Temperature is lowered so short, synthetic DNA pieces called **primers** can bind (anneal) to their complementary sequences on each single strand
- Primers flank the target region – they define exactly which segment will be copied
- Think of primers as "bookmarks" that tell the enzyme where to start copying

Step 3: Extension/Elongation (72°C)

- A heat-stable enzyme called **Taq polymerase** (from the hot-spring bacterium Thermus aquaticus) adds free nucleotides to the primers, building new complementary DNA strands
- The enzyme reads the template strand and synthesizes a matching new strand
- Result: One double-stranded DNA becomes two double-stranded copies

Each cycle doubles the DNA. After 30 cycles, 1 copy becomes over 1 billion copies.

Important Variants:

1. RT-PCR (Reverse Transcription PCR)

- **Purpose:** Amplify RNA instead of DNA
- **How:** First, an enzyme called reverse transcriptase converts RNA into complementary DNA (cDNA). Then standard PCR amplifies the cDNA.
- **Applications:** Detecting RNA viruses (SARS-CoV-2, HIV, dengue), studying gene expression (which genes are active in a tissue), cancer research

2. qPCR / Real-Time PCR

- **Purpose:** Quantify how much DNA was present initially, not just detect its presence
- **How:** Uses fluorescent dyes or probes that emit light as DNA is amplified. The machine measures fluorescence in real time – earlier signal = more starting DNA.
- **Applications:** Viral load testing (HIV, hepatitis), monitoring cancer treatment response, gene expression quantification, GMO detection

3. Multiplex PCR

- **Purpose:** Amplify multiple different DNA targets in a single reaction tube
- **How:** Uses multiple primer sets, each specific to a different target sequence, labeled with different markers for identification
- **Applications:** Pathogen panels (testing for multiple viruses/bacteria at once), genetic disorder screening, forensic DNA profiling, food authenticity testing

4. Nested PCR

- **Purpose:** Increase specificity and sensitivity when target DNA is very rare or sample is contaminated
- **How:** Two rounds of PCR – first with outer primers, then a second round with inner primers that bind inside the first product
- **Applications:** Detecting low-level infections, ancient DNA analysis, forensic samples with degraded DNA

5. Digital PCR (dPCR)

- **Purpose:** Absolute quantification of DNA without needing a standard curve
- **How:** Sample is partitioned into thousands of tiny reactions; each is scored positive or negative, and statistics calculate exact copy number

- **Applications:** Rare mutation detection, liquid biopsy for cancer, precise GMO quantification, monitoring minimal residual disease

Key Applications:
Medical diagnostics (HIV, TB, COVID), forensic DNA fingerprinting, paternity testing, GMO detection, archaeological DNA analysis, and as the first step in almost all genetic engineering workflows.

INDIAN CONTEXT AND EXAMPLES

- **COVID-19 Response:** India scaled up RT-PCR testing capacity to over 3,000 labs by 2021; indigenous kits developed by companies like Mylab, Transasia, and CSIR-IGIB reduced costs and dependency on imports
- **Tuberculosis Control:** CBNAAT (Cartridge-Based Nucleic Acid Amplification Test), a form of PCR, is used nationwide for rapid, accurate TB diagnosis and detection of drug-resistant strains
- **Agricultural Biotechnology:** PCR is used to confirm gene insertion in GM crops like Bt cotton and to screen for unauthorized GM varieties in markets
- **Wildlife Conservation:** PCR-based DNA barcoding helps identify species in illegal wildlife trade and monitor biodiversity in protected areas
- **Startups and Innovation:** Indian biotech startups like Bugworks, MedGenome, and Strand Life Sciences use advanced PCR variants for precision diagnostics and personalized medicine

ADVANTAGES OF PCR

- **High Sensitivity:** Can detect a single DNA molecule
- **High Specificity:** Primers ensure only the target sequence is amplified
- **Speed:** Results in 2–4 hours versus days for culture-based methods
- **Versatility:** Adaptable to DNA, RNA, quantification, multiplexing, and field use
- **Scalability:** From research labs to point-of-care devices

LIMITATIONS AND CHALLENGES

- **Contamination Risk:** Amplifying even tiny contaminants can cause false positives; requires strict lab protocols
- **Requires Prior Knowledge:** Primers must be designed based on known target sequences; cannot discover entirely unknown genes

- **Inhibition by Sample Impurities:** Substances in blood, soil, or food can block the reaction; requires careful DNA extraction
- **Cannot Distinguish Live vs Dead Pathogens:** Detects DNA regardless of whether the organism is alive (important for treatment decisions)
- **Equipment Dependency:** Thermal cyclers and real-time detectors require stable electricity and maintenance

2. GEL ELECTROPHORESIS AND BLOTTING TECHNIQUES

A method to separate DNA, RNA, or proteins based on their size using an electric field and a gel matrix (usually agarose or polyacrylamide).

How It Works:
Samples are loaded into wells at one end of the gel. When electric current is applied, negatively charged molecules move toward the positive end. Smaller molecules move faster through the gel's pores, while larger ones lag behind. After running, the gel is stained to visualize distinct bands.

Applications:
Checking PCR results, DNA fingerprinting, verifying gene cloning, quality control in labs.

Blotting Techniques (The "DNA → RNA → Protein" Trio)

Blotting is a two-step process: First, separate molecules by size on a gel. Second, transfer them onto a solid membrane for targeted detection using probes or antibodies.

1. Southern Blotting (DNA Detection):

DNA is cut, separated, transferred to a membrane, and exposed to a labeled DNA probe that binds only to a matching sequence. Used to confirm gene insertion, detect genetic mutations, and study DNA structure.

Named after: Edwin Southern (1975)

Purpose: Detect specific DNA sequences in a complex mixture

Step-by-Step Process:

1. **DNA Extraction & Digestion:**
    - DNA is extracted from cells
    - Cut into fragments using restriction enzymes
2. **Gel Electrophoresis:**
    - Fragments separated by size on agarose gel

3. **Denaturation:**
    - Gel treated with alkali (NaOH) to separate double-stranded DNA into single strands
4. **Transfer (Blotting):**
    - Gel placed on a sponge in a buffer-filled tray
    - Nitrocellulose or nylon membrane placed on top of gel
    - Paper towels placed on membrane to create capillary action
    - Buffer flows upward, carrying DNA from gel onto membrane
    - DNA sticks permanently to membrane
5. **Fixation:**
    - Membrane baked at 80°C or UV-crosslinked to permanently attach DNA
6. **Hybridization:**
    - Membrane incubated with a labeled DNA probe (radioactive or fluorescent)
    - Probe binds only to complementary DNA sequence
7. **Detection:**
    - Unbound probe washed away
    - Membrane exposed to X-ray film (autoradiography) or scanned for fluorescence
    - Bands appear where probe bound to target DNA

Applications:

- Confirming gene insertion in GM crops
- Detecting genetic mutations
- DNA fingerprinting
- Studying gene structure and organization
- Diagnosing genetic disorders

2.Northern Blotting (RNA Detection):

Similar to Southern, but starts with RNA. The probe binds to specific mRNA, showing which genes are actively being expressed in a tissue or under certain conditions. Used in gene regulation studies and cancer research.

Applications:

- Confirming protein expression from a cloned gene
- Diagnosing diseases (HIV test, prion diseases)
- Studying protein modifications (phosphorylation, glycosylation)
- Drug development and quality control
- Cancer biomarker detection

Simple Memory Trick:
Southern = **S**equence (DNA)
Northern = **N**ucleic acid but RNA
Western = **W**orking protein (antibodies detect proteins)

3. TRANSFORMATION AND DELIVERY METHODS

Transformation is the process of getting foreign DNA inside a host cell so it can be copied, expressed, or edited. Cells naturally resist outside DNA, so scientists use biological, physical, or chemical tricks to bypass their defenses.

In Simple terms:
Getting foreign DNA inside a host cell so it can be copied, expressed, or edited. Cells naturally resist outside DNA, so scientists use biological, physical, or chemical tricks to bypass their defenses.

Biological Methods

- **Agrobacterium-Mediated Transfer (Plants):**
  Agrobacterium tumefaciens is a soil bacterium that naturally injects part of its DNA into plants, causing crown gall disease. Scientists remove the disease-causing genes, replace them with useful genes (e.g., pest resistance), and let the bacterium deliver them safely. Highly efficient for dicot plants (tomato, cotton, mustard).
- **Viral Transduction (Animals/Humans):**
  Viruses are natural gene delivery vehicles. Scientists remove viral disease genes, insert therapeutic genes, and use the modified virus to infect target cells. Widely used in gene therapy (e.g., treating inherited blindness, blood disorders) and vaccine development.

Physical Methods

- **Electroporation:**
  A short electric pulse creates temporary tiny pores in the cell membrane. DNA slips through before the membrane reseals. Fast, works for bacteria, yeast, plant protoplasts, and mammalian cells.
- **Microinjection:**
  A glass needle thinner than a hair directly injects DNA into a cell or its nucleus. Highly precise but slow and skill-intensive. Used in creating transgenic animals and IVF-based genetic modifications.
- **Gene Gun (Biolistics):**
  DNA is coated onto microscopic gold or tungsten particles and shot into cells at high velocity using pressurized helium. Like a microscopic shotgun. Bypasses tough plant cell walls; widely used for cereals (rice, wheat, maize) where Agrobacterium struggles.

Chemical Methods

- **PEG-Mediated Uptake:**
  Polyethylene glycol makes the cell membrane sticky and temporarily leaky, allowing DNA to enter. Commonly used for plant protoplasts (cells without walls) and some mammalian cells.
- **Calcium Phosphate Precipitation:**
  DNA mixes with calcium to form tiny crystals. Cells naturally "eat" these crystals through endocytosis, releasing DNA inside. Simple, cheap, but lower efficiency; used in mammalian cell culture.
- **Lipofection:**
  DNA is wrapped in tiny fat bubbles (liposomes) that naturally fuse with the cell membrane, delivering DNA inside. Gentle, low toxicity, widely used in lab research and clinical trials.

Key Applications:
Creating transgenic crops, developing gene therapies, producing recombinant proteins, lab-scale genetic research, and vaccine manufacturing.

Genome Editing Techniques and Methods

Recombinant DNA, Gene Cloning, Gene Silencing, CRISPR-Cas9, Gene Splicing and Applications for Mains Examination

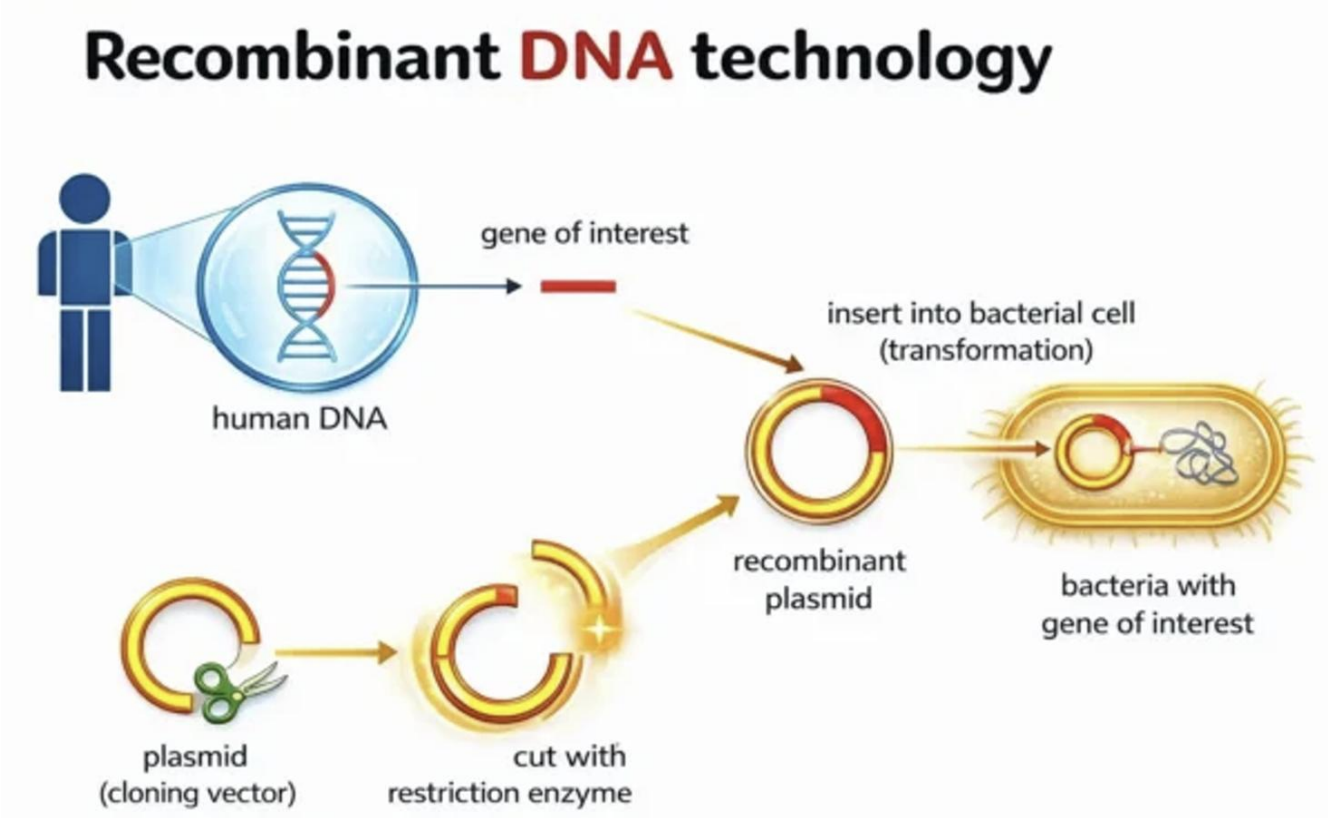
INTRODUCTION

Genetic engineering refers to the direct manipulation of an organism's genetic material using biotechnology to introduce, delete, or modify specific traits. The core techniques include recombinant DNA technology, gene cloning, gene transfer, gene silencing, and precision editing tools like CRISPR-Cas9. These methods have revolutionized medicine, agriculture, industry, and environmental management.

A. RECOMBINANT DNA TECHNOLOGY (**V.IMP**)

Recombinant DNA (rDNA) technology is like "molecular cut-and-paste". It involves taking a specific gene from one organism, inserting it into the DNA of another organism, and making the host produce the desired trait or protein.

Think of it as: Copying a useful recipe (gene) from one cookbook (organism) and pasting it into another cookbook (host) so the new book can now make that dish too.



Step-by-Step Process

Step 1: Isolation of DNA

- Extract pure DNA from the source organism (bacteria, plant, animal)
- Break open cells using enzymes or mechanical methods
- Purify DNA by removing proteins, RNA, and other cellular debris

Step 2: Fragmentation of DNA

- Use restriction enzymes (molecular scissors) to cut DNA at specific recognition sites
- Example: EcoRI cuts at GAATTC sequence
- Creates fragments with "sticky ends" (overhanging single strands) or "blunt ends"

Step 3: Isolation of Desired DNA Fragment

- Separate fragments by size using gel electrophoresis
- Identify and extract the fragment containing the gene of interest
- Confirm identity using probes or sequencing

Step 4: Ligation into Vector

- Cut a vector (plasmid, virus, or artificial chromosome) with the same restriction enzyme
- Mix gene fragment and vector; sticky ends match and bind
- Use DNA ligase (molecular glue) to seal the bond
- Result: Recombinant DNA molecule ready for delivery

Step 5: Transfer into Host Cell (Transformation)

- Introduce recombinant vector into host organism
- Methods: Chemical treatment, electroporation, Agrobacterium (plants), viral vectors (animals)
- Host cell takes up the foreign DNA

Step 6: Culturing and Product Extraction

- Grow transformed host cells in large-scale bioreactors
- Host cells replicate the recombinant DNA and express the foreign gene
- Harvest and purify the desired product (protein, enzyme, metabolite)

- **Future:** CRISPR-based therapies for sickle cell, thalassemia, inherited blindness

Diagnostics:

- **PCR-based tests:** Detect pathogens (TB, HIV, SARS-CoV-2), genetic mutations
- **Recombinant antigens:** Used in ELISA tests for accurate disease detection
- **Genetic screening:** Identify carriers of inherited disorders before symptoms appear

2. Agriculture and Food Security

Genetically Modified Crops:

- **Bt Cotton:** Contains bacterial gene for bollworm resistance; adopted on 90% of Indian cotton area; reduced pesticide use by 40-50%
- **Bt Brinjal:** Developed for fruit and shoot borer resistance; under regulatory review
- **GM Mustard (DMH-11):** Hybrid variety for higher yield; GEAC-approved, under Supreme Court consideration
- **Herbicide-Tolerant Crops:** Allow weed control without damaging crop; not yet approved in India

Nutritional Enhancement (Biofortification):

- **Golden Rice:** Produces beta-carotene (Vitamin A precursor); addresses blindness in deficient populations
- **Iron-rich Pearl Millet (Dhanshakti):** Combats anemia in women and children
- **High-lysine Maize:** Improves protein quality for livestock and human consumption

Stress Tolerance:

- **Drought-resistant varieties:** Maintain yield under water scarcity
- **Salt-tolerant crops:** Grow in saline soils, expanding cultivable land
- **Flood-tolerant rice (Sub1):** Survives submergence; protects farmers in flood-prone areas

Edible Vaccines:

- **Concept:** Plants produce vaccine antigens; eating the plant triggers immunity
- **Examples:** Transgenic potatoes, bananas expressing cholera or hepatitis B antigens
- **Advantage:** No needles, low cost, easy distribution in remote areas

Food Processing:

- **Recombinant enzymes:** Chymosin (cheese making), amylase (bread), pectinase (juice clarification)
- **Probiotics:** Engineered beneficial bacteria for gut health
- **Fermentation improvement:** Yeast strains for better flavor, texture, shelf-life

3. Industrial Biotechnology

Biofuels:

- **Bioethanol:** Engineered yeast/bacteria convert biomass to fuel; India targets 20% blending (E20) by 2025-26
- **Biobutanol:** Higher energy density than ethanol; compatible with existing engines
- **Algal biofuels:** Microalgae engineered for high lipid production; sustainable aviation fuel potential

Enzymes and Chemicals:

- **Industrial enzymes:** Detergent proteases, textile cellulases, paper xylanases produced via rDNA
- **Bioplastics:** PHA, PLA from engineered microbes; biodegradable alternatives to petroleum plastics
- **Specialty chemicals:** Flavors, fragrances, pharmaceuticals produced sustainably

Bioremediation:

- **Oil-degrading bacteria:** Engineered to clean up spills (e.g., Oilzapper in India)
- **Heavy metal absorbers:** Plants/microbes that concentrate toxic metals for safe removal
- **Plastic-eating enzymes:** PETase from engineered bacteria breaks down plastic waste

4. Environmental Protection

Pollution Control:

- **Biosensors:** Recombinant organisms detect pollutants in water/soil
- **Waste treatment:** Engineered microbes improve efficiency of sewage and industrial effluent treatment
- **Carbon capture:** Algae engineered to absorb CO2 and produce useful biomass

Conservation:

- **Disease resistance:** Protect endangered species from emerging pathogens

- **Single-window clearance:** Integrate DBT, GEAC, CDSCO, FSSAI approvals with statutory timelines
- **Transparent decision-making:** Publish biosafety data and rationale for public trust

Innovation Ecosystem Strengthening

- **Patient capital:** Expand BIRAC-like funding for translational rDNA research
- **Public-private partnerships:** Shared infrastructure for scale-up and commercialization
- **Startup support:** Regulatory sandboxes and incubation for biotech entrepreneurs

Capacity Building and Inclusion

- **Skill development:** Train biosafety officers, regulatory scientists, and bioprocess engineers
- **Regional hubs:** Decentralize advanced facilities to tier-2 cities for inclusive growth
- **Farmer and patient engagement:** Participatory research and clear communication of benefits/risks

Global Engagement

- **International harmonization:** Align with Codex, OECD standards while safeguarding national interests
- **South-South cooperation:** Share frugal innovations and regulatory best practices with developing countries
- **Technology diplomacy:** Position India as responsible leader in equitable biotechnology governance

B. GENE CLONING

Gene cloning is the creation of exact genetic copies of a DNA sequence or an entire organism. It produces organisms or cells with identical genetic makeup to the original.

Two Main Approaches:

1. Somatic Cell Nuclear Transfer (SCNT)

Simple Concept: Take the nucleus (containing DNA) from a body cell and insert it into an egg cell that has had its own nucleus removed. The egg then develops into an embryo genetically identical to the nucleus donor.

Step-by-Step Process:

1. **Collect Somatic Cell:**
    - Take any body cell (skin, muscle, etc.) from the organism to be cloned

- This cell contains the complete set of DNA

2. **Prepare Egg Cell:**
   - Collect an unfertilized egg from a female donor
   - Remove and discard the egg's nucleus (enucleation)
3. **Transfer Nucleus:**
   - Insert the somatic cell nucleus into the enucleated egg
   - The egg's cytoplasm "reprograms" the somatic nucleus to act like an embryonic nucleus
4. **Activate Development:**
   - Apply electric shock or chemical stimulus to trigger cell division
   - The egg begins dividing like a fertilized embryo
5. **Embryo Development:**
   - After multiple divisions, a blastocyst (early embryo) forms
   - This embryo has nearly identical DNA to the original somatic cell donor
6. **Implantation:**
   - Transfer embryo into a surrogate mother's uterus
   - If successful, pregnancy proceeds and a cloned offspring is born

Famous Example: Dolly the Sheep (1996) – first mammal cloned from an adult somatic cell

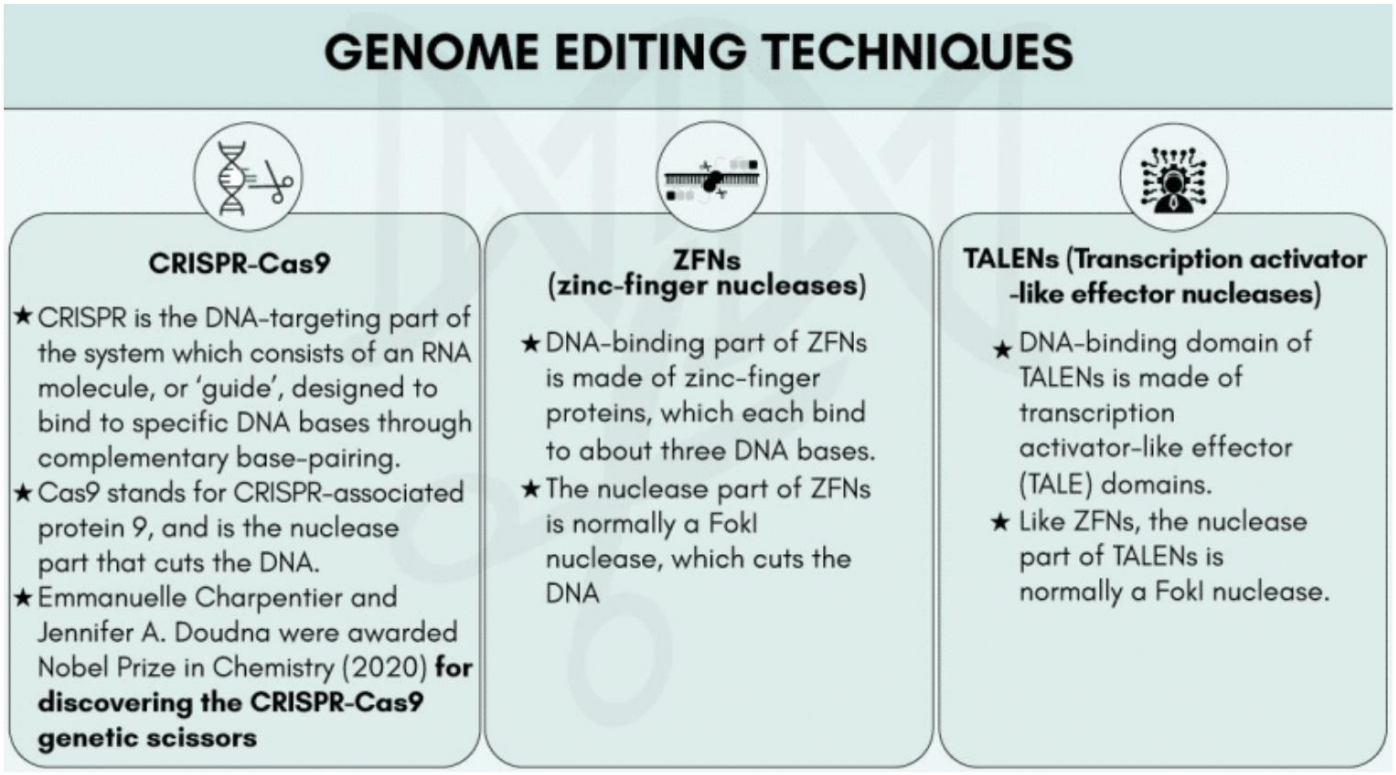
Two Possible Uses of SCNT:

Type	Purpose	Ethical Status
Therapeutic Cloning	Create cloned embryos for research and stem cell therapy; embryos not implanted	Permitted in many countries with strict regulation
Reproductive Cloning	Create cloned embryos intended for implantation and birth of a cloned individual	Banned in most countries including India for humans

2. Artificial Embryo Twinning

Simple Concept: Mimics the natural process that creates identical twins. An early embryo is manually split into separate cells, each of which develops into a complete, genetically identical individual.

D. GENOME EDITING



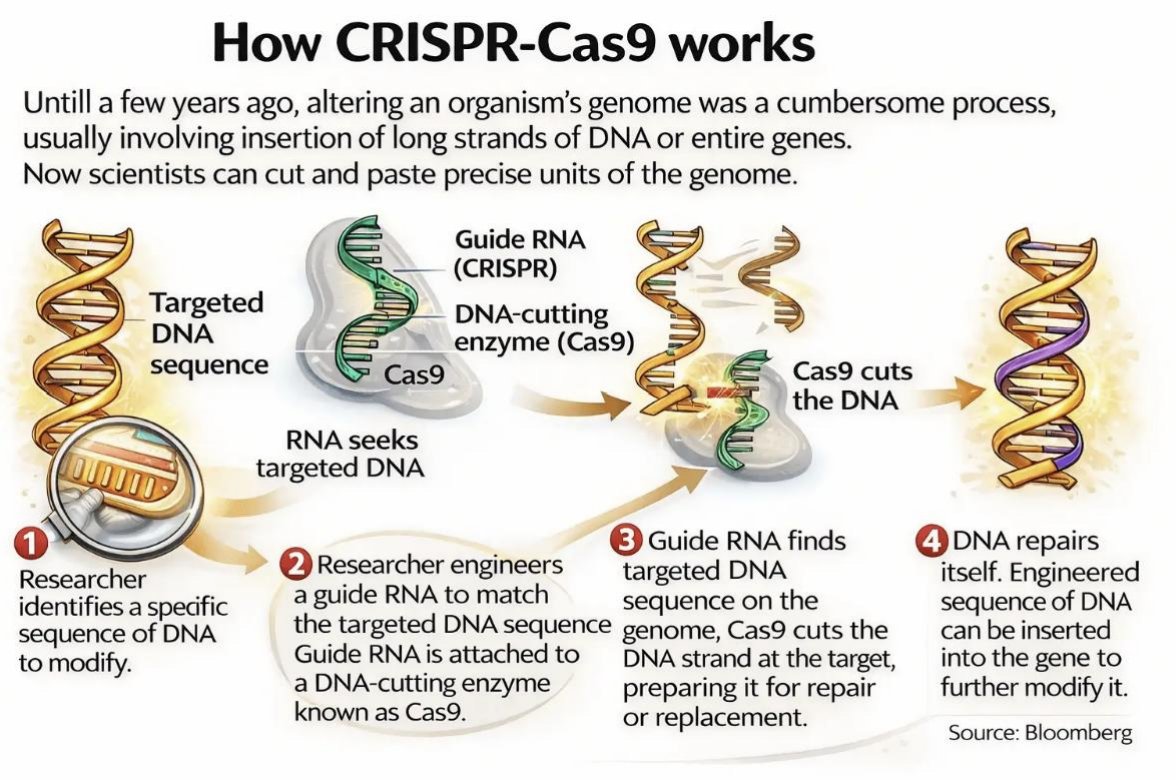
CRISPR-Cas9

CRISPR-Cas9 is like a "search-and-replace" function for DNA. It uses a guide RNA to find the exact location in the genome, and the Cas9 enzyme cuts the DNA at that spot. The cell's repair machinery then fixes the cut, allowing scientists to insert, delete, or change genes with high precision.

Components and Mechanism

Two Key Molecules:

1. **Cas9 Enzyme (Molecular Scissors)**
    - Cuts both strands of DNA at a specific location
    - Creates a double-strand break that triggers cellular repair mechanisms
2. **Guide RNA (gRNA) (GPS Navigator)**
    - Designed to be complementary to the target DNA sequence
    - Binds to Cas9 and leads it to the exact location in the genome



Step-by-Step Process:

1. **Design gRNA:**
    - Scientists identify the target DNA sequence to edit
    - Design a short RNA sequence that matches the target
2. **Form Complex:**
    - gRNA binds to Cas9 protein, forming a ribonucleoprotein complex
3. **Target Binding:**
    - Complex scans the genome until gRNA finds its matching sequence
    - Cas9 binds to the DNA at that location
4. **DNA Cutting:**
    - Cas9 makes a precise double-strand break in the DNA
5. **Cellular Repair:**
    - Cell detects the break and activates repair machinery
    - Two repair pathways:

APPLICATIONS OF GENETIC ENGINEERING TECHNIQUES

A. Three-Parent Babies (Mitochondrial Donation Treatment)

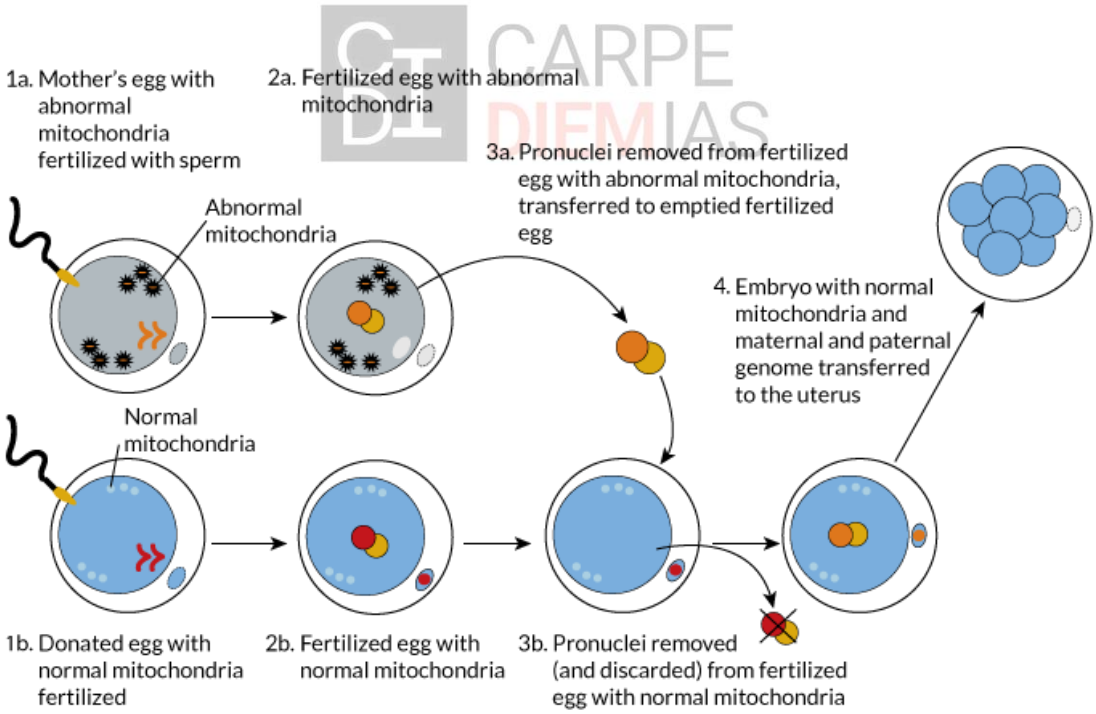
Simple Concept: A technique to prevent transmission of mitochondrial diseases from mother to child by using genetic material from three people: nuclear DNA from both parents + healthy mitochondria from a donor.

Why needed:

- Mitochondria have their own small DNA (mtDNA)
- Mutations in mtDNA cause severe, incurable diseases (muscle weakness, neurological disorders)
- mtDNA is inherited only from the mother
- Replacing defective maternal mitochondria with healthy donor mitochondria prevents disease transmission

Two Versions:

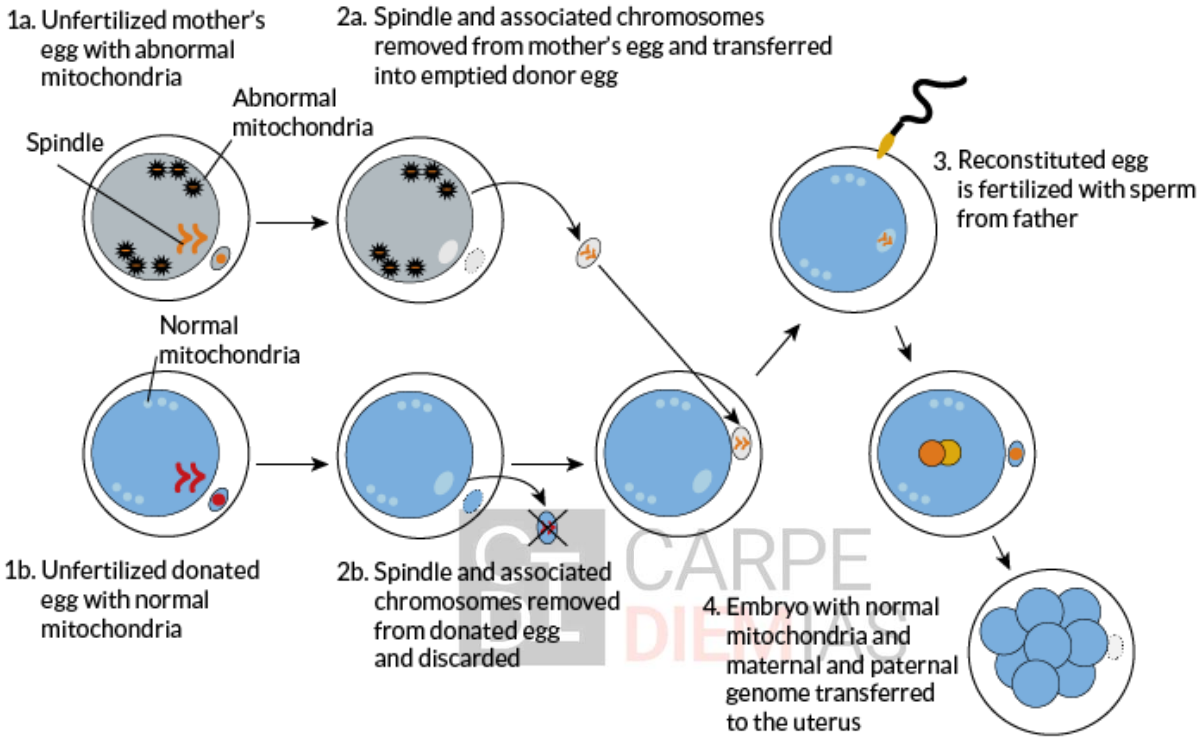
1. Pronuclear Transfer



1. Fertilize both the mother's egg (with defective mitochondria) and a donor egg (with healthy mitochondria) with father's sperm
2. Remove the pronuclei (containing parental nuclear DNA) from both fertilized eggs
3. Discard the pronucleus from the donor egg (with healthy mitochondria)

4. Insert the parents' pronuclei into the donor egg's cytoplasm (with healthy mitochondria)
5. The reconstructed embryo now has: nuclear DNA from both parents + healthy mtDNA from donor
6. Implant into mother's uterus for pregnancy.

2. Maternal Spindle Transfer



1. Remove the maternal spindle (chromosomes before fertilization) from mother's egg
2. Insert it into a donor egg that has had its own nucleus removed but retains healthy mitochondria
3. Fertilize the reconstructed egg with father's sperm
4. The resulting embryo has: nuclear DNA from both parents + healthy mtDNA from donor
5. Implant for pregnancy.

Global Status:

- First three-parent baby born in Mexico (2016) to Jordanian parents
- UK became first country to license the technique (2017) for preventing inherited mitochondrial diseases
- India: Not currently permitted; requires regulatory framework and ethical review

Ethical Considerations:

- Child carries genetic material from three people; questions about identity and donor rights
- Long-term health effects unknown; requires careful monitoring
- Risk of "slippery slope" toward non-therapeutic genetic enhancement

B. Gene Therapy

Simple Concept: Treating genetic diseases by introducing a functional copy of a defective gene into a patient's cells, or by editing the defective gene directly.

Historical Milestone:

- First gene therapy patient: Four-year-old girl with ADA deficiency (1990)
- ADA deficiency weakens the immune system; therapy introduced functional ADA gene into her white blood cells

Two Types:

Type	Target Cells	Heritability	Applications	Ethical Status
Somatic Gene Therapy	Body cells (blood, muscle, liver, etc.)	NOT inherited by offspring	Treating cancer, blood disorders, metabolic diseases	Widely accepted with regulation
Germline Gene Therapy	Reproductive cells (sperm, eggs) or early embryos	INHERITED by future generations	Theoretical prevention of inherited diseases	Banned in most countries including India

Delivery Methods:

- Viral vectors (retrovirus, lentivirus, AAV) for efficient gene delivery
- Non-viral methods (liposomes, electroporation) for safer but less efficient delivery
- Ex vivo: Cells removed, modified in lab, then returned to patient
- In vivo: Vector injected directly into patient's body

Indian Context:

- NexCAR19 (2023): India's first indigenous CAR-T cell therapy (a form of somatic gene therapy) for blood cancers
- CSIR-IGIB: Developing CRISPR-based therapies for sickle cell anemia and thalassemia

- Regulatory framework: ICMR guidelines permit somatic gene therapy trials with strict oversight; germline editing prohibited

C. Genetic Modification of Plants (GM Crops)

Using genetic engineering to introduce desirable traits into crop plants, such as pest resistance, herbicide tolerance, drought tolerance, or enhanced nutrition.

Two Primary Approaches:

Approach	Definition	Example	Regulatory Treatment
Cisgenic	Transfer genes between sexually compatible plants (could be done by conventional breeding, but faster)	Disease resistance gene from wild potato transferred to cultivated potato	Often treated similarly to conventionally bred crops
Transgenic	Transfer genes from unrelated species or sexually incompatible plants (horizontal gene transfer)	Bt gene from bacterium inserted into cotton for pest resistance	Regulated as GMO; requires rigorous biosafety assessment

Key Technologies in GM Crop Development:

1. Terminator Technology (Genetic Use Restriction Technology - GURT)

- **Concept:** Engineer plants to produce sterile seeds, preventing farmers from saving and replanting seeds
- **Purpose:** Protect intellectual property of biotech companies; ensure farmers purchase new seeds each season
- **Controversy:** Concerns about farmer dependency, seed sovereignty, and impact on traditional farming practices
- **Status:** Not commercialized anywhere; banned in India under the Protection of Plant Varieties and Farmers' Rights Act

2. Traitor Technology (Trait-GURT)

- **Concept:** Engineer plants so that desirable traits (e.g., pest resistance, high yield) can be switched on or off using a proprietary chemical trigger
- **Purpose:** Allow companies to control expression of traits; create market for chemical activators
- **Controversy:** Ethical concerns about corporate control over food production; potential for misuse
- **Status:** Research stage; not commercialized; faces strong public and regulatory opposition

PART 3: TISSUE CULTURE METHODS AND APPLICATIONS

Definition and Core Principles

Tissue Culture (Micropropagation/Cell Culture)

- The in vitro growth of cells, tissues, or organs under sterile, nutritionally optimized, and environmentally controlled conditions
- Foundational for genetic engineering, clonal propagation, and conservation biology

Key Principles

- Cellular Totipotency: Every plant cell contains the complete genetic blueprint and can regenerate a whole organism under appropriate hormonal and nutritional conditions
- Pluripotency/Multipotency: Animal stem cells can differentiate into multiple cell types but not entire organisms
- Aseptic Technique: Laminar airflow, sterilization of explants, media, and equipment using autoclave, ethanol, or sodium hypochlorite to prevent microbial contamination

Culture Media Composition

- Macronutrients: Nitrogen, phosphorus, potassium, calcium, magnesium, sulfur
- Micronutrients: Iron, manganese, zinc, copper, boron, molybdenum
- Vitamins: Thiamine, pyridoxine, nicotinic acid, myo-inositol
- Carbon Source: Sucrose (2-3%) as energy source since in vitro tissues have limited photosynthetic capacity
- Growth Regulators: Auxins (IAA, NAA, 2,4-D) for rooting and callus induction; Cytokinins (BAP, Kinetin) for shoot proliferation; Gibberellins for elongation
- Gelling Agent: Agar or phytagel (0.6-1%) for solid media; liquid media used for suspension cultures

Why Do We Need It?

- Grow plants faster than traditional methods
- Produce disease-free, genetically identical copies (clones)
- Save endangered or rare plant species
- Create new plant varieties through genetic engineering
- Produce valuable medicines and compounds from plant cells

Basic Requirements for Any Tissue Culture:

- Sterile environment (laminar airflow cabinet, autoclaved tools)
- Nutrient medium with sugars, vitamins, minerals, and plant hormones
- Controlled temperature (usually 22-28°C) and light conditions
- Healthy starting material (explant) from a disease-free plant

MAJOR TISSUE CULTURE METHODS

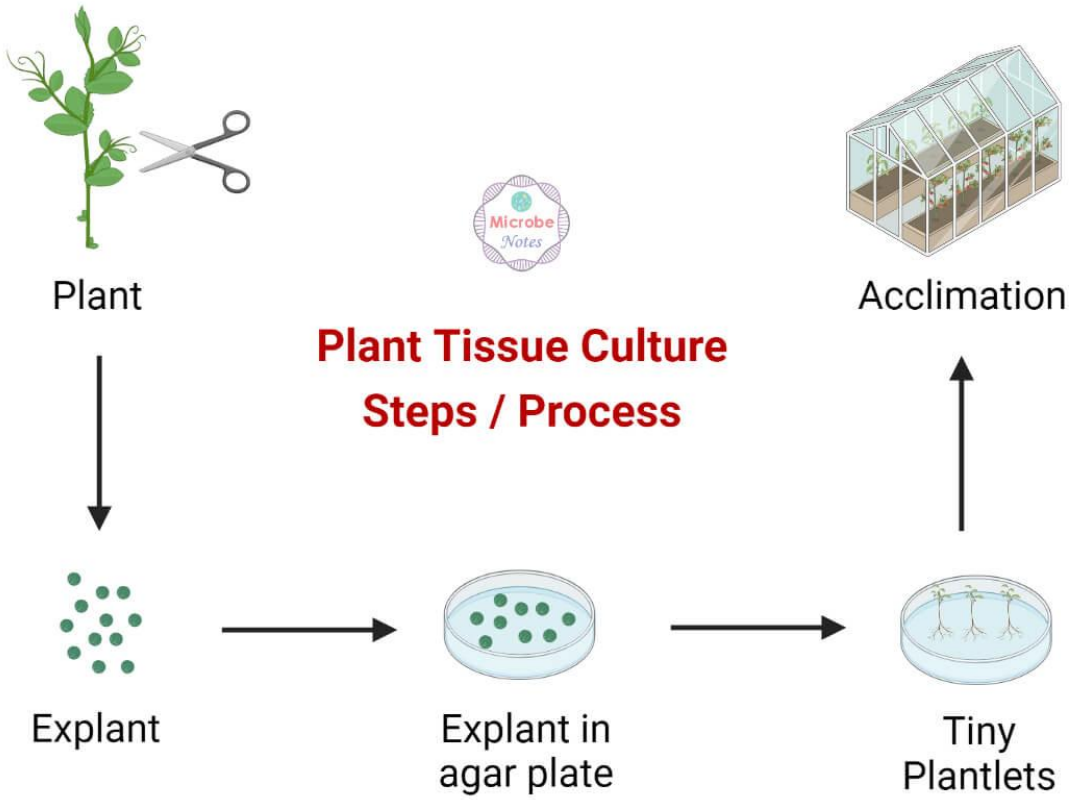
Concept, Process, Applications, and Indian Examples for Mains Examination

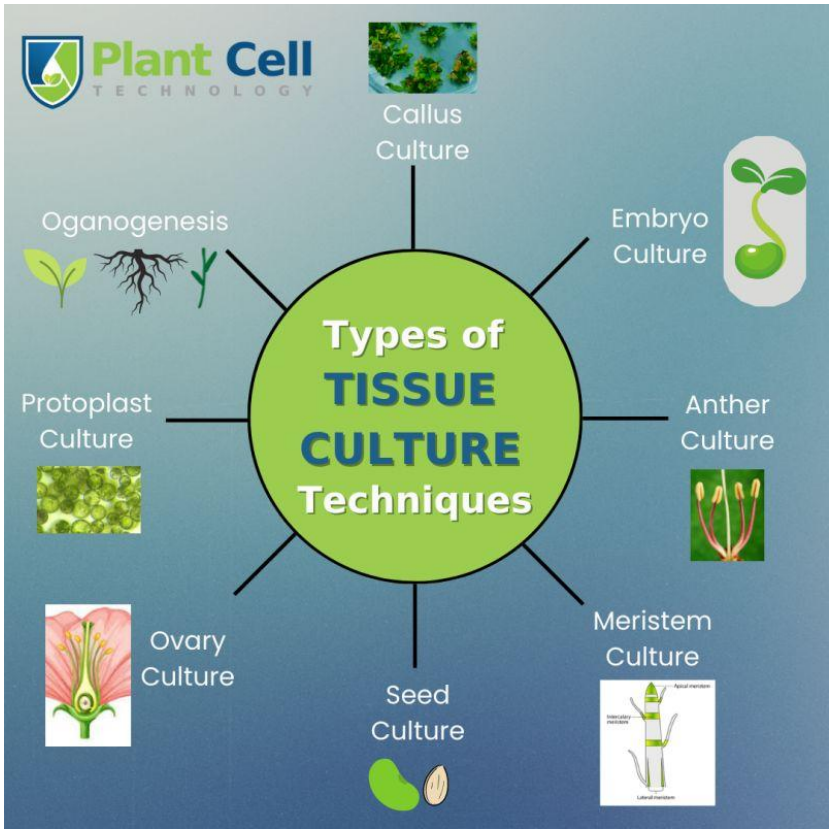
INTRODUCTION: WHAT IS TISSUE CULTURE?

The in vitro growth of cells, tissues, or organs under sterile, nutritionally optimized, and environmentally controlled conditions.

Foundational for genetic engineering, clonal propagation, and conservation biology.

In simple terms , Tissue culture is like growing plants in a test tube or petri dish instead of in soil. Scientists take a tiny piece of a plant (called an explant), place it in a sterile nutrient-rich medium with specific hormones, and control temperature, light, and humidity to help it grow into a complete plant.





1. ORGAN CULTURE

The cultivation of excised plant organs (such as shoot tips, roots, leaves, embryos, or ovules) on a nutrient medium under sterile conditions, maintaining their natural histological organization and structural integrity.

In simple terms,

Growing a whole plant organ (like a shoot tip, root, leaf, or embryo) outside the plant, while keeping its natural structure intact.

How It Works:

1. A small piece of the organ is carefully cut from a healthy plant under sterile conditions
2. It is placed on a nutrient gel containing sugars, vitamins, and hormones
3. The organ continues to grow and develop as it would inside the plant, but in a controlled lab environment
4. For shoot tips: The tip grows into a small plantlet that can later be transferred to soil

Why It Is Useful:

- **Clonal Propagation:** Produces exact copies of elite plants (e.g., high-yielding varieties)

- **Virus Elimination:** Shoot tips often lack viruses; growing them produces clean plants
- **Research:** Studying how organs develop, respond to stress, or interact with hormones
- **Conservation:** Preserving rare plant organs when whole plants cannot be collected

Simple Analogy:
Think of organ culture like growing a cutting from a rose bush in water until it develops roots, but in a highly controlled, sterile lab setting with added nutrients and hormones.

Indian Example:
Shoot tip culture is widely used for producing virus-free banana and potato plants, which has increased yields by 20-30% in states like Tamil Nadu, Karnataka, and Uttar Pradesh.

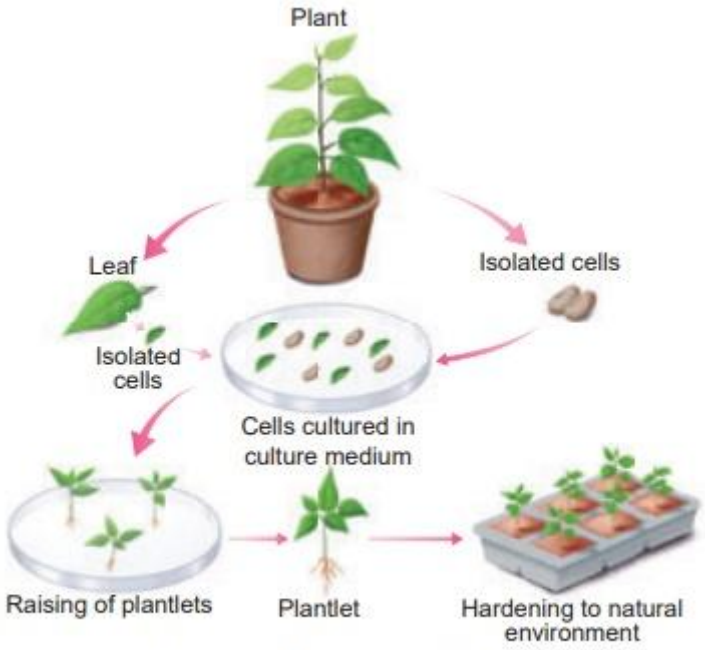


Figure 5.6: Organ Culture

2. CELL CULTURE/ Callus Culture

The growth of an unorganized, proliferating mass of plant cells (callus) induced from an explant on a solid nutrient medium containing high auxin-to-cytokinin ratio, used for genetic transformation and secondary metabolite production.

In simple terms
Growing a mass of unorganized, dividing plant cells (like a blob of cells) from a piece of plant tissue.

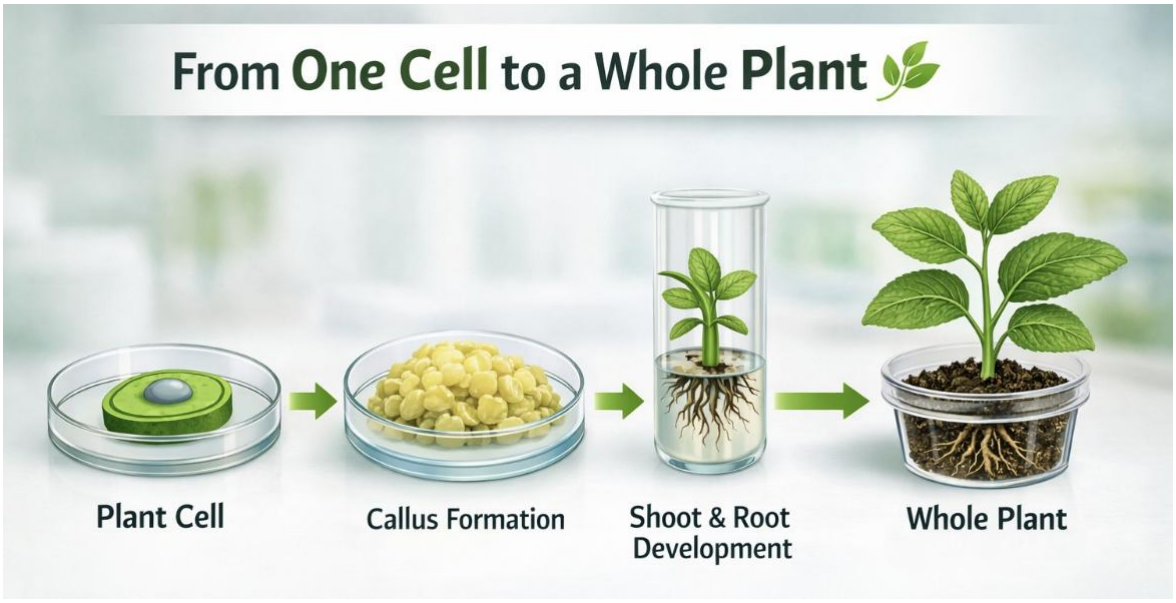
How It Works:

1. A small explant (leaf, stem, root piece) is placed on a nutrient medium with high levels of auxin hormones and low cytokinin

- **Uniformity:** Cells grow in a homogeneous environment, giving consistent product quality
- **Pharmaceutical Production:** Used to produce vaccines, monoclonal antibodies, and plant-derived drugs
- **Enzyme Production:** Industrial enzymes like amylase, protease, and cellulase are produced this way

Simple Analogy:
Suspension culture is like brewing beer or yogurt – cells grow in a liquid "soup" that is constantly mixed, but instead of yeast or bacteria, we grow plant cells for medicines or enzymes.

Indian Example:
Advanced Enzyme Technologies in Maharashtra uses suspension culture to produce industrial enzymes. CSIR labs are developing suspension cultures of Catharanthus roseus to produce anti-cancer compounds vincristine and vinblastine.

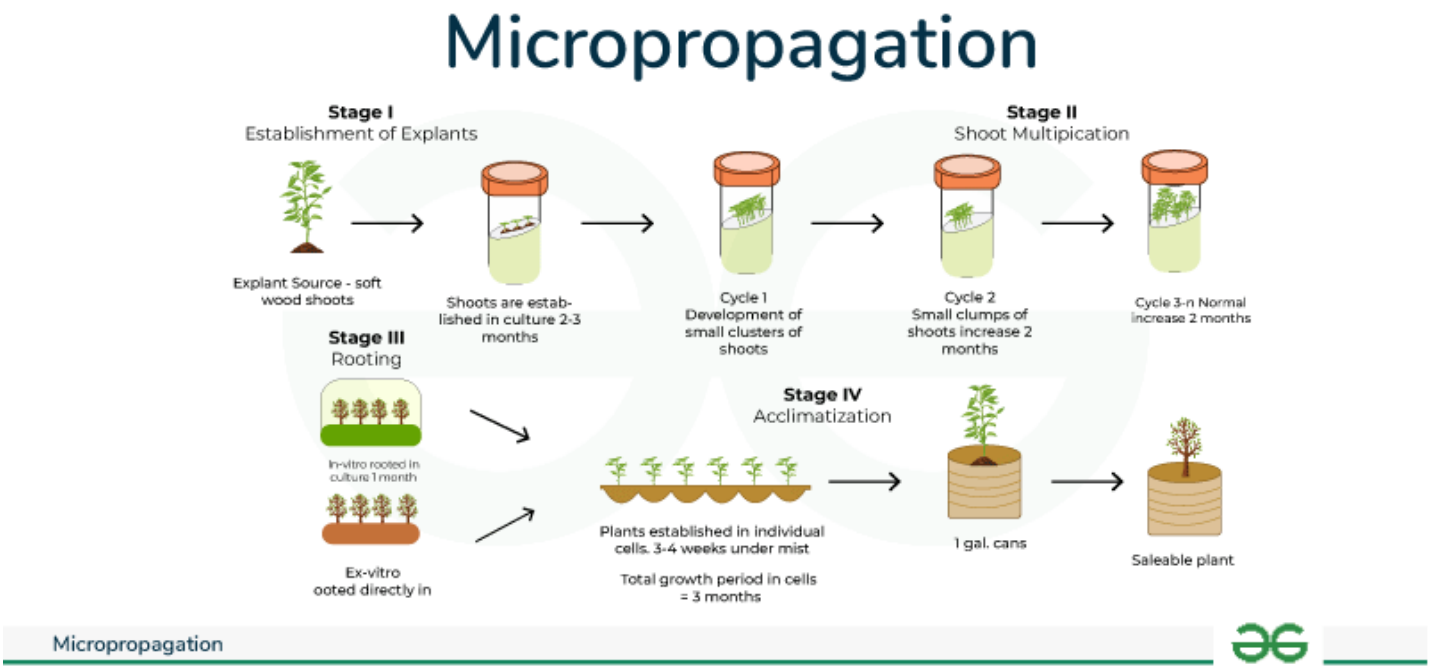


Callus culture is a specific *type* of cell culture. While a callus culture consists of an unorganized, solid mass of dividing cells, general "cell culture" usually refers to individual cells suspended and freely moving in a liquid medium.
The key differences between the two methods involve their physical states, uses, and growth media:
• **Callus Culture:** Cells grow as a solid, compact, or crumbly mass (lump) on a gel-based nutrient medium (like agar). It is frequently used as an intermediate step to regenerate entire plants or extract specific compounds.
• **Cell Suspension Culture:** Cells float and multiply individually or in very small clumps in a moving liquid nutrient medium. It is typically used for large-scale production of secondary metabolites and biochemical experiments.
• **Relationship:** A cell suspension culture is almost always started by taking a piece of a callus and placing it into a liquid, shaking flask so it breaks apart into individual cells.

- **Year-Round Production:** Not dependent on seasons or weather

Simple Analogy:
Micropropagation is like photocopying a plant – you start with one original and make thousands of perfect copies, all free from diseases and ready to grow.

Indian Example:
India produces over 50 crore (500 million) tissue-cultured plantlets annually. Banana (Grand Naine variety) is the most propagated crop, followed by potato (Kufri series), sugarcane, orchids, and teak. States like Tamil Nadu, Karnataka, and Maharashtra have large commercial micropropagation units.



4. MERISTEM CULTURE

The aseptic cultivation of the apical meristem (0.1-0.5 mm shoot tip) which is typically free from systemic pathogens due to lack of vascular connections, used to produce virus-free, pathogen-free planting material for crops like potato, banana, and sugarcane.

In Simple terms:
Growing plants from the very tip of a shoot (the meristem), which is a tiny region of actively dividing cells that is often free from viruses and other systemic diseases.

How It Works:

1. Under a microscope, the apical meristem (0.1-0.5 mm in size) is carefully dissected from a shoot tip using fine needles

2. This tiny piece, containing only a few cells and no vascular tissue, is placed on a nutrient medium
3. The meristem grows into a small plantlet, which is then multiplied through micropropagation
4. Because viruses and bacteria typically spread through vascular tissues (which the meristem lacks), the resulting plants are often pathogen-free

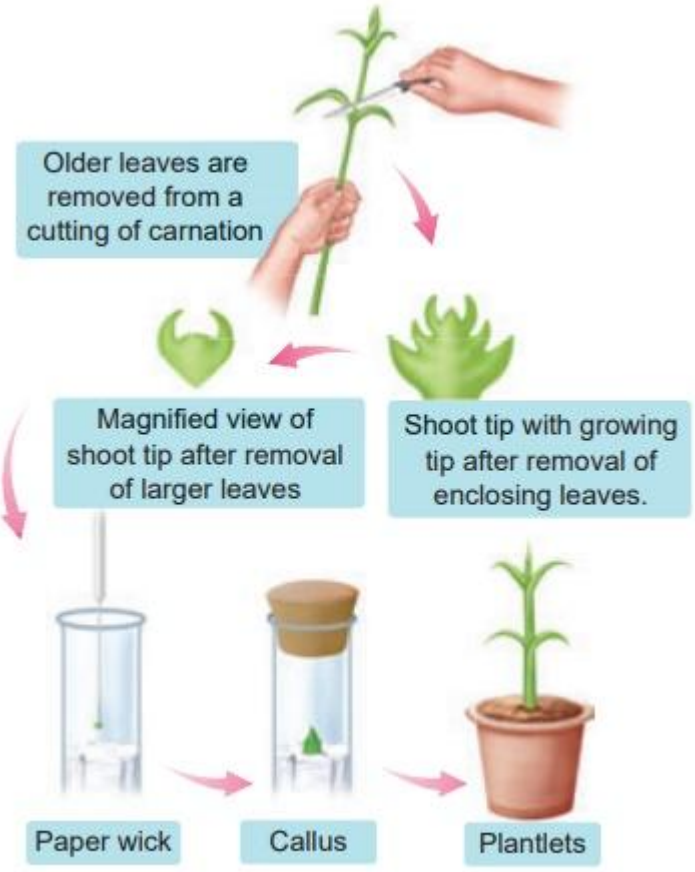


Figure 5.7: Meristem Culture

Why It Is Useful:

- **Virus Elimination:** The most reliable method to produce virus-free planting material for crops like potato, banana, sugarcane, and citrus
- **Germplasm Exchange:** Disease-free plants can be safely shared between countries without spreading pathogens
- **Preserving Elite Varieties:** High-yielding or unique varieties can be cleaned of diseases and multiplied
- **Research:** Studying virus-plant interactions and developing resistant varieties

Simple Analogy:
Think of a virus-infected plant like a building with infected pipes. The meristem is like the architect's office at the very top – the infection hasn't reached there yet. By growing a new plant from just that office, you get a clean building.

Impact in India:
Virus-free banana and potato cultivation through meristem culture has increased yields by 20-30% in southern and northern states respectively. The Central Potato Research Institute (CPRI) in Shimla produces millions of virus-free seed potato tubers annually using this technique.

Simple Analogy:

Protoplast fusion is like removing the shells from two different eggs, mixing their contents, and letting them form a new egg with traits from both parents – something impossible with normal reproduction.

Indian Example:

Research at the National Botanical Research Institute (NBRI), Lucknow, has created somatic hybrids between cultivated and wild species of Brassica (mustard family) to transfer disease resistance and oil quality traits. While commercial "Pomato" has not been successful due to growth incompatibilities, the technique remains valuable for research and pre-breeding.

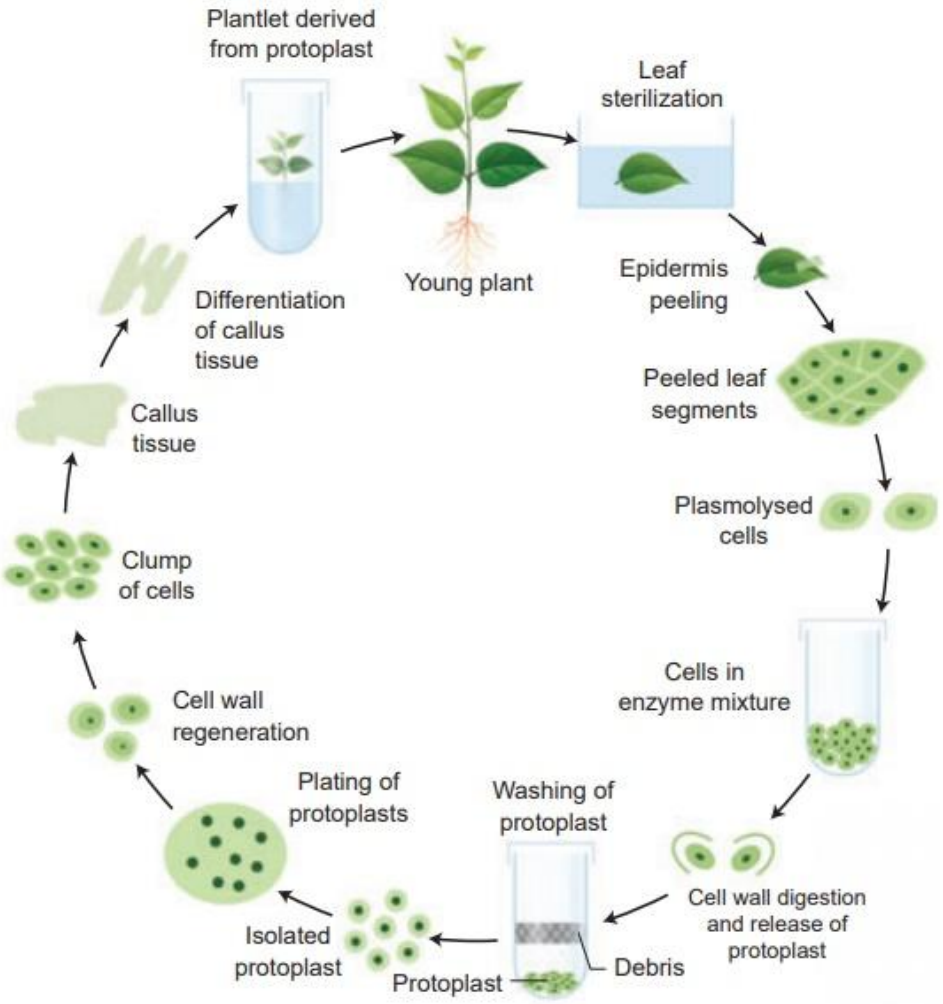


Figure 5.8: Protoplast Culture

COMMON EXAM QUESTIONS AND HOW TO APPROACH THEM

Q: Differentiate between micropropagation and meristem culture.

- Micropropagation is a broad term for rapid clonal multiplication using various explants (shoot tips, nodes, buds); meristem culture is a specific type that uses only the tiny apical meristem (0.1-0.5 mm) primarily for virus elimination
- Micropropagation focuses on quantity (thousands of plants); meristem culture focuses on quality (disease-free plants)
- Both use similar media and stages, but meristem culture requires more skill in dissection and has lower initial success rates

Q: Why is somatic embryogenesis preferred over organogenesis for synthetic seed production?

- Somatic embryos are bipolar (have shoot and root ends) and structurally similar to zygotic embryos, making them ideal for encapsulation and direct sowing
- Organogenesis produces shoots that require separate rooting, making encapsulation and field establishment more complex
- Somatic embryogenesis allows higher multiplication rates and better synchronization for industrial-scale production

Q: How does cryopreservation help in climate change adaptation?

- Preserves genetic diversity of crop wild relatives that may contain traits for drought, heat, or flood tolerance
- Provides a secure backup of elite varieties and breeding lines against extreme weather events that could destroy field collections
- Enables global sharing of climate-resilient germplasm without quarantine delays, accelerating breeding programs worldwide

KEY TERMS TO REMEMBER

- Explant: The small piece of plant tissue used to start a culture
- Totipotency: The ability of a single plant cell to regenerate into a whole plant
- Callus: An unorganized mass of dividing plant cells
- Somatic: Refers to non-reproductive body cells (as opposed to egg or sperm)
- Haploid: Having one set of chromosomes (n); Diploid: Having two sets (2n)
- Cryoprotectant: Chemical that protects cells from ice damage during freezing

- Acclimatization: The gradual process of adapting lab-grown plantlets to normal outdoor conditions

PART 4: BIOTECHNOLOGY IN AGRICULTURE

Bio-Pesticides

Definition and Concept

- Bio-pesticides are biological agents derived from natural materials such as animals, plants, bacteria, fungi, or certain minerals used to control pests, diseases, and weeds
- Environmentally friendly alternative to chemical pesticides; target-specific with minimal non-target effects

Types of Bio-Pesticides

1. **Microbial Pesticides**
    - Bacillus thuringiensis (Bt): Produces crystal proteins toxic to lepidopteran, coleopteran, and dipteran pests; used in Bt cotton and as spray formulation
    - Beauveria bassiana, Metarhizium anisopliae: Entomopathogenic fungi controlling aphids, whiteflies, and locusts
    - Nuclear Polyhedrosis Viruses (NPV): Species-specific viruses controlling Helicoverpa armigera in cotton and chickpea
2. **Biochemical Pesticides**
    - Plant extracts: Neem (azadirachtin), pyrethrum, rotenone with insecticidal properties
    - Pheromones: Species-specific chemical signals for mating disruption and pest monitoring
    - Growth regulators: Juvenile hormone analogs disrupting insect development
3. **Plant-Incorporated Protectants (PIPs)**
    - Genetically engineered plants producing pesticidal substances (e.g., Bt cotton producing Cry proteins)
    - Regulated under GEAC Rules, 1989 in India

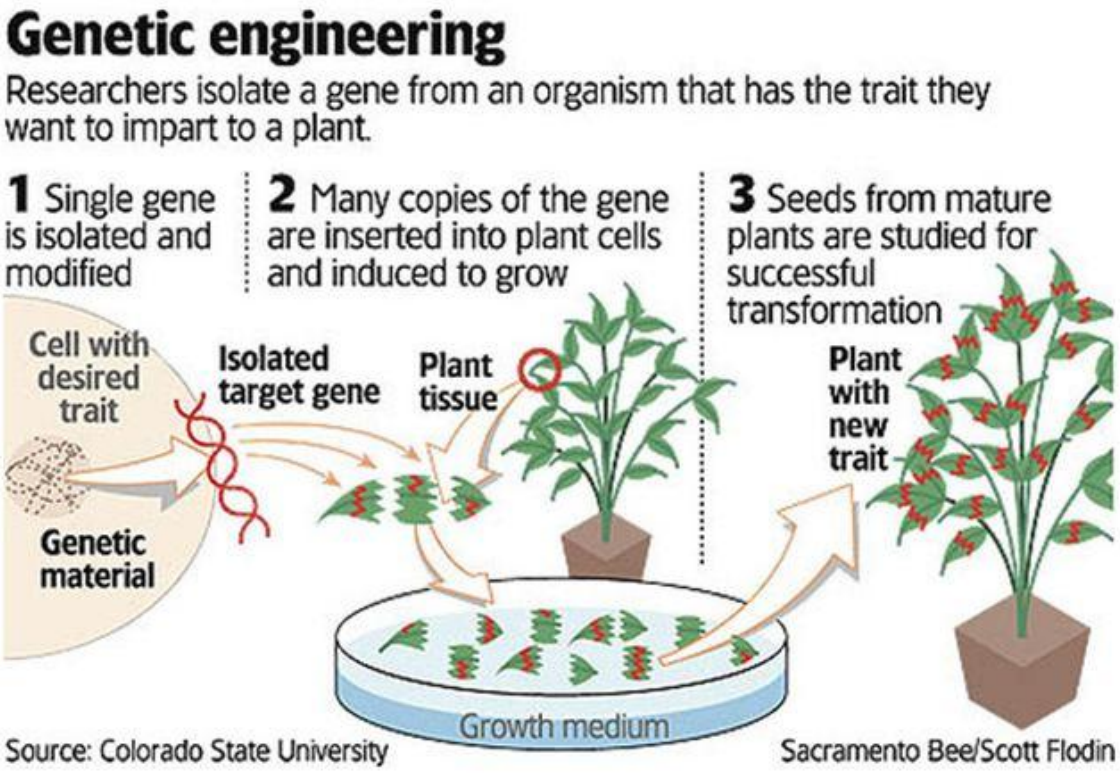
Advantages

- Target-specific action reduces harm to beneficial insects, pollinators, and natural enemies
- Minimal residue concerns; shorter pre-harvest intervals; compatible with organic farming
- Lower risk of pest resistance development when used in integrated pest management (IPM)

Genetically Modified (GM) Crops

Definition and Concept

- GM crops are plants whose genetic material has been altered using genetic engineering techniques to introduce desirable traits such as pest resistance, herbicide tolerance, drought tolerance, or enhanced nutritional content
- Also termed transgenic crops when genes from different species are introduced.



Key Techniques for Developing GM Crops

1. BIOLOGICAL GENE TRANSFER: AGROBACTERIUM-MEDIATED

This method uses a naturally occurring soil bacterium, Agrobacterium tumefaciens, often called "nature's genetic engineer", to deliver desired genes into plant cells.

How It Works

- **Step 1: Gene Insertion into Plasmid**
  - Scientists isolate the desired gene (e.g., Bt gene for pest resistance)
  - The gene is inserted into a modified Ti (Tumor-inducing) plasmid from Agrobacterium
  - Disease-causing genes in the plasmid are removed; only the gene transfer machinery is retained
- **Step 2: Bacterial Infection**
  - Plant tissue (leaf discs, callus, or embryos) is exposed to engineered Agrobacterium
  - Bacterium attaches to plant cells and activates its gene transfer system
- **Step 3: T-DNA Transfer**
  - A segment of the plasmid called T-DNA (Transfer DNA), carrying the desired gene, is transferred into the plant cell nucleus
  - T-DNA integrates randomly into the plant genome
- **Step 4: Selection and Regeneration**
  - Transformed cells are selected using marker genes (e.g., antibiotic or herbicide resistance)
  - Selected cells are grown on nutrient medium with plant hormones to regenerate whole plants

Best For

- Broadleaf crops (dicots) like cotton, tomato, potato, soybean, mustard
- Less efficient in monocots (cereals) though improved strains now work with rice and maize

Advantages

- Natural, efficient system with low copy number integration
- Stable expression of inserted gene
- Relatively low cost and well-understood mechanism

Limitations

- Historically inefficient for monocots (rice, wheat, maize), though newer methods have improved this
- Random integration may disrupt important plant genes
- Requires tissue culture capability for plant regeneration

Indian Example Bt cotton development used Agrobacterium-mediated transfer to insert Cry1Ac and Cry2Ab genes into Indian cotton varieties, leading to commercial approval in 2002.

2. PHYSICAL GENE TRANSFER: PARTICLE BOMBARDMENT (GENE GUN)

This direct approach bypasses biological vectors and physically forces DNA-coated particles into plant cells using high velocity.

How It Works

- **Step 1: Particle Preparation**
  - Microscopic gold or tungsten particles (0.5-1 micrometer) are coated with the desired DNA fragment
  - DNA precipitates onto particle surface through chemical treatment
- **Step 2: Loading and Firing**
  - DNA-coated particles are placed on a macrocarrier (plastic disk)
  - Compressed helium gas builds pressure behind a rupture disk
  - When pressure reaches threshold, disk bursts, accelerating particles down a barrel
- **Step 3: Penetration and Integration**
  - Particles penetrate plant cell walls and membranes at high speed
  - DNA detaches from particles inside the cell
  - Some DNA integrates into plant chromosomes through natural repair mechanisms
- **Step 4: Recovery and Selection**
  - Bombarded tissue is cultured on nutrient medium to recover
  - Transformed cells are selected using marker genes
  - Regenerated into whole plants through tissue culture

Best For

- Crops resistant to Agrobacterium infection, especially monocots like corn, wheat, rice, sorghum
- Organelle transformation (chloroplast, mitochondria)
- Species where tissue culture protocols are well-established

Advantages

- No host range limitations; works for almost any plant species
- Can deliver large DNA fragments or multiple genes simultaneously
- Useful for chloroplast transformation (maternal inheritance reduces gene flow risk)

Limitations

- Can cause physical damage to cells, reducing transformation efficiency
- Random integration with multiple copies may cause gene silencing
- Expensive equipment and consumables
- Requires optimization for each plant species and tissue type

Indian Example Used in research on transgenic rice, wheat, and chickpea at institutions like IARI, ICAR-NRCPB, and various state agricultural universities.

3. ADDITIONAL DIRECT TECHNIQUES

Electroporation

- **Concept:** Brief electric pulses create temporary pores in plant cell membranes, allowing DNA to enter
- **Process:** Protoplasts (wall-less plant cells) are mixed with DNA and subjected to controlled electric field; pores form, DNA enters, membranes reseal
- **Best For:** Plant protoplasts, some monocot species, and research applications
- **Limitation:** Protoplast regeneration into whole plants is difficult for many species

Microinjection

- **Concept:** Direct injection of DNA into plant cell nucleus using an ultra-fine glass needle under microscope
- **Process:** Cell held by suction pipette; needle penetrates membrane and nucleus; tiny volume of DNA solution injected
- **Best For:** Large-celled plant tissues, oocytes, embryos; precise delivery for research

- **Limitation:** Extremely labor-intensive, low throughput, requires high skill and expensive equipment

4. MODERN ADVANCEMENTS: GENOME EDITING

Unlike traditional transgenics that often introduce foreign DNA, genome editing allows precise alterations to the plant's existing DNA without necessarily adding alien genes.

CRISPR-Cas9 in Plants

- **How It Works:**
    - Guide RNA is designed to match the target plant gene sequence
    - Cas9 enzyme + guide RNA complex is delivered into plant cells (via Agrobacterium or gene gun)
    - Complex finds and cuts the target DNA sequence
    - Plant's repair machinery fixes the break; scientists can introduce desired changes during repair
- **Types of Edits:**
    - **SDN-1:** Small deletions or insertions at cut site; no foreign template
    - **SDN-2:** Small changes using a short DNA template; still no foreign genes
    - **SDN-3:** Insertion of larger foreign DNA segments; regulated as conventional GMO
- **Advantages Over Traditional GM:**
    - Precise targeting reduces unintended effects
    - SDN-1/2 edits may be exempt from full GMO regulations in some countries
    - Faster development cycle; no linkage drag
    - Can improve existing varieties without introducing foreign DNA

Indian Context

- Draft Guidelines for Genome Edited Plants (2022) propose exempting SDN-1 and SDN-2 edits from full GEAC approval if no foreign DNA is present
- Research ongoing on CRISPR-edited rice for drought tolerance, wheat for disease resistance, and banana for nutritional enhancement

Benefits of GMOs	Risks of GMOs
Nutritional value of foods could be improved (e.g. by introducing proteins, vitamins or vaccines)	New traits could cause adverse health reactions (e.g. new proteins may cause allergic responses)
Crops can be produced that lack known allergens	Removal of traits could have unknown effects
Crops can grow in arid conditions for better yield (e.g. by introducing drought resistant genes)	Crops may limit biodiversity of local environment (increased competition with native species)
GM crops can produce herbicides to kill pests	Cross pollination could lead to 'super weeds'
Improve food supply / agriculture in poor countries (GM crops can be engineered for improved yields)	Patents restrict farmers from accessing GM seeds (biotech companies hold monopolies over crop use)
GM crops may have longer shelf lives (less spoil)	Foods with GM components may not be labeled
Reduces economic costs and carbon footprint – less need for land clearing and pesticide usage	Different governments may have conflicting regulatory standards concerning safe usage

Regulatory Framework in India (Already covered in other pdf)

- Six-Tier Biosafety System: IBSC → RCGM (DBT) → GEAC (MoEFCC) → SBCC → DLC → Expert Committees
- Primary Legislation: Environment Protection Act 1986 + GEAC Rules 1989; Biological Diversity Act 2002; Food Safety and Standards Act 2006
- Recent Developments: Draft Guidelines for Genome Edited Plants (2022); BioE3 Policy 2024 proposing single-window clearance and regulatory sandboxes

Indian Initiatives and Success Stories

- Bt Cotton Success: Transformed India from cotton importer to net exporter; benefited millions of smallholder farmers
- Public-Private Partnerships: DBT-BIRAC funding for GM crop research; collaboration between ICAR, state universities, and private seed companies
- Farmer Participatory Research: On-farm trials and demonstrations build trust and generate location-specific data
- Capacity Building: Training programs for biosafety officers, regulatory scientists, and extension personnel

CHALLENGES AND WAY FORWARD

Persistent Challenges

- Regulatory Complexity: Overlapping jurisdictions between DBT, MoEFCC, CDSCO, FSSAI causing delays; average 7-10 years for GM crop approval versus 3-5 years globally
- Public Trust Deficit: Misinformation and polarized debates on GM crops, bio-pesticides, and bio-fuels; need for transparent risk communication
- Infrastructure Gaps: Limited biosafety testing facilities, bioreactor capacity, and cold chain for bio-fertilizers and bio-pesticides
- Skill Shortage: Mismatch between industry requirements and academic training; need for interdisciplinary expertise in bio-nano-AI convergence
- Economic Viability: High capital costs for 2G biofuel plants, bio-refineries, and tissue culture units; need for patient capital and policy support

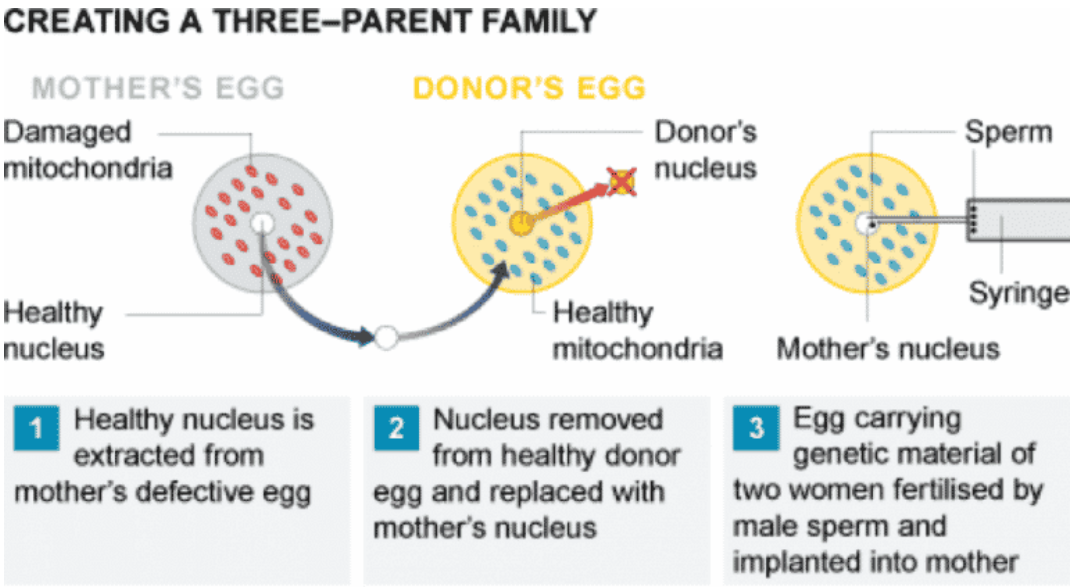
Strategic Recommendations

- Regulatory Modernization: Risk-proportionate, product-based frameworks; single-window digital clearance with statutory timelines (180 days for trials, 360 days for commercialization)
- Innovation Ecosystem Strengthening: Enhanced industry-academia collaboration; patient capital for translational research; expand BIRAC-like models
- Inclusive Capacity Building: Decentralized infrastructure; digital platforms for knowledge sharing; regional innovation hubs in tier-2 cities
- Responsible Innovation: Embedding ethical, social, legal considerations from research design to commercialization; mandatory social impact assessments
- Global Engagement: Active participation in international standard-setting; South-South cooperation on frugal innovations; leverage best practices from robust bioeconomy frameworks

Future Outlook

- Convergence Technologies: Integration of biotechnology, nanotechnology, AI, and robotics creating transformative applications
- Sustainable Development Alignment: Leveraging technologies for SDGs – zero hunger (SDG 2), clean energy (SDG 7), climate action (SDG 13)
- Atmanirbhar Bharat: Indigenous development of critical technologies reducing import dependence; enhancing strategic autonomy in seeds, bio-inputs, and biofuels

- Mitochondrial DNA (mtDNA) makes up about **0.1% of a cell's total DNA and does not affect individual characteristics such as appearance and personality.**
- MGT techniques essentially **swap a woman's defective mitochondrial DNA with that of a donor**. The resulting **embryo's DNA will mostly come from the two parents who supplied the egg and sperm, but a tiny proportion – a fraction of a percentage – will come from the donor.**
- All cells have mitochondria, which are like power packs for the cells and create the energy that keeps cells alive. While a child's DNA is a mixture from both the mother and father, mitochondria are separate "packages of genetics" that come solely from the mother.
- Some people have a mitochondrial disease — a problem with the genetics in their mitochondria — which can lead to severe, life-threatening conditions, although this is rare. **One treatment for a woman who might have one of these diseases is to replace the mitochondria in her eggs via IVF.** This can be done via a process like the one used in Greece where the DNA is taken out of the woman's egg and put into a donor woman's egg once the DNA has been stripped from it, which is then fertilized with sperm to create an embryo.



Why is it so controversial?

- Some people don't like the idea of a baby having three biological parents, and argue that mitochondrial DNA goes some way to shaping important characteristics, such as personality. But the scientific consensus is that swapping mitochondria is similar to changing a battery – it's unlikely to have much, if any, influence over a person's behaviour.
- Others have argued that the technique is unnecessary. After all, it won't help those who have already been born with mitochondrial diseases. Parents often don't find out they are carriers of these diseases until they give birth to sick children. And those who do know they could pass on

a disease have other options, such as using a donor egg. The technique is specifically for people who carry genes for the disease, but want to have a child genetically related to them.

- Another concern is that, by creating a new mix of genetic material, embryologists are creating lasting genetic changes that will be passed down through generations, before we have a chance to find out if they are dangerous. Some argue that this starts us on a slippery slope of germ-line editing – one that could eventually lead to "**designer babies".**

Mitochondrial Diseases

- **Mitochondrial diseases are a group of disorders caused by dysfunctional mitochondria**. ***Mitochondria are found in every cell of the human body except red blood cells.***
- Mitochondrial disorders may be caused by **mutations (acquired or inherited)**, in mitochondrial DNA (mtDNA), or in nuclear genes that code for mitochondrial components.
- They may also be the result of acquired mitochondrial dysfunction due to adverse effects of **drugs, infections, or other environmental causes**.

Gene Sanctuaries(*in situ* conservation)

Gene sanctuaries are protected areas aimed at conserving the genetic diversity of flora and fauna. Their primary purpose is to safeguard endangered species and their habitats. This ensures the preservation of vital genetic resources.

In India, a "gene sanctuary" typically refers to an *in situ* conservation area designated to protect the wild relatives of cultivated crops and preserve their natural genetic diversity. The most prominent example is the **Citrus Gene Sanctuary** located in the **Nokrek Biosphere Reserve** in the Garo Hills of **Meghalaya**.

These sanctuaries are fundamentally important for ecosystem health, food security, and developing climate-resilient crops. Key details on how India protects its genetic heritage include: [1]

Notable Gene Sanctuaries

- **Citrus Gene Sanctuary (Meghalaya):** Established to preserve the natural diversity of wild citrus species, making it a crucial site for understanding the evolutionary history of oranges and lemons.

Other Potential Sites: Similar *in situ* pockets are managed across the biodiversity-rich North Eastern states to protect indigenous varieties of crops like bananas, rice, and mangoes.

Historical Context

The concept of gene sanctuaries in India arose in response to biodiversity loss and habitat destruction. The Biological Diversity Act of 2002 and the National Biodiversity Action Plan highlight the need to conserve genetic resources. These legal frameworks provide a foundation for establishing and managing gene sanctuaries.

Gene sanctuaries helps in

Biodiversity Conservation

Protection of Endangered Species

Gene sanctuaries serve as safe havens for species at risk of extinction. Notable examples include the Asiatic lion and the Indian rhinoceros. By providing a secure environment, these sanctuaries help increase their populations.

Habitat Preservation

These sanctuaries maintain critical habitats that support various ecosystems. They play a vital role in preserving the natural environment and the species that inhabit it.

Agricultural Benefits

Crop Diversity

Gene sanctuaries conserve indigenous crop varieties. These varieties are crucial for food security and resilience against climate change. They ensure that diverse genetic resources are available for future generations.

Breeding Programs

Sanctuaries act as reservoirs for breeding programs. These initiatives aim to improve crop yields and enhance disease resistance. They contribute to sustainable agricultural practices.

Ecological Stability

Ecosystem Services

Healthy gene sanctuaries contribute to ecosystem services. These include pollination, water purification, and soil fertility. Such services are essential for maintaining ecological balance.

Climate Resilience

By preserving diverse genetic resources, gene sanctuaries enhance ecosystem resilience to climate change. They help ecosystems adapt to changing environmental conditions.

Economic Advantages

Sustainable Livelihoods

National Gene Banks (*Ex Situ* Conservation)

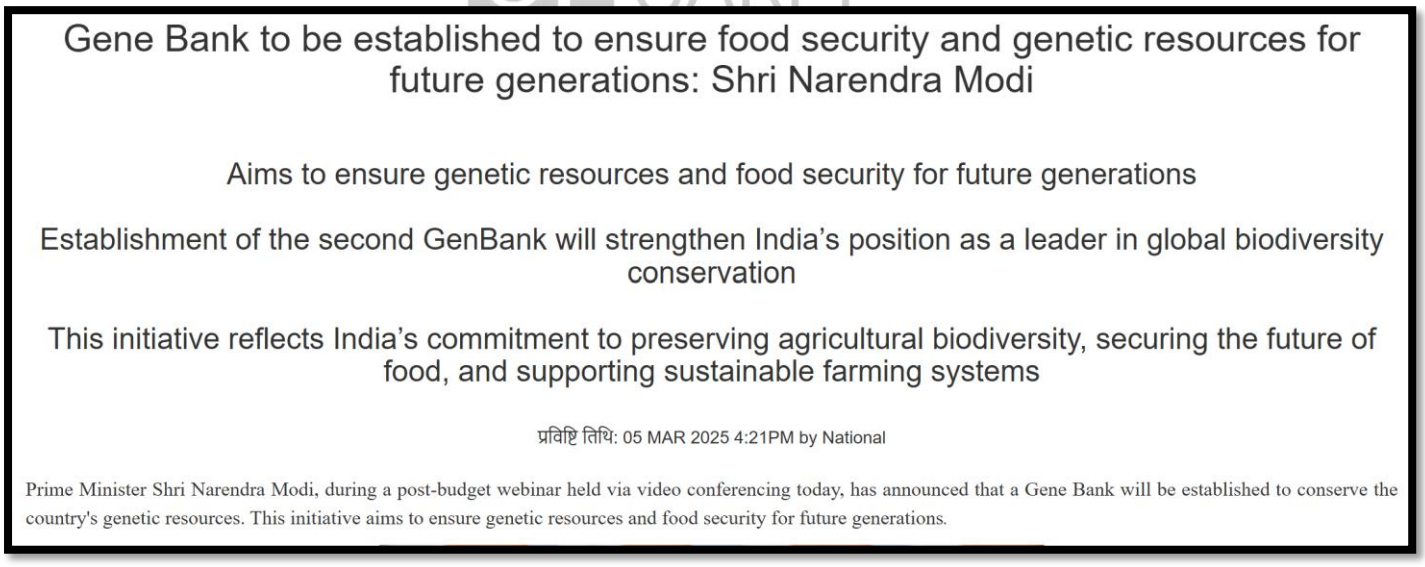
While gene sanctuaries protect plants in their natural habitats, India also maintains extensive *ex situ* (off-site) gene banks to safeguard agricultural and wildlife genetics:

- **National Gene Bank (New Delhi):** Operated by the ICAR-National Bureau of Plant Genetic Resources (NBPGR). It is one of the largest plant gene banks in the world, preserving hundreds of thousands of crop germplasm accessions.
- **National Wildlife Genetic Resource Bank (Hyderabad):** Located at the Nehru Zoological Park, this is India's first facility dedicated to storing genetic material—such as DNA, tissues, and embryos—from endangered and threatened wild animal species.

NATIONAL GENE BANK AND AGRICULTURAL BIODIVERSITY CONSERVATION

CURRENT CONTEXT

In the Union Budget 2025-26, the Government of India announced the establishment of a second National Gene Bank. Prime Minister Shri Narendra Modi highlighted this initiative during a post-budget webinar, emphasizing its role in conserving the country's genetic resources, ensuring food security for future generations, and strengthening India's leadership in global biodiversity conservation.



Gene Bank to be established to ensure food security and genetic resources for future generations: Shri Narendra Modi

Aims to ensure genetic resources and food security for future generations

Establishment of the second GenBank will strengthen India's position as a leader in global biodiversity conservation

This initiative reflects India's commitment to preserving agricultural biodiversity, securing the future of food, and supporting sustainable farming systems

प्रविष्टि तिथि: 05 MAR 2025 4:21PM by National

Prime Minister Shri Narendra Modi, during a post-budget webinar held via video conferencing today, has announced that a Gene Bank will be established to conserve the country's genetic resources. This initiative aims to ensure genetic resources and food security for future generations.

WHAT IS A GENE BANK?

Definition A gene bank is a specialized type of biobank designed to store and preserve genetic material (germplasm) of plants, animals, and microorganisms. The primary goal is to protect biological and genetic diversity from extinction, genetic erosion, or natural disasters.

Key Terminologies

- Germplasm: The living genetic resources (seeds, pollen, sperm, tissue cultures) used for research, conservation, and crop breeding.
- Accession: A distinct, uniquely identified sample of germplasm added to a gene bank's collection.
- Ex-situ Conservation: The conservation of components of biological diversity outside their natural habitats (e.g., in gene banks, botanical gardens).
- Cryopreservation: Storing biological material at ultra-low temperatures (usually in liquid nitrogen at minus 196 degrees Celsius) to halt all metabolic activity and preserve viability for decades or centuries.

INDIA'S GENE BANK INFRASTRUCTURE

The Existing National Gene Bank

- Nodal Agency: **Indian Council of Agricultural Research - National Bureau of Plant Genetic Resources (ICAR-NBPGR).**
- Location: Established in 1996 in New Delhi.
- Network: Comprises 12 regional stations across the country for the collection, characterization, and storage of crop germplasms.
- Current Capacity: As of January 2025, it stores approximately 4.7 lakh (0.47 million) accessions.
- Collection Breakdown: Cereals (1.7 lakh), Millets (over 60,600), Legumes (over 69,200), Oilseeds (over 63,500), and Vegetables (nearly 30,000).

The Proposed Second National Gene Bank

- Announcement: Union Budget 2025-26.
- Capacity: Designed to house 10 lakh (1 million) germplasm lines.
- Purpose: To act as a safety duplicate for the existing collection, ensuring a fail-safe against natural disasters, climate change, or infrastructure failures.
- Scope: Will offer critical conservation support to both public and private sectors involved in genetic resource management.

NEED AND SIGNIFICANCE OF GENE BANKS

1. Climate Change Resilience

- Strengthening Legal Frameworks: Strictly enforce the Protection of Plant Varieties and Farmers' Rights (PPV&FR) Act and the Biological Diversity Act to ensure that the commercial use of genetic resources results in equitable benefit-sharing with local farming communities.

QUICK REVISION POINTS

- Context: 2nd National Gene Bank announced in Budget 2025-26 by PM Modi.
- Purpose: Conserve genetic resources, ensure food security, support SAARC/BRICS nations.
- Existing Bank: ICAR-NBPGR, New Delhi (1996); stores 4.7 lakh accessions; 12 regional stations.
- New Bank Capacity: 10 lakh (1 million) germplasm lines; acts as a safety duplicate.
- India's Biodiversity: 811 cultivated crop species, 902 crop wild relatives.
- Key Terms: Germplasm (living genetic resource), Accession (unique stored sample), Ex-situ conservation (outside natural habitat).
- Global Vault: Svalbard Global Seed Vault in Norway acts as the ultimate global backup.
- Treaties: ITPGRFA (plant genetic resources), Nagoya Protocol (benefit sharing).
- Significance: Climate resilience, preventing genetic erosion, food security, combating biopiracy.
- Way Forward: Molecular characterization, community seed banks, digital integration, and strict enforcement of benefit-sharing laws.