Unit 13 · Biotechnology

STUDY NOTES

WHAT NEB'S GRADE 12 EXAM ASKS

- **CDC's grid (2078):** Biotechnology (Unit 13): 4 of 75 marks; 1 short question (4 marks: knowledge 1, application 1, higher ability 2)
- **The paper:** Botany 37 + Zoology 38; each part: MCQs × 1 (5 Botany, 6 Zoology) · 4 short × 4 · 2 long × 8; one OR in each short and long group; 3 hours; 1 mark per expected point
- **CDC's model paper:** short: micropropagation and its stages (4); MCQ: *Azolla* as a biofertilizer
- **MCQ (1 mark)**
  - cytokinin → shoots; laminar air flow cabinet
  - *Rhizobium* nodules; biofertilizers enrich soil
  - biopesticides from natural materials
  - washing hands; food security = access for all
  - unlabelled coloring breaks labeling
- **Short (in parts)**
  - totipotency; two GM crops; GMO
  - five disease-resistant crops of Nepal
  - micropropagation as a flowchart
  - hybridization

Key terms

Biotechnology: use of living organisms, cells, parts or processes to make or change products for a use.

Totipotency: a living plant cell's ability to grow into a whole plant.

Plant tissue culture: growing cells, tissues, organs on a sterile nutrient medium, controlled conditions.

Explant: plant piece used to start a culture.

Callus: unorganized mass of dividing cells.

Micropropagation: rapid multiplication of plants in sterile culture.

Hardening (acclimatization): moving plantlets gradually to open conditions.

Plant breeding: genetic improvement of useful plants on a scientific basis.

Hybridization: crossing genetically different parents; F_1 often shows **heterosis**.

Emasculation: removing anthers before they shed pollen.

Biofertilizer: living microbes that make nutrients available to plants.

Genetic engineering (rDNA technology, gene splicing): adding, removing or changing genes.

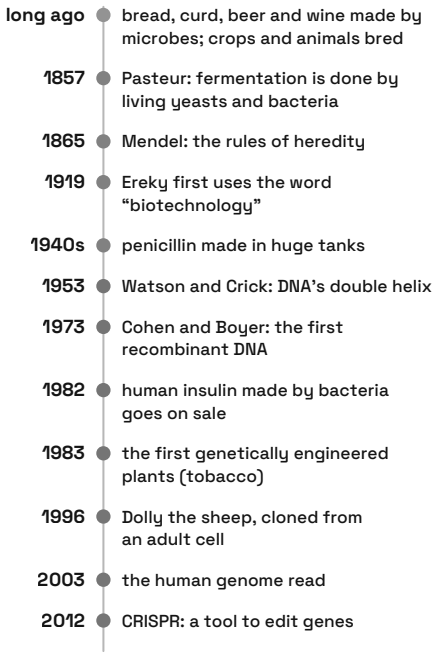
GMO: genetically modified organism; **transgenic**: carries another species' gene.

Biopesticide: pesticide from natural materials (animals, plants, microbes, minerals).

Food security: all people, at all times, have physical and economic access to enough safe, nutritious food.

Biotechnology

- **ancient**: fermentation (curd, bread, gundruk, kinema, jaand); selective breeding
- **19th c.**: **Pasteur**, fermentation by microbes (1857), pasteurization; **Mendel** (1865)
- **early 20th**: **penicillin** (Fleming 1928), factory-made from the 1940s
- **mid-20th**: DNA double helix (**Watson, Crick** 1953); 1970s **recombinant DNA** (Cohen, Boyer 1973)
- **now**: genomics, **CRISPR** gene editing, biomanufacturing
- draws on biology, chemistry, engineering, computer science



Milestones of biotechnology.

Color	Area	Examples
red	medical	insulin, hepatitis B vaccine, gene therapy, PCR tests
green	agricultural	tissue-cultured banana, Bt cotton, biofertilizers
white	industrial	enzymes in washing powder, biofuels, bioplastics
blue	aquatic	marine drugs, aquaculture, algal fuels
gold	bioinformatics, nanobiotech	comparing genomes; nanoparticles carrying drugs

Social and ethical issues

- **germline editing**: passed to descendants; "designer" children, inequality (He Jiankui, 2018); GMO effects on environment, health
- **privacy**: **genetic discrimination** in jobs, insurance → keep data confidential, consent
- **access and equity**: costly; rich–poor and global divide
- **animal welfare**: most cloned embryos die (Dolly, 1996)
- **patents** on genes, life (basmati patent, 1997)
- Nepal: **National Biosafety Framework** (2006)

Plant tissue culture

- **Haberlandt** (1902), father of PTC; **MS medium** (Murashige, Skoog 1962); auxin:cytokinin decides roots or shoots (Skoog, Miller 1957); whole tobacco plant from one cell (1965)

Principles: totipotency; aseptic conditions (microbes outgrow plant cells); defined medium; controlled environment

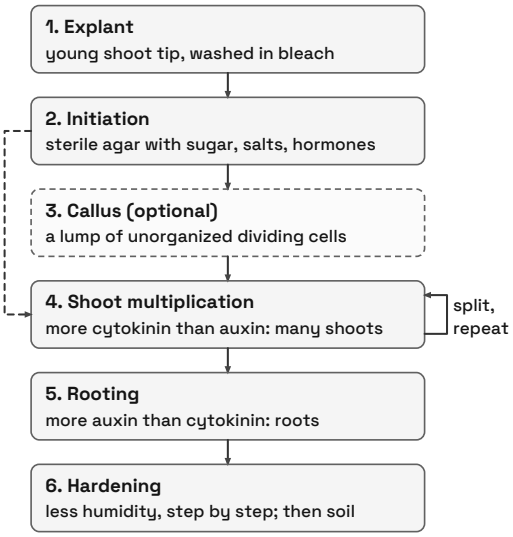
Requirements

1. **explant**: young, healthy tissue
2. **nutrient medium**: macro- and micronutrients; **sucrose** (can't photosynthesize enough); vitamins; **auxin, cytokinin**; **agar**
3. **aseptic**: **laminar air flow cabinet**; **autoclave** (121 °C); alcohol, bleach for the explant
4. culture vessels
5. growth chamber (temperature, light, humidity)

- **more auxin → roots; more cytokinin → shoots; balance → callus**

Micropropagation steps

1. **explant selection, sterilization**: shoot tip; bleach, alcohol, sterile water
2. **initiation**: on medium in a sterile vessel; contaminated jars discarded
3. **callus** formation, **subculture** (optional)
4. **shoot multiplication**: **high cytokinin : auxin**; split, subcultured
5. **rooting**: **high auxin : cytokinin** → plantlets
6. **hardening**: from ~100% humidity, sugar-fed, thin wax → greenhouse → field



Micropropagation.

Technique	Cultured	Use
micropropagation	shoot tips, buds	clones, fast
callus	callus	cell growth studies; regeneration
suspension	cells in shaken liquid	**secondary metabolites**
meristem	apical meristem	**virus-free** plants (no vascular tissue)
anther (pollen)	anthers, pollen	**haploids** → doubled → **pure lines** in one step
embryo	young embryos	**embryo rescue** of wide crosses; dormancy
protoplast	wall-less cells	**somatic hybridization**

Applications

1. fast duplication: orchids, bamboo, potato, banana
2. **disease-free** plants (meristem): potato, sugarcane, banana
3. **germplasm conservation**; **cryopreservation** at −196 °C
4. useful chemicals: **shikonin**, **taxol** (yew)
5. **somaclonal variation** → new types
6. step in making **transgenic plants**
7. plants all year

Plant breeding

Objectives: higher **yield**; **quality**; **disease resistance**; climate and soil fit; **maturity time** (early varieties); **lodging resistance** (short, stiff stems)

Selection

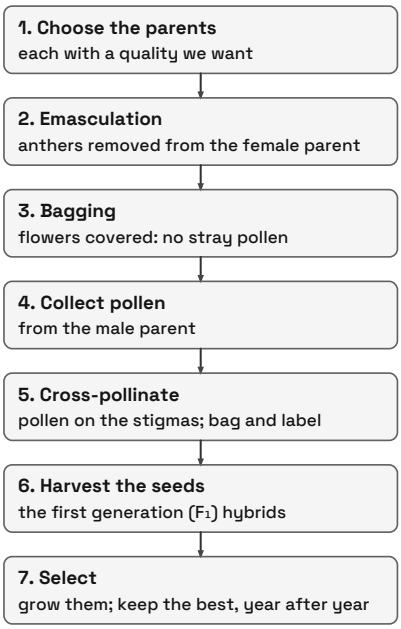
- **mass**: many similar good plants, seed **bulked**
- **pure line**: one superior plant of a **self-pollinated** crop (wheat, rice, barley, beans); homozygous progeny (Johannsen, 1903)
- **clonal**: best plant of an **asexually propagated** crop multiplied (potato, sugarcane, banana, apple, tea)

Hybridization: crossing different parents → desirable genes together; F_1 **heterosis** (fresh hybrid maize seed each year)

- **intraspecific**: two varieties of one species (commonest)
- **interspecific**: two species of a genus (wild tomato genes)
- **intergeneric**: two genera: **triticale** (*Triticum* × *Secale*), *Raphanobrassica*

Steps

1. **select parents**
2. **emasculation**: anthers removed before shedding (not for unisexual or male-sterile)
3. **bagging**
4. collect pollen from male parent
5. **cross-pollinate**; re-bag, **tag** (parents, date)
6. collect F_1 seed
7. **select** over generations → new variety



Steps of hybridization.

Modern methods

- **mutation breeding**: X-rays, gamma rays, chemical mutagens
- **polyploidy**: bigger, vigorous; triploid **seedless watermelon** (4n × 2n)
- **tissue culture**: micropropagation, embryo rescue
- **genetic engineering**: herbicide, insect resistance (Bt cotton, Bt maize)

Disease-resistant plants

- resist **pathogens** (bacteria, viruses, fungi, nematodes); genes to **recognize** and **defend**
- defences: **structural** (cuticle, wall), **chemical**, **R protein** → infected cells die, pathogen trapped
- raise yield, fewer pesticides, food security

Crop	Variety	Resistant to
rice	IR-20	bacterial leaf blight, blast
wheat	WK 1204	yellow rust
wheat	Vijay	stem rust (Ug99), leaf rust
potato	Kufri Jyoti	late blight (when released)
potato	Janakdev	late blight (moderate), wart

- often listed: rice CO-20, IR-8, IR-22, Basmati-370, Mansuli; maize Rampur, Kakani, Khumal Yellow; wheat Kalyan-227, NP-120, PO-18, UP 262, S-227; potato Kufri Red, Shakti, Sindhuri (several bred for yield, not resistance)
- **resistance breaks down** (new strains): **durable**, many-gene resistance preferred

Green manure and biofertilizers

Chemical fertilizers used heavily: soil acidity; harm soil microbes, earthworms; poorer structure, water holding; nitrate, phosphate **pollution**; costly, imported

Manure

1. **compost**: plant waste in pits, heaps
2. **farmyard manure**: dung, urine, bedding (commonest in Nepal)
3. **green manure**: legumes plowed in green, before flowering: **dhaincha** (*Sesbania*), khesari, soybean; before rice
4. **vermicompost**: earthworms (*Eisenia fetida*) on waste; **"nature's plowmen", "farmers' friends"**: aerate, mix soil, simplify nutrients

Green manure: importance: cheap; **nitrogen**; organic matter → structure, aeration; higher yield; reclaims **alkaline** soils; more microbes; eco-friendly

Biofertilizers

- **bacteria**: *Rhizobium* (**symbiotic**, legume nodules; seed inoculation); *Azotobacter*, *Paenibacillus polymyxa*

(free-living); *Azospirillum* (on grass roots: maize, sugarcane, wheat)

- **cyanobacteria**: fix N non-symbiotically in **heterocysts**: *Anabaena*, *Nostoc*, *Calothrix*; rice fields; *Anabaena azollae* in the fern *Azolla*
- **fungi**: **mycorrhiza**: ecto (sheath; pine, oak, sal), endo (inside cells; most crops); water, minerals, **phosphorus**; protect roots

Advantages: cheap; more yield; N and P; aeration, water holding; **sustainable** (multiply); some antibiotics; eco-friendly
Disadvantages: **slow**; not enough alone (no K); need care, right strain, storage, expiry

- not every nutrient: only **N** and **P**

Genetic engineering

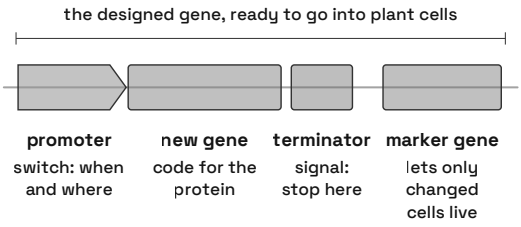
- adds a **foreign gene** or removes, changes one; **rDNA technology**, gene manipulation, gene splicing (gene therapy is a **use**)
- **one or a few chosen genes**; crosses the **species barrier**; precise

Tools (from bacteria)

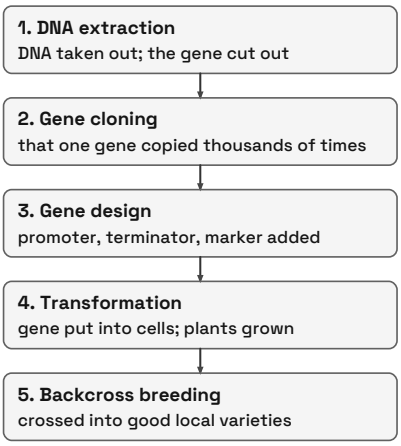
- **restriction endonucleases**: molecular scissors; *Eco***RI** cuts GAATTC → **sticky ends**
- **DNA ligase**: molecular glue
- **vectors**: **plasmids**, viruses, *Agrobacterium tumefaciens*
- same enzyme on plasmid and donor → matching sticky ends

Steps

1. **DNA extraction**; gene cut out by a restriction enzyme
2. **gene cloning**: in a plasmid in bacteria, thousands of copies (or **PCR**)
3. **gene design**: **promoter** (plant's), **terminator**, **marker gene** (antibiotic, herbicide resistance)
4. **transformation**: in callus cells: **gene gun** (gold, tungsten), *Agrobacterium* (Ti plasmid), **microfibers** (silicon carbide), **electroporation** (protoplasts); marked cells → **transgenic plants**
5. **backcross breeding** into elite local varieties



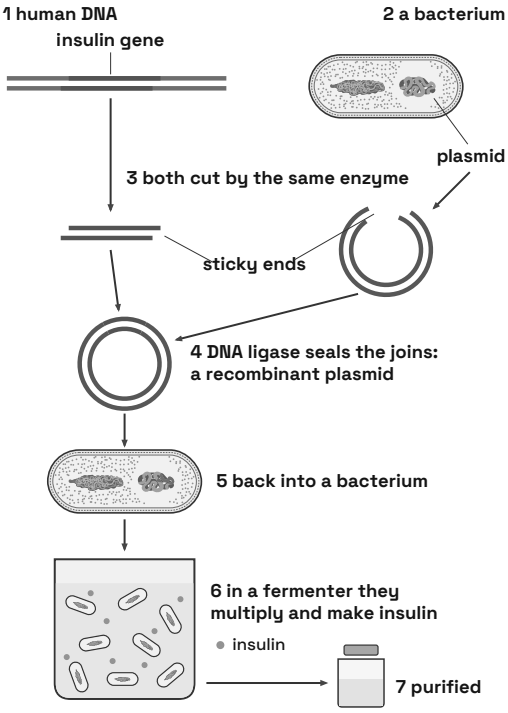
A designed gene.



Making a genetically engineered crop.

Applications

- **medicine**: human growth hormone, FSH, albumin, **monoclonal antibodies**, **factor VIII**, **insulin**; **hepatitis B vaccine** (yeast); model animals; **gene therapy**
- **research**: gene libraries in bacteria
- **industry**: insulin, tryptophan, **chymosin** (cheese), fuels
- **agriculture**: Bt cotton, Bt maize, Bt brinjal; herbicide tolerance; **Golden Rice**; virus-resistant papaya
- others: chestnut conservation; invasive pests; microbial art



Human insulin from bacteria.

Benefits: less insecticide; drought, disease resistance; **gene therapy** (CRISPR, sickle cell, 2023); engineered mosquitoes (dengue); therapeutic cloning (experimental)
Challenges: unforeseen results; upsets biodiversity (**superweeds**); allergens; bioethics

GMOs

- **plants**: Golden Rice (beta-carotene), **Flavr Savr tomato** (1994), Bt cotton, Bt brinjal
- **animals**: goats (antithrombin in milk, 2006), fast-growing salmon (2015)
- **microbes**: *E. coli* (insulin), yeast (hepatitis B vaccine), *Sinorhizobium meliloti*
- **advantages**: *nif* **genes** into cereals (aim, not done yet); more milk, meat, eggs; nutrition, pest resistance; model animals
- **disadvantages**: **gene flow** by pollen; allergy; organ transplant ethics; uniform crops → widespread failure, Bt-resistant insects

Biopesticides

1. **biochemical**: non-toxic: **sex pheromones** (traps, mating disruption), plant scents
2. **microbial**: *Bacillus thuringiensis* **(Bt)**: protein crystals → toxic in insect's **alkaline gut** → gut holes; specific larvae; harmless to us (acid stomach, no receptors)
3. **plant-incorporated protectants**: Bt gene in the plant (Bt cotton, maize, brinjal)

- **botanical**: neem, pyrethrum

Advantages: less toxic; **target-specific**; small amounts, **break down fast**; key in **IPM**
Disadvantages: slower; less effective, short-lasting; heat, UV sensitive; know-how, cost; availability; too specific; resistance; regulation

Bioengineering

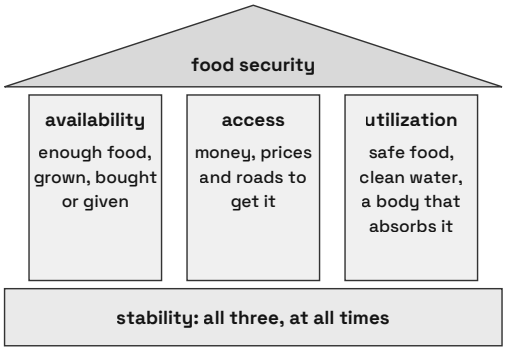
- engineering principles applied to living systems: models, devices, **biocompatible** materials, **scaffolds** (tissue engineering)
- medical devices, biomaterials, **bioprocessing**, GMOs, **bioremediation**
- gives: synthetic bone, dental implants, **pacemakers**, hearing aids, cochlear implants; dialysis, heart valves, hip and knee joints; robotic, laser surgery; monitors; ultrasound, CT, **MRI**

Food safety and security

- **food safety**: handling, preparing, storing food to prevent **foodborne illness** (*Salmonella*, harmful *E. coli*, *Listeria*)
- **toxicity**: ability to harm; "the dose makes the poison"
- **hazard**: biological (microbes, **aflatoxin**), chemical (pesticides, colors, lead), physical (glass, stone)
- **risk**: likelihood and seriousness of harm; needs exposure
- WHO **five keys**: keep clean; separate raw and cooked; cook thoroughly; safe temperatures (< 5 °C or > 60 °C, ≤ 2 h out); safe water and raw materials
- honest **labels**; Nepal: **Department of Food Technology and Quality Control**

Food security (World Food Summit, 1996)

1. **availability**: production, imports, aid
2. **access**: income, prices, distribution
3. **utilization**: nutritious, safe, healthy body (water, sanitation)
4. **stability**: over time, through shocks, lean seasons



Four components of food security.

COMMON MISTAKES

✗ Auxin gives shoots in culture
✓ **cytokinin**; auxin gives roots.

✗ Sucrose is added for taste
✓ cells **can't photosynthesize enough**.

✗ Meristem culture removes viruses by killing them
✓ the meristem is **often virus-free** (no vascular tissue).

✗ Bread wheat = einkorn × *Aegilops*
✓ **emmer** (AABB) × *Aegilops tauschii*; triticale is the safe intergeneric example.

✗ Resistant varieties stay resistant
✓ resistance **breaks down**.

✗ Biofertilizers supply all nutrients
✓ only **N**, **P**.

✗ *Azotobacter* is symbiotic
✓ **free-living**; *Rhizobium* is symbiotic.

✗ Gene therapy is another name for genetic engineering
✓ it is a **use** of it.

✗ A hazard is the likelihood of harm
✓ that is **risk**; a hazard is the agent.