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HANDOUT

BT613T

Agriculture Biotechnology (Theory)

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BT613T

Agriculture Biotechnology (Theory)

Lecture Handouts

Prepared for the students of Virtual University of Pakistan

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1. Introduction to Agricultural Biotechnology

LEARNING OBJECTIVES

- Define agricultural biotechnology and its scope
- Trace the history from traditional to modern biotechnology
- Identify applications in crop improvement

Agricultural biotechnology is the application of biological techniques - from traditional breeding to recombinant DNA technology - to improve crops, livestock productivity and agricultural sustainability. Traditional biotechnology includes selection, hybridization and fermentation (used for thousands of years); **modern biotechnology** adds tissue culture, molecular markers and **genetic engineering** (transfer of defined genes across species barriers).

Milestones: Mendel's laws (1865) gave breeding a scientific basis; Watson and Crick's DNA structure (1953) opened molecular biology; the first transgenic plant - antibiotic-resistant tobacco - was produced in 1983; **Bt cotton** commercialized in 1996 now covers most of Pakistan's cotton area. Agricultural biotechnology aims at higher yield, pest/disease resistance, abiotic-stress tolerance, improved nutrition and reduced chemical inputs.

GMO / Transgenic plant

An organism carrying a foreign gene (transgene) stably integrated into its genome by genetic engineering, e.g. Bt cotton carrying the cry gene from *Bacillus thuringiensis*.

SECTION SUMMARY

Agricultural biotechnology spans traditional breeding to genetic engineering. Its goal: better, hardier, more nutritious crops with fewer inputs - exemplified by Bt cotton in Pakistan.

2. The Plant Genome: Organization and Structure

LEARNING OBJECTIVES

- Describe plant genome organization
- Explain nuclear, chloroplast and mitochondrial genomes
- Discuss genome size variation and polyploidy

Plant nuclear genomes vary enormously - from ~125 Mb in *Arabidopsis* to ~16,000 Mb in wheat. Most of this variation is repetitive DNA (transposons, retrotransposons), not gene number: rice has ~40,000 genes in 430 Mb; wheat has ~100,000+ genes in a hexaploid 16 Gb genome. **Polyploidy** (multiple chromosome sets) is common in crops - wheat is hexaploid (AABBDD), cotton allotetraploid - and often increases vigor and adaptability.

Plants have three genomes: the **nuclear genome** (biparental inheritance), the **chloroplast genome** (plastome, ~120-160 kb, usually maternally inherited - useful for transgene containment since pollen rarely transmits it) and the **mitochondrial genome** (variable size, maternal; source of cytoplasmic male sterility used in hybrid seed production). Understanding genome organization guides marker development, gene cloning and transformation strategies.

- **Repetitive DNA:** transposable elements make up >80% of maize and wheat genomes.
- **Chloroplast transformation:** high transgene copy number, maternal inheritance = biocontainment; used for edible vaccines research.
- **Cytoplasmic male sterility (CMS):** mitochondrial trait exploited in hybrid rice, maize and sunflower seed production.

SECTION SUMMARY

Plant genomes are large, repetitive and often polyploid, with three semi-independent genomes. Chloroplast engineering offers containment; CMS enables hybrids.

3. Plant Gene Expression and Regulation

LEARNING OBJECTIVES

- Describe plant gene structure
- Explain transcriptional and post-transcriptional regulation
- Discuss promoters used in plant biotechnology

A plant gene has a **promoter** (with TATA box, CAAT box and enhancers), 5' UTR, coding exons interrupted by introns, and a 3' terminator with polyadenylation signal. **Transcription factors** (e.g. WRKY, NAC, bZIP families) bind cis-elements to switch genes on/off in response to development or stress - e.g. DREB factors activate drought-responsive genes.

Key promoters in biotechnology: **CaMV 35S** - strong constitutive promoter from cauliflower mosaic virus, the workhorse of plant transformation; **ubiquitin/actin promoters** (monocot-preferred); **tissue-specific promoters** (e.g. seed-specific glutelin promoter for rice endosperm - used in Golden Rice); **inducible promoters** (e.g. drought-inducible rd29A) that activate only under stress, avoiding yield drag. Post-transcriptional control includes alternative splicing and **RNA silencing (RNAi)** - exploited to make virus-resistant papaya and low-acrylamide potatoes.

RNA interference (RNAi)

A natural gene-silencing mechanism where small RNAs guide degradation of matching mRNAs; engineered as hairpin constructs to silence plant genes or viral RNAs.

SECTION SUMMARY

Gene expression is controlled at promoter, transcript and protein levels. Constitutive (35S), tissue-specific and inducible promoters - plus RNAi - are the expression toolkit of crop engineering.

4. Plant Tissue Culture: Foundations

LEARNING OBJECTIVES

- State the principle of totipotency
- List components of culture media
- Explain the role of plant growth regulators

Totipotency - the capacity of a single plant cell to regenerate a whole plant - is the foundation of all plant tissue culture (Haberlandt proposed it in 1902; Steward proved it with carrot cells in 1958). An **explant** (leaf, stem, embryo, meristem) is surface-sterilized and placed on sterile nutrient medium under controlled light and temperature.

MS medium (Murashige and Skoog, 1962) is the standard: macronutrients (N, P, K, Ca, Mg, S), micronutrients (Fe, Mn, Zn, B, Cu, Mo), vitamins (thiamine, nicotinic acid), sucrose (carbon source, 2-3%), agar for solidification and **plant growth regulators**. The **auxin:cytokinin ratio** decides fate: high auxin → roots; high cytokinin → shoots; balanced → undifferentiated **callus**. Gibberellins aid shoot elongation; abscisic acid promotes embryo maturation and dormancy.

- Culture types: callus, cell suspension, meristem, embryo, anther, protoplast culture.
- Sterilization: sodium hypochlorite/ethanol for explants; autoclaving for media; laminar-flow hood for handling.
- pH ~5.7-5.8; temperature 25 C; 16-h photoperiod are typical.

■ VERY IMPORTANT

The auxin:cytokinin ratio rule (Skoog and Miller) is the single most examined fact in tissue culture: high auxin/cytokinin = roots, low ratio = shoots, intermediate = callus.

SECTION SUMMARY

Totipotent explants grow on MS medium; the auxin:cytokinin ratio directs root, shoot or callus formation. Sterile technique is non-negotiable.

5. Micropropagation: Clonal Multiplication

LEARNING OBJECTIVES

- Describe the stages of micropropagation
- Explain meristem culture for virus elimination
- List commercial applications

Micropropagation mass-produces genetically uniform, disease-free plants through five stages: (0) mother-plant selection; (1) **initiation** - establishing sterile shoot cultures; (2) **multiplication** - repeated subculture on high-cytokinin medium giving exponential shoot numbers; (3) **rooting** - transfer to auxin medium; (4) **acclimatization** - hardening plantlets from humid culture vessels to greenhouse/field (the main loss stage).

Meristem culture (0.2-0.5 mm shoot tip) eliminates viruses because the meristem lacks vascular connections through which viruses travel - combined with thermotherapy it freed potato, citrus, sugarcane and banana from devastating viruses. Commercial crops micropropagated: banana, orchids, date palm, sugarcane, potato (microtubers), strawberry. **Somaclonal variation** - mutations arising in culture - is a drawback for uniformity but a source of new variants for breeding.

SECTION SUMMARY

Micropropagation gives rapid, uniform, virus-free clones in five stages; meristem-tip culture plus thermotherapy eradicates viruses. Acclimatization is the critical bottleneck.

6. Somatic Embryogenesis and Organogenesis

LEARNING OBJECTIVES

- Distinguish somatic embryogenesis from organogenesis
- Explain synthetic seeds
- Describe haploid production via anther culture

In **somatic embryogenesis**, somatic cells form bipolar embryos (with shoot and root poles, no vascular connection to parent tissue) - via direct or callus-mediated routes. Because embryos are complete propagules, they enable **synthetic seeds**: somatic embryos encapsulated in alginate gel with nutrients - used experimentally in carrot, alfalfa and sandalwood. In **organogenesis**, shoots or roots form unipolarly from callus under cytokinin or auxin dominance.

Anther/microspore culture produces **haploid** plants; chromosome doubling (colchicine) gives instant **doubled haploids** - 100% homozygous lines in one generation instead of 6-8 years of selfing. This revolutionized breeding of rice, wheat, barley and tobacco. **Ovule culture** rescues embryos from wide crosses (e.g. in brassicas), and **embryo rescue** saves otherwise aborting hybrid embryos.

SECTION SUMMARY

Somatic embryos (bipolar) enable synthetic seeds; organogenesis makes shoots/roots. Anther culture delivers doubled haploids - instant pure lines for breeders.

7. Protoplast Culture and Somatic Hybridization

LEARNING OBJECTIVES

- Explain protoplast isolation and culture
- Describe somatic hybridization and cybrids
- State applications and limits

Protoplasts - plant cells stripped of walls by cellulase + pectinase - can be cultured to regenerate whole plants (first achieved in tobacco, 1971). They are ideal for transformation (PEG-mediated DNA uptake), fusion and single-cell studies, but regeneration remains genotype-dependent and difficult in cereals.

Somatic hybridization fuses protoplasts (PEG or electrofusion) of sexually incompatible species, combining whole genomes - e.g. 'pomato' (potato + tomato), or transferring disease resistance from wild relatives into crops. **Cybrids** (cytoplasmic hybrids) combine one parent's nucleus with another's cytoplasm - used to transfer CMS for hybrid breeding. Limits: chromosome instability, sterility and unpredictable segregation of fused genomes.

SECTION SUMMARY

Protoplasts enable DNA uptake and cell fusion. Somatic hybrids and cybrids bypass sexual incompatibility - powerful but genetically unruly tools.

8. Agrobacterium-Mediated Transformation

LEARNING OBJECTIVES

- Explain the Ti plasmid and T-DNA mechanism
- Describe binary vector systems
- Outline the transformation workflow

Agrobacterium tumefaciens naturally transfers T-DNA from its **Ti plasmid** into plant genomes, causing crown gall. Disarmed vectors delete the tumor genes (auxin/cytokinin biosynthesis) but keep the **border repeats** and **vir genes** that mobilize T-DNA. In **binary vectors**, T-DNA (with gene of interest + selectable marker like nptII for kanamycin resistance) sits on a small plasmid while vir genes stay on a helper plasmid - the standard system since the 1980s.

Workflow: clone gene into binary vector -> transform *Agrobacterium* -> co-cultivate with explants (leaf discs) -> select on antibiotic/herbicide -> regenerate transgenic shoots -> confirm by PCR/Southern blot -> self to homozygosity. Advantages: low copy number, defined insertions, high efficiency in dicots. Cereals were recalcitrant until super-virulent strains (e.g. AGL1) and optimized protocols enabled rice, maize and wheat transformation in the 1990s.

T-DNA

The transferred DNA segment of the Ti plasmid, delimited by 25-bp border repeats, that integrates into the plant nuclear genome.

SECTION SUMMARY

Agrobacterium's T-DNA system, tamed via disarmed binary vectors, is the dominant transformation method: precise, low-copy transgene integration into the nuclear genome.

9. Direct Gene Transfer Methods

LEARNING OBJECTIVES

- Describe biolistics, electroporation and PEG methods
- Compare direct vs *Agrobacterium* methods
- Explain chloroplast transformation

The **gene gun (biolistics)** fires DNA-coated gold/tungsten microparticles into cells - genotype-independent and the key to transforming cereals, chloroplasts and organelles before *Agrobacterium* protocols matured. **Electroporation** (electric pulses) and **PEG-mediated** uptake work on protoplasts; **microinjection** and silicon-carbide whiskers are niche methods. Direct methods often give multi-copy, rearranged insertions versus *Agrobacterium*'s cleaner single-copy events.

Chloroplast (plastid) transformation by biolistics uses homologous recombination - giving precise insertion, very high expression (up to 70% of soluble protein), and **maternal inheritance** (pollen containment). It produced experimental edible vaccines (cholera, anthrax antigens in tobacco) and insecticidal proteins. Limitation: routine only in tobacco and a few species; monocot plastid transformation remains hard.

SECTION SUMMARY

Biolistics and other direct methods complement *Agrobacterium*, especially for cereals and plastids. Plastid engineering offers high expression plus natural biocontainment.

10. Molecular Markers in Plant Breeding

LEARNING OBJECTIVES

- Classify molecular markers
- Explain marker-assisted selection (MAS)
- Describe QTL mapping basics

Molecular markers are DNA landmarks: **RFLP** (hybridization-based, first generation), **RAPD** (random PCR, dominant), **AFLP**, **SSR/microsatellites** (co-dominant, highly polymorphic - the breeder's favorite), and **SNPs** (single-nucleotide polymorphisms - the modern high-throughput standard enabling genomic selection). Markers must be polymorphic, reproducible and linked to the trait.

Marker-assisted selection (MAS): select plants carrying a marker linked to a desired gene (e.g. bacterial blight resistance Xa21 in rice, submergence tolerance Sub1) without waiting for phenotype - accelerating breeding 2-3x and enabling selection for recessive or late-expressed traits. **QTL mapping** locates chromosome regions controlling quantitative traits (yield, drought tolerance) using segregating populations and linkage maps; **genomic selection** predicts breeding value from genome-wide SNPs, now routine in maize and wheat programs.

■ VERY IMPORTANT

SSR = co-dominant, multiallelic, PCR-based (great for diversity); SNP = biallelic but ultra-high-throughput (great for genomic selection). MAS selects the MARKER, not the trait - tight linkage (<5 cM) is essential.

SECTION SUMMARY

From RFLP to SNPs, markers let breeders select genes directly. MAS and genomic selection have cut breeding cycles dramatically - e.g. Sub1 flood-tolerant rice.

11. Insect-Resistant Crops: The Bt Story

LEARNING OBJECTIVES

- Explain the Bt mode of action
- Describe Bt cotton and Bt brinjal
- Discuss resistance management (refuge strategy)

Bacillus thuringiensis (Bt) produces **Cry proteins** (delta-endotoxins): ingested by susceptible larvae, the protoxin is activated in the alkaline midgut, binds cadherin receptors, forms pores, and the insect stops feeding and dies. Cry proteins are specific to insect orders (Cry1Ac -> lepidopterans like bollworm) and harmless to mammals - the basis of Bt's safety record and its prior use as an organic spray.

Bt cotton (cry1Ac, and stacked cry1Ac+cry2Ab 'Bollgard II') controls bollworms and cut Pakistan's pesticide sprays dramatically after its spread in the 2000s. **Bt brinjal** (fruit-and-shoot borer resistant) was approved in Bangladesh (2013) - the first Bt food crop in South Asia. **Refuge strategy**: planting 5-20% non-Bt cotton maintains susceptible insects, delaying resistance evolution - mandatory in regulated systems; where refuges were ignored (e.g. pink bollworm in parts of India/Pakistan), resistance emerged.

SECTION SUMMARY

Bt Cry proteins kill target pests specifically and safely. Bt cotton transformed South Asian agriculture; refuges are essential to preserve the technology against resistance.

12. Herbicide Tolerance and Abiotic-Stress Crops

LEARNING OBJECTIVES

- Explain glyphosate-tolerance mechanisms
- Describe drought/salinity tolerance engineering
- Discuss Golden Rice and biofortification

Herbicide-tolerant crops carry e.g. the **CP4 EPSPS** gene (glyphosate-insensitive enzyme from *Agrobacterium*) or **bar** gene (phosphinothricin detoxification) - 'Roundup Ready' soybean/maize/cotton. They simplify weed control but drove glyphosate overuse and resistant weeds - a lesson in stewardship. Pakistan has not commercialized HT crops.

Abiotic-stress engineering: DREB/CBF transcription factors confer drought/cold tolerance; NHX antiporters improve salinity tolerance; Sub1A (from MAS, not transgenic) gives flood tolerance. **Golden Rice** expresses phytoene synthase (daffodil/maize) + carotene desaturase in endosperm, producing pro-vitamin A - developed to combat vitamin-A deficiency blinding 250,000+ children yearly; approved in the Philippines (2021). **Biofortification** also includes iron-rich and high-lysine crops.

SECTION SUMMARY

HT crops simplify weed management but need stewardship; stress-tolerance genes (DREB, NHX) and biofortification (Golden Rice) target climate resilience and hidden hunger.

13. Biopharming, Biofertilizers and Biopesticides

LEARNING OBJECTIVES

- Define molecular farming
- Explain biofertilizers and biopesticides
- Assess their role in sustainable agriculture

Molecular farming (biopharming) uses plants as factories for pharmaceuticals - antibodies, vaccines, industrial enzymes - in tobacco, rice or maize; plant-made pharmaceuticals avoid animal-pathogen contamination and scale cheaply. Containment (chloroplast transformation, non-food crops) addresses gene-flow concerns.

Biofertilizers: Rhizobium/Bradyrhizobium inoculants fix nitrogen in legumes; Azospirillum and Azotobacter benefit cereals; **mycorrhizal fungi** extend phosphorus uptake; phosphate-solubilizing bacteria unlock bound P. **Biopesticides:** Bt sprays, Trichoderma (fungal antagonist), neem extracts, and nucleopolyhedroviruses (NPV) for caterpillar pests. Integrated with IPM, they cut chemical loads - Pakistan's cotton IPM programs combine Bt cotton with Trichogramma releases.

SECTION SUMMARY

Plants can manufacture drugs (biopharming); microbes can fertilize (Rhizobium, mycorrhizae) and protect (Bt, Trichoderma) crops - the biological toolkit for sustainable intensification.

14. Biosafety, Regulation and Ethics

LEARNING OBJECTIVES

- Outline biosafety concerns and the Cartagena Protocol
- Describe Pakistan's biosafety framework
- Discuss ethical and socioeconomic debates

Concerns: **gene flow** to wild relatives (canola, rice), effects on non-target organisms (the debated monarch-butterfly/Bt-pollen study), resistance evolution, and food safety (allergenicity/toxicity testing per Codex). The **Cartagena Protocol on Biosafety** (2000) governs transboundary movement of LMOs with advance informed agreement. Risk assessment is case-by-case: trait x crop x environment.

Pakistan's framework: the **Pakistan Biosafety Rules (2005)** under EPA, with the National Biosafety Committee and Institutional Biosafety Committees; Bt cotton varieties passed through field trials to commercialization. Debates continue: labeling, coexistence of GM/non-GM, corporate control of seed (terminator/GURT technology was never commercialized after backlash), and whether public-sector biotech serves smallholders. The scientific consensus (WHO, NAS, EU reviews) finds approved GM foods as safe as conventional counterparts.

SECTION SUMMARY

Biosafety = case-by-case risk assessment under Cartagena and Pakistan's 2005 Rules. The technology's safety record is strong; the debates are about governance, equity and stewardship.

15. Exam Pointers

High-yield topics

- Definitions: totipotency, explant, callus, transgenic, MAS, QTL, cybrid, somaclonal variation.
- The auxin:cytokinin ratio rule; MS medium components; five stages of micropropagation.
- Agrobacterium: Ti plasmid, T-DNA borders, vir genes, binary vectors - vs. biolistics.
- Markers: RFLP/RAPD/SSR/SNP properties; MAS workflow; Sub1 and Xa21 examples.
- Bt mode of action (Cry protoxin -> alkaline midgut -> pores); refuge strategy; CP4 EPSPS mechanism.
- Salam of biotech: Golden Rice genes (psy + crtI); DREB for drought; chloroplast transformation advantages.
- Pakistan context: Bt cotton impact, Biosafety Rules 2005, Cartagena Protocol.

SECTION SUMMARY

Master the tissue-culture rules, the two transformation systems, marker types, the Bt story and the biosafety framework - with one Pakistan example for each.