Al-Farabi Kazakh National University (KazNU)

Faculty of Biology and Biotechnology

Department of Molecular Biology and Genetics

Discipline: «Modern Problems of Plant Genetics»

Lecture 6

Risk and Safety Assessment of GM and Gene-Edited Plants

Comparative safety paradigms, Environmental Risk Assessment (ERA), Bt/RNAi pest resistance, meta-analytic outcomes, and global biosafety frameworks

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Aim and Structure of the Lecture

Aim: master scientific principles of environmental and food safety assessment for transgenic and genome-edited crops

KEY FOCUS

Lecture Plan & Modules

Key sections:
1. Regulatory Paradigms
2. Environmental Risk Assessment
3. Food & Feed Safety Assessment
4. Bt Proteins & RNAi Biopesticides
5. Empirical Global Impacts
6. Post-Market & Biosafety Law

- **Regulatory Paradigms:** Process-based (EU) vs product-based (USA, Canada, Argentina, Japan) frameworks and SDN-1/2/3 categories
- **Environmental Risk Assessment:** Six-step ERA workflow: hazard & exposure characterization, gene flow, non-target organisms, and resistance
- **Food & Feed Safety Assessment:** OECD/Codex comparative approach, substantial equivalence, allergenicity weight-of-evidence, and toxicology
- **Bt Proteins & RNAi Biopesticides:** Molecular mode of action of Cry/Vip toxins, HIGS/SIGS silencing, and high-dose/refuge resistance management
- **Empirical Global Impacts:** Long-term meta-analyses of crop yield, pesticide reduction, farmer income, and greenhouse gas savings
- **Post-Market & Biosafety Law:** PMEM monitoring, Cartagena Protocol, Codex standards, and Republic of Kazakhstan biosafety legislation

Two Contrasting Regulatory Paradigms for Biotech Crops

Does regulatory oversight trigger on the breeding technique used or the novel traits of the final plant?

Process-Triggered Model (e.g., EU, New Zealand)

- Triggered strictly by recombinant DNA or directed mutagenesis techniques under Directive 2001/18/EC
- Follows the Precautionary Principle via centralized EFSA safety evaluation plus member-state voting
- Requires mandatory GMO labeling above 0.9% adventitious presence and event-specific traceability
- Historically treats SDN-1/SDN-2 edits similarly to transgenics, though EU NGT-1 reforms are under debate

Product-Triggered Model (USA, Canada, Argentina, Japan)

- Triggered by the characteristics of the final plant product (e.g., Canada's «Plants with Novel Traits»)
- Same oversight applies whether a novel trait arises from mutagenesis, wide crosses, or genetic engineering
- Excludes targeted knockout mutations (SDN-1) lacking foreign DNA from full GMO regulation (USA, Japan, UK, India)
- Accelerates public-sector and university breeding by avoiding multi-million-dollar transgenic deregulation costs

Classification of Genetic Modifications and Regulatory Triggers

Site-directed nuclease (SDN) precision levels determine foreign-DNA status and global exemption eligibility

KEY FOCUS

Key Classification

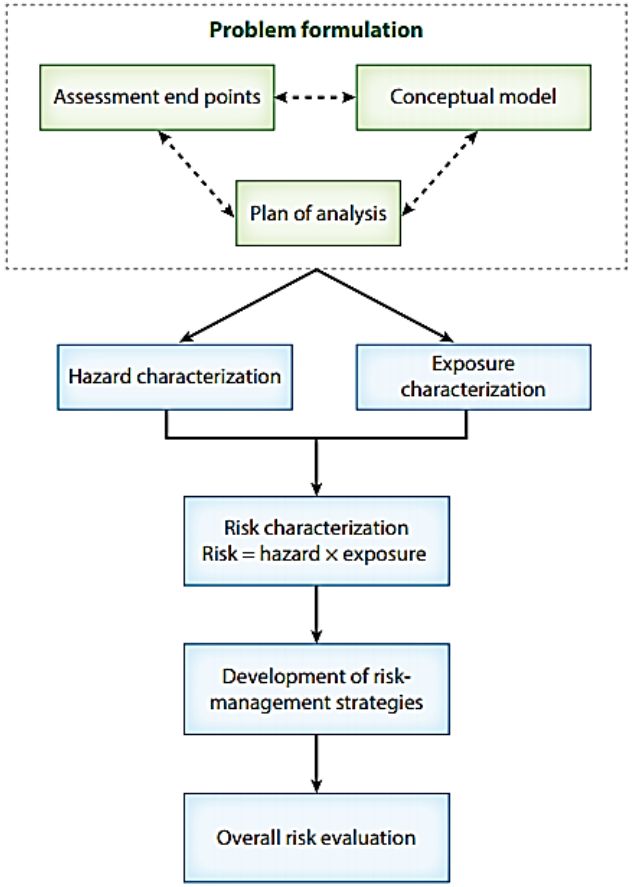
Focus on the biological mechanisms and practical significance of this topic.

- **SDN-1:** Error-prone NHEJ indels; targeted gene knockout without a repair template — None in final plant — Exempt from GMO rules in USA, Japan, Argentina, Brazil, India, UK; EU NGT-1 proposed
- **SDN-2:** HDR/base/prime editing of 1 to a few bp using a short homologous template — None in final plant — Exempt in USA, Argentina, Japan (no template left); case-by-case review elsewhere
- **SDN-3:** Targeted insertion of a cisgene, intragene, or transgene at a genomic locus — Present (cis/trans) — Regulated as GMO if foreign DNA inserted; eased if purely cisgenic/intragenic
- **Cisgenesis:** Transfer of complete native gene + regulatory regions from crossable relative — Crossable pool only — Reduced data package in Canada/EFSA NGT proposals; still labeled GMO in current EU law
- **Transgenesis:** Integration of gene cassette across species barriers (e.g., Bt Cry genes) — Present (heterologous) — Full ERA and food/feed safety dossier required globally prior to commercial release

Six-Step Environmental Risk Assessment (ERA) Framework

EFSA and OECD harmonized methodology balancing hazard potential against environmental exposure

- **Steps 1–3:** Problem to Exposure: Step 1: Problem formulation identifies plausible harm pathways vs non-GM comparator
- **Step 2:** Hazard characterization measures severity of potential adverse biological effects
- **Step 3:** Exposure characterization estimates spatial/temporal contact in receiving habitats
- **Steps 4–6:** Risk to Monitoring: Step 4: Risk characterization integrates Risk = Hazard × Exposure with uncertainty bounds
- **Step 5:** Risk management specifies isolation distances, insect refuges, or volunteer control
- **Step 6:** Overall risk evaluation guides release approval and post-market monitoring (PMEM)



EFSA Problem Formulation & Six-Step ERA Logic: Risk = Hazard × Exposure

Core Ecological Areas Evaluated in Plant ERA

Systematic testing prevents unintended impacts on wild relatives, agroecosystems, and beneficial fauna

Part I: Core Mechanisms

Gene Flow & Wild Relatives

- Assesses pollen-mediated outcrossing distance and compatibility with sexually compatible wild flora
- Evaluates fitness advantage or weediness of F1 hybrids under non-agricultural selection pressures

Persistence & Invasiveness

- Tests seed dormancy, shattering, overwintering, and volunteer survival outside cultivated plots
- Compares germination and competitiveness against conventional near-isogenic lines across multi-site trials

Part II: Applications & Balance

Non-Target Organisms (NTOs)

- Tiered laboratory and field bioassays on pollinators (Apis mellifera), predators, parasitoids, and soil decomposers
- Confirms narrow taxonomic specificity of insecticidal proteins and intact rhizosphere microbial functions

Resistance Evolution

- Models selection pressure for target-pest resistance (Bt toxins) or glyphosate/glufosinate-tolerant weeds
- Mandates Integrated Pest Management (IPM), stacked modes of action, and structured spatial refuges

OECD & Codex Comparative Food Safety Assessment

«Substantial Equivalence» is the starting point—not the endpoint—identifying differences requiring targeted safety testing

1 Molecular Profile

- Insert copy number, flanking borders, ORF check
- Multi-generation inheritance stability

2 Agronomic Trials

- Multi-location field growth vs isogenic control
- Yield, phenology & stress responses

3 Compositional Match

- Macro/micronutrients, anti-nutrients, toxins
- Compared within natural ILSI ranges

Step 4 Allergenicity Screen

- Bioinformatics (>35% / 80 aa) & pepsin digestion
- Human IgE serum testing if warranted

Step 5 Toxicology & Nutrition

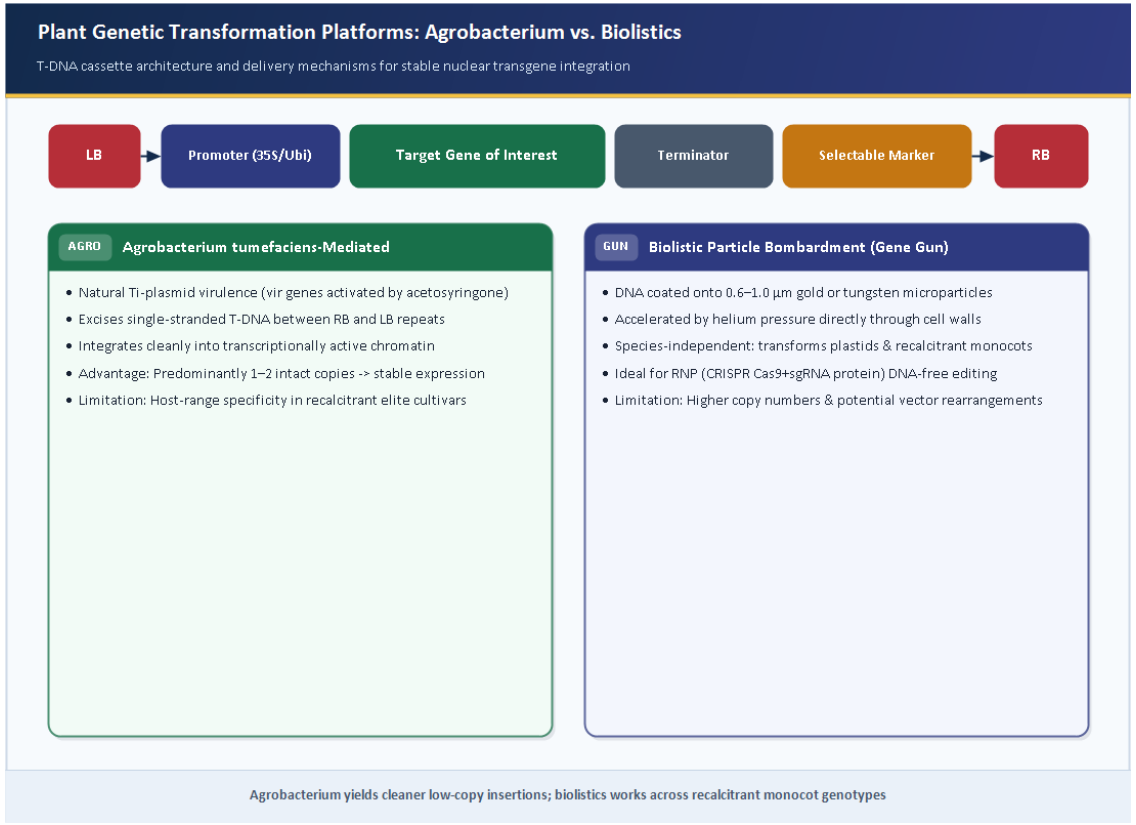
- Acute oral & 28/90-day rodent feeding trials
- Whole-food nutritional equivalence in livestock

Substantial equivalence assumes GM foods are compared against conventional counterparts with a safe history of food use.

Bt Insecticidal Proteins & Resistance Management

Why Bacillus thuringiensis Cry and Vip3A proteins spare mammals and beneficial insects

- **Selective Molecular Mechanism of Cry & Vip3A:** Protoxins solubilize only in alkaline lepidopteran/coleopteran midgut (pH 8–10), unlike acidic mammalian stomachs
- Midgut serine proteases cleave protoxin into active 60–65 kDa core fragment
- High-affinity binding to specific insect cadherin, aminopeptidase N, and ABCC2 receptors forms lytic pores
- **Result:** osmotic cell lysis, midgut paralysis, and rapid larval death; zero receptor binding in vertebrates
- **Delaying Pest Resistance in Field Deployment:** High-Dose Strategy: in planta toxin expression kills >99% of heterozygous resistant (RS) larvae
- **Structured Refuge:** 5–20% non-Bt crop strips (or «refuge-in-a-bag» seed blends) sustain susceptible (SS) mates



Bt insecticidal transgene design and resistance management vectors

Next-Generation RNAi Crop Protection: HIGS & SIGS

Sequence-specific double-stranded RNA (dsRNA) silences essential pest genes with minimal ecological footprint

Host-Induced Gene Silencing (HIGS)

- Plant transcribes hairpin dsRNA matching an essential insect mRNA (e.g., dvSnf7 in SmartStax PRO maize)
- Insect ingests dsRNA -> cellular Dicer-2 processes into 21-nt siRNAs -> RISC cleaves target pest mRNA
- Bioinformatics screens remove ≥21-nt homology to honeybees, ladybugs, soil fauna, and human transcripts
- Provides non-protein backup mode of action against Bt-resistant western corn rootworm

Spray-Induced Gene Silencing (SIGS)

- Exogenous bio-manufactured dsRNA applied as foliar spray—no genetic modification of the crop genome
- **Example:** Ledprona (EPA-registered 2023) targets PSMB5 proteasome subunit in Colorado potato beetle
- Rapid environmental biodegradation in soil microbes and UV within 2–7 days minimizes residue persistence
- Nanocarrier / clay-nanosheet formulations (BioClay) stabilize foliar RNA stability without chemical toxicants

Empirical Meta-Analysis of GM Crop Performance

Peer-reviewed synthesis of global commercial experience (Klümper & Qaim meta-analysis; Brookes environmental accounting)

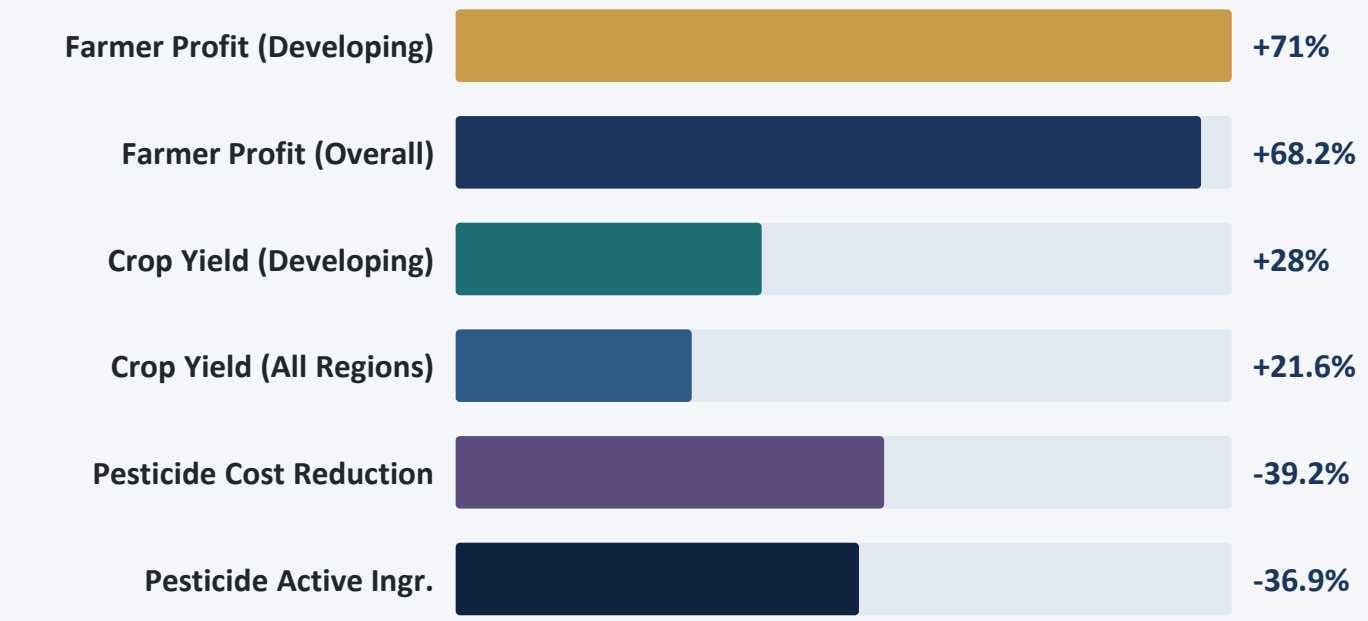
+22%
Average Crop Yield Gain

-37%
Chemical Pesticide Use

+68%
Farmer Profit Increase

>30 yrs
Safe Commercial Record

Global Meta-Analysis Outcomes (%, Klümper & Qaim)



Key Environmental Dividends

- Insect-resistant (Bt) crops cut insecticide sprays up to 50% more sharply than HT traits alone
- Cumulative active-ingredient reduction exceeded ~748M kg globally (1996–2020, Brookes 2022)
- No-till adoption lowered fuel burn & soil carbon loss (~23.6M t CO_2 eq saved annually)

Sources: Klümper & Qaim (PLoS ONE, 147 studies); Brookes (2022) GM Crops & Food; National Academies of Sciences (2016).

Codex Weight-of-Evidence Allergenicity & Toxicity Testing

No single test predicts allergenicity; regulators integrate five complementary molecular and biochemical criteria

Assessment Pillar	Experimental Method	Safety Threshold / Criterion	Action if Triggered
Source History	Review donor organism allergenicity (e.g., peanut, soy, Brazil nut)	Known IgE-mediated allergy triggers heightened scrutiny	Conduct targeted human serum IgE binding assays
Sequence Homology	FASTA/BLAST against AllergenOnline & COMPARE databases	<35% identity over any 80-aa window; <8 exact matches	Reject protein or verify absence of cross-reactive IgE
Pepsin Stability	Simulated Gastric Fluid (SGF, pH 1.2–2.0, 0.5–60 min SDS-PAGE)	Rapid degradation within 0.5–2 min correlates with low risk	Resistant proteins require intact stability + abundance check
Heat & Processing	Thermal denaturation curves & simulated intestinal fluid (SIF)	Loss of conformational epitopes upon cooking/processing	Evaluates dietary exposure form (raw vs processed)
In Vivo Toxicology	Acute oral gavage (mouse) & 28/90-day subchronic rodent studies	NOAEL margin >100–1000× estimated human intake	Whole-food feeding studies confirm absence of unexpected effects

Post-Market Environmental Monitoring (PMEM) and Traceability

Continuous surveillance ensures early detection of unforeseen ecological trends after commercial approval

KEY FOCUS

Core Analytical Focus

Key sections:
1. Case-Specific Monitoring
2. General Surveillance
3. Molecular Traceability
4. Threshold Labeling Rules

- **Case-Specific Monitoring:** Tests explicit ERA hypotheses such as Bt resistance allele frequencies in target pest populations; Managed via mandatory annual resistance monitoring reports by authorization holders
- **General Surveillance:** Screens broad agroecosystems via farmer questionnaires, national weed surveys, and plant-health networks; Detects unanticipated long-term or cumulative regional changes across rotations
- **Molecular Traceability:** Junction-specific real-time qPCR & digital PCR (dPCR) target unique plant–insert boundary sequences; Unique Identifiers (UIDs, OECD format e.g. MON-00810-6) standardize cross-border laboratory tracking
- **Threshold Labeling Rules:** Low-Level Presence (LLP) thresholds balance consumer choice against unavoidable supply-chain mixing; Benchmark tolerances: 0.9% (EU & EAEU), 1% (Brazil/Australia), 3% (South Korea), 5% (Japan)

International Treaties and Kazakhstan Biosafety Governance

Multilateral binding instruments coordinate cross-border movement, food safety benchmarks, and liability rules

Instrument / Body	Adoption & Status	Core Mandate	Relevance to Kazakhstan & Trade
Cartagena Protocol on Biosafety	2000 (in force 2003), 173 Parties	Safe transboundary movement of Living Modified Organisms (LMOs) under CBD	Establishes Advanced Informed Agreement (AIA) & Biosafety Clearing-House
Nagoya–Kuala Lumpur Protocol	2010 (in force 2018)	International rules on liability & redress for environmental LMO damage	Defines operator response duties for conservation & sustainable use
Codex Alimentarius Commission	FAO/WHO Guidelines (2003/2008)	Global benchmark for safety & nutritional assessment of rDNA foods	Reference standard under WTO Sanitary & Phytosanitary (SPS) Agreement
EAEU Technical Regulations	TR TS 021/2011 & 022/2011	Mandatory safety assessment, state registration, and >0.9% GMO labeling	Applies directly to food/feed circulation across Kazakhstan & EAEU market
RK National Biosafety Law	RK Law on Genetic Engineering & Seeds	Confined R&D standards, ecological expertise, and variety testing	Protects national agrobiodiversity while enabling accredited laboratory R&D

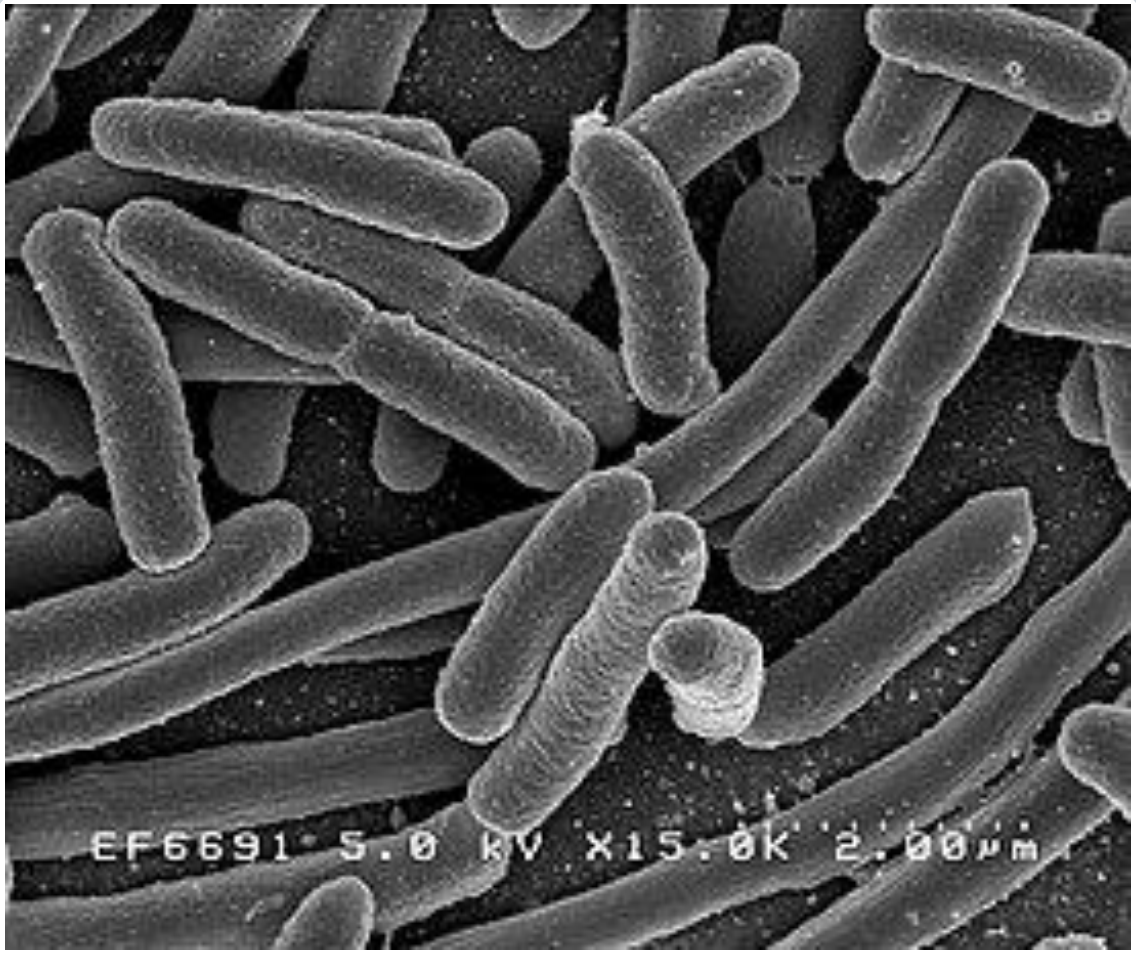
★ Key Takeaway

- Multilateral binding instruments coordinate cross-border movement, food safety benchmarks, and liability rules
- **Comparison scope (5):** Cartagena Protocol on Biosafety; Nagoya–Kuala Lumpur Protocol; Codex Alimentarius Commission; EAEU Technical Regulations; RK National Biosafety Law
- **Priority synthesis:** RK National Biosafety Law — Protects national agrobiodiversity while enabling accredited laboratory R&D

Biological and Physical Containment Strategies

Engineering safeguards and facility protocols prevent unintended escape from laboratory to ecosystem

- **Biological & Genetic Containment:** Plastid (chloroplast) transformation: maternal inheritance prevents transgene spread via pollen in most angiosperms
- Inducible or tissue-specific promoters avoid constitutive metabolic burden and expression in edible tissues
- Male sterility or cleistogamy (closed self-pollination) minimizes pollen outcrossing
- CRISPR clean-segregation eliminates Cas9/sgRNA cassettes in T1 generation before field trials
- **Physical Containment & Field Protocols:** Biosafety Levels for Plants (BSL-1P to BSL-4P): insect-proof mesh, filtered air, HEPA/spore traps, autoclave waste
- **Confined Field Trials (CFTs):** spatial isolation zones, temporal flowering offset, and border trap rows



Biological containment strategies and microbial genetics context

Control Questions and Key Takeaways

Synthesizing evidence-based risk evaluation for modern plant breeding

? Control & Review Questions

- 1. How do process-based (EU) and product-based (USA/Canada/Japan) biotech regulations differ in treating SDN-1 crops?
- 2. Explain the six sequential steps of Environmental Risk Assessment (ERA) using Risk = Hazard × Exposure.
- 3. What is «substantial equivalence» in OECD/Codex food safety testing, and what additional assays follow it?
- 4. How do high-dose/refuge planting and gene pyramiding delay insect resistance to Bt Cry and Vip3A proteins?
- 5. Compare the ecological safety profiles of HIGS transgenic RNAi and foliar SIGS biopesticides.

✓ Key Takeaways

- Regulators worldwide distinguish transgenesis from transgene-free SDN-1/SDN-2 edits lacking foreign DNA.
- Comparative ERA integrates molecular stability, non-target impacts, gene flow, and resistance management.
- Global meta-analyses verify +22% yields, -37% chemical pesticide use, and +68% farmer profit with GM crops.
- International treaties (Cartagena, Codex) and Kazakhstan/EAEU standards balance biosafety with innovation.

Recommended Literature and Authoritative Resources

Primary regulatory guidance documents and peer-reviewed safety syntheses

1 Main Literature

- 1. EFSA GMO Panel. Guidance for risk assessment of food and feed from genetically modified plants & ERA of GM plants. EFSA Journal.
- 2. OECD (2023). Consensus Documents on the Biology of Crops and Safety Assessment of Transgenic & Genome-Edited Organisms.
- 3. Codex Alimentarius Commission (FAO/WHO). Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003).
- 4. National Academies of Sciences, Engineering, and Medicine (2016). Genetically Engineered Crops: Experiences and Prospects.

2 Additional Literature & Databases

- 1. Klümper, W., Qaim, M. (2014). A Meta-Analysis of the Impacts of Genetically Modified Crops. PLoS ONE 9(11): e111629.
- 2. Brookes, G. (2022). Genetically Modified (GM) Crop Use 1996–2020: Environmental Impacts Associated with Pesticide Use Change. GM Crops & Food.
- 3. FAO GM Foods Platform & CBD Biosafety Clearing-House (bch.cbd.int) — Kazakhstan National Regulatory Profiles.
- **4. Taning, C.N.T. et al. (2020). RNAi-based pest control:** HIGS and SIGS risk assessment perspectives. Trends in Biotechnology.

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