Al-Farabi Kazakh National University (KazNU)

Faculty of Biology and Biotechnology

Department of Molecular Biology and Genetics

Discipline: «Modern Problems of Plant Genetics»

Lecture 5

Genetic Resources, Chromosome Engineering, and Crop Improvement

Vavilov centers & Kazakhstan biodiversity, in situ/ex situ conservation, Sears' wheat aneuploids, Ph1 manipulation, alien introgressions & GISH/FISH

Author: Amirova Aigul Kuzembayevna, Candidate of Biological Sciences, Associate Professor
E-mail: aigul_amir@mail.ru

Aim and Structure of the Lecture

Aim: connect conservation of Plant Genetic Resources (PGRFA) with classical and molecular chromosome engineering in cereals

KEY FOCUS

Lecture Plan & Modules

Key sections:
1. Plant Genetic Resources (PGRFA)
2. Vavilov & Kazakhstan Hotspots
3. In Situ vs Ex Situ Conservation
4. Aneuploidy & Sears' Series
5. Chromosome Engineering
6. GISH, FISH & Pre-Breeding

- **Plant Genetic Resources (PGRFA):** Primary, secondary, and tertiary gene pools; combating genetic erosion and uniformity
- **Vavilov & Kazakhstan Hotspots:** Centers of origin and Kazakhstan's global role for Malus sieversii, tulips, alliums, and wild Triticeae
- **In Situ vs Ex Situ Conservation:** Biosphere reserves, seed genebanks, slow-growth tissue culture, LN2 cryopreservation & Svalbard Vault
- **Aneuploidy & Sears' Series:** Monosomics, nullisomics, trisomics, tetrasomics, and ditelosomics in wheat cv. Chinese Spring
- **Chromosome Engineering:** Manipulating the Ph1 locus to transfer stem rust & yield traits via addition, substitution & translocation lines
- **GISH, FISH & Pre-Breeding:** Fluorescent painting of alien chromatin (1BL.1RS) and synthetic hexaploid wheat introgression

Structure of Crop Gene Pools (Harlan & de Wet) and Genetic Erosion

Classifying wild and cultivated germplasm by crossability and ease of gene transfer into elite varieties

Part I: Core Mechanisms

Free Sexual Recombination

- Consists of elite commercial cultivars, historic landraces, and immediate wild progenitors of the same biological species
- Hybrids are fully fertile with regular chromosomal pairing (e.g., cultivated vs wild rice Oryza rufipogon, Triticum dicoccoides)

Fertility Barriers & Alien Chromosomes

- Related species that cross with the crop with difficulty, yielding partially sterile hybrids requiring embryo rescue
- **Example:** rye (Secale cereale), Aegilops tauschii, or wild wheat relatives sharing one homologous genome

Part II: Applications & Balance

Extreme Barriers Requiring Engineering

- Distant genera requiring embryo rescue, chromosome doubling, bridging species, Ph1 mutation, or protoplast fusion
- **Example:** Thinopyrum ponticum, Dasypyrum villosum, Elymus — rich reservoirs of Fusarium and rust immunity

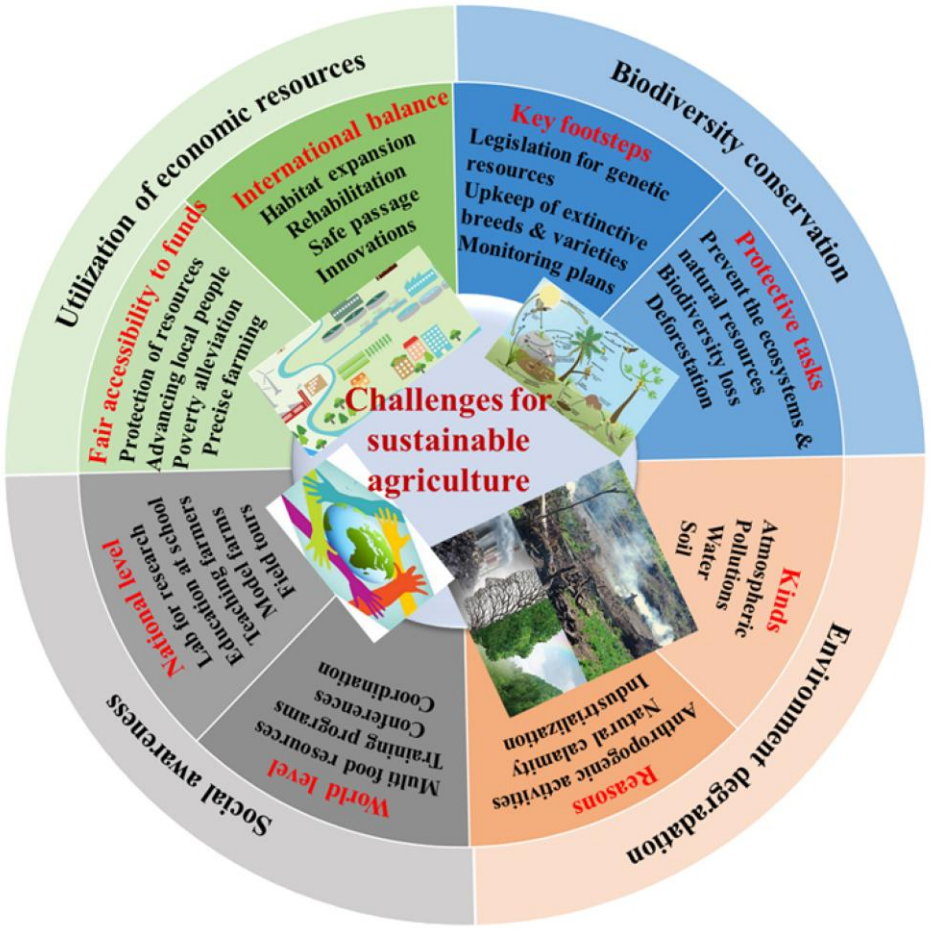
Loss of Diversity Through Monoculture

- Since 1900, FAO estimates ~75% of historic crop landrace diversity has disappeared as farmers adopted uniform elite lines
- Uniformity invites catastrophic epidemics (e.g., 1970 US Southern Corn Leaf Blight on T-cytoplasm)

Vavilov's Centers of Diversity and Kazakhstan as a Global Germplasm Hotspot

Wild progenitors in Central Asia harbor unique adaptive alleles for drought, frost, and fungal pathogens

- **N.I. Vavilov's Gene Centers & Law of Homologous Series:** Nikolai Vavilov identified 8 primary centers of crop origin where maximal allelic and phenotypic diversity concentrates
- **Key centers:** Southwest Asian / Fertile Crescent (wheat, barley, lentil), East Asian (soybean, millet), Mesoamerican (maize, beans), Andean (potato, tomato)
- Law of Homologous Series in Hereditary Variation predicts parallel trait mutations across closely related genera (wheat, barley, rye)
- **Kazakhstan's Unique Wild Flora (Central Asian Center):** Jungar and Ile Alatau mountains preserve intact ancestral forests of Malus sieversii — the primary genetic progenitor of all domesticated apples worldwide!
- Center of diversity for wild Tulipa species (T. greigii, T. kaufmanniana), wild Allium, apricot (Prunus armeniaca), and medicinal flora
- Kazakhstan's steppes contain drought- and cold-adapted populations of wild Triticeae grasses (Aegilops, Agropyron, Elymus, wild Hordeum)



Vavilov's Centers of Crop Diversity and Kazakhstan germplasm

In Situ and Ex Situ Conservation of Plant Genetic Resources

Complementary strategies preserving both living evolving ecosystems and long-term static seed/tissue collections

In Situ & On-Farm Conservation

- Protects wild crop relatives inside native ecosystems (biosphere reserves, national parks such as Ile-Alatau and Aksu-Zhabagly)
- On-farm conservation preserves traditional farmer landraces in agroecosystems under active selection
- **Key strength:** preserves ongoing natural co-evolution between host plants, pests, pollinators, and climate
- **Risk:** vulnerable to urban expansion, overgrazing, wildfires, and invasive species

Ex Situ Conservation (Genebanks & Cryopreservation)

- **Orthodox seeds (wheat, maize, rice):** dried to 3–7% moisture and stored at −18 to −20 °C for decades to centuries
- **Clonal / recalcitrant crops (apple, potato, banana):** maintained in field orchards, in vitro slow-growth culture, or liquid nitrogen (−196 °C cryo-banking of shoot tips)
- **Global safety backup:** Svalbard Global Seed Vault (Norway) stores >1.3 million duplicate accessions in Arctic permafrost
- **Legal governance:** International Treaty (ITPGRFA) and Nagoya Protocol ensure fair access and benefit sharing

Global Landscape of Ex Situ Crop Germplasm Conservation (~7.4 Million Accessions)

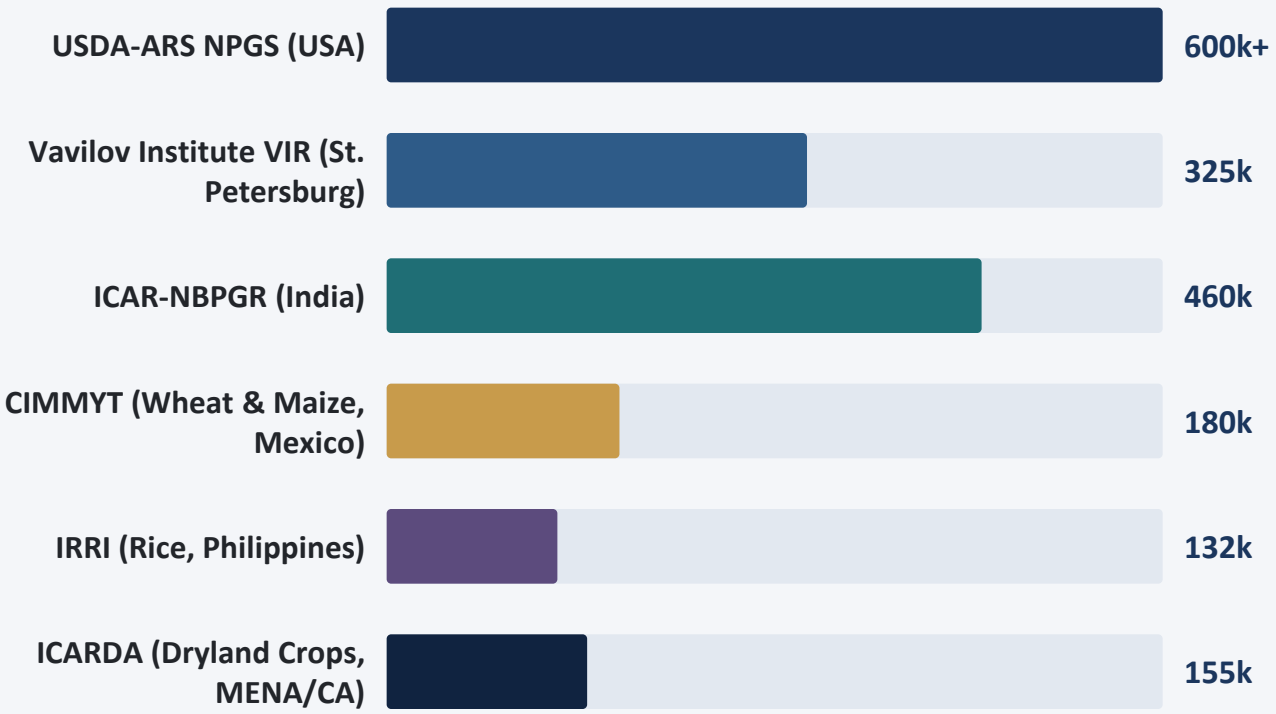
Major international & national genebanks safeguarding crop genetic diversity for future climate breeding

7.4M+
Accessions in ~1,750 genebanks

1.3M+
Samples in Svalbard Vault

4–5%
Accessions of wheat/barley wild rel.

Representative Accession Volumes Across Leading Crop Genebanks (thousands)



The «Dark Diversity» Opportunity

- Less than 10% of global genebank accessions have been deeply phenotyped or sequenced (FIGS / SpeedGS initiatives)
- Core collections (10% of bank holding ~70–80% of alleles) focus genomics and pre-breeding on the richest genetic variance

Cytogenetic Foundations: Euploidy and Aneuploidy Classifications

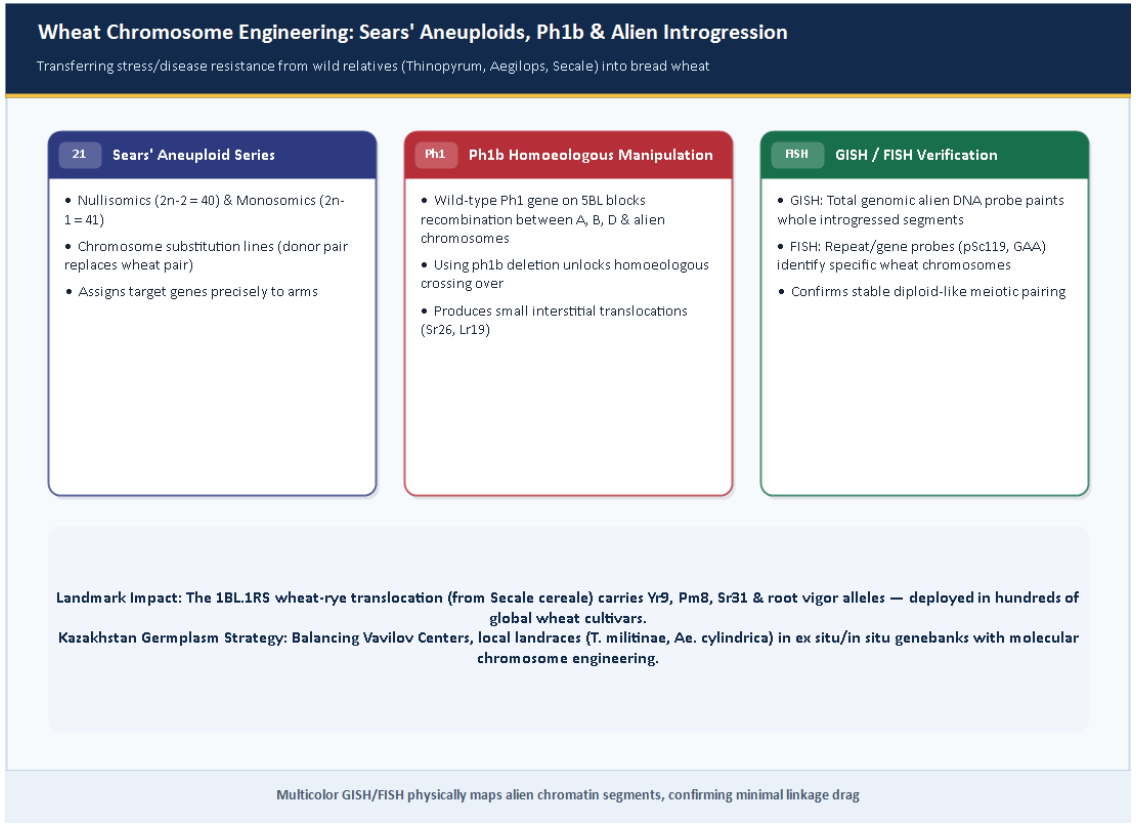
Polyploid cereals (like 6x wheat) tolerate missing or extra individual chromosomes thanks to homeologous buffering (A, B, D)

Cytogenetic Class	Chromosome Formula (Wheat 2n=42)	Meiotic / Phenotypic Behavior	Analytical & Breeding Utility
Monosomic (2n – 1)	41 chr. (20'' + 1')	Single unpaired chromosome becomes a univalent; transmits 20 or 21 gametes	Assigning recessive or dominant genes to specific chromosomes (monosomic F2 shift from 3:1)
Nullisomic (2n – 2)	40 chr. (20'')	Entire pair of homologous chromosomes deleted; reduced vigor/fertility	Directly proves essential traits encoded on the absent chromosome
Trisomic (2n + 1) / Tetrasomic (2n + 2)	43 or 44 chr.	Extra chromosome copies increase gene dosage; compensated tetrasomy possible	Nullisomic-tetrasomic compensation proves A/B/D homeologous homology (Sears)
Ditelosomic (2n – 2 arm)	40 + t'' (two telocentric arms)	Entire Short (S) or Long (L) arm of a specific chromosome pair missing	Maps genes accurately to a specific chromosome arm (e.g., 1BS vs 1BL)

E.R. Sears' Milestone: The Chinese Spring Aneuploid Series in Bread Wheat

Ernest Sears (1954–1966) assembled all 21 monosomics, nullisomics, and nulli-tetrasomics, establishing wheat chromosomal genetics

- **21 Chromosomes = 3 Subgenomes × 7:** Sears organized wheat chromosomes into Groups 1 through 7 across subgenomes A, B, and D (1A, 1B, 1D ... 7A, 7B, 7D); Loss of both copies of chromosome 2A is lethal or sterile unless compensated by doubling chromosome 2B or 2D (Nulli-2A Tetra-2D)!
- **Detecting Linkage Deviation in F2:** Cross a target variety carrying a mutant allele onto all 21 Chinese Spring monosomic lines; Only in the critical F1 monosomic cross does F2 segregation deviate drastically from 3:1 (near 100% mutant), pinpointing the chromosome
- **Chromosome 5B Pairing Homoeologous-1:** Nullisomic 5B plants exhibit massive multivalent synapsis between A, B, and D homeologous chromosomes; Riley & Chapman / Sears & Okamoto (1958) proved Ph1 (ZIP4 paralog on 5BL) forces strict homologous bivalent pairing
- **Unlocking Alien Recombination:** Deleting Ph1 (ph1b mutant) temporarily switches off pairing restriction in F1 wide hybrids; Forces physical crossing-over between alien donor chromosomes and wheat chromosomes to create tiny disease-resistance introgressions



E.R. Sears' Chinese Spring aneuploid series & Ph1b chromosome engineering

Step-by-Step Chromosome Engineering: Introgressing Wild Relative Traits

Progressively shrinking donor alien chromatin from an extra whole chromosome down to a tiny, drag-free translocation segment

1 1. Amphiploid / F1 Hybrid

Cross wheat × wild relative (e.g., rye or Thinopyrum) + embryo rescue + colchicine

2 2. Alien Addition Line (2n=44)

Backcross to retain 42 wheat chromosomes + 1 extra alien chromosome pair (DA)

3 3. Substitution Line (2n=42)

Replace a homeologous wheat pair (e.g., 1B) with the alien pair (e.g., 1R rye)

4 4. Centric/ph1b Translocation

X-ray break (Sears, 1956), centric misdivision, or ph1b crossing-over joins alien arm/segment to wheat

5 5. GISH + KASP Selection

Recover minimal alien introgression carrying target resistance without yield or quality drag

Landmark example: E.R. Sears (1956) irradiated wheat × Aegilops umbellulata lines to transfer leaf rust resistance (Lr9) cleanly onto wheat chromosome 6B.

Types of Alien Introgression Lines in Cereal Chromosome Engineering

Evaluating cytogenetic stability, yield penalty, and commercial breeding adoption

Line Type	Chromosome Composition (Wheat)	Cytogenetic Stability & Drag	Agronomic Example
Disomic Alien Addition (DA)	42 wheat + 2 alien (2n = 44)	Unstable (univalent loss); severe yield/fertility drag from extra ~5,000 genes	Used mainly as chromosome-specific donor stock for trait screening
Disomic Alien Substitution (DS)	40 wheat + 2 alien (2n = 42)	Stable if homeologous (e.g., 1R replacing 1B), moderate linkage drag	Wheat–rye 1R(1B) substitution donor for disease resistance
Whole-Arm Translocation	Centric fusion of 1 wheat arm + 1 alien arm	Meiotically stable; widely commercialized despite occasional end-use quality trade-offs	1BL.1RS wheat–rye translocation (Yr9, Lr26, Sr31, Pm8, drought root vigor)
Interstitial Ph1-Mediated Segment	Small (<10–30 Mb) donor DNA inside wheat arm	Ideal stability & minimal yield/quality drag — target of modern breeding	Lr19/Sr25 Thinopyrum segment; Sr26 shortened translocations

★ Key Takeaway

- Evaluating cytogenetic stability, yield penalty, and commercial breeding adoption
- **Comparison scope (4):** Disomic Alien Addition (DA); Disomic Alien Substitution (DS); Whole-Arm Translocation; Interstitial Ph1-Mediated Segment
- **Priority synthesis:** Interstitial Ph1-Mediated Segment — Lr19/Sr25 Thinopyrum segment; Sr26 shortened translocations

Two High-Impact Pre-Breeding Successes in Wheat Improvement

Harnessing both alien translocations (Secale cereale) and resynthesized wheat (T. durum × Ae. tauschii)

The Historic 1BL.1RS Wheat–Rye Translocation

- Spontaneous centric breakage-fusion replaced wheat short arm 1BS with rye short arm 1RS (present in cultivars Kavkaz, Aurora, Seri 82, Bezostaya derivatives)
- **Delivered a four-disease resistance cluster:** stem rust (Sr31), leaf rust (Lr26), stripe rust (Yr9), and powdery mildew (Pm8)
- Additionally increased above-ground biomass, root proliferation, and grain yield under drought across millions of hectares globally
- **Challenge:** 1BS loss replaced wheat gliadins/glutenins with rye secalins (sticky dough quality) & Ug99 broke Sr31 — motivating shortened 1RS re-engineering

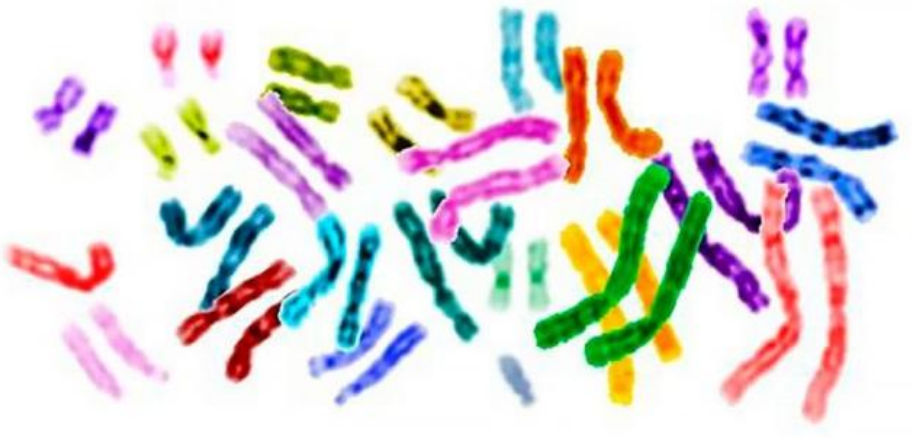
Synthetic Hexaploid Wheat (SHW — D-Genome Rescue)

- Modern bread wheat experienced a tight genetic bottleneck in its D-subgenome (few Ae. tauschii crosses occurred ~8,000 years ago)
- CIMMYT & breeders recreate hexaploid wheat de novo by crossing elite durum (AABB) × diverse wild Aegilops tauschii (DD) accessions followed by colchicine doubling
- Unlocks vast genetic variation for heat/drought tolerance, Fusarium head blight, Septoria, and grain Zn/Fe density
- Over 20% of newly released irrigated and dryland bread wheat cultivars globally carry Synthetic Hexaploid pedigrees

Molecular Cytogenetics: Visualizing Alien Chromatin via GISH and FISH

Transitioning from classical acetocarmine/C-banding to fluorescent multicolor chromosome painting

- **GISH (Genomic ISH) — Whole-Genome Chromosome Painting:** Uses total genomic DNA from the alien species (labeled with fluorescein/digoxigenin) as probe + excess unlabeled wheat blocking DNA; Uniquely paints alien chromosomes, telocentrics, and 1BL.1RS or Thinopyrum translocation breakpoints in bright green vs red/blue wheat
- **FISH (Fluorescence ISH) — Specific Repeat & Locus Mapping:** Hybridizes fluorescent probes targeting tandem repeats (pSc119.2, pAs1, GAA) or synthetic oligonucleotides (Oligo-FISH); Unambiguously identifies all 21 individual bread wheat chromosome pairs and arm rearrangements in a single metaphase spread
- **EXOME / KASP VALIDATION — High-Throughput Introgression Tracking:** Once GISH confirms an introgression, breeders convert alien junction sequences into cheap high-throughput KASP SNP markers; Allows screening thousands of F2/BC progeny without time-consuming root-tip microscopy
- **CRISPR Ph1b / CHROMOSOME EDITING —** Next-Generation Targeted Translocations: Using CRISPR-Cas9 to cut two non-homologous chromosomes simultaneously can engineer synthetic reciprocal translocations directly; Knockout of ZIP4 (Ph1) via CRISPR enables inducible homeologous recombination in any elite cultivar, not only Chinese Spring



Multicolor cytogenetics visualizing alien chromatin via GISH and FISH

Applying PGRFA and Genomics to Kazakhstan's Strategic Crops

Connecting wild germplasm in Central Asia to resilient wheat, barley, and apple breeding

Steppe Cereals: Drought, Heat & Stem Rust (Ug99)

- Kazakhstan is among the world's top wheat exporters, farming under harsh continental drought, frost, and yellow/stem rust pressure
- Using synthetic hexaploid wheats, Thinopyrum/Agropyron introgressions, and GWAS on local collections isolates new root and heat-tolerance alleles
- Combining alien Sr genes (Sr2, Sr25, Sr26, Sr33 from Ae. tauschii) provides durable resistance against virulent Ug99 races

Malus sieversii (Wild Apple of Tian Shan)

- Kazakhstan's mountain forests hold the center of origin and maximum genetic richness of cultivated apple
- Unlike genetically narrow commercial clones ('Gala', 'Fuji'), wild M. sieversii accessions resist apple scab (Venturia), fire blight, and extreme cold
- Genomic selection, cryopreservation of dormant winter buds in LN2, and GWAS preservation protect this irreplaceable global heritage

Modern Germplasm-to-Cultivar Pipeline (Pre-Breeding 4.0)

Bridging genebank wild accessions and commercial elite breeding programs without losing yield potential

1 1. FIGS / Eco-Mining

Focused Identification of Germplasm Strategy picks wild accessions from stressful climates

2 2. Sequence & Pangenome

High-density genotyping identifies unique haplotypes and presence–absence resistance genes

3 3. Wide Cross / Editing

Bridge cross, ph1b chromosome engineering, or somatic fusion transfers target trait

4 4. MABC + GISH / KASP

Speed-breeding marker-assisted backcrossing cuts out donor linkage drag in 2–3 years

5 5. Elite Deployment

Deploy pre-bred bridge lines directly into regional commercial cultivar development

Control Questions and Key Takeaways

Consolidating Lecture 5 concepts

? Control & Review Questions

- 1. Define Primary, Secondary, and Tertiary gene pools (Harlan & de Wet) and explain the causes of genetic erosion.
- 2. Why is Kazakhstan globally crucial for Vavilovian agrobiodiversity (Malus sieversii, wild Triticeae), and how do in situ and ex situ conservation complement each other?
- 3. Distinguish monosomics, nullisomics, trisomics, tetrasomics, and ditelosomics, and explain Sears' Chinese Spring aneuploid series.
- 4. What is the role of the wheat Ph1 locus on chromosome 5BL, and how does ph1b help shrink alien chromatin introgressions?
- 5. Compare alien addition, substitution, and translocation lines (like 1BL.1RS), and explain how GISH and FISH verify them.

✓ Key Takeaways

- Wild relatives and landraces preserve vital resistance and climate-resilience alleles lost during domestication bottlenecks.
- Integrated conservation pairs evolving in situ reserves with −20 °C seed genebanks, LN2 cryopreservation, and Svalbard backups.
- Wheat's polyploid buffering enabled Sears' aneuploid series and Ph1-mediated chromosome engineering of alien translocations.
- GISH, Oligo-FISH, Synthetic Hexaploid Wheat, and CRISPR now rapidly unlock tertiary gene pools without linkage drag.

Recommended Literature

Sources on plant genetic resources, wheat aneuploidy, and chromosome engineering

1 Main Literature

- 1. Sears E.R. The aneuploids of common wheat // Missouri Agricultural Experiment Station Research Bulletin. — 1954. — No. 572.
- 2. Friebe B., Jiang J., Raupp W.J., McIntosh R.A., Gill B.S. Characterization of wheat-alien translocations conferring resistance to diseases and pests // Euphytica. — 1996. — Vol. 91.
- 3. Hajjar R., Hodgkin T. The use of wild relatives in crop improvement: A survey of developments over the last 20 years // Euphytica. — 2007. — Vol. 156.
- 4. Rey M.-D. et al. Exploiting the ZIP4 homologue within the wheat Ph1 locus has identified two lines exhibiting homoeologous crossover in wheat wild relative hybrids // Molecular Breeding. — 2017.

2 Additional Literature & Databases

- 1. FAO Commission on Genetic Resources for Food and Agriculture & Genesys PGR Database: https://www.genesys-pgr.org
- **2. Crop Trust & Svalbard Global Seed Vault:** https://www.croptrust.org
- 3. Cornille A. et al. The domestication and evolutionary ecology of apples (Malus sieversii) // Trends in Genetics. — 2014.
- 4. International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA): https://www.fao.org/plant-treaty

Thank you for your attention! • Department of Molecular Biology and Genetics, KazNU