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Molecular Breeding and Biotechnology: Principles, Tools, and Transformative Applications in Crop Improvement

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The convergence of molecular genetics, genomics, and biotechnology has fundamentally transformed crop improvement. Molecular breeding — the strategic integration of DNA-based tools with classical plant breeding — now encompasses a continuum of technologies ranging from simple sequence repeat (SSR) markers and quantitative trait locus (QTL) mapping to genome-wide association studies (GWAS), genomic selection (GS), CRISPR/Cas9 genome editing, doubled haploid (DH) technology, speed breeding, RNA interference (RNAi), and artificial intelligence (AI)-assisted prediction models. Together, these innovations have compressed breeding cycles, enhanced selection precision, and enabled trait improvements in crop species that were previously intractable by conventional methods. This chapter provides a comprehensive, mechanistic, and critically evaluated account of the major tools and strategies of modern molecular breeding and biotechnology, illustrating their applications in developing disease-resistant, stress-tolerant, high-yielding, and biofortified crop varieties. Regulatory frameworks, biosafety considerations, and emerging frontiers — including pan-genomics, epigenomic breeding, and AI-driven multi-omics integration— are also discussed. A central thesis is that the synergistic deployment of these technologies, rather than reliance on any single approach, constitutes the most productive path toward climate-resilient, food-secure agriculture.

Introduction: From Phenotype to Genome-The Molecular Breeding Revolution

Crop improvement has been practiced for millennia, beginning with empirical selection of superior phenotypes by early farmers. The formalization of genetics following Mendel's rediscovery in 1900, and the subsequent development of quantitative genetics, hybridization programs, and mutation breeding, drove extraordinary gains in crop productivity throughout the twentieth century. However, phenotype-based selection is fundamentally constrained by the plasticity of phenotypic expression under environmental variation, and by the impossibility of directly selecting for alleles at multiple loci simultaneously without genetic markers. The advent of recombinant DNA technology, DNA markers, and, later, whole-genome sequencing catalyzed a paradigm shift. Plant breeding has gradually changed from phenotype-to-genotype based to genotype-to-phenotype-based selection — a transition that has dramatically improved the efficiency, accuracy, and speed of crop improvement programs. As Krishna, Veeramuthu, Maharajan, and Soosaimanickam (2023) articulate, the availability of high-throughput molecular markers followed by genomic-assisted approaches has significantly contributed to advancing plant breeding, and integration of speed breeding with genomic and phenomic facilities has allowed rapid QTL/gene identifications and ultimately accelerated crop improvement programs. Concurrently, plant biotechnology has provided transformative tools for directly engineering crop genomes — from *Agrobacterium*-mediated transformation and biolistic gene delivery to precision genome editing via

CRISPR/Cas9 and its derivatives. Regulatory landscapes are evolving globally to accommodate these innovations: Kumar, Das, Choudhury, Kumar, Prakash, Verma, Chakraborti, Devi, Bhattacharjee, Das, Das, Devi, Das, Rawat, and Mishra (2024) note that the Government of India issued an Office Memorandum in March 2022 exempting SDN-1 and SDN-2 genome-edited plants without foreign DNA from the stringent GMO regulatory pathway — a landmark policy shift that reflects growing recognition of the fundamental distinction between gene editing and transgenic approaches.

This chapter systematically reviews the landscape of molecular breeding and plant biotechnology tools, tracing their conceptual foundations, mechanistic principles, crop applications, and future trajectories. Four overarching sections address: (i) the progression from conventional to molecular markers; (ii) QTL mapping, GWAS, and genomic selection; (iii) biotechnological tools including transgenic approaches, genome editing, RNAi, and doubled haploidy; and (iv) integrative and emerging technologies including speed breeding, AI, multi-omics, and pan-genomics.

Table: The four eras of plant breeding from empirical phenotypics to intelligent genomics.

Era / Period	Paradigm	Core Tools	Limiting Factor
Breeding 1.0 (Pre-1900s)	Empirical phenotypic selection	Visual trait selection; hybridisation; landraces	No genetic understanding; slow; environment-dependent
Breeding 2.0 (1900–1980)	Mendelian & quantitative genetics	Pedigree methods; hybridisation; mutation breeding; cytogenetics	Still phenotype-limited; multi-year cycles
Breeding 3.0 (1980–2010)	Molecular genetics	RFLP, SSR, SNP markers; QTL mapping; MAS; transgenics; tissue culture	Marker cost; limited genomic coverage
Breeding 4.0 (2010–present)	Intelligent precision breeding	WGS; GWAS; GS; CRISPR; DH; speed breeding; AI/ML; multi-omics	Data integration; regulatory hurdles; trait complexity

Molecular Markers: The Foundation of Genomics-Assisted Breeding

From Morphological to DNA-Based Markers

Morphological and biochemical markers — seed colour, plant architecture, isozyme polymorphisms — were the first tools used to track genetic variation. While informative, these markers are few in number, sensitive to developmental and environmental effects, and provide limited genome coverage. The development of DNA-based molecular markers in the 1980s and 1990s transformed the field by providing virtually unlimited polymorphisms directly at the DNA level, unaffected by the environment. **Hasan, Choudhary, Naaz, Sharma, and Laskar (2021)** — from Aligarh Muslim University and Bahona College, Assam — provide a comprehensive account of the transition: restriction fragment length polymorphisms (RFLPs), the first DNA markers, were followed by random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), and simple sequence repeats (SSRs). Each generation offered improvements in throughput, reproducibility, and informativeness.

SSR and SNP Markers

SSR (microsatellite) markers became the workhorse of molecular breeding in the 1990s and 2000s, owing to their high polymorphism information content (PIC), co-dominant inheritance, and amenability to PCR-based detection. The Q-TARO database alone currently contains 114 cloned genes with natural variants affecting various rice agronomic traits, largely identified through SSR-based QTL mapping (Hasan et al., 2021). Single nucleotide polymorphisms (SNPs) subsequently emerged as the dominant marker class with the advent of next-generation sequencing. Kumar et al. (2024) — from ICAR Indian Agricultural

Statistics Research Institute and partner institutions — note that SNPs are crucial for identifying genomic regions linked to important traits, enhancing breeding accuracy and efficiency. High-density SNP arrays (50K, 90K, 660K) have enabled genome-wide scanning of plant populations at resolutions impossible with SSRs.

Genotyping-by-Sequencing and High-Throughput Platforms

The declining cost of sequencing has made genotyping-by-sequencing (GBS) and whole-genome resequencing economically viable for breeding programs. These approaches simultaneously discover and genotype SNPs across entire populations, bypassing the need for pre-designed arrays. **Anand, Subramanian, and Kar (2023)** — from Ramaiah University of Applied Sciences, Bangalore — highlight that high-throughput phenomic platforms have played a significant role in the modern breeding program and are considered an essential part of precision breeding, complementing high-throughput genotyping with rapid, accurate phenotypic data collection.

QTL Mapping, GWAS, and Genomic Selection: From Markers to Breeding Value Prediction

QTL Mapping: Linking Genomic Regions to Agronomic Traits

Quantitative trait locus (QTL) mapping identifies chromosomal regions that influence complex, polygenic traits such as yield, drought tolerance, and grain quality. Mapping populations — backcross (BC), F2, doubled haploid (DH), near-isogenic lines (NILs), and recombinant inbred lines (RILs) — segregate for target traits and are genotyped at dense marker intervals. Statistical models then associate marker genotype with phenotype, revealing QTL intervals. Krishna et al. (2023) summarize that integration of speed breeding with genomic and phenomic facilities allowed rapid QTL/gene identifications, while advances in sequencing technology have helped with genomic selection in crop breeding. Classical QTL studies have identified major resistance genes (e.g., Xa21 for bacterial blight in rice, Lr34 for leaf rust resistance in wheat) that have since been deployed in breeding programs worldwide via marker-assisted selection.

Marker-Assisted Selection (MAS) and Gene Pyramiding

Marker-assisted selection (MAS) uses DNA markers tightly linked to QTLs or candidate genes to select plants carrying desired alleles without the need for laborious phenotyping. MAS is particularly powerful for traits that are difficult or expensive to phenotype directly — such as disease resistance, nematode resistance, or quality traits requiring chemical analysis.

Hasan et al. (2021) note that molecular marker-assisted selection has considerably shortened the time for new crop varieties to be brought to the market. The four primary MAS schemes are: (i) Marker-Assisted Backcross Selection (MABC) for introgression of one to few major genes; (ii) Marker-Assisted Gene Pyramiding (MAGP) for stacking multiple resistance genes; (iii) Marker-Assisted Recurrent Selection (MARS) for accumulation of QTLs with smaller effects; and (iv) Genomic Selection (GS) for genome-wide prediction of breeding values.

Gene pyramiding — stacking multiple resistance or quality genes in a single genetic background — illustrates the power of MAS. Watson and Singh (1952) first conceptualized gene pyramiding, but it was only with the advent of molecular markers that the approach became practically feasible, enabling breeders to track multiple genes simultaneously and avoid the linkage drag associated with conventional backcrossing.

Genome-Wide Association Studies (GWAS)

While QTL mapping is conducted in biparental populations derived from two parental lines, genome-wide association studies (GWAS) exploit the natural diversity of diverse panels of accessions, landraces, and elite lines. GWAS leverages linkage disequilibrium (LD) between SNP markers and causative variants across many thousands of loci simultaneously, offering higher resolution and broader allele discovery than biparental QTL mapping. Kumar et al. (2024) — from ICAR–RCER and partner institutions — emphasize that GWAS, combined with genomic resources such as reference genomes, transcriptomes, and gene expression profiles, is vital in plant breeding and aids in the identification of key traits, understanding genetic diversity, and speeding up breeding programs. Multi-trait GWAS approaches are increasingly being adopted to identify pleiotropic SNPs that simultaneously influence several agronomic traits such as yield components in rice.

Genomic Selection (GS): Predicting Breeding Values Genome-Wide

Genomic selection, proposed by Meuwissen, Hayes, and Goddard (2001) and widely adopted from animal breeding, extends marker-assisted approaches by using all genome-wide markers simultaneously to estimate genomic estimated breeding values (GEBVs) for individuals in a breeding population — without the need to identify individual QTLs of significance. Budhlakoti, Kushwaha, Rai, Chaturvedi, Kumar, Pradhan, Kumar, Kumar, Juliana, Mishra, and Kumar (2022) — from ICAR–Indian Agricultural Statistics Research Institute, New Delhi — provide a thorough review of GS applications for climate-resilient crops. They demonstrate that while marker-assisted selection has proven its potential for qualitative traits controlled by one to few genes with large effects, its role in improving quantitative traits controlled by several genes with small effects is limited. In contrast, GS that utilizes GEBVs obtained from genome-wide markers to choose candidates for the next breeding cycle is a powerful approach to improve quantitative traits. GS has been widely adopted in animal breeding programs globally and is now being integrated into wheat, maize, and rice breeding pipelines. Key statistical models for GS include Ridge Regression-BLUP (RR-BLUP), GBLUP, Bayesian methods (BayesA, BayesB, BayesCpi), and machine learning approaches. The choice of model influences prediction accuracy, particularly for traits with different genetic architectures. Improved statistical models that leverage the genomic information across all loci improve prediction accuracy for complex, polygenic traits such as grain yield, which remain challenging for MAS. Anand et al. (2023) note that one way to reduce the costs of GS would be to use genomic selection every other generation or to choose candidates only if they meet certain requirements for traits like disease resistance that can be accurately phenotyped — a practical recommendation that illustrates the cost-benefit optimization now being pursued in resource-limited breeding programs.

Plant Biotechnology: Transgenic Approaches, Genome Editing, and Gene Silencing

Transgenic Crop Development

Agrobacterium-Mediated Transformation: *Agrobacterium tumefaciens* is a naturally occurring soil bacterium that transfers part of its Ti-plasmid DNA (T-DNA) into the plant genome. Scientists exploit this mechanism by replacing the native T-DNA with the desired transgene(s). As described by Su, Xu, Radani, and Yang (2023) — from Nanjing Forestry University — phenolics or acidic sugars released from the injured plant tissue are sensed by the VirA kinase, which activates VirG via phosphorylation. VirG induces expression of the Vir operon; VirD1/VirD2 then cleave the T-DNA at left and right border sequences, and the resulting single-stranded T-DNA complex is transported into the plant nucleus for integration. This process facilitates stable integration of the transgene, makes it cost-effective, and generally results in low-copy-number insertions. However, Agrobacterium-based transformation has historically been less efficient in monocots — particularly maize, wheat, and sorghum — due to recalcitrance to infection and tissue culture regeneration.

Biolistic (Gene Gun) Method: The particle bombardment method addresses the monocot recalcitrance problem by physically delivering DNA-coated gold or tungsten microparticles into plant cells using high-pressure helium gas. As reviewed by Su et al. (2023), some particles penetrate the nuclei of plant cells and integrate the DNA into chromosomes — a process independent of Agrobacterium's host range limitations. Biolistics has been particularly important in the transformation of cereals, sugarcane, and soybean. Key limitations of biolistics include multiple transgene copy insertions (leading to silencing and instability), higher rates of transgene rearrangement, and the potential for integration of non-T-DNA vector backbone sequences. Recent advances such as nanoparticle-mediated delivery have begun to address some of these limitations. Kumar et al. (2024) describe nanoparticle-mediated delivery as protecting CRISPR components from degradation and enhancing cellular uptake, significantly improving trait enhancement in maize.

Applications: Bt Crops, Golden Rice, and Beyond: Transgenic crops have delivered substantial agricultural and nutritional benefits globally. Bt crops expressing insecticidal crystal proteins from Bacillus thuringiensis provided durable protection against Lepidopteran and Coleopteran pests, reducing pesticide use and improving farmer income across millions of hectares. Herbicide-tolerant soybean, canola, and maize streamlined weed management. More recently, Golden Rice — engineered to accumulate provitamin A (beta-carotene) in the endosperm— represents a landmark biofortification achievement. Garg, Sharma, Sharma, Kapoor, Kumar, Chunduri, and Arora (2018) — from the National Agri-Food Biotechnology Institute (NABI), Mohali — documented that biofortified crops generated by transgenic approaches are improving the lives of millions globally by addressing micronutrient deficiencies, particularly iron and zinc, through molecular farming strategies. The transgenic approach entails identifying and characterizing suitable genes for desired nutritional qualities — an approach that is particularly valuable when limited genetic variability among crops makes conventional breeding biofortification infeasible.

RNA Interference (RNAi) and Virus-Induced Gene Silencing (VIGS)

RNA interference (RNAi) operates through double-stranded RNA (dsRNA) molecules that are processed by DICER-LIKE enzymes into 21–23 nt small interfering RNAs (siRNAs), which are loaded into the RNA-induced silencing complex (RISC) to target complementary mRNA for degradation or translational arrest. This mechanism can be exploited either to silence endogenous plant genes (host-induced gene silencing, HIGS) or to silence pathogen/pest genes in planta. In soybean, as reviewed by authors of recent molecular breeding approaches, RNAi has successfully modulated gene expression to optimize nutritional properties — including reducing saturated fatty acid content and increasing oleic acid proportions in seed oil — as well as enhancing responses to biotic stresses. Su et al. (2023) note that the combination of Agrobacterium tumefaciens vectors with virus-induced gene silencing (VIGS) provides a powerful genetic resource for rapid functional validation of candidate genes — without requiring stable transformation.

VIGS uses viral vectors to deliver dsRNA triggers, inducing transient silencing of target genes in young tissues within days or weeks. While VIGS results are transient and not heritable, it serves as an invaluable tool for rapid functional genomics screens, particularly in crop species where stable transformation is slow or genotype-specific.

CRISPR/Cas9 and Next-Generation Genome Editing

Mechanism and Variants: The CRISPR/Cas9 system — repurposed from the adaptive immune system of Streptococcus pyogenes uses a programmable single-guide RNA (sgRNA) to direct the Cas9 nuclease to a complementary genomic target sequence (protospacer), where it generates a double-strand break (DSB). Cellular DNA repair then proceeds via non-homologous end joining (NHEJ) — introducing small insertions/deletions (indels) that disrupt gene function — or via homology-directed repair (HDR) using a provided donor template, enabling precise gene replacement or insertion. Kumar et al. (2024) enumerate the extensive family of Cas9 variants that have evolved since the discovery of SpCas9: the most popular, SpCas9, contains 1,368 amino acids, recognizes NGG PAMs, and supports both sgRNAs and crRNA/tracrRNA pairings with 20-nt spacers, but exhibits a higher off-target editing rate. Smaller variants (SaCas9), high-fidelity variants (eSpCas9, SpCas9-HF1), and Cas12a (Cpf1) — which generates staggered cuts and recognizes T-rich PAMs — have expanded the targetable sequence space and improved specificity.

Base Editing and Prime Editing: Base editors (cytosine base editors, CBEs; adenine base editors, ABEs) enable programmable single nucleotide conversions without DSBs or donor templates, dramatically expanding the precision of point mutation introduction. Prime editing, using a reverse transcriptase fused to a Cas9 nickase and a pegRNA, further expands editing capabilities to include all 12 types of point mutations, small insertions, and small deletions — all without DSBs.

Applications in Stress Tolerance and Disease Resistance: Li, Wu, Zhang, and Zhang (2022) — from Shandong Agricultural University and Hunan Tobacco Research Institute — comprehensively review CRISPR/Cas genome editing for abiotic and biotic stress tolerance, demonstrating that these techniques have achieved remarkable results in resistance breeding of cereal crops (maize, rice, wheat), vegetable and fruit crops. Key applications include:

- Drought tolerance: Editing of ABA signalling genes (e.g., OsEra1 in rice, TaNAC in wheat) to enhance stomatal closure and root architecture under water deficit.
- Blast resistance in rice: Disruption of susceptibility genes (OsSEC3A, OsERF) to confer broad-spectrum resistance to Magnaporthe oryzae without yield penalties.
- Powdery mildew resistance in wheat: Simultaneous editing of TaMLO homoeologs (A, B, D genomes) using multiplex CRISPR to generate durable resistance.
- Virus resistance: Knockout of susceptibility genes such as TaPDIL5 or OsDjA2 to provide broad-spectrum resistance to Wheat streak mosaic virus and Rice tungro virus respectively.
- Improved nutritional quality: Editing of fatty acid desaturase genes in soybean to improve oil composition (increased oleic acid) and editing of alpha-gliadin genes in wheat to reduce immunogenic epitopes for coeliac patients.

Regarding delivery, Kumar et al. (2024) describe recent methodologies including nanoparticle-mediated delivery, viral vector delivery (leveraging natural infection mechanisms), and ribonucleoprotein (RNP) complex delivery — the latter being particularly effective in reducing off-target effects because the protein-RNA complex is transient and does not persist in edited cells.

Regulatory Landscape for Gene-Edited Crops: The regulatory treatment of CRISPR-edited crops — particularly those with no foreign DNA, falling under SDN-1 and SDN-2 categories — is evolving rapidly. Japan, the United States, and India have developed or are developing streamlined regulatory pathways for gene-edited crops that do not contain exogenous DNA. Japan is working towards a more tailored approach to genome editing. Kumar et al. (2024) report that the Government of India's 2022 Office Memorandum exempted SDN-1 and SDN-2 genome-edited plants from the standard GMO regulatory pathway, streamlining approval for gene-edited crops without foreign DNA. This policy shift is expected to significantly accelerate the deployment of CRISPR-edited varieties in India.

Doubled Haploid Technology: Accelerating Homozygosity

Principles of Haploid Induction

Doubled haploid (DH) technology provides 100% homozygous lines in one or two seasons, bypassing the 6–8 generations of selfing required by conventional inbreeding. Haploid plants — containing only the gametic (n) chromosome number — are produced through anther culture, microspore culture, ovule/embryo culture, or in vivo haploid induction via cross with inducer lines. Chromosome doubling with colchicine or oryzalin then restores the diploid state as a fully homozygous DH line.Mabuza, Mchunu, Crampton, and Swanevelder (2023) — from the Agricultural Research Council of South Africa and University of Pretoria — review DH technology in the context of sunflower breeding, noting that DH technologies can facilitate the production of true breeding lines faster and in a more efficient manner than the traditional back crossing and selection strategies. Hybrids often require homozygous parents that may require up to eight generations of back crossing and selection — a timeline compressed to one or two seasons with DH.

Androgenesis and Gynogenesis

Androgenesis-based DH production — using anther or microspore culture — is the most widely used approach in cereals, canola, and tobacco. Isolated microspores, reprogrammed by stress treatments (cold, heat, osmotic shock), undergo embryogenesis in defined media, producing haploid embryoids that are subsequently regenerated into plants. A key recent finding is the role of epigenetic mechanisms in this process: microRNAs appear to regulate early microspore responses to external stimuli, and trichostatin-A (a histone deacetylase inhibitor) acts as an epigenetic additive that enhances androgenic efficiency in several species.

In Vivo Haploid Induction and CRISPR-Assisted DH

In vivo haploid induction (HI) — using haploid inducer lines that carry genetic constructs causing genome elimination after pollination — has revolutionized DH production in maize and is being extended to other crops. Ye and Han (2024) — from the Chinese Academy of Agricultural Sciences and Chinese Academy of Sciences — describe how DH production through in vivo maternal haploid induction provides an effective way to generate homozygous genetic and breeding materials over a short period, widely applied in maize breeding programs. The integration of CRISPR/Cas9 with DH technology is enabling novel approaches. In durum wheat, homozygous TtMTL gene-edited mutants (mtl-a, mtl-b, and mtl-ab) produced by CRISPR/Cas9 function as haploid inducers, extending the in vivo HI system to polyploid wheats. Recent work has also combined DH production with Agrobacterium-mediated transformation of haploid immature embryos, generating doubled haploid transgenic and gene-edited plants confirmed by molecular analysis — an approach that markedly shortens the breeding cycle.

Speed Breeding: Compressing the Generation Cycle

Speed breeding (SB) accelerates the generation cycle by manipulating the photoperiod (typically extending light to 22 hours), temperature, and planting density to achieve rapid-cycling growth. Under optimal SB conditions, cereals such as wheat, barley, pea, and canola can complete up to 4–6 generations per year compared with 1–2 under glasshouse or field conditions. Krishna et al. (2023) note that the integration of speed breeding with genomic and phenomic facilities has allowed rapid QTL/gene identifications and ultimately accelerated crop improvement programs. Speed breeding is particularly powerful when combined with genomic selection: one way to reduce costs is to use genomic selection every other generation or to choose candidates only if they meet certain requirements for traits like disease resistance (Anand et al., 2023). This hybrid SB+GS strategy has been shown to reduce inbreeding compared to purely phenotypic or purely genomic selection. Light quality is a critical parameter in SB systems. Studies in soybean have defined optimal conditions using LED light sources, advancing one generation within 73 days — enabling five generations per year. Far-red light (>700 nm) should be avoided as it causes elongated petioles and lodging; low red/blue light ratio with green or cool white LEDs (4000 K) is recommended for robust soybean plants in high-throughput systems (Anand et al., 2023). One challenge related to SB approaches that use artificial greenhouses is their limited scale and high infrastructure cost — a factor that currently restricts their broad adoption in resource-limited breeding programs in developing countries.

Biofortification Through Molecular Breeding and Biotechnology

Biofortification — the enhancement of nutritional quality within edible crop tissues — addresses the global burden of micronutrient malnutrition affecting over three billion people, with particular severity in iron and zinc nutrition in developing countries. Three strategic approaches are deployed: agronomic biofortification (mineral fertilizers), conventional breeding biofortification (exploiting natural genetic variation), and transgenic/genome-editing biofortification.

Shohael, Kelly, Venkataraman, and Hefferon (2025) — from Jahangirnagar University (Bangladesh), Cornell University, and the University of Toronto — review opportunities and challenges in genetically engineered biofortification. They note that conventional breeding selects and cross-breeds plants with high natural levels of desired nutrients over generations, but this method is not always feasible due to limited genetic variability. The transgenic approach, by contrast, enables introduction of genes of interest involved in nutritional improvement directly into crops where natural genetic variation is insufficient.

Garg et al. (2018) document diverse biofortification outcomes: Golden Rice accumulates ~37 micrograms of beta-carotene per gram of dry weight in the endosperm; iron-enriched beans carrying the ferritin gene from soybean show significantly increased seed iron content; and zinc-enriched wheat lines developed through marker-assisted introgression of Gpc-B1 (a NAC transcription factor) show improvements in grain protein, zinc, and iron concentration simultaneously. Cereal crops — prominent targets of biofortification — include wheat, rice, and maize; the limited genetic variability in oilseeds makes them particularly suited to the transgenic approach.

Medina-Lozano and Díaz (2022) — from the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Zaragoza, Spain — review genomic tools for biofortification, emphasizing metabolic GWAS (mGWAS) for identifying loci controlling nutritional metabolites, cisgenesis and intragenesis as alternatives to transgenesis that avoid regulatory burdens, and CRISPR-based approaches for precision nutritional trait modification.

Artificial Intelligence, Multi-Omics, and the Frontier of Intelligent Breeding

Machine Learning for Genomic Prediction

The integration of artificial intelligence and machine learning (ML) into plant breeding represents the transition to 'Breeding 4.0' — or intelligent breeding. Deep learning models, including convolutional neural networks (CNNs) and recurrent neural networks (RNNs), offer the ability to capture non-additive (epistatic and dominance) genetic variance that is missed by linear genomic selection models such as GBLUP and BayesB. Anand et al. (2023) highlight that speed breeding coupled with AI can significantly facilitate understanding of genomic architecture by using a machine learning approach for genomic selection model development. ML algorithms such as DeepGS (an R package predicting phenotypes from genotypes using deep learning) and DNNGP (deep neural network for genomic prediction) are actively being benchmarked against classical models for yield prediction in wheat, rice, and maize. Explainable AI (XAI) methods are increasingly important in this context: being able to explain predictions allows for rapid development of genetic markers for breeding and can lead to more robust and resilient crop varieties. It also develops the confidence of breeders in AI-based decisions, which is critical for their adoption in breeding programs.

Multi-Omics Integration

Modern crop improvement increasingly integrates data streams from genomics, transcriptomics, proteomics, metabolomics, and phenomics — a multi-omics approach that provides a systems-level understanding of trait biology. Kumar et al. (2024) note that genomic resources including reference genomes, sequence and protein databases, transcriptomes, and gene expression profiles are vital in plant breeding and aid in the identification of key traits, understanding genetic diversity, assisting in genomic mapping, and supporting marker-assisted selection. Telomere-to-Telomere (T2T) complete genomes represent the latest advance in reference assembly quality, providing accurate identification of genetic diversity and enhancing functional genomics and genetic improvement.

Pan-Genomics and Haplotype-Based Breeding

A single reference genome fails to capture the full complement of genetic variation within a species — particularly presence/absence variation (PAV) of genes, structural variation, and rare alleles. Pan-genomics — constructing graph-based reference structures representing the genomic sequences of multiple diverse accessions — addresses this limitation. Pan-genome analyses have revealed thousands of dispensable genes absent from the primary reference that contribute to trait variation in rice, maize, wheat, and soybean. Pan-genomics combined with artificial intelligence and precision breeding can accelerate crop improvement by enabling haplotype-based selection — a strategy in which haplotype blocks spanning multiple favourable SNPs are selected simultaneously, capturing linkage-phase information that single-SNP analysis misses. Long-read sequencing technologies (PacBio HiFi, Oxford Nanopore) and graph-based assembly tools have made pan-genome construction increasingly practical even for large polyploid genomes such as wheat and sugarcane.

Comparative Overview of Major Molecular Breeding and Biotechnology Tools

Table: Comparison of major molecular breeding and biotechnology tools: principle, advantages, limitations, and key references.

Technology	Principle	Key Advantage	Limitation	Key Reference(s)
RFLP / RAPD	DNA hybridisation / random PCR amplification	First DNA markers; revealed polymorphisms	Low throughput; often dominant	Hasan et al. (2021)
SSR (Microsatellites)	PCR amplification of tandem repeats	Highly polymorphic; co-dominant; reproducible	Requires prior sequence knowledge	Krishna et al. (2023)
SNP Arrays	Hybridisation to allele-specific probes on chips	High-density; automation-friendly; genome-wide	SNP ascertainment bias in non-model crops	Kumar et al. (2024)
QTL Mapping	Statistical association of markers with traits in mapping populations	Identifies chromosomal regions controlling traits	Low resolution; population-specific	Krishna et al. (2023)
GWAS	Linkage disequilibrium between SNPs and traits across diverse panels	High resolution; harnesses natural diversity	Population structure confounding; rare alleles missed	Kumar et al. (2024)
MAS / MABC	Foreground/background selection with linked markers	Accelerates introgression; reduces linkage drag	Effective mainly for few major QTLs	Hasan et al. (2021); Budhlakoti et al. (2022)
Genomic Selection (GS)	Genome-wide GEBV prediction with statistical models	Captures small-effect loci; reduces cycle time	Requires large training population; costly genotyping	Budhlakoti et al. (2022)
CRISPR / Cas9	RNA-guided site-specific DSB and repair	Precise; multiplex; no foreign DNA needed (SDN-1)	Off-target cuts; delivery recalcitrance in some crops	Li et al. (2022); Kumar et al. (2024)
RNAi / VIGS	dsRNA-triggered post-transcriptional gene silencing	Rapid functional validation; no stable transformation	Transient; not heritable without stable insertion	Su et al. (2023)
Speed Breeding	Extended photoperiod / controlled environment for rapid cycling	Up to 6 generations/year; combinable with GS	Species-specific; high infrastructure cost	Anand et al. (2023); Krishna et al. (2023)
Doubled Haploidy (DH)	Anther/microspore culture + colchicine doubling	100% homozygosity in 1–2 seasons	Genotype recalcitrance; albino problem	Mabuza et al. (2023)
AI / Deep Learning in GS	Neural networks / ML for genomic prediction	Captures non-additive variance; integrates multi-omics	Interpretability; large data requirements	Anand et al. (2023); Kumar et al. (2024)

Regulatory Frameworks and Biosafety Considerations

The deployment of transgenic and genome-edited crops is subject to regulatory oversight in virtually all jurisdictions, though the frameworks vary considerably in stringency and scope. Transgenic crops expressing exogenous DNA are generally regulated as GMOs and require full biosafety dossiers demonstrating food, feed, and environmental safety. Gene-edited crops — particularly those containing no exogenous DNA — are increasingly treated differently. Kumar et al. (2024) provide a survey of the global regulatory landscape: initially, genome-edited crops were assessed under the existing GMO framework, which involves rigorous safety evaluations. Japan is working towards creating a more tailored approach to genome editing. CRISPR-edited soybeans with improved nutritional properties are currently under review in Japan, with recent discussions focused on developing regulations that distinguish between genome editing and traditional GMOs, potentially streamlining the approval process. In India, the Government's 2022 Office Memorandum represents a significant liberalization, exempting SDN-1 and SDN-2 gene-edited plants — those producing targeted sequence changes without incorporating foreign DNA from other species — from the Genetic Engineering Appraisal Committee (GEAC) process. This streamlining is expected to facilitate more rapid testing and release of gene-edited crop varieties suited to Indian agro-climatic conditions. Biosafety assessments for transgenic crops evaluate potential allergenicity and toxicity of expressed proteins, gene flow to wild relatives, effects on non-target organisms, and impacts on biodiversity. The principle of substantial equivalence — comparing the transgenic crop with its conventional counterpart— remains central to risk assessment frameworks internationally.

Open Questions and Future Directions

Despite extraordinary progress, several challenges and frontiers remain:
Off-target editing and specificity: While high-fidelity Cas9 variants and improved guide RNA design tools have reduced off-target editing, whole-genome sequencing verification of edited plants remains essential before variety release. The development of computational tools for precise guide RNA design using ML models is an active area of research. Delivery recalcitrance in crop species: Transformation efficiency remains a bottleneck for many important crop species, particularly for large-genome polyploids and species where tissue culture regeneration is genotype-specific. Developmental regulator-based overcoming of transformation recalcitrance (e.g., using Baby boom and Wuschel transcription factors) represents a promising recent advance. Complex trait improvement: Traits such as yield potential and adaptation to compound stresses (simultaneous drought + heat) are governed by complex gene networks with significant G×E interactions. Multi-trait, multi-environment genomic selection models, combined with high-throughput phenotyping and remote sensing, offer a path forward. Epigenomic breeding: Epigenetic variation — heritable changes in gene expression not encoded in DNA sequence — contributes significantly to phenotypic variation and stress adaptation. Epigenome-aware breeding, exploiting epialleles and epigenetic priming, is an emerging paradigm that could complement genomic selection approaches. Equity and access: The benefits of molecular breeding and biotechnology have disproportionately accrued to crops and regions with strong private sector investment. Expanding genomic resources, DH technology, and speed breeding infrastructure for orphan crops (teff, pearl millet, pigeonpea, cassava) in developing countries remains a critical global priority.

Conclusion

Molecular breeding and plant biotechnology have collectively transformed crop improvement from an art guided by phenotypic intuition into a precision science guided by genomic data, computational models, and targeted molecular tools. The progression from restriction fragment length polymorphism mapping to whole-genome pan-genomics, from single-gene transgenic events to multiplex CRISPR base editing, and from simple marker-assisted backcrossing to AI-powered genomic selection embodies one of the most rapid technological revolutions in the history of agricultural science. As global food demand is projected to increase by 50–70% by 2050, driven by population growth and dietary transitions, and as climate change reduces the productivity and stability of major cropping systems, the tools reviewed in this chapter offer a scientifically credible and practically achievable path toward climate-resilient, nutritionally improved, and high-yielding crop varieties. Their deployment at scale will require not only continued scientific innovation, but also enabling regulatory frameworks, equitable access to genomic infrastructure, and integration with farmer-centered participatory approaches to ensure that the benefits of the molecular breeding revolution reach all who depend on agriculture for food security.

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