Review

Genetics Has Enabled the Improvement of Breeding Strategies: Single-Cell Analysis, Epigenetics, *cis*-Regulation and Gene Editing to Exploit Genetic Variants Underlying Phenotypes

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ABSTRACT

The study of genetics seeks to understand the molecular relationships that ensure the proper patterning of gene expression necessary for plant development and its responses to the environment that determine crop performance. Improvements in molecular genetics, biotechnology, and a deeper understanding of gene expression have given breeders access to major sources of phenotypic novelty in plants and allowed them to accelerate genetic gains by breeding locally adapted crops. Advances in genetics, and more recently the edition of epigenetic marks, *cis*-regulatory elements and gene editing have gained significant importance in crop breeding. Single-cell level studies have helped to elucidate cell heterogeneity and intercellular regulation in key tissues, enabling a more precise comprehension of the effects of plant developmental processes on phenotypes. The aim of this review is to highlight how genetic knowledge has fostered progress in crop improvement and selection. After a summary of the historical use of genetics in breeding strategies over the past 100 years, we discuss how the latest technological developments in genetics can be leveraged in complex quantitative trait improvement. Recent progress in single-cell analyses allow us to identify the subtle relationship between the genomic blueprint and cellular diversity, connecting this cellular genetic heterogeneity of a plant to specific phenotypes, that would be otherwise overlooked when using bulk cell analyses. High-throughput access to numerous epigenetic marks has also

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opened up new opportunities and challenges for crop genetic improvement. This progress has led to a better understanding of the role of *cis*-regulatory elements in determining some aspects of crop performance. Here we also discuss whether it is possible to leverage reliable variations in these regions in crop breeding, using targeted selection to manipulate traits in a wide range of crops. Finally, we outline advances in gene editing tools in crop improvement, and discuss how these could be implemented to accelerate breeding for crops adapted to new environments.

KEYWORDS: genetics; breeding strategies; single-cell; epigenetics; *cis*-regulation; gene editing; biotechnology

GENETICS AS A TOOL FOR IMPROVING BREEDING STRATEGIES

Plant breeding has profoundly evolved throughout the history of crop improvement and has incorporated timely co-evolving genetic progress knowledge, molecular biology and innovative techniques (Figure 1). Before Mendel's law of heredity [1] and its rediscovery in the 1900s [2–4], breeding relied on classical phenotypic selection and pedigree methods. For instance, Vilmorin Co. in France introduced the pedigree method in the 1800s [5], where a few plants were selected to provide seeds to sow in rows for the next generation based on progeny testing principles. The development of genetics improved our understanding of chromosome and loci re-arrangement with a substantial impact on breeding methodologies.

Pure line selection theory [6], a system where the progeny of a single self-fertilized homozygous plant is obtained, helped demonstrate that phenotypic variation is present within natural accession, intrinsically separating out between-lines phenotypic variance by reducing within-line variation. One successful example of a variety developed using pure line selection was the sorghum line resistant to Dwarf Yellow Milo caused by root ascomycete fungus *Periconia circinate* [7] (pages 112–113). In 1930, F. A Wagner, at the Kansas Agriculture Research Station (USA), realized that two apparently phenotypically identical plants withstood the devastating Dwarf Yellow Milo disease differently and led to progeny-free offspring of the disease progeny. However, problems with the resistance mechanism were identified [8,9], and, despite the efforts of molecular geneticists [10,11], the resistance genes have not yet been characterized.

Bulk-population was originally proposed by Swedish geneticist Nilsson-Ehle [12]. This method takes advantage of natural selection: a large population of F2 segregants are sowed in a large plot, from which seeds are collected and sowed in subsequent years. Some artificial selection normally occurs by suppressing off-types plants. With his research on oats, Nilsson-Ehle shed light on a quantitative genetics theory at the same time as American geneticist East's discoveries on maize [13]. Single seed descent (SSD) [14] is a method were segregants from a hybridization event are

rapidly fixed by advancing one randomly selected seed per plant at the early stages through subsequent generations, as a modified pedigree method [15]). SSD is used to develop recombinant inbred lines (RILs), a breeding resource that takes advantage of several recombination events resulting from self-fertilization. Their analysis in applied quantitative genetic studies [16,17] has led to the identification of quantitative trait loci (QTLs) used for improving crops and developing resistant varieties. RILs are a powerful tool for genetic variation mapping. Together with next-generation sequencing (NGS), they have enabled rapid identification of candidate genes, for example, in maize for traits associated with the mimic lesion [18], in foxtail millet for morphological traits [19], in cucumber using genotyping-by-sequencing for downy mildew resistance [20] or as a RILs resource for diploid potato [21].

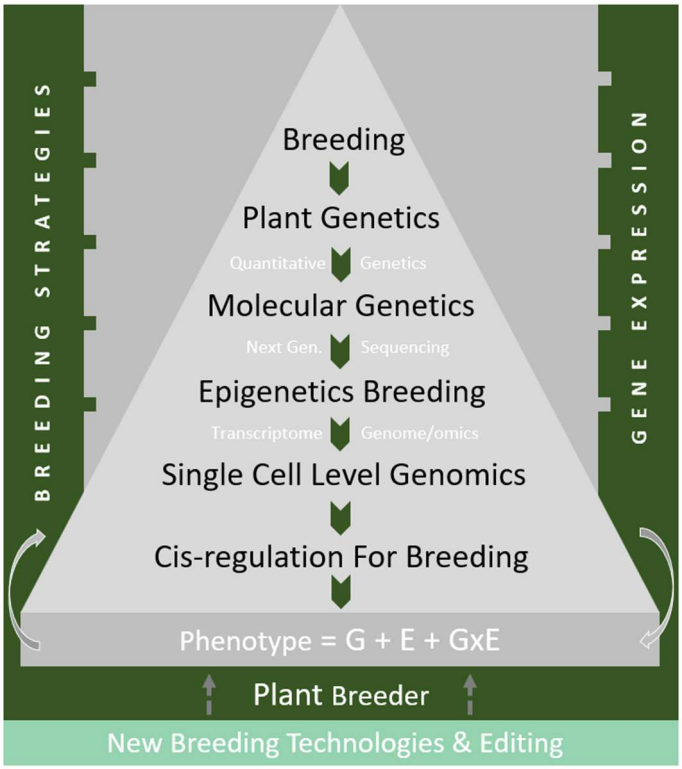

Figure 1. Diagram representing advances in genetics, molecular biology, epigenetics, single-cell level analysis, and gene editing, to better understand the layers of gene regulation for phenotypic expression as a result of a plant performance for variety development. Geneticists have elucidated and optimized traditional breeding. Similarly, breeding methods have provided a means to create population structures helping practical and theoretical quantitative genetic advances and gene mapping. At the same time, a breeding strategy is a breeder's decision based on combined knowledge of all these genetic layers of information that lead to a complex regulatory interaction observed in a phenotype expressed within an environment. P = phenotype, G = genotype, E = environment; G × E = genotype by environment.

Backcross breeding [22,23] is another example of this co-evolution where plant breeders increase the genetic control of the populations in their breeding programs. In this method, a donor parent, generally with a specific trait (allele) to transfer, is backcrossed several times to an adapted

variety to be improved, a process eventually ending with a self-pollination event to obtain a truly homozygous plant. Successful examples of backcross breeding include the US spring wheat cultivars, 'Linkert', resistant to the stem rust pathogen, *Puccinia graminis* f. sp. Tritici. Traditional backcross breeding has also benefited from incorporating the application of molecular biotechnologies (DNA markers) to practical breeding, which is known as marker assisted backcross (MABC). The use of MABC led to a five percent higher recurrent parent genome recovery in a BC2 generation compared to conventional backcross [24]. Continuous advances in understanding the genetic mechanisms underlying desirable traits, together with the development of molecular biology tools, have enabled the use of molecular markers in linkage disequilibrium with specific traits for marker-assisted selection (MAS). This approach has provided a major advantage in classical breeding because it is more cost-effective than conventional phenotypic selection. MAS has successfully improved crop breeding. In wheat, it has enabled the identification and use of markers associated with physiological traits [25], drought tolerance [25], and biotic resistance such as to Fusarium Head Blight [26]. It has also facilitated the simultaneous pyramiding of disease resistance and quality traits (e.g., glutenin) [27], the introgression of clubroot resistances in *Brassica napus* using Kompetitive Allele Specific PCR (KASP) markers [28], and the improvement of yield-related traits [29]. However, further genetics studies were needed to identify and characterize these genes (e.g., [30]) to better understand their defense mechanisms for optimal implementation in commercial breeding programs.

Hybrid breeding methods were initially proposed to breed cross-pollinated crops by combining two pure lines of maize [31], and later [32] suggested the use of double crosses to obtain a hybrid maize product. Hybrids attain high overall performance in the produced lines evaluated in a progeny after a cross with a set of testers. These crosses provide information on both specific and general combining abilities [33], reflecting the benefits of heterosis as a result of the response due to dominance effects [34].

Recurrent selection [35,36] can be used to improve inbred lines of each heterotic pool, or to develop hybrid varieties. The basis of this system relies on the self-pollination of an initial set of heterozygous lines that are evaluated for a desirable trait; plants with lower performance are discarded and the superior individual plants are sowed from the selfed seed. Then, all possible intercrosses are made, which serve as a source of the additional 'recurrent' cycles of selection. Genetic discoveries and advances in genomic capabilities have brought extraordinary improvement to hybrid development efficacy with the use of methods including cytoplasm male sterility [37,38], double haploid line development (e.g., in wheat [39,40] or maize [41]), or hybrid prediction performance of potential crosses (e.g., in rice [42,43] or *Brassica napus*

[44]). These methods have been widely used to improve crops in the seed industry.

Bulk Segregant Analysis (BSA) is an effective method for identifying molecular markers associated with a phenotype of interest, and, compared with conventional mapping, is more cost-effective as it does not require genotyping and phenotyping of a complete mapping population [45]. In BSA two extreme phenotypes are characterized, and DNA bulks (groups) are pooled from individuals with similar phenotypes. These two groups can be derived from F2, F2:3 family populations, backcrossing (BC) populations, RILs, double haploids (DH) or multiparental populations [46]. With the advent of Next Generation Sequencing (NGS) technologies, BSA technology coupled with whole-genome sequencing (WGS) has been proven a powerful approach. By sequencing the two extreme phenotypes, BSA can identify genes located on different chromosomal regions associated with complex traits, for example, the control of amylose content in rice [47]. Advances in RNA-seq applied to the BSA breeding method, known as BSA-RNAseq, provides precise information on gene transcript abundance, offering stronger evidence of a gene's causative role when its expression correlates with the phenotype of interest under specific conditions. Bulked segregant RNA-seq (BSA-RNA seq) has been successfully applied in different crops: e.g., to validate a stem resistance locus in a wild relative of wheat, *Aegilops umbellulata* [48], to identify a single variant (*Pm5*) conferring powdery mildew resistance in wheat [49], to study stay-green in maize [50] and to map *Chilli veinal mottle virus* resistance 4 (*cvr4*) in pepper [51]. This research has major implications for understanding crop adaptation and crop improvement.

Variations in chromosome number and structure from the basic crop genomes of a normal plant have been used to a lesser extent in cultivar development and have often been overlooked. Common types of aneuploidies include the addition or deletion of entire chromosomes or chromosome segments from the basic somatic number (2n) of a species. The disruption of chromosome pairing and segregation caused by the addition or deletion of a chromosome has been intentionally implemented for breeding purposes [52]. Examples include: hybrid seed production exploiting tertiary trisomic individuals in barley that contain the nuclear restorer locus male sterile (MS) in linkage with the breakpoint at the chromosome exchange position [53]; using monosomic lines in chromosome mapping, e.g., in *Allium* genetic studies [54,55]; or chromosome substitution, e.g., the transfer of rust resistance from *Aegilops umbellulata* to wheat [56], or the transfer of powdery mildew resistance into common wheat (*Triticum aestivum* L.) from its *Aegilops geniculata* relative [57]. Furthermore, another common type of aneuploidy is caused by the alteration of the chromosome structure known as translocation, where pieces of DNA segments move places within or between chromosomes. Translocations have also been suggested for inbred lines and hybrid seed production. Inbred lines can be obtained by

crossing parents carrying different translocation events within a stock of multiple translocation lines [58,59]. For instance, in maize, hybrid seed production has exploited male sterility arising from heterozygous individuals of duplicate-deficient (Dp-Df) chromosomes [60], while a similar strategy was tested in hybrid wheat using the 4E-ms system [61]. Overall, chromosomal rearrangements can drive gene expression differences, leading to manifest phenotypic and metabolic differences [62], and are thus a source of genetic variation that can be exploited for crop breeding.

Advances in genetics and in particular, whole-genome sequencing (WGS) and the genome-wide mapping of genetic variations have helped improve breeding strategies and the development of powerful methods to predict yield/crop performance in the past 20 years. However, it has also shown its limits, and brought attention to the fact that how the genome is expressed is also an important component of crop performance (Figure 1). The cutting-edge technologies discussed in this article have given researchers access to gene expression patterns at single-cell level (see below) to partially decipher the epigenetic code governing gene regulation expression. Moreover, the integration of several data types has revealed complex regulatory networks underlying traits and highlighted the role of *cis*-regulatory elements. Finally, rapidly progressing editing techniques will allow breeders to integrate, in already elite germplasm, some advantageous (epi-)mutations, not only in genes but also in regulatory elements, and thus accelerate the timeframe for creating new, locally adapted genotypes (Figure 1).

LEVERAGING EPIGENETICS IN EPI-BREEDING

Most phenotypic differences in heritable traits are considered to be associated with DNA structure variants that differ between individuals. Trait variations can, more than is generally believed, be the result of gene expression consequences of epi-genetic regulation that have long-term effects in terms of mitotic inheritance and meiotic inheritance (between generations) [63,64]. Thus, in addition to inheriting genetic information, cells receive information that is not encoded in the DNA's nucleotide sequence. A key element is DNA packing in the nucleus and epigenetic marks intrinsically arranged in a hierarchical three-dimensional conformation [65]. Epigenetic marks facilitate interactions between proximal and distal DNA sequences [66], as well as between RNA and DNA, leading to transcriptional and translational regulations of gene expressions [67,68]. These epigenetic marks, which stably modify gene expression and subsequently contribute to phenotypic plasticity, generally include: (1) DNA methylation; (2) histone modifications (post-translational modification of histone proteins) and remodeling of chromatin; and (3) RNA processing and non-coding RNAs (ncRNAs).

DNA methylation entails the addition of a methyl group at the 5'carbon of cytosine. In plants, methylation has a tendency to concentrate at

contexts followed by guanines forming symmetric CG and CHG, as well as in asymmetric CHH (where H denotes A, C, or T) contexts. CG dinucleotides in plants are uniformly distributed and not depleted genome-wide compared to mammals. This is because of a distinct DNA methylation architecture with additional 5mC contexts (CHG, CHH) that reduces mutational pressure across many contexts other than CG, in addition to active demethylation via the ROS1 and Demeter pathway [69]. DNA methylation is generally associated with repressive marks that mediate tissue-specific gene silencing. However, genome-wide DNA methylation maps have revealed unexpected local dynamics, particularly at regulatory regions, where active demethylation occurs [70]). Methylation can alter transcriptional processes by modifying CpG contexts of gene promoters, interfering with transcription factors and other DNA-binding proteins, and ultimately inhibiting gene expression by recognizing methyl-CpG binding protein (MBD) [71,72]. Methylation marks can have strong phenotypic effects and have influenced different crop domestication events, e.g., maize, cotton or lettuce [73–75]. A significant correlation has also been found between the methylated state and the geographic origins of a wheat collection [76]. Methylation also affects genes related to yield and other agronomically important traits. In maize, differentially methylated regions (DMR) in the *ZMET2* (Zea methyltransferase2) gene affects yield and the number of husk layers and may have been a target of selection [77,78]. In rice, demethylated patterns of the *DWARF1* (D1) gene promote the transition from dwarf to normal stature [63], while in wheat, overexpression of the histone acetyltransferase TaHAG1 improves wheat tolerance to heat [79]. Han et al. (2021) [80] showed that hyper DNA methylation of *Teosinte branched1/cycloidea/proliferating cell factor* (TCP) were linked to inbreeding depression in maize. DNA methylation can also occur in gene bodies, epi-marks where gene exons are methylated, termed as Gene Body Methylation (GBM). GBM primarily manifests as CG methylation as opposed to CHH or CHG contexts [81]. GBM occurs in evolutionary conserved and ubiquitously expressed genes, however, it is causal relationship is still unresolved.

Histone modifications are chemical modifications on N-terminal tails of histones that induce post-translational gene regulation by determining chromatin configuration, either promoting an open (active) or closed (repressive) configuration [82]. The main role of histone modifications is to provide a memory of transcriptional activity [83] as well as to stabilize/reinforce transcriptional state [84]. These modification sites can be acetylated (Hac), methylated (Hme), phosphorylated, ubiquitinated, glycosylated, and sumoylated [85]. Histone modifications are permissive/suppressive to a particular transcriptional outcome, and the various combinations of these marks will form what is often referred as the ‘histone code’. For instance, Hac promotes transcription by neutralizing the positive charge of histones, weakening the binding interaction with negatively charged DNA [86]. Hac regulating dynamics

involve histone acetyltransferases (HATs) and histone deacetylases (HDACs), enzymes that respectively drive the expression and inhibition of a gene [86,87]. On the other hand, Hme occur primarily at the lysine (K) and arginine (R) residues on the N-terminal tails of histones H3 and H4 [88]; they are laid down by histone methyltransferases (HMTs) and are removed by histone demethylases (HDM). Histone modifications play key roles in shaping agronomic phenotypes and crop performance. For example, acetylation of lysine residues on histone H3 and H4 suggest that HATs were involved in drought stress responses in rice [89], wheat [90], and several other crops and vegetables [91,92]. Histones are also associated with complex traits such as heterosis, which is a cornerstone of hybrid breeding (Section “Genetics as a tool for improving breeding strategies”). In rice, inter-subspecific hybrids displayed allele-specific differences in histones H3K4me3 and H3K27me3 [93]. Similar findings in Arabidopsis identified epigenetic quantitative trait loci (QTLs) explaining up to 90% of heritable variation [94]. In addition, stress-induced changes of histone modifications have been shown to enhance plant adaptation, and could thus be targeted by breeding programs [95].

Finally, RNA processing and interplay with non-coding RNAs (ncRNAs) can shape the epigenetic landscape of genomes impacting genome integrity, and gene expression. For instance, modulation of RNA m^6A methylation has improved crop yield in rice and potato [96], and has been associated with biotic and abiotic stresses [97,98]. RNA-processing epigenetic modifications contribute to the post-transcriptional regulation of gene expression and represent a promising strategy for crop improvement.

LEVERAGING SINGLE-CELL SEQUENCING IN BREEDING

Advances in genetics driven by the use of NGS technologies, allowing sequencing of multiple plant genomes and transcriptomes, have led to impactful plant biology progress applied to agriculture. RNAseq, in particular, has become a powerful tool for investigating transcriptional variations across plant tissues and conditions, such as biotic and abiotic stresses [99,100]. However, RNAseq faces limitations, as traditional bulk methods often mask cell-specific responses due to tissue heterogeneity and developmental gradients. To overcome this limitation, single-cell RNA sequencing (scRNAseq, from protoplasts) and single-nucleus RNA sequencing (snRNAseq, from isolated nuclei) enable precise transcriptomic profiling of thousands of individual cells. These techniques reveal subtle biological variations often masked by bulk RNAseq methods [101] and have a bright future as complementary breeding methods.

Originally applied to mammalian systems, scRNAseq and snRNAseq have recently been adapted for plants. However, plant cells pose unique challenges due to their rigid cell walls, which complicate protoplast and nuclei isolation procedures from dense tissues. The first successful applications of single-cell sequencing in plants emerged in 2019 with

Arabidopsis thaliana [102–106], followed by crops like maize (*Zea mays*) [107–109] and rice (*Oryza sativa*) [110,111]. Recent protocol improvements now facilitate studies in non-model plants, including orphan crops. In practice, isolated nuclei or protoplasts are typically processed using 10X Genomics droplet-based systems, although alternative platforms from other providers are also available [112]. After library preparation, the samples undergo sequencing, and their data are visualized using techniques like Uniform Manifold Approximation and Projection (UMAP) and t-distributed stochastic neighbor embedding (t-SNE). These methods cluster cells or nuclei with similar transcriptomic profiles. Cluster annotations, based on known marker genes, are then used to construct gene regulatory networks (GRNs) by cell type. As with other RNA-seq methods, identifying candidate genes typically requires additional validation through genetic experiments, such as knocking out or knocking down the candidate genes or their regulatory factors (Figure 2).

The applications of single-cell studies in plant breeding are expanding, notably in yield improvement and tissue-specific responses to environmental stresses. Cereals like maize and rice have been focal points for single-cell research, particularly in roots and leaves, where studies have identified key genes involved in tissue development [107–109,113]. For example, Li et al. (2022) [113] used scRNA-seq to characterize maize root cell populations responding to nitrate availability and identified cell-type-specific regulators associated with nitrogen uptake efficiency. Because nitrogen-use efficiency is a highly quantitative trait traditionally evaluated at the whole-plant level, single-cell approaches may improve the precision of breeding strategies targeting nutrient acquisition and resource allocation. Similarly, Xu et al. (2025) [114] discovered stem cell regulators associated with heritable yield traits such as cob diameter and ear length in maize. These findings illustrate how cellular-resolution analyses can uncover developmental programs contributing to complex agronomic phenotypes. Other studies have revealed regulators that could enhance nitrogen uptake efficiency by targeting root responses to nitrate [113] or for physiological traits such as fruit senescence [115,116].

Single-cell studies also extend to plant-biotic interactions, including beneficial relationships like root nodules and arbuscular mycorrhizal symbioses. Research in model legumes, such as *Medicago truncatula* [117–120] and *Lotus japonicus* [121], and crops like soybean [122,123] have identified regulators that could enable cereals to form symbiotic structures or improve their capacity for beneficial microbial associations [124]. Conversely, single-cell techniques are also applied to study negative interactions. For instance, Yue et al. (2024) [125] used scRNAseq to characterize tomato leaf responses to tomato chlorosis virus infection and identified ERF4 as a master regulator involved in chlorosis development. Functional validation demonstrated that disruption of ERF4 mitigated chlorosis symptoms, highlighting the potential of single-cell-guided gene editing approaches for crop improvement.

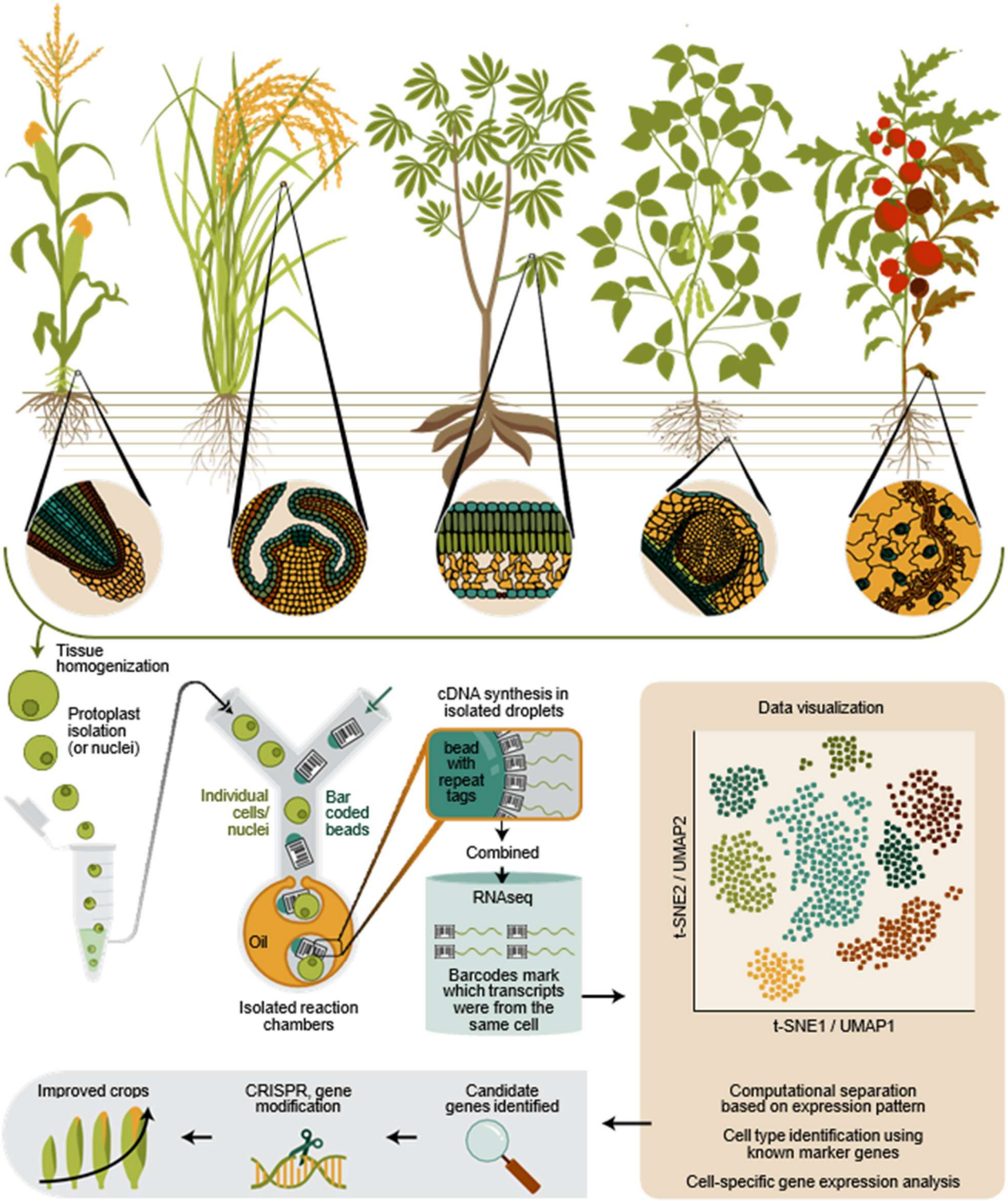

Figure 2. Single-cell omics in breeding. Workflow for asc/snRNAseq experiment in crop research. Plant tissue from economically important or orphan crops is used to isolate protoplasts or nuclei. mRNA transcripts from individual cells are captured using a microfluidic-based system, which encapsulate single cells with unique barcodes for high- throughput sequencing e.g., 10× Genomics. Downstream analysis identifies cell clusters, annotates them using known marker genes, and experimentally validates candidate genes. These genes can be utilized to enhance agronomically important traits through conventional breeding or precise tools like CRISPR. This approach can uncover novel genes involved in biological processes or abiotic and biotic interactions, advancing applications in crop improvement.

Orphan crops have also benefited from single-cell insights. Zhang et al. (2023) [126] profiled *Manihot esculenta* (Cassava) leaves, uncovering antioxidant compounds like flavonoids and carotenoids in mesophyll cells, while Li et al. (2024) explored metabolic regulation in *Hylocereus undatus* [115]. Other studies have highlighted single-cell applications in stress responses in *Brassica rapa* (Chinese cabbage) [127] and medicinal compound biosynthesis in *Catharanthus roseus* [128].

Beyond transcript abundance, single-cell technologies are increasingly integrated with epigenomic and *cis*-regulatory analyses. These approaches now allow the characterization of chromatin accessibility, transcription factor binding, and regulatory landscapes at cellular resolution. For example, Marand et al. (2021) generated a single-cell *cis*-regulatory atlas in maize that linked chromatin accessibility patterns to cell-type-specific gene expression programs [108]. Such studies demonstrate how single-cell approaches can identify *cis*-regulatory elements (CREs) and regulatory networks underlying agronomically important traits. These datasets provide valuable targets for both genome editing and *cis*-regulatory breeding strategies discussed in later sections of this review.

The challenge is whether single-cell analysis can be leveraged to improve complex quantitative traits. The classical single-locus genetic model has been expanded into a quantitative genetics framework [129–131], including epistasis interaction effects [132–135]. Integrating this framework with single-cell data offers the opportunity to account for cellular heterogeneity and intercellular regulatory effects in plants. In a single cell context, narrow sense heritability, defined as the proportion of additive genetic variance relative to total phenotypic variance would, in principle, be 'narrower' as the genetic variance would reflect differences within a population of individual cells rather than among bulk tissues or whole plants typically analyzed in population genetics. From a breeder's equation perspective (Equation (1)), this refined resolution could potentially increase the response to selection, or genetic gain (ΔG), per breeding cycle (t). On the other hand, traditional quantitative genetics models that describe polygenic effects on phenotypic variance may represent an oversimplification, much like earlier single or multilocus models, when compared to the cellular complexity revealed by single-cell approaches. Experimental computational approaches on single-cell based quantitative genetics, such as Perturb-seq or MPRA approaches (e.g., Queitsch lab at University of Washington) will likely provide substantial advances to the multilocus quantitative model established in breeding.

$$R = h^2 S = i\, r\, \sigma_g \;\rightarrow\; \Delta G = i\, r\, \sigma_g / t \quad (1)$$

Breeder's equation, where R = response realized by selection, S = selection differential, i = standardized selection differential, selection accuracy (r), h^2 = heritability, σ_g = genotypic standard deviation, and genetic gain (ΔG).

Single-cell technologies also provide an important conceptual bridge between genetics, epigenetics, *cis*-regulation, and gene editing. By resolving developmental trajectories and regulatory networks at cellular resolution, these approaches facilitate the identification of candidate genes, epigenetic states, and regulatory elements associated with agronomically important traits. These discoveries can subsequently be exploited through modern genome editing and molecular breeding strategies.

Despite its promise, single-cell omics technology in plants has limitations. Genes identified under controlled conditions require validation in field environments, and the high complexity and heterogeneity of plant tissues demand continued advances in computational and statistical methods. Future studies will benefit from integrating multi-omics approaches, including spatial transcriptomics and epigenomics, to better capture the dynamic cell-type cellular landscapes underlying plant environmental and developmental responses [136]. As these technologies continue to mature, hypothesis-driven single-cell studies will likely become an essential component of next-generation crop breeding programs.

CIS-REGULATION: CIS-REGULATORY ELEMENTS AS OPPORTUNITIES FOR CROP BREEDING

Many attempts at identifying the mutations responsible for phenotypes of agronomic interest have historically focused on coding genes (Figure 1 and Section “Genetics as a tool for improving breeding strategies”). However, genome-wide association studies have, in the second part of the 2010s, highlighted the key role of non-coding genetic variation at CREs in determining yield and other traits of agronomical interest in many crop species [137–139], mainly influencing regulation of plant development [140,141]. The main candidates as carriers of causal mutations in such regions are *cis*-regulatory elements (CREs), short non-coding DNA sequence motifs bound by sequence-specific transcription factors (TFs) [142]. They include promoters that fix the transcription machinery, and distal elements (dCREs), also called enhancers and silencers, that interact with their target gene’s promoter in *cis* through a chromatin 3D loop, stabilizing or preventing the fixation of the transcription machinery.

Crop responses to biotic and abiotic stresses are also often largely determined by CRE [143]. Beyond promoters and distal CRE, genetic variations in the untranslated regions (UTRs) and introns can also affect integrated phenotypes through modification of the gene expression levels [139]. Targeting genetic variations within regulatory elements in crop breeding programs is thus a promising path to select for genotypes presenting enhanced yield, and most importantly, for phenotypes better adapted to future environments in the context of climate change (Table 1). This is partially reflected in the fact that breeding methods described in Section “Genetics as a tool for improving breeding strategies”, such as

hybrid breeding or other types of population structure, lead to changes in allele frequencies at *cis*-expression quantitative traits loci (*cis*-eQTLs), genetic variants associated with nearby gene expression levels.

Recent advances in new breeding technologies, particularly CRISPR-based approaches have demonstrated that it is indeed possible to identify and modify functional CREs with reliable effect, resulting in measurable effects on target phenotype [144]. Overexpressing or deleting promoters of genes involved in developmental traits have enabled significative improvements to be measured in yield-related traits such as fruit size [145,146] and inflorescence type in tomatoes [147], heading date in rice [148] or grain yield in rice and maize [110,149]. By modifying promoters or 5'UTRs, it has been possible to improve other traits of agronomical interest in various crops, including modifying tomato fruit color [150], rice chalkiness [151], creating gradients of amylose content in rice [152–154], increasing sugar content in strawberries [155], and ascorbic acid levels in lettuce and lettuce [156]. These approaches have also been successful in improving stress-response-related phenotypes such as water use efficiency of rice [157], drought tolerance in maize [158,159], pest resistance in rice [160,161] and citrus [162]. Beyond genetic variations, epigenetic variations, particularly DNA methylation patterns at CRE (see Section "Leveraging epigenetics in epi-breeding"), play a role in important agronomical traits in several crops [163–165] and may serve as stress-response memory [166]. Thus, targeting CREs and harnessing these epigenetic variations represent promising avenues that deserve further investigation.

Table 1. Summary of *cis*-regulatory elements (CREs) described by crops, target gene, and their phenotypic effect.

Organism	Element type	Target gene	Phenotype	Ref.
Maize	Promoter	*CLE*	Grain yield traits (cob diameter, kernel row number, kernel depth)	[110]
Maize	Promoter	*Zmm28*	Drought tolerance; low N stress tolerance	[159]
Maize	Promoter	*ARGOS8*	Drought tolerance	[158]
Rice	Promoter	*HD1, Ghd7 and DTH8*	Heading date	[148]
Rice	Promoter	*IPA1*	Grain yield traits (grains per panicle and tiller number)	[149]
Rice	Promoter	*SLG7*	Chalkiness	[151]
Rice	Promoter	*Wx*	Amylose content	[153]
Rice	Promoter	*PSBS1*	Water use efficiency	[157]
Rice	Promoter	*Xa13*	Blight resistance	[160]
Rice	Promoter	*OsSWEET14*	*Xanthomonas oryzae pv. Oryzae* (Xoo) resistance	[161]
Wild Tomato	Promoter	*SP5G, SP, SlCLV3 and SlWUS, SlGGP1*	Day neutrality, Shoot architecture, Fruit size, Ascorbic acid levels	[148]
Tomato	Promoter	*SlCLV3*	Fruit size	[145]
Tomato	Promoter	*WOX9*	Inflorescence architecture	[147]
Tomato	5' UTR	*CRTISO*	Fruit color	[150]
Strawberry	uORFs	*FvebZIPs1.1*	Sugar content	[155]
Lettuce	uORF	*LsGGP2*	Ascorbic acid levels	[156]

What challenges do we face in our efforts to consistently implement *cis*-regulation in breeding? Most developmental traits and stress-response phenotypes often display a highly polygenic architecture and may involve a multitude of CREs. Two main concerns need to be overcome to improve breeding by targeting CREs. First, genetic variations at CREs may have a significant effect on the expression of their target gene(s), but they may have a small impact on the final phenotype value [140]. To this end, it will be necessary to select for beneficial alleles at different CREs at once. Second, pervasive pleiotropy may be a barrier. New CRISPR approaches (see Section "Gene editing technologies in breeding") are aimed at modifying several regulatory regions at once to overcome the first caveat [167]. In addition, geneticists' attempts at unveiling crops' regulatory code will help breeders to identify the most relevant CREs [168,169]. Traits of agronomical interest are often partially correlated and share genetic bases [170], which means that it may be complex to select for one trait while not bringing deleterious consequences to another. Furthermore, progress in quantitative genetics approaches have allowed us to map these partial correlations between traits and the identification of their genetic basis [171,172]. Advances in machine learning have enabled the extraction of meaningful patterns from large and diverse datasets ranging from genomics to phenomics [169], coupled with progresses in long-read sequencing technologies [173,174] that provide better assemblies of intergenic regions and facilitate comparative genomics analyses of distal CREs [143], have greatly enhanced the identification of genomic targets of interest. Inference of these gene regulatory networks underlying the trait expression has also reduced the candidate pool of CREs [175,176]. Among the structured regulatory networks, we should narrow the candidate pools to a subset of CREs that are well-connected in pathways of interest but fairly isolated from others [177,178]. This increasing knowledge in determining, filtering and deciphering the importance of CREs in the molecular bases of crop phenotypes has allowed the selection of genomic and epigenetic alleles for breeding purposes. Therefore, *cis*-regulation CREs offer good future potential for genetic crop improvement, particularly in variety development in the context of climate change.

GENE EDITING TECHNOLOGIES IN BREEDING

All of these advances in the understanding of the molecular bases of the complex phenotypes that determine crop performance are now beginning to be included in breeding programs. As highlighted in Section "Genetics as a tool for improving breeding strategies", one of the aims of genetics in breeding is to combine these beneficial genetic variants to select for desired traits. However, dependence on spontaneous beneficial mutations is very time-consuming [179,180]. Consequently, methods for inducing genetic variation are gaining attention, as they have the capacity to rapidly generate novel mutations [181]. Traditional physical and chemical mutagenesis treatments can generate random mutations, but

both require large-scale screening to discover which ones are valuable [182,183].

The emergence of gene editing technology has overcome the unpredictability of mutations by enabling precise, targeted modifications within specific genomic sequences [184,185]. Technologies such as single-cell sequencing (discussed in Section "Leveraging single-cell sequencing in breeding") have elevated plant breeding from tissue level to single-cell level, greatly enhancing the resolution of molecular mechanisms underlying target traits. This provides targets for gene editing in breeding, which are then achieved through programmable molecular tools that recognize and interact with predetermined DNA sites. Early platforms, such as Zinc Finger (ZF) and Transcription Activator-Like Effector (TALEN) DNA-binding domains, required complex protein engineering for each new target. In contrast, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system is distinguished by its simplicity and powerful programmability, facilitated by a guide RNA (gRNA) that is complementary to the target DNA sequence and a CRISPR-associated (Cas) nuclease. The Cas-gRNA complex locates its target by scanning for a protospacer adjacent motif (PAM) sequence, enabling highly specific DNA binding [186–189].

The canonical Cas nuclease primarily induces random small insertions and deletions (indels) at the target site, often resulting in gene knockouts that are valuable for functional genetics and breeding [190]. Gene editing technology has since evolved beyond simple nucleases. By fusing the CRISPR system with diverse effector domains such as deaminases, transposases, and recombinases, researchers can now introduce a wide spectrum of genomic variations. For instance, base editing permits precise single-nucleotide substitutions, while prime editing mediates all 12 possible base-to-base conversions, in addition to targeted insertions or deletions [191,192]. These advances are driving innovation in breeding procedures, offering superior efficiency and precision over conventional techniques (Table 2). Notably, these highly precise tools are well-suited for modifying the *cis*-regulatory elements detailed in Section "*cis*-regulation: *cis*-regulatory elements as opportunities for crop breeding", allowing breeders to fine-tune gene expression for complex traits like quality and disease resistance rather than completely knocking out gene function.

Although gene editing has markedly enhanced the efficiency of genetic breeding, it has typically been limited to modifying a small number of genes per experiment. In the context of global climate change, which threatens crop resilience, there is a growing need for more transformative strategies. One promising approach is the de novo domestication of wild species, which naturally harbor robust resistance to biotic and abiotic stresses. Leveraging powerful gene-editing toolkits [193], breeders can now rapidly introduce domestication traits into wild relatives. Successful examples in tomato and rice demonstrate that gene editing can

recapitulate centuries of natural domestication in a laboratory setting by simultaneously altering multiple key genes [194–196].

Table 2. Comparison of genetic editing tools, key mechanisms and main advantages.

Tool/Technology	Key Mechanism	Main Advantages
Zinc Fingers (ZF) & TALEs	Protein-based DNA-binding domains designed to target specific sequences.	• Were the first platforms to enable targeted genome editing.
CRISPR-Cas9	RNA-guided (gRNA) DNA cleavage by Cas nuclease.	• Highly programmable to design (via gRNA). • Effective for generating gene knockouts (indels).
Base Editing	CRISPR-guided deaminase enzyme that chemically converts one base into another.	• Enables precise single-nucleotide changes without double-strand DNA breaks. • Broader editing scope than standard knockouts.
Prime Editing	CRISPR-guided reverse transcriptase that uses a prime editing guide RNA (pegRNA).	• Can mediate all 12 base-to-base conversions. • Can perform targeted insertions and deletions.

A significant advantage of gene-edited crops is that their genomes can be modified without integrating foreign genetic material, resulting in products that may be indistinguishable from those developed through conventional breeding. Nevertheless, a primary concern for clinical and agricultural applications remains off-target effects—the introduction of unintended, potentially deleterious mutations at non-target genomic sites [197]. Significant progress has been made in mitigating this risk through the development of high-fidelity Cas variants, optimized sgRNA designs, prime editors, and anti-CRISPR proteins [198,199]. A future direction involves integrating the concepts of epi-breeding described in Section “Leveraging epigenetics in epi-breeding”. For instance, by exploiting epigenetic marks, “reader” proteins could be recruited to specific loci to modulate gene transcription by rewriting epigenetic states, thereby directing phenotypic outcomes without altering the underlying DNA sequence [88,200].

Regulatory frameworks are evolving in tandem with technological improvements. An increasing number of countries and regions have established policies that exempt certain gene-edited products from the stringent regulations applied to traditional genetically modified organisms (GMOs) [201]. For example, the United States and Japan have approved the commercial marketing of gene-edited crops, including high-GABA tomatoes and non-bitter mustard greens. This policy shift facilitates a potential global adoption of gene-edited crops. As technology continues to advance, it holds the promise of developing crop varieties that meet the long-standing demands for enhanced adaptability and resilience in a changing global environment.

DISCUSSION

Breeding methods have undoubtedly been essential in crop improvement. The increasing knowledge of plant genetics, together with advances in molecular technologies, has not only deepened our understanding of complex genetic relationships underpinning phenotypes but has also greatly enhanced the potential of traditional breeding methods. Since the rediscovery of Mendel's laws, genetics has increasingly focused first on the efficient identification of genes, and then on their functional characterization (Figure 1). This progress, leveraged by quantitative genetics and functional genomics, has improved our understanding of key scientific questions such as domestication, hybrid breeding, heterosis, and molecular breeding. As an example, the SSD method [14] has greatly contributed to line development of self-pollinated crops and also laid the foundations for RIL development. Genome sequencing tools coupled with RIL populations shed light on genetic questions underlying a single gene and more complex traits [16,17,19,20].

Current crop breeding should shift, and in some cases is shifting quickly, from unpredictable discovery to a more predictable, engineering-oriented discipline. As discussed in this review, the core principles of traditional genetic breeding (Section "Genetics as a tool for improving breeding strategies") have been improved with intrinsic genetic discoveries and are nowadays greatly enhanced by next-generation multi-omics and molecular tools. We believe that single-cell sequencing (Section "Leveraging single-cell sequencing in breeding") will play an important role in the future of breeding, since it provides much higher cellular resolution and will allow breeders to pinpoint novel, cell-specific functional targets that will otherwise be missed by bulk tissue analyses (Figure 2). This approach will synergize with established single-locus and classical quantitative genetics to further unveil the relationships behind polygenic trait heritability. The expansion of classical quantitative models will be twofold. The ability to partition additive genetic variance nested at cellular population level offers new opportunities, as single-cell sequencing of a mutant can capture rare cell response with specific traits (e.g., rhizobia interactions, [120]). The possibility of optimizing resource allocation taking the cell into consideration rather than the confounding effect of the plant level phenotype should, in principle, enable more precise estimates of a trait's narrow sense heritability. Thus, incorporating single-cell data into quantitative models could optimize the estimation of additive genetic variance within an individual's cell population relative to total phenotypic variance, ultimately leading to increasing genetic gains over time. We believe that this strategy could enhance trait identification, as single-cell data can uncover key genes [107,108,113] and reveal distinct molecular phenotypes [114,115] that have remained hidden at population level across the genome.

Meanwhile, our growing understanding of dynamic epigenetic landscapes (Section “Leveraging epigenetics in epi-breeding”) creates further possibilities for breeding. Characterizing the genetic structure of a population is a requirement for crop improvement, in particular in hybrid breeding (see Section “Genetics as a tool for improving breeding strategies”). Similarly, the epigenetic structure characterization of a population will be a highly relevant factor to consider for the crossing matrix of breeding programs. In addition, significant research has focused on understanding the contribution of epigenetic marks’ to explain the mechanisms of heritable transcriptional states independently of DNA sequence [202] that lead to a better understanding of complex traits [94,203]. It is known that some epi-mutations are stable and heritable in offspring [64]. High levels of heritability are ideal for QTL detection; therefore, QTL identification of epigenetic traits (epiQTL) would have the properties to become targets of natural or artificial selection, and serve as markers for marker-assisted selection. On the other hand, specific DNA methyltransferases may have single targets. However, the potential partial-to-complete redundancy of different DNA methyltransferases for establishing and maintaining DNA methylation patterns in a locus or in a tissue-specific manner [204] can present a challenge. Finally, provided that DNA methylation patterns can be stably transmitted through meiosis and that large phenotypic variations may be due to epimutations, epigenome editing holds great promise by creating additional phenotypic variability in the same genetic background. One powerful but challenging possibility could be to breed cultivars with the potential to trigger a high response to certain environmental cues (e.g., drought), exhibiting a stand-by epigenomic state that would relax chromatin packing and induce favorable gene expression. We could couple gene editing tools by targeting specific epigenetic marks by reprograming epigenetic *de novo* functions in cells [88,200]. As an alternative to gene editing, we propose the direct application of proteins and RNAs to permanently modify the methylation status of a locus in a plant cell [205,206].

Importantly, single-cell approaches should not be viewed solely as transcriptomic tools, but also as extensions of classical quantitative genetics. Traditional breeding and quantitative genetic models typically evaluate phenotypes at the whole-plant or tissue level, often overlooking cellular heterogeneity and rare developmental responses. Single-cell sequencing provides an opportunity to partition biological variation at the cellular level and identify specific cell populations contributing disproportionately to quantitative traits. In principle, integrating single-cell information into quantitative genetic frameworks could improve estimates of additive genetic variance and narrow-sense heritability by reducing phenotypic noise generated by tissue heterogeneity.

cis-regulatory mechanisms (Section "*cis*-regulation: cis-regulatory elements as opportunities for crop breeding") can change breeding strategies that have in some cases relied on gene knockouts. Instead, researchers are increasingly focusing on precise, spatiotemporal fine-tuning of gene expression, which helps optimize complex traits without causing unwanted linkage drag effects, e.g., yield penalties. We also envisage feasible and effective use of *cis*-regulation in modern breeding as an alternative to gene knockout approaches. Breeding leverages natural diversity as a source of resilience (abiotic and biotic stresses) for high-yielding varieties. However, the effective use of such natural variation resources and intrinsically the fine-tuning of the gene expression regulation underlying this natural phenotypic variation at the genome-wide level remains a challenge. Similarly, the identification of loci to target and also the functional variants that bring *cis*-element efficiency within the whole genome of *cis*-acting regulatory loci are key from a genome editing standpoint. Thus, there is a need for comprehensive maps of functional variation at transcription factor binding sites (TFBS), for example by quantifying haplotype-specific TF footprints [207]. A growing body of literature finds a likely relationship between siRNAs (via the RdDM pathway), inbreeding, and transcriptional activity of growth regulatory networks which would be interesting to investigate further for crop breeding purposes. A recent study of F1 parents-offspring in maize identified differentially trans-regulated accessible chromatin regions (ACR) related to heterosis inheritance [208], highlighting the importance of the *cis*-regulatory element in terms of chromatin accessibility inheritance and the expression patterns of heterotic phenotypes. The gap presented by these challenges is being closed both through advances in long-read sequencing technologies that more accurately assemble the intergenic regions in often complex genomes; and also, due to advances in molecular biology techniques that allow us to more precisely identify these *cis*-regulatory elements. As described in Section "*cis*-regulation: cis-regulatory elements as opportunities for crop breeding", CREs are particularly relevant to regulating abiotic environmental stresses, and thus, selecting for genetic and epigenetic alleles at CREs will be a prospective breeding strategy to consider for crop adaptation to climate change.

Finally, gene-editing technologies have rapidly evolved in the past decade (see Section "Gene editing technologies in breeding") and have proven their effectiveness in turning all these molecular possibilities into practical outcomes in favor of sustainable agriculture. For instance, we outlined their potential to modify entire *cis*-regulatory elements to show reliable regulatory variants as alternative 'allele' sources of crop improvement even when introduced in different genetic backgrounds.

In this review we revisited and proposed scientific advances in genetics, single-cell analysis, epigenetic marks, *cis*-regulation, and gene editing offering remarkable value both now and in future to improve traditional breeding strategies by combining genomic and functional knowledge with cutting-edge biotechnology to ensure a sustainable future for our society.

DATA AVAILABILITY

All data is used and presented in the manuscript.

AUTHOR CONTRIBUTIONS

EDM: Conceptualization, funding acquisition, writing—review and editing; MF: writing—review and editing; REV: writing—review and editing; JS: writing—review and editing.

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES

1. Mendel G. Verhandlungen des naturforschenden vereines in Brünn. Available from: https://www.biodiversitylibrary.org/bibliography/70769. Accessed on 2026 Jan 15.
2. Correns C. G. Mendel’s Regal uber das Verhalten der Nach-kommenschaft der Rassenbasterde. In: Gesammelte Abhandlungen zur Vererbungswissenschaft aus Periodischen Schriften 1899–1924. Berlin (Germany): Springer; 1900. doi: 10.1007/978-3-642-52587-2_2
3. Bateson W, Bateson W, Mendel G, Leighton AG. Mendel’s principles of heredity, by W. Bateson. Cambridge (UK): University Press; 1909. doi: 10.5962/bhl.title.1057
4. de Vries H. Das Spaltungsgesetz der Bastarde. Bet Dtsch Bet Ges. 1900;18(3):83-90.
5. Gayon J, Zallen DT. The Role of the Vilmorin Company in the Promotion and Diffusion of the Experimental Science of Heredity in France, 1840–1920. J Hist Biol. 1998;31(2):241-62. doi: 10.1023/A:1004335619901

6. Johannsen W. Heredity in populations and pure lines—A Contribution to the Solution of the Outstanding Questions in Selection. In: Classic Papers in Genetics. Englewood Cliffs (NJ, US): Prentice-Hall, Inc.; 1959. pp. 20-6.
7. Allard RW. Principles of Plant Breeding. Hoboken (NJ, US): John Wiley & Sons; 1999.
8. Melchers LE, Lows AE. The development of Sorghums resistant to Milo disease. Technical Bulletin 55. 1943. Available from: https://www.extension.k-state.edu/historicpublications/pubs/STB055.pdf. Accessed on 2026 Jan 15.
9. Odvody GN, Dunkle LD. Periconia root rot. Available from: https://oar.icrisat.org/804/1/RA_00066.pdf#page=61. Accessed on 2026 Jan 15.
10. Brown PJ, Paterson AH. Bridging classical and molecular genetics of sorghum plant stature and maturity. In: Genomics of the Saccharinae. New York (NY, US): Springer; 2013; pp. 333-45. doi: 10.1007/978-1-4419-5947-8_14.
11. Ordonio R, Ito Y, Morinaka Y, Sazuka T, Matsuoka M. Molecular breeding of Sorghum bicolor, a novel energy crop. Int Rev Cell Mol Biol. 2016;321:221-57. doi: 10.1016/bs.ircmb.2015.09.001
12. Nilsson-Ehle H. Kreuzungsuntersuchungen an hafer und weizen. Z.Ver-erbungslehre. 1910;3:290-1. doi: 10.1007/BF02047779
13. East EM. A Mendelian Interpretation of Variation that is Apparently Continuous. Am Nat. 1910;44(518):65-82. doi: 10.1086/279117
14. Goulden CH. Problems in plant selection. CABI Databases. Available from: https://www.cabidigitallibrary.org/doi/full/10.5555/19411600504. Accessed on 2026 Jan 15.
15. Brim CA. A modified pedigree method of selection in soyabeans. Crop Sci. 1966;6:220.
16. Lander ES, Botstein D. Mapping Complex Genetic Traits in Humans: New Methods Using a Complete RFLP Linkage Map. Cold Spring Harb Symp Quant Biol. 1986;51:49-62. doi:10.1101/SQB.1986.051.01.007
17. Broman KW, Wu H, Sen Ś, Churchill GA. R/qtl: QTL mapping in experimental crosses. Bioinformatics. 2003;19(7):889-90.
18. Adak A, Murray SC, Calderón CI, Infante V, Wilker J, Varela JI, et al. Genetic mapping and prediction for novel lesion mimic in maize demonstrates quantitative effects from genetic background, environment and epistasis. Theor Appl Genet. 2023;136(7):155. doi: 10.1007/s00122-023-04394-y
19. Fukunaga K, Abe A, Mukainari Y, Komori K, Tanaka K, Fujihara A, et al. Recombinant inbred lines and next-generation sequencing enable rapid identification of candidate genes involved in morphological and agronomic traits in foxtail millet. Sci Rep. 2022;12:218. doi: 10.1038/s41598-021-04012-1
20. Wang Y, VandenLangenberg K, Wen C, Wehner TC, Weng Y. QTL mapping of downy and powdery mildew resistances in PI 197088 cucumber with genotyping-by-sequencing in RIL population. Theor Appl Genet. 2018;131(3):597-611. doi: 10.1007/s00122-017-3022-1
21. Jansky S, Hamernik A, Endelman JB. Diploid Interspecific Recombinant Inbred Lines for Genetic Mapping in Potato. Am J Potato Res. 2024;101(2):153-61. doi: 10.1007/s12230-024-09953-7

22. Harlan HV, Pope MN. The use and value of back-crosses in small-grain breeding. J Hered. 1922;13(7):319-22.
23. Briggs FN. The use of the backcross in crop improvement. Am Nat. 1938;72(740):285-92.
24. Hospital F. Marker-assisted breeding. In: Plant molecular breeding. Oxford (UK): Blackwell Publishing; Boca Raton (FL, US): CRC Press; 2003.
25. Kumar PKC, Bellundagi A, Krishna H, Mallikarjuna MG, Thimmappa RK, Rai N, et al. Development of bread wheat (*Triticum aestivum* L) variety HD3411 following marker-assisted backcross breeding for drought tolerance. Front Genet. 2023;14:1046624. doi: 10.3389/fgene.2023.1046624
26. Arruda MP, Brown P, Brown-Guedira G, Krill AM, Thurber C, Merrill KR, et al. Genome-Wide Association Mapping of Fusarium Head Blight Resistance in Wheat using Genotyping-by-Sequencing. Plant Genome. 2016;9(1):1-14. doi: 10.3835/plantgenome2015.04.0028
27. Zheng W, Li S, Liu Z, Zhou Q, Feng Y, Chai S. Molecular marker assisted gene stacking for disease resistance and quality genes in the dwarf mutant of an elite common wheat cultivar Xiaoyan22. BMC Genet. 2020;21(1):45. doi: 10.1186/s12863-020-00854-2
28. Guo Y, Li B, Li M, Zhu H, Yang Q, Liu X, et al. Efficient marker-assisted breeding for clubroot resistance in elite Pol-CMS rapeseed varieties by updating the PbBa8.1 locus. Mol Breed. 2022;42(7):41. doi: 10.1007/s11032-022-01305-9
29. Ma F, Xu Y, Wang R, Tong Y, Zhang A, Liu D, et al. Identification of major QTLs for yield-related traits with improved genetic map in wheat. Front Plant Sci. 2023;14. doi: 10.3389/fpls.2023.1138696
30. Zhang X, Han F, Li Z, Wen Z, Cheng W, Shan X, et al. Map-based cloning and functional analysis of a major quantitative trait locus, BolC.Pb9.1, controlling clubroot resistance in a wild Brassica relative (Brassica macrocarpa). Theor Appl Genet. 2024;137(2):41. doi: 10.1007/s00122-024-04543-x
31. Shull GH. A pure-line method in corn breeding. J Hered. 1909;(1):51-8.
32. Jones D. The effects of inbreeding and corssbreeding on development. Proc Natl Acad Sci U S A. 1918;4(8):246-50.
33. Sprague GF, Tatum LA. General vs. specific combining ability in single crosses of corn. Agron. J. 1942;34:923-32. doi: 10.2134/agronj1942.00021962003400100008x
34. Comstock RE, Robinson HF, Harvey PH. A breeding procedure designed to make maximum use of both general and specific combining ability. Agron. J. 1949;41(8):360-7. doi: 10.2134/agronj1949.00021962004100080006x
35. Jenkins MT. The segregation of genes affecting yield of grain in maize. J Am Soc Agron. 1940;26:199–204.
36. Hull FH. Recurrent selection and overdominance. In: Heterosis: A Record of Researches Directed Toward Explaining and Utilizing the Vigor of Hybrids. Ames (IA, US): Iowa State College Press; 1952.

37. Havey MJ. The Use of Cytoplasmic Male Sterility for Hybrid Seed Production. In: Daniell H, Chase C, editors. Molecular Biology and Biotechnology of Plant Organelles: Chloroplasts and Mitochondria. Dordrecht (the Netherlands): Springer; 2004. p. 623-34. doi: 10.1007/978-1-4020-3166-3_23
38. 39. Jaqueth JS, Li B, Vollbrecht E. Pentatricopeptide repeat 153 (PPR153) restores maize C-type cytoplasmic male sterility in conjunction with RF4. PLOS ONE. 2024 juil;19(7):e0303436. doi:10.1371/journal.pone.0303436
39. Liu W, Zheng MY, Polle EA, Konzak CF. Highly Efficient Doubled-Haploid Production in Wheat (*Triticum aestivum* L.) via Induced Microspore Embryogenesis. Crop Sci. 2002;42(3):686-92. doi: 10.2135/cropsci2002.6860
40. Kim KM, Baenziger PS. A simple wheat haploid and doubled haploid production system using anther culture. CellDevBiol-Plant. 2005;41(1):22-7. doi: 10.1079/IVP2004594
41. Gallais A, Bordes J. The Use of Doubled Haploids in Recurrent Selection and Hybrid Development in Maize. Crop Sci. 2007;47(S3):S-190-S-201. doi: 10.2135/cropsci2007.04.0019IPBS
42. Labroo MR, Ali J, Aslam MU, de Asis EJ, dela Paz MA, Sevilla MA, et al. Genomic Prediction of Yield Traits in Single-Cross Hybrid Rice (*Oryza sativa* L.). Front Genet. 2021;12:692870. doi: 10.3389/fgene.2021.692870
43. Fritsche-Neto R, Ali J, De Asis EJ, Allahgholipour M, Labroo MR. Improving hybrid rice breeding programs via stochastic simulations: number of parents, number of hybrids, tester update, and genomic prediction of hybrid performance. Theor Appl Genet. 2023;137(1):3. doi: 10.1007/s00122-023-04508-6
44. Knoch D, Werner CR, Meyer RC, Riewe D, Abbadi A, Lücke S, et al. Multi-omics-based prediction of hybrid performance in canola. Theor Appl Genet. 2021;134(4):1147-65. doi: 10.1007/s00122-020-03759-x
45. Michelmore RW, Paran I, Kesseli RV. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci U S A. 1991;88(21):9828-32. doi: 10.1073/pnas.88.21.9828
46. Li Z, Xu Y. Bulk segregation analysis in the NGS era: a review of its teenage years. Plant J. 2022;109(6):1355-74. doi: 10.1111/tpj.15646
47. Wambugu P, Ndjiondjop MN, Furtado A, Henry R. Sequencing of bulks of segregants allows dissection of genetic control of amylose content in rice. Plant Biotechnol J. 2018;16(1):100-10. doi: 10.1111/pbi.12752
48. Edae EA, Rouse MN. Bulked segregant analysis RNA-seq (BSR-Seq) validated a stem resistance locus in Aegilops umbellulata, a wild relative of wheat. PLOS ONE. 2019;14(9):e0215492. doi: 10.1371/journal.pone.0215492
49. Xie J, Guo G, Wang Y, Hu T, Wang L, Li J, et al. A rare single nucleotide variant in Pm5e confers powdery mildew resistance in common wheat. New Phytol. 2020;228(3):1011-26. doi: 10.1111/nph.16762
50. Zheng R, Deng M, Lv D, Tong B, Liu Y, Luo H. Combined BSA-Seq and RNA-Seq Reveal Genes Associated with the Visual Stay-Green of Maize (*Zea mays* L.). Int J Mol Sci. 2023;24(24):24. doi: 10.3390/ijms242417617

51. Lee JH, Kim JM, Kwon JK, Kang BC. Fine mapping of the Chilli veinal mottle virus resistance 4 (cvr4) gene in pepper (Capsicum annuum L.). Theor Appl Genet. 2025;138(1):19. doi: 10.1007/s00122-024-04805-8
52. Burnham CR. Discussions in Cytogenetics. Minneapolis (MN, US): Burgess Publ. Co.; 1962. p. 66-116.
53. Ramage RT. Balanced Tertiary Trisomics for Use in Hybrid Seed Production. Crop Sci. 1965;5(2):177-8. doi: 10.2135/cropsci1965.0011183X000500020026x
54. Fujito S, Akyol TY, Mukae T, Wako T, Yamashita K ichiro, Tsukazaki H, et al. Construction of a high-density linkage map and graphical representation of the arrangement of transcriptome-based unigene markers on the chromosomes of onion, *Allium cepa* L. BMC Genomics. 2021;22(1):481. doi: 10.1186/s12864-021-07803-y
55. Nakajima T, Yaguchi S, Hirata S, Abdelrahman M, Wada T, Mega R, et al. Effects of Drought Stress on Abscisic Acid Content and Its Related Transcripts in Allium fistulosum—A. cepa Monosomic Addition Lines. Genes. 2024;15(6):754.
56. Fehr W. Principles of cultivar development: theory and technique. New York (NY, US): Macmillan Publishing Company; 1991.
57. Wang Y, Cheng X, Yang X, Wang C, Zhang H, Deng P, et al. Molecular cytogenetics for a wheat–Aegilops geniculata 3Mg alien addition line with resistance to stripe rust and powdery mildew. BMC Plant Biol. 2021;21(1):575. doi: 10.1186/s12870-021-03360-4
58. Burnham CR. An “Oenothera” or multiple translocation method of establishing homozygous lines. Agron. J. 1946;38(8):702-7. doi: 10.2134/agronj1946.00021962003800080005x
59. Brar DS, Minocha JL. Multiple chromosomal interchanges in pearl millet. Theor Appl Genet. 1982;61:105-8. doi: 10.1007/BF00273875
60. Patterson EB. Properties and uses of duplicate-deficient chromosome complements in maize. In: Walden DB, editor. Maize breeding and genetics. New York (NY, US): John Wiley and Sons; 1978.
61. Zhou K, Wang S, Feng Y, Liu Z, Wang G. The 4E-ms System of Producing Hybrid Wheat. Crop Sci. 2006;46(1):250-5. doi: 10.2135/cropsci2005.0029
62. Lv R, Gou X, Li N, Zhang Z, Wang C, Wang R, et al. Chromosome translocation affects multiple phenotypes, causes genome-wide dysregulation of gene expression, and remodels metabolome in hexaploid wheat. Plant J. 2023;115(6):1564-82.
63. Miura K, Agetsuma M, Kitano H, Yoshimura A, Matsuoka M, Jacobsen SE, et al. A metastable DWARF1 epigenetic mutant affecting plant stature in rice. Proc Natl Acad Sci U S A. 2009;106(27):11218-23.
64. Hofmeister BT, Lee K, Rohr NA, Hall DW, Schmitz RJ. Stable inheritance of DNA methylation allows creation of epigenotype maps and the study of epiallele inheritance patterns in the absence of genetic variation. Genome Biol. 2017;18(1):155.
65. van Emmerik CL, van Ingen H. Unspinning chromatin: Revealing the dynamic nucleosome landscape by NMR. Prog Nucl Magn Reson Spectrosc. 2019;110:1-19.

66. Di Giammartino DC, Polyzos A, Apostolou E. Transcription factors: building hubs in the 3D space. Cell Cycle. 2020;19(19):2395-410.
67. Fu Y, Dominissini D, Rechavi G, He C. Gene expression regulation mediated through reversible m6A RNA methylation. Nat Rev Genet. 2014;15(5):293-306.
68. Tremblay BJ, Santini CP, Cheng Y, Zhang X, Rosa S, Qüesta JI. Interplay between coding and non-coding regulation drives the Arabidopsis seed-to-seedling transition. Nat Commun. 2024;15(1):1724.
69. Penterman J, Zilberman D, Huh JH, Ballinger T, Henikoff S, Fischer RL. DNA demethylation in the Arabidopsis genome. Proc Natl Acad Sci U S A. 2007;104(16):6752-7.
70. Schübeler D. Function and information content of DNA methylation. Nature. 2015;517(7534):321-6.
71. Boyes J, Bird A. DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein. Cell. 1991;64(6):1123-34.
72. Ravi S, Sharma A, Sharma AK. Roles of methyl-CpG-binding-domain (MBD) protiens in regulation of biological processes in plants. Physiol Mol Biol Plants. 2025;31(2):163-71.
73. Xu G, Lyu J, Li Q, Liu H, Wang D, Zhang M, et al. Evolutionary and functional genomics of DNA methylation in maize domestication and improvement. Nat Commun. 2020;11(1):5539.
74. Cao S, Sawettalake N, Li P, Fan S, Shen L. DNA methylation variations underlie lettuce domestication and divergence. Genome Biol. 2024;25(1):158
75. Zhao T, Guan X, Hu Y, Zhang Z, Yang H, Shi X, et al. Population-wide DNA methylation polymorphisms at single-nucleotide resolution in 207 cotton accessions reveal epigenomic contributions to complex traits. Cell Res. 2024;1-14.
76. Gardiner LJ, Joynson R, Omony J, Rusholme-Pilcher R, Olohan L, Lang D, et al. Hidden variation in polyploid wheat drives local adaptation. Genome Res. 2018;28(9):1319-32.
77. Papa CM, Springer NM, Muszynski MG, Meeley R, Kaeppler SM. Maize Chromomethylase Zea methyltransferase2 Is Required for CpNpG Methylation. Plant Cell. 2001;13(8):1919-28. doi: 10.1105/TPC.010064
78. Wang Z, Xia A, Wang Q, Cui Z, Lu M, Ye Y, et al. Natural polymorphisms in ZMET2 encoding a DNA methyltransferase modulate the number of husk layers in maize. Plant Physiol. 2024;195(3):2129-42. doi: 10.1093/plphys/kiae113
79. Lin J, Song N, Liu D, Liu X, Chu W, Li J, et al. Histone acetyltransferase TaHAG1 interacts with TaNACL to promote heat stress tolerance in wheat. Plant Biotechnol J. 2022;20(9):1645.
80. Han T, Wang F, Song Q, Ye W, Liu T, Wang L, et al. An epigenetic basis of inbreeding depression in maize. Sci Adv. 2021;7(35):eabg5442.
81. Muyle AM, Seymour DK, Lv Y, Huettel B, Gaut BS. Gene Body Methylation in Plants: Mechanisms, Functions, and Important Implications for Understanding Evolutionary Processes. Genome Biol Evol. 2022;14(4):evac038. doi: 10.1093/gbe/evac038

82. Pfluger J, Wagner D. Histone modifications and dynamic regulation of genome accessibility in plants. Curr Opin Plant Biol. 2007;10(6):645-52.
83. Kouskouti A, Talianidis I. Histone modifications defining active genes persist after transcriptional and mitotic inactivation. EMBO J. 2004;24(2):347.
84. Ng RK, Gurdon JB. Epigenetic memory of an active gene state depends on histone H3. 3 incorporation into chromatin in the absence of transcription. Nature Cell Biol. 2008;10(1):102-9.
85. Kouzarides T. Chromatin modifications and their function. Cell. 2007;128(4):693-705.
86. Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genet. 2016;17(8):487-500.
87. Brownell JE, Zhou J, Ranalli T, Kobayashi R, Edmondson DG, Roth SY, et al. Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell. 1996;84(6):843-51.
88. Berger SL. The complex language of chromatin regulation during transcription. Nature. 2007;447(7143):407-12.
89. Kumar V, Thakur JK, Prasad M. Histone acetylation dynamics regulating plant development and stress responses. Cellular and Molecular Life Sciences. 2021 May;78(10):4467-86.
90. Li H, Liu H, Pei X, Chen H, Li X, Wang J, et al. Comparative genome-wide analysis and expression profiling of histone acetyltransferases and histone deacetylases involved in the response to drought in wheat. J Plant Growth Regul. 2022;41(3):1065-78.
91. Eom SH, Hyun TK. Histone acetyltransferases (HATs) in Chinese cabbage: Insights from histone H3 acetylation and expression profiling of HATs in response to abiotic stresses. J Am Soc Hortic Sci. 2018;143(4):296-303.
92. Nunez-Vazquez R, Desvoyes B, Gutierrez C. Histone variants and modifications during abiotic stress response. Front Plant Sci. 2022;13:984702.
93. Qi T, Wang M, Wang P, Wang L, Wang J. Insights into heterosis from histone modifications in the flag leaf of inter-subspecific hybrid rice. BMC Plant Biol. 2024;24(1):767.
94. Cortijo S, Wardenaar R, Colomé-Tatché M, Gilly A, Etcheverry M, Labadie K, et al. Mapping the epigenetic basis of complex traits. Science. 2014;343(6175):1145-8.
95. Wan H, Cao L, Wang P, Hu H, Guo R, Chen J, et al. Genome-wide mapping of main histone modifications and coordination regulation of metabolic genes under salt stress in pea (*Pisum sativum* L). Epigenomes. 2024;11(12):1-40.
96. Yu Q, Liu S, Yu L, Xiao Y, Zhang S, Wang X, et al. RNA demethylation increases the yield and biomass of rice and potato plants in field trials. Nat Biotechnol. 2021;39(12):1581-8. doi: 10.1038/s41587-021-00982-9
97. Fu D, Wang H, Jiang B. Molecular mechanisms and crop improvement potential of RNA N6-methyladenosine in plants. aBIOTECH. 2025;6(3):489-509. doi: 10.1007/s42994-025-00228-1
98. Tang J, Chen S, Jia G. Detection, regulation, and functions of RNA N6-methyladenosine modification in plants. Plant Comm. 2023;4(3):1-15. doi: 10.1016/j.xplc.2023.100546

99. Martin L, Fei Z, Giovannoni J, Rose JKC. Catalyzing plant science research with RNA-seq. Front Plant Sci. 2013;4(66):1-10. doi: 10.3389/fpls.2013.00066
100. Wang H, Xu Y, Zhang Z, Zhang G, Tan C, Ye L. Development and application of transcriptomics technologies in plant science. Crop Design. 2024;3(2):100057. doi: 10.1016/j.cropd.2024.100057
101. Hwang B, Lee JH, Bang D. Single-cell RNA sequencing technologies and bioinformatics pipelines. Exp Mol Med. 2018;50(8):1-14. doi: 10.1038/s12276-018-0071-8
102. Denyer T, Ma X, Klesen S, Scacchi E, Nieselt K, Timmermans MCP. Spatiotemporal Developmental Trajectories in the Arabidopsis Root Revealed Using High-Throughput Single-Cell RNA Sequencing. Dev Cell. 2019;48(6):840-852.e5. doi: 10.1016/j.devcel.2019.02.022
103. Jean-Baptiste K, McFaline-Figueroa JL, Alexandre CM, Dorrity MW, Saunders L, Bubb KL, et al. Dynamics of Gene Expression in Single Root Cells of Arabidopsis Thaliana. Plant Cell. 2019;31(5):993-1011. doi: 10.1105/tpc.18.00785
104. Ryu KH, Huang L, Kang HM, Schiefelbein J. Single-Cell RNA Sequencing Resolves Molecular Relationships Among Individual Plant Cells. Plant Physiol. 2019;179(4):1444-56. doi: 10.1104/pp.18.01482
105. Shulse CN, Cole BJ, Ciobanu D, Lin J, Yoshinaga Y, Gouran M, et al. High-Throughput Single-Cell Transcriptome Profiling of Plant Cell Types. Cell Rep. 2019;27(7):2241-7. doi: 10.1016/j.celrep.2019.04.054
106. Zhang TQ, Xu ZG, Shang GD, Wang JW. A Single-Cell RNA Sequencing Profiles the Developmental Landscape of Arabidopsis Root. Mol Plant. 2019;12(5):648-60. doi: 10.1016/j.molp.2019.04.004
107. Bezrutczyk M, Zöllner NR, Kruse CPS, Hartwig T, Lautwein T, Köhrer K, et al. Evidence for phloem loading via the abaxial bundle sheath cells in maize leaves. Plant Cell. 2021;33(3):531-47. doi: 10.1093/plcell/koaa055
108. Marand AP, Chen Z, Gallavotti A, Schmitz RJ. A cis-regulatory atlas in maize at single-cell resolution. Cell. 2021;184(11):3041-55. doi: 10.1016/j.cell.2021.04.014
109. Ortiz-Ramírez C, Guillotin B, Xu X, Rahni R, Zhang S, Yan Z, et al. Ground tissue circuitry regulates organ complexity in maize and Setaria. Science. 2021;374(6572):1247-52. doi: 10.1126/science.abj2327
110. Liu L, Gallagher J, Arevalo ED, Chen R, Skopelitis T, Wu Q, et al. Enhancing grain-yield-related traits by CRISPR–Cas9 promoter editing of maize CLE genes. Nat Plants. 2021;7(3):287-94. doi: 10.1038/s41477-021-00858-5
111. Wang Y, Huan Q, Li K, Qian W. Single-cell transcriptome atlas of the leaf and root of rice seedlings. J Genet Genomics. 2021;48(10):881-98. doi: 10.1016/j.jgg.2021.06.001
112. Vandereyken K, Sifrim A, Thienpont B, Voet T. Methods and applications for single-cell and spatial multi-omics. Nat Rev Genet. 2023;24(8):494-515. doi: 10.1038/s41576-023-00580-2

113. Li X, Zhang X, Gao S, Cui F, Chen W, Fan L, et al. Single-cell RNA sequencing reveals the landscape of maize root tips and assists in identification of cell type-specific nitrate-response genes. Crop J. 2022;10(6):1589-600. doi: 10.1016/j.cj.2022.02.004
114. Xu X, Passalacqua M, Rice B, Demesa-Arevalo E, Kojima M, Takebayashi Y, et al. Large-scale single-cell profiling of stem cells uncovers redundant regulators of shoot development and yield trait variation . bioRxiv [Preprint]. 2025 Apr 17. doi: 10.1101/2024.03.04.583414
115. Li X, Li B, Gu S, Pang X, Mason P, Yuan J, et al. Single-cell and spatial RNA sequencing reveal the spatiotemporal trajectories of fruit senescence. Nat Commun. 2024;15(1):3108. doi: 10.1038/s41467-024-47329-x
116. Munaiz ED, Martínez S, Kumar A, Caicedo M, Ordás B. The senescence (stay-green)—an important trait to exploit crop residuals for bioenergy. Energies. 2020 Feb 11;13(4):790.
117. Cervantes-Pérez SA, Thibivilliers S, Laffont C, Farmer AD, Frugier F, Libault M. Cell-specific pathways recruited for symbiotic nodulation in the Medicago truncatula legume. Mol Plant. 2022;15(12):1868-88. doi: 10.1016/j.molp.2022.10.021
118. Ye Q, Zhu F, Sun F, Wang TC, Wu J, Liu P, et al. Differentiation trajectories and biofunctions of symbiotic and un-symbiotic fate cells in root nodules of Medicago truncatula. Mol Plant. 2022;15(12):1852-67. doi: 10.1016/j.molp.2022.10.019
119. Liu Z, Yang J, Long Y, Zhang C, Wang D, Zhang X, et al. Single-nucleus transcriptomes reveal spatiotemporal symbiotic perception and early response in Medicago. Nat Plants. 2023;9(10):1734-48. doi: 10.1038/s41477-023-01524-8
120. Pereira WJ, Boyd J, Conde D, Triozzi PM, Balmant KM, Dervinis C, et al. The single-cell transcriptome program of nodule development cellular lineages in Medicago truncatula. Cell Rep. 2024;43(2):113747. doi: 10.1016/j.celrep.2024.113747
121. Frank M, Fechete LI, Tedeschi F, Nadzieja M, Nørgaard MMM, Montiel J, et al. Single-cell analysis identifies genes facilitating rhizobium infection in Lotus japonicus. Nat Commun. 2023;14(1):7171. doi: 10.1038/s41467-023-42911-1
122. Liu Z, Kong X, Long Y, Liu S, Zhang H, Jia J, et al. Integrated single-nucleus and spatial transcriptomics captures transitional states in soybean nodule maturation. Nat Plants. 2023;9(4):515-24. doi: 10.1038/s41477-023-01387-z
123. Cervantes-Pérez SA, Zogli P, Amini S, Thibivilliers S, Tennant S, Hossain MS, et al. Single-cell transcriptome atlases of soybean root and mature nodule reveal new regulatory programs that control the nodulation process. Plant Comm. 2024;5(8):100984. doi: 10.1016/j.xplc.2024.100984
124. Serrano K, Tedeschi F, Andersen SU, Scheller HV. Unraveling plant–microbe symbioses using single-cell and spatial transcriptomics. Trends Plant Sci. 2024;29(12):1356-67. doi: 10.1016/j.tplants.2024.06.008

125. Yue H, Chen G, Zhang Z, Guo Z, Zhang Z, Zhang S, et al. Single-cell transcriptome landscape elucidates the cellular and developmental responses to tomato chlorosis virus infection in tomato leaf. Plant Cell Environ. 2024;47(7):2658-72. doi: 10.1111/pce.14906
126. Zang Y, Pei Y, Cong X, Ran F, Liu L, Wang C, et al. Single-cell RNA-sequencing profiles reveal the developmental landscape of the Manihot esculenta Crantz leaves. Plant Physiol. 2024;194(1):456-74. doi: 10.1093/plphys/kiad500
127. Sun X, Feng D, Liu M, Qin R, Li Y, Lu Y, et al. Single-cell transcriptome reveals dominant subgenome expression and transcriptional response to heat stress in Chinese cabbage. Genome Biol. 2022;23(1):262. doi: 10.1186/s13059-022-02834-4
128. Li C, Wood JC, Vu AH, Hamilton JP, Rodriguez Lopez CE, Payne RME, et al. Single-cell multi-omics in the medicinal plant Catharanthus roseus. Nat Chem Biol. 2023 Aug;19(8):1031–41. doi:10.1038/s41589-023-01327-0
129. Fisher RA. XV.—The Correlation between Relatives on the Supposition of Mendelian Inheritance. Earth and Environmental Science Trans R Soc Edinb. 1919;52(2):399-433. doi: 10.1017/S0080456800012163
130. Cockerham CC. An Extension of the Concept of Partitioning Hereditary Variance for Analysis of Covariances among Relatives When Epistasis Is Present. Genetics. 1954;39(6):859-82. doi: 10.1093/genetics/39.6.859
131. Falconer DS, Mackay TFC. Introduction to quantitative genetics. London (UK): Longman Group; 1996.
132. Hayman BI, Mather K. The Description of Genic Interactions in Continuous Variation. Biometrics. 1955;11(1):69-82. doi: 10.2307/3001481
133. Van Der Veen JH. Tests of non-allelic interaction and linkage for quantitative characters in generations derived from two diploid pure lines. Genetica. 1959;30:201-32. doi: 10.1007/BF01535675
134. Henderson CR. Best linear unbiased estimation and prediction under a selection model. Biometrics. 1975;423-47.
135. Zeng ZB, Wang T, Zou W. Modeling Quantitative Trait Loci and Interpretation of Models. Genetics. 2005;169(3):1711-25. doi: 10.1534/genetics.104.035857
136. Bawa G, Liu Z, Yu X, Tran LSP, Sun X. Introducing single cell stereo-sequencing technology to transform the plant transcriptome landscape. Trends Plant Sci. 2024;29(2):249-65. doi: 10.1016/j.tplants.2023.10.002
137. Rodgers-Melnick E, Vera DL, Bass HW, Buckler ES. Open chromatin reveals the functional maize genome. Proc Natl Acad Sci U S A. 2016;113(22):3177-84.
138. Ricci WA, Lu Z, Ji L, Marand AP, Ethridge CL, Murphy NG, et al. Widespread long-range cis-regulatory elements in the maize genome. Nat Plants. 2019;5(12):1237-49.
139. Crisp PA, Bhatnagar-Mathur P, Hundleby P, Godwin ID, Waterhouse PM, Hickey LT. Beyond the gene: epigenetic and cis-regulatory targets offer new breeding potential for the future. Curr Opin Biotechnol. 2022;73:88-94. doi: 10.1016/j.copbio.2021.07.008

140. Wallace JG, Bradbury PJ, Zhang N, Gibon Y, Stitt M, Buckler ES. Association Mapping across Numerous Traits Reveals Patterns of Functional Variation in Maize. Borevitz JO, editor. PLoS Genet. 2014;10(12):e1004845. doi: 10.1371/journal.pgen.1004845
141. Weber B, Zicola J, Oka R, Stam M. Plant enhancers: a call for discovery. Trends Plant Sci. 2016;21(11):974-87.
142. Marand AP, Jiang L, Gomez-Cano F, Minow MA, Zhang X, Mendieta JP, et al. The genetic architecture of cell type–specific cis regulation in maize. Science. 2025;388(6744):eads6601.
143. Marand AP, Eveland AL, Kaufmann K, Springer NM. cis -regulatory elements in plant development, adaptation, and evolution. Annu Rev Plant Biol. 2023;74(1):111-37. doi: 10.1146/annurev-arplant-070122-030236
144. Tuncel A, Pan C, Clem JS, Liu D, Qi Y. CRISPR–Cas applications in agriculture and plant research. Nat Rev Mol Cell Biol. 2025;26(6):419-41. doi: 10.1038/s41580-025-00813634-3.
145. Rodríguez-Leal D, Lemmon ZH, Man J, Bartlett ME, Lippman ZB. Engineering Quantitative Trait Variation for Crop Improvement by Genome Editing. Cell. 2017;171(2):470-480.e8. doi: 10.1016/j.cell.2017.08.030
146. Li T, Yang X, Yu Y, Si X, Zhai X, Zhang H, et al. Domestication of wild tomato is accelerated by genome editing. Nat Biotechnol. 2018;36(12):1160-3. doi: 10.1038/nbt.4273
147. Hendelman A, Zebell S, Rodriguez-Leal D, Dukler N, Robitaille G, Wu X, et al. Conserved pleiotropy of an ancient plant homeobox gene uncovered by cis-regulatory dissection. Cell. 2021;184(7):1724-39.
148. Zhou S, Cai L, Wu H, Wang B, Gu B, Cui S, et al. Fine-tuning rice heading date through multiplex editing of the regulatory regions of key genes by CRISPR-Cas9. Plant Biotechnol J. 2024;22(3):751-8. doi: 10.1111/pbi.14221
149. Song X, Meng X, Guo H, Cheng Q, Jing Y, Chen M, et al. Targeting a gene regulatory element enhances rice grain yield by decoupling panicle number and size. Nat Biotechnol. 2022;40(9):1403-11. doi: 10.1038/s41587-022-01281-7
150. Lakshmi Jayaraj K, Thulasidharan N, Antony A, John M, Augustine R, Chakravartty N, et al. Targeted editing of tomato carotenoid isomerase reveals the role of 5' UTR region in gene expression regulation. Plant Cell Rep. 2021;40(4):621-35. doi: 10.1007/s00299-020-02659-0
151. Tan W, Miao J, Xu B, Zhou C, Wang Y, Gu X, et al. Rapid production of novel beneficial alleles for improving rice appearance quality by targeting a regulatory element of SLG7. Plant Biotechnol J. 2023;21(7):1305-7.
152. Sano Y. Differential regulation of waxy gene expression in rice endosperm. Theor Appl Genet. 1984;68(5):467-73. doi:10.1007/BF00254822
153. Huang L, Li Q, Zhang C, Chu R, Gu Z, Tan H, et al. Creating novel Wx alleles with fine-tuned amylose levels and improved grain quality in rice by promoter editing using CRISPR/Cas9 system. Plant Biotechnol J. 2020;18(11):2164.

154. Zeng D, Liu T, Ma X, Wang B, Zheng Z, Zhang Y, et al. Quantitative regulation of Waxy expression by CRISPR/Cas9-based promoter and 5'UTR-intron editing improves grain quality in rice. Plant Biotechnol J. 2020;18(12):2385-7. doi: 10.1111/pbi.13427
155. Xing S, Chen K, Zhu H, Zhang R, Zhang H, Li B, et al. Fine-tuning sugar content in strawberry. Genome Biol. 2020;21(1):230. doi: 10.1186/s13059-020-02146-5
156. Zhang H, Si X, Ji X, Fan R, Liu J, Chen K, et al. Genome editing of upstream open reading frames enables translational control in plants. Nat Biotechnol. 2018;36(9):894-8. doi: 10.1038/nbt.4202
157. Patel-Tupper D, Kelikian A, Leipertz A, Maryn N, Tjahjadi M, Karavolias NG, et al. Multiplexed CRISPR-Cas9 mutagenesis of rice PSBS1 noncoding sequences for transgene-free overexpression. Sci Adv. 2024;10(23):eadm7452. doi: 10.1126/sciadv.adm7452
158. Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, et al. ARGOS 8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol J. 2017;15(2):207-16. doi: 10.1111/pbi.12603
159. Schussler JR, Weers B, Wu J, Mo H, Lafitte RH, Coles ND, et al. Novel genetic variation through altered zmm28 expression improves maize performance under abiotic stress. Field Crops Res. 2022;281:108486. doi: 10.1016/j.fcr.2022.108486
160. Li C, Li W, Zhou Z, Chen H, Xie C, Lin Y. A new rice breeding method: CRISPR/Cas9 system editing of the Xa13 promoter to cultivate transgene-free bacterial blight-resistant rice. Plant Biotechnol J. 2020;18(2):313-5. doi: 10.1111/pbi.13217
161. Zafar K, Khan MZ, Amin I, Mukhtar Z, Yasmin S, Arif M, et al. Precise CRISPR-Cas9 mediated genome editing in super basmati rice for resistance against bacterial blight by targeting the major susceptibility gene. Front Plant Sci. 2020;11:575. doi: 10.3389/fpls.2020.00575
162. Peng A, Chen S, Lei T, Xu L, He Y, Wu L, et al. Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene Cs LOB 1 promoter in citrus. Plant Biotechnol J. 2017;15(12):1509-19. doi: 10.1111/pbi.12733
163. Butelli E, Licciardello C, Zhang Y, Liu J, Mackay S, Bailey P, et al. Retrotransposons Control Fruit-Specific, Cold-Dependent Accumulation of Anthocyanins in Blood Oranges. Plant Cell. 2012;24(3):1242-55. doi: 10.1105/tpc.111.095232
164. Kobayashi S, Goto-Yamamoto N, Hirochika H. Retrotransposon-Induced Mutations in Grape Skin Color. Science. 2004;304(5673):982. doi: 10.1126/science.1095011
165. Stam M, Belele C, Dorweiler JE, Chandler VL. Differential chromatin structure within a tandem array 100 kb upstream of the maize b1 locus is associated with paramutation. Genes Dev. 2002;16(15):1906-18. doi: 10.1101/gad.1006702
166. Lämke J, Bäurle I. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. Genome Biol. 2017;18(1). doi: 10.1186/s13059-017-1263-6

167. Pan C, Li G, Malzahn AA, Cheng Y, Leyson B, Sretenovic S, et al. Boosting plant genome editing with a versatile CRISPR-Combo system. Nat Plants. 2022;8(5):513-25. doi: 10.1038/s41477-022-01151-9
168. Azodi CB, Lloyd JP, Shiu SH. The cis-regulatory codes of response to combined heat and drought stress in Arabidopsis Thaliana. NAR Genom Bioinform. 2020;2(3):lqaa049. doi: 10.1093/nargab/lqaa049
169. Peleke FF, Zumkeller SM, Gültas M, Schmitt A, Szymański J. Deep learning the cis-regulatory code for gene expression in selected model plants. Nat Commun. 2024;15(1):3488. doi: 10.1038/s41467-024-47744-0
170. Hallgrimsson B, Mio W, Marcucio RS, Spritz R. Let's Face It—Complex Traits Are Just Not That Simple. PLOS Genet. 2014;10(11):e1004724. doi: 10.1371/journal.pgen.1004724
171. Lemoine T, Violle C, Montazeaud G, Isaac ME, Rocher A, Fréville H, et al. Plant trait relationships are maintained within a major crop species: lack of artificial selection signal and potential for improved agronomic performance. New Phytol. 2023;240(6):2227-38. doi: 10.1111/nph.19279
172. Zhou J, Cieraad E, Van Bodegom PM. Global analysis of trait–trait relationships within and between species. New Phytol. 2022;233(4):1643-56. doi: 10.1111/nph.17879
173. Lee YG, Choi SC, Kang Y, Kim KM, Kang CS, Kim C. Constructing a Reference Genome in a Single Lab: The Possibility to Use Oxford Nanopore Technology. Plants. 2019;8(8):270. doi: 10.3390/plants8080270
174. Wenger AM, Peluso P, Rowell WJ, Chang PC, Hall RJ, Concepcion GT, et al. Accurate circular consensus long-read sequencing improves variant detection and assembly of a human genome. Nat Biotechnol. 2019;37(10):1155-62. doi: 10.1038/s41587-019-0217-9
175. Jones DM, Vandepoele K. Identification and evolution of gene regulatory networks: insights from comparative studies in plants. Curr Opin Plant Biol. 2020;54:42-8. doi: 10.1016/j.pbi.2019.12.008
176. Staut J, Pérez NM, Ferrando AM, Dissanayake I, Vandepoele K. A map of integrated cis-regulatory elements enhances gene regulatory analysis in maize. Plant Commun. 2025;6(7):101376. doi: 10.1016/j.xplc.2025.101376
177. Fagny M, Austerlitz F. Polygenic Adaptation: Integrating Population Genetics and Gene Regulatory Networks. Trends Genet. 2021;37(7):631-8. doi: 10.1016/j.tig.2021.03.005
178. Stone KL, Platig J, Quackenbush J, Fagny M. The Importance of Regulatory Network Structure for Complex Trait Heritability and Evolution. Mol Biol Evol. 2025;42(8):1-15. doi: 10.1093/molbev/msaf174
179. Sikora P, Chawade A, Larsson M, Olsson J, Olsson O. Mutagenesis as a Tool in Plant Genetics, Functional Genomics, and Breeding. Int J Plant Genom. 2011;2011(1):314829. doi: 10.1155/2011/314829
180. Wortel MT, Agashe D, Bailey SF, Bank C, Bisschop K, Blankers T, et al. Towards evolutionary predictions: Current promises and challenges. Evol Appl. 2023;16(1):3-21. doi: 10.1111/eva.13513

181. Martínez-Fortún J, Phillips DW, Jones HD. Natural and artificial sources of genetic variation used in crop breeding: A baseline comparator for genome editing. Front Genome Ed. 2022;4. doi: 10.3389/fgeed.2022.937853
182. Stockwell BR. Chemical genetics: ligand-based discovery of gene function. Nat Rev Genet. 2000;1(2):116-25. doi: 10.1038/35038557
183. Comai L, Henikoff S. TILLING: practical single-nucleotide mutation discovery. Plant J. 2006;45(4):684-94. doi: 10.1111/j.1365-313X.2006.02670.x
184. Abdallah NA, Prakash CS, McHughen AG. Genome editing for crop improvement: Challenges and opportunities. GM Crops Food. 2015;6(4):183-205. doi: 10.1080/21645698.2015.1129937
185. Songstad DD, Petolino JF, Voytas DF, Reichert NA. Genome Editing of Plants. Crit Rev Plant Sci. 2017;36(1):1-23. doi: 10.1080/07352689.2017.1281663
186. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity. Science. 2012;337(6096):816-21. doi: 10.1126/science.1225829
187. Kim H, Kim JS. A guide to genome engineering with programmable nucleases. Nat Rev Genet. 2014;15(5):5. doi: 10.1038/nrg3686
188. Hayes RP, Xiao Y, Ding F, van Erp PBG, Rajashankar K, Bailey S, et al. Structural basis for promiscuous PAM recognition in type I–E Cascade from E. coli. Nature. 2016;530(7591):499-503. doi: 10.1038/nature16995
189. Ikram M, Rauf A, Rao MJ, Maqsood MFK, Bakhsh MZM, Ullah M, et al. CRISPR-Cas9 based molecular breeding in crop plants: a review. Mol Biol Rep. 2024;51(1):227. doi: 10.1007/s11033-023-09086-w
190. Scheben A, Wolter F, Batley J, Puchta H, Edwards D. Towards CRISPR/Cas crops–bringing together genomics and genome editing. New Phytologist. 2017;216(3):682-98. doi: 10.1111/nph.14702
191. Anzalone AV, Koblan LW, Liu DR. Genome editing with CRISPR–Cas nucleases, base editors, transposases and prime editors. Nat Biotechnol. 2020;38(7):824-44. doi: 10.1038/s41587-020-0561-9
192. Averina OA, Kuznetsova SA, Permyakov OA, Sergiev PV. Current Knowledge of Base Editing and Prime Editing. Mol Biol. 2024;58(4):571-87. doi: 10.1134/S0026893324700195
193. Gasparini K, Moreira J dos R, Peres LEP, Zsögön A. De novo domestication of wild species to create crops with increased resilience and nutritional value. Current Opinion in Plant Biology. 2021;60:102006. doi: 10.1016/j.pbi.2021.102006
194. Zsögön A, Čermák T, Naves ER, Notini MM, Edel KH, Weinl S, et al. De novo domestication of wild tomato using genome editing. Nat Biotechnol. 2018;36(12):1211-6. doi: 10.1038/nbt.4272
195. Yu H, Lin T, Meng X, Du H, Zhang J, Liu G, et al. A route to de novo domestication of wild allotetraploid rice. Cell. 2021;184(5):1156-1170.e14. doi: 10.1016/j.cell.2021.01.013
196. Correia PMP, Najafi J, Palmgren M. De novo domestication: what about the weeds? Trends in Plant Science. 2024;29(9):962-70. doi: 10.1016/j.tplants.2024.03.001

197. Hahn F, Nekrasov V. CRISPR/Cas precision: do we need to worry about off-targeting in plants? Plant Cell Rep. 2019;38(4):437-41. doi: 10.1007/s00299-018-2355-9
198. Feng S, Wang Z, Li A, Xie X, Liu J, Li S, et al. Strategies for High-Efficiency Mutation Using the CRISPR/Cas System. Front Cell Dev Biol. 2022;9:803252. doi: 10.3389/fcell.2021.803252
199. Li B, Sun C, Li J, Gao C. Targeted genome-modification tools and their advanced applications in crop breeding. Nat Rev Genet. 2024;25(9):603-22. doi: 10.1038/s41576-024-00720-2
200. Park M, Patel N, Keung AJ, Khalil AS. Engineering epigenetic regulation using synthetic read-write modules. Cell. 2019;176(1-2):227-38. doi: 10.1016/j.cell.2018.11.002
201. Turnbull C, Lillemo M, Hvoslef-Eide TAK. Global Regulation of Genetically Modified Crops Amid the Gene Edited Crop Boom–A Review. Front Plant Sci. 2021;12:630396. doi: 10.3389/fpls.2021.630396
202. Bonasio R, Tu S, Reinberg D. Molecular Signals of Epigenetic States. Science. 2010;330(6004):612-6. doi: 10.1126/science.1191078
203. Zou M, Lu C, Zhang S, Chen Q, Sun X, Ma P, et al. Epigenetic map and genetic map basis of complex traits in cassava population. Sci Rep. 2017;7(1):41232. doi: 10.1038/srep41232
204. Gouil Q, Baulcombe DC. DNA Methylation Signatures of the Plant Chromomethyltransferases. PLOS Genet. 2016;12(12):e1006526. doi: 10.1371/journal.pgen.1006526
205. Que Q, Chilton MDM, Elumalai S, Zhong H, Dong S, Shi L. Repurposing macromolecule delivery tools for plant genetic modification in the era of precision genome engineering. In: Transgenic plants: methods and protocols. New York (NY, US): Humana Press; 2019. doi: 10.1007/978-1-4939-8778-8_1
206. Dubrovina AS, Kiselev KV. Exogenous RNAs for gene regulation and plant resistance. Int J Mol Sci. 2019;20(9):2282. doi: 10.3390/ijms20092282
207. Engelhorn J, Snodgrass SJ, Kok A, Seetharam AS, Schneider M, Kiwit T, et al. Genetic variation at transcription factor binding sites largely explains phenotypic heritability in maize. Nat Genet. 2025;57(9):2313-22. doi: 10.1038/s41588-025-02246-7
208. Minow MA, Marand AP, Zhang X, Meyer CA, Pelkmans J, Schmitz RJ. Hybrids reveal accessible chromatin trans genetic associations. BioRxiv 681407. [Preprint]. 2025 Oct 10. doi: 10.1101/2025.10.09.681407

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