January 2025. Horticultural Plant Journal, 11 (1): 15–41

Horticultural Plant Journal

Available online at www.sciencedirect.com

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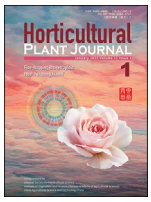

Review

Molecular breakthroughs in modern plant breeding techniques

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Received 20 September 2023; Accepted 5 January 2024; Available online 8 March 2024

A B S T R A C T

Advancements in molecular approaches have been utilized to breed crops with a wide range of economically valuable traits to develop superior cultivars. This review provides a concise overview of modern breakthroughs in molecular plant production. Genotyping and high-throughput phenotyping methods for predictive plant breeding are briefly discussed. In this study, we explore contemporary molecular breeding techniques for producing desirable crop varieties. These techniques include cisgenesis, clustered regularly interspaced short palindromic repeat (CRISPR/Cas9) gene editing, haploid induction, and de novo domestication. We examine the speed breeding approach—a strategy for cultivating plants under controlled conditions. We further highlight the significance of modern breeding technologies in efficiently utilizing agricultural resources for crop production in urban areas. The deciphering of crop genomes has led to the development of extensive DNA markers, quantitative trait loci (QTLs), and pangenomes associated with various desirable crop traits. This shift to the genotypic selection of crops considerably expedites the plant breeding process. Based on the plant population used, the connection between genotypic and phenotypic data provides several genetic elements, including genes, markers, and alleles that can be used in genomic breeding and gene editing. The integration of speed breeding with genomic-assisted breeding and cutting-edge genome editing tools has made it feasible to rapidly manipulate and generate multiple crop cycles and accelerate the plant breeding process. Breakthroughs in molecular techniques have led to substantial improvements in modern breeding methods.

Keywords: Plant breeding; Molecular approaches; Genotype; Phenotype; Crop traits

1. Introduction

Shifts in the climatic patterns and the constrained availability of cultivable land have caused a plateau in agricultural production. Projections indicate that existing global crop yields must be doubled to sustain ten billion people (Campbell et al., 2023). Consequently, scientific and technological breakthroughs in crop cultivation are important for the continuous development of high-yield resilient crop varieties. The practice of enhancing plant cultivars is an ancient custom rooted in agriculture. It encompasses the deliberate crossbreeding of plants to combine favorable attributes through sexual recombination. Traditional breeding relies on the selection of plant phenotypes with favorable characteristics, such as semi-dwarf plants with high nutrient efficiency (Jeon et al., 2023). Several generations are required to develop stable lines and eliminate undesirable traits (Sinha et al., 2023). Thus, this inefficiency associated with developing new plant varieties using traditional breeding methods has prompted a gradual shift toward contemporary breeding technologies.

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Peer review under responsibility of Chinese Society of Horticultural Science (CSHS) and Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS)

https://doi.org/10.1016/j.hpj.2024.01.004

This has led to the rapid evolution of functional genomics and gene manipulation methodologies, culminating in the acquisition of comprehensive genomic information for diverse crop species. DNA sequencing techniques represent an exceptional genomic approach. The origin of DNA sequencing can be traced to the introduction of two fundamental methods: Sanger sequencing and Maxam and Gilbert's approach (Heather and Chain, 2016). Subsequent developments in polymerase chain reaction (PCR), the availability of high-quality enzymes for DNA modification, and the advent of fluorescent automated sequencing have enabled the sequencing of various plant species (Pervez et al., 2022). Such advancements have revolved around molecular principles concerning the manipulation and utilization of genetic variations that govern plant traits (Cortés and Du, 2023). Sequencing techniques have led to the emergence of several next-generation sequencing (NGS) methods that provide novel ways to explore plant genomes and develop genome assemblies (Yang et al., 2024).

These strategies play a pivotal role in the investigation of the genetic and molecular mechanisms underlying crop enhancement, encompassing variations in DNA, transcripts, proteins, metabolites, and minerals. The function of these approaches lies in the reinforcement of genetic diversity and the identification of novel genes for use in plant breeding (Sinha et al., 2023). Progressions in molecular techniques have prompted a transition in plant breeding from reliance on phenotypically observable characteristics to selection based on genotypic makeup.

A pivotal advancement in the genotypic breeding of crops has emerged with the advent of transgenic breeding, wherein specific genetic elements and traits of distinct organisms are transferred to plants, resulting in crops exhibiting the sought-after attributes (Caradus, 2023). Nevertheless, the utilization of transgenic crops remains limited owing to the haphazard integration of foreign DNA into plant genomes, subjecting them to rigorous governmental regulations. Therefore, transgenesis methods have not kept pace with rapid advancements in high-throughput genotyping tools. In response to this challenge, the scientific community has embarked on rigorous efforts to integrate novel technologies that enable the efficient exploitation of genomic data and the application of high-throughput breeding techniques (Sandhu et al., 2022). The objective of these methods is to accelerate the development of crop varieties with enhanced safety, precision, speed, and accuracy (Lamichhane and Thapa, 2022).

Thus, breeding techniques have transformed through the integration of advanced biotechnological methodologies, such as cisgenesis, genome editing, and speed breeding. These advances have enabled efficient breeding by synergistically incorporating genotypic and phenotypic traits (Sanchez et al., 2023). These molecular breeding techniques focus on identifying optimal combinations of alleles or haplotypes, gene networks, and specific genomic regions for strategic breeding, resulting in the rapid production of crops possessing the desired traits.

Ongoing advancements in biotechnology, in conjunction with contemporary breeding methodologies, signify an evolutionary phase in breeding practices tailored to enhancing 21st-century crop cultivation. Despite the continuous evolution of molecular plant breeding techniques, little research has been conducted on them, which remains a point of keen interest among plant breeders. These nascent breeding methodologies must be further explored to address escalating food demands, shifting consumer dietary preferences, and fluctuating climatic conditions.

Consequently, the present review aims to provide an in-depth understanding of fundamental molecular crop breeding approaches. This review addresses contemporary biotechnology tools that complement breeding methodologies by introducing novel ways to ameliorate crop and food production. In this study, technological progress in crop improvement was investigated, focusing on the shift from conventional breeding techniques toward more complex molecular techniques. This emphasizes modern tools that contribute to the discovery of genes, their functions, and the modification of plants. This review enumerates various contemporary approaches to breeding that have been implemented for crop trait enhancement and the development of superior cultivars. It elucidates the integration of state-of-the-art genomic approaches with speed breeding methods to improve the pace and precision of breeding cultivars. Moreover, we provide a comprehensive elaboration of the potential role of these advanced breeding technologies in enhancing food production and aligning it with consumer demand in urban regions.

2. Genetic mutations in the domestication and breeding of crops

The menace posed by the shifts in the Plant domestication commenced approximately ten-thousand years ago, with the practice of crop breeding in local environments. Breeders had to visually assess the phenotypic characteristics of wild crops and selectively domesticate them according to trait requirements. Over time, this process evolved into a renowned practice for enhancing crop yield, nutritional attributes, and other commercially valuable traits. In 1865, the inception of the traditional era of crop breeding was marked by the discovery of Mendel's laws of inheritance, which involved the meticulous selection and cultivation of crops for five to six generations (Mendel, 1865). During several crop generations, mutations that confer advantageous agronomic traits were identified in the offspring by observing morphological features, such as plant height, branch number, and yield (Bateson, 1909).

Non-sense mutations are largely responsible for the domestication of plant phenotypes (Meyer and Purugganan, 2013). These mutations often result in the premature truncation of open reading frames (ORFs), which are caused by induced frameshifts, insertion of STOP codons, alterations in splicing signals, or variations in amino acids, leading to the loss of protein function. An example of this phenomenon is the domestication of rice. During the shift from ancestral wild rice plants, prostrate growth to erect growth in common rice cultivars was due to a single mutation in prostrate growth 1 (*PROG1*) gene. This mutation caused an alteration in one of the amino acids within the zinc finger nuclear transcription factor produced by *PROG1*, resulting in a loss of functional capacity (Tan et al., 2008). This single genetic change was instrumental in shaping the evolution of rice and contributed to its domestication.

Mutations that induce variations in the sequences of cis-regulatory elements or amino acids, causing changes in functional activity, have been relatively common during plant evolution and domestication. For instance, significant domestication

changes in maize involving the reduction of auxiliary branches and enhancement of apical dominance have been linked to a genetic mutation influencing teosinte branched 1 (*tb1*) gene expression (Wang et al., 1999). The causal mutation in this particular instance was identified as the insertion of a Hopscotch retrotransposon approximately 60 kb upstream of the coding region, which led to increased expression of *tb1* gene (Meihls et al., 2013). This gene encodes a transcriptional regulator known for its growth-repressing activity, and its overexpression hinders plant development in auxiliary branches.

Spontaneous mutations include null alleles and alterations in gene expression. has led to extensive modifications in various crop traits to enhance cultivation, improve product quality, and increase the diversity of derived goods. An intriguing example of the remarkable adaptability of plant genomes is a mutation linked to the *SUN* gene in tomatoes that is responsible for the elongated phenotype. This mutation is characterized by a gene duplication event mediated by retrotransposons that relocate the duplicated gene under the influence of a distinct promoter within the genome (Xiao et al., 2008). This structural rearrangement results in an elongated fruit shape, as observed in modern tomato varieties. Such instances demonstrate the diverse ways in which mutations contribute to the genetic diversity and adaptability of crops, leading to valuable improvements in agriculture and derived products.

Mutations or variations in plant populations are induced through sexual hybridization. This is considered more realistic and practical than traditional domestication methods for the development of hybrids with superior agronomic traits (Crews and Cattani, 2018). Comprehensive hybridization approaches have been employed to improve several perennial crops, including wheat (*Triticum* spp. × *Thinopyrum* spp.), sorghum (*Sorghum bicolor* × *S. halepense*), rice (*Oryza sativa* × *O. longistaminata*), and buckwheat (*Fagopyrum* spp. × *F.* spp.) (Crews and Cattani, 2018). Plant breeding through the hybridization paradigm has been enormously successful on a global scale, spawning the Green Revolution.

Over the past century, plant breeding has undergone continuous evolution from traditional to modern methods, driven by the need to address human requirements more effectively. Traditional breeding techniques lack precision in the manipulation and selection of specific genes, often resulting in unintended consequences. Nevertheless, with the progress of molecular approaches, plant breeders have begun utilizing several molecular and genetic markers, enabling a robust and rapid assessment of genetic variation among crop progenies (Wu et al., 2023; Zhang et al., 2023b; You et al., 2024). The integration of biotechnological and molecular interventions has revealed the hidden genetic potentials of wild relatives, common landraces, and crop varieties. This will allow for a more precise and efficient selection of desirable traits, facilitating the development of improved plant varieties that better meet the needs of agriculture and society.

3. Molecular interventions for the elucidation of plant traits

Substantial progress in the development of molecular instrumentation and software has enabled the analysis of plant traits using high-throughput methods. These interventions have enhanced the process of screening and revealed several genomic and molecular aspects of plants that are needed for efficient breeding.

3.1. *Plant whole-genome sequencing*

Modern molecular breeding methods depend extensively on sequencing technologies to enhance breeding efficiency and overcome plant population bottlenecks. The initial method employed in genome sequencing was Sanger sequencing, utilizing a sequencing-by-synthesis (SBS) approach (Cheng et al., 2023). This method involves the use of a radioactively labeled DNA strand complementary to the template strand by employing the dideoxy chain termination technique. The resulting fragments were analyzed by polyacrylamide gel electrophoresis. This approach, known as first-generation sequencing, has undergone subsequent modifications, automation, and commercialization (Heather and Chain, 2016). Prominent enhancements include the adoption of capillary electrophoresis in conjunction with gel electrophoresis, the substitution of radioactively labeled DNA with fluorescently labeled DNA, recombinant DNA, and the integration of polymerase chain reaction (PCR) technologies (Smith et al., 1986). However, a significant limitation of first-generation sequencing is that it generates or analyzes only one sequence per electrophoresis lane or capillary tube. This constraint resulted in DNA fragmentation. Other drawbacks of this technique include its low throughput and high cost (Pervez et al., 2022).

Second-generation sequencing techniques, particularly Illumina, have emerged to address the constraints of first-generation sequencing methods. This sequencing method is more practical for population-level breeding because of its higher throughput (Othman et al., 2023). Next-generation sequencing, high-throughput sequencing, and massively parallel sequencing are the terms used for second-generation sequencing. Unlike first-generation sequencing, this approach does not segregate sequencing mixtures into lanes, capillaries, or tubes (Alekseyev et al., 2018). NGS enables the simultaneous occurrence of billions of sequencing processes on a slide surface such as glass or beads in parallel, thereby representing significant advancements in throughput and cost efficiency. However, a significant drawback of second-generation sequencing is the need for PCR amplification of the template DNA, which leads to potential sequencing errors (Cheng et al., 2023). Additionally, the time required to obtain the results was prolonged because of the need for multiple scanning and washing cycles. The asynchronous addition of each nucleotide can result in noisy sequencing data and shorter reads (Anderson and Schrijver, 2010).

Third-generation sequencing, also known as Single-Molecule Sequencing (SMS), utilizes synthetic chemistry similar to certain second-generation sequencing methods (Athanasopoulou et al., 2021). However, it requires less starting material and eliminates the need for PCR amplification of template DNA, resulting in a lower error rate and the generation of long reads in a shorter run time. A prominent third-generation sequencing approach is single-molecule real-time sequencing (SMRT) by Pacific Biosciences (PacBio) (Ardui et al., 2018). SMRT can produce reads tens of kilobases in length. For instance, the PacBio sequencer

generates 4 million reads with over 99% accuracy within 30 h (Pervez et al., 2022). The introduction of third-generation sequencing technologies such as PacBio and Nanopore has significantly improved reading lengths, enabling the assembly of whole genomes. Notable drawbacks of this technique include the need for fresh DNA to ensure the quality of ultra-long reads, and the scarcity of database systems and algorithm tools for analysis.

Fourth-generation sequencing incorporates nanopore technology into the SMS. This approach enables real-time sequencing without the need for amplification and recurrent cycles, removing synthesis, and is referred to as 4G sequencing. In situ sequencing technology has revolutionized DNA sequencing by enabling the identification of nucleotide orders in fixed cells and tissues (Ke et al., 2016). This distinguishes itself from other sequencing generation approaches in two ways. First, it enables observation of the spatial distribution of DNA reads across the sample, providing valuable information for highlighting tissue heterogeneity based on known markers (Feng et al., 2015). The second difference is the capacity to analyze a large number of cells simultaneously. For instance, cost-effective single-cell RNA-sequencing approaches have been developed, capable of sequencing numerous cells with minimal starting material (pictograms) (Bawa et al., 2022). However, tissue material typically consists of several thousand cells and the sequencing of single cells poses technical and computational challenges.

Sequencing whole plant genomes using these technologies has enabled the structural and functional characterization of coding and non-coding genes, promoters, and transposons, which are essential for understanding the diversification and evolution of genes (Fig. 1). High-throughput sequencing, in combination with multi-omics assays, such as epigenomics, transcriptomics, metabolomics, and proteomics, has led to the identification of differential genes, proteins, and metabolites. This functional characterization aids in the connection of genomic variations and provides a powerful means of mapping important traits. After a gene–trait association was identified, superior varieties were developed by recombining the candidate genes or alleles (Fig. 1).

Starting with the genome sequencing of *Arabidopsis thaliana*, the pursuit of sequencing has progressively broadened its scope to encompass numerous agronomically important plant species. Rice was the first crop to undergo comprehensive genome sequencing (Goff et al., 2002; Yu et al., 2002), followed by *Zea mays*, sorghum, and soybeans (Paterson et al., 2009; Schnable et al., 2009; Schmutz et al., 2010). In addition, high-quality annotation-grade complete genome sequence data have been developed for wheat plants by the International Wheat Genome Sequencing Consortium (2018).

Within the realm of third-generation sequencing, the telomere-to-telomere (T2T) genome sequencing technique stands out for its high accuracy and continuity (Bi et al., 2023). This approach is valuable for overcoming the challenges associated with the assembly of centromeres or highly repetitive regions, ultimately enhancing the continuity and integrity of chromosomes. Zhang et al. (2023a) developed the first comprehensive genome for the Chinese soybean cultivar T2T, utilizing

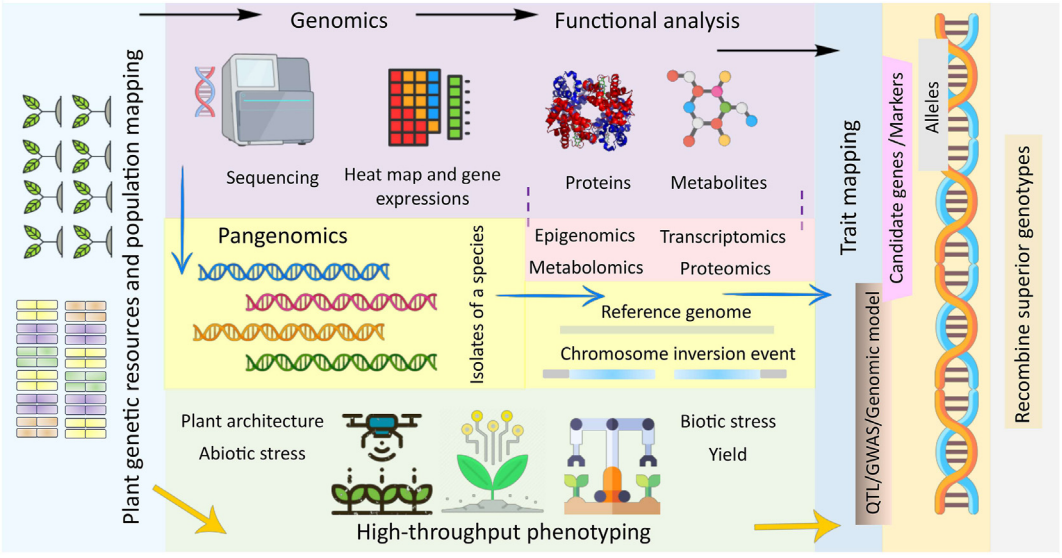

Fig. 1 Overview of modern molecular tools to deliver superior genotypes

The contemporary sequencing platforms use different plant resources to generate genomic variations, which facilitates functional characterization of the genes using different omics approaches. Alternatively, sequencing information is utilized in pangenome development of the plant species through analysing genomes of several plants. Another technique involves the investigation of plant resources through high-throughput tools that provide phenotypic information of the plants through the acquisition of precise phenotypic data by image and sensor technologies. The attained information from the three methods and their combination leads to trait mapping and identification of genotypic information related to the plant phenotypic character. The identified candidate genes and markers related to the favorable trait found from the sequencing and phenotyping data analysis will allow for the recombination of superior plant genotypes.

the genome of the cultivar Zhonghuang 13 (ZH13). The full T2T assembly encompassing all 20 chromosomes indicated that approximately 57.07% of the soybean genome consists of annotated repetitive elements. The analysis revealed that retrotransposons comprised 38.16% of these elements, whereas DNA transposons constituted 6.72% (Zhang et al., 2023a). Moreover, various T2T genomes have recently been identified in several crop species including *A. thaliana* (Naish et al., 2021), rice (Song et al., 2021), maize (Chen et al., 2023), watermelon (Deng et al., 2022), banana (Belser et al., 2021), and kiwi (Yue et al., 2022).

Genome sequencing not only involves deciphering the genetic code of an organism but also encompasses the analysis of gene functions, their regulatory mechanisms, and interactions within biological networks to influence various traits. These approaches are mainly aimed at identifying allelic variations or genetic differences responsible for the enhanced or improved phenotypic traits of crops. Zhang et al. (2012) sequenced the genome of foxtail millet and identified approximately 1 500 specific genes. Among these genes, 580 were annotated as involved in biological responses to water. Through re-annotation efforts, valuable insights have been gained into the classification of these genes, with certain stress-tolerance genes functionalized at the whole-genome level (Muthamilarasan and Prasad, 2017). Furthermore, a genome-wide RNA-seq investigation was conducted on two different sorghum genotypes, drought-susceptible (IS20351) and drought-tolerant (IS22330), to examine the association between their physiological responses to drought stress and differential gene expression (Fracasso et al., 2016). Transcript abundance of drought-related genes was higher in the drought-sensitive genotype. Under drought conditions, 1036 genes were upregulated and 809 genes were downregulated, with 428 upregulated and 393 downregulated genes specifically for the drought-tolerant genotype. This study compares the approaches employed by both genotypes to cope with drought stress conditions. The resilient genotype initiated the production of secondary metabolites, such as glycinbetaine and glutathione, whereas the vulnerable genotype hydrolyzed carbohydrates and sugars. The impact of the imposition and sensing of drought stress was more pronounced in the vulnerable genotype than in the tolerant genotype. The drought-tolerant genotype can act as a genetic donor to enhance drought-tolerance traits in sorghum germplasm. Similarly, in wheat, high temperatures and drought resulted in the differential expression of 29 395 genes (Liu et al., 2015). Rice contains 696 and 808 differentially expressed genes (DEGs) during the conditions of low and high nitrogen levels, respectively (Xin et al., 2013). Additionally, tomato roots responded with 267 and 1 421 DEGs during hypoxia in both the short and long terms, respectively (Safavi-Rizi et al., 2020). The introduction of NGS for gene characterization has facilitated the development of large-scale molecular markers.

Numerous techniques have been developed for sequencing, including plant resequencing, transcriptomics, and contrasting genomic investigations. These approaches have been employed to explore molecular markers, quantitative trait loci (QTLs), and pangenomes related to important agronomic traits.

3.2. *Molecular markers in the process of genotype selection*

Molecular markers have attracted significant interest from breeders. These are powerful tools for detecting genetic variations related to valuable agronomic properties among different cultivars within a species (Fig. 1). They are characterized by an even distribution throughout the genome, low mutation rates, and high heritability (Amiteye, 2021). These qualities make them pivotal in classical genetics and plant breeding, offering the ability to select and breed the traits of interest. Several molecular markers have been developed, including microsatellites, simple sequence repeats (SSRs), insertion-deletions (InDels), and single nucleotide polymorphisms (SNPs).

A genome-wide marker study conducted on Chinese spring wheat found 364 347 SSR markers from a vast dataset of 10 603 760 genome sequences, yielding a density of 36.68 SSR markers per megabase (Mb) (Han et al., 2015). Among the detected SSR motifs, 488 different forms were observed, with dinucleotide repeats being the most prevalent, followed by trinucleotide, hexanucleotide, tetranucleotide, and pentanucleotide repeats. Similarly, a genome-wide study identified 28 324 microsatellite repeat motifs in foxtail millet covering a total sequence length of 405.3 Mb (Pandey et al., 2013). Trinucleotide repeats were more abundant than dinucleotide repeats. Furthermore, Zhou et al. (2012) identified 436 640 indels throughout the barley genome by aligning DNA sequences from two different accessions, Morex and Barke. From these, 1 140 InDel markers were integrated with 383 SSRs, 1544 diversity array technologies (DarT), and 3 909 gene-based SNP markers, creating a comprehensive barley genetic map. In addition, different categories of markers, including transposable element-related and miRNA-associated markers, have been developed and displayed in various crops such as foxtail millet (Muthamilarasan and Prasad, 2015). These markers are crucial for wider genotyping applications in cereal, millet, and bioenergy grass species.

Throughout the genome, SNPs are widely dispersed and are detectable by comparative analyses of whole-genome sequences and transcriptomic data from diverse accessions (Habash et al., 2014). Approximately 20 million SNPs have been identified in rice by aligning reads from 3 000 genome sequences with the Nipponbare reference genome (Alexandrov et al., 2015). In wheat, 46 977 gene-associated SNPs have been genetically mapped using a high-density SNP array comprising 90 000 markers and eight mapping populations (Wang et al., 2014). Furthermore, 6 385 011 high-density SNPs were detected in 15 contrasting drought-responsive inbred maize lines and compared with the reference B73 genome. The majority of SNPs were identified in the intergenic region and a few in the intronic regions, upstream regions, exons, untranslated regions (UTRs), and splice sites. Moreover, 271 candidate genes associated with non-synonymous single SNPs (nsSNPs) that play a role in conferring drought tolerance in maize have been identified (Xu et al., 2012).

Sequencing of 588 *Brassica napus* accessions from 21 countries led to the discovery of approximately 5 294 158 SNPs and 1 307 151 indels. Genome-wide association studies (GWAS) that 60 genetic loci are associated with different agronomic traits, including seed quality, oil content, and stress tolerance (Lu et al., 2019). This study indicated that the origin of *B. napus* resulted from domesticated *Brassica rapa* and *Brassica oleracea* hybridization. These genetic resources hold immense importance for diversity studies and for understanding the genetic basis of trait variation within populations. Molecular markers are genetically linked to quantitative trait loci (QTL) in crops.

3.3. QTLs mapping for the agronomic traits

The primary objective of molecular markers is to map and identify QTLs. It is a technique for selecting favorable plant species in a breeding scheme depending on the presence of a molecular marker or essential alleles (Fig. 1). When employed in appropriate situations, it can result in the recombination of crops with superior traits. The foundational principles of QTL mapping trace back to the association between seed weight segregation and a seed coat color marker in *Phaseolus vulgaris* L. (Sax, 1923). Sax postulated that a single gene controlling seed color could be genetically linked to one or more polygenes that govern seed size. The framework of genetic linkage mapping depends on the examination of an organism's genome using DNA markers, establishing the comparative positions of these markers in linkage categories, and elucidating their genetic correlation with QTLs (van Ooijen, 1992). Two principal approaches are used for genetic mapping. The first, referred to as linkage analysis or QTL mapping, has become a classical technique (Jansen, 1993). This method relies on experimental populations of F_n generations, which include backcrosses (BC_n), recombinant inbred lines (RIL), and doubled haploid lines (DHL) (Lewis, 2012). The second approach involved examining linkage disequilibrium (LD) and conducting association mapping. The second approach involves scrutiny of linkage disequilibrium (LD) and association mapping. LD mapping employs diverse lines sourced from germplasm or natural populations (Asíns, 2002; Glazier et al., 2002). Consequently, during the mapping process, individuals are grouped into genetic categories based on specific DNA markers used (van Ooijen, 1992). Moreover, several other QTLs, such as expression, proteomic, metabolic, and phenomic QTL, have gained prominence for crop enhancement. eQTLs are associated with genetic variations that influence gene expression characteristics, whereas pQTLs are linked to genetic polymorphisms and protein abundance (Nica and Dermitzakis, 2013).

Numerous genetic maps formulated have recognized a multitude of QTLs linked to economically valuable attributes through genome-wide association studies (GWAS) based on SNP markers. In a study by Zhu et al. (2020), a GWAS was performed using 57 413 high-quality SNPs within a group of 316 *Gossypium hirsutum* accessions cultivated under four distinct salinity stress conditions across a span of two years. In this study, 42, 91, and 25 stable QTLs were identified for single-boll weight, lint percentage, and 25 stable QTLs for boll number per plant, respectively. Furthermore, QTL analysis was conducted for preharvest sprouting (PHS) using populations of F_8 RILs derived from japonica weedy rice (Lee et al., 2023). QTL analysis revealed two consistent QTLs, qPH7 and qPH2, linked to PHS resistance and situated on chromosomes 7 and 2. These QTLs collectively explained approximately 38% of the phenotypic variance. Moreover, employing targeted metabolome profiling in wheat kernels through liquid chromatography-tandem mass spectrometry (LC-MS/MS) in tandem with linkage analysis (Shi et al., 2020) found a total of 1 005 mQTLs distributed non-uniformly across the genome. Among these mQTLs, twenty-two candidate genes governing the modulation of diverse metabolite levels were functionally annotated. Advanced QTL genomics strategies have facilitated a deeper understanding of gene regulatory networks and have proven instrumental in enhancing the potential for developing reference genomes for different crop species.

3.4. Crop pangenomics development

The presence of reference genomes for most contemporary crops has enabled a comprehensive analysis of single nucleotide polymorphisms (SNPs), facilitating subsequent investigations into associations between genetic and phenotypic variations on a genomic scale. Fig. 1 shows that pangenomes can be developed using genome sequences. This technique is used to sequence genomic isolates from several plants of the same species. Pangenomics compares the genes of these plants and identifies specific gene clusters responsible for the trait of interest. This resulted in the development of superior plants through the recombination of these gene clusters (Fig. 1). However, the exploration of genetic variation has been limited by reliance on resequencing methodologies that focus on a solitary reference genome.

These methods have shown limited effectiveness in detecting structural variations (SVs) that are crucial for determining the genetic foundation of agronomic traits (Lu et al., 2015; Zhao et al., 2018). Genetic variations, particularly SVs, can yield significant diversity in the composition and content of functional genes among individuals within the same species. The pangenome concept was introduced to comprehensively encompass this diversity in gene content (Tettelin et al., 2005). It focuses on investigating the complete set of genes within a species by sequencing several individuals representing that species (Abdul Aziz and Masmoudi, 2023a, 2023b). In *Cucumis sativus*, a pangenome has been constructed using a graph-based approach involving the analysis of 12 genome assemblies at the chromosome-scale (Li et al., 2022). Leveraging chromosome-level assemblies enabled the identification of approximately 4.3 million genetic variants, encompassing 56 214 SVs. The integration of variant information and reference genome sequences into the pangenome graph facilitates the identification of SVs linked to important agronomic traits, such as warty fruits, flowering times, and root growth (Li et al., 2022). Furthermore, chromosome-level genome assembly of a specific landrace melon accession (*Cucumis melo*) was examined. A comprehensive melon pangenome atlas was created by integrating various sequenced melon genome datasets (Lyu et al., 2023). Comparative genomic analysis indicated a total of 3.4 million genetic variations. A previous study found that the prevalence of numerous SVs is closely linked to significant melon agronomic traits, including sucrose content, flesh color, and rind stripes (Lyu et al., 2023). Moreover, a pangenome was constructed using the genomes of 10 genetically diverse *Malus* accessions, encompassing the evergreen cultivar Granny Smith and the largely cultivated Red Fuji (Wang et al., 2023). A previous study found 20 220 copy number variations (CNVs) and 317 393 structural variations (SVs) in *Malus* species, which revealed an SV within *MMK2* that correlated with fruit coloration (Wang et al., 2023).

Recent advancements in molecular and analytical tools have made it feasible to sequence multiple accessions of a particular crop species, thereby expanding the capacity to study genetic diversity within that species (Golicz et al., 2016). Pangenomic assemblies initially involved only a restricted number of accessions and primarily relied on short-read sequencing methods. This was observed in the initial assemblies of *Glycine max*, *O. sativa*, and Brassicaceae species (Li et al., 2014; Schatz

et al., 2014; Golicz et al., 2016). However, long-read sequencing and hybrid methodologies, which combine sequences of long and short reads, have recently enabled the reconstruction of pangenome assemblies with a larger set of accessions. Pangenomic investigations have been conducted on major crops and their closely related species. The pangenome of *Helianthus annuus* was established using a compilation of 493 cultivars, landraces, and compatible wild species. This study aimed to elucidate the influence of introgression from wild relatives on the composition of unessential genomic elements in crop cultivars (Hübner et al., 2019). The study reported that approximately 1.5% of these genes were uniquely attributed to introgression by wild relatives. Dolatabadian et al. (2020) further conducted a pangenome analysis of rapeseed and found 753 variable resistance gene analogs, of which 106 candidates were predicted to confer resistance against blackleg, a major disease affecting Brassica species (Howlett et al., 2001; Dolatabadian et al., 2020). Similarly, the genomes of 725 tomato plant accessions were sequenced and compared to those of the Heinz 1706 reference assembly. This facilitated the establishment of a pangenome assemblage encompassing tomato plants and their wild variants (Gao et al., 2019; Alonge et al., 2020). Investigation of the pangenome revealed a truncated, non-reference allele in the *TomLoxC* promoter region. This allele, which was found predominantly in wild relatives, underwent negative selection during domestication, causing reduced *TomLoxC* expression levels and an altered flavor profile in the tomato cultivars. Certain contemporary elite breeding lines chosen for stress resilience exhibit an inadvertent resurgence of this rare allele, accompanied by elevated production levels of apocarotenoids in these lineages (Gao et al., 2019). Moreover, the construction of a pangenome for *S. bicolor* serves as a platform for identifying drought-responsive genes from the transcriptomics of both the resistant and susceptible genotypes. This analysis revealed the absence of 79 genes from the reference genome that were differentially expressed during drought stress. Notably, two resistance-specific genes co-mapped with traits related to plant height and leaf pigments (Varoquaux et al., 2019; Abdel-Ghany et al., 2020; Ruperao et al., 2021). Utilizing pangenomes instead of single-genome sequences as references can help identify valuable genes retained or lost due to the artificial selection and breeding of crop cultivars. Moreover, pangenomics analysis expedites the use of accessions of wild crop relatives and cultivated species to enhance tolerance to biotic and abiotic stresses, improve nutritional characteristics, and enhance other economically valuable traits in commonly grown cultivars. Pangemics have been developed for different crops using various strategies to decipher valuable crop traits (Table 1). These molecular approaches can be further enhanced by using plant phenotypic data.

Table 1 Pangenome development in different crop species

Plant species	Accession used	Genetic resources	Method	Source
Tomato	100	Early domesticated species, wild red-fruit species, commercial varieties, and vintage cultivars	Long-read sequencing with ONT technology was performed for each accession, and the resulting sequences were aligned to a top-tier reference genome (SL4.0). Unmatched reads were combined to construct the structural variation pangenome	Alonge et al. (2020)
Rice	3 010	Different Asian cultivated rice accessions including *Oryza sativa*	Illumina reads aligned to the Nipponbare reference genome were combined with unique, previously unseen sequences to augment the reference dataset	Wang et al. (2018)
Sunflower	493	Sunflower accessions, which included wild relatives, landraces, and modern cultivars	Illumina short reads were aligned to the reference genome, and a pangenome was constructed by performing de novo assembly on the reads that didn't align	Hubner et al. (2019)
Tomato	725	Tomato accessions, which included *Solanum lycopersicum*, *Solanum pimpinellifolium*, *Solanum cheesmaniae*, and *Solanum galapagoense*	A de novo assembly was conducted using a combination of previously gathered and newly generated sequence data for each accession. The resulting contigs were then aligned with the reference genomes. Any nonredundant sequences that did not aligned were integrated into the reference genome to create the pangenome	Gao et al. (2019)
Sesamum indicum	5	Landraces, modern varieties, and cultivars	New assemblies were generated for previously published genomes and aligned with the reference. The pangenome was constructed by aligning entire genomes using Mugsy, and it included regions that were common among different varieties in the core genome	Yu et al. (2019a, 2019b)
Soybean	26	Plant accessions	A de novo genome assembly was performed for each accession utilizing a combination of SMRT sequencing, optical mapping, Hi-C sequencing, and Illumina short reads to derive the pangenome	Liu et al. (2020b)

(*continued on next page*)

Table 1 – (*continued*)

Plant species	Accession used	Genetic resources	Method	Source
Rice	66	Modern cultivars, and accessions from the complex of *Oryza sativa* and *Oryza rufipogon* species	*De novo* assemblies were constructed using Illumina short reads for individual accessions, and structural variations (SVs) were detected by aligning each of these assemblies to the Nipponbare reference genome	Zhao et al. (2018)
Brachypodium distachyon	54	Inbred lines	The genome assembly was performed using Illumina short reads. The genes that showed homology across all 54 lines were categorized into either the core genome or the dispensable genome, depending on their presence or absence across these lines	Gordon et al. (2017)
Peach	336	Accessions from *Prunus persica*	Short Illumina reads combined with long PacBio reads from six accessions were aligned to the reference genome, and structural variations (SVs) were identified using the LUMPY tool	Guo et al. (2020a, 2020b)
Gossypium spp.	5	Different species including *Gossypium darwinii*, *Gossypium hirsutum*, *Gossypium mustelinum*, *Gossypium barbadense*, and *Gossypium tomentosum*,	Whole-genome sequencing was performed using a comprehensive approach that included short reads (Illumina), long reads (PacBio), and chromatin conformation capture (Hi-C seq) for five different species. The data were analyzed to evaluate collinearity, as well as the presence or absence of genes, among these species	Chen et al. (2020)
Poplar	10	Different species including *Populus alba*, *Populus devidiana*, *Populus simonii*, *Populus lasiocarpa*, *Populus ussuriensis*, *Populus nigra*, *Populus cathayana*, *Populus maximowiczii*, *Populus deltoides*, and *Populus euphratica*	Illumina short reads were aligned to the reference genome (*P. trichocarpa* v3.0), and structural variations (SVs) were detected using the BreakDancer tool	Zhang et al. (2019b)
Brassica napus	8	Accessions from the winter and spring-based oilseed rapes	The de novo assembly process combined PacBio-SMRT long reads, short Illumina reads, and BioNano optical mapping data. The pangenome was constructed by comparing and incrementally incorporating Presence-Absence Variations (PAVs) into the reference genome	Song et al. (2020)
Maize	4	Inbred lines	The entire genome, organized at the chromosome level, was sequenced using Illumina short reads. To identify both the common core genome and the structural variations specific to different groups, all potential combinations of genome alignments were utilized	Haberer et al. (2020)
Brachypodium hybridum	9	*Brachypodium hybridum* lines, and *Brachypodium stacei* line	The primary method employed for genome assembly relied predominantly on de novo assemblies of Illumina short reads. PacBio sequencing at a depth of 60x was utilized for a single *B. hybridum* line for assembling the pangenome	Gordon et al. (2020)
Foxtail millet	1 844	Wild species, landraces, and modern cultivars	Illumina NovaSeq 6000 and PacBio RSII platforms were used for sequencing of genomes. subsequently de novo assembled these genomes using CANU24 and HERA25 pipelines. Aligned the CDS of all annotated genes If a gene was aligned with >99% coverage and identity, it was considered present in the corresponding genome. Performed a pangenome analysis based on a Markov clustering approach.	He et al. (2023)

4. High-throughput phenotyping (HTP) advances crop genomics for precise trait selection

The advent of NGS and the availability of large-scale marker information have led to an increased application of Genomic Selection (GS) in plant breeding. However, the primary obstacle encountered by breeders when using GS models is imprecise or unreliable phenotypic data. The accuracy of the phenotypic information is crucial for building robust GS models. Inaccurate phenotypic data can reduce the predictive ability of GS models. Moreover, existing methods for phenotypic measurement are labor-intensive, time-consuming, and costly.

To address these challenges, High-throughput Phenotyping (HTP) has emerged as an advanced approach for obtaining precise phenotypic data. In addition to the genomic analysis of crop species, plant resources can be alternately examined through high-throughput phenotyping to identify traits of interest, such as tolerance to biotic and abiotic stress, plant architecture, and higher yield quality (Fig. 1). This phenotypic information can be related to the genomic data for trait mapping to elucidate the QTLs, candidate genes, and associated markers to recombine the superior genotypes (Fig. 1). It is a non-destructive, rapid, and precise approach for measuring various favorable traits in plants (Pabuayon et al., 2019). HTP involves automated sensing, data acquisition, and processing to assess stress tolerance, yield, and plant growth. These tools have been employed to phenotype physiological traits in a substantial number of breeding lines using several plant images obtained daily with a high degree of automation. By utilizing HTP, breeders can accelerate and efficiently screen several plants at different stages of growth (Yadav, 2021).

HTP platforms encompass a range of technologies including cameras, sensors, spectrometers, and Light Detection and Ranging (LiDAR) technologies (Shabannejad et al., 2020). Numerous studies have used GS in conjunction with HTP in plants. For instance, GS was successfully applied using NDVI data in wheat, resulting in a 7%–33% increase in prediction accuracy, in contrast to the standard univariate model (Crain et al., 2018). Similarly, utilizing RGB data through HTP in wheat improved the prediction precision for days to maturity by three to four times (Shabannejad et al., 2020). Another study demonstrated that measuring secondary traits in wheat using an Unmanned Aerial Vehicle (UAV) enhanced the precision of genetic prediction of grain yield by an average of 146% (Sun et al., 2019). Krause et al. (2020) examined the potential of predicting wheat grain yield by utilizing vegetation indices that incorporate measurements of canopy structure and photosynthetic activity derived from the amount of light reflected by the crop canopy. Vegetation indices were collected in the early generations using aerial HTP systems. The study showed that the HTP methodology enhanced selection in the early generations. Furthermore, five spectral reflectance indices were applied using HTP platforms and 11 089 SNPs to evaluate the effectiveness of incorporating secondary traits to improve the accuracy of predicting high-yield potential in winter wheat lines (Lozada et al., 2019). The spectral characteristics displayed moderate to high phenotypic and genetic correlations, indicating a substantial enhancement in the accuracy of grain yield prediction. This study shows that the integration of HTP tools with genomic prediction is advantageous for improving genetic gains in winter wheat breeding. These findings indicate the benefits of employing HTP to improve the performance of GS models and enable accurate plant selection.

The combined use of advanced genotyping and phenotyping approaches will enable greater opportunities for breeders to enhance the accuracy and efficiency of GS in plant breeding. HTP tools provide several means of measuring plant development and determining the genomic basis of quantitative traits to control the association between plant genotypes and the environment in predictive models. This has the potential to change the method of selecting plant materials. These tools are useful for phenotyping several thousand plots, which reduces costs and manual labor. It has been indicated that combining molecular approaches, environmental information, and HTP techniques will result in a substantial enhancement of prediction precision in contrast to conventional breeding practices (Jeon et al., 2023). The convergence of biotechnological interventions and modern molecular breeding approaches has increased the potential for developing better-performing crop varieties that can address agricultural challenges.

5. Progression in plant breeding practices using the modern molecular approaches

The process of generating a stable inherited trait in a new plant cultivar through conventional plant breeding is difficult and largely depends on the crop and agronomic trait of interest, and faces the issue of the introduction of undesirable DNA fragments. However, these issues can be minimized using advanced molecular approaches for genomic breeding.

5.1. *Marker-assisted backcrossing (MABC) of crops: reducing the proportion of undesirable genes*

Modern molecular breeding methods depend extensively on sequencing technologies to enhance breeding efficiency and overcome plant population bottlenecks. (MABC) is a molecular approach used to transfer a specific genetic region, such as a QTL or gene while avoiding the introduction of undesirable genetic material through multiple rounds of backcrossing. The outcome of MABC is a hybrid containing the entire genome of the elite parent and the genetic material of the donor plant, which leads to the desired phenotype (Pukalenthy et al., 2020) (Fig. 2, a). The efficiency of MABC is influenced by factors such as the quantity of molecular markers used, strength of the marker associated with the phenotype, presence of undesirable linkage drags, and size of the population used during each generation of backcrossing.

This method has proven to be highly effective in generating superior crop lines with enhanced tolerance to biotic and abiotic stressors. In rice, MABC has been utilized to create Near-Isogenic Lines (NILs) with drought tolerance by introducing root QTLs from a drought-tolerant japonica upland variety (CT9993) into a lowland rice cultivar (IR20) susceptible to drought (Suji et al., 2012). Similarly, Yang et al. (2019a) successfully introduced *Pi2*, a blast-resistance gene, into a high-performance variety by using MABC combined with genomics-based background selection. Furthermore, Kang et al. (2019) employed MABC to rapidly develop virus-resistant lines from parents with different levels of resistance against the rice stripe virus. In peanuts, MABC contributes to the development of superior lines with favorable oleic and linoleic fatty acid ratios (Bera et al., 2019). Moreover, in *Cicer arietinum*, Mannur et al. (2019) developed Fusarium wilt-resistant, highly productive *C. arietinum* cultivars, Super Annigeri 1, and enhanced JG 74 lines in a remarkable period of ten years. MABC has been widely applied to various crops, including rice (Hasan et al., 2015), cotton (Wu et al., 2017), wheat (Yadawad et al., 2017), barley (Xu et al., 2018), soybeans (Rawal et al., 2020), and tomatoes (Osei et al., 2018).

The use of markers for pyramiding multiple genes or QTLs presents challenges; in such cases, recurrent selection has emerged as a potential tool for enhancing polygenic traits. It was

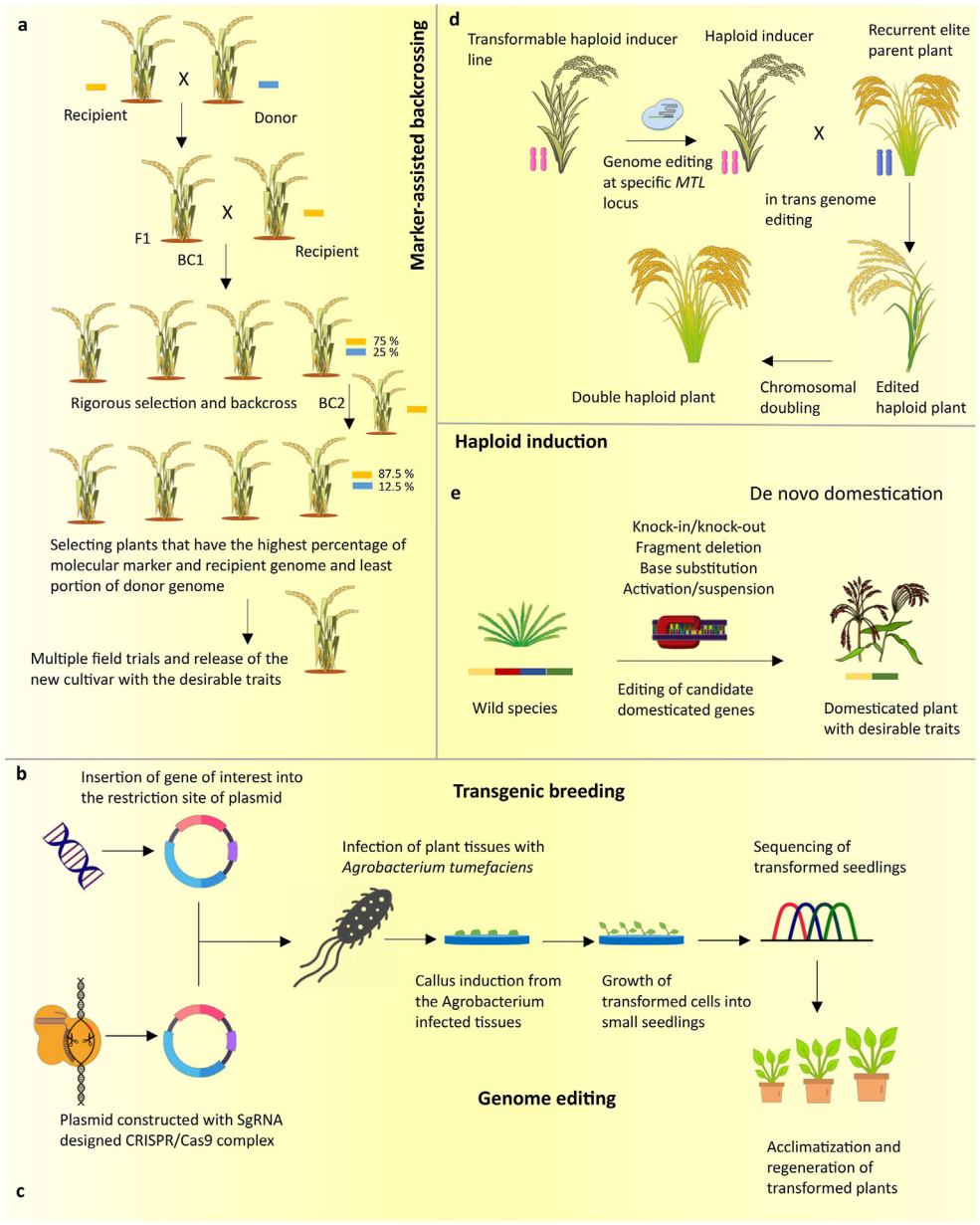

Fig. 2 Illustration of the molecular breeding approaches (a) Marker assisted backcrossing The undesirable traits are reduced through backcrossing with the recipient plant. The selected offspring plant will have the highest portion of the molecular marker that will be regenerated. (b) Transgenic crop breeding. The gene of interest is transferred into the plants through appropriate media and the plants are transformed and regenerated. (c) Genome-editing of crops. The plants are edited with the Agrobacterium-mediated delivery of CRISPR/Cas 9 complex for specific traits. (d) Haploid induction. The haloid plants are produced by editing the gene locus on the chromosome that are doubled in the plants through various means (e) De novo domestication of crops. The desirable traits are domesticated in the wild species using the genome-editing techniques.

reported in a study that the Marker-Assisted Recurrent Selection (MARS) strategy in MAS (Marker-Assisted Selection) involves genotypic selection and intercrossing within the single cropping season for a single breeding cycle (Jiang, 2013). Furthermore, it has been outlined that the primary objective of the MARS scheme is to identify and select multiple genomic regions associated with the expression of complex quantitative characteristics, bringing them together within a single cross or across interconnected populations (Ribaut et al., 2002). The MARS program was successfully applied to enhance essential agronomic traits in maize. In addition, this strategy yielded superior results compared to traditional selection, which focuses on developing an enhanced drought-tolerant germplasm (Beyene et al., 2016). Another investigation within the maize improvement program achieved a high grain yield by employing the SNP marker system in MARS with a biparental population (Bankole et al., 2017). In addition,

through MABC, six soybean breeding lines devoid of Kunitz trypsin inhibitor (KTI) were generated in the genomic context of DS9712 and DS9814 (Kumar et al., 2015). In another study, the marker-assisted introgression of a gene encoding a null phenotype version of the β-conglycinin α-subunit (*cgy-2*) from the RiB donor line into the genetic makeup of the Chinese cultivar Dongnong47 (DN47). This generates seeds that are free from allergens and possess enhanced nutritional value and quality for food processing (Song et al., 2014). Moreover, in *Arachis hypogaea*, MABC lines with enhanced oleic acid content were developed through the transfer of two FAD2 mutant alleles from SunOleic 95R to the genetic backgrounds of ICGV 06110, ICGV 06142, and ICGV 06420 (Janila et al., 2016).

5.2. *Candidate gene expression for targeted phenotypic outcomes*

The accessibility of genome sequence information in available databases has greatly facilitated the extensive analyses of genes, gene families, and promoter elements. These analyses provided valuable insights into potential candidate genes that can be overexpressed in target organisms to enhance specific desirable traits (Fig. 2, b). Functional characterization of these candidate genes is important for expanding our understanding of gene functions and regulatory mechanisms, which have significant implications for breeding applications (Abdul Aziz et al., 2021). T-DNA insertion lines in *A. thaliana* are valuable resources for determining gene function. With the function elucidation, the next step is to employ these genes in crop improvement programs, with gene overexpression being one of the most widely utilized methods. A prime example of this application is the expression of the *Cry* gene from *Bacillus thuringiensis*.

Isolating and expressing genes from diverse plant species has been employed to enhance the agronomic traits and stress tolerance of target crops. For instance, the elevated expression of *ARGOS* genes in *Zea mays* reduces ethylene sensitivity, resulting in enhanced drought resistance and increased grain yield under both well-watered and drought conditions (Shi et al., 2015). Similarly, transgenic *G. max* plants overexpressing *GmWRI1b* exhibit improved oil content and enhanced plant architecture under field conditions (Guo et al., 2020a, 2020b). Moreover, PTE-2, a Stoway-like MITE, has been identified, characterized, and activated in transgenic Chinese cabbage lines (Jeon et al., 2022). In soybean, the cyst nematode *Heterodera glycines* Ichinohe) resistance and two sources of soybean cyst nematode (SCN) resistance have been widely used, from accessions PI 88788 (rhg1-b) and Peking (rhg1-a and Rhg4). Soybean varieties with enhanced SCN resistance have been developed by introgressing these resistance genes (Santana et al., 2014). Moreover, soybean ILs with enhanced resistance to Phytophthora rot and powdery mildew diseases have been developed through the insertion of *Rps2* and *Rmd-c* genes, respectively (Ramalingam et al., 2020). Different transgenic plants were generated via gene overexpression (Table 2).

5.3. *Cisgenesis and intragenesis manipulation of plants*

Cisgenesis refers to the genetic modification of plants using genes that originate from recipient species or closely related species that can be crossbred (Schneider et al., 2023). The primary advantage of cisgenesis is its ability to rapidly introduce desirable characteristics, including disease resistance, from wild relatives, without the need for extensive crossing. In cisgenesis, the introduced gene is considered an additional copy of a naturally occurring variation, with its introns adjacent to the native promoter and terminator arranged in their normal typical sense orientation (Lusser and Davies, 2013). Cisgenic crops have demonstrated increased public acceptance compared with traditional transgenic genetically modified (GM) crops (Saleh et al., 2021). Intragenesis involves the creation of a novel gene combination by assembling naturally isolated elements, including the promoter and terminator regions, through in vitro methods using borders resembling the left and right T-DNA borders extracted from sexually compatible DNA pools (Holme et al., 2013).

The effectiveness of cisgenesis has been demonstrated in both cereal crops and trees. For example, in barley, a marker-free cisgenic variety has been created by introducing an additional phytase gene to improve phosphate bioavailability through the

Table 2 Genetically modified crops with improved traits

Host plant	Gene	Transgenic plant	Trait improved	Source
Oryza sativa	*OVP1*	Rice	Salinity tolerance	Kim et al. (2020)
Oryza sativa	*OSMYB6*	Rice	Salt and drought stress tolerance	Tang et al. (2019)
Salicornia europea	*SeVP*	Wheat	Salt stress tolerance and nitrogen deficiency	Lv et al. (2015)
Solanum torvum	*P5CS*	Soybean	Salinity tolerance	Zhang et al. (2015)
Zea mays	*ZmbZIP4*	Maize	Salinity, drought, and osmotic stress tolerance	Ma et al. (2018)
Solanum tuberosum	*StDREB2*	Cotton	Drought tolerance	El-Esawi and Alayafi (2019)
Arabidopsis thaliana	*AtJUB1*	Tomato	Drought tolerance and reduced oxidative damage	Thirumalaikumar et al. (2018)
Arabidopsis thaliana	*AtWRKY30*	Wheat	Drought and heat tolerance	El-Esawi et al. (2019)
Oryza sativa	*OsSIZ1*	Cotton	Heat and drought tolerance	Mishra et al. (2017)
Spinacia oleracea	*CMO*	Rice	Heat and salt stress tolerance	Shirasawa et al. (2006)
Arthrobacter globiformis	*CodA*	Tomato	Drought tolerance	Cheng et al. (2013)
Arabidopsis thaliana	*AVP1*	Cotton	Salt and drought tolerance	Pasapula et al. (2011)
Arabidopsis thaliana	*DREB1A*	Peanut	Drought tolerance	Bhatnagar-Mathur et al. (2007)
Agave sisalana	*AsHSP70*	Cotton	Heat tolerance	Batcho et al. (2021)
Helianthus annuus	*Hahb-4*	Wheat	Yield increase and drought tolerance	González et al. (2019)

enzymatic breakdown of phytic acid (Holme et al., 2012). Field trials of this cisgenic barley were conducted during the release period from 2012 to 2016. Gadaleta et al. (2008) used biolistic transformation to improve the bread-making properties of durum wheat by cloning and transferring genes encoding specific glutenin subunits. The *Escherichia coli* phosphomannose isomerase (pmi), which is used as a positively selectable marker, was subsequently removed through genetic segregation and positive selection. In addition, Han et al. (2011) conducted a study that investigated the impact of integrating five cisgenes responsible for proteins involved in gibberellin metabolism or signaling into poplar trees. Every component of these cisgenes, including their promoter and terminator regions, was introduced into the genome of *Populus trichocarpa*. Remarkably, three of these alterations had substantial effects on plant growth rate, morphology, and wood characteristics. Moreover, in *Malus domestica*, cisgenic lines were successfully developed to enhance resistance toward fire blight (Kost et al., 2015) and apple scab (Würdig et al., 2015), leading to enhanced resistance levels. One specific study aimed to evaluate the performance of the cisgenic *Mallus domestica* line (C11.1.53) under greenhouse conditions owing to its higher scab resilience, minimal foreign DNA, and single genome insertion (Jänsch et al., 2014). Moreover, allergen genes that exhibited varying levels of expression in the cisgenic line did not have a significant effect on protein levels. However, this leads to minor unintentional alterations in growth. Subsequent field trials verified the resistance of the clone to apple scab. However, this study did not assess its effects on tree growth or fruit traits (Krens et al., 2015).

To introduce fire blight resistance into a susceptible domesticated apple, a gene identified as *FbMR5*, which encodes a CC-NLS-LRR protein found in *Malus* × *Robusta* population 5, was introduced through cisgenesis (Flachowsky et al., 2011). The safety of these cisgenic plants was assessed in contrast to their non-GM counterparts, Schlathölter et al. (2022) specifically examined the C44.4.146 line, assessing potential unintended effects on key leaf components and the fitness of decomposers such as *Drosophila melanogaster* (fruit fly) and *Folsomia candida* (collembolan). However, no discernible differences were observed, and the survival, growth, and reproductive capabilities of decomposers remained unaffected. Moreover, an in-depth five-year field assessment confirmed that there were no apparent differences in tree growth, flower characteristics, or fruit phenotype between non-GM controls and the cisgenic C44.1.146 apple line (Schlathölter et al., 2023). Similarly, during a twelve-year field trial involving GM apple lines that produced the antimicrobial protein attacin E from *Hyalophora cecropia* pupae for fire blight resistance, there were no observed alterations in fruit, flower, or tree morphology compared with the non-GM Galaxy apple cultivar (Borejsza-Wysocka et al., 2010).

5.4. RNA interference (RNAi) of target genes in plants

RNA interference (RNAi) is a regulatory process that involves targeted gene silencing using sequence-specific double-stranded RNA (dsRNA) molecules. These dsRNA precursors vary in length and origin (Koeppe et al., 2023). In plant cellular systems, dsRNAs are processed into three primary categories: short interfering RNAs (siRNAs), microRNAs (miRNAs), and piwi-interacting RNAs (piRNAs). Collectively, siRNAs and miRNAs are termed small RNAs (sRNAs) (Chen et al., 2021). The cellular pathway of dsRNA-induced RNAi in plants begins with the cellular uptake of dsRNAs. These dsRNAs are rapidly cleaved by DICER-LIKE (DCL) endonucleases resulting in the production of siRNAs that are typically 20 to 25 nucleotides long, with 2-nucleotide 3′ overhangs at both ends. One strand of the siRNA duplex was integrated into an ARGONAUTE (AGO) protein to form the RNA-induced silencing complex (RISC). siRNA molecules direct RISC to examine complementary target transcripts in the cytosol After recognition, RISC mediates the cleavage or degradation of target transcripts, leading to post-transcriptional gene silencing (PTGS). By expressing RNAi constructs in plants, specific target genes can be suppressed, ultimately resulting in a range of beneficial effects.

RNAi technology has been successfully used to generate desirable plants. One such application involves the introduction of a dsRNA unit that targets the 1-Aminocyclopropane-1-carboxylate (*ACC*) oxidase gene, which is involved in ethylene production (Xiong et al., 2005). The resulting transgenic tomatoes exhibited altered ACC activity, leading to significantly reduced ethylene production and extended shelf life. Furthermore, RNAi has been used to increase the content of artemisinin, a valuable medicinal compound, in transgenic *Artemisia annua* plants by targeting squalene synthase (SQS) in the sterol pathway (Zhang et al., 2009). In addition to regulating fruit ripening, RNAi technology has been applied to modify plant metabolite profiles for nutritional improvement, biofortification, and the elimination of allergens and toxins (Saurabh et al., 2014). For example, RNAi has been used to enhance carotenoids and flavonoids (Davuluri et al., 2005). Similarly, RNAi has been harnessed to augment the carotenoid concentration in *B. napus* by downregulating the expression of the lycopene epsilon cyclase (ε-CYC) gene. Transgenic Brassica seeds originating from this approach exhibited enhanced levels of zeaxanthin, violaxanthin, β-carotene, and lutein (Yu et al., 2008). Moreover, RNAi-mediated suppression of the *OsDWARF4* gene in rice resulted in reduced plant stature with upright leaf architecture, consequently leading to heightened photosynthetic activity in the lower leaves. This modified plant phenotype demonstrates the potential for increased crop yield when cultivated under high-density planting conditions (Feldmann, 2006). RNAi has been shown to play pivotal roles in the generation of seedless fruits, regulation of plant biomass, modification of flower color, and enhancement of tolerance to biotic and abiotic stress. Various crops have been further modified using RNAi for different traits (Table 3). These achievements demonstrate the versatility and potential of RNAi technology for the advancement of plant breeding.

5.5. Gene and genome editing of plants for precise development of crop traits

Conventional plant breeding methods face constraints in meeting the demands of the continuously expanding global population. Recent advances in marker-assisted selection and genomics-assisted breeding have extended the boundaries of conventional breeding practices. Genome editing has introduced a groundbreaking set of tools for plant breeding, enabling unprecedented accuracy, efficiency, and cost-effectiveness (Abdul Aziz et al., 2022). With this new toolkit, plant breeding is poised

Table 3 RNAi for the enhancement of crop traits

Plant	Gene	Trait improved	Source
Solanum lycopersicum	ACC synthase (*ACS*)	Decreased ethylene	Gupta et al. (2013a)
Oryza sativa	*OsGA20ox2*	Grain yield improvement	Qiao et al. (2007)
Glycine max	*GmNFYA3*	Drought tolerance	Ni et al. (2013)
Lycopersicon esculentum	Polygalacturonase	Delayed fruit ripening	Sheehy et al. (1988)
Oryza sativa	*SPL*	Panicle branching	Yang et al. (2019b)
Glycine max	*GmFNSII, F3H*	Isoflavone enhancement	Jiang et al. (2014)
Solanum lycopersicum and *Solanum melongena*	*Pad-1*	Parthenocarpy induction	Matsuo et al. (2020)
Gossypium hirsutum	*GhADF1*	Drought tolerance	Qin et al. (2022)
Triticum sp.	*WZY2*	Osmotic stress tolerance	Yu et al. (2019a, 2019b)
Oryza sativa	*OsABA8ox1*	Saline–alkaline stress tolerance	Liu et al. (2022)
Zea mays	*CgbHLH001*	Drought tolerance	Zhao et al. (2021)
Glycine max	*eIF4E1*	Soybean mosaic virus (SMV) resistant	Gao et al. (2019)
Zea mays	*Amy1*	Aspergillus flavus resistance	Gilbert et al. (2018)
Lycopersicon esculentum	*LeCOR413PM2*	Cold stress tolerance	Zhang et al. (2021a)
Solanum lycopersicum	*PolA1*	Meloidogyne incognita resistance	Chukwurah et al. (2019)
Triticum aestivum	*TaPUB-1*	Salt tolerance	Wang et al. (2020)
Oryza sativa	*OsDIS1*	Drought tolerance	Ning et al. (2011)
Cucumis sativus	*CsGolS4*	Cold and Drought tolerance	Ma et al. (2021)

to transcend the current limitations and enter the next generation of crop cultivation.

This breakthrough can be traced back to 1996 when it was demonstrated that zinc finger domains in combination with Fok1 endonuclease domains could precisely cut DNA at particular locations, functioning as site-specific nucleases (SSNs) (Kim et al., 1996). Meganucleases (MegaNs), which possess endonuclease activity, recognize long DNA sequences ranging from 14 to 40 nucleotides, enabling them to create double-stranded cleaves at recognition sites. However, their application in genome editing has been limited due to the scarcity of available MegaNs, making them unsuitable for editing every genomic locus (Abdallah et al., 2021). Subsequent investigations resulted in the creation of transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR) with CRISPR-associated protein 9 (Cas9) (Fig. 2, c). Compared to other genome-editing technologies, CRISPR/Cas9 has gained widespread popularity because of its user-friendly nature.

The CRISPR/Cas9 system comprises two essential elements: a customizable single-guide RNA (sgRNA) and a Cas9 endonuclease. Additionally, a specific DNA sequence called the protospacer adjacent motif (PAM) (5′NGG3′) is required for inducing double-stranded breaks at the targeted genomic sites. These breaks can be repaired using homology-directed repair (HoDR) or non-homologous end joining (NHEJ). NHEJ is error-prone and often leads to insertions or deletions at the target site (Khatodia et al., 2016). CRISPR/Cas9 has emerged as a powerful tool for enhancing crop traits, including nutritional, biotic, and abiotic stress tolerance (Guo et al., 2023).

Targeted mutagenesis has facilitated crop improvement by enabling the transfer of desired traits with reduced reliance on wide genetic crossing and extensive progeny genotyping. Genome editing allows precise genetic modifications through targeted mutagenesis. Specific knockout of the betaine aldehyde dehydrogenase 2 (*BADH2*) gene using TALENs disrupts the production of 2-acetyl-1-pyrroline, a key aromatic compound in rice, leading to the development of a fragrant variety of rice (Shan et al., 2015). Genome editing was used to introduce specific mutations in the P171 and G628 codons of *OsALS* using a cytidine base editor (CBE). This has resulted in the development of rice plants that exhibit broad-spectrum tolerance to herbicides that inhibit acetolactate synthase (ALS), providing enhanced weed management solutions for rice producers (Zhang et al., 2021b). Another significant benefit of genome editing in plant breeding is the ability to eliminate deleterious genes. CRISPR-Cas9 was employed to inactivate *OsERF922*, a gene that negatively regulates fungal blast resistance in plants. This has led to the development of rice plants that are resistant to fungal blasts (Wang et al., 2016). Because of the deleterious load typically resulting from numerous minor-effect mutations exhibiting a polygenic nature, efficient multiplex genome editing shows great promise as an approach for removing these undesirable alleles. Moreover, mutations produced using the CRISPR/Cas9 system are stable and inheritable in subsequent generations following classical Mendelian segregation. Using CRISPR/Cas9-targeted mutagenesis of the *OsRR22* gene, rice salinity stress tolerance was enhanced (Zhang et al., 2019). Bao et al. (2019) further demonstrated improved plant architecture in soybean through the CRISPR/Cas9-mediated targeted mutagenesis of *GmSPL9* genes. An herbicide-resistant watermelon variety was developed by base editing the *als* gene using a CRISPR/Cas9-mediated system (Tian et al., 2018).

The ability of CRISPR/Cas9 to edit multiple genomic sites concurrently is enabled by the expression of several sgRNAs within the same cell. Various methods such as transfer RNA processing, ribozyme self-cleavage, crRNA arrays, and Csy4 ribonuclease cleavage have been employed for multiplex editing (Minkenberg et al., 2017). Approximately 25% of vascular plants exhibit polyploidy, a condition in which they possess multiple sets of chromosomes. Many of these polyploid plants hold significant agricultural importance, including *Gossypium* spp., *S. tuberosum*, hexaploid bread wheat (Gardiner et al., 2019), and tetraploid Brassica (Abe et al., 2019). Members of a gene family typically exhibit overlapping structures and functions, leading to functional redundancy and genetic robustness. Consequently, merely knocking out one paralog may not be adequate to produce a noticeable phenotypic effect, often requiring the mutation of

two or more paralogues. Multiplex editing techniques can be employed using CRISPR/Cas9. For instance, the gluten gene family in wheat consists of genes that encode at least 29 a-gliadins, 18 g-gliadins, and 10 u-gliadins, in addition to 16 low molecular weight and 6 high molecular weight glutenins (Jouanin et al., 2020). Certain immunogenic epitopes present in a-, g-, and u-gliadins, and in low-molecular-weight glutenins to a lesser extent, can trigger celiac disease, an autoimmune condition that affects approximately 1%–2% of the human population (Jouanin et al., 2020). Conventional breeding methods for obtaining gluten-free wheat face challenges due to the genetic complexity of this trait. However, CRISPR/Cas9 technology has proven successful in simultaneously editing hexaploid bread wheat with different a- and g-gliadin genes. Remarkably, a single wheat line with 35 successfully mutated genes showed an 85% reduction in immunoreactivity (Sánchez-León et al., 2018).

Genome editing methods based on the genetic and biological information of model plants provide a direct means of enhancing complex traits in diverse crop species. This principle enables the sharing of significant traits across species. A compelling example of cross-species trait sharing is the editing of the mildew resistance locus (*MLO*), a recessive gene that was initially found in barley. Disabling this gene results in durable, wide-ranging resistance to powdery mildew (Jørgensen, 1992). This resistance has been effectively attained in various plant varieties such as wheat (Wang et al., 2014), tomato (Nekrasov et al., 2017), and grape (Wan et al., 2020), through genome editing techniques. In addition, *OsNP1* and *ZmIPE1*, which encode a potential glucose-methanol-choline oxidoreductase, have significant functions in inducing male sterility. Using the CRISPR-Cas9 technology, modifications have been made to the counterparts of these genes in wheat, specifically TaNP1, leading to full male sterility (Li et al., 2020).

In the context of crossbreeding, overcoming the tight genetic linkages between beneficial and deleterious loci can be challenging. Traditional breeding methods often involve extensive backcrossing to separate such loci, which is time-consuming and unfeasible. Genome editing offers two promising strategies to address this issue. First, genome editing techniques such as SSNs can be employed to trigger chromosomal rearrangements, leading to an upsurge in recombination incidents. SSNs can generate mutual chromosomal translocations and inversions within the same chromosome in plant species, which can help break genetic linkages (Shan et al., 2013; Beying et al., 2020). Alternatively, a direct editing approach can be used to modify or remove unwanted alleles, thereby eliminating the need for traditional introgression. This has been successfully demonstrated in various plants, including tomatoes, where the undesired allele has been knocked out (Roldan et al., 2017). Furthermore, in maize, two closely linked genetic alleles have been combined using genome editing (Gao et al., 2020a, 2020b), demonstrating the potential of this approach to overcome linkage drag.

Another approach for domesticating new species involves engineering established domestication genes to recreate the characteristics of a domesticated plant ideotype. This has been successfully attained through genome editing techniques, such as CRISPR/Cas9, which can act on domestication-related genes in orphan crops and wild species. By creating engineered neo-domesticates, it is possible to use them directly as crops or cross them with superior lines to introduce novel traits without the time delay associated with the use of wild germplasm.

5.6. *De novo domestication of crops using wild relative genetic resources*

The domestication and selection of modern cultivars have led to a significant loss of genetic diversity in ancestral crops and wild germplasm. Several staple crops are unable to meet their micronutrient requirements, leading to deficiencies, and crop production is further challenged by the impacts of changing climatic conditions (Jiang et al., 2013). To address these challenges, it is essential to explore and utilize the genetic resources present in wild relatives and landraces assembled through de novo domestication (Fig. 2, e). This process depends on knowledge-based breeding and involves the integration of a limited number of genes from wild relatives (Li and Yan, 2020). In this approach, well-examined genes associated with domestication were selected as focal genes. These genes are typically easier to modify than beneficial genes with unknown functions, which are the focus of alternative genetic enhancement approaches.

De novo domestication can be categorized into four primary stages. These include choosing an appropriate initial material, setting up an effective transformation system and a well-annotated reference genome, editing genes linked to domestication-related traits, and subsequently engaging in breeding and field assessments before introducing the new cultivar to the market. Although the process of de novo domestication is clear, each of these four steps, or all of them combined, could present technical bottlenecks, especially for wild species and cultivated crops (Atkins and Voytas, 2020). This is evident in the case of soybeans, where establishing transformation and genome editing systems remains a considerable challenge, resulting in either very difficult establishment or low efficiency. Similarly, for numerous wild species, the absence of a well-annotated reference genome, limited understanding of genes related to domestication, and the lack of a robust genome editing system hinder the progress of de novo domestication efforts. These technical obstacles must be addressed to fully harness the potential of de novo domestication strategies for improving crops.

Nevertheless, advancements in genomics and bioinformatics provide a promising route for the examination and identification of existing genetic structures and variations that can then be integrated into elite crop lines through introgression. Investigations of rice landraces obtained from several agroecological regions in Northern India have revealed substantial differences in mineral contents, such as iron and zinc, demonstrating a wide range of potential gains (Roy and Sharma, 2014). Recent breakthroughs in genomics and bioinformatics have enabled the identification of pangenomic R genes linked to rust resistance in wild diploid wheat (Arora et al., 2019). This indicates that novel technologies for de novo domestication or re-domestication of wild relatives of crop species will enhance crop yield and, more crucially, provide yield stability under less-than-ideal conditions (Zhang and Batley, 2020).

The utilization of the CRISPR-Cas9 technology has enabled the successful introduction of domestication-related traits into stress-tolerant *Solanum pimpinellifolium* wild tomato accessions,

achieving the objective of re-wild breeding with high efficiency (Zsögön et al., 2018; Li et al., 2018). Similarly, *Physalis pruinosa*, an orphan crop distantly associated with tomatoes, was significantly enhanced through the editing of genes analogous to those involved in tomato domestication (Lemmon et al., 2018). Furthermore, in a recent breakthrough, the de novo domestication of wild allotetraploid rice, *Oryza alta*, was performed (Yu et al., 2021). In this study, agronomically important genes in *O. alta* were annotated using a high-quality genome assembly and data obtained from the domestication of diploid rice. Multiple edited lines were generated using genome editing techniques, each exhibiting different improvements in essential agronomic traits, such as plant height, reduced seed shattering, increased stem thickness, and altered heading date. Although modern molecular breeding of crops demonstrates greater efficiency than traditional breeding, several challenges need to be addressed.

5.7. *Haploid induction for crops trait manipulation*

In traditional plant breeding, the process of obtaining highly homozygous and stable cultivars involves six–seven generations of self-pollination. However, the production of double haploids provided a more efficient alternative by solidifying the recombinant haploid genomes within two generations (Fig. 2, d). To achieve this, direct editing of endogenous plant genes is an effective approach in generating haploid inducer lines. For example, in maize, rice, and wheat, the disruption of *MTL*, *PLA1*, or *NLD*, which encodes a phospholipase specific to sperm cells, results in the generation of impaired male gametophytes and triggers a maternal haploid induction trait (Dong et al., 2018; Wang et al., 2019; Liu et al., 2020). CRISPR/Cas9 technology has been successfully used to generate haploid inducer lines in various plant species. In maize, manipulation of the DAP2-like MADS-box transcription factor (*DMP*) using CRISPR-mediated mutagenesis produced similar results (Zhong et al., 2019). Furthermore, the deletion of the N-terminal a-helix of CENH3 in *A. thaliana* resulted in the production of a haploid inducer line (Kuppu et al., 2020). Similarly, in wheat, genome editing of *TaCENH3a* led to a haploid induction rate of 7%, and restoration of frameshift alleles for heterozygous genotypes resulted in higher rates of paternal haploid induction compared to homozygous combinations (Lv et al., 2020).

Angiosperm seed development is initiated by a process called double fertilization. In this process, haploid egg cells fuse with the central diploid sperm cells, leading to the formation of a diploid embryo and triploid endosperm (Hisanaga et al., 2019). However, in certain plant species, seeds can be reproduced asexually through a phenomenon known as apomixis. This mechanism results in the generation of genetically identical seeds and has great potential for plant breeding applications (Sailer et al., 2016). Although apomixis occurs naturally in over 400 plant species, it is not commonly found in major crops and is difficult to produce using traditional breeding methods (Willmann, 2019). One approach to inducing apomixis involves the disruption of meiosis and the generation of unreduced gametes through genome editing. Knockout of the meiotic genes *REC8*, *PAIR1*, and *OSD1* through CRISPR/Cas9 induces apomeiosis in rice, resulting in the production of unreduced gametes and the potential for clonal seed formation (Khanday et al., 2019; Wang et al., 2019). Another method of triggering apomixis is to promote embryonic development in unfertilized egg cells without actual fertilization. The improper expression of *BBM1* in unfertilized rice egg cells triggers embryogenesis, and when combined with MiMe mutations achieved through genome editing, it gives rise to synthetic apomixis (Khanday et al., 2019). However, challenges remain before synthetic apomixis can be practically used in contemporary agriculture, such as achieving hybrid vigor. Modulation of endosperm fertilization may hold the key to further advancements in the generation of synthetic apomixis in various crops.

6. Speed breeding: an emerging approach to accelerate plant breeding

Numerous beneficial genes have been identified in diverse plant species that play crucial roles in enhancing plant characteristics. These genes have become prime targets in breeding programs aimed at creating improved cultivars by employing both conventional and contemporary breeding methods, such as marker-assisted selection, genetic engineering, and genome editing (Jain, 2012). Nevertheless, a significant drawback in the development of improved varieties is the time consumption. Traditional approaches take more than a decade from the initial cross to the final release of an improved variety. Given these challenges, breeders must integrate various technologies to accelerate crop development. The introduction of speed breeding technology represents a breakthrough concept wherein plants are cultivated in a manner that keeps pace with advancements in genomic technology.

Speed breeding is the collection of methodologies that involve the manipulation of environmental factors in which crop genotypes are cultivated to accelerate the processes of flowering and seed production (Fig. 3). This will rapidly and efficiently advance the subsequent breeding generation to conserve time and resources. Speed breeding yields approximately three to nine generations per year, leading to the accelerated development and release of new crop cultivars compared to one to two generations per year through traditional selection methods (Ochatt et al., 2002; Ghosh et al., 2018). Furthermore, rapid breeding allows for the rapid development of homozygous and stable genotypes (Watson et al., 2018). Moreover, speed breeding techniques harmonize well with marker-assisted selection (MAS) and high-throughput phenotyping techniques for the simultaneous selection of multiple desired traits.

This approach has been applied to several important crops. Controlled growth chambers equipped with adjustable factors such as light intensity, temperature regime, photoperiod, soil moisture, carbon dioxide level, and nutrient supply have a profound impact on the growth processes of plants. These modifications have been incorporated into crop cultivation. For instance, in the IR 64 rice variety, early flowering was induced by manipulating the light exposure in a growth chamber (Köhl 2015). Similarly, in groundnuts, continuous exposure to 450 W lamps for 24 h for 25 days after germination triggers early flowering (O'Connor et al., 2013). Altering the temperature regime and photoperiod of groundnuts results in rapid plant development (O'Connor et al., 2013). In soybean, a breeding cycle of up to five

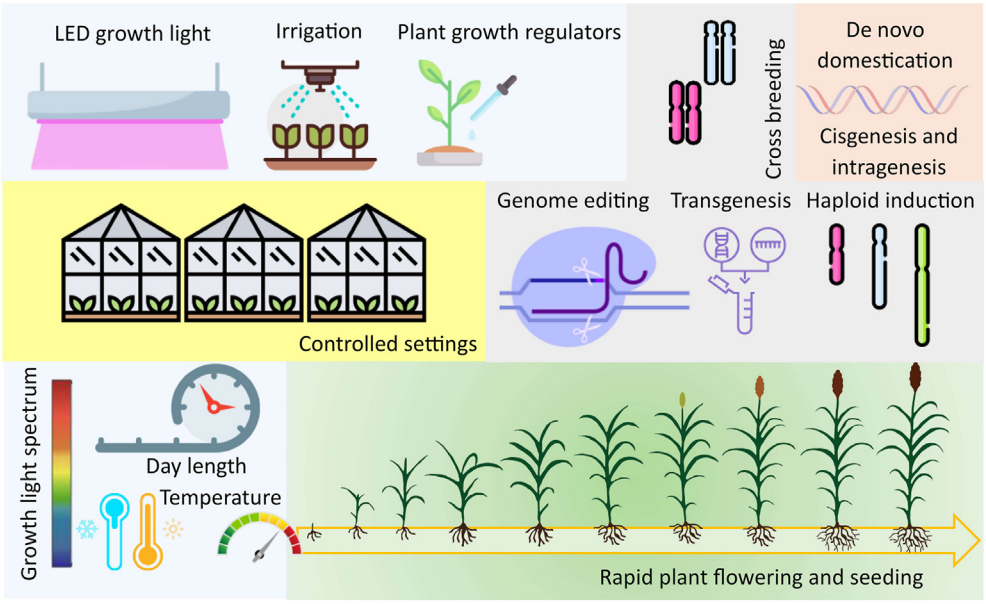

Fig. 3 Speed breeding accelerates the rate of crop flowering and seeding progress

The technique of speed breeding utilizes precise environmental conditions in a controlled setting for the growth and development of crops. It can be integrated with advanced crop breeding methods to enhance the speed of genomic and phenotypic selection, which will increase the genetic gains.

generations per year has been attained by a combination of more than 400 ppm of CO_2 with a 14-h light exposure cycle in a growth chamber (Nagatoshi and Fujita, 2019). Additionally, in soybeans, immature seeds from plants grown under CO_2 supplementation exhibited a high germination rate, similar to that under control conditions (Nagatoshi and Fujita, 2019). Moisture stress causes many crop species to exhibit early flowering and seed set (Shavrukov et al., 2017). In barley, wheat, and chickpeas, a significant increase in grain filling and maturation occurs when the moisture status decreases gradually toward the end of the flowering stage (Watson et al., 2018). Lentil and faba bean achieved around eight generations per year by employing plant growth regulators, such as auxin, cytokinin, and flurprimidol, under modified temperature conditions with 18 h of light at 22 °C and 6 h of dark at 18 °C in the growth chambers (Mobini and Warkentin, 2016). Lentils can reach approximately seven to eight generations per year by integrating early harvest practices with the application of plant growth regulators (Mobini et al., 2014).

Speed breeding has been effectively combined with MABC to develop salt-tolerant rice (Rana et al., 2019). This integration resulted in achieving six backcross generations within 17 months, with an average of 85 days per generation. The BC_3F_3 lines obtained through this process were subjected to salinity stress and exhibited significantly lower Na^+ accumulation, higher survival rates, and increased biomass compared to the recipient parent (Rana et al., 2019). Furthermore, numerous phenotyping protocols have been adapted to accelerate breeding systems, enabling the characterization and selection of essential traits. For instance, various traits have been identified in wheat and barley through speed breeding, including seminal root traits for drought adaptation (Richard et al., 2015), grain dormancy for tolerance to preharvest sprouting (Hickey et al., 2009, 2010), adult plant resistance (APR) to leaf rust (LR) (Riaz et al., 2016), yellow spot resistance (Dinglasan et al., 2016), and stripe rust resistance (Hickey et al., 2011). This acceleration facilitates efficient assessment and identification of desirable traits in crops, contributing to the improvement of crop varieties with enhanced resilience and productivity.

Domestication is frequently associated with polyploidy and several crops exhibit this condition. However, polyploidy poses challenges for crop improvement, owing to barriers in sexual reproduction in related species and the complex inheritance patterns associated with multiple sets of chromosomes (Renny-Byfield and Wendel, 2014). When polyploidy is closely associated with domestication, a swift re-domestication approach involving the deliberate induction of polyploids becomes a direct pathway for introducing new genes and alleles from wild relatives. Speed breeding is a valuable technique for accelerating re-domestication process. This approach can benefit polyploid crops such as peanuts and bananas. In the case of peanut, an allotetraploid can be recreated through a process involving the crossing of wild diploids with AA and BB genomes. Chromosome doubling is induced by colchicine, multiple cycles of backcrossing, and the selection of desirable agronomic characteristics (Leal-Bertioli et al., 2018). Speed breeding is effective at hastening the re-domestication process at multiple selection stages (O'Connor et al., 2013). Furthermore, in bananas, the occurrence of polyploidy aligns with the domestication of various cultivars. This domestication process involves crosses between wild AA and BB species, accompanied by chromosome elimination and the introduction of genetic material from other species, which has led to the genome constitutions of AAA, AAB, and ABB, which are seedless cultivated forms (Simmonds and Rind, 1962). The limited number of foundational polyploid events coupled with the widespread clonal propagation of perennial varieties

susceptible to devastating diseases exacerbates the challenges posed by restricted genetic diversity (Ploetz, 2015). In both peanuts and bananas, the recreation of domesticated polyploid varieties using different diploid species and speed breeding techniques allows access to new traits, such as disease resistance, and facilitates the rapid development of novel crop varieties. Additionally, in the case of bananas, direct editing of existing elite triploid cultivars can lead to the rapid deployment of improved lines in the short term, avoiding the time and costs associated with re-synthesizing triploids (Naim et al., 2018; Tripathi et al., 2019).

To overcome the challenges posed by polysomic genetics, certain breeders employ a breeding strategy that involves the use of diploids as donors with the desired traits. Subsequently, the polyploid state was reconstituted using unreduced gametes and crosses between individuals with different ploidy levels. This approach is an essential way to generate new varieties as it requires less time and resources than the direct breeding of polyploids. This method has been proven to be effective for specific crops, including bananas and potatoes (Ortiz, 1995; Jansky et al., 2016). In banana, the breeding process involves crossing diploid elite lines with wild relatives and selecting specific diploids (diploid hybrids). These selected diploids undergo chromosome doubling, resulting in interploid crosses, such as 4× × 2×, which lead to the production of seedless triploids (Ortiz and Swennen, 2014). The large size of banana plants and lengthy breeding cycles, lasting up to three years from hybrid creation to initial evaluation, can be addressed with the potential implementation of speed breeding techniques. Speed breeding can significantly accelerate the production of hybrids for evaluation and streamline subsequent crossbreeding and selection processes.

To expedite the realization of the benefits associated with gene editing and genetically modified (GM) traits, their incorporation into speedy breeding is crucial. Initial gene-editing applications rely on a limited number of non-elite genotypes that can be regenerated from plant tissue cultures and transformed. However, certain techniques have shown significant progress, offering high transformation efficiency even for elite genotypes (Richardson et al., 2014). Despite these advancements, the application of gene editing still requires the use of tissue culture and specific facilities equipped for genetic manipulation involving the Cas9 gene and sgRNA sequences (Doudna and Charpentier, 2014). Thus, the integration of rapid breeding accelerates crop domestication and ensures the rapid generation of improved varieties. These varieties have great potential for the development of urban food production systems.

7. Breeding molecular directed crops for urban resource utilization and crop production

The integration and enhancement of multiple complex traits within crop species play a crucial role in urban production systems. The significance of plant breeding has shown a notable increase over the years, contributing to approximately 20% of the yield growth between 1960 and 1980, which rose to 50% between 1980 and 2000 (Evenson and Gollin, 2003; Qaim, 2016). Ultimately, this approach will lead to the development of designer crops that improve natural resource use and cater to the specific needs of various consumer food demands, thereby ensuring efficient production practices in urban production systems (Fig. 4).

Although breeding for various traits remains essential in the projected agricultural scenarios, there is a growing emphasis on prioritizing plants that actively aid in mitigating global climate change. For instance, efforts can be directed toward developing crops that promote deep soil carbon sequestration. The organic carbon content of soils varies significantly, with at least a 15-fold

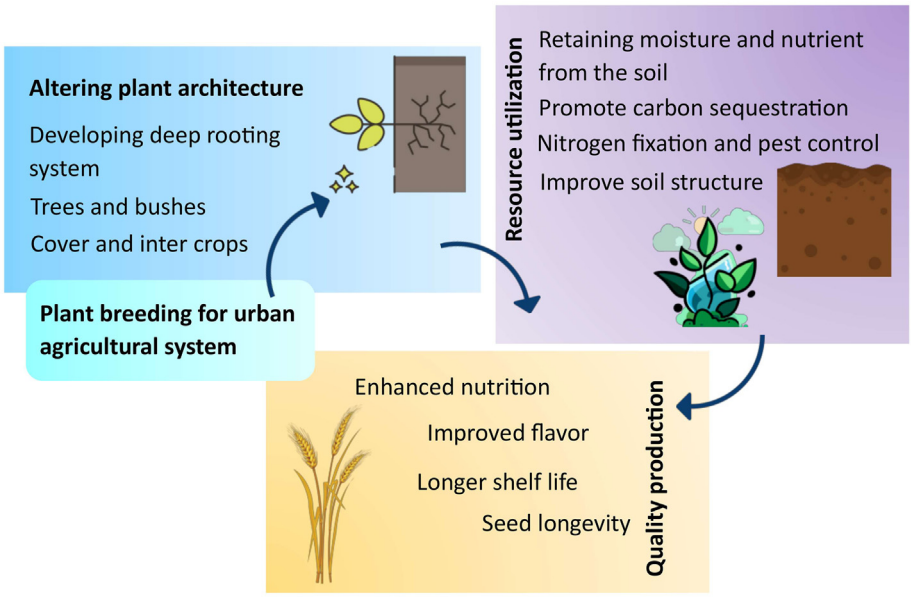

Fig. 4 Plant breeding in the urban production systems

The crops can be bred for the development of varieties with improved architecture including rooting system adapted to urban soils and enhancing its properties. The improvement of plant architecture and soil characteristics will lead to the high-quality products for the consumers.

difference observed in the UK (Bradley et al., 2005; Ostle et al., 2009). Enhancing crop plants with deeper and bushy root systems can improve the soil structure and capacity for retaining carbon, water, and nutrients while promoting sustainable plant yields. Several crops, such as *Panicum virgatum* (switchgrass), *Chrysopogon zizanoides* (vetiver), and sugarcane, have been shown to contribute significantly to SOC sequestration through their belowground biomass (Collins et al., 2010; Galdos et al., 2010). The breeding of these crop plants presents a promising approach for enhancing soil quality and carbon sequestration in urban soils that are degraded by anthropogenic activities.

Additionally, to combat rising temperatures, the breeding focus can be extended to trees and shrubs that are well suited for urban planting or for use in green walls (Fineschi and Loreto, 2020). Researchers identified a stem length regulator in tomatoes and developed a trait-stacking strategy using CRISPR/Cas9 genome editing to create compact, early-yielding tomato plants suitable for urban agriculture (Kwon et al., 2020). The same approach was successful for groundcherry, indicating its potential to expand the range of crops suitable for urban farming (Kwon et al., 2020). Furthermore, this approach presents valuable opportunities for the rapid and efficient breeding of non-food species that can serve as cover crops, relay crops, and intercrops. These non-food species can be selected based on their ability to provide ecosystem services, including nitrogen fixation, soil coverage, pollination, and pest control. Prioritizing eco-friendly species is aligned with sustainable agricultural practices in urban farming.

Breeding efforts have placed an equal emphasis on urban consumer choices. This has led to the development of crops with new properties, specific compounds for enhanced nutrition, and functional traits, such as longer shelf life. Molecular breeding has generated rice lines with reduced amylose content (Tao et al., 2016), and improved cooking and eating qualities (Ni et al., 2011). In addition, several enhanced cultivars of Quality Protein Maize (QPM), such as Vivek QPM-9, Pusa HM-4, Pusa HM-8, Pusa HM-9, CML244Q, CMl246Q, CML349Q, CML354Q, HQPM-1, HQPM-4, HQPM-5, and HQPM-7, have been successfully introduced for commercial production (Gupta et al., 2013b; Prasanna et al., 2020). Similar to cereals, efforts to improve quality and enhance value addition have been initiated for various legume crops. For example, in soybeans, the presence of a Kunitz trypsin inhibitor (KTI) in seeds requires a pre-heat treatment of soy flour, which increases the processing costs for soy-based products and feeds that impact the quality and solubility of seed proteins. To address this issue, six KTI-free soybean breeding lines have been successfully developed using a molecular marker-assisted backcross breeding approach, with DS9712 and DS9814 serving as the parent lines (Kumar et al., 2015). Likewise, in the soybean cultivar JS97-52, efforts were made to remove lipoxygenase-2 (Lox2), which leads to an off-flavor in soy products. Substantial improvements in seed longevity have been achieved using marker-assisted introgression of the null allele of Lox2 from PI596540 (lox2lox2) (Rawal et al., 2020). These developments highlight the potential of molecular breeding techniques to enhance the quality and market value of legume crops, thereby providing benefits to both producers and consumers in the agri-food industry.

In urban production systems, breeding for controlled environments shifts the emphasis to enable the rapid growth and performance of plants under low light conditions and the active manipulation of plant stature. Compared with breeding for phenotypic stability, plants can be bred to maximize genetic plasticity, allowing the occurrence of particular traits as a function of the quality of the ambient light spectrum (Folta, 2019). Thus, plant varieties can be developed with optimal size and focus on consumer traits, such as flavor and the accumulation of health-associated compounds. Gene editing is a central molecular tool for producing designer plants in controlled urban environments.

Although traditional breeding methods can be employed to acquire desired traits in crops, employing advanced biotechnological breeding techniques is a more efficient approach. For instance, the levels of healthy omega-3 long-chain polyunsaturated fatty acids in oilseeds have been augmented using Next-Generation Techniques (NGTs). These advancements have the potential to enhance human nutrition directly or indirectly through animal and fish feed (Napier et al., 2019). This enhancement has significant environmental advantages, as it reduces the strain on fisheries capturing and sustaining marine ecosystems, which operate at their maximum productivity levels in various regions. Despite the considerable advantages that nutritional improvement may provide, such crops have not yet been adopted by the agricultural sector, nor have they been sought after by consumers, as demonstrated by the lack of acceptance of Golden rice fortified with Vitamin A (Kettenburg et al., 2018). However, considerable emphasis should be placed on developing breeding programs that use advanced molecular breeding technologies for alternative cropping systems. This promotes a diverse and sustainable agricultural landscape driven by consumer preferences for healthier and more environmentally friendly food choices.

8. Future perspectives

Biotic and abiotic stresses on crops and the rise in food prices with looming economic consequences have disrupted the global food supply chain and aggravated poverty and hunger. This situation highlights the urgent need to employ modern molecular techniques in crop breeding to achieve sustainable food security. The current imperative is to utilize genomics-assisted breeding, along with high-throughput phenomics, for rapid genomic selection. This approach is crucial for the timely enhancement of agricultural practices and control of invasive species by developing plant cultivars that are resistant to pests, pathogens, and herbicides (Sabeem et al., 2022). In cases where germplasm exhibits favorable traits, such as disease resistance and environmental adaptation, but faces yield stagnation, or vice versa, modern molecular technologies are promising tools for suppressing undesirable genes in crops and attaining desirable genes (Masmoudi et al., 2021). Moreover, access to the pangenomes of crops facilitates improved breeding strategies, particularly for crops with complex genomes, elucidating common or unique gene combinations for different traits.

The efficient use of modern molecular technologies necessitates the adept computing and parallelization of genomic data to phenomic resources. Integrating genome editing with speed breeding can accelerate crop breeding, allowing favorable plants to be cultivated under accelerated conditions and yielding

desirable seeds in a shorter timeframe than under conventional greenhouse conditions. This method not only accelerates the development of stable homozygous phenotypes but also reduces the generation time, a process that typically takes several years. The implementation of efficient breeding strategies and the use of modern molecular technologies provide a more practical and sustainable force for agronomic development.

9. Conclusion

The rapid emergence of modern biotechnological tools has shown promise for crop breeding. These tools provide a means of introducing genomic variations and chromosomal alterations into plants with high precision and accuracy. It empowers breeders to direct and expedite crop selection in an unparalleled way and allows for limitless possibilities in terms of combining new alleles by removing breeding barriers. These tools can enable an effective selection of elite genotypes and lines with novel traits. Furthermore, speed breeding can accelerate the generation of superior high-performance cultivars with desirable traits. This is achieved by minimizing the time, space, and resources required to genetically select and enhance superior crop varieties. The breeding of crops has a profound impact on urban production systems, the development of crops suitable for environmental conditions, and consumer choices. Nonetheless, the primary uncertainty concerning the use of these techniques in plant breeding is related to the regulatory framework governing the commercial products produced. The utilization of high-throughput technologies is crucial for overcoming regulations and for employing modern plant breeding techniques to develop new crop cultivars.

Declaration of competing 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.

Acknowledgments

This research was funded by the United Arab Emirates University, Research Office, grant number 12F041 to KM.

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