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**Kanchan Kumari Gupta**  
Department of Horticulture,  
Assam Agricultural University,  
Jorhat, Assam, India

**Nitesh Kushawha**  
Division of Genetics, ICAR-Indian  
Agricultural Research Institute,  
Pusa, New Delhi, 110012, India

## Modern breeding approaches in vegetable crops: A comprehensive review of tools and applications

**Kanchan Kumari Gupta and Nitesh Kushawha**

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### Abstract

The global demand for nutritious, resilient, and high-yielding vegetable crops is steadily increasing in response to population growth, climate variability, and changing dietary patterns. Conventional breeding methods, while foundational, are often time-consuming and limited in their ability to address complex traits such as abiotic stress tolerance, disease resistance, and quality attributes. The integration of advanced breeding tools-including marker-assisted selection (MAS), genomic selection (GS), genome editing (e.g., CRISPR/Cas9), speed breeding and phenomics, etc. has ushered in a new era of precision breeding in vegetable crops. This review synthesizes recent advancements in these tools and highlights their application across a range of vegetable species, with illustrative case studies. We discuss the underlying principles, practical implementation, and comparative advantages of each method. Furthermore, we examine the major challenges impeding widespread adoption, such as limited genomic resources, high costs, regulatory uncertainties, and the need for skilled human capital. The paper concludes by outlining future prospects for democratizing breeding technologies and promoting global collaboration to accelerate genetic gains in vegetable crops. This integrative approach holds significant potential to enhance food and nutritional security while ensuring the sustainability of vegetable production systems worldwide.

**Keywords:** Marker-assisted selection (MAS), genomic selection (GS), genome editing, speed breeding, phenomics, doubled haploid

### Introduction

Vegetable crops constitute an essential component of global agriculture, contributing significantly to human nutrition, health, and economic security. They are rich sources of dietary fiber, vitamins (such as A, C, and folate), minerals (including potassium and magnesium), antioxidants, and phytonutrients that help prevent chronic diseases and malnutrition (Dias, 2011; Nicolle *et al.*, 2004) <sup>[10, 27]</sup>. According to the Food and Agriculture Organization (FAO), global vegetable production reached over 1.1 billion metric tonnes in 2022, with crops such as tomato, onion, cabbage, and leafy vegetables forming the bulk of this output (FAO, 2023) <sup>[11]</sup>. However, productivity in vegetable crops remains highly variable across regions due to genetic, environmental, and management constraints.

Traditional breeding techniques-including mass selection, pedigree breeding, and backcrossing-have historically been effective in improving yield, disease resistance, and quality traits. Nonetheless, these methods are time-consuming, labor-intensive, and limited by the genetic complexity of traits and environmental interactions (Bai *et al.*, 2016) <sup>[12]</sup>. Furthermore, the narrow genetic base in many elite cultivars, driven by repeated selection and inbreeding, has resulted in increased vulnerability to biotic and abiotic stresses.

The global demand for vegetable crops is expected to increase by at least 30-40% by 2050, driven by population growth, urbanization, and the shift toward plant-based diets (Tilman *et al.*, 2011; Willett *et al.*, 2019) <sup>[34, 40]</sup>. This escalating demand places immense pressure on breeding programs to accelerate the development of improved varieties with higher productivity, resilience to climate change, enhanced nutritional profiles, and reduced input requirements. However, conventional breeding alone is insufficient to meet these multidimensional goals within the required timeframe.

In response, recent advances in molecular genetics, genomics, and data science have led to the

**Corresponding Author:**  
**Kanchan Kumari Gupta**  
Department of Horticulture,  
Assam Agricultural University,  
Jorhat, Assam, India

development of next-generation breeding tools that allow for more efficient, targeted, and predictive approaches to crop improvement. These include marker-assisted selection (MAS), genomic selection (GS), genome editing (e.g., CRISPR/Cas9), speed breeding, and phenomics. Such tools facilitate the rapid identification and manipulation of genes or quantitative trait loci (QTLs) associated with complex traits, thus enhancing selection accuracy and reducing breeding cycle time (Varshney *et al.*, 2021; Xu *et al.*, 2020)<sup>[36, 41]</sup>.

Vegetable crops are particularly suitable for the application of advanced breeding technologies due to their short life cycles, diverse reproductive systems, and availability of genomic resources. For instance, high-quality reference genomes and transcriptomic data are now available for major vegetable crops such as tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), carrot (*Daucus carota*), and lettuce (*Lactuca sativa*) (Kim *et al.*, 2014; Reyes-Chin-Wo *et al.*, 2017)<sup>[19, 30]</sup>. Coupled with high-throughput genotyping platforms and phenotyping technologies, these tools offer unprecedented opportunities to dissect complex traits and accelerate the development of climate-smart, nutrient-dense, and resilient vegetable varieties.

This review provides a comprehensive overview of the application of advanced breeding tools in vegetable crops, including recent developments, case studies, and future directions. It highlights how the integration of these technologies can help overcome current breeding limitations and contribute to sustainable vegetable production in the face of growing global challenges

### Marker-Assisted Selection (MAS)

Marker-assisted selection (MAS) is one of the earliest and most widely adopted molecular breeding tools, enabling indirect selection of desirable traits based on molecular markers tightly linked to genes or quantitative trait loci (QTLs). MAS offers significant advantages over conventional phenotypic selection, especially for traits that are difficult to measure, exhibit low heritability, or are expressed late in the plant's life cycle. It is particularly useful in vegetable crops, where many economically important traits—such as disease resistance, fruit quality, and shelf life—are governed by complex genetic interactions and are influenced by environmental factors.

In vegetable breeding, MAS has been effectively employed to accelerate the development of disease-resistant cultivars. For example, in tomato (*Solanum lycopersicum*), MAS has been instrumental in pyramiding resistance genes against Tomato Yellow Leaf Curl Virus (TYLCV). The *Ty-1*, *Ty-2*, and *Ty-3* resistance genes have been successfully introgressed into commercial varieties using tightly linked markers, reducing the need for time-consuming and labor-intensive phenotypic screening under natural disease pressure (Hanson *et al.*, 2000; Verlaan *et al.*, 2013)<sup>[14, 37]</sup>. Similarly, in cucumber (*Cucumis sativus*), the *dm1* locus conferring resistance to downy mildew has been mapped and used in marker-assisted backcrossing to develop resistant lines with high commercial potential (Cai *et al.*, 2019)<sup>[5]</sup>.

MAS is also widely used in brinjal (*Solanum melongena*) breeding programs. The introgression of resistance to the notorious fruit and shoot borer (*Leucinodes orbonalis*)—a major pest limiting brinjal productivity in Asia—has been facilitated using QTL-linked markers. These markers assist in the early-stage selection of resistant lines, reducing the need for pesticide applications and enabling sustainable crop production (Singh *et al.*, 2014)<sup>[32]</sup>. In addition, traits related to quality and nutrition have been improved using MAS in various vegetables. For

instance, in carrot (*Daucus carota*), markers linked to high provitamin A content ( $\beta$ -carotene) have been used to select biofortified lines suitable for addressing vitamin A deficiency (Just *et al.*, 2009)<sup>[17]</sup>.

MAS also plays a vital role in the improvement of complex traits like fruit shape, earliness, and shelf life in crops such as pepper (*Capsicum spp.*), melon (*Cucumis melo*), and onion (*Allium cepa*). In onion, where resistance to pink root (*Phoma terrestris*) is a key breeding objective, markers closely associated with resistance genes have enabled early selection in segregating populations, thus reducing breeding cycle duration (Havey & Galmarini, 2004)<sup>[15]</sup>.

Despite its success, MAS has certain limitations. Its efficiency depends on the availability of closely linked, reliable markers and a good understanding of trait genetics. For polygenic traits or those controlled by multiple minor-effect QTLs, MAS is often less effective than genomic selection (GS). However, when used in conjunction with other modern breeding tools such as double haploids and speed breeding, MAS remains a powerful and cost-effective strategy for improving traits in a targeted manner.

The continued refinement of molecular marker technologies, such as single nucleotide polymorphisms (SNPs), Kompetitive Allele-Specific PCR (KASP) assays, and genotyping-by-sequencing (GBS), has made MAS more precise, scalable, and accessible. As a result, MAS is expected to remain a cornerstone of precision breeding in vegetable crops, especially in regions where rapid deployment of improved varieties is essential to address emerging biotic and abiotic stresses.

### Genomic Selection (GS)

Genomic selection (GS) represents a paradigm shift in modern plant breeding, offering a more predictive, holistic, and efficient approach to crop improvement. Unlike marker-assisted selection (MAS), which focuses on a few markers associated with major genes or QTLs, GS uses genome-wide marker data to estimate the breeding value of individuals based on thousands of small-effect loci simultaneously. This allows for the selection of superior genotypes early in the breeding cycle—even before phenotypic data is available—thereby significantly reducing the time and cost required to develop new varieties (Meuwissen *et al.*, 2001; Heffner *et al.*, 2009)<sup>[16, 24]</sup>.

In vegetable crops, where many important traits such as yield, stress tolerance, fruit quality, and shelf life are quantitatively inherited and controlled by multiple minor-effect loci, GS is especially advantageous. It has been successfully applied in crops such as tomato, carrot, onion, and pepper, demonstrating enhanced accuracy in predicting complex traits over traditional phenotypic or MAS approaches. For instance, in tomato (*Solanum lycopersicum*), genomic prediction models incorporating single nucleotide polymorphisms (SNPs) have been used to accurately forecast traits such as fruit weight, sugar content ( $^{\circ}$ Brix), and shelf life, significantly accelerating the breeding cycle (Finkers *et al.*, 2021)<sup>[12]</sup>. In onion (*Allium cepa*), GS has been explored to predict traits like bulb shape and storability, which are difficult to measure accurately and have low heritability under field conditions (Scholten *et al.*, 2016)<sup>[31]</sup>.

A key advantage of GS is its capacity to handle genotype-by-environment interactions (G×E), which are particularly relevant in vegetable breeding due to diverse cultivation zones and seasonal variability. By incorporating multi-environment phenotypic data into genomic prediction models, breeders can better account for trait plasticity and stability across environments, thus improving selection accuracy and the development of broadly adapted varieties (Crossa *et al.*, 2017)<sup>[9]</sup>.

Recent advancements in machine learning and statistical algorithms have further enhanced the predictive power of GS. Approaches such as genomic best linear unbiased prediction (GBLUP), Bayesian ridge regression (BRR), and random forests have been tested in various vegetable crops, with GBLUP and Bayesian models often providing the best balance between computational efficiency and prediction accuracy (Zhang *et al.*, 2017)<sup>[44]</sup>. In carrot breeding, genomic selection has been used to improve nutritional quality traits like  $\beta$ -carotene content, alongside yield-related attributes, using high-density SNP arrays (Le Clerc *et al.*, 2021).

Despite its promise, the practical implementation of GS in vegetables still faces some challenges, including the need for high-quality, well-curated genomic and phenotypic datasets, high-throughput genotyping platforms, and appropriate statistical infrastructure. However, with the decreasing cost of next-generation sequencing and advances in data management, the integration of GS into routine breeding pipelines is becoming increasingly feasible, even for resource-limited breeding programs.

Moreover, GS can be effectively combined with other breeding tools such as doubled haploids and speed breeding to fast-track varietal development. This integration is particularly useful for vegetables with long generation times or complex breeding systems. For instance, in pepper and tomato, combining GS with early-generation selection and speed breeding has allowed for significant gains in yield, disease resistance, and market-preferred traits within fewer breeding cycles (Lin *et al.*, 2020)<sup>[23]</sup>.

In summary, genomic selection is revolutionizing vegetable crop breeding by enabling the accurate, early, and cost-effective identification of elite genotypes. As genomic resources continue to expand and computational tools evolve, GS is poised to become a standard component of precision breeding strategies aimed at addressing food security, climate resilience, and nutritional quality.

### Genome Editing

Genome editing has emerged as one of the most revolutionary tools in advanced plant breeding, offering unparalleled precision in modifying the genetic makeup of crops. Among the various genome-editing technologies, CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats and associated protein 9) has gained prominence due to its simplicity, efficiency, and versatility. Unlike traditional breeding or even marker-assisted selection (MAS), genome editing enables direct, targeted modifications at specific loci, allowing breeders to introduce or knock out genes responsible for desirable or undesirable traits without altering the overall genetic background of elite varieties (Zhang *et al.*, 2018; Chen *et al.*, 2019)<sup>[8, 45]</sup>.

In vegetable crops, genome editing has opened new avenues for improving traits such as disease resistance, shelf life, nutritional content, and abiotic stress tolerance. For example, in tomato (*Solanum lycopersicum*), CRISPR/Cas9 has been used to knock out the *SIMLO1* gene, conferring resistance to powdery mildew without the need for conventional resistance gene introgression (Nekrasov *et al.*, 2017)<sup>[26]</sup>. Similarly, the *SIALC* gene, involved in fruit softening and post-harvest deterioration, has been edited to extend fruit shelf life, which is highly beneficial for commercial supply chains and reducing post-harvest losses (Yu *et al.*, 2017)<sup>[43]</sup>.

Another successful application is in lettuce (*Lactuca sativa*), where CRISPR has been used to target genes involved in bolting

and flowering, enabling the development of lines with delayed bolting—a crucial trait for regions with fluctuating temperatures (Zhang *et al.*, 2018)<sup>[45]</sup>. Additionally, editing genes associated with leaf morphology and texture has allowed breeders to fine-tune aesthetic and functional traits that cater to consumer preferences.

In cucumber (*Cucumis sativus*), genome editing of the *CsWRKY20* transcription factor has improved resistance to downy mildew—a major fungal disease affecting cucurbits—demonstrating the potential of CRISPR to tackle biotic stress with high precision (Chandrasekaran *et al.*, 2016)<sup>[7]</sup>. Furthermore, targeted editing of genes like *CLV3* in leafy vegetables such as Chinese cabbage (*Brassica rapa*) has shown promise in improving plant architecture and yield through enhanced meristem activity and increased organ formation (Li *et al.*, 2018).

One of the most remarkable features of genome editing is its capacity to produce "non-transgenic" or "transgene-free" plants. By segregating out the editing machinery through conventional backcrossing or using transient expression systems, it is possible to develop edited lines that are indistinguishable from naturally bred varieties at the molecular level, thus bypassing stringent GMO regulations in several countries (Turnbull *et al.*, 2021)<sup>[35]</sup>. This characteristic holds enormous promise for the commercialization of genome-edited vegetable varieties, especially in markets with regulatory flexibility.

Despite its potential, genome editing in vegetables still faces challenges related to tissue culture regeneration, transformation efficiency, and off-target effects. Many vegetable species, particularly those with recalcitrant genomes or polyploidy (e.g., onion and garlic), are difficult to transform using current methods. However, advances in DNA-free editing, base editing, and prime editing are rapidly addressing these limitations and are expected to broaden the applicability of genome editing to a wider range of vegetable crops (Anzalone *et al.*, 2020)<sup>[1]</sup>.

In conclusion, genome editing is redefining vegetable breeding by enabling targeted improvements in key traits with unprecedented accuracy and speed. As regulatory frameworks evolve and technical barriers diminish, the integration of CRISPR and related technologies into mainstream breeding programs will accelerate the development of high-performing, resilient, and consumer-focused vegetable varieties.

### Speed Breeding and Doubled Haploids: Accelerating Genetic Gains in Vegetable Crops

The integration of speed breeding and doubled haploid (DH) technology into vegetable crop improvement programs has significantly accelerated genetic gains and varietal development. These tools, often used in tandem or alongside genomic selection and genome editing, have revolutionized breeding pipelines by reducing the time required to achieve homozygosity and evaluate successive generations.

#### Speed Breeding

**Speed breeding** refers to the manipulation of environmental conditions (light, temperature, photoperiod, and humidity) to hasten plant growth and reproductive cycles. By providing extended photoperiods (up to 22 hours of light per day), breeders can achieve up to 4-6 generations per year, compared to 1-2 under conventional conditions (Watson *et al.*, 2018)<sup>[39]</sup>. This accelerated breeding is particularly beneficial in vegetables with long juvenile phases, such as tomato, pepper, and cabbage.

In tomato (*Solanum lycopersicum*), for instance, speed breeding has enabled the completion of a full breeding cycle in just 75-90

days using controlled-environment growth chambers. Combined with early flowering mutants (e.g., *sft* or *sp*) and embryo rescue techniques, this method has been used successfully for both inbred line development and hybrid testing (Ghosh *et al.*, 2018)<sup>[13]</sup>. Similarly, in *Brassica oleracea* (e.g., broccoli and cauliflower), speed breeding has shortened the breeding cycle from 18 months to less than 6 months, facilitating rapid trait introgression and selection under controlled conditions (Mobini *et al.*, 2020)<sup>[25]</sup>. Speed breeding also synergizes well with other technologies. For example, combining speed breeding with CRISPR/Cas9 editing allows for rapid fixation of edited alleles within a few generations. Additionally, integrating genomic selection within speed-breeding programs enables early-generation selection, further reducing breeding timelines.

Doubled Haploids (DH)

**Doubled haploid technology** allows for the generation of completely homozygous lines in a single generation through chromosome doubling of haploid cells. In vegetables, this technology is particularly impactful for crops with complex or highly heterozygous genomes, such as onion, cucumber, and brassicas. In cabbage and cauliflower, DH lines are generated through microspore culture or another culture techniques, and have been successfully used to develop inbred lines with enhanced uniformity and stability for hybrid development (Barroco *et al.*, 2005)<sup>[3]</sup>. Similarly, in hot pepper (*Capsicum annuum*), gynogenesis-based DH production has proven efficient for accelerating inbred development, especially for disease

resistance breeding programs (Supena *et al.*, 2006)<sup>[33]</sup>. DH technology is also critical for QTL mapping, genome-wide association studies (GWAS), and trait dissection, as it provides genetically stable lines with minimal segregation. The fixed nature of DH lines makes them ideal for constructing core sets of reference populations for genomic prediction and phenotypic evaluation. However, challenges remain in implementing DH technology across all vegetables. Species-specific protocols are often required due to varying responses to *in vitro* culture and chromosome doubling agents. Additionally, spontaneous chromosome doubling rates may be low in some species, necessitating the use of chemicals like colchicine, which can affect plant health and regeneration. Despite these challenges, ongoing research into *in vivo* haploid induction systems and doubled haploid machine learning models holds promise for broader application in vegetable crops (Prasanna *et al.*, 2020)<sup>[28]</sup>.

Integration and Impact

Together, speed breeding and DH technology offer a powerful means to enhance the efficiency of breeding programs. Their integration facilitates rapid trait fixation, quick cycling, and development of genetically pure lines, which is particularly important for hybrid seed production and trait stacking. In the context of climate change and increasing global food demand, these tools are indispensable for developing resilient, high-yielding, and nutritionally superior vegetable cultivars in a timely manner.

Table 1: Overview of Advanced Breeding Tools and Their Applications in Vegetable Crops

| Breeding Tool                       | Key Features                                            | Application in Vegetables                        | Example Crops      | References                                                                |
|-------------------------------------|---------------------------------------------------------|--------------------------------------------------|--------------------|---------------------------------------------------------------------------|
| Marker-Assisted Selection (MAS)     | Use of DNA markers linked to target traits              | Disease resistance, quality trait improvement    | Tomato, Eggplant   | Collard <i>et al.</i> (2001); Hanson <i>et al.</i> (2000) <sup>[14]</sup> |
| Genomic Selection (GS)              | Whole-genome prediction of breeding value               | Yield, stress tolerance, complex trait selection | Onion, Carrot      | Kumar <i>et al.</i> (2015); Crossa <i>et al.</i> (2017) <sup>[9]</sup>    |
| Genome Editing (CRISPR/Cas9)        | Precise, site-directed modification of DNA sequences    | Disease resistance, shelf-life, parthenocarpy    | Tomato, Lettuce    | Ueta <i>et al.</i> (2017); Nekrasov <i>et al.</i> (2017) <sup>[26]</sup>  |
| Speed Breeding                      | Accelerated crop cycles using controlled environments   | Rapid generation advancement                     | Tomato, Pepper     | Watson <i>et al.</i> (2018) <sup>[39]</sup>                               |
| Phenomics                           | High-throughput phenotyping using imaging and sensors   | Trait analysis, QTL mapping                      | Cucumber, Lettuce  | Yang <i>et al.</i> (2020)                                                 |
| Doubled Haploid (DH)                | Rapid development of complete homozygous lines          | Hybrid breeding, genetic analysis                | Brassica, Capsicum | Ferrie & Möllers (2011)                                                   |
| Marker-Assisted Backcrossing (MABC) | Introgression of specific genes using molecular markers | Resistance breeding, abiotic stress traits       | Melon, Eggplant    | Mutlu <i>et al.</i> (2008); Nankar <i>et al.</i> (2020)                   |

Multi-Omics Integration: A Systems Approach to Vegetable Crop Improvement

The complexity of traits in vegetable crops-especially those related to yield, quality, disease resistance, and stress tolerance-often arises from intricate interactions between genes, proteins, metabolites, and environmental cues. To address this, modern breeding programs are increasingly turning to multi-omics integration, which involves combining genomics, transcriptomics, proteomics, metabolomics, and phenomics to gain a holistic understanding of plant biology and improve the efficiency of selection strategies.

Genomics and Transcriptomics

Genomics provides the blueprint of an organism, revealing the structural composition and variation of DNA sequences across individuals. When combined with transcriptomics, which

profiles gene expression under specific conditions or developmental stages, breeders can identify candidate genes and regulatory pathways responsible for key agronomic traits. For example, in tomato (*Solanum lycopersicum*), transcriptomic profiling under heat stress has revealed the upregulation of heat shock proteins and transcription factors like *HsfA1a*, *HsfB1*, and *DREB*, which play pivotal roles in thermotolerance (Bita *et al.*, 2011). In cucumber (*Cucumis sativus*), integrated genomic and transcriptomic studies identified *CsPIP* aquaporin genes associated with salinity stress response, aiding the development of salt-tolerant varieties (Wang *et al.*, 2020)<sup>[38]</sup>.

Proteomics

Proteomics involves large-scale profiling of proteins, the functional molecules in cells. In vegetables, proteomics has been instrumental in elucidating protein-level changes during disease

responses, ripening, or abiotic stress. For instance, comparative proteomics in pepper (*Capsicum annuum*) identified differentially expressed proteins involved in ROS scavenging and pathogenesis-related pathways during bacterial wilt infection (Kim *et al.*, 2014) <sup>[19]</sup>. This information supports selection for biotic stress resistance at the functional level.

### Metabolomics

Metabolomics complements transcriptomics and proteomics by identifying and quantifying metabolites—small molecules that act as substrates or products in plant biochemical pathways. In lettuce (*Lactuca sativa*), metabolomic analysis has been used to differentiate bitter vs. sweet genotypes based on lactucopicrin and other sesquiterpene lactones, providing targets for nutritional breeding (Kim *et al.*, 2016) <sup>[18]</sup>. In broccoli (*Brassica oleracea* var. *italica*), metabolomic profiling has uncovered glucosinolate and flavonoid pathways linked to anti-cancer properties, helping breeders select for lines with enhanced health-promoting compounds (Brown *et al.*, 2020) <sup>[4]</sup>.

### Phenomics

Phenomics refers to the high-throughput measurement of plant phenotypes using advanced imaging and sensor technologies. This field bridges the gap between genotype and phenotype, especially for traits like canopy architecture, root morphology, or photosynthetic efficiency. For example, high-throughput phenotyping platforms using RGB, thermal, and hyperspectral imaging have been employed in carrot, lettuce, and spinach breeding to assess drought tolerance, leaf area index, and growth dynamics under real-time field conditions (Rahaman *et al.*, 2015) <sup>[29]</sup>. These tools are invaluable for non-destructive, rapid trait screening across large populations.

### Integrated Case Study: Tomato Shelf Life

A notable case of multi-omics integration is in improving shelf life and fruit firmness in tomato. By combining genomic QTL mapping, RNA-seq, and metabolomic profiling, researchers identified the *NOR*, *RIN*, and *SIALC* genes as key regulators of ripening and firmness. Simultaneously, metabolomics revealed changes in cell wall-modifying enzymes like pectin methylesterases and polygalacturonases during fruit softening (Centeno *et al.*, 2011) <sup>[6]</sup>. These insights guided CRISPR-based editing of *SIALC* and *SIPG2a* genes to extend shelf life without compromising taste or texture (Yu *et al.*, 2017) <sup>[43]</sup>.

### Advantages and Future Prospects

Multi-omics integration enhances predictive breeding models, facilitates trait dissection, and enables precision selection for complex traits. With the advent of AI and machine learning, breeders can now model the interactions between omics layers to predict plant performance under diverse environmental conditions. The establishment of vegetable pan-omics databases (e.g., Tomato Expression Atlas, Cucurbit Genomics Database) further supports data sharing and collaborative research. As costs for omics technologies continue to decline, their routine integration into breeding pipelines for crops like eggplant, onion, and leafy greens is becoming increasingly feasible.

### Genome Editing Tools (CRISPR/Cas9 and Beyond)

The advent of genome editing technologies has revolutionized the landscape of modern plant breeding, offering unprecedented precision and efficiency in modifying targeted genomic regions. Among these tools, the CRISPR/Cas9 system has emerged as the most versatile and widely adopted platform in vegetable crop improvement. Unlike traditional breeding or transgenic

approaches, genome editing enables the induction of specific genetic mutations or the alteration of gene expression with minimal linkage drag and without the need for introducing foreign DNA, thus facilitating faster trait incorporation and potentially reducing regulatory constraints (Nekrasov *et al.*, 2017) <sup>[26]</sup>.

In vegetable crops, CRISPR/Cas9 has been successfully employed for trait improvement in several species. Tomato (*Solanum lycopersicum*) has served as a model for genome editing applications due to its well-characterized genome and amenability to transformation. Targeted knockout of *SIMLO1* conferred resistance to powdery mildew, while editing of *SlIAA9* and *SlAGAMOUS-LIKE6* induced parthenocarp, promoting fruit set under stress conditions (Ueta *et al.*, 2017). Furthermore, modifications in *SIALC* and *SIPG2a* significantly enhanced shelf life and fruit firmness without affecting organoleptic properties (Yu *et al.*, 2017) <sup>[43]</sup>. In cucumber (*Cucumis sativus*), the editing of *CsWRKY* transcription factors has been explored to enhance resistance to biotic and abiotic stresses (Chandrasekaran *et al.*, 2016) <sup>[7]</sup>. Similarly, lettuce (*Lactuca sativa*) has been edited at the *LsPDS* and *LsFT* loci to demonstrate applications in pigment modification and flowering time control (Woo *et al.*, 2015).

Recent advancements in genome editing have introduced base editing and prime editing as next-generation tools. Base editing enables targeted nucleotide conversions, such as cytosine-to-thymine or adenine-to-guanine, without inducing double-stranded breaks. This approach was successfully applied in tomato to modify the *ALS1* gene, conferring herbicide resistance (Veillet *et al.*, 2019). Prime editing, which expands the scope of precise genome modifications to include insertions, deletions, and all possible base substitutions, holds considerable promise, although its application in vegetable crops remains nascent. Effective delivery of genome editing reagents remains a critical aspect of the technology's success. While *Agrobacterium*-mediated transformation remains the predominant method in tomato, eggplant, and brassicas, protoplast-based transformation has shown promise in species such as lettuce and carrot, despite challenges in regeneration. Furthermore, the delivery of pre-assembled CRISPR-Cas9 ribonucleoproteins (RNPs) allows for transgene-free editing, aligning with consumer and regulatory preferences in regions such as the United States and Japan, where genome-edited crops without foreign DNA are not regulated as genetically modified organisms (GMOs).

Genome editing has also shown potential in nutritional enhancement and abiotic stress tolerance. In *Brassica oleracea*, CRISPR/Cas9 editing of the *FAD2* gene successfully increased oleic acid content, improving the nutritional profile of oilseed vegetables (Okuzaki *et al.*, 2018). In carrot (*Daucus carota*), genome editing is being explored to modify anthocyanin biosynthesis pathways and enhance pigment stability and root quality traits (Klimek-Chodacka *et al.*, 2021). Despite these advances, several challenges persist, including limited transformation protocols for many minor vegetables, off-target effects, and regulatory uncertainties in regions such as the European Union.

Looking forward, genome editing in vegetables is expected to benefit from developments in multiplex editing, artificial intelligence-assisted guide RNA design, and synthetic biology. These innovations will enable the simultaneous modification of multiple genes, enhance editing precision, and even allow the creation of novel traits not present in the natural gene pool. As such, genome editing stands at the forefront of a new era in vegetable breeding, offering a rapid and precise approach to addressing the pressing challenges of climate resilience, nutritional security, and sustainable agriculture.

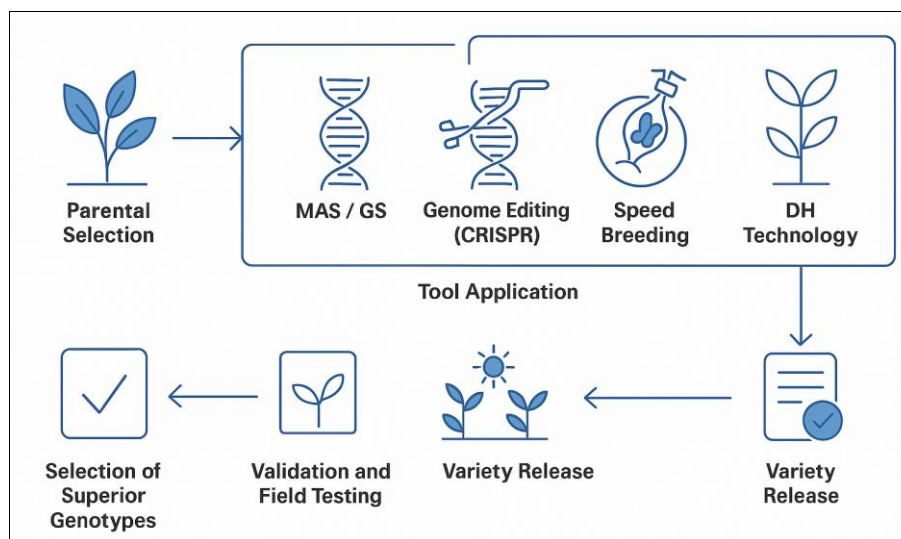


**Fig 1:** Diagrammatic representation of advanced breeding tools used in vegetable improvement

### Challenges and Future Prospects

Despite the transformative potential of advanced breeding tools in revolutionizing vegetable crop improvement, several significant challenges continue to impede their widespread adoption, especially in low- and middle-income countries. One of the foremost limitations is the lack of high-quality, annotated reference genomes for many economically important but under-researched vegetable species. Unlike major cereal crops, vegetables such as okra, ridge gourd, sponge gourd, and various indigenous leafy greens remain genetically less characterized, hindering effective application of genomic selection, gene editing, and marker-assisted breeding. Additionally, the high cost of establishing and maintaining genotyping and phenotyping infrastructure poses a considerable barrier. Advanced tools like next-generation sequencing (NGS), high-throughput phenotyping platforms, and bioinformatics pipelines often demand significant financial investment, making them less accessible to public sector breeding programs and small-scale private enterprises.

Another major concern revolves around the regulatory uncertainty associated with genome-edited crops. While countries such as the United States, Brazil, and Japan have adopted relatively progressive policies that differentiate genome-edited crops from traditional GMOs-particularly if no foreign DNA is introduced-many regions, including the European Union, continue to treat genome editing under the same stringent regulatory framework as transgenics. This lack of global harmonization in policy hinders research collaboration, technology transfer, and commercial deployment. Moreover, the successful implementation of advanced breeding technologies requires a multidisciplinary workforce skilled in molecular genetics, computational biology, quantitative genetics, and plant physiology. The shortage of trained personnel, particularly in developing countries, limits the scalability and impact of these technologies.

To overcome these hurdles, future research and development initiatives must prioritize the democratization of breeding technologies by investing in open-access genomic resources, low-cost genotyping platforms, and user-friendly decision support tools. Capacity-building programs focused on training young researchers in molecular breeding and bioinformatics will be essential to bridge the human resource gap. Furthermore, fostering global dialogues and partnerships among scientists, policymakers, and regulatory bodies is imperative to develop

clear, science-based, and proportionate regulations for genome-edited crops. Ultimately, ensuring equitable access to these tools-especially for smallholder farmers and breeders in resource-constrained regions-will be key to harnessing the full potential of advanced breeding in enhancing vegetable productivity, resilience, and nutritional quality in the face of climate change and food insecurity.

### Conclusion

Advanced breeding tools are revolutionizing vegetable crop improvement by enabling the rapid, precise, and efficient selection of complex traits, thereby overcoming many limitations of traditional breeding approaches. The integration of marker-assisted selection (MAS), genomic selection (GS), genome editing technologies such as CRISPR/Cas9, speed breeding, phenomics, and other molecular breeding strategies offers unprecedented opportunities to accelerate genetic gains and enhance resilience, productivity, and nutritional quality in vegetable crops. These innovations are particularly critical in the face of mounting challenges such as climate change, emerging pests and diseases, shrinking arable land, and growing demands for safe and nutritious food.

However, the successful deployment of these tools requires more than technological innovation-it necessitates enabling regulatory frameworks, investment in genomics infrastructure, skilled human resources, and cross-sectoral partnerships. Collaborative efforts between governments, research institutions, and private stakeholders will be essential to democratize access to these technologies and ensure their equitable application across diverse agroecological and socioeconomic contexts. With supportive policies and sustained interdisciplinary research, advanced breeding tools have the potential to transform vegetable agriculture and make a substantial contribution to global food and nutritional security.

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