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ADVANCES IN MOLECULAR BREEDING, GENOME EDITING, AND MULTI-OMICS FOR ACCELERATED IMPROVEMENT OF VEGETABLE CROPS

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ABSTRACT

Advances in molecular breeding, genome editing and multi-omics have transformed the improvement of vegetable crops, enabling accelerated development of varieties that combine high yield, superior quality and resilience to climate-induced stresses. Marker-assisted selection and genomic selection provide complementary frameworks for identifying and predicting favorable alleles, while CRISPR-based editing, RNA interference and epigenetic modulation offer precise tools for engineering desirable traits. Integrative multi-omics spanning genomics, transcriptomics, proteomics, metabolomics, ionomics and microbiomics unravels the regulatory networks and biochemical pathways underlying complex phenotypes, guiding rational ideotype design. Emerging single-cell and spatial omics further refine this understanding at cellular resolution. Together, these innovations support sustainable vegetable production, enhanced nutritional security and reduced dependence on synthetic inputs.

***Keywords* :** molecular breeding, genomic selection, CRISPR, RNAi, epigenetics, multi-omics, microbiome, vegetable crops, stress resilience, ideotype design.

Introduction

Rapid population growth, dietary transitions and accelerating climate variability are placing unprecedented pressure on global crop improvement programmes. Breeders must now deliver cultivars that simultaneously sustain or enhance yield, improve nutritional quality and withstand increasingly frequent episodes of heat, drought, salinity and disease pressure, often on shrinking and degraded arable land (Mirón *et al.*, 2023; Tuckeldoe *et al.*, 2023). In vegetable crops, which are central to dietary diversity and micronutrient supply, this challenge is especially acute: consumers and value chains demand superior sensory quality and extended shelf-life in addition to resilience and productivity (Kumari *et al.*, 2023; Kaiser *et al.*, 2020).

Classical breeding based on recurrent phenotypic selection has yielded substantial genetic gains over the past century, but it is inherently slow, labour-intensive and constrained by limited genetic resolution and the often-narrow genetic base of elite germplasm (Varshney *et al.*, 2014; Swarup *et al.*, 2021). The convergence of high-density molecular markers, reference genomes, pan-genomes and genome-wide variant catalogues has fundamentally changed this picture by allowing breeders to dissect the genetic architecture of complex traits with unprecedented precision (Wallace *et al.*, 2018; Qin *et al.*, 2021; Song *et al.*, 2021).

In parallel, advances in transcriptomics, proteomics, metabolomics, ionomics, microbiomics and epigenomics provide functional layers that connect DNA sequence polymorphisms to gene regulation, metabolic pathways and whole-plant phenotypes (Fiehn, 2002; Jamil *et al.*, 2020; Derbyshire *et al.*, 2022; Scossa *et al.*, 2021). When these multi-omics data streams are combined with genome editing, RNA interference (RNAi), trait pyramiding and predictive genomic selection (GS), they create an integrated molecular toolbox for the rational design of vegetable ideotypes that combine high yield, dense nutritional profiles, extended shelf-life and multi-stress resilience (Iqbal *et al.*, 2023a; Li *et al.*, 2023a,b; Fan *et al.*, 2025).

In vegetables, this toolbox is being applied to: (i) shelf-life and firmness in tomato; (ii) flavour and metabolite profiles in tomato and pepper; (iii) nutrient density in leafy vegetables; (iv) disease resistance in brassicas and cucurbits; and (v) abiotic stress tolerance in heat- and drought-sensitive species such as lettuce and spinach.

Box 1: Real-world molecular breeding examples in vegetable crops

1. **Marker-assisted selection (MAS) for tomato shelf-life and firmness**
   MAS tracking QTLs for ripening and cell-wall metabolism has been used to develop tomato lines with significantly improved firmness and delayed softening, translating into shelf-life extended by several days under ambient conditions. These MAS-derived lines maintain consumer-acceptable flavour while reducing post-harvest losses, and they have been validated across multiple production environments.
2. **CRISPR–Cas editing for disease resistance and fruit quality in tomato**
   CRISPR–Cas-mediated editing of susceptibility (S) genes and regulators of fruit traits has generated tomatoes with enhanced resistance to fungal diseases and finely tuned texture or shape, often with near-isogenic backgrounds relative to elite cultivars. Most studies to date are greenhouse or confined-field proof-of-concept trials, with clear direction of effect (reduced disease severity, improved quality) and minimal visible penalties on yield.
3. **Metabolomics-guided breeding for carotenoids and pungency in pepper**
   Integrated genomics–metabolomics analyses in Capsicum spp. have mapped metabolic QTLs for carotenoids, capsaicinoids and other flavour compounds. These studies have identified candidate genes and biomarkers that are now being used to select lines with intensified colour and tailored pungency, with substantial increases in target metabolites while preserving agronomic performance.
4. **Microbiome consortia to stabilise yield in leafy vegetables under stress**
   Plant growth-promoting rhizobacteria (PGPR) and microbial consortia formulated from core rhizosphere microbiomes have been tested in lettuce, spinach and other leafy vegetables under salinity or drought. Field and greenhouse trials report higher biomass, improved nutrient uptake and partial maintenance of yield and quality under stress compared with uninoculated controls, illustrating how microbiome-based tools can complement genetic improvement.

Marker-Assisted Selection in Contemporary Breeding Pipelines

Marker-assisted selection (MAS) uses DNA markers tightly linked to major genes or QTLs to track favourable alleles across breeding cycles, improving selection accuracy while reducing dependence on phenotype-only selection. MAS is especially valuable for targeted introgression and rapid stacking of resistance and quality loci into elite backgrounds, and has been widely deployed in cereals for multi-stress resistance breeding and trait fixation. In vegetable crops, MAS has supported improvements in tomato fruit firmness, shelf-life and nutritional traits by selecting loci associated with ripening regulation, cell-wall metabolism and carotenoid pathways, while also enabling faster deployment of resistance to viral and oomycete pathogens across tomato, brassicas, cucurbits and pepper. Its limitations are most evident for highly polygenic traits, where many small-effect loci collectively control performance, and where success depends on robust markers and affordable high-throughput genotyping (Dixit *et al.*, 2020; Nadeem *et al.*, 2018; Voss-Fels *et al.*, 2019).

Genomic Selection and Whole-Genome Prediction

Genomic selection (GS) advances beyond locus-focused methods by using genome-wide markers to predict genomic estimated breeding values, thereby capturing the aggregate contribution of numerous small- and moderate-effect loci. With the expansion of SNP platforms and sequencing-based genotyping, GS has become increasingly feasible for complex targets such as yield, quality, and multi-stress tolerance.

Predictive models commonly include genomic BLUP, ridge regression and Bayesian approaches, with machine-learning methods now frequently explored in parallel. In horticultural crops, GS has shown practical promise for accelerating improvement in yield components, fruit quality attributes and stress-response traits, particularly when used for early selection and shortened generation cycles. Predictive stability can be strengthened by integrating multi-environment phenotypes, environmental covariates and multi-omics signals into model training, enhancing genetic gain per unit time (Hickey *et al.*, 2019; Harfouche *et al.*, 2019; Fan *et al.*, 2025).

Gene Stacking and Trait Pyramiding for Durability

Gene stacking, or pyramiding, combines multiple resistance genes and/or quantitative loci within a single genetic background to improve durability and reduce the likelihood of pathogen adaptation. This strategy is widely used to strengthen resistance longevity and maintain performance consistency under disease pressure. In vegetables, pyramiding often targets both resistance and market-defining traits (shelf-life, flavour, fruit morphology), generating genotypes that sustain productivity and quality more reliably under biotic stress. However, successful pyramids require careful management of epistasis and pleiotropy, and robust multi-environment validation to avoid hidden trade-offs in yield or quality, making pyramiding an iterative, evidence-driven process (Dormatey *et al.*, 2020; Zheng *et al.*, 2023).

Genome Editing Technologies for Precision Crop Improvement

Genome editing, especially CRISPR–Cas systems, enables targeted modification of genes regulating agronomic, quality and stress-response traits with precision beyond conventional mutagenesis. In vegetable crops, editing has been applied to improve nutritional composition, enhance firmness and post-harvest stability, and increase resistance by modifying susceptibility genes. Genome-edited products that segregate free of foreign DNA may face simplified regulatory review in some contexts, yet rigorous assessment of off-target edits and ecological consequences remains essential for responsible deployment. As regulatory frameworks and validation standards evolve, genome editing is expected to expand as a central tool in precision breeding pipelines (Chen *et al.*, 2019; Hafeez *et al.*, 2023; Kalaitzandonakes *et al.*, 2023).

RNA Interference and Host-Induced Gene Silencing

RNA interference (RNAi) enables post-transcriptional gene silencing through sequence-specific degradation of targeted transcripts, allowing reprogramming of pathways relevant to resistance, quality and metabolism. In vegetables, RNAi-based suppression of cell-wall enzymes has delayed softening and improved post-harvest behaviour, while host-induced gene silencing has shown potential for controlling insects and viruses via highly specific targeting. Despite versatility, adoption is constrained by delivery challenges under field conditions, off-target concerns and uneven regulatory acceptance across jurisdictions (Bradshaw, 2019; Gautam *et al.*, 2022).

Epigenetic Breeding and Chromatin Dynamics

Epigenetic regulation mediated through DNA methylation, histone modifications and chromatin accessibility contributes to phenotypic variation and stress responsiveness without altering DNA sequence. Genome-scale epigenomic profiling has demonstrated that chromatin states can modulate key traits, including ripening, flowering transitions and stress tolerance, and that some epigenetic states may persist across generations. These findings have strengthened the concept of epigenetic breeding, which seeks to harness stable epialleles or induced epigenomic diversity to enhance resilience and resource-use efficiency, particularly under climate variability (Cokus *et al.*, 2008; Sun *et al.*, 2023; Tiwari *et al.*, 2022).

Multi-Omics Integration for Yield, Quality and Resilience

Multi-omics frameworks integrate genomics with transcriptomics, proteomics, metabolomics and epigenomics to resolve trait architecture and identify regulatory nodes, biomarkers and pathways associated with performance. In vegetable crops, integrative analyses have clarified the molecular basis of flavour and nutritional quality, improved understanding of stress-responsive networks, and supported ideotype design by linking molecular profiles with phenotype expression. As computational integration methods advance, multi-omics-driven breeding is increasingly positioned to complement GS and genome editing by improving trait prediction, mechanistic interpretation and selection precision (Fiehn, 2002; Scossa *et al.*, 2021; Derbyshire *et al.*, 2022).

Table 1: Representative multi-omics applications relevant to vegetable crop improvement

Trait/Focus	Omics layers integrated	Crop / System	Outcomes	References
Nutritional quality and metabolite profiles	Genomics, transcriptomics, metabolomics	Tomato, pepper, leafy vegetables	Identification of metabolic QTLs and candidate genes for carotenoids, flavonoids and vitamins; discovery of biomarkers for high-quality ideotypes	Fiehn, 2002; Scossa *et al.*, 2021; Guo *et al.*, 2021
Abiotic stress resilience (drought, salinity, heat)	Transcriptomics, proteomics, metabolomics, ionomics	Cereals and vegetables (model systems extrapolated to vegetables)	Reconstruction of stress-responsive networks; identification of transcription factors, transporters and osmolyte pathways associated with tolerance	Jamil *et al.*, 2020; Derbyshire *et al.*, 2022; Fan *et al.*, 2025
Yield and complex agronomic traits	Genomics (GWAS/QTL), transcriptomics (expression QTL, eQTL), metabolomics (metabolite QTL, mQTL)	Major field crops, with growing work in vegetables	Integration of eQTL, pQTL and mQTL reveals causal genes and regulatory modules for yield and stability	Wallace *et al.*, 2018; Hickey *et al.*, 2019; Scossa *et al.*, 2021
Post-harvest behaviour and shelf-life	Transcriptomics, proteomics, metabolomics	Fruit vegetables (tomato, cucurbits, solanaceous crops)	Identification of cell wall-modifying enzymes, ethylene-related and oxidative pathways associated with firmness and storage life	Kaiser *et al.*, 2020; Ming *et al.*, 2023; Zhang *et al.*, 2020

Integrative Molecular Analysis and Breeding Pipelines

Modern molecular breeding is most effective when genomics, phenomics and multi-omics are assembled into a single decision pipeline rather than used as stand-alone tools. Well-curated, multi-environment phenotypes provide the anchor for QTL mapping, GWAS and genomic prediction, enabling robust locus discovery and genome-wide breeding value estimation across diverse genetic backgrounds and environments (Korte and Farlow, 2013; Wallace *et al.*, 2018; Hickey *et al.*, 2019). Functional layers then add mechanistic resolution: transcriptomics and proteomics identify regulatory shifts and network modules that separate contrasting phenotypes, helping to prioritize pathways and candidate regulators that are likely to be causal rather than merely correlated (Wang *et al.*, 2009; Zhang *et al.*, 2020). Metabolomics and ionomics complement these layers by capturing biochemical and elemental states that frequently mediate genotype–environment interactions, thereby linking DNA-level variation to trait expression through measurable physiological intermediates (Fiehn, 2002; Salt *et al.*, 2008; Huang and Salt, 2016).

Analytically, integration is strengthened by approaches that connect genotype to intermediate molecular traits such as eQTL and metabolite/ion QTL mapping and by association frameworks that relate trait values to gene- or metabolite-level variation while accounting for population structure, improving causal inference and reducing confounding (Scossa *et al.*, 2021; Derbyshire *et al.*, 2022). In vegetable crops, these pipelines increasingly support practical prioritization of ripening and shelf-life regulators for marker-assisted selection or genome editing, help resolve the genetic–biochemical basis of flavour and pigmentation, and connect stress-responsive metabolic or ionomic signatures to tolerance phenotypes that can be exploited in genomic prediction and introgression strategies (Scossa *et al.*, 2021; Fan *et al.*, 2025). Once candidates are prioritized, validation through reverse genetics, targeted editing or RNA-based silencing closes the loop, enabling an iterative “design–test–learn” cycle that accelerates ideotype development and deployment (Chen *et al.*, 2019).

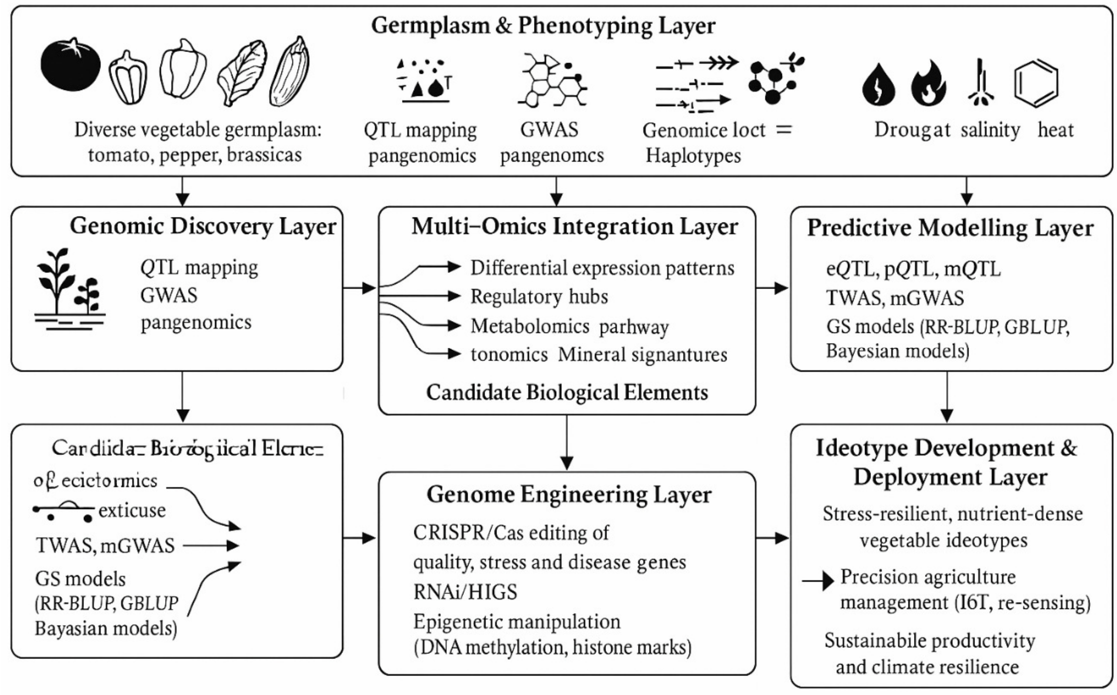

Fig. 1: Integrated Molecular Breeding Pipeline for Vegetable Crop Improvement

Plant Microbiomics in a Multi-Omics Context

Plant-associated microbiomes in the rhizosphere, endosphere and phyllosphere are now widely recognized as functional components of the extended plant phenotype, influencing nutrient acquisition, growth regulation and tolerance to biotic and abiotic stresses (Berg *et al.*, 2014; Compant *et al.*, 2019; Glick, 2021). Progress in community profiling and functional inference has enabled the identification of core taxa and microbial functions associated with yield stability and stress buffering, and the integration of microbiome signals into breeding and management frameworks is increasingly viewed as a route to stabilize performance in heterogeneous environments (Wang and Haney, 2020).

Mechanistically, microbiome effects are best interpreted when microbial profiles are integrated with plant metabolomic and ionomic states, because root exudation chemistry, microbial metabolism and nutrient availability form a coupled system that shapes defence metabolites, hormone balance and mineral nutrition under stress (Huang and Salt, 2016; Scossa *et al.*, 2021). In vegetable systems, such integrated evidence is increasingly translated into microbial consortia, biofertilizers and biocontrol strategies that complement host genetics, reduce reliance on synthetic inputs and improve resilience, particularly where nutrient-use efficiency and disease suppression are major production constraints (Compant *et al.*, 2019; Wang and Haney, 2020; Glick, 2021).

Table 2 : Genome editing, RNAi and microbiome-based strategies relevant to vegetable improvement

Approach	**Target / Goal**	**Example outcome (crop or model)**	**Relevance for vegetable breeding**	**Representative references**
Genome editing of susceptibility genes	Knockout or modification of susceptibility loci to improve disease resistance	*OsSWEET14* editing confers bacterial blight resistance in rice; susceptibility gene editing for powdery mildew resistance in tomato	Transferable concept for editing disease susceptibility genes and stress pathways in vegetables (e.g. cucurbits, solanaceous crops)	Chen *et al.*, 2019; Zeng *et al.*, 2020; Li *et al.*, 2023b
Editing metabolic regulators	Modification of enzymes and transcription factors in quality-related pathways	Fine-tuning carotenoid, aroma and texture traits in fruit crops	Direct application for enhancing nutrient density, flavour and shelf-life in tomato, pepper and leafy vegetables	Ahmad *et al.*, 2021; Kaiser *et al.*, 2020; Hafeez *et al.*, 2023

RNAi for quality traits	Silencing of endogenous genes controlling anti-nutrients or undesirable metabolites	Reduced acrylamide precursors in potato; altered oil composition in oilseeds	Potential to reduce anti-nutritional factors, browning or off-flavours in vegetable products	Bradshaw, 2019; Mondal *et al.*, 2020; Abdellatef *et al.*, 2021
Host-induced gene silencing (HIGS)	Silencing of pest/pathogen genes via plant-expressed dsRNA	RNAi-based resistance to insect pests and viruses	Provides a highly specific biotechnological route to insect and virus resistance in vegetable crops	Gautam *et al.*, 2022; Routhu *et al.*, 2020
Microbiome-based consortia	Use of beneficial microbial communities for nutrition and stress mitigation	PGPR and microbial consortia enhancing nutrient uptake and stress tolerance	Complements genetic improvement by stabilising vegetable yields under variable climates and low-input systems	Berg *et al.*, 2014; Compant *et al.*, 2019; Iqbal *et al.*, 2023b; Hafeez *et al.*, 2023

Single-Cell and Spatial Multi-Omics: Emerging Frontiers

Bulk omics averages signals across mixed cell populations and can mask cell-type-specific regulation that drives development, defence and stress adaptation. Single-cell RNA sequencing provides transcriptome profiles at cellular resolution, while spatial transcriptomics preserves positional context in intact tissues, enabling the reconstruction of developmental trajectories and spatially structured stress responses (Hwang *et al.*, 2018; Giacomello *et al.*, 2017; Stuart and Satija, 2019). Despite plant-specific technical constraints, these approaches have already generated cell atlases and regulatory maps in model plants and are increasingly extending into crops, creating a foundation for cell-resolved interpretation of complex traits (Wu and Wagner, 2021; Xia *et al.*, 2022). Parallel progress in single-cell proteomics and epigenomic profiling is expected to deepen these maps by capturing protein-level states and chromatin regulation that often determine phenotype more directly than transcript abundance alone (Slavov, 2020; Sun *et al.*, 2023).

For vegetable crops, the most immediate value lies in resolving cell-type programs controlling meristem activity, fruit development and vascular differentiation, and in dissecting guard-cell and epidermal responses that shape drought, salinity and heat tolerance in leafy vegetables. As costs fall and protocols mature, coupling cell- and spatial-resolved layers with established breeding tools (genomic prediction, MAS and genome editing) is likely to enable more targeted, mechanistically informed interventions and improve the precision of trait design (Hickey *et al.*, 2019; Chen *et al.*, 2019).

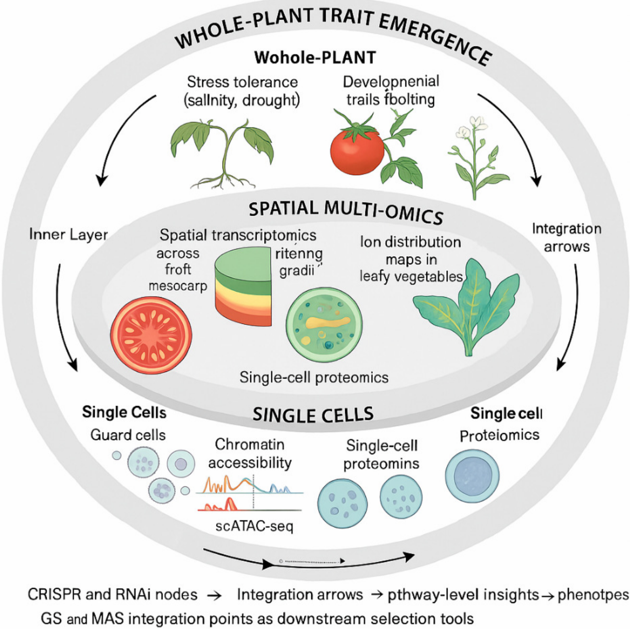

Fig. 2: Cell-to-Systems Multi-Omics Architecture from Single Cells to Whole-Plant Traits in Vegetable Crops

Conclusion

Modern vegetable breeding is being reshaped by the convergence of genome-scale technologies and mechanistic biological insight. Integrating molecular markers, genomic prediction, genome editing and multi-omics enables precise dissection and manipulation of complex traits, supporting the development of cultivars that remain productive and nutritionally rich under intensifying climatic and biotic pressures. Microbiome-based strategies and advanced phenotyping further complement genetic improvement, promoting resource-efficient and resilient production systems. As single-cell and spatial omics mature, they will unlock new opportunities for cell-type-specific trait engineering. Continued refinement of analytical pipelines, regulatory clarity and wider adoption of FAIR data practices will be essential for translating these advances into scalable, sustainable solutions for global vegetable production and food security.

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