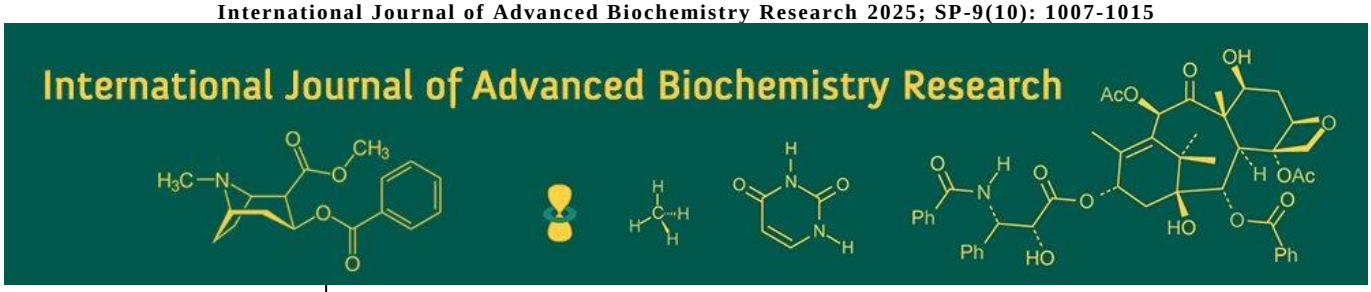

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Revolutionizing vegetable breeding: Integrating CRISPR/Cas9 and genomic selection for precision improvement

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Abstract
Modern vegetable breeding is undergoing a revolutionary transformation through the integration of advanced genome-editing and genomic prediction tools. This article reviews how the CRISPR/Cas9 gene-editing system and genomic selection strategies are being combined to achieve precision improvements in vegetable crops. We outline historical developments in plant genetics and breeding from Mendel's foundational pea experiments and the Green Revolution to the rise of genomic technologies setting the stage for these innovations. We then explain how CRISPR/Cas9 enables targeted alterations of specific genes affecting yield, nutrition, and stress resilience, while genomic selection uses genome-wide marker data to predict breeding values for complex traits. The synergistic workflow of combining both methods is detailed, and key examples are presented. Notable advances include CRISPR-edited tomatoes with enhanced size and shelf-life, nutrient-fortified lettuce, disease-resistant peppers, and virus-resistant cucumbers. The fusion of these tools promises greatly accelerated development of superior vegetable cultivars, although challenges in regulation and deployment remain. We conclude that harnessing both gene editing and genomic selection will dramatically improve breeding precision, enabling crops with higher yield, better nutrition and greater resilience.

Keywords: CRISPR/Cas9, genomic selection, vegetable breeding, precision agriculture, genome editing, plant genetics, crop improvement

Introduction
The science of plant breeding has transformed agriculture over centuries. Humans have practiced selection of desirable plant traits since antiquity, but a turning point came in 1865 when Gregor Mendel, an Augustinian monk, reported the first law of inheritance in pea plants [1]. Mendel's work established that heritable traits are controlled by discrete "factors" (genes), laying the cornerstone for modern genetics. In the decades that followed, pioneers such as Charles Darwin and later geneticists (rediscovering Mendel around 1900) advanced our understanding of variation and selection [2]. By the 20th century, applied breeding took dramatic leaps: the development of hybrids (for example, in corn by George Shull and Donald Jones) and the Green Revolution spearheaded by Norman Borlaug in the 1960s [3]. Borlaug's work in dwarf wheat varieties exemplified how genetic knowledge could enormously boost yields and food security, a lesson soon applied to vegetables as well [4]. Institutions like those founded by Luther Burbank and Nikolai Vavilov collected and exploited vegetable germplasm worldwide, demonstrating the importance of genetic diversity. Nobel laureates like Barbara McClintock (who discovered mobile DNA elements in maize) and geneticists around the globe showed that new traits could arise from genetic change, encouraging further exploration of mutagenesis and hybridization [5].
In the late 20th century, the advent of molecular biology accelerated progress in vegetable breeding. The discovery of DNA's structure by Watson and Crick (1953) and subsequent molecular markers (RFLPs, SSRs, SNPs) allowed breeders to identify and track genes of interest. The polymerase chain reaction and genome sequencing made it possible to pinpoint genetic variation underlying yield, flavor, nutrient content and disease resistance [6]. In 2001, Meuwissen and colleagues proposed genomic selection a revolutionary strategy using genome-wide DNA markers to predict a plant's breeding value even before field testing. This approach, drawing on advances in statistics and genomics, promised to greatly accelerate the

breeding cycle for complex traits like yield. Simultaneously, efforts to directly edit genes progressed [7]. Early methods like EMS mutagenesis or ZFNs/TALENs proved useful, but a watershed moment came in 2012 when Emmanuelle Charpentier and Jennifer Doudna described the CRISPR/Cas9 system for genome editing. This technology, awarded the 2020 Nobel Prize in Chemistry, uses a programmable RNA molecule to guide the Cas9 enzyme to cut DNA at a precise location. CRISPR/Cas9 has since been rapidly adopted in plant research due to its simplicity and versatility [8]. For vegetable breeding, these tools genomic selection and CRISPR represent the latest chapter in a long legacy of innovation that began with simple garden crosses and now continues in the genomics era. Throughout history, notable scientists have guided these advances [9]. Mendel's laws are now being exploited by breeders, Norman Borlaug's principles of genetic gain are echoed in every yield trial, and today's breakthroughs stand on the shoulders of visionaries like Doudna and Charpentier. In parallel, the work of statisticians and geneticists (for example, Meuwissen, Goddard and Hayes) brought genomic selection into practice [10]. Together, these interdisciplinary efforts have now given rise to an integrated approach: using gene editing to create precise trait variants and genomic selection to choose the best gene combinations. This article will examine how CRISPR/Cas9 and genomic selection are being combined to revolutionize vegetable breeding, leading to unprecedented precision in creating improved crop varieties [11].

Traditional and Molecular Approaches in Vegetable Breeding

Before the genomics era, vegetable breeding relied on classical methods. Breeders selected parent plants with desirable traits (such as large fruit or disease resistance) and performed controlled crosses to combine those traits. Hybridization among diverse lines generated variation, but also required many backcrosses and extensive field testing to recover elite performance while introducing the trait of interest [12]. Mutagenesis was another tool: chemical mutagens or radiation were used to create random genetic changes, from which novel traits (e.g. virus resistance in peppers) could sometimes emerge. These methods, while productive, were slow and imprecise. A breeder introducing a single gene might have to grow successive generations over a decade to achieve a stable cultivar. Moreover, conventional breeding often involved shuffling thousands of genes at once, making it difficult to predict outcomes and combine multiple traits efficiently [13]. The late 20th century saw the introduction of molecular marker technologies. DNA markers linked to specific genes allowed breeders to perform marker-assisted selection (MAS). For example, if a gene controlling aphid resistance in lettuce was known, breeders could test seedlings for that gene's marker without waiting for plant maturation or insect exposure [14]. MAS shortened breeding cycles and improved accuracy for traits controlled by a few major genes (such as resistances or quality attributes). However, many important traits like yield, nutrient content or complex disease resistance are controlled by dozens or hundreds of genes, each with small effect. For such traits, MAS was less effective [15].

The concept of genomic selection (GS) addressed this limitation. In GS, a broad panel of breeding lines is both genotyped (using thousands of DNA markers spread across the genome) and phenotyped for key traits. Statistical models are then trained to predict the performance (breeding values) of lines based on their genomic profiles [16]. Once validated, this model can predict the genetic merit of new candidate plants just from their DNA, without full field trials. In practice, GS allows breeders to screen and select progeny in early generations or seedling stages, greatly shortening the time needed per breeding cycle. The power of GS has been demonstrated in major crops: for instance, wheat yield gains of ~15% have been reported using GS over traditional methods [17]. For vegetables, which often have high-value traits and intense research, GS is beginning to show similar promise. Researchers have developed GS models in crops like tomato and cucumber to predict fruit traits, sugar content and disease responses. When integrated into breeding programs, genomic selection can raise the accuracy and speed of developing improved varieties, especially for quantitative traits [18]. Meanwhile, genome editing has evolved as another molecular leap. As mentioned, CRISPR/Cas9 enables breeders to introduce specific changes in the DNA sequence at will. Unlike conventional transgenics, which often involve inserting foreign genes, CRISPR can create mutations or alterations directly in a plant's own genome. A classic example is knocking out a susceptibility gene: if a lettuce gene allows a pathogen to infect, CRISPR can disable that gene, yielding a resistant plant [19]. The ability to target a gene known to affect a trait means that much less trial-and-error is needed compared to random mutagenesis or selection. In vegetables, CRISPR has been used in recent years to modify plant height, fruit characteristics, nutrient pathways and resistance genes. Early lab-scale successes include longer shelf life tomatoes by editing a pectinase gene, heat-tolerant peppers, and virus-resistant cucumbers. These examples highlight how genome editing adds precision to breeding: rather than shuffling whole genomes, breeders change just the key letters in the DNA code for a trait [20].

CRISPR/Cas9 in Vegetable Crop Improvement

- The CRISPR/Cas9 system revolutionized molecular plant breeding by enabling targeted and efficient editing of crop genomes. In vegetables, this technology has been applied to a wide range of traits. CRISPR works by designing a short guide RNA that matches a gene of interest; this guide RNA directs the Cas9 enzyme to the matching DNA location, where Cas9 makes a cut. The plant cell then repairs the break, often introducing small insertions or deletions (a "knockout"). Alternatively, by supplying a DNA template, precise edits or insertions can be made (homology-directed repair), though this is more challenging in plants [21].
- One advantage of CRISPR/Cas9 is its relative simplicity compared to earlier gene-editing methods. It is easily programmable: changing the guide RNA sequence retargets Cas9 to new genes. This flexibility has enabled plant scientists to rapidly target multiple genes or gene families simultaneously (multiplexing). In tomato, for example, CRISPR has been used to knock out several genes at once to alter fruit shape, plant architecture and nutrient content. The technology also works in polyploid species (like potato) which have multiple copies of each gene, by designing guides to hit all copies [22].

- CRISPR has accelerated the pace of trait introduction. Under traditional breeding, transferring a single gene from a wild relative into an elite vegetable cultivar might require years of backcrossing. With CRISPR, researchers can create that gene variant directly in an elite line. For example, if a wild pepper species has a version of a disease-resistance gene, CRISPR can be used to introduce the same resistant allele into cultivated pepper without any crossing, preserving the rest of the cultivar's genetics intact. This precision avoids linkage drag (unwanted traits that are brought along with the target gene) and reduces the breeding timeline dramatically [23].
- Many CRISPR applications in vegetables aim at improving yield, quality and stress tolerance. In tomato, knockouts of genes involved in fruit softening have produced firmer fruits and longer shelf-life without affecting taste. Editing key regulatory genes like the CLAVATA3 (CLV3) family has been shown to expand meristems, resulting in larger fruits or more fruit clusters (raising potential yield). Nutritional quality has also been enhanced: for instance, CRISPR has been used to increase the content of health-promoting compounds such as GABA (gamma-aminobutyric acid) in tomato or anthocyanins in purple pepper, by editing metabolic enzymes. In leafy vegetables like lettuce, CRISPR can target pathways that produce vitamins and antioxidants; recent studies have reported gene-edited lettuce lines with several-fold higher levels of provitamin A (beta-carotene), vitamin C, and beneficial phytonutrients, all without yield penalties [23].
- CRISPR/Cas9 is also a powerful tool for conferring resistance to stresses. Many vegetable crops suffer from viral diseases, and often a single host gene (such as a translation initiation factor) is critical for virus infection. By using CRISPR to disrupt that gene, breeders have generated cucumber and melon lines that are broadly resistant to viruses like mosaic viruses. Similarly, editing susceptibility genes in potato has conferred resistance to late blight fungus. Abiotic stress tolerance has been addressed as well: genes involved in heat and drought response have been modified in tomato and pepper to produce plants that set fruit under higher temperatures [24].
- Beyond trait improvement, CRISPR has a strong research role. It enables rapid functional genomics in vegetables: by knocking out or modifying a gene of unknown function, scientists can observe the effect on the plant and thereby link genes to traits. This accelerates gene discovery, which in turn feeds into breeding. For example, CRISPR screens in tomato have identified new genes controlling flowering time, fruit size and metabolic composition. The knowledge gained can guide breeders to edit just the right gene in a targeted variety [25].
- Despite its power, CRISPR/Cas9 editing must be applied with care. Off-target cuts (unintended edits elsewhere in the genome) are a technical concern, although improved versions of Cas9 (high-fidelity or PAM-flexible variants) are reducing this risk. Also, because many vegetables are vegetatively propagated or highly heterozygous, regenerating and breeding out undesired edits can be challenging. Nevertheless, the speed and precision advantages have already made CRISPR an indispensable tool in the vegetable breeder's toolbox [26].

Genomic Selection in Vegetable Breeding

- Where CRISPR acts at the level of individual genes, genomic selection (GS) operates on the entire genome of the breeding population. GS is a predictive breeding approach in which statistical models trained on DNA marker data forecast the performance of untested plants. In practice, breeders first assemble a training population that is both genotyped (typically by SNP arrays or sequencing) and phenotyped for key traits (such as yield, fruit size, nutrient content). Advanced algorithms then relate the dense marker data to observed performance, yielding a genomic prediction model. Once the model is established, new plants are genotyped and their "genomic estimated breeding values" (GEBVs) are computed immediately, without waiting for full field trials [27].
- For vegetables, which often involve long juvenile phases or labor-intensive trait measurements, GS can greatly speed selection. Leafy greens and herbs, for example, may have short cycles already, but fruiting vegetables like tomato, pepper or eggplant benefit from skip-year selection. By using GS, breeders can select superior seedlings (even in greenhouses) before costly field trials, or in parallel with early testing. GS also improves the accuracy of selection when traits are expensive or difficult to measure. For instance, carotenoid levels in carrots or lycopene in tomato require lab assays; a GS model can predict these quality traits in many individuals from genomic data alone, focusing attention on the most promising lines [28].
- An important strength of GS is capturing small effects of many genes simultaneously. Many yield-related traits and stress tolerances in vegetables are polygenic. Traditional QTL mapping might identify a handful of major loci, but ignore hundreds of minor genes. GS embraces this complexity by using all marker information. Early studies in cucumber, for example, have shown that GS models can predict fruit yield and quality with much higher accuracy than selecting by phenotype in early generations. Similarly, in tomato, GS has been applied to improve multiple fruit traits (size, sugar content, firmness) at once. These studies indicate that genomic selection can accelerate genetic gain (the improvement per cycle) in vegetable breeding [29].
- However, GS does require substantial data and resources. A large, diverse training population must be developed with thorough phenotyping across relevant environments. Vegetables with available reference genomes and high-quality markers (tomato, lettuce, pepper) are now benefiting most. Low-cost genotyping and computational tools have made GS more accessible even to smaller vegetable breeding programs. The key tradeoff is between genotyping costs and phenotyping costs: in many cases, it pays off to genotype large numbers of plants and phenotype fewer, since lab or field assays can be slow [30].
- In practice, integrating genomic selection into vegetable breeding often follows a cycle: first, use GS to choose the best parents or early-generation individuals; then cross and produce progeny; then genotype these

progeny and use GS to predict their values; then field-test only the top predictions. This can effectively condense multiple generations of backcrossing or selection into a single "genomic generation." Importantly, GS is complementary to CRISPR: while CRISPR can introduce new variation at key loci, GS can help determine which edited lines (or which crosses of edited lines) will perform best overall. Together, they enable precision breeding at both gene and genome levels [31].

Comparison of Breeding Approaches

Vegetable breeders today have multiple tools at their disposal. Table 1 summarizes the characteristics of conventional breeding, genomic selection and CRISPR/Cas9 gene editing in the context of crop improvement. Traditional breeding involves crossing and selection based on phenotype; it is a proven method but often slow and less precise [32]. Genomic selection, a form of marker-assisted breeding, uses statistical prediction from dense marker data to accelerate selection, especially for complex traits. CRISPR/Cas9 is a gene-editing approach that directly alters DNA sequences of target genes [33]. From Table 1 and practice, the choice of method depends on the breeding goal. For traits controlled by a few genes (such as a single disease resistance), CRISPR offers the fastest route to create the desired allele in a preferred cultivar. For highly polygenic traits like overall yield or multi-trait improvement (e.g. yield + quality), GS can enhance selection intensity and accuracy across the genome. Conventional breeding remains useful when resources for molecular tools are limited or for traits not easily defined genetically. In many modern programs, these approaches are combined: for example, CRISPR might be used to introduce or fix a valuable allele at a specific gene, while GS ensures that the edited lines are combined optimally for background genetics and other traits. In this way, breeders harness the precision of gene editing and the predictive power of genomics simultaneously [34].

The key differences are evident in Table 1

Table 1: Comparison of conventional breeding, genomic selection and CRISPR/Cas9 editing for crop improvement. Each approach has unique advantages and constraints.

Breeding Approach	Mechanism	Generation Time	Precision	Strengths	Limitations
Conventional Breeding	Cross plants and select offspring by traits	Often multiple years/generation	Low (genes are shuffled randomly)	Well-established; no molecular tools needed	Slow; requires large trials; difficult to fix specific alleles
Genomic Selection (GS)	Predict breeding value from genome-wide markers	1-2 years (shortens cycles)	Medium (uses many markers)	Accelerates complex trait gain; uses existing variation	Requires large genotyped training population; dependent on model accuracy
CRISPR/Cas9 Gene Editing	Direct DNA modification via targeted nucleases	1-3 years (once plants are transformed)	Very high (specific genes targeted)	Very precise; can create novel alleles; speeds introgression	Off-target edits possible; regulatory hurdles; needs known target gene

Integrating CRISPR and Genomic Selection: A Workflow

The integration of CRISPR/Cas9 editing with genomic selection can create a high-throughput pipeline for vegetable breeding (Figure 1). The key steps in this workflow are:

- **Define Breeding Objectives:** Clearly identify the target traits (e.g. higher yield, nutrient content, stress tolerance) and the species or germplasm to improve.
- **Phenotype and Genotype a Training Population:** Assemble a representative set of breeding lines or varieties, and measure the traits of interest across relevant environments. Genotype these lines with genome-wide markers (e.g., SNP chips or genotyping-by-sequencing).
- **Develop a Genomic Prediction Model:** Use the training data to build a statistical model that predicts trait values (genomic estimated breeding values, GEBVs) from marker data. Validate the model's accuracy in predicting independent lines .
- **Use GS to Select Top Parents:** Apply the model to a larger breeding population (e.g. progeny from initial crosses) and select the best individuals based on predicted performance. These become parents for the next generation or candidates for editing.
- **Identify Candidate Genes/QTL:** Within the selected lines or from parallel studies (e.g. GWAS), pinpoint genes or genomic regions that strongly influence the target traits. These become targets for CRISPR editing.
- **Design and Implement CRISPR Edits:** For each candidate gene, design guide RNAs to introduce beneficial edits (knockouts, base changes, etc.) in elite breeding lines or selected parents. Transform plants (e.g. via Agrobacterium) and regenerate edited plants.
- **Genotype and Screen Edits:** Confirm successful edits by sequencing the target locus. Ensure off-target effects are minimal.
- **Phenotype Edited Lines:** Grow the edited plants and measure the traits of interest. This verifies that the CRISPR-induced mutation had the intended effect (e.g. disease resistance achieved, nutrient level increased).
- **Reintegrate into Breeding via GS:** Add the phenotypes of edited lines to the training set and update the GS model. Use GS again to combine the edited gene(s) with other superior alleles across the genome and select the best progeny for advancement.
- **Iterate and Deploy:** Continue iterating GS and gene editing steps across breeding generations. Eventually, select final lines carrying the desired edited genes and high overall breeding values for release or further testing.

The above workflow can be summarized in a table

Table 2: Conceptual workflow for integrating genomic selection (GS) and CRISPR/Cas9 in a vegetable breeding program. GS drives selection at the population level, while CRISPR edits specific genes.

Step	Genomic Selection Role	CRISPR/Cas9 Role
1. Objectives	Define key traits; gather phenotypic data for goals	Identify precise gene targets associated with traits
2. Training Population	Genotype diverse lines and measure phenotypes to train GS model	-
3. Model Development	Build prediction model (GEBVs) for traits using markers	-
4. Parent Selection	Use model to select top candidates from breeding populations	-
5. Candidate Gene Identification	Use GS results and other data to flag QTL/genes	Select genes (e.g. via literature or GS marker effect)
6. CRISPR Design	-	Design guide RNAs targeting candidate genes
7. Plant Editing	-	Perform transformations and regenerate edited plants
8. Screening	Genotype lines for genome-wide markers	Sequence target gene to confirm edits
9. Phenotyping	Evaluate edited lines for whole-trait performance (GS)	Verify targeted trait improvement (functional test)
10. Model Update	Incorporate edited line data into GS model	-
11. Advanced Selection	Use updated GS model to choose best combinations of alleles, including edits	-
12. Variety Release	Final selection of lines with edited genes and high GEBVs	-

Laboratory Innovations and Field Applications

Recent advances in gene editing have moved rapidly from the laboratory into field testing and even commercial products. In the lab, researchers have demonstrated CRISPR edits in virtually every major vegetable species [36]. For example, tomato, pepper, potato, cucumber, lettuce and cauliflower have all been genetically edited under controlled conditions to alter traits ranging from fruit shape and nutrition to stress resistance. Many of these studies are proof-of-concept (knocking out genes and observing traits) and have established protocols for efficient transformation and editing [37]. Innovations such as DNA-free editing (delivering CRISPR components as ribonucleoproteins) have also emerged, potentially easing regulatory burdens. In parallel, some CRISPR-edited vegetable products are reaching the farm and market [38]. A landmark case is the GABA-enriched tomato approved and sold in Japan in 2021. This variety, called "Sicilian Rouge High GABA," was created by CRISPR knockout of a gene in the GABA metabolic pathway, resulting in fruits with substantially higher levels of the amino acid γ-aminobutyric acid (beneficial for nutrition). It became one of the first gene-edited foods commercialized anywhere. Similarly, in China researchers have reported the development of gene-edited white button mushrooms and long-lived white peaches (both technically fruits) which also show how these tools can reduce waste [39].

Several countries have now allowed field trials of gene-edited crops under regulatory frameworks. For vegetables specifically, field experiments have been documented for peppers (e.g. editing for fungal resistance in Indonesian chili pepper) and for Brassica vegetables in the UK. In 2021, for example, UK scientists conducted field trials of CRISPR-edited Brassica oleracea (broccoli/cauliflower family) targeting glucosinolate genes for modifying flavor compounds [40]. They showed that the intended metabolic changes observed in the lab persisted in field-grown plants, demonstrating feasibility outside greenhouses. Potatoes edited for virus resistance and improved processing qualities have been trialed in Europe [41]. Even though many of these trials focus on genetic and agronomic testing rather than commercial release, they indicate that CRISPR improvements can perform under realistic growing conditions. On the other hand, regulatory acceptance varies by region. The United States and Japan have taken relatively permissive stances, often allowing CRISPR-edited plants (without foreign DNA) to be cultivated with fewer restrictions than traditional GMOs [42]. In Europe, gene-edited plants are currently regulated under the same strict rules as GMOs, though this may change with ongoing policy debates. These regulations affect how quickly edited vegetables can move from lab to farm. In practical terms, laboratory successes abound, but only a handful of CRISPR-edited vegetables have reached growers so far [43]. To maximize real-world impact, breeders are combining genome editing with more conventional methods. For instance, one major seed company uses CRISPR in conjunction with haploid induction: they induce haploids (seedlings with a single genome copy) that are then edited at target genes and "doubled" to create new breeding lines in just one generation. Others use marker-assisted backcrossing alongside CRISPR to ensure edited alleles move into elite germplasm [44]. The rise of gene-editing has also spurred field-relevant innovations like speed breeding (accelerated greenhouse conditions) and high-throughput phenotyping, which feed data into genomic selection models. In this way, both lab-born innovations and field-scale selection are working hand-in-hand to bring improved vegetables to market [45].

Examples of Trait Improvements in Major Vegetables

The combination of CRISPR editing and genomic selection is already yielding tangible improvements in specific vegetable crops [46]. Below we highlight a few prominent cases that illustrate the range of benefits:

- **Tomato (Solanum lycopersicum):** Tomato has been a leading model for gene editing. CRISPR has modified dozens of traits in tomato, including fruit development

and quality. For example, editing the *SlPL* gene (which encodes pectate lyase) leads to firmer fruit with greatly extended shelf life, reducing post-harvest losses [47]. In another study, researchers targeted the CLAVATA signaling pathway (e.g. *CLV3* gene) to enlarge meristem size; edited plants produced dramatically larger and more numerous fruits, offering a route to higher yield per plant. Nutritional enhancement is another focus: knockout of *GAD2*, a gene in the GABA shunt, resulted in tomatoes with much higher GABA content, marketed in Japan for its potential health benefits [48]. Disease resistance has also been improved; for instance, editing a susceptibility gene conferred resistance to bacterial speck disease without affecting yield. These trait modifications were largely guided by genomic knowledge (e.g. known QTLs) and often validated by GS predictions. As a result, several CRISPR-edited tomato lines now serve as references or even commercial lines in some countries [49].

- **Lettuce (Lactuca sativa):** Leafy greens like lettuce are valued for nutritional quality and shelf life. Recent gene-editing work has focused on boosting vitamins and antioxidants. One high-profile example is CRISPR editing of the carotenoid pathway: knockout of the *LCY-ε* (lycopene ε-cyclase) gene in lettuce led to an astonishing 2.7-fold increase in provitamin A (beta-carotene) and a nearly 7-fold increase in vitamin C, without any drop in plant vigor or yield [50]. This multiplex approach (affecting multiple biochemical steps) created a lettuce variety whose nutritional profile rivals that of far more expensive vegetables. In addition, edits in ethylene or polyphenol oxidase genes are being explored to delay wilting and browning, potentially extending shelf life by days. In general, lettuce breeding programs are beginning to combine GS for yield-related traits with CRISPR changes in quality genes, aiming for crisp, nutritious heads that store longer [51].
- **Capsicum Pepper (Capsicum spp.):** Peppers are both vegetable and spice crops, and CRISPR has been used for disease resistance and capsaicin content. In Indonesia, CRISPR was deployed to knock out an ethylene-responsive transcription factor (*CaERF28*), which conferred strong resistance to anthracnose (a fungal disease) in hot peppers. The edited plants showed dramatically reduced fruit rot under greenhouse conditions [52]. Other efforts have targeted the capsaicin biosynthesis pathway: by fine-tuning genes like *Pun1*, breeders can potentially create milder or hotter varieties with precise levels of pungency. For peppers, genomic selection models have also been developed for yield and pungency traits, allowing breeders to predict the performance of new hybrid combinations. Thus, combining GS to select the best genetic background with CRISPR tweaks in key capsaicinoid genes or disease genes yields improved pepper lines more efficiently than conventional breeding alone [53].
- **Cucumber (Cucumis sativus):** Viral diseases such as cucumber mosaic virus are major constraints. Researchers used CRISPR to disrupt the *eIF4E* gene in cucumber (a translation initiation factor that many plant viruses hijack). The edited cucumber lines showed broad-spectrum resistance to several potyviruses (including ZYMV, WMV, PRSV) that normally devastate cucurbit crops. Since the gene edit was precisely introduced, the plants remained non-transgenic [54]. This work demonstrated how a single targeted edit can improve a complex trait like virus resistance. In terms of yield, edited cucumbers with improved virus resistance can fruit normally where wild-type plants fail, effectively increasing marketable yield. Cucumber breeding is now using GS to combine such resistance with fruit quality traits (size, shape, skin texture) by predicting the best hybrids, ensuring that the CRISPR-edited resistance can be deployed in high-performance backgrounds [55].
- **Potato (Solanum tuberosum):** In this tetraploid vegetable, CRISPR/Cas9 has been applied to traits like disease resistance and nutritional content. For example, gene-edited potatoes lacking all copies of the *DND1* susceptibility gene showed strong resistance to late blight. Another success is herbicide tolerance: by introducing a specific point mutation in the *ALS* gene (targeting a known herbicide-binding site), breeders created potato lines that tolerate a certain broadleaf herbicide without yield loss [56]. This edit was done without introducing foreign DNA, potentially simplifying regulation. On the nutritional side, CRISPR was used to reduce the precursors of neurotoxic solanine (a glycoalkaloid) by editing a key gene in its biosynthetic pathway, making the tubers safer to eat. Genomic selection in potato breeding is also advancing, with models predicting tuber yield and cooking quality. By integrating GS, breeders can ensure that CRISPR-edited potatoes are not only resistant or nutrient-rich, but also maintain high yield and taste qualities [57].
- **Cruciferous Vegetables (e.g., Brassica oleracea broccoli/cauliflower):** These vegetables produce health-promoting glucosinolates, but high levels can cause excessively bitter flavor. In field trials, scientists used CRISPR to knock out the *MYB28* transcription factor, a master regulator of glucosinolate biosynthesis. The edited broccoli had significantly lower glucosinolate content in florets, yielding a milder taste [58]. Importantly, this edit did not hurt yield or overall plant health. Such precise control over nutrient compounds is uniquely enabled by CRISPR. Parallel efforts in kale and Chinese cabbage are underway, targeting other transcription factors (like *MYB29*, *MYB76*) to fine-tune nutritional profiles. For breeding overall, genomic selection models in Brassicas focus on yield, head size and disease tolerance, so the CRISPR-induced flavor modifications can be introduced into high-yielding varieties with minimal fuss [60].

These examples, summarized in Table 3, illustrate the tangible benefits achieved so far by integrating gene editing and advanced breeding

Table 3: Representative examples of CRISPR/Cas9 edits in vegetable crops and their beneficial effects. The outcomes listed were achieved without detriment to other key traits, demonstrating precise improvement.

Crop	Trait Improved	Gene(s) Edited	Outcome
Tomato	Fruit size and yield	*CLV3* (meristem regulator)	Larger fruits; potential yield increase
Tomato	Shelf life, firmness	*SlPL* (pectate lyase)	Firmer fruit, much longer shelf life
Tomato	Nutritional GABA content	*GAD* (glutamate decarboxylase)	Elevated GABA levels in fruit
Lettuce	Provitamin A (β-carotene) & Vit C	*LCY-ε* (carotenoid enzyme)	~2.7× β-carotene, ~6.9× vitamin C; normal growth
Pepper (Capsicum)	Disease resistance (anthracnose)	*CaERF28* (ethylene response factor)	Strong resistance to fungal fruit rot
Cucumber	Viral disease resistance	*eIF4E* (initiation factor)	Immunity to multiple potyviruses
Potato	Late blight resistance	*DND1* (susceptibility gene)	High resistance to Phytophthora infestans
Potato	Herbicide tolerance	*ALS* (acetolactate synthase)	Tolerant to specific herbicide; normal yield
Broccoli (Brassica)	Bitterness (glucosinolate levels)	*MYB28* (transcription factor)	Reduced glucosinolates; milder flavor, normal yield

Conclusion

Vegetable breeding is entering a new precision era through the combined use of CRISPR/Cas9 gene editing and genomic selection. Historical giants like Mendel and Borlaug laid the conceptual foundations by showing how genetics and selection can raise yields. Today's scientists from Doudna and Charpentier, who gave us CRISPR, to the developers of GS models are carrying that legacy forward. By editing genes known to affect yield, quality or stress tolerance, and by using genome-wide data to predict the best plant combinations, breeders can now sculpt vegetable genomes with unprecedented control. The result is a much faster pipeline from gene discovery to field-ready variety. The practical gains are already evident. CRISPR-edited tomatoes, peppers, lettuce and other vegetables demonstrate improved yields, nutrition and resilience. Field trials confirm that these gains translate into real-world conditions. Combining these lab successes with genomic selection means that every small gene edit is placed into an optimal genetic background, stacking the deck in favor of superior performance. This synergy dramatically shrinks breeding timelines: changes that once took decades of crossing and backcrossing can now be accomplished in a few years. Looking ahead, the integration of CRISPR and genomic selection will likely become standard practice in vegetable improvement. This precision breeding approach not only accelerates genetic gain but also allows breeders to address the multiple challenges of sustainable agriculture higher yield, greater nutritional density, climate resilience and reduced chemical inputs all at once. Challenges remain, particularly in regulation and public perception of gene-edited foods, but the technical pathway is clear.

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