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CRISPR-Cas9-Driven Innovations for Improving Quality and Productivity in Floricultural Crops

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Floricultural crops represent a high-value segment of horticulture where aesthetic attributes (flower colour, form, fragrance), post-harvest durability, stress resilience and growth synchrony determine economic success. Conventional breeding approaches for ornamentals are limited by long breeding cycles, polyploidy and complex genetics. CRISPR-Cas9 and derivative genome-editing platforms have revolutionized trait engineering by enabling precise, efficient and targeted alterations of genetic pathways that underpin key phenotypes. This review synthesizes mechanistic foundations of CRISPR technologies, trait classes edited in ornamentals, delivery systems and associated challenges representative case studies in floriculture, regulatory and commercialization landscapes and prospects for future research.

Introduction

Floriculture is a global industry that contributes substantially to ornamental horticulture through cut flowers, potted plants, and landscaping materials. The market value of floricultural commodities is defined by traits that are visually and sensorially discernible flower colour, shape, pattern, fragrance, vase life and stress resilience (Mekapogu *et al*., 2023; Sirohi *et al*., 2022). Traditional breeding in ornamentals has achieved significant successes, but its pace is constrained by biological barriers including long juvenile phases, self-incompatibility systems, high heterozygosity and complex polyploid genomes (Partap *et al*., 2023). Genome editing particularly CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9 has emerged as a powerful tool for precise modification of specific genes or regulatory sequences. Unlike conventional transgenic approaches, CRISPR enables targeted mutations that can improve aesthetic and production traits without necessarily introducing foreign DNA, thereby accelerating breeding cycles and potentially easing regulatory hurdles (Nishihara *et al*., 2018). CRISPR systems facilitate the functional dissection of ornamental genetics and can help meet evolving consumer preferences and production demands.

CRISPR-Cas9 and Genome-Editing Principles

1. Mechanism of Action

The CRISPR-Cas9 system uses a synthetic single-guide RNA (sgRNA) to direct the Cas9 endonuclease to a specific genomic locus adjacent to a conserved protospacer adjacent motif (PAM), typically NGG for *Streptococcus pyogenes* Cas9 (SpCas9). Upon recognition, Cas9 introduces a double-strand break (DSB) at the targeted site. Endogenous repair pathways non-homologous end joining (NHEJ) or homology-directed repair (HDR) determine the final outcome (Sirohi *et al*., 2022). NHEJ commonly results in insertions or deletions (indels) that

can knock out gene function, whereas HDR can be exploited for precise insertions or allelic replacements when donor templates are provided (Ma *et al*., 2023).

2. Base and Prime Editing

Recent advances extend CRISPR beyond simple knockouts. Base editors (cytosine or adenine deaminases fused to Cas9 nickases) mediate single-base conversions without DSBs, reducing unintended mutations. Prime editors combine a Cas9 nickase with reverse transcriptase, enabling precise nucleotide replacements or small insertions/deletions valuable for fine-tuning functional alleles (Chapparo, 2024). These precision editing strategies increase the repertoire of editable biochemical pathways relevant to ornamental traits.

3. Multiplex Editing

Multiplex CRISPR leverages multiple sgRNAs to target several gene copies or paralogs simultaneously. This is critical in polyploid ornamentals (e.g., chrysanthemum, rose), where redundant alleles reside across homologous genomes (Mekapogu *et al*., 2023). Multiplex editing enhances the likelihood of achieving complete phenotypic modifications.

Delivery Systems and Regeneration Challenges

A pivotal requirement for successful genome editing is delivery of CRISPR components into plant cells and subsequent regeneration of whole plants from edited cells.

1. Agrobacterium-mediated Transformation

Agrobacterium tumefaciens is widely used for delivering T-DNA constructs that express Cas9 and sgRNAs. It is established in several ornamentals such as petunia, torenia and lily allowing stable integration and selection of transformed tissues (Mekapogu *et al*., 2023). However, this method often results in transgene integration which can complicate regulatory classification.

2. Biolistic (Particle Bombardment)

Particle bombardment delivers DNA or RNA through high-velocity microprojectiles. It is applicable to crops recalcitrant to *Agrobacterium*, but may cause complex insertion patterns and tissue damage (Partap *et al*., 2023).

3. Protoplast Transfection and Ribonucleoprotein (RNP) Delivery

Protoplasts cells without cell walls can be transfected with CRISPR reagents including Cas9-sgRNA ribonucleoproteins (RNPs). RNP delivery minimizes transgene footprint and reduces off-target risks (Chapparo, 2024). However, many ornamentals are recalcitrant to protoplast regeneration, limiting practical adoption.

4. Viral Vectors

Virus-based delivery systems (e.g., TRV, geminiviruses) can disseminate CRISPR components systemically for transient editing, though achieving heritable edits remains challenging (Partap *et al*., 2023).

5. Regeneration Bottlenecks

Many ornamentals exhibit genotype-dependent responses to tissue culture conditions. Optimization of hormonal regimes, explant selection and culture environments is essential for recovering whole plants from edited tissues. Regeneration remains a major technical hurdle for genome editing across diverse floricultural taxa (Mekapogu *et al*., 2023).

Trait Classes and CRISPR Targets in Floricultural Crops

Genome editing via CRISPR has been deployed or proposed for several trait categories of commercial significance in ornamentals.

1. Flower Colour and Pattern

Flower colour is governed by pigments including anthocyanins, flavonoids, carotenoids and their regulatory networks (Mekapogu *et al*., 2023). Key enzymes and transcription factors such as dihydroflavonol-4-reductase (DFR), flavonoid 3′,5′-hydroxylase (F3′5′H) and MYB regulators are established targets for editing (Nishihara *et al*., 2018; Sirohi *et al*., 2022). CRISPR-generated mutations in pigment biosynthesis genes can yield colour shifts, intensity changes or pattern alterations expanding phenotypic diversity. Multiplex editing facilitates

simultaneous targeting of paralogs in polyploid genomes necessary for clear phenotypes in complex ornamental species (Mekapogu *et al*., 2023).

2. Floral Scent and Volatile Profiles

Fragrance arises from volatile organic compounds (VOCs) derived from terpenoid, phenylpropanoid and benzenoid pathways. Editing of terpene synthases, methyltransferases or regulatory genes affects the bouquet composition, enabling enhancement, suppression, or novel synthesis of scent profiles (Sirohi *et al*., 2022). Though fewer floral scent editing reports exist than colour modification, conceptual frameworks support CRISPR-based scent engineering.

3. Vase Life and Senescence

Post-harvest longevity is governed by cellular senescence pathways and ethylene biosynthesis/signalling networks (Sirohi *et al*., 2022; Mekapogu *et al*., 2023). Genes in the ethylene pathway such as 1-aminocyclopropane-1-carboxylic acid synthase (ACS), oxidase (ACO) and ethylene response factors (EIN2/EIN3) are prime targets for editing to delay senescence and extend vase life. Delayed senescence has substantial economic impact by reducing post-harvest losses.

4. Flowering Time and Architecture

Precise control of flowering time and plant architecture enhances production scheduling and market timing. CRISPR-mediated modifications in floral regulators like flowering locus t (ft), terminal flower 1 (tfl1), leafy (lfy) and constans (CO) provide avenues to adjust flowering onset, synchronize inflorescence or shorten juvenile phases (Partap *et al*., 2023). Edited architecture can also reduce labour requirements and improve aesthetic uniformity.

5. Disease Resistance and Stress Tolerance

Pathogen susceptibility genes (S genes) that facilitate viral, bacterial or fungal infection can be disrupted to confer resistance (Bhalerao *et al*, 2025). Similarly, editing stress-responsive regulators (e.g., drought/salinity pathways) can improve resilience in field or greenhouse environments. These approaches mirror successful CRISPR applications in food crops and help reduce yield losses.

6. De-novo Domestication and Novel Trait Creation

De-novo domestication involves editing wild relatives for desirable traits an approach that expands the ornamental palette by converting wild species with unique phenotypes into commercially attractive cultivars (Verma *et al*., 2024). This strategy enables rapid diversification of available ornamental varieties.

Representative Case Studies in Floriculture

1. Torenia: Nishihara *et al*. (2018) demonstrated CRISPR-Cas9 editing of anthocyanin biosynthesis genes in *Torenia fournieri*, achieving visible alterations in flower colour with ~80% editing efficiency. This study provided one of the first successful proofs of concept for aesthetic trait editing in ornamentals.

2. Petunia and Other Solanaceous Ornamentals: Petunia serves as a model for CRISPR-mediated manipulation of pigment pathways. Targeted disruption of transcription regulators and biosynthetic enzymes has produced altered colour profiles underscoring the tractability of Solanaceous ornamentals to genome editing (Partap *et al*., 2023).

3. Chrysanthemum and Rose: Highly polyploid species like chrysanthemum and rose present challenges for editing due to multiple homologous gene copies. Nevertheless reviews report progress in multiplex CRISPR strategies and outline pipelines integrating allele-specific genotyping to ensure full conversion of target genes (Mekapogu *et al*., 2023 Bhalerao *et al*., 2025).

Off-Target Effects, Polyploidy, and Precision Editing

1. Off-Target Mutations: Unintended edits at genomic regions resembling target sites "off-targets" can alter phenotypes or destabilize genomes. Mitigation strategies include refined sgRNA design, use of high-fidelity Cas9 variants and transient RNP delivery to reduce Cas9 persistence (Ma *et al*., 2023; Chapparo, 2024).

2. Polyploidy Challenges: Polyploid ornamentals (e.g., rose, chrysanthemum) contain multiple gene copies across homologous chromosomes. To achieve phenotypic changes editing must targ*et* all homeologs necessitating multiplex CRISPR systems and careful allele tracking via genotyping (Mekapogu *et al*., 2023).

3. Precision and Base Editing: Base editing and prime editing allow targeted nucleotide conversions without DSBs. Such precision is advantageous when modulating regulatory motifs, enzyme active sites, or promoter elements associated with ornamental traits (Chapparo, 2024).

Regulatory, IP, and Commercialization Landscape

Regulation of genome-edited crops varies globally. Some jurisdictions (e.g., United States, parts of South America) exempt DNA-free, site-specific edits from stringent GMO regulations if the outcome could occur through conventional breeding. Other regions (e.g., European Union) maintain GMO categorization regardless of transgene presence (Ma *et al*., 2023; Chapparo, 2024). Commercialization strategies for edited ornamentals therefore depend on regulatory classification, intellectual property (IP) landscapes and product differentiation objectives. Varieties with novel colour extended vase life or enhanced disease resistance may attain strong market acceptance if regulatory barriers are minimized.

Socioeconomic Impacts

Edited floricultural crops can reduce production costs (less disease loss, reduced waste) expand variety diversity and enable geographic adaptation. Market studies suggest high return on genetic investments for traits with direct consumer visibility (e.g., vase life, fragrance) reinforcing CRISPR's value proposition (Beese *et al*., 2023).

Challenges and Future Research Priorities

Despite significant promise, several gaps remain:

- **Transformation and Regeneration**: Species-specific protocols for efficient transformation and plant regeneration remain a top priority (Mekapogu *et al*., 2023).
- **High-Quality References**: Genomic resources (reference genomes, gene models) are limited for many ornamentals, impeding target identification and off-target prediction.
- **Phenotype Evaluation**: Field validation and multi-environment phenotypic assessments are needed to confirm trait stability and commercial performance.
- **Consumer and Regulatory Engagement**: Supporting adoption requires clear communication of safety, benefits and policy frameworks.

Conclusions

CRISPR-Cas9 and advanced genome-editing platforms have transformed the potential of floricultural crop improvement by enabling precise alterations of key aesthetic and productivity traits. From colour engineering and scent modification to vase life extension and disease resistance, CRISPR technologies offer unprecedented control over ornamental phenotypes. Technical challenges (delivery, off-targets, polyploidy) and regulatory diversity remain, but coordinated research and policy efforts can accelerate the translation of genome editing into commercial floriculture, expanding genetic diversity and enhancing sustainability.

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