



Harnessing *Agrobacterium rhizogenes* *rol* Genes for Ornamental Plant Improvement: Case Studies and Perspectives

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Abstract

The *rol* (root locus) genes derived from *Agrobacterium rhizogenes* (syn. *Rhizobium rhizogenes*) have emerged as powerful genetic tools for modifying plant architecture in ornamental crops. Located on the Ri plasmid T-DNA, these genes—*rolA*, *rolB*, *rolC*, and *rolD*—induce a characteristic suite of morphological alterations collectively termed the "Ri phenotype," including dwarfism, enhanced lateral branching, wrinkled leaves, superior rooting capacity, and modified flowering patterns. This manuscript reviews the molecular mechanisms underlying *rol* gene action and synthesizes documented phenotypic outcomes across major ornamental genera, with emphasis on five detailed case studies: carnation (*Dianthus caryophyllus*), Kalanchoë (*Kalanchoë blossfeldiana*), gloxinia (*Sinningia speciosa*), rose (*Rosa hybrida*), and silver birch (*Betula pendula*). In carnation, *rolC* transformation dramatically improved cutting propagation and flowering stem yield without compromising floral quality. Kalanchoë Ri lines exhibited stable, heritable compact growth ideal for potted production. *Sinningia* studies confirmed segregation and inheritance of *rol*-linked traits across generations. Rose and birch transformations demonstrated applicability to woody ornamentals, albeit with species-specific trade-offs in leaf aesthetics and root biomass. Additional examples across Chrysanthemum, Petunia, Antirrhinum, Begonia, and other genera underscore the broad taxonomic utility of *rol* genes. While challenges such as fertility reduction, flower size diminution, and regulatory constraints persist, integration with modern breeding technologies—including CRISPR-based genome editing, tissue-specific promoters, and phenomic selection—offers pathways to refine *rol*-mediated trait expression. As the floriculture industry increasingly prioritizes sustainability and resource efficiency, *rol* gene technology provides a genetically encoded alternative to chemical growth retardants, positioning itself as a cornerstone strategy for developing next-generation ornamental cultivars with enhanced commercial and environmental value.

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Introduction

Ornamental plant breeding has traditionally relied on conventional hybridization, mutation breeding, and chemical growth regulation to achieve desirable horticultural traits such as compact growth, prolific flowering, and ease of vegetative propagation. However, the advent of genetic transformation technologies has opened new avenues for precise trait modification. Among these, the *rol* (root locus) genes derived from *Agrobacterium rhizogenes* (syn. *Rhizobium rhizogenes*) have garnered significant attention for their ability to induce profound and commercially valuable phenotypic alterations across diverse plant taxa (Christey, 2001; Zdravković-Korać *et al.*, 2004)^[9, 22].

A. rhizogenes is a soil-borne bacterium that naturally infects plants, causing hairy root disease characterized by excessive, highly branched root proliferation (Caplan *et al.*, 1983) ^[1]. This pathogenicity is mediated by the root-inducing (Ri) plasmid, which transfers a segment of its T-DNA into the plant genome. The T-DNA harbors several open reading frames, including the four principal *rol* genes—*rolA*, *rolB*, *rolC*, and *rolD*—each contributing to the distinctive "Ri phenotype" observed in transformed plants (Evtushenko & Lutkov, 2014, 2023) ^[12, 13].

In floriculture and ornamental horticulture, where aesthetic appeal, production efficiency, and sustainability are paramount, *rol* gene transformation offers a genetic alternative to chemical growth retardants such as paclobutrazol and daminozide (Lütken *et al.*, 2020) ^[15]. The compact, bushy architectures induced by *rol* genes are particularly valuable for potted plants, while enhanced rooting capacity benefits clonally propagated cut-flower crops (Casanova *et al.*, 2005; Kumar *et al.*, 2026) ^[14]. This manuscript provides a comprehensive review of *rol* gene structure and function, documents phenotypic alterations across ornamental species through detailed case studies, and discusses the prospects and limitations of this technology in modern ornamental breeding programs.

Molecular Basis and Functional Roles of *rol* Genes Structure and Organization of the Ri Plasmid T-DNA

The Ri plasmid of *A. rhizogenes* contains two main T-DNA regions: the left T-DNA (TL-DNA) and the right T-DNA (TR-DNA), separated by a central region (Tepfer, 1984) ^[20]. The TL-DNA, which is primarily responsible for the Ri phenotype, carries the *rol* genes along with other open reading frames such as *aux1* (also designated *orf13* and *orf14*) (Christey, 2001) ^[9]. The TR-DNA contains genes involved in agropine synthesis and plasmid maintenance but contributes minimally to phenotypic alterations (Caplan *et al.*, 1983) ^[1].

The four principal *rol* genes are arranged sequentially on the TL-DNA and are transcribed from their own promoters, though they can also be expressed under heterologous regulatory sequences such as the constitutive cauliflower mosaic virus (CaMV) 35S promoter (Evtushenko & Lutkov, 2023) ^[13]. Each *rol* gene encodes a protein with distinct biochemical properties and physiological effects, though their precise molecular mechanisms remain incompletely elucidated.

Functional Characterization of Individual *rol* Genes

***rolA*:** The *rolA* gene encodes a 20 kDa protein that localizes to the nucleus and may function as a transcriptional regulator (Evtushenko & Lutkov, 2014) ^[12]. Expression of *rolA* alone typically induces dwarfism, shortened internodes, and leaf wrinkling, often accompanied by larger flowers and condensed inflorescences (Schmulling *et al.*, 1988; Zdravković-Korać *et al.*, 2004) ^[22]. The nuclear localization suggests a role in modulating gene expression networks governing cell expansion and differentiation.

***rolB*:** The *rolB* gene encodes a 28 kDa protein with tyrosine phosphatase activity implicated in auxin signal transduction (Pitzschke *et al.*, 2019) ^[16]. *rolB* expression reduces apical dominance, alters leaf morphology, increases adventitious root formation on stems, and can enlarge stigma and flower size (Zdravković-Korać *et al.*, 2004; Casanova *et al.*, 2005) ^[22, 4]. Biochemical studies suggest that *rolB* may

dephosphorylate key components of the auxin signaling pathway, thereby enhancing auxin sensitivity in transformed tissues.

***rolC*:** The *rolC* gene, the most widely utilized in ornamental transformation, encodes a 33 kDa cytokinin-specific β -glucosidase (Casanova *et al.*, 2003) ^[2]. This enzyme hydrolyzes inactive cytokinin conjugates (e.g., zeatin riboside-O-glucoside), thereby elevating levels of active cytokinins in transformed tissues (Zdravković-Korać *et al.*, 2004) ^[22]. The resulting cytokinin-like activity suppresses apical dominance, promotes lateral shoot proliferation, accelerates flowering, and often reduces flower and pollen size (Lütken *et al.*, 2020) ^[15].

***rolD*:** The *rolD* gene encodes ornithine cyclodeaminase, a 40 kDa enzyme involved in proline metabolism (Evtushenko & Lutkov, 2023) ^[13]. *rolD* contributes to the Ri phenotype, particularly in combination with other *rol* genes, and has been associated with enhanced pathogen resistance and stress tolerance (Pitzschke *et al.*, 2019) ^[16]. Proline accumulation is a well-documented stress response in plants, suggesting that *rolD* may prime transformed plants for adverse environmental conditions.

Mechanisms of Phenotypic Alteration

The phenotypic alterations driven by *rol* genes stem primarily from their modulation of plant hormone networks, particularly auxin and cytokinin pathways (Evtushenko & Lutkov, 2014) ^[12]. *rolB* and *rolD* influence auxin transport and perception, leading to altered root architecture, reduced apical dominance, and changes in organ size (Pitzschke *et al.*, 2019) ^[16]. *rolC*'s β -glucosidase activity releases active cytokinins from conjugates, promoting cell division in axillary buds (increased branching), delaying senescence, and suppressing apical dominance (Zdravković-Korać *et al.*, 2004; Casanova *et al.*, 2005) ^[22, 4]. *rolA* may act as a nuclear regulator, affecting expression of downstream genes involved in cell expansion and differentiation, thereby contributing to dwarfism and leaf wrinkling (Evtushenko & Lutkov, 2023) ^[13].

The interplay of these mechanisms results in the integrated Ri phenotype, with the relative contribution of each *rol* gene varying by species, promoter strength, and expression level (Lütken *et al.*, 2020) ^[15]. Importantly, the Ri phenotype is not merely the sum of individual *rol* gene effects but emerges from complex interactions among the gene products and endogenous plant signaling networks (Christey, 2001) ^[9].

General Phenotypic Alterations Induced by *rol* Genes in Ornamentals

Across diverse ornamental taxa, *rol* gene transformation consistently yields a core set of morphological modifications that align with breeding objectives for potted and cut-flower crops (Zdravković-Korać *et al.*, 2004; Kumar *et al.*, 2026) ^[22, 14]. These alterations include:

Dwarfism and compact growth: Reduced plant height and internode length produce bushier, more compact architectures desirable for potted plants, reducing or eliminating the need for chemical growth retardants (Rugini *et al.*, 2015; Lütken *et al.*, 2020) ^[15]. This trait is particularly valuable in species where excessive height compromises aesthetic appeal or shelf stability.

Increased lateral branching: Suppression of apical dominance leads to enhanced axillary budbreak and proliferation of side shoots, improving plant fullness and

cutting yield (Casanova *et al.*, 2005; Christensen *et al.*, 2021)^[4, 7]. In cut-flower crops, this translates directly to increased production per mother plant.

Leaf morphology changes: Leaves often become smaller, thicker, wrinkled, or chlorotic, contributing to the distinctive Ri phenotype (Schmulling *et al.*, 1988; Zdravković-Korać *et al.*, 2004)^[22]. While these changes may be acceptable or even desirable in some contexts (e.g., succulent foliage plants), they can reduce ornamental value in species prized for large, smooth leaves.

Flowering modifications: Effects on flowering time (earlier or delayed), flower number (increased), flower size (often reduced), and inflorescence architecture (condensed or altered) are common and species-dependent (Casanova *et al.*, 2005; Desmet *et al.*, 2020)^[4, 10]. The direction and magnitude of these effects vary widely, necessitating empirical evaluation for each crop.

Enhanced rooting capacity: Transgenic lines frequently exhibit superior adventitious root formation, facilitating vegetative propagation—a critical trait for clonally propagated ornamentals (Christey, 2001; Casanova *et al.*, 2001)^[9, 3]. This trait alone can justify *rol* gene deployment in propagation-intensive crops.

Fertility reductions: Many *rol*-transgenic plants show decreased pollen viability, smaller seed capsules, and fewer seeds, which can be advantageous for preventing unwanted self-seeding in commercial settings but problematic for breeding programs (Schmulling *et al.*, 1988; Casanova *et al.*, 2005)^[4].

These traits are not merely cosmetic; they translate directly into horticultural and economic benefits, including reduced chemical inputs, higher propagation efficiency, and improved shelf appeal (Lütken *et al.*, 2020; Kumar *et al.*, 2026)^[15, 14]. The following sections detail representative case studies that exemplify the successful application of *rol* genes in specific ornamental crops.

Case Study 1: Carnation (*Dianthus caryophyllus*) – *rolC* for Enhanced Cutting and Flower Yield

Carnation (*Dianthus caryophyllus* L.), a premier cut-flower species worldwide, has been a flagship model for *rol* gene application in floriculture (Zdravković-Korać *et al.*, 2004)^[22]. Researchers introduced the *rolC* gene under the constitutive CaMV 35S promoter into the cultivar 'White Sim' to improve rooting and production yield (Casanova *et al.*, 2001, 2003; Christey, 2001)^[3, 9].

Phenotypic Outcomes

Greenhouse evaluations over two years revealed that *rolC*-transgenic carnation lines exhibited dramatically improved rooting, with transgenic stem cuttings rooting faster and more uniformly than controls (Casanova *et al.*, 2001; Zdravković-Korać *et al.*, 2004)^[3, 22]. This trait is critical for commercial propagation, where rooting efficiency directly impacts production costs and plant quality.

Under standard greenhouse conditions, *rolC* plants showed significantly enhanced development of lateral shoots, leading to greater cutting production per mother plant (Casanova *et al.*, 2001)^[3]. During the flowering season, *rolC* lines produced up to three times more flowering stems per mother plant compared to non-transgenic controls (e.g., 9.4 ± 1.8 vs. 3.2 ± 1.0 stems in one line) (Casanova *et al.*, 2001; Zdravković-Korać *et al.*, 2004)^[3, 22].

Despite increased branching, the height of flowering stems (85–95 cm) and key floral traits (petal number, size, dry weight, color, fragrance) remained statistically indistinguishable from controls, ensuring market acceptability (Casanova *et al.*, 2001)^[3]. The improved traits persisted over multiple vegetative generations and two years of greenhouse testing, confirming phenotypic stability (Zdravković-Korać *et al.*, 2004)^[22].

Commercial Implications

The *rolC*-carnation represents one of the most successful translations of *rol* gene technology into practical horticulture (Casanova *et al.*, 2001)^[3]. By boosting cutting and flower stem yield without compromising floral quality or requiring chemical growth regulators, this transgenic line offers a sustainable, cost-effective alternative to conventional production methods (Lütken *et al.*, 2020)^[15]. The stability of traits over time further supports its potential for commercial deployment, pending regulatory approval (Zdravković-Korać *et al.*, 2004)^[22].

Case Study 2: Kalanchoë (*Kalanchoë blossfeldiana*) – Natural and Engineered Ri Lines for Compact Potted Plants

Kalanchoë blossfeldiana, a popular potted succulent, has been transformed both naturally (via spontaneous *A. rhizogenes* infection) and through targeted genetic engineering to exploit the compacting effects of *rol* genes (Christensen *et al.*, 2008, 2021; European Patent Office, 2013)^[7, 11].

Phenotypic Outcomes

Multiple independent Ri lines of *Kalanchoë* displayed significantly reduced height compared to controls, with condensed internodes and a bushier appearance—ideal traits for potted ornamentals (Christensen *et al.*, 2008; Desmet *et al.*, 2020)^[10]. Enhanced branching improved plant fullness and aesthetic appeal, reducing the need for manual pinching or chemical growth retardants (Christensen *et al.*, 2008).

Leaves in some lines were smaller and thicker, consistent with the canonical Ri phenotype (Zdravković-Korać *et al.*, 2004; Christensen *et al.*, 2022)^[22, 8]. In T1 progeny, the compact phenotype segregated with the presence of *rol* genes, confirming genetic control and enabling selection of stable lines (Christensen *et al.*, 2008; Desmet *et al.*, 2020)^[8, 10].

Breeding Applications

Kalanchoë Ri lines have been advanced as pre-breeding material for developing compact cultivars (Desmet *et al.*, 2020)^[10]. The natural occurrence of Ri-transformed plants in commercial settings underscores the stability and adaptability of the *rol*-induced phenotype in this genus (Christensen *et al.*, 2022)^[8]. Moreover, the ability to regenerate fertile shoots from hairy roots allows for further genetic stacking (e.g., combining *rol* genes with color or scent modifiers) (Lütken *et al.*, 2020)^[15].

Case Study 3: *Sinningia speciosa* (Gloxinia) – Segregation and Stability of *rol* Phenotypes Across Generations

Sinningia speciosa, a tuberous ornamental in the Gesneriaceae family, has served as a model for studying the inheritance and stability of *rol*-induced traits (Desmet *et al.*, 2020)^[10]. Researchers regenerated plants from hairy roots

induced by wild-type *R. rhizogenes* and tracked *rol* gene segregation over two generations (R1 and R2).

Phenotypic Outcomes

Ri lines of *Sinningia* exhibited significantly shorter peduncles and delayed flowering compared to non-transformed regenerants (Desmet *et al.*, 2020)^[10]. Classic Ri symptoms, including leaf rugosity and prolific rooting, were consistently observed in *rol*-positive lines (Desmet *et al.*, 2020)^[10]. Floral size was diminished in *rol*+ R2 plants, a trade-off for compactness that may be acceptable in potted contexts (Desmet *et al.*, 2020)^[10]. Spontaneously regenerated shoots lacking *rol* genes reverted to wild-type morphology, while lines retaining *rolA*, *rolB*, *rolC*, and *rolD* (plus *aux1*) maintained the altered phenotype, confirming gene-phenotype linkage (Desmet *et al.*, 2020)^[10].

Genetic Insights

Copy number analysis revealed variation in *rol* gene dosage among lines (e.g., Reg2 carried multiple copies of *rolA*, *rolB*, *rolC*, and *rolD*), correlating with phenotypic intensity (Desmet *et al.*, 2020)^[10]. This highlights the importance of molecular characterization in selecting optimal lines for breeding (Lütken *et al.*, 2020)^[15]. The *Sinningia* system demonstrates that *rol*-induced traits are heritable and selectable, supporting their use in long-term breeding programs.

Case Study 4: Rose (*Rosa hybrida*) – *rolC* for Dwarfing and Branching

Rose (*Rosa hybrida*), the quintessential ornamental, has been transformed with *rolC* to produce compact, highly branched phenotypes suitable for potted or landscape use (Silva *et al.*, 2018; Zdravković-Korać *et al.*, 2004)^[19, 22].

Phenotypic Outcomes

rolC-transgenic rose bushes displayed reduced overall height and shortened internodes, yielding a compact growth form (Silva *et al.*, 2018)^[19]. Enhanced axillary shoot production improved plant density and floral display (Silva *et al.*, 2018)^[19].

Leaves were chlorotic and wrinkled, typical of Ri phenotypes, though this may require management in high-value cut-flower contexts (Zdravković-Korać *et al.*, 2004)^[22]. Contrary to expectations, some *rolC*-rose lines showed diminished root biomass, suggesting species-specific interactions between *rol* genes and root architecture (Silva *et al.*, 2018)^[19].

Horticultural Trade-offs

While the compact, bushy habit is advantageous for potted roses, the chlorotic leaves and reduced rooting may limit applicability in certain production systems (Zdravković-Korać *et al.*, 2004)^[22]. Nonetheless, the rose case illustrates the potential for *rol* genes to replace chemical growth retardants in ornamental shrub breeding (Lütken *et al.*, 2020)^[15].

Case Study 5: Silver Birch (*Betula pendula*) – *rol* Genes in Woody Ornamentals

Though primarily a timber species, silver birch (*Betula pendula*) serves as a model for understanding *rol* gene effects in woody perennials, with implications for ornamental tree breeding (Rugini *et al.*, 2015).

Phenotypic Outcomes

Transgenic birch lines carrying *rolC* and *rolD* (or all four *rol* genes) exhibited marked dwarfism, with plants significantly shorter and bearing smaller leaves than controls (Rugini *et al.*, 2015). Xylem fibers and vessels were shorter, and the proportional vessel area was reduced, indicating *rol* genes influence secondary growth (Rugini *et al.*, 2015). Phenological shifts, such as later bud burst, were observed, which could affect cold hardiness or seasonal display in ornamental trees (Rugini *et al.*, 2015). Biomass allocation favored roots over stems, consistent with the Ri phenotype's resource reallocation (Rugini *et al.*, 2015).

Broader Implications

The birch study confirms that *rol* genes function in woody species, opening avenues for creating compact ornamental trees and shrubs without growth regulators (Rugini *et al.*, 2015). However, effects on wood quality and phenology must be carefully evaluated for each application (Lütken *et al.*, 2020)^[15].

Additional Documented Cases Across Ornamental Genera

Beyond the detailed case studies above, *rol* genes have been successfully deployed in numerous other ornamentals, each exhibiting characteristic Ri phenotypes (Zdravković-Korać *et al.*, 2004; Kumar *et al.*, 2026)^[22, 41]:

- **Chrysanthemum (*Chrysanthemum morifolium*):** *rolC* transformation induced dwarfism, increased branching, and altered leaf morphology, with some lines showing improved vase life (Zdravković-Korać *et al.*, 2004; Casanova *et al.*, 2005)^[22, 41].
- **Petunia (*Petunia* spp.):** *rol* gene expression led to reduced height, enhanced rooting, and modified flower architecture, though flower size was often diminished (Casanova *et al.*, 2005; Zdravković-Korać *et al.*, 2004)^[4, 22].
- **Antirrhinum majus (Snapdragon):** *rolC* lines displayed compact growth, earlier flowering, and increased flower number, albeit with smaller individual blooms (Casanova *et al.*, 2005; Zdravković-Korać *et al.*, 2004)^[4, 22].
- **Begonia (*Begonia tuberhybrida*):** Transgenic plants showed dwarfism, wrinkled leaves, and enhanced rooting capacity (Zdravković-Korać *et al.*, 2004)^[22].
- **Lisianthus (*Eustoma grandiflorum*):** *rol* genes promoted compactness and branching, valuable for potted production (Zdravković-Korać *et al.*, 2004)^[22].
- **Lilium (*Lilium longiflorum*):** Altered morphology included reduced height and modified leaf shape, though fertility was often compromised (Zdravković-Korać *et al.*, 2004)^[22].
- **Osteospermum (*Osteospermum ecklonis*) and Limonium (*Limonium* spp.):** Both genera exhibited dwarfism and increased branching post-transformation (Zdravković-Korać *et al.*, 2004)^[22].
- **Populus (*Populus tremula* × *P. tremuloides*):** As in birch, *rol* genes induced compact growth and altered wood properties, relevant for ornamental poplars (Rugini *et al.*, 2015; Zdravković-Korać *et al.*, 2004)^[22].
- **Datura (*Datura arborea*, *D. sanguinea*):** *rol* transformation yielded stunted plants with wrinkled leaves and modified flowering (Zdravković-Korać *et al.*,

2004)^[22].

- **Pelargonium (Pelargonium × domesticum):** Geranium lines showed reduced stature and enhanced lateral shoot formation (Zdravković-Korać *et al.*, 2004)^[22].
- **Malus (Malus prunifolia):** Ornamental crabapple exhibited dwarfism and altered branching, useful for landscape breeding (Zdravković-Korać *et al.*, 2004)^[22].
- **Gypsophila (Gypsophila spp.):** *rol* genes enhanced rooting and compactness, beneficial for cut-flower production (Zdravković-Korać *et al.*, 2004)^[22].

These examples underscore the broad taxonomic applicability of *rol* genes and their consistent induction of horticulturally desirable traits across diverse ornamental families.

Advantages and Limitations of *rol* Gene Technology in Ornamental Breeding

Advantages

Sustainability: *rol* gene transformation replaces chemical growth retardants (e.g., paclobutrazol, daminozide) with a genetic solution, aligning with eco-friendly production goals (Lütken *et al.*, 2020; Kumar *et al.*, 2026)^[15, 14].

Enhanced propagation: Superior rooting accelerates cutting production and reduces losses, lowering costs for clonally propagated crops (Christey, 2001; Casanova *et al.*, 2001)^[9].

Trait stability: *rol*-induced phenotypes are often stable over generations and environments, as demonstrated in carnation and Kalanchoë (Casanova *et al.*, 2001; Christensen *et al.*, 2008).

Broad applicability: Effective across monocots and dicots, herbaceous and woody species, enabling wide deployment in ornamental breeding (Zdravković-Korać *et al.*, 2004; Lütken *et al.*, 2020)^[22, 15].

Limitations

Fertility reduction: Many *rol*-transgenic plants exhibit decreased pollen viability and seed set, complicating further breeding efforts (Schmulling *et al.*, 1988; Casanova *et al.*, 2005).

Flower size trade-offs: Increased flower number often comes at the expense of individual flower size, which may be unacceptable in cut-flower markets (Casanova *et al.*, 2005; Desmet *et al.*, 2020)^[10].

Leaf aesthetics: Wrinkled, chlorotic, or small leaves may reduce ornamental value in foliage-focused crops (Zdravković-Korać *et al.*, 2004; Silva *et al.*, 2018)^[22, 19].

Regulatory hurdles: Transgenic status limits commercialization in regions with stringent GMO regulations, despite the absence of foreign DNA in some *Ri* lines (e.g., cisgenic approaches) (Lütken *et al.*, 2020; Desmet *et al.*, 2020)^[15, 10].

Future Directions and Integration with Modern Breeding Tools

The future of *rol* gene technology in ornamentals lies in its integration with advanced breeding and genome editing platforms (Wang *et al.*, 2023; Chandler & Lu, 2005)^[21, 5].

Precision editing: CRISPR/Cas systems could be used to fine-tune endogenous hormone pathways to mimic *rol* effects without transgene insertion, circumventing regulatory barriers (Wang *et al.*, 2023; Chandler & Lu, 2005)^[21, 5].

Promoter engineering: Tissue-specific or inducible promoters could restrict *rol* gene expression to desired organs (e.g., roots for enhanced propagation, shoots for compactness), minimizing unwanted side effects (Zdravković-Korać *et al.*, 2004; Pitzschke *et al.*, 2019)^[22, 16].

Gene stacking: Combining *rol* genes with modifiers of flower color, scent, or vase life could create multifunctional cultivars tailored to market demands (Zdravković-Korać *et al.*, 2004; Chandler & Lu, 2005)^[22, 5].

Cisgenic approaches: Using *rol* genes from endogenous or closely related species may ease regulatory approval and public acceptance (Lütken *et al.*, 2020; Desmet *et al.*, 2020)^[15, 10].

Phenomic selection: High-throughput phenotyping coupled with genomic selection could accelerate the identification of optimal *rol* expression levels for target traits (Lütken *et al.*, 2020; Wang *et al.*, 2023)^[15, 21].

Conclusion

The *rol* genes of *Agrobacterium rhizogenes* represent a transformative tool for ornamental plant breeding, offering a genetic route to compact growth, enhanced propagation, and modified flowering—traits of paramount commercial importance (Christey, 2001; Zdravković-Korać *et al.*, 2004)^[9, 22]. Case studies in carnation, Kalanchoë, Sinningia, rose, and birch, among others, demonstrate the robustness and versatility of *rol*-induced phenotypes across diverse taxa (Casanova *et al.*, 2001; Christensen *et al.*, 2008; Desmet *et al.*, 2020; Silva *et al.*, 2018; Rugini *et al.*, 2015)^[3, 10, 19]. While challenges such as fertility reduction and regulatory constraints persist, ongoing advances in genome editing, promoter design, and phenomic selection promise to unlock the full potential of *rol* gene technology in sustainable ornamental crop improvement (Wang *et al.*, 2023; Lütken *et al.*, 2020)^[21, 15]. As the floriculture industry increasingly prioritizes environmental stewardship and resource efficiency, *rol*-based strategies are poised to play a central role in shaping the next generation of ornamental cultivars.

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