

DESIGN BRIEF

Extending carnation lifespan through modulation of ethylene and ACC deaminase activity

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Ethylene is a critical plant hormone that regulates the ripening, wilting, and senescence of many fruits, vegetables, and floricultural crops. In *Dianthus caryophyllus* (carnations), premature ethylene-induced senescence significantly shortens vase life and contributes to economic loss within the floral industry. This project aims to develop a synthetic biology-based strategy to delay senescence in carnations by reducing ethylene biosynthesis and signaling. Due to the technical challenges of direct plant transformation, *Escherichia coli* will be used as an accessible model system to test a modular plasmid design inducible by isopropyl β -D-1-thiogalactopyranoside (IPTG). The system includes gene constructs intended to reduce ethylene activity. It employs RNA interference (RNAi) mechanisms, through small interfering RNA (siRNA) or short hairpin RNA (shRNA), to silence key ethylene-related genes as a proof of concept. Successful plasmid incorporation is verified by blue-white colony screening. This study demonstrates a foundational approach for regulating the ethylene pathways in carnations and presents a framework adaptable to other ethylene-sensitive crops. Ultimately, this project highlights the potential for synthetic biology to extend shelf life, reduce postharvest waste, and improve sustainability in agricultural and floricultural industries.

Keywords: Genetic engineering, senescence, ethylene pathway, carnations, ACC deaminase pathway



The authors were mentored by Ana White, Cross Keys High School, Brookhaven, Georgia, United States. Please direct correspondence to: Ana White, ana_m_white@dekalbschoolsga.org. This is an Open Access article, which was copyrighted by the authors and published by BioTreks in 2026. It is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

SENESCENCE is a natural stage in a plant's life cycle, marking the aging and deterioration of tissues such as leaves and flowers. However, in the floral and agricultural industries, premature senescence results in substantial economic losses by reducing the shelf and vase life of crops and ornamentals. In cut flowers such as carnations, rapid wilting leads to significant waste during shipping and retail display, causing financial losses for growers, wholesalers, and florists (Wang et al., 2024). Extending flower longevity can reduce vegetation waste, lower cold-storage energy consumption, and decrease reliance on chemical preservatives. Similarly, in agriculture, delaying senescence can help maintain the firmness, color, and nutritional quality of fruits, vegetables, and flowers for long periods. The techniques developed in this project may serve as a prototype for other ethylene-sensitive species—such as roses, tomatoes, and bananas—where regulating ethylene can slow ripening and extend shelf life (Kinha, 2024).

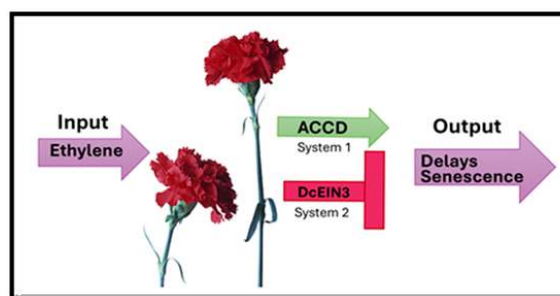


Figure 1. Overview. This diagram illustrates how ethylene input triggers the upregulation of ACCD (ACC deaminase) and the suppression of EIN3, leading to delayed senescence and extended floral longevity in carnations.

D. caryophyllus (carnations) is a globally cultivated cut flower valued for its color and vase appeal, yet its commercial lifespan is limited by high sensitivity to the plant hormone ethylene. Even slight increases in ethy-

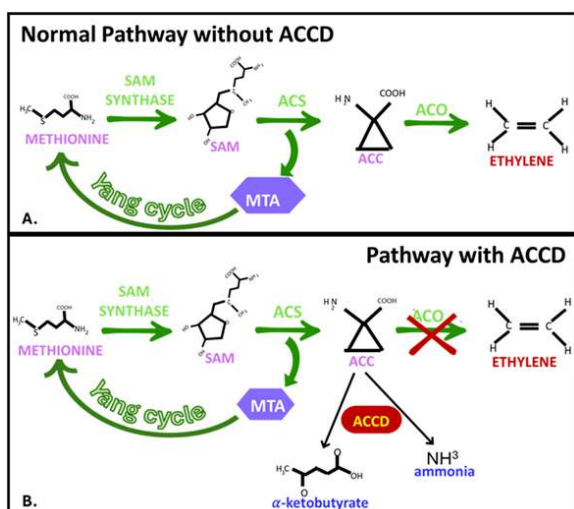


Figure 2. 2A: Normal Pathway without ACC deaminase. This diagram shows the Yang cycle and ethylene biosynthesis pathway in plants, in which methionine is converted to SAM, then to ACC, which is oxidized by ACO to produce ethylene. MTA is recycled to methionine through the Yang cycle. 2B: Senescence with ACC deaminase (ACCD). This diagram shows the Yang cycle and the ethylene biosynthesis pathway, in which ACC deaminase (ACCD) converts ACC to α -ketobutyrate and ammonia, thereby reducing ethylene levels and delaying senescence. The production of α -ketobutyrate and ammonia serves as a negative feedback loop that lowers ACC levels and is known to be a nutrient that is typically added in fertilizers.

ylene levels can induce petal wilting and discoloration, ultimately shortening vase life and reducing market value (Wang et al., 2024; Zhu et al., 2023).

Systems level

This project employs a two-step molecular strategy to reduce ethylene-induced senescence in carnations. Ethylene biosynthesis proceeds from S-adenosyl methionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) via ACC synthase (ACS), and then to ethylene via ACC oxidase (ACO) (**Figure 2**). By introducing the bacterial *acdS* gene, which encodes ACC deaminase (ACCD), ACC can be degraded into ammonia and α -ketobutyrate, reducing substrate availability for ethylene formation. This approach has been shown to decrease ethylene production and extend floral longevity in other floral genera, such as *Petunia* (Baek et al., 2024; Shahid et al., 2023).

Even when ethylene is present, its downstream signaling cascade determines the progression of senescence. Ethylene perception inactivates the negative

regulator CTR1, allowing the EIN2–EIN3 pathway to activate senescence-associated genes. In carnations, genes such as DcEBF1/2 and DcEIN/DcEIL regulate EIN3 stability and petal aging; thus, targeting these signaling components can effectively delay senescence (Zhu et al., 2023; Wang et al., 2023). To suppress this pathway, our project employs RNA interference (RNAi) to silence key ethylene-signaling genes, reducing the plant's response to ethylene.

RNAi functions by targeting specific mRNA transcripts, preventing their translation into proteins. Dicer enzymes process double-stranded RNA (dsRNA) into siRNAs or microRNAs (miRNAs), which are incorporated into the RNA-Induced Silencing Complex (RISC). Guided by sequence complementarity, RISC binds to target mRNAs, inducing cleavage or translational repression (Koeppe et al., 2023; Gebremichael et al., 2021). For this project, RNAi constructs will be designed to target DcEIN3 and DcEBF transcripts, thereby dampening ethylene signal transduction (Guo & Ecker, 2003).

Our approach utilizes *acdS* expression and RNA interference to target ethylene at two levels: suppressing its biosynthesis and inhibiting its signaling pathways. This dual strategy simultaneously reduces hormone concentrations and cellular sensitivity. This integrated strategy has strong potential to extend carnation lifetime, minimize agricultural byproducts, and reduce storage costs, ultimately improving sustainability and profitability within the floral industry (Wang et al., 2024).

Device level

Challenges and Model System

A major challenge is that plant genes are often difficult to express in bacterial systems due to differences in codon usage, promoter compatibility, and post-translational modification requirements (Baek et al., 2024). Consequently, our high school laboratory setting limits access to the specialized tools and biosafety facilities necessary for direct plant genetic transformation.

To overcome these constraints, we selected *E. coli* as a safe and accessible model organism for proof-of-concept experiments. This bacterial system allows us to test inducible gene expression and targeted gene suppression using synthetic regulatory elements, demonstrating the core logic of our ethylene-control framework without the need for plant transformation. This adaptation highlights the educational and practical versatility of synthetic biology.

The experimental model employs an IPTG-inducible lac operon system in *E. coli*. Upon IPTG induction, the

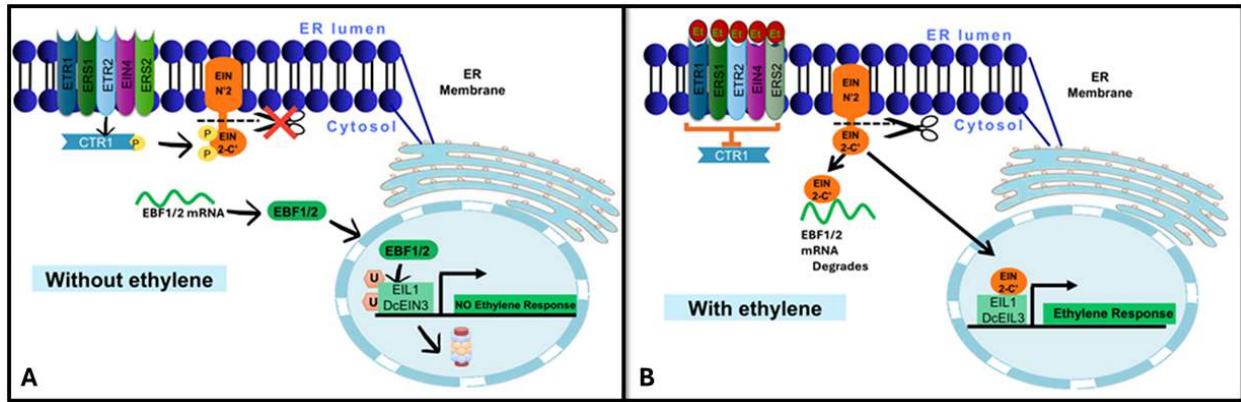


Figure 3. 3A: Absence of Ethylene: Active ER-bound receptors (e.g., ETR1) keep CTR1 kinase active, which phosphorylates and inhibits EIN2. EBF1/2 then targets EIN3/EIL1 for degradation, suppressing ethylene-responsive gene expression (Guo & Ecker, 2003; Potuschak et al., 2003). 3B: Presence of Ethylene: Ethylene binding inactivates the receptors and CTR1, allowing EIN2 cleavage and translocation of its C-terminal fragment to the nucleus and cytoplasm. This suppresses EBF1/2 translation, stabilizing EIN3/EIL1 and activating ethylene-responsive genes. As feedback, EIN3/EIL1 later induces EBF1/2 expression to attenuate signaling. In our experiment, siRNA will silence EIN3 (red circle), blocking the ethylene response (Ju, 2012; Li, 2015).

lac promoter activates *acdS* expression, mimicking the reduction in ethylene biosynthesis observed in plants. The same inducible system also drives a silencing construct targeting *lacZ*—a bacterial analog used to model RNAi-like repression of ethylene-signaling genes.

This study demonstrates interference with ethylene biosynthesis and signaling pathways in carnations to delay senescence. Because direct plant genetic modification is complex and resource-intensive, *E. coli* is used as a simplified model system to test and visualize gene regulation.

Proof-of-concept design

In this design, *E. coli* expresses the *acdS* gene under the control of an IPTG-inducible lac promoter to simulate the reduced ethylene biosynthesis observed in plants. Simultaneously, a small interfering RNA (siRNA) construct targets *lacZ* to model RNAi-like gene silencing of ethylene-signaling components (Figure 6).

Upon IPTG induction, the lac promoter activates *acdS* expression while suppressing *lacZ* expression. Gene regulation will be confirmed through blue-white colony screening on IPTG plates:

- Blue colonies indicate *lacZ* expression (no silencing)
- White colonies indicate *lacZ* repression (successful gene silencing)

This dual system offers a clear, visual demonstration of controllable gene activation and repression, serv-

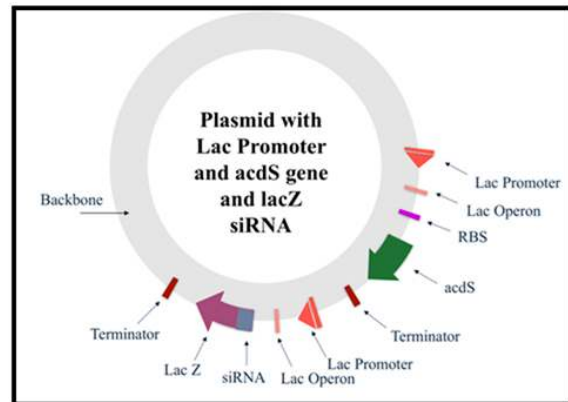


Figure 4. The plasmid construct for *E. coli* is designed to delay ethylene-induced senescence. The design begins with the overexpression of the *acdS* gene under the control of the Lac promoter; as gene transcription ends, *lacZ* gene silencing using siRNA driven by a second Lac promoter begins. This design is expected to produce low levels of ethylene, therefore delaying senescence (Hasan Sowrav et al., 2024).

ing as a powerful educational tool for understanding ethylene regulation and gene network engineering in plants.

Parts level

Two systems will be engineered to regulate carnation ethylene levels by leveraging the ethylene-inducible ERF1 promoter. The ERF1 promoter acts as a stress-

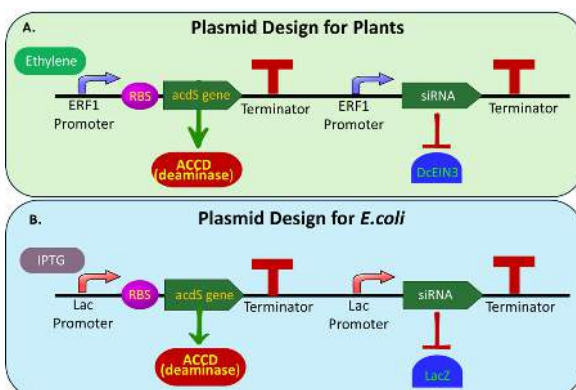


Figure 5. System 1. 5A. Plasmid Design Constructs: This diagram illustrates a conceptual plasmid construct designed to regulate ethylene signaling and senescence in plants. An ethylene-inducible ERF1 promoter drives the expression of the *acdS* gene, which encodes ACC deaminase (ACCD). This enzyme reduces ethylene production by catalyzing the breakdown of the precursor ACC. A second ERF1 promoter controls the expression of siRNA targeting *DcEIN3*, which silences ethylene-related genes (Glick B.R., 2014). **5B. Plasmid Design in *E. coli*:** This schematic details a dual-function plasmid construct for *E. coli*. The *acdS* gene is expressed under the control of an IPTG-inducible *lac* promoter, simulating the reduced ethylene biosynthesis observed in plants. Simultaneously, a small interfering RNA (siRNA) construct targets the *lacZ* gene to model RNAi-like gene silencing of ethylene-signaling components. Functional success is determined via blue-white screening: Blue colonies indicate active *lacZ* expression (no silencing). White colonies indicate *lacZ* repression (successful gene silencing). (Glick B.R., 2014).

responsive switch, It is activated by ethylene itself and by abiotic stresses such as drought and salt (Solano et al., 1998). Its activation triggers a genetic response that lowers overall ethylene levels, forming a negative feedback loop.

Both systems include a terminator sequence to ensure proper transcriptional termination (Figure 5).

System 1: cytosolic ACC deamination

The first system functions in the cytosol, where it is engineered to upregulate *acdS* expression. The ethylene-inducible ERF1 promoter drives the transcription of this gene, and the resulting messenger RNA (mRNA) includes a ribosome-binding site (RBS) to ensure efficient translation. This process produces the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. This enzyme actively reduces ethylene biosynthesis by catalyzing the cleavage of ACC, ethylene's

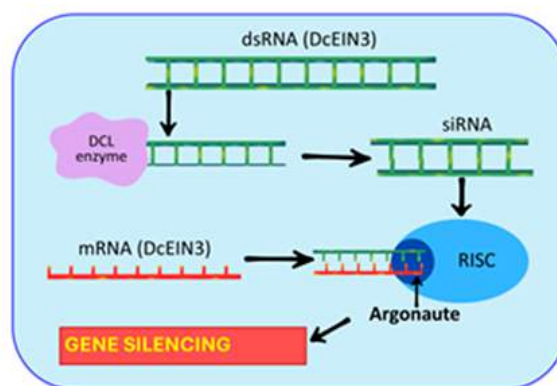


Figure 6. Plasmid Design for Plants: RNA interference (RNAi) involves Dicer processing double-stranded RNA into siRNAs, loading of the guide strand into Argonaute, removal of the passenger strand, and sequence-specific cleavage of target mRNA (Bologna & Voinnet, 2014).

direct precursor, into α -ketobutyrate and ammonia. (Figure 5).

System 2: nuclear gene silencing

The second system operates in the nucleus and is designed to downregulate the *DcEIL3-1* gene. It also uses the ERF1 promoter, but in this configuration, it drives expression of siRNA. The siRNA is specifically designed to target *DcEIL3-1* mRNA transcripts, initiating gene silencing. By suppressing *DcEIL3-1* expression, this system interferes with the plant's ethylene biosynthesis and signaling pathways (Xu et al., 2021). (Figure 5).

RNA interference (RNAi) is a cellular defense and regulatory pathway in which plants respond to double-stranded RNA (dsRNA) by generating short guide molecules that silence matching genes. When dsRNA is present in the cell, Dicer-like enzymes cleave it into small interfering RNAs (siRNAs). These siRNAs are then transferred to an Argonaute protein, which keeps only one strand—the guide strand—while the partner (passenger) strand is discarded. The guide-loaded Argonaute complex scans the cell for RNA sequences with complementary bases. Once it finds a matching messenger RNA, Argonaute cleaves that transcript, preventing its translation into a protein (Bologna & Voinnet, 2014).

Safety

E. coli is classified as a model organism due to its rapid growth and reproduction. These traits, along with their genetic simplicity and ease of manipulation, allow *E. coli* to transform and express foreign plasmids and genes. A Biosafety Level 1 (BSL-1) precaution

is advised when handling *E. coli* in a lab setting. To maintain safety standards, lab safety equipment will be worn, including gloves, closed-toe shoes, lab coats, and appropriate eye protection. In addition, any outside material, such as food, will be removed from the lab. Once the experiment is complete, a bleach solution will be applied to disinfect all equipment used. The lab will then be wiped down with cleaning agents, and all equipment used will be sanitized and disposed of (Rutgers, retrieved 2025).

Discussions

This project presents a sustainable synthetic biology approach to reduce postharvest waste in the floriculture industry by extending the vase life of ethylene-sensitive flowers, such as carnations. Premature senescence caused by ethylene shortens flower longevity, resulting in significant financial losses for growers, wholesalers, and retailers (Wang et al., 2024). By regulating ethylene biosynthesis and signaling, this system aims to reduce wilting, improve product quality, and decrease dependence on chemical preservatives (Oluwaseun S.A., 2024).

Next steps

Protein expression levels will be analyzed by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) to verify upregulation and silencing effects. This provides a clear visual demonstration of controllable gene activation and repression, serving as a proof-of-concept for the regulation of the ethylene pathway.

Beyond carnations, this synthetic biology framework can be adapted to other ethylene-sensitive species—such as roses, tomatoes, and bananas—to extend shelf life and preserve both nutritional and aesthetic quality. This work demonstrates how biological engineering can drive eco-friendly agricultural innovation that aligns economic efficiency with environmental sustainability.

In future applications, this RNA interference strategy can be adapted to silence specific plant genes, offering a sustainable approach to delay flower aging and extend vase life in ethylene-sensitive species.

Plant growth-promoting bacteria (PGPB), including genera such as *Pseudomonas*, *Rhizobium*, and *Azospirillum*, naturally express proteins that facilitate symbiotic relationships with host plants. Research suggests that certain strains of *E. coli* can also exhibit PGPB-like characteristics, supporting plant development through similar symbiotic mechanisms.

To leverage these properties, we designed a plasmid construct for expression in *E. coli*. This system enables

the production of the PGPB-derived *acdS* gene (encoding ACC deaminase) while simultaneously incorporating a gene-silencing strategy. In this model, the construct targets the *lacZ* gene, allowing us to validate the silencing mechanism before applying it to plant systems.

Author contributions

MB, AM, EG, NP, and HU came up with the project concept. MB focused her research on the first system for ethylene production, and EG and HU worked on the second system for ethylene response. NP focused on the siRNA research for EIN3. MB developed the systematic design and proof of concept. AM and NP worked on editing. All authors contributed to the final manuscript and the Google Slides final product.

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