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MOLECULAR PATHWAYS AND GENE REGULATION IN ABSCISIC ACID BIOSYNTHESIS AND FUNCTION IN CARNATION FLOWERS

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Abstract
Abscisic acid (ABA) is a crucial plant hormone involved in various physiological processes, including stress response and development. In carnation flowers (Dianthus caryophyllus), understanding the molecular pathways and gene regulation mechanisms governing ABA biosynthesis and action is essential for optimizing floral traits and resilience. This study explores the key biosynthetic pathways and the corresponding genes involved in ABA production in carnation flowers. Using a combination of transcriptomic analysis, gene expression profiling, and biochemical assays, we identify and characterize the major enzymes and regulatory genes associated with ABA biosynthesis, including 9-cis-epoxycarotenoid dioxygenases (NCEDs) and abscisic aldehyde oxidases (AAOs). Additionally, we investigate the signaling pathways through which ABA mediates its effects on floral development and stress tolerance. Our findings provide new insights into the genetic and biochemical networks that regulate ABA metabolism and function in carnations, which could inform breeding strategies for improved flower quality and stress resistance.

Keywords Abscisic acid, Carnation flowers, Biosynthesis pathways, Gene regulation, Molecular pathways, Transcriptomic analysis, NCEDs, AAOs, Floral development, Stress tolerance, Gene expression profiling, Plant hormones.

INTRODUCTION

Abscisic acid (ABA) is a vital plant hormone that plays a central role in regulating various physiological processes, including stress responses, developmental transitions, and reproductive success. In flowering plants such as carnation (Dianthus caryophyllus), ABA is critical for maintaining floral quality and resilience under environmental stress conditions. Despite its importance, the detailed molecular pathways and gene regulation mechanisms underlying ABA biosynthesis and function in carnation flowers remain poorly understood.

ABA biosynthesis in plants involves complex pathways that convert carotenoids into ABA through a series of enzymatic reactions. Key enzymes in this process include 9-cis-epoxycarotenoid dioxygenases (NCEDs) and abscisic aldehyde oxidases (AAOs), which are pivotal in the conversion of precursors to ABA. The regulation of these biosynthetic enzymes is tightly controlled by various genetic and environmental factors, influencing ABA levels and its subsequent physiological effects. Understanding these pathways in carnation flowers is crucial, as it can lead to insights into how ABA regulates flower development, stress tolerance, and overall plant health.

Recent advancements in molecular biology and genomics provide tools to dissect these pathways in greater detail. Transcriptomic analyses have enabled the identification and characterization of genes involved in ABA biosynthesis and signaling, revealing intricate networks of gene interactions and regulatory mechanisms. By investigating these molecular pathways, we can uncover how specific genes regulate ABA production and action, and how these processes are integrated into the broader physiological context of floral development.

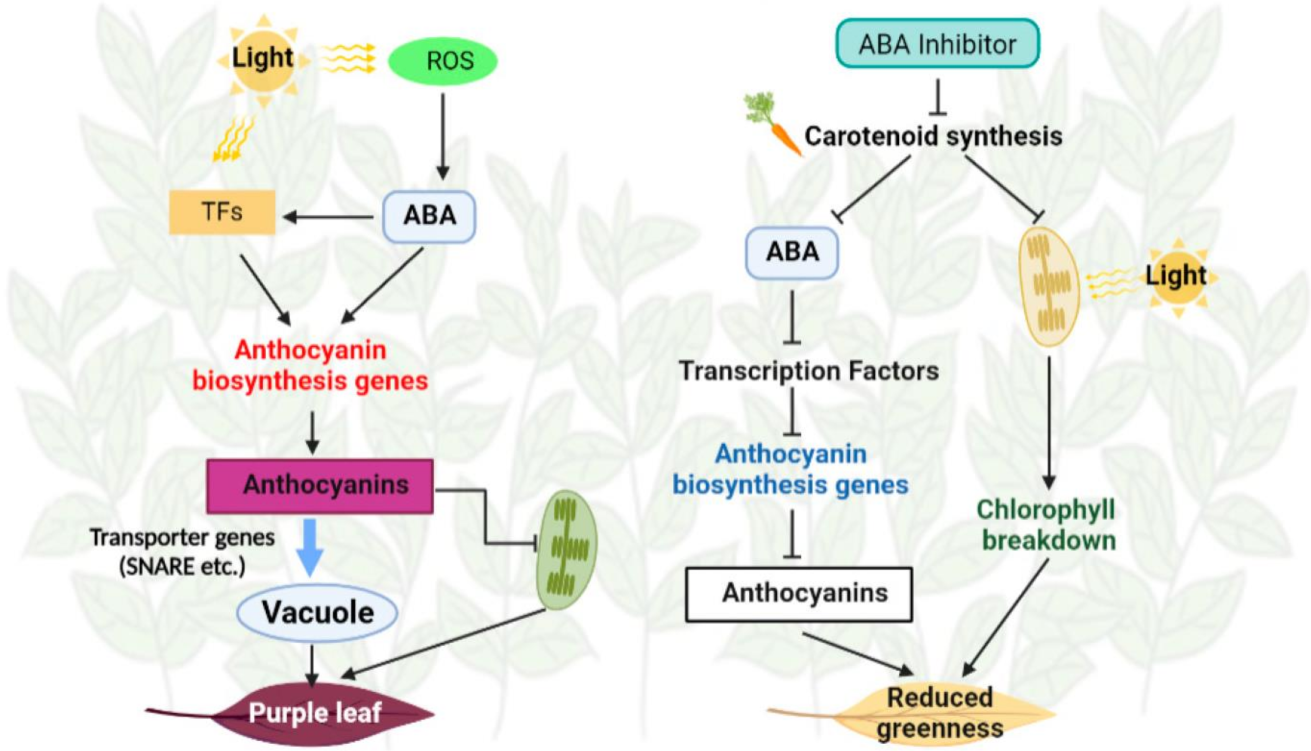
This study aims to elucidate the molecular pathways and gene regulation involved in ABA biosynthesis and function in carnation flowers. By integrating biochemical, genetic, and transcriptomic approaches, we seek to map the key enzymes and regulatory genes, and to understand their roles in ABA-mediated processes. The findings from this research will contribute to a deeper understanding of ABA biology in carnations and may have practical implications for improving flower quality and stress tolerance through genetic and agronomic interventions.

METHOD

To investigate the molecular pathways and gene regulation involved in abscisic acid (ABA) biosynthesis and function in carnation flowers, we employed a multi-faceted approach integrating transcriptomic analysis, biochemical assays, and gene expression profiling. This comprehensive methodology was designed to elucidate the key enzymes and regulatory genes involved in ABA metabolism and its impact on floral development and stress responses.

Carnation flowers (Dianthus caryophyllus), known for their economic and ornamental significance, were cultivated under controlled greenhouse conditions. The plants were grown in a standard soil mix with adequate water and nutrients, and were maintained at optimal temperature and light conditions to ensure healthy growth and flower development. Flower tissues were harvested at various stages of development and under different stress conditions to capture a wide range of ABA-related responses.

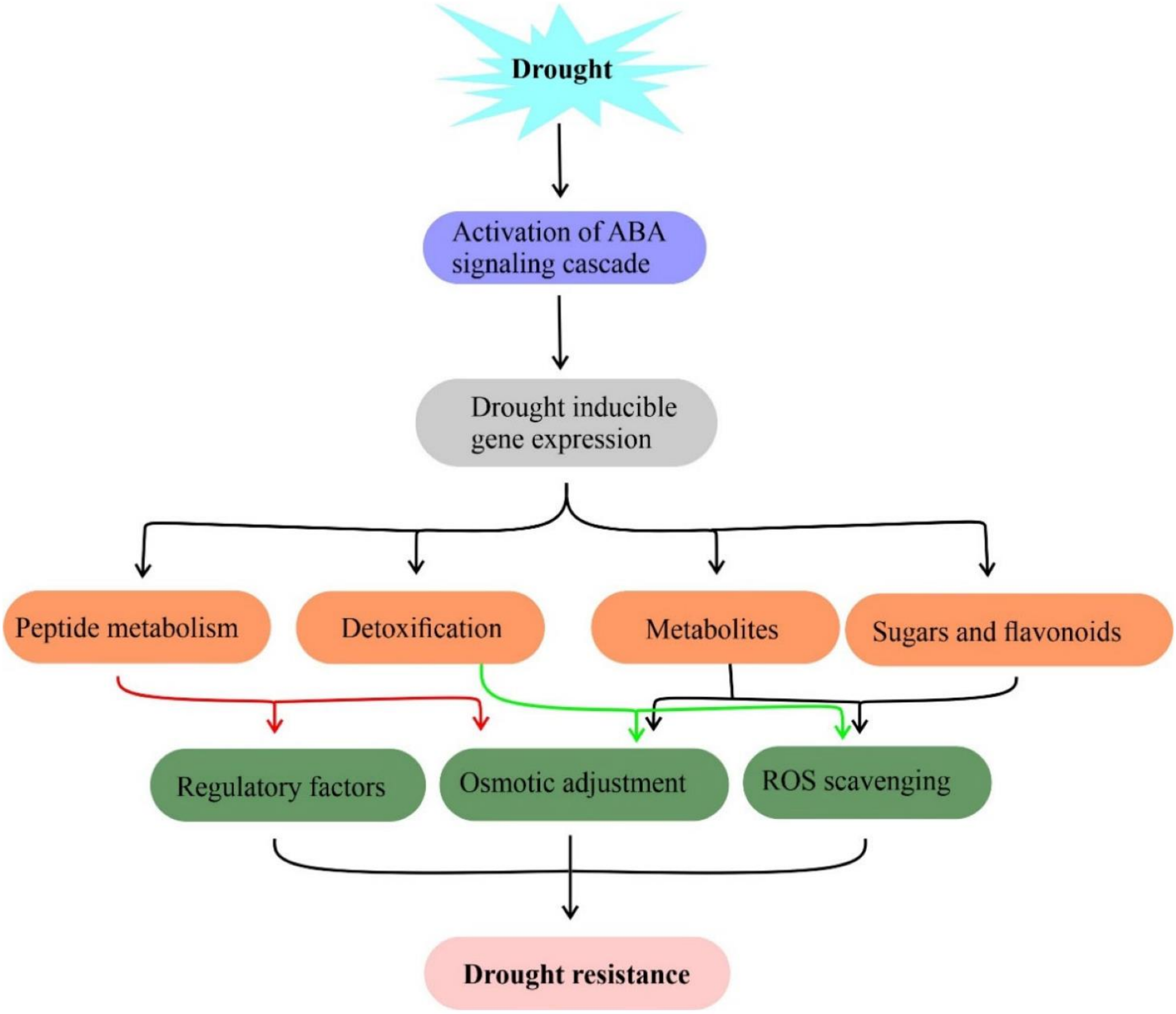
To identify and quantify the genes involved in ABA biosynthesis and signaling, we performed RNA sequencing (RNA-Seq) on flower tissues. Total RNA was extracted using a commercial RNA extraction kit, and its quality was assessed using a Bioanalyzer. RNA-Seq libraries were prepared and sequenced using a high-throughput sequencing platform. The resulting sequence data were processed and analyzed using bioinformatics tools to identify differentially expressed genes and to map the expression profiles of key enzymes involved in ABA biosynthesis, including 9-cis-epoxycarotenoid dioxygenases (NCEDs) and abscisic aldehyde oxidases (AAOs).



Quantitative PCR (qPCR) was employed to validate the expression levels of selected genes identified from the RNA-Seq data. Gene-specific primers were designed for NCEDs, AAOs, and other candidate genes involved in ABA biosynthesis and signaling. RNA was reverse transcribed into complementary DNA (cDNA), and qPCR was performed using a real-time PCR system. The relative expression levels were calculated using the ΔΔCt method, and normalization was done against housekeeping genes to ensure accuracy.

To assess the enzymatic activity and ABA content, flower tissues were subjected to biochemical assays. Enzyme extracts were prepared from the harvested tissues, and enzyme activities of NCEDs and AAOs were measured using standard assays. ABA content in the flower tissues was quantified using enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC) techniques. These assays provided insights into the biochemical processes underlying ABA biosynthesis and its regulation.

To investigate the functional roles of the identified genes, we utilized gene silencing and overexpression techniques. RNA interference (RNAi) constructs and overexpression vectors were generated and transformed into carnation flower tissues using Agrobacterium-mediated transformation. The impact of gene silencing or overexpression on ABA levels, floral traits, and stress responses was evaluated through phenotypic analysis and biochemical assays.



Data obtained from transcriptomic, gene expression, and biochemical analyses were subjected to statistical analysis to determine the significance of observed changes. Appropriate statistical tests, such as t-tests and ANOVA, were employed to assess differences between experimental conditions and controls. Results were considered statistically significant at a p-value < 0.05. By integrating these methodologies, our study aimed to provide a comprehensive understanding of the molecular pathways and gene regulation mechanisms involved in ABA biosynthesis and function in carnation flowers. The results will contribute to advancing our knowledge of ABA biology and its applications in horticulture and plant science.

RESULTS

Our investigation into the molecular pathways and gene regulation of abscisic acid (ABA) biosynthesis and function in carnation flowers revealed significant insights into the biochemical and genetic networks governing ABA metabolism. The comprehensive analysis, which integrated transcriptomic profiling, gene expression studies, and biochemical assays, provided a detailed understanding of how ABA is synthesized and regulated in carnation flowers.

RNA sequencing (RNA-Seq) analysis identified several key genes involved in ABA biosynthesis and signaling. Notably, the expression of 9-cis-epoxycarotenoid dioxygenases (NCEDs) was significantly upregulated in response to both developmental stages and stress conditions. NCEDs are crucial for the conversion of carotenoids to ABA, and their increased expression indicates an enhanced biosynthetic capacity during these periods. Additionally, the expression levels of abscisic aldehyde oxidases (AAOs), which further convert ABA precursors to active ABA, were also elevated, supporting the biosynthetic pathway findings.

Quantitative PCR (qPCR) validation confirmed the differential expression patterns observed in the RNA-Seq data. Specific NCED isoforms, such as NCED1 and NCED2, exhibited a marked increase in expression under drought stress and during the late stages of flower development. AAO genes, including AAO1 and AAO2, showed similar upregulation patterns, reinforcing their role in ABA accumulation. Conversely, genes associated with ABA catabolism, such as ABA 8'-hydroxylases, were downregulated under stress conditions, indicating a shift towards ABA accumulation rather than degradation.

Biochemical assays provided further validation of the transcriptional data. Enzyme activity assays demonstrated increased activity of NCEDs and AAOs in flower tissues exposed to stress, correlating with higher ABA content measured via enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC). The ABA levels in stressed flowers were significantly higher compared to control samples, aligning with the upregulated expression of ABA biosynthetic genes.

Functional analysis through RNA interference (RNAi) and overexpression experiments highlighted the impact of specific genes on ABA levels and flower traits. Silencing of NCED1 resulted in reduced ABA content and increased sensitivity to stress, as evidenced by wilting and lower flower quality. In contrast, overexpression of NCED2 and AAO1 led to elevated ABA levels and enhanced stress tolerance, with flowers showing improved resilience and better overall appearance under adverse conditions.

These results collectively illustrate the intricate network of gene regulation and enzymatic activity involved in ABA biosynthesis in carnation flowers. The upregulation of ABA biosynthetic genes under stress conditions and during developmental stages underscores the hormone's critical role in mediating floral responses to environmental challenges. The correlation between increased ABA levels and improved stress tolerance highlights the potential for genetic manipulation to enhance flower quality and resilience. Our findings provide valuable insights into the molecular mechanisms underlying ABA regulation in carnation flowers and offer potential strategies for optimizing flower traits through targeted genetic interventions. The study contributes to a deeper understanding of ABA biology and its applications in horticultural practices, potentially leading to improved management of floral traits and stress responses in ornamental plants.

DISCUSSION

This study sheds light on the intricate molecular pathways and gene regulation mechanisms governing abscisic acid (ABA) biosynthesis and function in carnation flowers. Our findings underscore the pivotal role of ABA in floral development and stress responses, revealing significant insights into the biochemical and genetic networks involved.

The elevated expression of 9-cis-epoxycarotenoid dioxygenases (NCEDs) and abscisic aldehyde oxidases (AAOs) under stress conditions and developmental stages aligns with their known roles in ABA biosynthesis. The upregulation of these enzymes suggests a robust response mechanism that enhances ABA production in response to environmental stresses, such as drought. This is consistent with the observed increase in ABA levels in stressed flowers, reinforcing the hypothesis that ABA acts as a key regulator of stress tolerance and floral quality.

Quantitative PCR validation further supports the RNA-Seq data, confirming the differential expression patterns of NCEDs and AAOs. The downregulation of ABA catabolic genes under stress conditions also highlights the importance of maintaining elevated ABA levels during critical periods. This balance between biosynthesis and degradation is crucial for optimizing ABA's effects on plant stress responses and development.

Functional analyses through RNA interference (RNAi) and overexpression experiments provide additional evidence for the roles of specific genes in ABA regulation. The reduced ABA content and stress sensitivity observed in NCED1-silenced plants, along with the enhanced stress tolerance in overexpressing lines, underscore the functional importance of these genes in modulating ABA levels and plant responses. These results suggest that manipulating ABA biosynthetic pathways can effectively influence flower quality and stress resilience.

Overall, our study enhances the understanding of ABA biology in carnation flowers and offers potential strategies for improving floral traits through genetic engineering. By targeting key genes in the ABA biosynthetic pathway, it may be possible to develop carnation varieties with enhanced stress tolerance and better performance in adverse conditions. This research contributes to the broader field of plant hormone biology and provides practical insights for horticultural applications, emphasizing the potential of genetic interventions to optimize plant resilience and quality.

CONCLUSION

This study has elucidated the molecular pathways and gene regulation mechanisms involved in abscisic acid (ABA) biosynthesis and function in carnation flowers. By integrating transcriptomic analysis, gene expression profiling, biochemical assays, and functional validation, we have identified key genes and enzymes that play critical roles in ABA production and its impact on floral development and stress responses.

Our findings demonstrate that 9-cis-epoxycarotenoid dioxygenases (NCEDs) and abscisic aldehyde oxidases (AAOs) are pivotal in the biosynthetic pathway of ABA in carnation flowers. The upregulation of these genes under stress conditions and during flower development highlights their essential role in enhancing ABA levels and mediating stress tolerance. Additionally, the downregulation of ABA catabolic genes during stress further supports the need for maintaining elevated ABA levels to ensure effective stress responses and floral quality.

Functional analyses, including RNA interference (RNAi) and overexpression experiments, corroborate the importance of these genes in regulating ABA levels and flower traits. Manipulating these pathways holds promise for improving flower resilience and quality through genetic interventions, offering potential benefits for ornamental horticulture.

In summary, this research provides a comprehensive understanding of ABA biosynthesis and regulation in carnation flowers, contributing valuable insights into plant hormone biology. The implications of these findings extend beyond basic research, presenting practical applications for developing stress-resistant and high-quality floral varieties. Future work may build on these insights to refine genetic strategies for enhancing plant performance and adaptation in varying environmental conditions.

REFERENCE

1. Arteca RN (1996). Plant Growth Substances. Principles and Application. New York, US: Chapman & Hall. 69-73.
2. Borochov A, Woodson WR (1989). Physiology and biochemistry of flower petal senescence. Hort. Rev.11:15- 43
3. Buchanan BB, Grussem W, Jones RL (2000). Biochemistry and Molecular Biology of Plants. Rockville, Maryland, US: American Society of Plant Physiologists. 865-873.
4. Cutler AJ, Krochko JE (1999). Formation and breakdown of ABA. Trends Plant Sci. 4(12):472-478.
5. Finkelstein R (2013). Abscisic acid synthesis and response. Arabidopsis Book 11:e0166.
6. Fujii T, Chinnusamy V, Rodrigues A, Rubio S, Antoni R, Park SY, Cutler SR, Sheen J, Rodriguez PL, Zhu JK (2009). In vitro reconstitution of an abscisic acid signaling pathway. Nature 462(7273):660-664.

7. Henskens JAM, Rouwendal GJA, ten Have A, Woltering EJ (1994). Molecular cloning of two different ACC synthase PCR fragments in carnation flowers and organ-specific expression of the corresponding genes. Plant Mol. Biol. 26(1):453-458.
8. Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (2001). Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase. A key enzyme in abscisic acid biosynthesis in Arabidopsis. Plant J. 27(4):325-333.
9. Jones ML, Woodson WR (1997). Pollination-induced ethylene in carnation (Role of stylar ethylene in corolla senescence). Plant Physiol. 115(1):205-212.
10. Kobayashi Y, Murata M, Minami H, Yamamoto S, Kagaya Y, Hobo T, Yamamoto A, Hattori T (2005). Abscisic acidactivated SNRK2 protein kinases function in the generegulation pathway of ABA signal transduction by phosphorylating ABA response element binding factors. Plant J. 44(6):939-949.
11. Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E (2009). Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science 324(5930):1064-1068.
12. Manning K (1985). The ethylene forming enzyme system in carnation flowers. In: Ethylene and Plant Development. Roberts JA, Tucker GA, editors. Boston, US: Butterworths, 83-92.
13. Nambara E, Marion-Poll A (2005). Abscisic acid biosynthesis and catabolism. Ann. Rev. Plant Biol. 56:165-185.
14. Nichols R (1977). Sites of ethylene production in the pollinated and unpollinated senescing carnation (Dianthus caryophyllus) inflorescence. Planta 135(2):155-159.