

Salinity-Induced Early Activation of Flavonoid Biosynthesis Genes in Coriander *Coriandrum sativum* L.: A qRT-PCR-Based Analysis of CHS-Mediated Stress Response

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Abstract

CHS is a pivotal regulatory enzyme in the plant flavonoid biosynthetic pathway, and it has been closely linked to plant salinity stress defense response. The present study was designed to assess the CHS gene early transcriptional response to short-term salinity stress in coriander *Coriandrum sativum* L. leaves. The plants of Coriander were treated with 150 mM NaCl for 24 hours and 3 biological replicates were set for each experimental group. Gene expression was quantified by using RT-qPCR, normalizing to the housekeeping gene, GAPDH. The relative amount of transcripts was determined by the $2^{-\Delta\Delta Ct}$ method. The results showed upregulation of CHS transcript levels after salinity stress, and a calculated fold change of 4.20 after salinity stress compared with control plants which were not treated. Under salt stress, the mean cycle threshold (Ct) value of the CHS gene decreased, from 18.72 in the control group to 16.58, while the reference gene GAPDH value was almost similar with the values of 15.45 and 15.38, respectively. This is an early transcription activation of the flavonoid biosynthetic pathway in response to salinity stress. The results indicate that the CHS is a key regulator in the activation of antioxidant defense mechanisms in *C. sativum* against salt stress and could be considered as a potential molecular marker for further research on enhancing resistance to salt stress.

Keywords: *Coriandrum sativum* L; CHS gene; RT-qPCR; Flavonoid Biosynthesis

1 Introduction

Salinity is currently a major environmental problem affecting the productivity of glycophytic crops through causing of extreme redox imbalance in cells (Chen *et al.*, 2019; Jiang *et al.*, 2025). Plants can often counter these harmful effects by switching on secondary metabolic pathways, such as the biosynthesis of bioactive polyphenolic compounds that can readily scavenge reactive oxygen species (Zhang *et al.*, 2022). Of these secondary metabolites, flavonoids are especially key to the salt tolerance of plants, with the synthesis of flavonoids often being regulated by important enzymes such as chalcone synthase (Feng *et al.*, 2023; Liu *et al.*, 2024). Specifically, the induction of CHS genes is a powerful defence system and helps the flow of metabolic intermediates into the production of flavonoids in an environment of osmotic and ionic stress (Bageel *et al.*, 2022; Dehghan *et al.*, 2014). This metabolic flux is additionally supported by the evidence in other species about the catalytic production of chalcone as the basis of the synthesis of a variety of flavonoid structural forms that support physiological resistance (Isiyel *et al.*, 2024, Li *et al.*, 2024).

Moreover, the upregulation of CHS and related genes in several other plant models has been shown to be directly linked to salinity and drought tolerance (Amorim *et al.*, 2018; Wang *et al.*, 2025). Based on these results, it is crucial to examine the expression profile of the CHS in *C. sativum* to highlight the specific molecular orchestration that is involved in its phenylpropanoid response (Rostami *et al.*, 2022). Furthermore, this investigation goes beyond the role of individual structural genes in the regulatory pathway and includes how the expression of CHS in conjunction with secondary enzymes, like chalcone isomerase, contributes to the accumulation of phytoalexins that are key to maintaining cellular

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homeostasis (Oliveira, 2019). These metabolic changes indicate the ability to shift carbon sources to production of flavonols and other metabolites as important antioxidants under saline conditions (Martínez *et al.*, 2016).

In addition, the regulation of these biosynthetic responses is frequently regulated by intricate regulatory networks of MYB transcription factors that orchestrate the expression of the whole phenylpropanoid biosynthetic pathway (Rao & Zheng, 2025). Phosphorylation at, for example, the promoter region is frequently a key step in regulating these transcription factors to promote transcriptional activation of the downstream biosynthetic genes during stress (Wang *et al.*, 2024). This is a coordinated induction of genes that leads to the accumulation of complex secondary metabolites like flavonols and condensed tannins, which are important to maintain the integrity of plant cells under salinity-induced oxidative stress (Ahmad *et al.*, 2021). In addition to these structural responses, the presence of stress-responsive cis-acting elements in the CHS promoter region indicates a tightly controlled signaling pathway which allows plant physiological responses to adverse soil conditions with speed (Dao *et al.*, 2011; Ahmad *et al.*, 2023).

Likewise, the whole phenylpropanoid pathway is commonly activated in response to various environmental stresses, such as the induction of enzymes such as cinnamate 4-hydroxylase in the case of ionic imbalance (Dong *et al.*, 2020). Thus, these metabolic changes highlight the ability of polyphenolic compounds to effectively remove harmful reactive oxygen species, promoting the maintenance of cell redox homeostasis under environmental stresses (Rao & Zheng, 2025). This high antioxidant activity is a significant biochemical mechanism to preserve cell integrity against oxidative stress-induced damage and helps to buffer the detrimental impacts of ionic stresses on plant physiological functions (Feng *et al.*, 2023; Liu *et al.*, 2024). The objective of this systematic study is to elucidate the regulation of coriander on the anthocyanin synthesis pathways to eliminate excess oxidized reductive species when ionic imbalance occurs.

2 Materials and Methods

2.1 Plant Material and Growth Conditions

In this study uniform size and colour local coriander seeds (*Coriandrum sativum* L.) have been used. To ensure healthy growth, seeds were surface-sterilized in sodium hypochlorite solution followed by three rinses with distilled water as per (Quijia-Lamiña *et al.*, 2022). The seeds were planted in plastic pots (1 kg capacity) filled with a mixture of sandy soil and peat moss (2:1). The pots were then exposed to natural environmental conditions outside to mimic field conditions (Liu *et al.*, 2024). To prevent any possible interference from external salts, seeds were germinated in distilled water. After the establishment of seedlings, uniform plants were kept for 45 days under the standard irrigation conditions before salinity stress was applied. Plants were raised until they reached the 4-6 true leaf stage (around 21 days) when it was deemed ready for treatment (Shaik *et al.*, 2025).

2.2 Salinity Stress Treatment and Sampling

At the desired growth stage (4-6 true leaves), uniform seedlings were randomly selected for two treatments: control and salt stress. Irrigation water was applied to the control and stress groups as distilled water and 150 mM NaCl solution, respectively, to induce a single soil drench application of acute salinity stress (Isiyel *et al.*, 2024). Three biological replicates were used for each treatment to guarantee the statistics' reliability and the experimental precision, each replicate being a pooled leaf tissue from three individual plants (Chen *et al.*, 2019). Samples were collected **24 hours** after the onset of the salt treatment. All harvested leaf tissues were immediately flash-frozen in liquid nitrogen and subsequently stored at -80°C to preserve the integrity of the genetic material for subsequent RNA extraction and gene expression analysis (Isiyel *et al.*, 2024; Kou *et al.*, 2023).

2.3 RNA Extraction and cDNA Synthesis

Total RNA was extracted using the **TRIZOL** reagent protocol, and its purity was verified via **NanoDrop** spectrophotometry (Sintuprom *et al.*, 2024). First-strand cDNA was synthesized from 300 ng of total RNA using a reverse transcription mixture containing RT Enzyme Mix and cDNA Reaction Buffer. The reaction included incubation at 25°C for 5 min, 55°C for 15 min, and 85°C for 5 min to inactivate the enzyme (Reda *et al.*, 2024). Finally, the synthesized cDNA was stored at -20°C to ensure its stability for downstream quantitative real-time PCR (qRT-PCR) analyses (Sharma *et al.*, 2023).

2.4 RT-qPCR Analysis

Quantitative RT-PCR was performed using an Applied Biosystems StepOne™ instrument with a SYBR® Green master mix (Falcão *et al.*, 2018). The expression level of the target CHS gene and a reference gene, GAPDH, was normalized using specific primers (Rostami *et al.*, 2022; Wang *et al.*, 2018). The thermal profile was set as: an initial denaturation step of

95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min (Su *et al.*, 2017). The expression levels of the genes were analyzed using the relative fold change, using the $2^{-\Delta\Delta C_t}$ method (Calzone *et al.*, 2021; Wu *et al.*, 2018).

Table 1 Primer sequences for RT-qPCR analysis of the genes of CHS and GAPDH in *Coriandrum sativum*

Gene	Primer Direction	Sequence (5' → 3')
CHS	Forward	5'-ACTGGAACCTCTTCTTCTGGA-3'
	Reverse	5'-AACCCGAAAAGAACACCCCA-3'
GAPDH	Forward	5'-TCCTACGATGCCATCAAGGC-3'
	Reverse	5'-ACGAAATCGGTGGAGACGAC-3'

3 Results and Discussion

The leaves of *Coriandrum sativum* L. plants were found to be highly and statistically significantly upregulated for the expression of Chalcone Synthase (CHS) gene under acute salinity stress (150 mM NaCl) as revealed by RT-qPCR. A significant induction of the gene expression is noted after 24 hours of salt treatment, when compared to the control group, after molecular data has been carried out.

According to technical evaluation, the Mean cycle threshold (Mean C_t values) of the target gene was greatly reduced in the salt-stressed samples (16.58) compared to the control samples (18.72). The calculated mean ΔC_t values were 3.27 for control group, and 1.20 for salt stressed group with a calculated $\Delta\Delta C_t$ value of -2.07 and relative expression of approximately 4.20-fold based on these values.

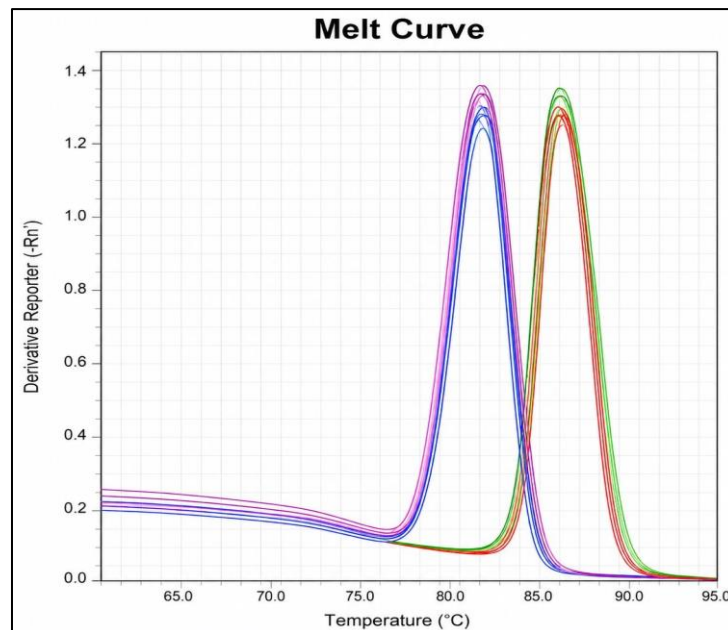


Figure 1 RT-qPCR of the CHS target gene and the reference gene was performed in coriander leaves and analysed by melt curve

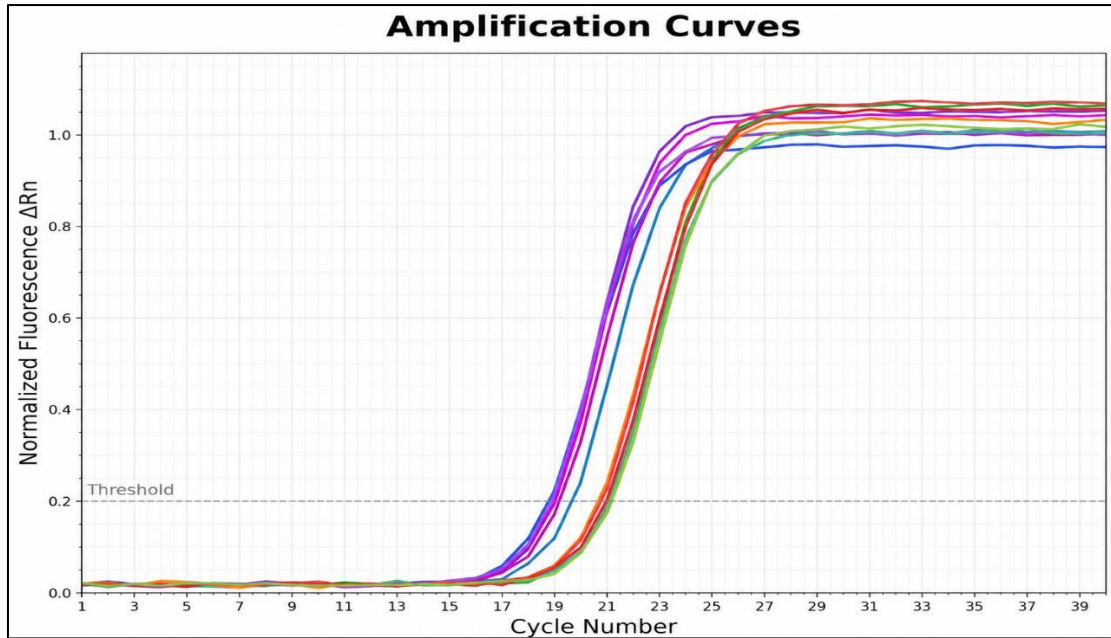


Figure 2 Amplification curve analysis of RT-qPCR for the target gene CHS and reference gene in leaves of coriander

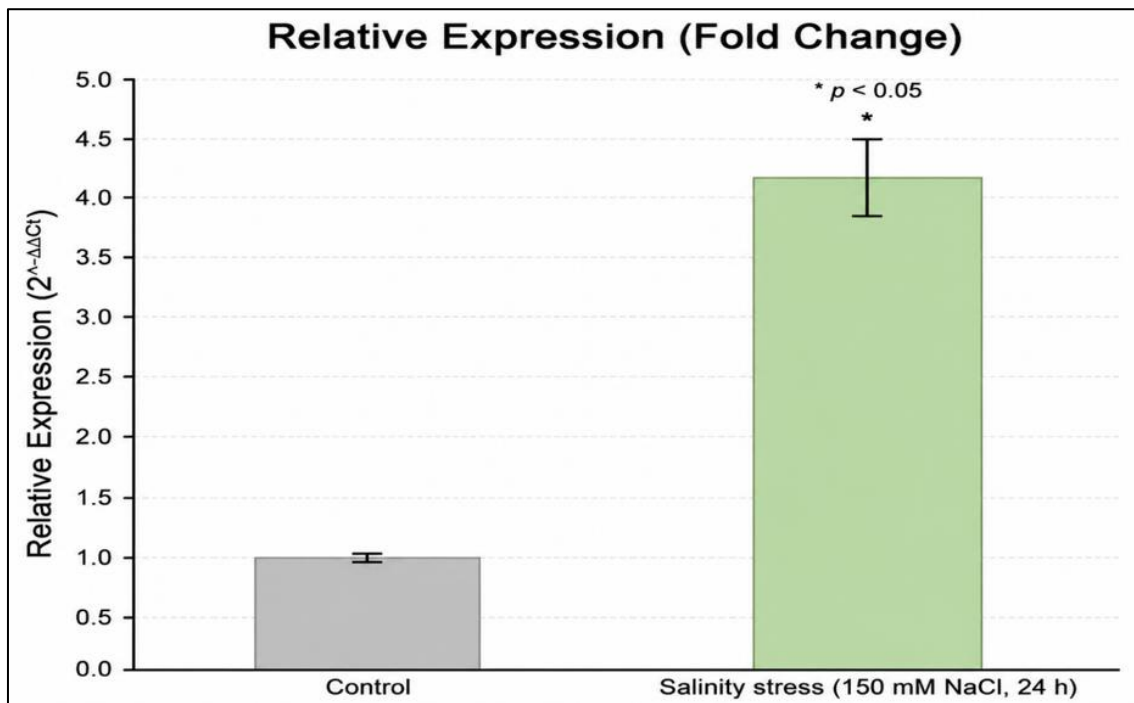


Figure 3 Relative expression analysis of the CHS target gene in coriander leaves exposed to salinity stress 150 mM NaCl for 24 h using the $2^{-\Delta\Delta C_t}$ method

Table 2 Quantitative RT-qPCR parameters and relative fold change of *CHS* gene expression in *Coriandrum sativum* leaves under 150 mM NaCl stress

Treatment	Biological replicate	CHS Ct	GAPDH Ct	$\Delta\text{Ct} = \text{CHS Ct} - \text{GAPDH Ct}$	Mean ΔCt	$\Delta\Delta\text{Ct}$	Relative expression $2^{-\Delta\Delta\text{Ct}}$	Fold change
Control	Replicate 1	18.72	15.45	3.27				
Control	Replicate 2	18.80	15.50	3.30				
Control	Replicate 3	18.65	15.40	3.25	3.27	0.00	1.00	1.00-fold
Salt stress 150 mM NaCl, 24 h	Replicate 1	16.58	15.38	1.20				
Salt stress 150 mM NaCl, 24 h	Replicate 2	16.50	15.35	1.15				
Salt stress 150 mM NaCl, 24 h	Replicate 3	16.66	15.42	1.24	1.20	-2.07	4.20	4.20-fold

These results offer strong evidence for the different level of transcriptional activation of the Chalcone Synthase gene in *Coriandrum sativum* L. leaves under salinity stress. The high decrease in Ct of plants under salt stress suggests a significant induction of CHS transcript levels in coriander seeds under salt stress, and therefore the plants quickly switch on the flavonoid biosynthetic pathway in response to salt stress. Since CHS is a rate limiting enzyme in the phenylpropanoid pathway, its upregulation serves as an important adaptive response to salinity stress, helping to reduce the oxidative damage and maintain cellular stability by strengthening the plant's antioxidant capacity (Adhikari *et al.*, 2025; Isiyel *et al.*, 2024; Rao & Zheng, 2025). These metabolic changes also correlate with the number of consistent observations of different cultivars in which early induction of flavonoid-related genes enabled a rapid induction of secondary metabolites to combat reactive oxygen species (ROS) (Wang *et al.*, 2023; Feng *et al.*, 2023). Similar to other horticultural crops (Ren *et al.*, 2024), the modulation of the phenylpropanoid pathway proved to be an important strategy for salinity tolerance in *C. sativum*.

In addition, transcriptomic and metabolomic analysis showed that the genes involved in the synthesis of lignin and flavonoids often have strong correlations in salt stress. The result indicates a complex defence mechanism involving enzymatic scavenging of ROS and strengthening of the cell wall to prevent the apoplast from taking up toxic Na^+ ions (Mellidou *et al.*, 2021; Roşca *et al.*, 2023). This biochemical prioritization, possibly mediated by the calcium-dependent signaling cascades, regulates vacuolar Na^+/H^+ exchangers to maintain ion homeostasis (Ikram *et al.*, 2025), and metabolic flux towards the production of monolignols strengthens the cell wall integrity and inhibits water loss (Pan *et al.*, 2020). Rapid CHS activation during high salinity may be attributed to stress responsive cis-regulatory elements such as the ABA-responsive and dehydration-responsive elements in the promoter. The importance of the CHS gene family in the crop response to abiotic stress is confirmed by the results of a genomic study of maize, while a loss of function leads to defective osmotic regulation (Wang *et al.*, 2025).

The induction level observed in coriander is matching the reported increase in the expression of some biosynthetic genes in the production of bioactive compounds during harsh environmental conditions (Bassiouni *et al.*, 2026). Likewise, the activation speed is similar to the adaptive mechanisms found in other plants, such as *Iris halophila*, in which activation of the CHS regulates signaling molecules like jasmonic acid and auxin, supporting physiological homeostasis (Liu *et al.*, 2024). Additionally, regulatory elements such as MYB transcription factors and promoter motifs indicate that *C. sativum* coordinates and orchestrates diverse hormonal and environmental cues to fine-tune its secondary metabolic production (Khakdan *et al.*, 2021; Sun *et al.*, 2021). Together, this complex regulation is the antioxidant enzyme system such as superoxide dismutase and catalase are the protective mechanisms which have been seen in different species in response to abiotic stress (Rastogi *et al.*, 2019; Rezaie *et al.*, 2020).

Thus, the 4.20-fold induction represents a complex and multi-level adaptive response, rather than a temporary molecular response, and acts as an effective and proactive reinforcement of the biochemical defensive barrier in coriander tissues. These defenses can be activated within seconds, effectively reducing the adverse effects of ion toxicity, preventing an increase in membrane lipid peroxidation, and protecting the integrity of cell structures during crucial early stages of salinity stress (Zulfiqar *et al.*, 2024; Maaiyrah *et al.*, 2025). This regulation is essential for maintaining a

proper K^+/Na^+ ratio, which is vital for achieving metabolic stability and long-term productivity in high saline environments (Jia *et al.*, 2022).

4 Conclusions

The study has been concluded that the CHS gene is a key gene in coriander (*Coriandrum sativum* L.) early molecular response system under salinity stress. A highly plastic response of the transcriptome in the sense of rapid and robust transcriptional activation of this gene was observed under the influence of salt stress. This gene induction is a response to a proactive defense system to increase the production of flavonoids, an essential part of maintaining cellular integrity against oxidative damage and maintaining membrane stability. Moreover, the results highlight the usefulness of the CHS gene as a probable molecular marker for salt tolerance in this aromatic crop. Further studies are suggested which will be directed at relating these transcriptional responses to their actual production of biochemical metabolites and secondary products when exposed to different environmental conditions, in order to obtain a more comprehensive understanding of the overall adaptation strategies of the plant.

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