

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(5): 941-944
www.biochemjournal.com
Received: 25-02-2026
Accepted: 29-03-2026

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Expression analysis of drought responsive genes in tomato treated with green synthesized SiO₂ nanoparticles under drought stress

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5Sk.8491>

Abstract

Drought stress is one of the major abiotic stresses responsible for severe reduction in growth and productivity of tomato plants. Silicon dioxide nanoparticles (SiO₂NPs) have recently gained attention due to their beneficial role in improving plant stress tolerance. The present study was conducted to investigate the expression analysis of drought responsive genes in tomato plants treated with green synthesized SiO₂ nanoparticles under drought stress conditions. Green synthesized SiO₂ nanoparticles prepared from rice husk were applied as foliar spray at different concentrations (50, 100 and 150 ppm). Drought stress was imposed by withholding irrigation for 7 days after nanoparticle treatment. Quantitative Real-Time PCR (qRT-PCR) analysis was carried out using drought responsive genes *ERF-H7* and *P5CS*, while *Actin* gene was used as an internal reference gene. The results revealed enhanced expression of *ERF-H7* and *P5CS* genes in SiO₂ nanoparticle treated plants under drought stress conditions. Among all treatments, 100 ppm SiO₂ nanoparticle treatment exhibited maximum expression with 3.58±0.12 and 3.87±0.15 fold increase for *ERF-H7* and *P5CS* genes, respectively. The enhanced expression of drought responsive genes indicated improved stress signaling and osmotic adjustment in tomato plants treated with SiO₂ nanoparticles. The present findings demonstrated that green synthesized SiO₂ nanoparticles may improve drought tolerance through molecular regulation of stress responsive genes in tomato plants.

Keywords: Tomato, Drought stress, Silicon dioxide nanoparticles, Gene expression, qRT-PCR, *ERF-H7*, *P5CS*

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops cultivated worldwide because of its high nutritional and economic importance. However, tomato production is severely affected by several abiotic stresses, among which drought stress is considered one of the major limiting factors reducing plant growth, physiological performance and yield. Water deficit conditions adversely affect photosynthesis, nutrient uptake, membrane stability and metabolic activities in plants.

Under drought stress conditions, plants activate several physiological, biochemical and molecular defense mechanisms to survive unfavorable environmental conditions. Drought stress also induces production of reactive oxygen species (ROS), which cause oxidative damage to cellular structures and metabolic pathways. Therefore, activation of drought responsive genes plays an important role in stress adaptation and tolerance.

ERF-H7 belongs to AP2/ERF transcription factor family and plays a major role in regulation of stress responsive pathways under drought stress conditions. Similarly, *P5CS* is a key enzyme involved in proline biosynthesis. Proline accumulation contributes to osmotic adjustment, membrane stabilization and cellular protection during water deficit stress.

Recently, nanotechnology has emerged as a promising approach for improving crop productivity and stress tolerance in plants. Silicon dioxide nanoparticles (SiO₂NPs) have been reported to improve drought tolerance through enhancement of antioxidant defense systems, osmolyte accumulation and stress signaling pathways. Green synthesis of nanoparticles using rice husk is considered an eco-friendly and sustainable method due to high silica content present in rice husk.

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Although beneficial effects of SiO₂ nanoparticles under abiotic stress conditions have been reported in several crops, limited information is available regarding molecular response of tomato plants treated with green synthesized SiO₂ nanoparticles under drought stress conditions. Therefore, the present investigation was undertaken to study the expression analysis of drought responsive genes *ERF-H7* and *P5CS* in tomato plants treated with green synthesized SiO₂ nanoparticles under drought stress conditions.

Materials and Methods

Experimental Site

The present investigation was carried out in the Molecular Biology Laboratory of SDMVM'S College of Agricultural Biotechnology, Chh, Sambhajinagar, Maharashtra, India.

Experimental Design and Treatments

Healthy and uniform tomato seedlings (*Solanum lycopersicum* L.) were used for the present study. The experiment consisted of four treatments with three biological replications under controlled environmental conditions.

Treatment	Description
T ₀	Control
T ₁	SiO ₂ nanoparticles @ 50 ppm
T ₂	SiO ₂ nanoparticles @ 100 ppm
T ₃	SiO ₂ nanoparticles @ 150 ppm

Green synthesized silicon dioxide nanoparticles were applied as foliar spray. Drought stress was imposed by withholding irrigation for 7 days after nanoparticle application. Leaf samples were collected after stress imposition and stored for molecular analysis.

Green Synthesis of SiO₂ Nanoparticles

Green synthesized SiO₂ nanoparticles were prepared from rice husk following the method described by Wang *et al.* (2012) with slight modifications. Rice husk was washed thoroughly, treated with hydrochloric acid and dried at 100 °C. The dried material was pyrolyzed for silica extraction and ultrasonicated further to obtain semi-crystalline porous SiO₂ nanoparticles.

RNA Extraction

Fresh leaf samples were frozen in liquid nitrogen and stored at -80 °C until further analysis. Approximately 50 mg leaf tissue was ground into fine powder using pre-chilled mortar and pestle.

Total RNA was extracted using CTAB extraction method. The extraction buffer contained 2% CTAB, 100 mM Tris-HCl (pH 8.0), 25 mM EDTA, 2 M NaCl and 2% β-mercaptoethanol. The samples were incubated at 65 °C for 15-20 min followed by chloroform:isoamyl alcohol extraction and centrifugation at 12,000 rpm for 10 min at 4 °C. RNA was precipitated using chilled isopropanol and washed with 70% ethanol. RNase-free DNase I treatment was performed to remove genomic DNA contamination. RNA quantity and purity were analyzed using Nanodrop spectrophotometer and RNA integrity was confirmed by 1.5% agarose gel electrophoresis.

cDNA Synthesis

First-strand cDNA synthesis was carried out using purified RNA samples. Approximately 1 µg RNA was mixed with Oligo(dT) primer and incubated at 60 °C for 5 min followed by chilling on ice. The reaction mixture containing dNTPs, reverse transcriptase enzyme, RNase inhibitor and reaction buffer was added and incubated at 45 °C for 60 min followed by enzyme inactivation at 85 °C for 5 min. The synthesized cDNA was stored at -20 °C, while green synthesized SiO₂ nanoparticles were characterized and confirmed using FTIR and TEM analysis.

Primer Design

Gene specific primers were designed for *ERF-H7* and *P5CS* genes using NCBI mRNA sequences and Primer3 software. *Actin* gene was used as an internal reference gene for normalization of qRT-PCR data.

Primer Sequences Used

Gene	Primer Type	Sequence (5'-3')
<i>ERF-H7</i>	Forward	TTGAGTCGGAAGAATCGAAGAC
<i>ERF-H7</i>	Reverse	TACCCATCAAAGTCGCCATC
<i>P5CS</i>	Forward	CCCACACCGATAGCATCATTAC
<i>P5CS</i>	Reverse	TCCAAAGCGGAATCCATCAC
<i>Actin</i>	Forward	CTCTCTCTCTCTCATCTCTCTAC
<i>Actin</i>	Reverse	CCTTGACCATTCCAGTTCCA

The primers were selected based on primer length, GC content, melting temperature and amplification specificity.

Quantitative Real-Time PCR (qRT-PCR) Analysis

Quantitative Real-Time PCR analysis was performed using SYBR Green chemistry for determination of relative expression levels of *ERF-H7* and *P5CS* genes. *Actin* gene was used as internal reference gene.

The qRT-PCR reaction mixture consisted of 5 µL 2× SYBR Green Master Mix, 0.5 µL forward primer, 0.5 µL reverse primer, 1 µL cDNA template and nuclease-free water in a final volume of 10 µL.

Amplification was carried out under the following conditions:

Step	Temperature	Time
Initial denaturation	95 °C	2 min
Denaturation	94 °C	30 sec
Annealing	58 °C	30 sec
Extension	72 °C	20 sec

A total of 40 cycles were performed and relative fold expression was calculated using the 2^{-ΔΔCt} method after normalization against *Actin* gene.

All experiments were performed in triplicate and data were expressed as mean±standard error (SE).

Results and Discussion

Expression Stability of *Actin* Reference Gene

Table 1: Ct values of *Actin* gene under different treatments

Treatment	<i>Actin</i> Ct Value
T ₀ (Control)	22.10±0.11
T ₁ (50 ppm)	22.02±0.09
T ₂ (100 ppm)	21.98±0.08
T ₃ (150 ppm)	22.05±0.10

The *Actin* gene was used as an internal reference gene for normalization of qRT-PCR data. The C_t values of *Actin* gene remained nearly constant across all treatments under drought stress conditions, indicating stable expression of the housekeeping gene. The minimal variation observed in C_t values confirmed the suitability of *Actin* gene for normalization and relative fold expression analysis of drought responsive genes.

Relative Expression Analysis of *ERF-H7* Gene

Table 2: Relative fold expression of *ERF-H7* gene under different treatments

Treatment	<i>ERF-H7</i> Fold Expression
T ₀ (Control)	1.02±0.03
T ₁ (50 ppm)	1.84±0.08
T ₂ (100 ppm)	3.58±0.12
T ₃ (150 ppm)	2.39±0.10

The results revealed enhanced expression of *ERF-H7* gene in SiO₂ nanoparticle treated plants compared to untreated

control plants under drought stress conditions. Among different treatments, T₂ (100 ppm) exhibited maximum fold expression (3.58±0.12 fold), indicating enhanced activation of drought responsive signaling pathways under moderate nanoparticle concentration (Fig. 1).

ERF transcription factors are known to regulate several abiotic stress responsive genes involved in osmotic regulation and stress adaptation. Similar findings were reported by Mizoi *et al.* (2012), who observed moderate induction of AP2/ERF transcription factors under drought stress conditions in plants. The present findings are also supported by Almutairi (2016), who reported enhanced stress responsive gene expression in tomato plants treated with nano-silicon under abiotic stress conditions.

Moderate increase in *ERF-H7* expression was observed in T₁ treatment, while comparatively reduced expression was recorded in T₃ treatment compared to T₂ treatment. This may be associated with concentration dependent nanoparticle response under drought stress conditions.

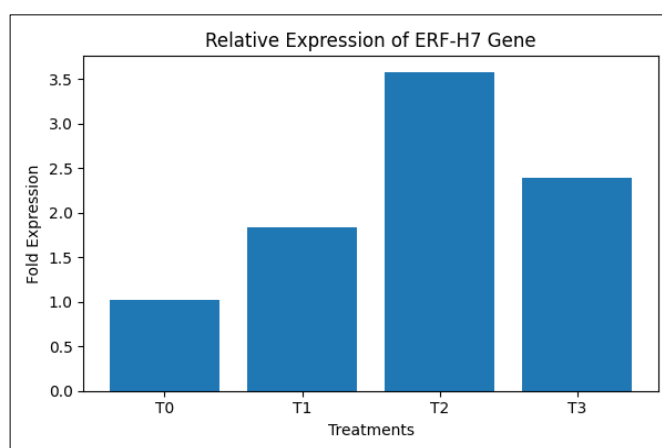


Fig 1: Relative expression of *ERF-H7* gene under different treatments

Relative Expression Analysis of *P5CS* Gene

Table 3: Relative fold expression of *P5CS* gene under different treatments

Treatment	<i>P5CS</i> Fold Expression
T ₀ (Control)	1.03±0.02
T ₁ (50 ppm)	1.76±0.06
T ₂ (100 ppm)	3.87±0.15
T ₃ (150 ppm)	2.54±0.11

The *P5CS* gene showed enhanced expression in SiO₂ nanoparticle treated tomato plants under drought stress

conditions. Among all treatments, T₂ (100 ppm) showed maximum fold expression (3.87±0.15 fold) compared to untreated control plants (Fig. 2).

P5CS is a key enzyme involved in proline biosynthesis and osmotic adjustment during drought stress. Increased expression of *P5CS* gene suggested enhanced proline accumulation and cellular protection in treated plants under water deficit conditions. Similar observations were reported by Szabados and Savaure (2010), who reported enhanced *P5CS* expression associated with proline accumulation under drought stress conditions.

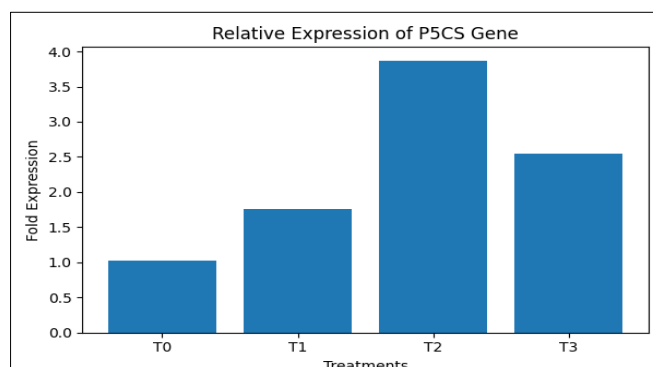


Fig 2: Relative expression of *P5CS* gene under different treatments

3.4 Overall Effect of SiO₂ Nanoparticles on Drought Responsive Gene Expression

The overall results of the present investigation demonstrated that green synthesized SiO₂ nanoparticles enhanced expression of drought responsive genes in tomato plants under drought stress conditions. Among different concentrations, 100 ppm SiO₂ nanoparticle treatment proved most effective in upregulating *ERF-H7* and *P5CS* genes.

Enhanced expression of *ERF-H7* indicated activation of drought stress signaling pathways, whereas increased expression of *P5CS* suggested improved osmotic adjustment through proline biosynthesis. The results strongly indicated that green synthesized SiO₂ nanoparticles may improve drought tolerance in tomato plants through molecular regulation of stress responsive pathways.

The observed gene expression pattern was found to be concentration dependent, where moderate nanoparticle concentration (100 ppm) exhibited maximum effectiveness, while higher concentration (150 ppm) showed comparatively reduced expression.

Conclusion

The present investigation demonstrated that green synthesized SiO₂ nanoparticles enhanced drought tolerance in tomato plants through upregulation of drought responsive genes under drought stress conditions. Among different treatments, 100 ppm SiO₂ nanoparticle treatment showed maximum expression of *ERF-H7* and *P5CS* genes, indicating improved stress signaling and osmotic adjustment. Therefore, green synthesized SiO₂ nanoparticles may be used as a sustainable approach for improving drought tolerance in tomato plants.

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