



# Characterization and Expression of the S-nitrosogluthathione Reductase (GSNOR) Gene from *Jatropha curcas* in Response to Azelaic Acid

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## Abstract

The 1421 bp gene encoding S-nitrosogluthathione reductase (GSNOR) was successfully cloned and characterized from *Jatropha curcas*. The gene contains an open reading frame (ORF) of 1140 base pairs, encoding a polypeptide of 379 amino acids. Expression analysis revealed that *JcGSNOR* is constitutively expressed across all major organs of *Jatropha curcas*. To further investigate the role of *JcGSNOR* in systemic acquired resistance (SAR) and its molecular response to azelaic acid, a key SAR signaling molecule, the expression patterns of *GSNOR* under exogenous azelaic acid treatment were examined. Expression levels of *GSNOR* in both *Zea mays* and *Jatropha curcas* decreased with increasing concentrations of azelaic acid up to 600  $\mu\text{g}\cdot\text{mL}^{-1}$ . Notably, while *ZmGSNOR* expression continued declining, *JcGSNOR* sharply increased at the 800  $\mu\text{g}\cdot\text{mL}^{-1}$  treatment, suggesting a species-specific regulatory response. Additionally, *JcGSNOR* was heterologously expressed in *Saccharomyces cerevisiae* and in the *gsnor* mutant of *Arabidopsis thaliana*. Our findings indicate that *JcGSNOR* enhances GSNOR enzymatic activity in *S. cerevisiae*, although it has minimal impact on yeast growth. Transgenic *Arabidopsis thaliana* expressing *JcGSNOR* demonstrated an improved tolerance to exogenous azelaic acid. This study provides the first characterization of *GSNOR* from *Jatropha curcas* and its response to azelaic acid. Our findings suggest that *JcGSNOR* contributes to defense-related signaling and may hold potential for enhancing plant resistance. These results deepen our understanding of the function of GSNOR in SAR signaling.

**Keywords** SAR · Azelaic acid · *Jatropha curcas* · GSNOR · Plant defense

## Introduction

To promote local economic development, *Jatropha curcas*, recognized as a potential biodiesel source, has been extensively cultivated in Asian countries such as India, Pakistan, and China (Khanam et al. 2021), (Ye et al. 2009). *Jatropha curcas* is often intercropped with economically important species like corn (*Zea mays*) and tobacco (*Nicotiana tabacum*). Despite its promise, concerns have arisen due to the presence of toxic compounds in *J. curcas*, which may exert allelopathic effects on neighboring crops (Ma et al. 2011) and its susceptibility to epidermal diseases under large-scale

cultivation (Mazumdar et al. 2018). Excessive reliance on chemical treatments further undermines the plant's innate resistance and intensifies environmental stress. In this context, systemic acquired resistance (SAR) represents a critical adaptive mechanism, enabling plants to mount whole-plant defense responses following pathogen exposure, herbivore attack, or environmental stress. (Fu and Dong 2013) SAR involves physiological adjustments and regulation of signaling molecules such as nitric oxide (NO) and S-nitrosogluthathione (GSNO) (Espunya et al. 2012a), which enhance survival by strengthening disease resistance. Functioning as a signal transduction pathway, SAR initiates plant immune responses, thereby offering opportunities to improve *J. curcas* resilience either through genetic engineering or by modulating the signaling molecules that are intrinsic to SAR.

NO is a versatile signaling molecule in plants, influencing numerous physiological processes. It regulates seed germination (Graziano et al. 2002), (Giba et al. 1998), root development (Ma et al. 2020), (Correa-Aragunde et al.

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2004), stomatal closure (Mata and Lamattina 2001), (Neill et al. 2002), and flowering by interacting with hormones such as auxin (Fu et al. 2022), abscisic acid (Khan et al. 2023), and ethylene (Khan et al. 2023). NO also modulates cellular redox status and participates in responses to abiotic stresses like drought and salinity (Khator et al. 2024). Beyond growth regulation, NO plays a pivotal role in plant defense. During pathogen attack, NO accumulates rapidly, triggering hypersensitive response (HR) and programmed cell death at infection sites (Govrin and Levine 2000). NO enhances salicylic acid signaling, activates pathogenesis-related genes, and promotes SAR. Additionally, NO mediates GSNO, altering the activity of defense enzymes and signaling components (Xu et al. 2025). Thus, NO acts as a central hub linking developmental processes with immune responses, enabling plants to adapt and survive under biotic stress. GSNO reductase (GSNOR) is a key enzyme regulating NO signaling in plants by controlling GSNO, a major reservoir of NO, at the cellular levels. GSNOR maintains NO homeostasis by degrading GSNO, thereby modulating GSNO level, which affects the activity of defense-related proteins (Yang 2025). Loss of GSNOR function often leads to excessive GSNO accumulation, causing nitrosative stress and impaired immune signaling (Li et al. 2025). Conversely, optimal GSNOR activity ensures balanced NO signaling, promoting effective pathogen recognition and activation of salicylic acid-dependent pathways.

In our previous study, we identified azelaic acid as one of the allelopathic compounds produced by *J. curcas* (Ma et al. 2011). Azelaic acid is recognized as a signaling molecule that mediates SAR, functioning similarly to salicylic acid (SA) and jasmonic acid (JA) (Haghpanah 2024). In *Arabidopsis thaliana*, exogenous SA treatment significantly upregulates GSNOR expression (Espunya et al. 2012a). Conversely, JA signaling shows minimal direct induction of GSNOR in *Arabidopsis*, indicating a preference for SA-mediated NO homeostasis during SAR (Jahnová 2019). In *Nicotiana tabacum*, the pattern is more pronounced. SA strongly induces GSNOR activity, whereas JA downregulates its expression, aligning with JA's role in necrotrophic pathogen and herbivory defense, which often suppresses SA-related components (Wünsche 2011). Therefore, investigating how azelaic acid affects GSNOR activity and SAR establishment will not only deepen our understanding of plant immune signaling but also provide strategies to enhance disease resistance in *J. curcas*, supporting its large-scale cultivation and sustainable agricultural applications.

In this study, we hypothesize that the expression of GSNOR in *J. curcas* (*JcGSNOR*) responds to exogenous azelaic acid for improved tolerance to environmental stresses. To test this hypothesis, we cloned and characterized the gene encoding GSNOR from *J. curcas*, expressed *JcGSNOR* in *Saccharomyces cerevisiae* and *Arabidopsis*, and

examined the effect of azelaic acid on GSNOR gene expression and its functional relationship. This study presents three novel contributions to the field of plant molecular biology and immunity. It reveals a previously uncharacterized interaction between azelaic acid and *JcGSNOR*, offering new insights into their roles within the SAR signaling network. And the research introduces a strategic approach to enhance the innate immune capacity of *J. curcas*, thereby supporting its potential for large-scale cultivation and improved resilience against both biotic and abiotic stresses.

## Material and Method

### Plant Materials

Seeds of *J. curcas* L. mature seeds were harvested from Hainan Province, China. Corn (*Zea mays* L.) seeds were lab-provided. *GSNOR* mutant (AT5G43940) *A. thaliana* seeds were bought from the Ohio State University (#CS66012) (Hewitt 1966). Wild-type (WT) *Arabidopsis* was purchased from Innovating Science (IS3731). Young seedlings were cultivated in greenhouse conditions and irrigated daily with Hoagland solution (Yeku and Frohman 2011).

### Azelaic Acid Treatment in This Study

For quantitative real-time polymerase chain reaction (qRT-PCR), seedlings were irrigated with azelaic acid (Sigma Aldrich, #246379) in Hoagland solution at concentrations of 200, 400, 600, and 800  $\mu\text{g} \times \text{mL}^{-1}$ , respectively. The treatment of Hoagland media without azelaic acid was used as the control. 1-month-old leaves of *J. curcas* from different treatments were collected, immediately frozen in liquid nitrogen, and stored at  $-80^\circ\text{C}$  for RNA isolation.

### JcGSNOR Cloning

Total RNA was extracted from leaves of 1-month-old *J. curcas* seedlings by using PureLink Plant RNA Reagent (Invitrogen, #12322012) and Qiagen's RNeasy Plant Mini Kit (74904) following the vendor's protocol. Next, cDNA was synthesized using SuperScript III Reverse Transcriptase (Invitrogen, #18080044). To obtain the full-length cDNA of *JcGSNOR*, two pairs of degenerated primers, F1/R1 and F2/R2 in Supplementary Table 1, were designed based on the conserved region of *GSNORs* from GenBank: XM\_002301800.1 (*Populus trichocarpa*), AK226412.1 (*Arabidopsis thaliana*), HQ620558.1 (*Solanum chmielewskii*), and BT042962.1 (*Zea mays* L.). Then, the 5' and 3' ends of *JcGSNOR* cDNA were obtained by the rapid amplification of cDNA ends (RACE) method (Hewitt 1966). Three outer adaptor primers (Oligod (T)<sub>18</sub>, AP1, AP2) and five

gene-specific inner primers (5R1, 5R2, 5R3, and 3R1, 3R2) were used to obtain the region of 5' and 3' ends of *JcGSNOR*. After that, the full-length cDNA sequence of *JcGSNOR* was obtained through PCR with specific primers JW5 and JW3, which encode the 5' and 3' ends of *JcGSNOR*, respectively. The PCR reaction was carried out according to the following protocols: initial denaturation at 94 °C for 3 min; 35 cycles of 94 °C for 40 s, 65 °C for 40 s, and 72 °C for 1 min; and an extension of 72 °C for 5 min. Finally, the full-length cDNA was purified with Qiagen's QIAquick PCR purification kit and cloned into the T vector pMD19 (Sino Biological) and sequenced.

### Phylogenetic Tree Construction

Full-length GSNOR sequences from *Jatropha curcas* and seven representative dicotyledonous species (*Ricinus communis*, *Populus trichocarpa*, *Linum usitatissimum*, *Arabidopsis thaliana*, *Nicotiana attenuata*, *Medicago truncatula*, and *Glycine max*) were retrieved from the NCBI GenBank database. Multiple sequence alignment of the acquired sequences was performed using the MUSCLE algorithm with default parameters. The evolutionary history was inferred by constructing a phylogenetic tree using the Maximum Likelihood (ML) method based on the Poisson correction model. The analysis was conducted using MEGA 11 software.

### Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The primers used in this section are listed in Supplementary Table 2. The transcriptional levels of *JcGSNOR* were evaluated by qRT-PCR. The total RNAs were extracted from roots, stems, and leaves of 1-month-old seedlings and ripe fruit from WT *J. curcas*. Specific primers of *QJcGSNORF* and *QJcGSNORR* were designed by the characterized sequence, with *QJcACTIN-F* and *QJcACTIN-R* being controls. 18S rRNA primers, *QJc18s-S* and *QJc18s-A*, were designed according to Gao et al. (Yeku and Frohman 2011) as an internal control. Then, the effects of azelaic acid concentration at transcriptional levels of *ZmGSNOR* and *JcGSNOR* in leaves of seedlings of *Z. mays* and *J. curcas* were also evaluated. Specific primers *QZmGSNORF* and *QZmGSNORR* were designed according to the corresponding sequence of *ZmGSNOR* (GenBank Accession NM\_001112015.1). Actin primers (*QZmACTIN-F*, *QZmACTIN-R*) were designed according to Ma et al. (Gao et al. 2012) as an internal control. The total reaction volume was set at 20 µl, containing appropriately diluted cDNA with a combination of 10 µl of SsoFast EvaGreen Supermix from Bio-Rad. The following qRT-PCR cycle is used: initial denaturation at 95 °C for 30 s; 40 cycles of 95 °C for 5 s, 60 °C

for 1 min, and a final melt curve profile (65–95 °C). Quantification was determined by the comparative C<sub>T</sub> method.

### Sequence Analysis

The sequence was searched for similarity with the BLAST algorithm at the web servers of NCBI (<http://www.ncbi.nlm.nih.gov/BLAST/>). The deduced amino acid sequence was analyzed by NCBI's ORF finder (<https://www.ncbi.nlm.nih.gov/orffinder/>). Multiple alignments of the sequences were performed by <http://www.expasy.org/> upon the amino acid sequences from NCBI.

### Expression of *JcGSNOR* in *Saccharomyces cerevisiae* and *Arabidopsis*

The primers used in this section are listed in Supplementary Table 3. The ORF of *JcGSNOR* was amplified by primers *JCGSNORBamHI* and *JCGSNORXhoI*, inserted into the *pYES2* expression vector from Invitrogen between BamHI and XhoI sites, and transformed into the *Saccharomyces cerevisiae* Y190 strain. An empty vector without *JcGSNOR* was also transformed as the control. The transformants were selected in SC-Ura media (Ma and L. 2010) at 30 °C for 48 h and induced in induction medium [20202020]. The ORF of *JcGSNOR* was amplified by *JCGSNORBamHI* and *JCGSNORXbaI* primers and inserted into pBI121 vectors (NovoPro Bioscience, Supplementary Fig. 1) by the CaMV 35S promoter (Jorgensen et al. 2002) to generate a 35S::*JcGSNOR* construct. Then the 35S::*JcGSNOR* construct was introduced into *GSNOR* mutant *Arabidopsis* through an *Agrobacterium tumefaciens* LBA4404 strain (Invitrogen) using the floral dip method (Amack and Antunes 2020). Three independent lines were used to ensure reproducibility and mitigate positional effects. Empty vectors without *JcGSNOR* were also transformed into wild type and *GSNOR* mutants of *Arabidopsis*. Transformed progenies (35S::*JcGSNOR*/Mutant, 35S/Wild, 35S/Mutant, named as *Arabidopsis*<sup>*JcGSNOR*+</sup>, *Arabidopsis*<sup>WT</sup>, and *Arabidopsis*<sup>mutant</sup>) were selected by germination in MS medium supplemented with 100 µg × mL Kanamycin. The T2 generation, which was obtained by self-pollination, was used to confirm the transgenic status by RT-PCR.

### Enzymatic and Cellular Assay for *JcGSNOR* + *S. cerevisiae*

GSNO was purchased from Sigma (Sigma, N4148). GSNOR activity was determined according to Sakamoto's method (Clough and Bent 1998). Briefly, the consumption of GSNO at ambient conditions in 0.1 M sodium phosphate (pH 7.5) was monitored by a UV-Vis spectrometer at 340 nm. One unit of activity is defined as the ability to decompose 1 µmol

of GSNO per minute. Additionally, the GSNO-hypersensitivity was evaluated by cell growth of *Saccharomyces cerevisiae* in YPD medium supplemented with 2 mM GSNO according to Liu et al. (Sakamoto et al. 2002). In general, cells grown in mid-log phase ( $OD_{600} = 0.5$ ) were diluted 20-fold in fresh YPD medium with or without GSNO. Then, the cell culture started again at 30 °C in the dark. Cell density was recorded every 2 h by monitoring the  $OD_{600}$  value.

### Germination and MDA Assay of Arabidopsis

All *Arabidopsis* were cultured in Hoagland medium, supplemented with  $600 \mu\text{g} \times \text{mL}^{-1}$  azelaic acid<sup>24</sup>. A treatment of Hoagland media without azelaic acid was set as the control. Briefly, 20 mL treatment medium was added to a petri dish (9 cm in diameter) containing 15 g of clean quartz sand, which was covered with a circle of filter paper (7-cm diameter, Whatman 1). Then, 30 seeds of *Arabidopsis thaliana* were placed in a prepared dish. All treatments were arranged in a completely randomized design and incubated in a growth chamber at  $27 \pm 1$  °C with an intensity of  $400 \mu\text{mol} \times \text{m}^{-2} \times \text{s}^{-1}$  and a 16 h photoperiod for 30 days. Germination rate was measured on Day 7. MDA content was measured on Day 30. In general, fresh leaf (1 g) without any browning was ground and homogenized with a chilled mortar and pestle in 5 mL of a chilled 0.05 M phosphate buffer (pH 7.8) at 4 °C. The homogenate was strained through four layers of cheesecloth. The liquid suspension was centrifuged at 1900 g for 20 min. Next, the supernatant was used for measurement of MDA content according to the method described by Pinhero et al. (Liu et al. 2001).

### Statistical Analysis

Five seeds per repeat, and five repeats were performed for germination experiments. For the rest of the experiments, five samples were analyzed per treatment. Comparison between two means was determined using Student's *t*-test, and statistical significance was defined as  $p < 0.05$ .

## Results and Discussion

### GSNOR Is Highly Conserved Across Species and Functions Similarly

The nucleotide sequence of the *JcGSNOR* gene and its deduced amino acid (AA) sequence are presented in Supplementary Fig. 2. A total of 11 ORFs were found, and ORF2 presents the longest read from the cloned full-length cDNA that spans 1421 base pairs (bp). *JcGSNOR* comprises an 80 bp 5' untranslated region (UTR), a 1140 bp open reading frame (ORF2), a 180 bp 3' UTR, and an 18 bp poly(A) tail.

The ORF encodes a polypeptide of 379 amino acids, with an estimated molecular mass of 40.484 kDa and a predicted isoelectric point (pI) of 6.51. The deduced protein sequence contains a PKS\_ER domain extending from AA positions 17 to 375, which is associated with enoyl-reductase activity, catalyzing acyl groups other than amino-acyl groups.

The nucleotide motifs within the *JcGSNOR* cDNA sequence (GeneBank Submission ID 3061865) act as essential structural and regulatory signals that govern how the cell processes and translates the RNA transcript. Spanning the 1421 base pair sequence, these motifs include the standard translation initiation signal (the ATG start codon at position 82) and the termination signal (the TAG stop codon at position 1218), which together perfectly define the open reading frame 2 for the ribosome. Beyond the coding region, the 3' untranslated region contains critical stability and processing elements, specifically an AT-rich putative polyadenylation signal (from 1407 to 1421). This motif directs the cellular machinery to cleave the transcript and attach a protective poly-A tail at the extreme 3' end, ensuring the mRNA remains stable, avoids degradation, and is properly exported from the nucleus.

We also aligned *JcGSNOR* cDNA sequence against the *Jatropha curcas* reference genome using the NCBI BLAST tool, as shown in Supplementary Fig. 3. The figure displays the resulting NCBI Sequence Viewer output, illustrating the mapping of the cDNA query onto the genomic scaffold NW\_023591767.1. The upper "Genes" track displays the annotated reference gene model for LOC105644457, where the solid green boxes denote exons and the connecting lines indicate introns. The in silico result tracks the alignment of the input of *JcGSNOR* cDNA (gray blocks), which perfectly spans the predicted exon boundaries of the reference genome model.

*JcGSNOR* enzyme, a member of the Class III zinc-dependent alcohol dehydrogenase family, is highly conserved, with specialized domains essential for enzymatic activity. These include a catalytic zinc-binding motif (AA99, 120, 105, snf 113) that creates an active site to form a secondary structure for zinc-binding (Pinhero et al. 1997). Additionally, the sequence features a classic Rossmann fold, which functions as an NAD-binding domain, allowing the enzyme to successfully dock with the essential cofactors necessary for electron transfer during the reduction process (Sowa and Lehtiö 2022).

Sequence analysis further revealed over 80% similarity to GSNORs from other plant species, confirming that GSNOR is highly conserved across species, as evidenced by alignment with GSNOR homologs in the NCBI database (Fig. 1 and Supplementary Table 4). We know GSNOR functions in priming SAR, such that we may be able to tune plant resistance of *J. curcas* to epidermal diseases on a large scale of cultivation by promoting the activity of GSNOR.

|           |                                          |     |           |                                           |     |
|-----------|------------------------------------------|-----|-----------|-------------------------------------------|-----|
| JcGSNOR   | MATCGQVITCKAAVAWEFNKPLVIEDVQVAPPQAGEVR   | 38  | JcGSNOR   | AEFGLGTVGLAVAEGAKAGASRIIGIDIDSKKFARAKDF   | 238 |
| AtGSNOR   | MATCGQVITCKAAVAWEFNKPLVIEDVQVAPPQAGEVR   | 38  | AtGSNOR   | AEFGLGTVGLAVAEGAKAGASRIIGIDIDSKKYETAKKF   | 238 |
| GmGSNOR   | MATCGQVITCKAAVAWEFNKPLTVQEVQVAPPQAGEVR   | 38  | GmGSNOR   | AEFGLGTVGLAVAEGAKAGASRVIGIDIDSKKFDVAKNF   | 238 |
| LuGSNOR   |                                          | 0   | LuGSNOR   | AEFGLGTVGLAVAEGAKAGASRIIGIDIDSKKFDIAKNF   | 149 |
| MtGSNOR   | MASSTFGQVITCKAAVAWEFNKPLTIEDVEVAPPQANEVR | 40  | MtGSNOR   | AEFGLGTVGLAVAEGAKAGASRIIGIDIDSKKFDIAKNF   | 240 |
| NaGSNRO   | ENKPLVIEDVQVAPPQAGEVR                    | 22  | NaGSNRO   | AEFGLGTVGLAVAEGAKAGASRIIGIDIDSKKFDIAKNF   | 222 |
| PtGSNOR   | MATCGQVITCKAAVAWEFNKPLVIEDVQVAPPQAGEVR   | 38  | PtGSNOR   | AEFGLGTVGLAVAEGAKAGASRIIGIDIDSKKFDIAKNF   | 238 |
| RcGSNOR   | MATCGQVITCKAAVAWEFNKPLVIEDVQVAPPQAGEVR   | 38  | RcGSNOR   | AEFGLGTVGLAVAEGAKAGASRIIGIDIDSKKFDIAKNF   | 238 |
| SlGSNOR   | MATCGQVITCKAAVAWEFNKPLVIEDVQVAPPQAGEVR   | 38  | SlGSNOR   | AEFGLGTVGLAVAEGAKAGASRIIGIDIDSKKFDIAKNF   | 238 |
| ZmGSNOR   | MASFTCGQVITCKAAVAWEFNKPLVIEDVQVAPPQAGEVR | 40  | ZmGSNOR   | AEFGLGTVGLAVAEGAKAGASRIIGIDIDSKKFDIAKNF   | 240 |
| Consensus |                                          |     | Consensus | a fglgtvglavaegak agasr                   |     |
| JcGSNOR   | IQLIFTALCHTDAYTWSGKDAEGLFPCILGHEAAGIVESV | 78  | JcGSNOR   | GVTEFVNPKDHDKFIQQVIV.LTDGGVDYSFECIGNVSVM  | 277 |
| AtGSNOR   | IKILYIALCHTDAYTWSGKDFEGLFPCILGHEAAGIVESV | 78  | AtGSNOR   | GVNEFVNPKDHDKFIQEVIV.LTDGGVDYSFECIGNVSVM  | 277 |
| GmGSNOR   | VQILFTALCHTDAYTWSGKDFEGLFPCILGHEAAGIVESV | 78  | GmGSNOR   | GVTEFINPNEHDKFIQQVII.RTDGGVDYSFECIGNVSVM  | 277 |
| LuGSNOR   |                                          | 5   | LuGSNOR   |                                           |     |
| MtGSNOR   | IQLIFTALCHTDAYTWSGKDFEGLFPCILGHEAAGIVESV | 80  | MtGSNOR   | GVTEFINPKDHEKFIQQVSD.LTDGGVDYSFECIGNVSVM  | 279 |
| NaGSNRO   | VKVLYIALCHTDAYTWSGKDFEGLFPCVLGHEAAGIVESV | 62  | NaGSNRO   | GVTEFINPKHEKFIQQVVDLTDGGVDYSFECIGNVSVM    | 261 |
| PtGSNOR   | VKILFAALCHTDAYTWSGKDFEGLFPCILGHEAAGIVESV | 78  | PtGSNOR   | GVTEFVNPKDHDKFIQQVLIDTDGGVDYSFECIGNVSVM   | 278 |
| RcGSNOR   | VQILFTALCHTDAYTWSGKDFEGLFPCILGHEAAGIVESV | 78  | RcGSNOR   | GVTEFVNPKDHDKFIQQVIV.LTDGGVDYSFECIGNVSVM  | 277 |
| SlGSNOR   | VKVLYIALCHTDAYTWSGKDFEGLFPCVLGHEAAGIVESV | 78  | SlGSNOR   | GVTEFINPKHEKFIQQVIV.LTDGGVDYSFECIGNVSVM   | 277 |
| ZmGSNOR   | VKILFTALCHTDHYTWSGKDFEGLFPCILGHEAAGIVESV | 80  | ZmGSNOR   | GVTEFVNPKEDKFIQQVVD.LTDGGVDYSFECIGNVSIM   | 279 |
| Consensus |                                          |     | Consensus |                                           |     |
| JcGSNOR   | GEGVTBVQEGDHVIPCQAECCECKECSGKTNLCGKVRL   | 118 | JcGSNOR   | RSALECCHKGWGTISVIVGVAASGQEISTREFQLVTGRVWK | 317 |
| AtGSNOR   | GEGVTBVQEGDHVIPCQAECCECKECSGKTNLCGKVRS   | 118 | AtGSNOR   | RAALECCHKGWGTISVIVGVAASGQEISTREFQLVTGRVWK | 317 |
| GmGSNOR   | GEGVTBVQEGDHVIPCQAECCECKECSGKTNLCGKVRS   | 118 | GmGSNOR   | RAALECCHKGWGTISVIVGVAASGQEISTREFQLVSGRVWK | 317 |
| LuGSNOR   | GEGVTBVQEGDHVIPCQAECCECKECSGKTNLCGKVRA   | 45  | LuGSNOR   |                                           |     |
| MtGSNOR   | GEGVTBVQEGDHVIPCQAECCECKECSGKTNLCGKVRA   | 120 | MtGSNOR   | RSALECCHKGWGTISVVVGVAASGQEISTREFQLVTGRVWK | 319 |
| NaGSNRO   | GEGVTBVQEGDHVIPCQAECCECKECSGKTNLCGKVRA   | 102 | NaGSNRO   | RAALECCHKGWGTISVIVGVAASGQEISTREFQLVTGRVWK | 301 |
| PtGSNOR   | GEGVTBVQEGDHVIPCQAECCECKECSGKTNLCGKVRL   | 118 | PtGSNOR   | RAALECCHKGWGTISVIVGVAASGQEISTREFQLVTGRVWK | 318 |
| RcGSNOR   | GEGVTBVQEGDHVIPCQAECCECKECSGKTNLCGKVRA   | 118 | RcGSNOR   | RSALECCHKGWGTISVIVGVAASGQEISTREFQLVTGRVWK | 317 |
| SlGSNOR   | GEGVTBVQEGDHVIPCQAECCECKECSGKTNLCGKVRA   | 118 | SlGSNOR   | RSALECCHKGWGTISVIVGVAASGQEISTREFQLVTGRVWK | 317 |
| ZmGSNOR   | GEGVTBVQEGDHVIPCQAECCECKECSGKTNLCGKVRS   | 120 | ZmGSNOR   | RAALECCHKGWGTISVIVGVAASGQEISTREFQLVTGRVWK | 319 |
| Consensus | gegvt v gdhvipcyqaec ck csgktnlcgkvr     |     | Consensus |                                           |     |
| JcGSNOR   | ATGCGMLSDRKSRSFSGNGEYHFMGTSTFSQYTVVHVDV  | 158 | JcGSNOR   | GTAFFGFKSRSQVPWLVDKYLKKEIKVDEYITHNLTLGEI  | 357 |
| AtGSNOR   | ATGCGMLSDRKSRSFSGNGEYHFMGTSTFSQYTVVHVDV  | 158 | AtGSNOR   | GTAFFGFKSRTQVPWLVERKYMKEIKVDEYITHNLTLGEI  | 357 |
| GmGSNOR   | ATGCGMLSDRKSRSFSGNGEYHFMGTSTFSQYTVVHVDV  | 158 | GmGSNOR   | GTAFFGFKSRSQVPWLVDKYLKKEIKVDEYITHNLTLSEI  | 357 |
| LuGSNOR   | ATGCGMLSDRKSRSFSGNGEYHFMGTSTFSQYTVVHVDV  | 85  | LuGSNOR   |                                           |     |
| MtGSNOR   | ATGCGMLSDRKSRSFSGNGEYHFMGTSTFSQYTVVHVDV  | 160 | MtGSNOR   | GTAFFGFKSRSQVPWLVERKYLKKEIKVDEYITHNLTLAEI | 359 |
| NaGSNRO   | ATGCGMLSDRKSRSFSGNGEYHFMGTSTFSQYTVVHVDV  | 142 | NaGSNRO   | GTAFFGFKSRSQVPWLVDKY                      | 321 |
| PtGSNOR   | ATGCGMLSDRKSRSFSGNGEYHFMGTSTFSQYTVVHVDV  | 158 | PtGSNOR   | GTAFFGFKSRSQVPWLVDKYMKEIKVDEYITHNLTLPEM   | 358 |
| RcGSNOR   | ATGCGMLSDRKSRSFSGNGEYHFMGTSTFSQYTVVHVDV  | 158 | RcGSNOR   | GTAFFGFKSRSQVPWLVDKYMKEIKVDEYITHNLTLVEI   | 357 |
| SlGSNOR   | ATGCGMLSDRKSRSFSGNGEYHFMGTSTFSQYTVVHVDV  | 158 | SlGSNOR   | GTAFFGFKSRSQVPWLVDKYLKKEIKVDEYITHNLTLADI  | 357 |
| ZmGSNOR   | ATGCGMLSDRKSRSFSGNGEYHFMGTSTFSQYTVVHVDV  | 160 | ZmGSNOR   | GTAFFGFKSRTQVPWLVDKYMKEIKVDEYITHNLTLADI   | 359 |
| Consensus | atg g m d rfs gk iyhfmgtstfsqyvvhdv      |     | Consensus |                                           |     |
| JcGSNOR   | SVAKIDPFAELDKVCLLGCGVETGLGAVWNTAKVEGSSIV | 198 | JcGSNOR   | NKAFDLMHEGSCIRCVLDA                       | 377 |
| AtGSNOR   | SVAKIDPFAELDKVCLLGCGVETGLGAVWNTAKVEGSSIV | 198 | AtGSNOR   | NKAFDLLHEGTCIRCVLDT                       | 377 |
| GmGSNOR   | SVAKIDPFAELDKVCLLGCGVETGLGAVWNTAKVEGSSIV | 198 | GmGSNOR   | NKAFDLLHEGGCIRCVLSTQ                      | 377 |
| LuGSNOR   | SVAKIDPFAELDKVCLLGCGVETGLGAVWNTAKVEGSSIV | 125 | LuGSNOR   |                                           |     |
| MtGSNOR   | SVAKIDPFAELDKVCLLGCGVETGLGAVWNTAKVEGSSIV | 200 | MtGSNOR   | NEAFDLMHEGKCLRCVLALH                      | 379 |
| NaGSNRO   | SVAKIDPFAELDKVCLLGCGVETGLGAVWNTAKVEGSSIV | 182 | NaGSNRO   |                                           |     |
| PtGSNOR   | SVAKIDPFAELDKVCLLGCGVETGLGAVWNTAKVEGSSIV | 198 | PtGSNOR   | NQAFDLMHEGSCIRCVLDMQ                      | 378 |
| RcGSNOR   | SVAKIDPFAELDKVCLLGCGVETGLGAVWNTAKVEGSSIV | 198 | RcGSNOR   | NKAFDLMHEGDCIRCVLVSH                      | 377 |
| SlGSNOR   | SVAKIDPFAELDKVCLLGCGVETGLGAVWNTAKVEGSSIV | 198 | SlGSNOR   | NKAFDLMHGDCLRVVDMF                        | 377 |
| ZmGSNOR   | SVAKIDPFAELDKVCLLGCGVETGLGAVWNTAKVEGSSIV | 198 | ZmGSNOR   | NDAFDLLHEGGCIRCVLAMQ                      | 379 |
| Consensus | svaki p a l kvcllgcgv tglgavwntakve gs v |     | Consensus |                                           |     |

**Fig. 1** Multiple alignment of the deduced amino acid sequences of *JcGSNOR* protein and GSNOR proteins from other species. Conserved amino acid sequences are boxed in black shadow. Similar amino acid sequences are shaded in pink, and no color indicates low conservation

To elucidate the evolutionary relationship of *JcGSNOR* with other plants, a phylogenetic tree was constructed using GSNOR protein sequences from *Jatropha curcas* and seven diverse dicotyledonous species. The phylogenetic analysis reveals that these plant GSNORs segregate into distinct clades that align closely with their established botanical lineages. As anticipated, *JcGSNOR* shares the highest evolutionary proximity with the ortholog from *Ricinus communis*

(castor bean), clustering together as sister taxa. This tight grouping is consistent with their shared taxonomy within the Euphorbiaceae family. This Euphorbiaceae clade forms a broader group with *Populus trichocarpa* and *Linum usitatissimum*, diverging earlier from the well-characterized *Arabidopsis thaliana* ortholog. Furthermore, the tree demonstrates that *JcGSNOR* is more evolutionarily distant from GSNORs found in the Fabaceae family (*Glycine max* and

*Medicago truncatula*) and the Solanaceae family (*Nicotiana attenuata*). This phylogenetic positioning strongly supports the conserved evolutionary trajectory of *JcGSNOR* among woody, oil-producing dicots (Fig. 2).

### Expression Level of *JcGSNOR* in Different Organs of *J. curcas* Was Quantified by qRT-PCR

To understand the role *JcGSNOR* plays in the growth of *J. curcas*, the mRNA expression of *JcGSNOR* in different organs was quantified by qRT-PCR, as shown in Fig. 3. According to Figure, the expression of *JcGSNOR* was observed in all organs of *J. curcas*. The average mRNA level of *JcGSNOR* was the highest in seed (1.8), the lowest in leaf (0.6), and medium in root (1) and stem (1.1). The seed exhibited significantly higher levels of *JcGSNOR* compared to the leaf, root, and stem ( $p < 0.05$ ).

We attribute this to the need to remove excessive NO, which is associated with germination (Medvedev et al. 2019), and seeds need to be more resistant to pathogens and allelopathy<sup>18</sup>. The pronounced expression of *JcGSNOR* in *Jatropha curcas* seeds likely functions to enforce and maintain dormancy by continually degrading GSNO, thereby preventing the premature accumulation of bioactive NO that would trigger precocious germination (Medvedev et al. 2019). The unique biological composition of *Jatropha curcas* seeds, rich in lipid reserves and high in oil content (Zhang et al. 2023), necessitates highly efficient biochemical protection. During the late stages of seed maturation and the severe cellular dehydration that accompanies deep desiccation, seeds naturally experience intense oxidative and nitrosative stress. If left unregulated, this burst of reactive nitrogen species would initiate severe lipid peroxidation, degrade the seed's essential oil reserves and causing irreversible macromolecular damage to the embryo (Jonas et al. 2020), (Ciacka et al. 2020). Therefore, the robust and constitutive activity of *JcGSNOR* serves as a critical detoxification mechanism. By rapidly clearing cytotoxic reactive nitrogen species and

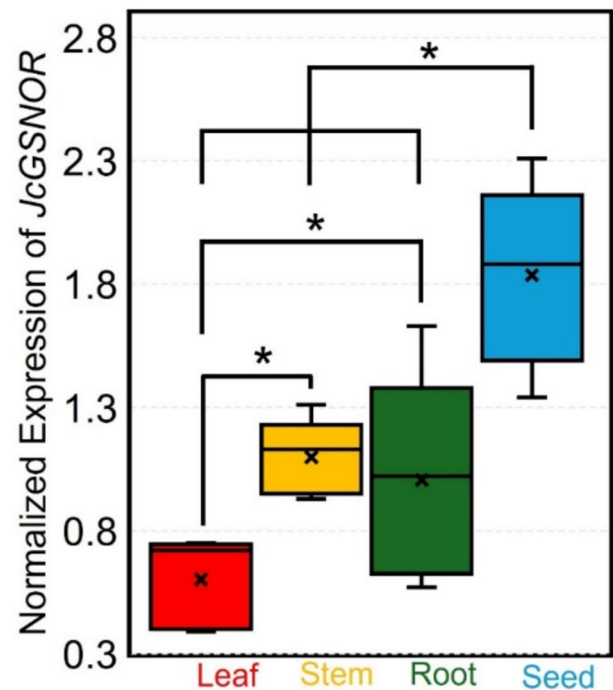
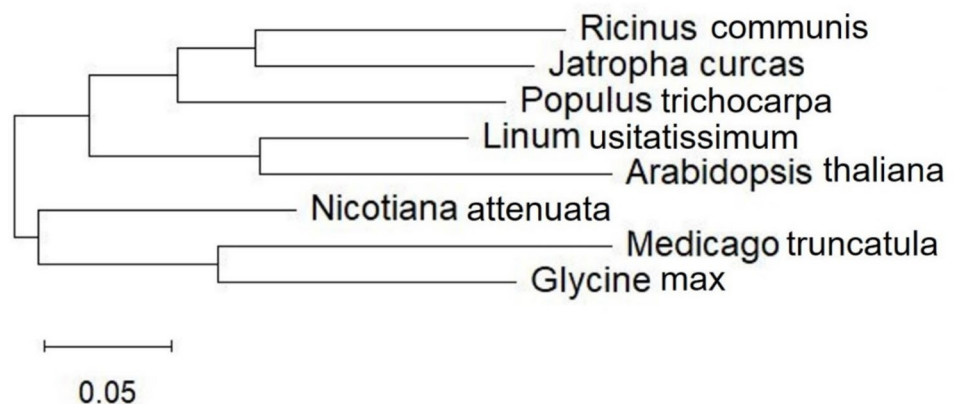


**Fig. 3** Expression levels of *JcGSNOR* in different organs of *J. curcas*. The seed exhibited significantly higher levels of *JcGSNOR* compared to the leaf, root, and stem. The leaf showed significantly lower levels of *JcGSNOR* than both the stem and root. However, the difference between the root and stem was not statistically significant. x marker represents mean, \* marker represents  $p < 0.05$ , and  $n = 5$

maintaining overall nitrosative homeostasis, *JcGSNOR* acts as a molecular shield, protecting the dense lipid reserves from degradation and preserving the viability of the embryo throughout the harsh desiccation phase. While our current data establishes the spatial dominance of *JcGSNOR* in seed tissues, future investigations are needed for the temporal profiling of *JcGSNOR* expression across distinct seed developmental stages to precisely delineate its regulatory role in dormancy and metabolic protection. By contrast, the leaf

**Fig. 2** Phylogenetic tree of plant GSNOR proteins show the evolutionary history was inferred using the GSNOR amino acid sequences from *Jatropha curcas* and seven representative dicotyledonous species



showed significantly lower levels of *JcGSNOR* than both the stem and root ( $p < 0.05$ ). Leaves are more susceptible to pathogen attacks and stressors compared to other plant organs due to their large surface area and constant exposure to environmental stressors. As a result, the concentration of plant defense markers such as azelaic acid, SA, and JA will increase. These metabolites could inhibit the synthesis of GSNOR (Reinrothe et al. 1993). However, the difference between the root and stem was not statistically significant ( $p > 0.05$ ). Our findings are in line with what has been found in *Arabidopsis* (Xing et al. 2025; Li et al. 2019; Martinez et al. 1996). Because *J. curcas* and *Arabidopsis* are not closely related but show similar expression of GSNOR, this strengthens that GSNOR functions in priming SAR across the species.

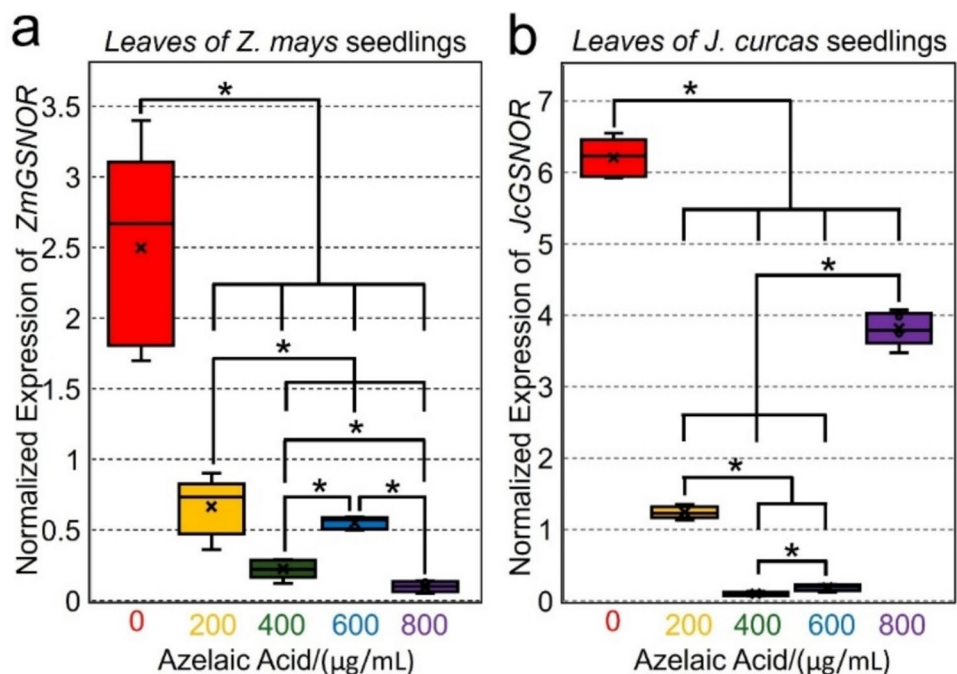
### Impact of Azelaic Acid on the Expression Level of GSNOR in Seedlings of *Z. mays* and *J. curcas* Was Quantified by qRT-PCR

To further understand the role of GSNOR in SAR triggered by azelaic acid, we investigated the transcriptional regulation of GSNOR under azelaic acid in two plant species: *Z. mays* and *J. curcas*. GSNOR has been previously implicated in priming SAR, and suppression of GSNOR may serve as a trigger for activating downstream immune pathways (Ventimiglia and Mutus 2020). Our interest in GSNOR's behavior under azelaic acid treatment is further supported by reports in other species. For instance, in *N. attenuata*, exogenous JA was shown to reduce GSNOR transcription (Jahnova et al. 2019), while in *Arabidopsis*, SA treatment

led to a dramatic increase in GSNOR expression (Espunya et al. 2012b). Our research stemmed from the observation that leaves exhibited the lowest GSNOR expression, suggesting SAR responding to environmental stress. Since GSNOR is known to modulate NO signaling and redox balance, its expression under azelaic acid treatment could reveal important insights into plant defense mechanisms, which are in line with findings by Deepak et al. and Rudolf et al. (Hurali 2022), (Rudolf et al. 2021)

Seedlings of *Z. mays* and *J. curcas* were treated with varying concentrations of azelaic acid (0, 200, 400, 600, and 800  $\mu\text{g/mL}$ ), and the transcriptional levels of *ZmGSNOR* and *JcGSNOR* were analyzed in leaf tissues. The results, illustrated in Fig. 4, show distinct expression patterns between the two species. In *Z. mays*, *ZmGSNOR* expression was highest in the control groups and progressively declined with increasing azelaic acid concentrations. In contrast, *J. curcas* displayed a more complex and nonlinear response. *JcGSNOR* expression decreased at intermediate concentrations (200–600  $\mu\text{g/mL}$ ) but rebounded sharply at 800  $\mu\text{g/mL}$ , forming a biphasic pattern. The reason for the divergent expression patterns of *JcGSNOR* and *ZmGSNOR* at 800  $\mu\text{g/mL}$  is unclear. We believe the difference may be due to varying tolerance levels to exogenous azelaic acid and intrinsic SA accumulation between the two species. *Z. mays* may be more susceptible to prolonged suppression. This dose-dependent suppression suggests that azelaic acid negatively regulates *ZmGSNOR* transcription in maize, potentially leading to altered NO homeostasis and reduced redox buffering capacity. The downward trend indicates a robust inhibitory effect of azelaic acid on *ZmGSNOR* expression,

**Fig. 4** qRT-PCR analysis of GSNOR expression in leaves of *Z. mays* and *J. curcas* in response to azelaic acid was analyzed



thus leading to the accumulation of GSNO. Elevated GSNO levels enhance NO signaling, which in turn promotes the accumulation of SA, a key hormone in SAR. This amplification of SA signaling strengthens plant defense responses. *J. curcas* may possess a more dynamic or resilient response to azelaic acid-induced stress. The rebound of GSNOR expression at 800  $\mu\text{g/mL}$  may reflect a compensatory mechanism or stress-induced activation of GSNOR at higher azelaic acid levels and is consistent with the SA-induced upregulation observed in *Arabidopsis* (Wang et al. 2014). The late-stage increase in GSNOR may be driven by SA accumulation resulting from azelaic acid treatment. As a result, GSNOR likely plays a dual role in the NO signaling pathway. Initially, GSNOR protects against oxidative stress by detoxifying reactive nitrogen species and prevents excessive SNO of intracellular thiol compounds. Then, compensative expression of GSNOR ensures that GSNO levels remain within a non-toxic range, especially under conditions of elevated NO production. To conclude, *J. curcas* may possess a higher threshold for azelaic acid-induced stress or a more efficient feedback mechanism to restore GSNOR expression. Our findings underscore the complexity of GSNOR regulation and its central role in coordinating plant defense responses through NO and SA signaling pathways.

### GSNOR Transfection Does Not Impact Cell Viability of *S. cerevisiae*

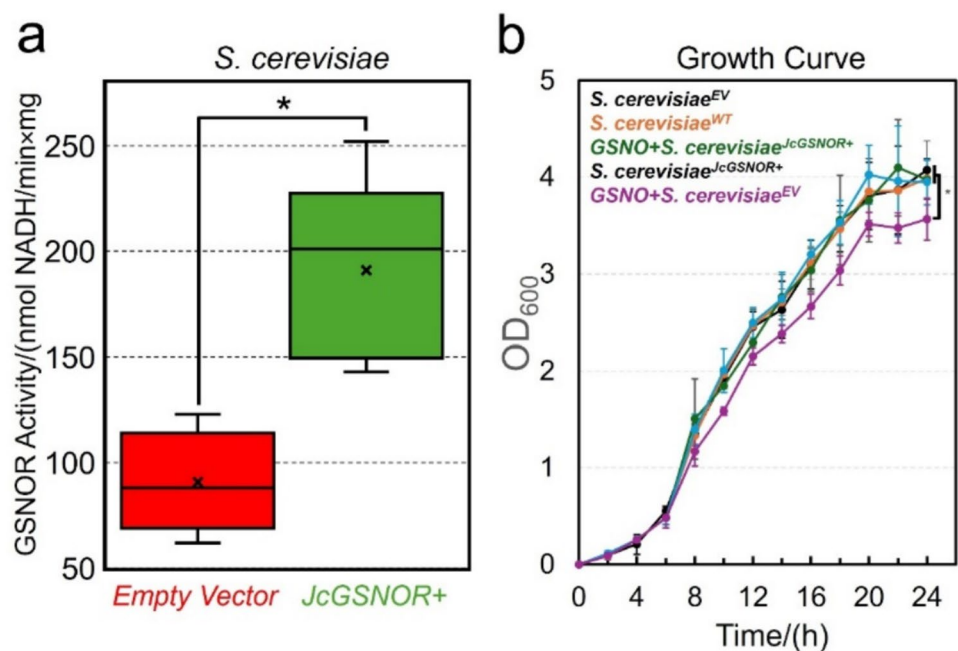
To further determine whether the *GSNOR* transfection would impact seed survivability, we first validated that the transfection of *GSNOR* in the model strain *S. cerevisiae* does

not impair cell viability. After the gene encoding *JcGSNOR* was successfully cloned into *S. cerevisiae*, the expression of *JcGSNOR* at the protein level was validated by the GSNOR activity assay. Our results shown in Fig. 5A demonstrate that GSNOR activity of *S. cerevisiae*<sup>*JcGSNOR*+</sup> has been elevated significantly to 182.6% compared to that of empty vector infected *S. cerevisiae*<sup>WT</sup>, which means *JcGSNOR* was successfully cloned and expressed at the protein level. We opted to evaluate the actual elevated function rather than the mRNA expression value because functional analysis demonstrates the actual activity of the *JcGSNOR* gene and its impact on metabolic pathways or stress responses. Next, we strive to understand how the transfection of *JcGSNOR* in *S. cerevisiae* would impact cell viability. Figure 5b shows the growth curve of *S. cerevisiae*<sup>*JcGSNOR*+</sup> and *S. cerevisiae*<sup>WT</sup>. *S. cerevisiae*<sup>*JcGSNOR*+</sup> with and without GSNO shows a similar level of growth curve to *S. cerevisiae*<sup>WT</sup> without GSNO supplement. This is likely due to the elevated *JcGSNOR* expression in *S. cerevisiae*<sup>*JcGSNOR*+</sup> accelerated the GSNO reduction, thus inhibiting toxicity caused by excessive GSNO because of the imbalance of GSNO metabolism in *S. cerevisiae*<sup>WT</sup> with GSNO supplements. Therefore, our data confirms that GSNOR is not the pivotal enzyme for regulating the growth of *S. cerevisiae*, indicating the cloning of *JcGSNOR* in *Arabidopsis* will not inhibit their growth rate.

### GSNOR Transfection

Azelaic acid not only has been identified as the allelopathy chemical of *J. curcas* by our previous study but also has been reported as a component of plant systemic immunity

**Fig. 5** **a** GSNOR activity of *S. cerevisiae* was examined. *S. cerevisiae*<sup>*JcGSNOR*+</sup> strain showed significantly higher GSNOR activity than empty vector-transfected *S. cerevisiae*, i.e., *S. cerevisiae*<sup>EV</sup>. **b** The growth curve of different *S. cerevisiae* strains with and without the addition of a supplement of GSNO. \* means  $p < 0.05$



involved in resistance to bacterial and fungal pathogens (Jung et al. 2009). However, the answer to how azelaic acid regulates plant defense is still not fully understood. To further investigate the function of azelaic acid in plant defense, we need to pinpoint the relationship between azelaic acid and GSNOR. Based on the previous dose response results, we induce the SAR in the concentration of 600  $\mu\text{g/mL}$ , which is approximately 3.2 mM.

First, *JcGSNOR* was transformed into the *GSNOR* mutant of *Arabidopsis*<sup>Mutant</sup>. Then, seeds of *Arabidopsis*<sup>Mutant</sup>, *Arabidopsis*<sup>WT</sup>, and *Arabidopsis*<sup>JcGSNOR+</sup> were treated with and without 600  $\mu\text{g/mL}$  (3.2 mM) of azelaic acid. We first confirmed that the empty vector does not impact the germination rate, as shown in Fig. 6a. These findings are in line with what we observed in the previous sections, that *JcGSNOR* transfection does not impair cellular growth at the cellular level using model strain *S. cerevisiae*. Then, Figs. 6b–d show the germination rate of *Arabidopsis*<sup>Mutant</sup>, *Arabidopsis*<sup>WT</sup>, and *Arabidopsis*<sup>JcGSNOR+</sup> were 96%, 84%, and 76%, respectively, without azelaic acid treatment. After exposure to 600  $\mu\text{g/mL}$  (3.2 mM) of azelaic acid, the germination rate of seeds of *Arabidopsis*<sup>Mutant</sup>, *Arabidopsis*<sup>WT</sup>, and *Arabidopsis*<sup>JcGSNOR+</sup> were 84%, 52%, and 56%, respectively. A decline of 12%, 32%, and 20% was observed. Smaller decline in germination after exposure to azelaic acid was observed in *Arabidopsis*<sup>JcGSNOR+</sup> and *Arabidopsis*<sup>Mutant</sup>.

MDA content is widely recognized as a reliable biomarker for oxidative stress because it is a major end product of lipid peroxidation. When reactive oxygen species attack polyunsaturated fatty acids in cell membranes, MDA is

generated, reflecting the extent of oxidative damage. While GSNOR regulates NO signaling by metabolizing GSNO. When GSNOR expression is suppressed, GSNO accumulates, leading to enhanced NO signaling, SAR, and increased production of reactive oxygen species. As a result of that, MDA levels increase. Conversely, when GSNOR activity is restored or upregulated, GSNO is decomposed, reducing excessive NO signaling and helping to limit oxidative damage, which in turn lowers MDA accumulation. Thus, we investigated how MDA content fluctuates in GSNOR-transformed seedlings to azelaic acid. In Fig. 7a, the MDA content of *Arabidopsis*<sup>Mutant</sup>, *Arabidopsis*<sup>WT</sup>, and *Arabidopsis*<sup>JcGSNOR+</sup> was 4.2, 6.28, and 5.74 nmol/min  $\times$  gFW, respectively. After exposure to 600  $\mu\text{g/mL}$  (3.2 mM) of azelaic acid, the MDA content of *Arabidopsis*<sup>Mutant</sup>, *Arabidopsis*<sup>WT</sup>, and *Arabidopsis*<sup>JcGSNOR+</sup> was 5.84, 7.57, and 8.12 nmol/min  $\times$  gFW, respectively. An increase of 35.6%, 42%, and 72.7%, respectively, was observed, as shown in Figs. 7b–d. Additionally, Fig. 7e shows *Arabidopsis*<sup>JcGSNOR+</sup> accumulated more MDA content than *Arabidopsis*<sup>Mutant</sup> and *Arabidopsis*<sup>WT</sup>. Under the exposure of azelaic acid, indicating better tolerance for environmental stimuli.

Our data demonstrate that all azelaic acid treated samples exhibited reduced germination rates and elevated MDA levels compared with untreated controls, indicating enhanced oxidative stress. Interestingly, *Arabidopsis*<sup>JcGSNOR+</sup> appears to confer greater tolerance to exogenous pressure. When GSNO or NO concentrations are elevated in *J. curcas*, GSNOR expression is upregulated, thereby strengthening its enzymatic activity. This enhanced activity helps regulate

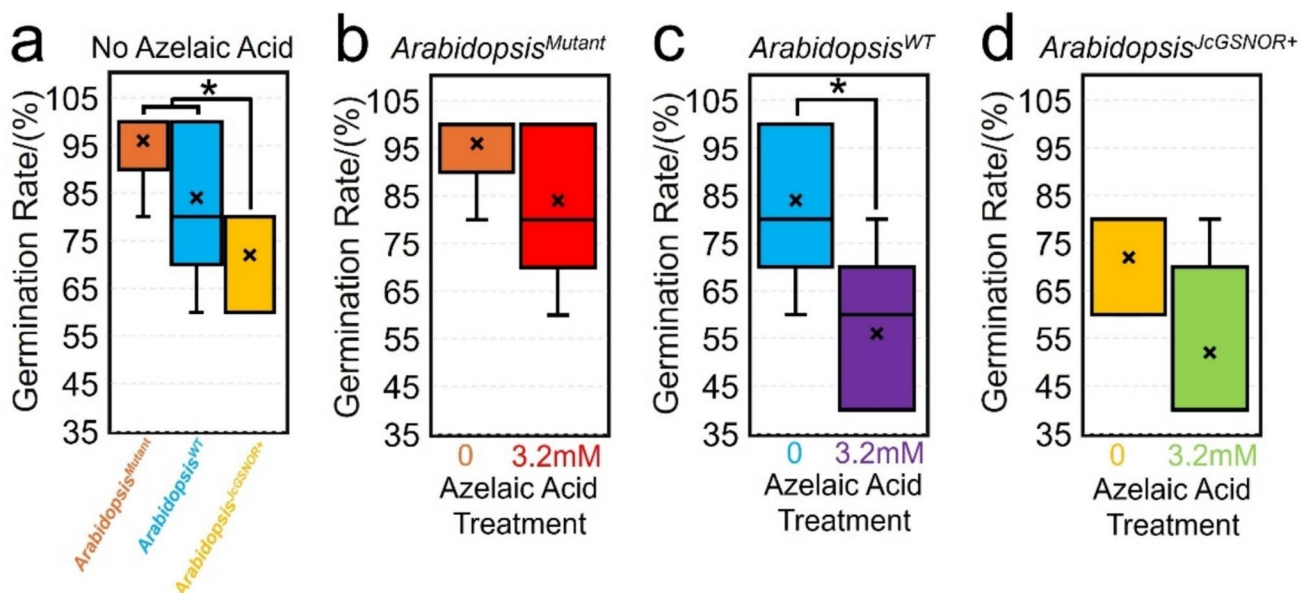


**Fig. 6** Germination of *Arabidopsis* was investigated in *Arabidopsis*<sup>Mutant</sup>, *Arabidopsis*<sup>WT</sup>, and *Arabidopsis*<sup>JcGSNOR+</sup> with and without 600  $\mu\text{g/mL}$  (3.2 mM) of azelaic acid treatment for \* means  $p < 0.05$

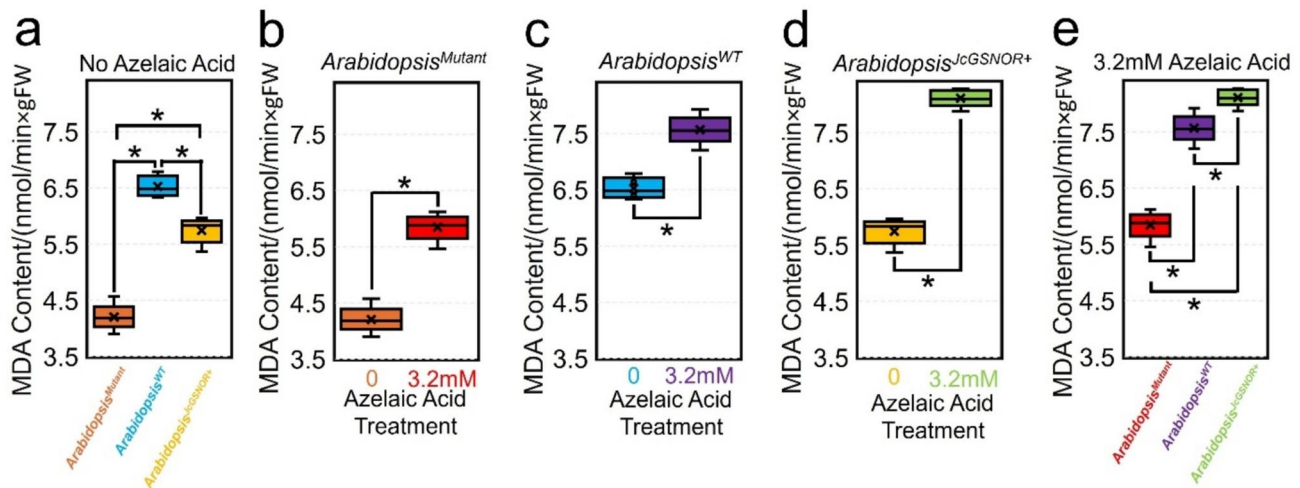


**Fig. 7** MDA content of *Arabidopsis* was investigated in *Arabidopsis*<sup>Mutant</sup>, *Arabidopsis*<sup>WT</sup>, and *Arabidopsis*<sup>JcGSNOR+</sup>, with and without 600 µg/mL (3.2 mM) of azelaic acid treatment. \* means  $p < 0.05$

NO and SNO at higher concentrations, protecting cells from nitrosative stress. Therefore, we believe that GSNOR functions not only as a negative regulator of inflammatory responses, safeguarding plants against nitrosative stress and tissue injury (Liu et al. 2004), but also as a positive regulator of early immune function that suppresses pathogen growth (Feechan et al. 2005). Consistent with this dual role, *Arabidopsis*<sup>Mutant</sup> seedlings displayed greater tolerance during early growth stages compared with *Arabidopsis*<sup>WT</sup>. These findings align with our results, which showed that GSNOR protects against oxidative damage while preventing excessive SNO of intracellular thiol compounds. Furthermore, *Arabidopsis*<sup>JcGSNOR+</sup> exhibited improved tolerance under azelaic acid treatment compared with both mutant and wild-type plants. Notably, *Arabidopsis*<sup>Mutant</sup> mutants also showed enhanced tolerance to environmental stress, suggesting the involvement of compensatory pathways, in agreement with published reports (Feechan et al. 2005).

## Conclusion

To summarize, GSNOR serves as a critical regulator of GSNO and NO metabolism in plant cells, playing a central role in maintaining redox balance and signaling during stress responses. Our findings indicate that exogenous azelaic acid at concentrations below 600 µg/mL leads to a significant downregulation of GSNOR expression. This suppression suggests that azelaic acid modulates NO signaling by altering GSNO turnover, thereby influencing defense activation. In *J. curcas*, *JcGSNOR* is expressed across all plant organs and demonstrates stronger tolerance to exogenous stress compared with homologous enzymes previously

characterized in model plants, such as *Arabidopsis* and *Solanum lycopersicum* (Lee et al. 2008), (Gong 2015). This broad expression pattern highlights its potential application in enhancing plant resistance against pathogens, diseases, and environmental challenges. Importantly, the ability of *JcGSNOR* to maintain activity under stress conditions suggests that it may contribute to both protection against nitrosative damage and regulation of immune signaling.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11105-026-01706-6>.

**Author Contribution** Q.C. conceptualized the project, analyzed data, and drafted the manuscript. Y.M. designed and performed the experiments, collected and analyzed data, and drafted the manuscript.

**Data Availability** Data is available via email request to the corresponding author.

## Declarations

**Conflict of Interest** The authors declare no competing interests.

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