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Peng Xu , Zheyi Zhou , Yiguo Li , Chunyan Zhou , [Hongjuan Nian](#) , [Yuanshuang Wu](#) * , [Xing Zhao](#) * , [Kunzhi Li](#) *

Posted Date: 20 January 2026

doi: 10.20944/preprints202601.1483.v1

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Article

Overexpression of the Plant Manganese Superoxide Dismutase Gene *GeSOD7* Enhances Cold Stress Tolerance in *Armillaria mellea*

Peng Xu, Zheyi Zhou, Yiguo Li, Chunyan Zhou, Hongjuan Nian, Yuanshuang Wu *, Xing Zhao * and Kunzhi Li *

Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, Yunnan, China

* Correspondence: wuys@kust.edu.cn (Y.W.); zhaoxing328328@163.com (X.Z.); likzkm@163.com (K.L.)

Abstract

Background: Fungal symbionts are critical for host plant survival but are vulnerable to abiotic stresses such as low temperature, which limits their agricultural utility. *Armillaria mellea* is an essential fungal partner for the cultivation of the valuable medicinal orchid *Gastrodia elata* (*G. elata*). Enhancing its cold tolerance is a key step toward stabilizing *G. elata* production. **Methods:** Based on *G. elata* transcriptome data, a manganese superoxide dismutase gene (*GeSOD7*) was identified and heterologously expressed in *Escherichia coli* for enzymatic characterization. The gene was then overexpressed in *A. mellea* via *Agrobacterium tumefaciens*-mediated transformation. Transgenic and wild-type strains were subjected to cold stress (13 °C for 45 days), after which physiological, biochemical, and molecular responses were analyzed. **Results:** Recombinant *GeSOD7* showed optimal activity at pH 6.0 and 60 °C, with inhibition under high concentrations of metal ions, especially Mn^{2+} and Cu^{2+} . Overexpression of *GeSOD7* in *A. mellea* significantly improved hyphal growth and fresh weight under cold stress. Transgenic strains exhibited higher activities of catalase and glutathione peroxidase, increased accumulation of glutathione and proline, and reduced levels of hydrogen peroxide and malondialdehyde. Expression of genes involved in glutathione synthesis and peroxide detoxification was coordinately upregulated. **Conclusions:** This study demonstrates that heterologous expression of a plant-derived Mn-SOD can effectively enhance the cold stress tolerance of a symbiotic fungus by boosting its enzymatic and non-enzymatic antioxidant systems. These findings provide a novel genetic strategy for improving stress resilience in agriculturally important fungi and contribute to the sustainable cultivation of *G. elata*.

Keywords: *Armillaria mellea*; superoxide dismutase (SOD); cold stress; antioxidant defense; fungal stress tolerance; symbiosis

1. Introduction

Fungal symbionts play indispensable roles in terrestrial ecosystems and agriculture, forming mutualistic, parasitic, or commensal relationships with a vast range of host plants [1]. Among these, the root-inhabiting fungus *Armillaria mellea* (*A. mellea*) is of particular ecological and economic importance as an obligate symbiont of the achlorophyllous orchid *Gastrodia elata* (*G. elata*), providing essential nutrients for its growth throughout a three-year life cycle [2,3]. The vigor and stress resilience of *A. mellea* directly determine the yield and quality of *G. elata*, a highly valued medicinal herb in traditional Chinese medicine [4,5]. However, like many fungi, *A. mellea* is highly sensitive to low-temperature stress, which frequently occurs during early spring and late autumn in its cultivation areas. This sensitivity constitutes a major bottleneck for the stable production of *G. elata* [6]. Therefore, enhancing the cold tolerance of this symbiotic fungus is an urgent agricultural objective.

Under abiotic stress, including chilling, cells experience an accelerated production of reactive oxygen species (ROS), leading to oxidative damage to lipids, proteins, and nucleic acids [7]. To mitigate such damage, organisms have evolved sophisticated antioxidant systems, in which superoxide dismutase (SOD) acts as the first line of defense by catalyzing the dismutation of superoxide anions ($O_2^{\bullet-}$) into hydrogen peroxide and oxygen [8,9]. Based on their metal cofactors, SODs are classified into Cu/Zn-SODs, Fe-SODs, Mn-SODs, and Ni-SODs [10,11]. In plants, the critical role of SODs in conferring tolerance to drought, salinity, and extreme temperatures has been well documented through transgenic approaches [12–14]. In contrast, the functional significance of SODs in fungal symbionts, particularly in the context of host-derived gene resources, remains largely unexplored. Investigating whether a plant-derived SOD can function within a fungal partner to enhance its stress resilience represents a novel frontier in symbiotic biology and applied mycology.

The *G. elata*–*A. mellea* system offers a unique model to address this question. *G. elata*, lacking photosynthetic capacity, has likely evolved robust molecular mechanisms to cope with environmental stress, potentially including an efficient antioxidant repertoire [15]. Recent transcriptomic studies of *G. elata* have enabled the genome-wide identification of gene families involved in stress responses [16]. Among these, the SOD gene family represents a key candidate for biotechnological exploitation to improve the stress tolerance of its fungal partner.

In this study, we identified 10 SOD genes from the *G. elata* transcriptome and focused on *GeSOD7*, a highly expressed mitochondrial *Mn*-SOD, for functional characterization. We hypothesized that heterologous expression of this plant-derived *GeSOD7* in *A. mellea* would enhance its antioxidant capacity and improve its tolerance to low-temperature stress. To test this, we (1) characterized the biochemical properties of the recombinant *GeSOD7* protein, (2) generated *GeSOD7*-overexpressing transgenic strains of *A. mellea*, and (3) evaluated their physiological, biochemical, and molecular responses under cold stress. Our findings demonstrate that *GeSOD7* overexpression significantly enhances cold tolerance in *A. mellea* by modulating antioxidant metabolism and redox homeostasis, providing a novel genetic strategy for engineering stress-resilient fungal symbionts to support sustainable cultivation of medicinal plants.

2. Materials and Methods

2.1. Plant and Fungal Materials

Tubers of *G. elata* f. *glauca* were collected from Zhaotong City, Yunnan Province, China. The *A. mellea* strain AM02, previously isolated and preserved in our laboratory, was used throughout this study. Fungal cultures were maintained on potato dextrose agar (PDA) at 23 °C in the dark.

2.2. Identification of the SOD Gene Family in *G. elata*

Hidden Markov models (HMMs) corresponding to SOD family domains (Pfam: PF00080, PF00081, PF02777) were retrieved from the Pfam database (<http://pfam.xfam.org/>). Candidate SOD genes were identified from the *G. elata* transcriptome using TBtools v2.0 [17]. Domain validation was performed using SMART (<http://smart.embl-heidelberg.de/>) and the NCBI Conserved Domain Database (CDD; <https://www.ncbi.nlm.nih.gov/cdd/>). Redundant sequences and those lacking complete SOD domains were excluded.

2.3. Bioinformatics Analysis

Amino acid sequences were analyzed using the ExPASy ProtParam tool (<https://web.expasy.org/protparam/>) to predict molecular weight, theoretical isoelectric point (pI), and grand average of hydropathicity (GRAVY). Subcellular localization was predicted using Euk-mPLoc 2.0 (<http://www.csbio.sjtu.edu.cn/bioinf/euk-multi-2/>). Conserved motifs were identified with MEME Suite v5.5.2 (<http://meme-suite.org/tools/meme>), with the maximum number of motifs set to 10.

2.4. Phylogenetic Analysis

Reference SOD protein sequences from *Arabidopsis thaliana* and *Dendrobium candidum* were downloaded from the NCBI protein database. Multiple sequence alignment was performed using ClustalW in MEGA-X [18]. A phylogenetic tree was constructed using the neighbor-joining method with 1000 bootstrap replicates.

2.5. Expression Profiling and Gene Selection

Transcriptomic expression data (FPKM values) of GeSOD genes were obtained from three tissue types: immature tubers at 13 °C (NS), immature tubers symbiotic with *A. mellea* at 23 °C (TB), and mature tubers (SS). Expression values were log₂-transformed, and a heatmap was generated using TBtools. GeSOD7 was selected for functional analysis based on its high expression and predicted mitochondrial localization.

2.6. Gene Cloning and Vector Construction

The coding sequence (CDS) of GeSOD7 was amplified from *G. elata* cDNA using gene-specific primers (Table S1). The purified PCR product was ligated into the pET-32a⁽⁺⁾ vector (Novagen) for prokaryotic expression. For fungal transformation, the GeSOD7 CDS was recombined into the Gateway[®]-compatible binary vector pH7WG2.0 using LR Clonase II (Invitrogen), generating pH7WG2.0-35S-GeSOD7. The construct was introduced into *A. tumefaciens* strain PMP90 via electroporation.

2.7. Recombinant Protein Expression and Purification

The recombinant plasmid pET-32a-GeSOD7 was transformed into *Escherichia coli* BL21(DE3). Protein expression was induced with 0.1–1.0 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 37 °C for 8 h. His-tagged GeSOD7 was purified under native conditions using Ni-NTA affinity chromatography (Qiagen), following the manufacturer's protocol. Protein purity was assessed by SDS-PAGE.

2.8. Enzyme Activity Assay and Biochemical Characterization

SOD activity was measured using the ABTS method [19]. The optimal temperature was determined by assaying activity between 30 °C and 90 °C. Thermostability was evaluated by pre-incubating the enzyme at 40–70 °C for 0–80 min before activity measurement. The optimal pH was determined in buffers ranging from pH 2.0 to 10.0. The effects of metal ions (Fe²⁺, Ca²⁺, Mg²⁺, Zn²⁺, K⁺, Na⁺, Mn²⁺, Cu²⁺) at 5 and 9 mM were also tested. All assays were performed in triplicate.

2.9. Genetic Transformation of *A. mellea*

A. tumefaciens PMP90 harboring pH7WG2.0-35S-GeSOD7 was co-cultured with wild-type *A. mellea* mycelia on induction medium (IM) at 25 °C for 10 h. After co-culture, mycelia were washed with sterile water containing cefotaxime (500 µg/mL) and transferred to PDA medium supplemented with hygromycin B (50 µg/mL). Positive transformants were selected after 10–14 days and subcultured on selective media. Integration of *GeSOD7* and the *hph* (hygromycin phosphotransferase) gene was confirmed by PCR using specific primers (Table S1).

2.10. Cold Stress Treatment and Physiological Assays

Wild-type and transgenic strains were pre-cultured on PDA until rhizomorph formation. Rhizomorph fragments were inoculated into semi-solid medium and incubated at 13 °C for 45 days. Mycelia were harvested for physiological analysis. Glutathione content was measured using a commercial assay kit (Nanjing Jiancheng Bioengineering Institute). Malondialdehyde (MDA) content was determined by thiobarbituric acid colorimetry [20], soluble sugar by anthrone colorimetry [21],

hydrogen peroxide (H₂O₂) by xylenol orange colorimetry [22], and free proline by the acid ninhydrin method [23]. All measurements were performed with three biological replicates.

2.11. RNA Extraction and Quantitative Real-Time PCR (RT-qPCR)

Total RNA was extracted using the RNAprep Pure Plant Kit (Tiangen). First-strand cDNA was synthesized from 1 µg RNA using HiScript III RT SuperMix (Vazyme). RT-qPCR was performed on a QuantStudio 5 Real-Time PCR System (Applied Biosystems) using SYBR Green master mix (Takara). Gene-specific primers for target genes (Glutamate dehydrogenase, Catalase, Glutathione reductase, Glutathione peroxidase, Trehalose phosphorylase) and the reference gene (Elongation Factor 1- α , EF-1 α) are listed in Table S2. Relative expression levels were calculated using the 2^{- $\Delta\Delta C_t$} method [24].

2.12. Statistical Analysis

Data are presented as mean \pm standard deviation (SD) of three biological replicates. Statistical significance was determined using Student's t-test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Identification and Bioinformatics Analysis of the SOD Gene Family in *G. elata*

A total of 18 putative SOD genes were initially retrieved from the *G. elata* transcriptome using HMM searches. After removing redundant and incomplete sequences through domain validation, 10 non-redundant SOD genes were confirmed and designated *GeSOD1* to *GeSOD10* (Table 1). Bioinformatic analysis revealed that the encoded proteins ranged from 145 to 237 amino acids in length, with molecular weights between 14.39 and 26.53 kDa and theoretical isoelectric points (pI) from 5.29 to 8.56. Subcellular localization predictions indicated that *GeSOD1*–*GeSOD6* are likely chloroplast-targeted, whereas *GeSOD7*–*GeSOD10* are predicted to localize to mitochondria.

Table 1. Characteristics of the SOD gene family in *Gastrodia elata*.

Gene Name	Number of amino acids	Molecular weight (Da)	PI	Instability index prediction	GRAVY	Instability index	Subcellular localization
<i>GeSOD1</i>	145	14558.00	5.29	12.10	-0.126	79.86	Chloroplast
<i>GeSOD2</i>	175	17778.83	6.30	24.45	-0.155	79.03	Chloroplast
<i>GeSOD3</i>	206	20672.20	5.63	20.79	0.120	91.46	Chloroplast
<i>GeSOD4</i>	164	16757.70	6.01	22.06	-0.121	79.09	Chloroplast
<i>GeSOD5</i>	152	15076.80	5.71	23.27	-0.026	82.11	Chloroplast
<i>GeSOD6</i>	211	21944.12	8.56	31.15	0.048	95.21	Chloroplast
<i>GeSOD7</i>	237	26526.18	7.17	44.20	-0.398	88.57	Mitochondrion
<i>GeSOD8</i>	194	21520.25	6.21	40.11	-0.323	82.06	Mitochondrion
<i>GeSOD9</i>	197	21971.85	6.43	29.48	-0.433	89.70	Mitochondrion
<i>GeSOD10</i>	203	22176.93	5.94	38.02	-0.250	87.59	Mitochondrion

3.2. Phylogenetic and Conserved Motif Analysis

Phylogenetic analysis classified the 10 GeSOD proteins into two distinct clades (Figure 1). GeSOD1–GeSOD6 clustered with known Cu/Zn-SODs, while GeSOD7–GeSOD10 formed a separate clade closely related to mitochondrial Mn-SODs from *A. thaliana* and *Dendrobium candidum*. Domain architecture analysis confirmed that GeSOD1–6 contain the Cu/Zn-SOD domain, whereas GeSOD7–10 harbor the Fe/Mn-SOD domain (Figure 2A). MEME motif analysis further supported this classification, revealing distinct conserved sequence patterns between the two groups (Figure 2B).

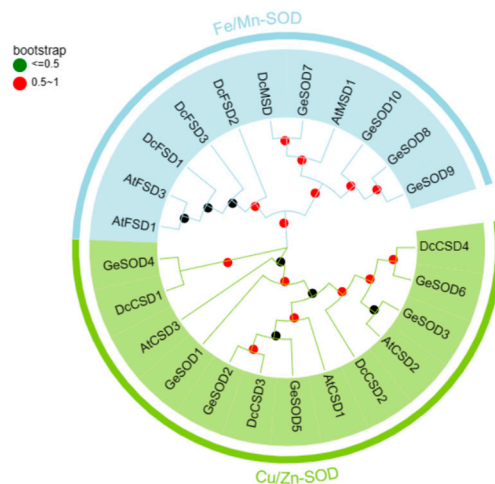


Figure 1. Phylogenetic analysis of GeSOD proteins.

The phylogenetic tree was constructed using the neighbor-joining method with 1000 bootstrap replicates, comparing GeSOD proteins with AtSOD (*Arabidopsis thaliana*) and DcSOD (*Dendrobium candidum*).

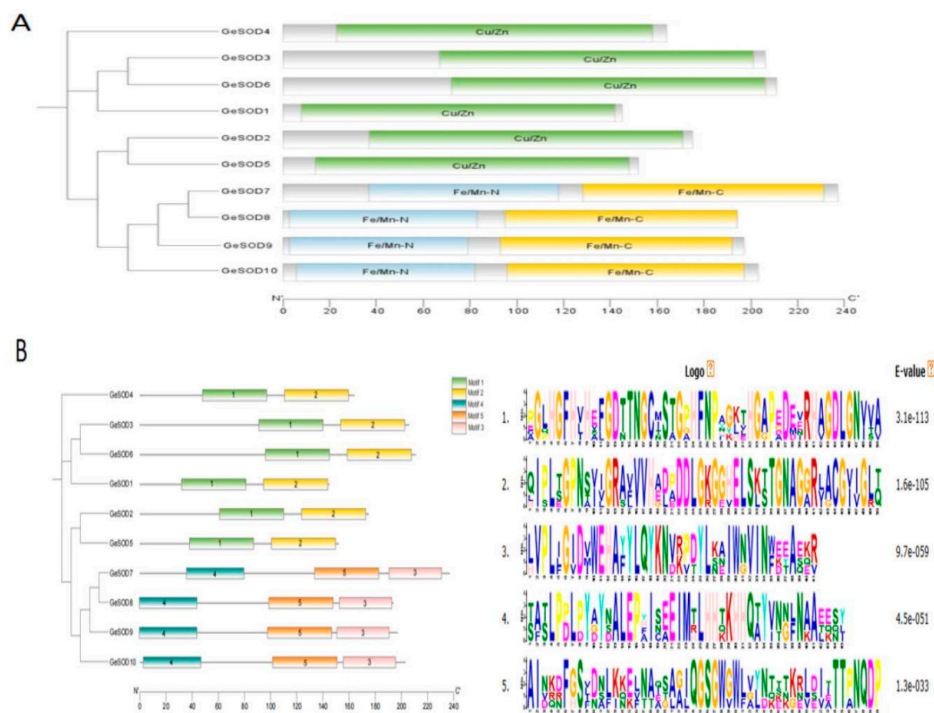


Figure 2. Domain architecture and conserved motif analysis of GeSOD proteins. (A) Domain analysis of the 10 SOD genes in *G. elata*. (B) Conserved motifs and sequence logos of the 10 SOD genes.

3.3. Expression Profiling and Selection of GeSOD7

Analysis of transcriptomic expression data across different tissues and temperatures showed that GeSOD2 and GeSOD7 were the most highly expressed genes within the family (Figure 3). Both genes exhibited notably high expression in immature tubers at 13 °C, as well as in symbiotic and mature tuber tissues at 23 °C. Given the achlorophyllous nature of *G. elata* tubers and the mitochondrial localization prediction, GeSOD7 was selected as the prime candidate for further functional investigation related to low-temperature stress.

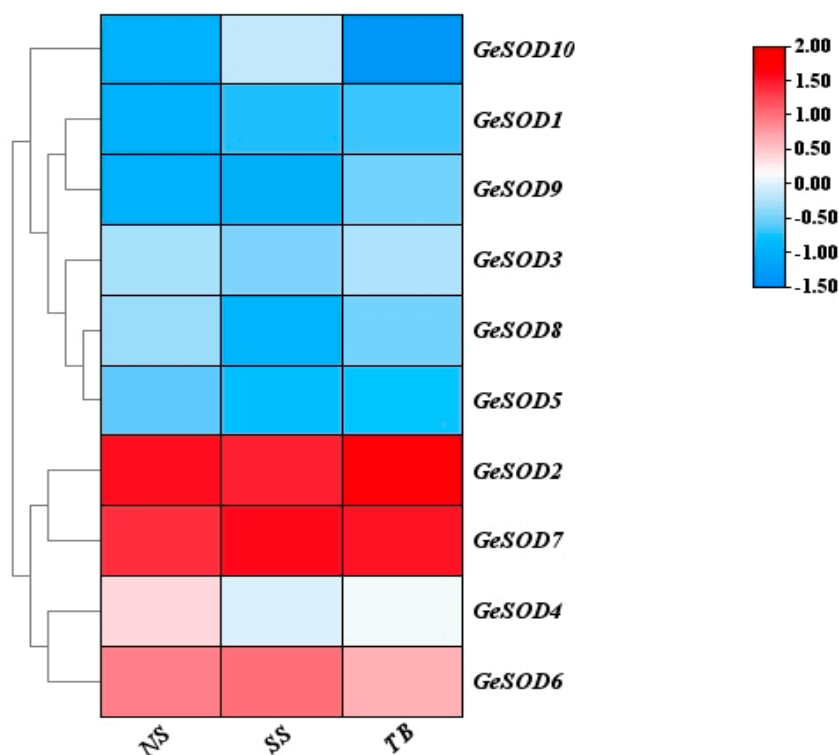


Figure 3. Expression profiles of the GeSOD gene family under different temperatures and tissues. Expression levels are shown in immature tubers at 13 °C (NS), immature tubers symbiotic with *A. mellea* at 23 °C (TB), and mature tubers (SS).

3.4. Heterologous Expression and Enzymatic Characterization of GeSOD7

The GeSOD7 coding sequence was successfully expressed in *E. coli* BL21(DE3). SDS-PAGE confirmed the induction and purification of the recombinant His-tagged GeSOD7 protein with an apparent molecular weight of approximately 40 kDa (Figure S1). Biochemical characterization revealed that the purified GeSOD7 exhibited maximum activity at 60 °C and pH 6.0 (Figure 4A,C). The enzyme showed moderate thermostability at 60 °C but was rapidly inactivated at 70 °C (Figure 4B). Metal ion assays indicated that enzyme activity was significantly inhibited by high concentrations (9 mM) of Mn^{2+} and Cu^{2+} , while Ca^{2+} , Mg^{2+} , and K^{+} had mild effects at lower concentrations (5 mM) (Figure 4D).

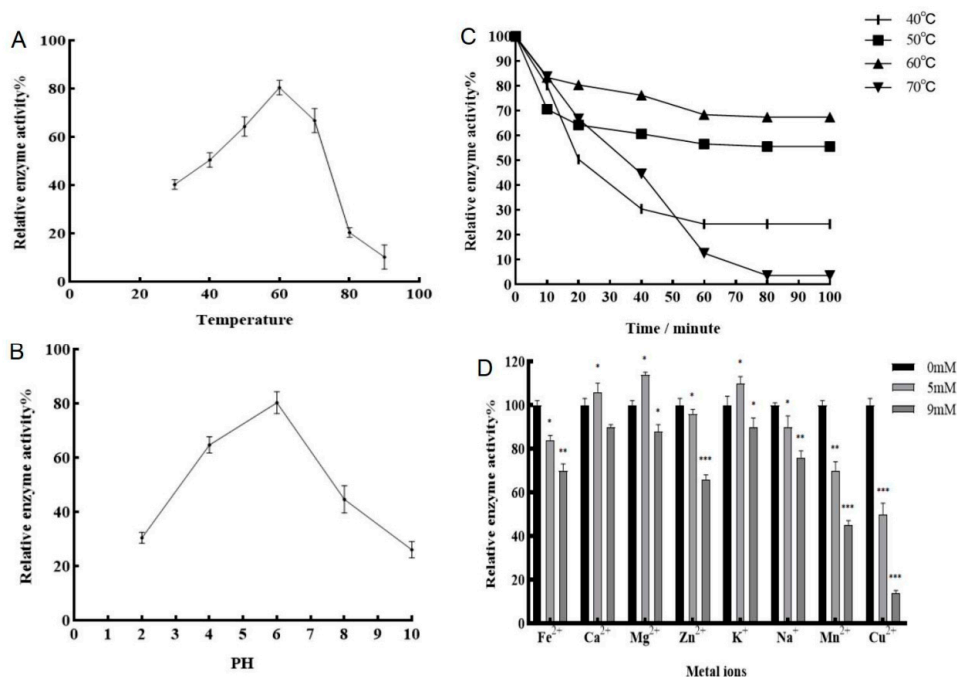


Figure 4. Biochemical characterization of recombinant GeSOD7. (A) Effect of temperature on enzyme activity. (B) Thermostability analysis. (C) Effect of pH on enzyme activity. (D) Effect of metal ions on enzyme activity. Data are presented as mean \pm SD (n = 3). Statistical significance was determined by Student's t-test (*p < 0.05; **p < 0.01; ***p < 0.001).

3.5. Overexpression of GeSOD7 Enhances Cold Tolerance in *A. mellea*

The binary vector pH7WG2.0-35S-GeSOD7 was successfully introduced into wild-type *A. mellea* via *A. tumefaciens*-mediated transformation. Positive transformants were selected on hygromycin-containing media and validated by PCR amplification of both the GeSOD7 insert and the *hph* marker gene (Figure 5A,B). Under prolonged low-temperature stress (13 °C for 45 days), the GeSOD7-overexpressing (OE) strains displayed significantly more robust rhizomorph development and a higher fresh weight compared to the wild-type (WT) control (Figure 5C,D).

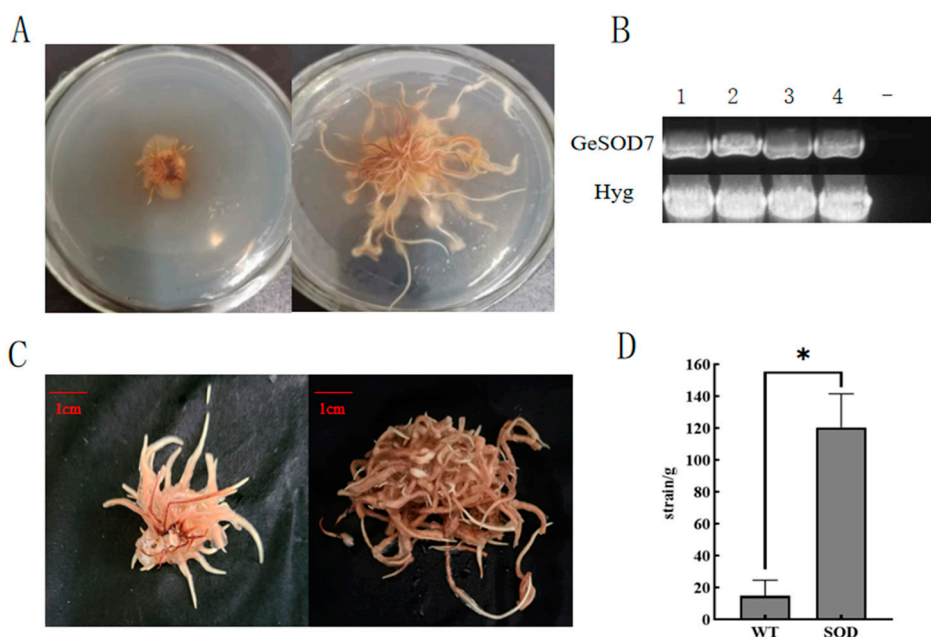


Figure 5. Generation and phenotypic characterization of GeSOD7-overexpressing *A. mellea* strains. (A) Growth of wild-type (WT, left) and transgenic (right) *A. mellea* on hygromycin selection plates. (B) PCR confirmation of GeSOD7 integration (lanes 1-4: transgenic strains; -: WT control; HYG: hygromycin resistance gene). (C) Morphology of WT (left) and transgenic (right) strains after 45 days of cold stress at 13 °C. (D) Fresh weight comparison after 45 days of cold stress. Data are mean \pm SD ($n \geq 3$). * $p < 0.05$ (Student's t-test).

3.6. GeSOD7 Overexpression Modulates Antioxidant Metabolism and Alleviates Oxidative Damage

Physiological analysis revealed that the OE strains accumulated significantly higher levels of glutathione (GSH) and proline compared to the WT under cold stress (Figure 6A,B). Concurrently, the contents of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), a marker of lipid peroxidation, were markedly reduced in the transgenic strains (Figure 6C,D). Consistent with these physiological changes, RT-qPCR analysis showed that the expression of key antioxidant genes—including Glutamate dehydrogenase (GDH), Catalase (CAT), Glutathione reductase (GR), and Glutathione peroxidase (GPX)—was significantly upregulated in the OE strains, whereas Trehalose phosphorylase (TPP) expression remained unchanged (Figure 7).

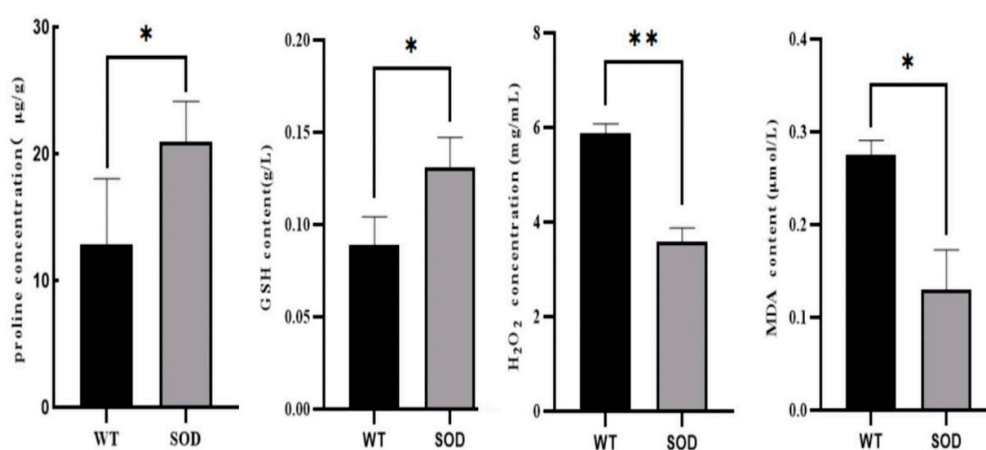


Figure 6. Physiological and biochemical indices of GeSOD7-overexpressing *A. mellea* under cold stress. (A) Proline content. (B) Glutathione (GSH) content. (C) Hydrogen peroxide (H_2O_2) content. (D) Malondialdehyde (MDA) content. Data are mean \pm SD ($n = 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student's t-test).

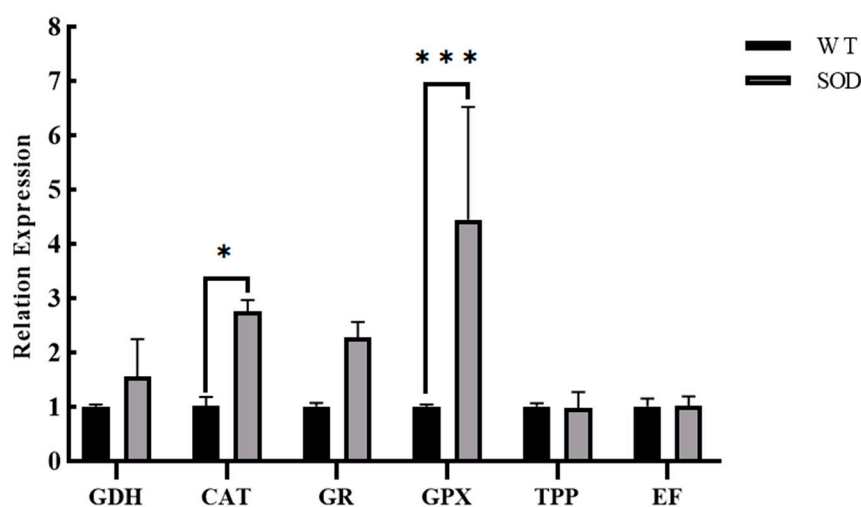


Figure 7. Expression levels of genes related to glutathione metabolism and peroxide detoxification. GDH, glutamate dehydrogenase; CAT, catalase; GR, glutathione reductase; GPX, glutathione peroxidase; TPP, trehalose-6-phosphate phosphatase; EF-1 α , elongation factor 1-alpha (reference gene). Data are mean \pm SD (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 (Student's t-test).

4. Discussion

Enhancing the stress resilience of symbiotic fungi is a critical step toward securing the productivity of economically important plant–fungus systems. This study demonstrates, for the first time, that the heterologous expression of a plant-derived manganese superoxide dismutase gene, GeSOD7, can significantly improve the cold tolerance of the symbiotic fungus *A. mellea*. Our findings reveal that this improvement is mechanistically underpinned by a reinforced antioxidant defense system and improved redox homeostasis, offering a novel genetic strategy for engineering stress-tolerant fungal symbionts.

4.1. GeSOD7 as a Functional Mn-SOD with Distinct Biochemical Properties

The 10 GeSOD proteins identified in this study were phylogenetically classified into Cu/Zn-SOD and Fe/Mn-SOD subfamilies, a categorization consistent with those reported in other plant species [10,25]. GeSOD7 clustered specifically with mitochondrial Mn-SODs, and its predicted subcellular localization to mitochondria aligns with its presumed role in scavenging superoxide anions (O₂•⁻) generated in the electron transport chain—a major site of ROS production under stress [26]. The recombinant GeSOD7 protein exhibited classic enzymatic characteristics of Mn-SODs, with an optimal activity at 60 °C and pH 6.0. The pronounced inhibition of its activity by high concentrations of Mn²⁺ and Cu²⁺ is noteworthy. While Mn²⁺ serves as an essential cofactor, excess ions can disrupt enzyme conformation or induce non-productive binding, a phenomenon observed in other metalloenzymes [27]. This property highlights the importance of intracellular metal ion homeostasis for optimal SOD function in vivo.

4.2. GeSOD7 Overexpression Confers a Growth Advantage Under Cold Stress by Activating a Coordinated Antioxidant Response

The core finding of this work is that GeSOD7 overexpression conferred a clear growth advantage to *A. mellea* under prolonged low-temperature (13 °C) stress (Figure 5). We propose a mechanistic model wherein enhanced SOD activity serves as the initial trigger for a broader defensive reprogramming (Figure 8). The primary increase in SOD-mediated H₂O₂ production likely functions not only as a detoxification step but also as a signaling molecule, orchestrating the upregulation of downstream antioxidant components [13,28]. This is supported by the concurrent increase in the activities and gene expression of H₂O₂-scavenging enzymes (CAT and GPX) and the key glutathione-cycle enzyme GR in our transgenic strains (Figure 7). The resultant significant reduction in both H₂O₂ and MDA levels unequivocally indicates mitigated oxidative damage to cellular membranes and other components [29].

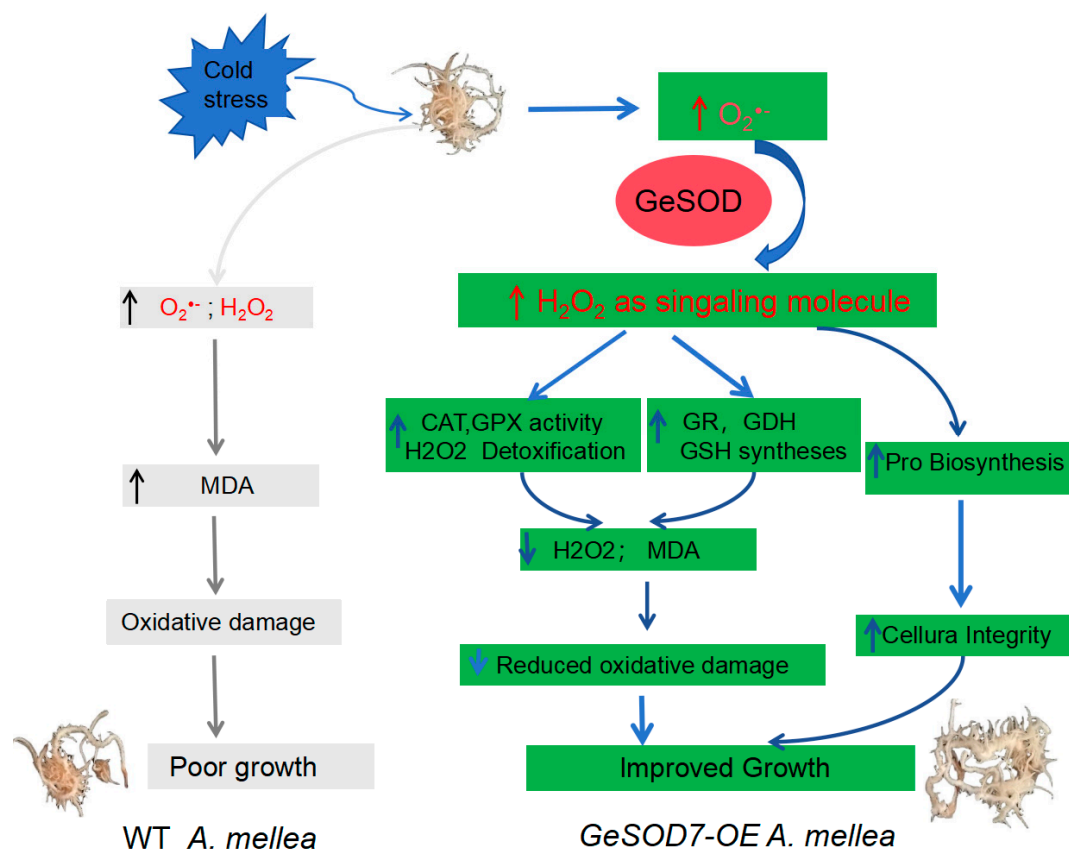


Figure 8. Proposed Mechanism of GeSOD7-Enhanced Cold Tolerance in *Armillaria mellea*.

4.3. Synergistic Enhancement of Non-Enzymatic Antioxidant Pools

Beyond enzymatic defenses, the transgenic strains exhibited a marked accumulation of the compatible solutes proline and glutathione (GSH) (Figure 6). Proline is a multifunctional molecule known to act as an osmoprotectant, a ROS scavenger, and a stabilizer of cellular structures under abiotic stress [30]. Glutathione, a major cellular redox buffer and antioxidant, serves as a crucial substrate for GPX and directly neutralizes reactive species [31]. The upregulation of Glutamate Dehydrogenase (GDH), which bridges nitrogen metabolism with the synthesis of these compounds, suggests that GeSOD7 overexpression may rewire central metabolic pathways to support the biosynthesis of protective metabolites. This synergistic enhancement of both enzymatic and non-enzymatic antioxidant systems creates a robust defense network, enabling the fungus to maintain cellular integrity under cold stress.

4.4. Implications for Fungal Biotechnology and Sustainable Agriculture

While SOD overexpression has been shown to enhance abiotic stress tolerance in various transgenic plants [17,32], its application in improving the resilience of fungal symbionts remains underexplored. Our study effectively bridges this gap. The *G. elata*–*A. mellea* symbiosis is not only a fascinating model for basic research on cross-kingdom molecular interactions but also a system of high agricultural value [3,5]. The cold sensitivity of *A. mellea* is a primary limiting factor for *G. elata* cultivation in temperate regions. The strategy demonstrated here—using a host plant’s own genetic resource to fortify its fungal partner—presents a targeted and potentially sustainable approach to alleviate this bottleneck. Future work should focus on evaluating the performance of these engineered fungal strains in actual symbiotic cultivation settings and assessing their long-term genetic stability and environmental safety.

As summarized in the proposed model (Figure 8), under cold stress, the heterologous expression of GeSOD7 in *A. mellea* initiated a cascade of antioxidant responses. The primary increase in SOD

activity not only directly scavenged $O_2\bullet$ but also generated a modulated level of H_2O_2 , which likely acted as a signaling molecule to upregulate downstream defenses. This coordinated response ultimately resulted in reduced oxidative damage and improved growth, in stark contrast to the overwhelmed antioxidant system and poor growth observed in the wild-type strain under the same conditions.

5. Conclusions

In conclusion, this study provides comprehensive evidence that the *G. elata* Mn-SOD gene GeSOD7 functions as a key regulator of antioxidant defense. Its heterologous expression in the symbiotic fungus *A. mellea* significantly enhanced cold stress tolerance through a multi-faceted mechanism involving the coordinated upregulation of enzymatic antioxidants and the accumulation of protective osmolytes. These findings advance our understanding of antioxidant network regulation in symbiotic fungi and establish a proof-of-concept for using plant-derived genes to engineer stress-resilient fungal partners, with direct implications for improving the sustainable production of valuable medicinal plants like *G. elata*.

Supplementary Materials: The following supporting information can be downloaded at: [link to be inserted by publisher]. Figure S1: SDS-PAGE analysis of recombinant GeSOD7 expression and purification. Table S1: Primers used for cloning and PCR confirmation. Table S2: Primers used for RT-qPCR analysis.

Author Contributions: Conceptualization, X.Z. and K.L.; methodology, P.X., Z.Z., C.Z. and H.N.; validation, Y.L. and Z.Z.; formal analysis, P.X.; investigation, P.X. and Z.Z.; resources, Y.L.; data curation, H.N.; writing—original draft preparation, P.X.; writing—review and editing, K.L. and X.Z.; visualization, P.X.; supervision, X.Z. and K.L.; project administration, K.L.; funding acquisition, K.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China, grant number 31960071.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available within the article and its Supplementary Materials.

Acknowledgments: The authors thank the Faculty of Life Science and Technology, Kunming University of Science and Technology, for providing laboratory facilities and technical support.

Conflicts of Interest: The authors declare no conflict of interest.

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