Medicinal Plant Research 2024, Vol.14, No.4, 223-233
http://hortherbpublisher.com/index.php/mpr

Research Insight **Open Access**

Regulation of Secondary Metabolite Pathways in *Ganoderma lucidum* under Environmental Stress

Weidong Zhu
Zhejiang Shouxiangu Pharmaceutical Co., Ltd., Jinhua, 321200, Zhejiang, China
Corresponding email: 1427396212@qq.com

Medicinal Plant Research, 2024, Vol.14, No.4 doi: 10.5376/mpr.2024.14.0019
Received: 05 Jul., 2024
Accepted: 09 Aug., 2024
Published: 25 Aug., 2024

Preferred citation for this article:
Zhu W.D., 2024, Regulation of secondary metabolite pathways in *Ganoderma lucidum* under environmental stress, Medicinal Plant Research, 14(4): 223-233 (doi: 10.5376/mpr.2024.14.0019)

Abstract This study explores the regulatory mechanisms of secondary metabolic pathways, particularly the biosynthesis of ganoderic acid (GA), in *Ganoderma lucidum* under various environmental stress conditions. The research identified several key environmental factors influencing GA biosynthesis, including water stress, heat stress, and nitrogen limitation. Water stress was found to increase intracellular reactive oxygen species (ROS) levels and GA content, mediated by the interaction between aquaporin (GlAQP) and NADPH oxidase (NOX). Heat stress was shown to inhibit mycelium growth and induce GA biosynthesis through increased cytosolic Ca^{2+} concentration and membrane fluidity. Additionally, the mitochondrial pyruvate carrier (MPC) regulated by GCN4 was found to play a crucial role in GA biosynthesis under nitrogen limitation. Phospholipase D (PLD) and phosphatidic acid (PA) were also implicated in heat stress-induced GA biosynthesis. The findings highlight the complex regulatory mechanisms of GA biosynthesis in *G. lucidum* under environmental stress, involving multiple signaling pathways and metabolic adjustments. These insights provide a foundation for further research on fungal secondary metabolism and potential biotechnological applications.

Keywords *Ganoderma lucidum*; Ganoderic acid; Environmental stress; Secondary metabolism; Reactive oxygen species; Cytosolic Ca^{2+}; Mitochondrial pyruvate carrier; Phospholipase D

1 Introduction

Ganoderma lucidum, commonly known as "Lingzhi" in Chinese, is a highly esteemed medicinal mushroom that has been utilized in traditional Chinese medicine for over two thousand years. It is renowned for its potential health benefits, which include promoting longevity, enhancing immune function, and reducing the risk of chronic diseases such as cancer and heart disease (Wachtel-galor et al., 2004; Lu et al., 2020). The mushroom's bioactive compounds, particularly polysaccharides and triterpenoids, have been the focus of extensive research due to their diverse therapeutic properties, including antioxidant, antitumor, anti-inflammatory, and immunomodulatory activities (Lu et al., 2020). The industrial significance of *G. lucidum* is also notable, as it is widely cultivated and processed into various health supplements and functional foods, contributing to a growing global market.

Secondary metabolites in *Ganoderma lucidum*, such as polysaccharides and triterpenoids, play crucial roles in its medicinal properties. These compounds are not directly involved in the primary metabolic processes of growth and reproduction but are essential for the organism's interaction with its environment and defense mechanisms. Polysaccharides from *G. lucidum*, for instance, have been shown to exhibit significant biological activities, including antioxidant, antitumor, and immunomodulatory effects (Lu et al., 2020). The therapeutic potential of these secondary metabolites has spurred considerable interest in understanding their biosynthesis and regulation, as well as optimizing their production for medicinal use.

Environmental stress factors, such as temperature fluctuations, UV radiation, and nutrient availability, can significantly influence the biosynthesis and accumulation of secondary metabolites in *Ganoderma lucidum*. These stress conditions can trigger complex regulatory mechanisms within the mushroom, leading to enhanced production of bioactive compounds as a defensive response. Understanding how environmental stress affects secondary metabolite pathways is crucial for developing strategies to optimize the cultivation and extraction of these valuable compounds. This knowledge can lead to improved yields of bioactive metabolites, thereby enhancing the medicinal and commercial value of *G. lucidum*.

By elucidating how different stress factors influence the production and accumulation of key bioactive compounds, this study aims to provide insights that can be applied to optimize the cultivation and processing of *G. lucidum* for enhanced medicinal efficacy. The scope of the research includes a comprehensive analysis of the molecular and biochemical responses of *G. lucidum* to environmental stress, with a focus on identifying key regulatory genes and pathways involved in secondary metabolite biosynthesis. This research will contribute to the broader understanding of fungal secondary metabolism and its applications in biotechnology and medicine.

2 Secondary Metabolites in *Ganoderma lucidum*

2.1 Classification and key types of secondary metabolites

Ganoderma lucidum, commonly known as Lingzhi or Reishi, is renowned for its diverse array of secondary metabolites, which are primarily classified into triterpenoids, polysaccharides, and other bioactive compounds. Triterpenoids, such as ganoderic acids, are a significant class of compounds known for their pharmacological properties, including anti-inflammatory and anticancer activities (Cao et al., 2017; Čižmáriková, 2017). Polysaccharides, particularly *Ganoderma lucidum* polysaccharides (GLPs), are another major group, recognized for their immunomodulatory, antioxidant, and antitumor effects (Lu et al., 2020). Other secondary metabolites include sterols, alkaloids, and phenols, which contribute to the overall therapeutic potential of *G. lucidum* (Wachtel-galor et al., 2011).

2.2 Biosynthetic pathways of major metabolites

The biosynthesis of triterpenoids and polysaccharides in *G. lucidum* involves complex metabolic pathways. Triterpenoids, such as ganoderic acids, are synthesized through the mevalonate pathway. Key enzymes in this pathway include squalene synthase (sqs), lanosterol synthase (osc), and hydroxy-3-methylglutaryl-coenzyme A reductase (hmgr). The expression of these genes can be significantly upregulated by environmental factors, such as the application of salicylic acid, which enhances ganoderic acid accumulation (Cao et al., 2017; Ren et al., 2019).

Polysaccharides are synthesized through a series of glycosylation reactions, where glucose units are polymerized to form complex carbohydrate structures. The molecular mechanisms underlying the biosynthesis of GLPs involve various enzymes that regulate the polymerization and branching of glucose units, contributing to their diverse biological activities (Lu et al., 2020).

2.3 Role of these metabolites in therapeutic applications

The secondary metabolites of *G. lucidum* play crucial roles in various therapeutic applications. Triterpenoids, particularly ganoderic acids, exhibit potent anticancer properties by inhibiting cancer cell proliferation and inducing apoptosis. They also possess anti-inflammatory and hepatoprotective effects, making them valuable in the treatment of liver diseases and inflammatory conditions (Čižmáriková, 2017; Zhang, 2017).

Polysaccharides from *G. lucidum* are well-known for their immunomodulatory effects, enhancing the body's immune response against infections and tumors. They also exhibit antioxidant properties, protecting cells from oxidative stress, and have been shown to improve cardiovascular health by lowering cholesterol levels (Wachtel-galor et al., 2004; Lu et al., 2020).

3 Environmental Stress and Its Impact on *Ganoderma lucidum*

3.1 Types of environmental stress

Ganoderma lucidum, a medicinal mushroom known for its bioactive secondary metabolites, is subject to various types of environmental stress that can significantly impact its growth and metabolite production. The primary types of environmental stress include:

Temperature Stress: Both high and low temperatures can affect the physiological processes of *G. lucidum*. Heat stress, for instance, has been shown to inhibit mycelium growth, reduce hyphal branching, and induce the accumulation of heat shock proteins (HSPs) and ganoderic acid (GA) (Zhang et al., 2016).

Water Stress (Drought): Water scarcity is another critical stressor that affects *G. lucidum*. Water stress increases intracellular reactive oxygen species (ROS) levels, which in turn influences the biosynthesis of ganoderic acid and the activity of NADPH oxidase (NOX) (Zhu et al., 2022).

Salinity Stress: Although not extensively covered in the provided data, salinity stress generally affects the osmotic balance and can lead to similar physiological responses as drought stress.

Oxidative Stress: This type of stress is often a secondary effect of other environmental stressors like drought and temperature extremes. Increased ROS levels under oxidative stress can lead to cellular damage but also play a role in signaling pathways that regulate secondary metabolite production (Zhang et al., 2016; Zhu et al., 2022).

3.2 Physiological responses of *Ganoderma lucidum* to stress

The physiological responses of *G. lucidum* to environmental stress are multifaceted and involve various cellular mechanisms:

Heat Stress Response: Under heat stress, *G. lucidum* exhibits inhibited mycelium growth and reduced hyphal branching. There is also an induction of HSPs and an increase in cytosolic Ca^{2+} concentration, which is crucial for heat shock signal transduction (Zhang et al., 2016) (Figure 1).

Water Stress Response: Water stress leads to an increase in ROS levels, which subsequently enhances the biosynthesis of ganoderic acid. The expression of the aquaporin gene (GlAQP) is induced to facilitate water transfer, aiding in microbial growth under water-limited conditions (Zhu et al., 2022).

Oxidative Stress Response: Elevated ROS levels under oxidative stress can activate signaling pathways that modulate the production of secondary metabolites like ganoderic acid. The interaction between GlAQP and NOX plays a significant role in this process (Zhu et al., 2022).

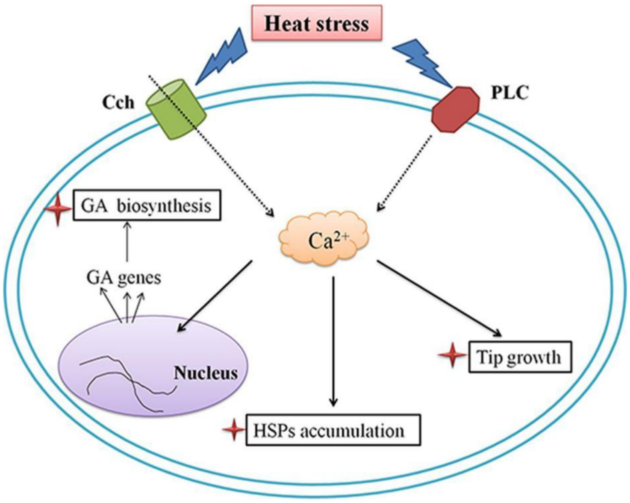

Figure 1 Schematic representation showing that HS regulates HSP expression, hyphal branching, and GA biosynthesis via cytosolic Ca^{2+} in *Ganoderma lucidum* (Adopted from Zhang et al., 2016)

Image caption: HS-induced cytosolic Ca^{2+} regulates GA biosynthesis, HSP accumulation, and hyphal branching. The black solid arrows indicate data supported by our own experiments, and the dotted arrows indicate data experimentally supported in other fungal systems (Adopted from Zhang et al., 2016)

3.3 Changes in metabolite production under stress conditions

Environmental stressors can significantly alter the production of secondary metabolites in *G. lucidum*. Both heat and water stress have been shown to increase the production of ganoderic acid. Under heat stress, the accumulation of ganoderic acid is mediated by increased cytosolic Ca^{2+} levels and the expression of HSPs (Zhang et al., 2016). Water stress, on the other hand, enhances ganoderic acid production through increased ROS levels and NOX activity (Zhu et al., 2022). While the provided data does not extensively cover the impact of stress on polysaccharides, it is known that environmental stress can influence the overall metabolic profile of *G. lucidum*, potentially affecting the production of other bioactive compounds like polysaccharides and triterpenoids (Sharma et al., 2019; Swallah et al., 2023).

4 Molecular Mechanisms Regulating Secondary Metabolite Pathways

4.1 Gene expression changes in response to stress

Environmental stress significantly impacts the gene expression profiles in *Ganoderma lucidum*, leading to alterations in secondary metabolite pathways. For instance, nitrogen limitation induces the expression of the transcription factor GCN4, which in turn promotes the synthesis of ganoderic acid (GA) by activating genes involved in antioxidant enzyme biosynthesis, such as glutathione reductase, glutathione S-transferase, and catalase (Lian et al., 2021). Similarly, heat stress activates the AMP-activated protein kinase (AMPK)/Sucrose-nonfermenting serine-threonine protein kinase 1 (Snf1), which mediates metabolic rearrangement to cope with oxidative stress and influences GA biosynthesis (Hu et al., 2019). Water stress also triggers changes in gene expression, notably increasing the levels of reactive oxygen species (ROS) and GA content through the modulation of NADPH oxidase (NOX) activity and aquaporin (GlAQP) expression (Zhu et al., 2022).

4.2 Key transcription factors and signaling pathways involved

Several key transcription factors and signaling pathways are involved in regulating secondary metabolite pathways in *G. lucidum*. The MADS-box transcription factor GlMADS1 has been shown to negatively regulate GA and flavonoid accumulation, as silencing this gene enhances the content of these secondary metabolites (Meng et al., 2020). Additionally, the general control non-derepressible 4 (GCN4) transcription factor plays a crucial role under nitrogen limitation conditions by binding to the promoter region of the mitochondrial pyruvate carrier (GlMPC) and activating its expression, thereby regulating the tricarboxylic acid (TCA) cycle and GA biosynthesis (Wang et al., 2023) (Figure 2). Furthermore, transcriptome and metabolome analyses have identified homeobox transcription factors and velvet family proteins as significant regulators of GA biosynthesis during the development of *G. lucidum* (Meng et al., 2022b).

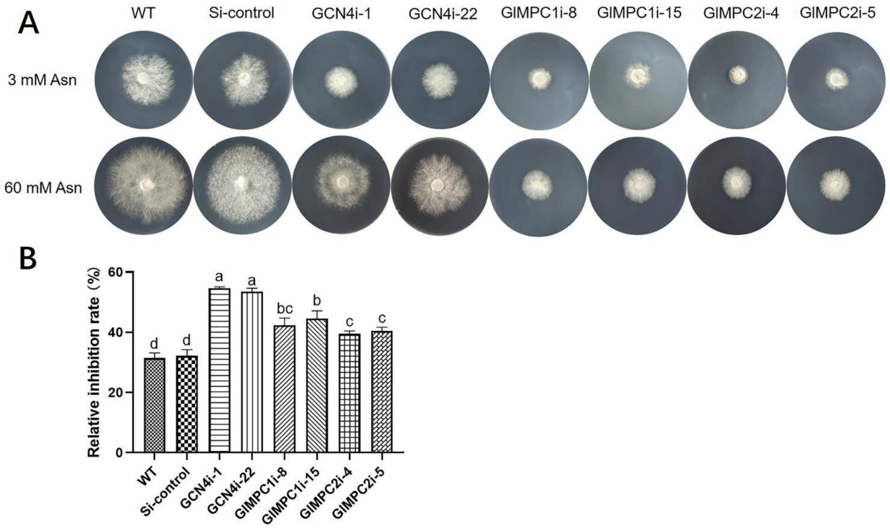

Figure 2 Mitochondrial pyruvate transport was significantly increased under nitrogen limitation conditions (Adopted from Wang et al., 2023)

Image caption: (A) Images of mycelial growth in WT, Si-control, GCN4-silenced, and GlMPC1/2-silenced strains under 3 mM and 60 mM Asn. (B) Relative inhibition rates under 3 mM and 60 mM Asn in WT, Si-control, GCN4-silenced, and GlMPC1/2-silenced strains. The growth inhibition rate in each strain was calculated as follows: [diameter (60 mM Asn) - diameter (3 mM Asn)]/diameter (60 mM Asn)]. Data are presented as the mean ± SD (n = 3). Statistical significance is represented by different letters corresponding to $P < 0.05$ based on Tukey's multiple range test (Adopted from Wang et al., 2023)

4.3 Role of enzymes and post-translational modifications

Enzymes and post-translational modifications play vital roles in the regulation of secondary metabolite pathways in *G. lucidum*. The mitochondrial pyruvate carrier (MPC) is essential for transporting pyruvate into mitochondria and maintaining carbon metabolic homeostasis, which is crucial for secondary metabolism under environmental stress (Wang et al., 2023). Nicotinamide adenine dinucleotide phosphate oxidases (NOX) are also central to this process, as they generate ROS that elevate cytosolic Ca^{2+} levels, thereby inducing the Ca^{2+} signaling pathway to regulate GA biosynthesis (Mu et al., 2014). Additionally, post-translational modifications such as phosphorylation mediated by AMPK/Snf1 under heat stress conditions help in metabolic remodeling, which is a protective mechanism against oxidative stress and influences GA biosynthesis (Hu et al., 2019).

5 Omics Approaches to Studying Stress Responses

5.1 Genomic and transcriptomic tools for understanding stress response

Genomic and transcriptomic analyses have been pivotal in elucidating the stress response mechanisms in *Ganoderma lucidum*. High-throughput RNA sequencing (RNA-Seq) has been employed to identify differentially expressed genes (DEGs) under various stress conditions. For instance, under heat stress, a comprehensive transcriptome analysis revealed 2 790 DEGs, including genes involved in stress resistance, protein assembly, signal transduction, and carbohydrate metabolism (Tan et al., 2018). Similarly, transcriptomic studies under ethylene treatment identified 4 070 DEGs, highlighting the upregulation of genes in the tricarboxylic acid (TCA) cycle and polyamine metabolic pathways, which are crucial for secondary metabolite biosynthesis (Meng et al., 2022) (Figure 3). These genomic and transcriptomic tools provide a detailed understanding of the molecular networks activated in response to environmental stresses.

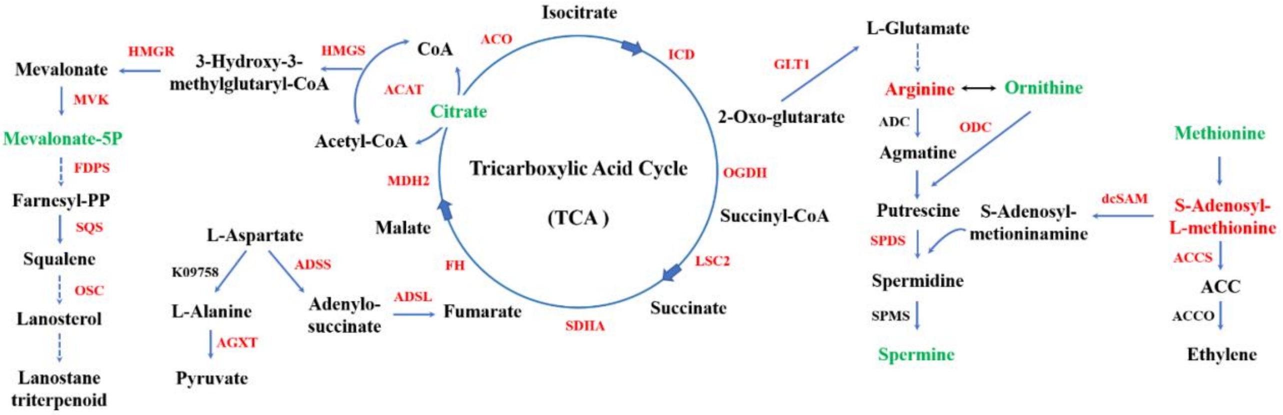

Figure 3 The expression pattern of genes and metabolites involved in key metabolism pathway (Adopted from Meng et al., 2022)

Image caption: HMGS, hydroxymethylglutaryl-CoA synthase; HMGR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; MVK, mevalonate kinase; FDPS, farnesyl diphosphate synthase; SQS, squalene synthase; OSC, lanosterol synthase; ACAT, acetyl-CoA acetyltransferase; AGXT, alanine-glyoxylate transaminase; ADSS, adenylosuccinate synthase; ADSL, adenylosuccinate lyase; ACO, aconitate hydratase; ICD, isocitrate dehydrogenase; OGDH, 2-oxoglutarate dehydrogenase E1 component; LSC2, succinyl-CoA synthetase beta subunit; SDHA, uccinate dehydrogenase (ubiquinone) flavoprotein subunit; FH, fumarate hydratase; MDH2, malate dehydrogenase; GLT1, glutamate synthase; ADC, arginine decarboxylase; ODC, ornithine decarboxylase; SPDS, spermidine synthase; SPMS, Spermine synthase; dcSAM, decarboxylated S-adenosylmethionine; ACC, 1-Aminocyclopropane-1-carboxylate; ACCS, ACC synthase; ACCO, ACC oxidase. The red color indicates up-regulation, green indicates down-regulation, and black indicates not detected (Adopted from Meng et al., 2022)

5.2 Proteomic and metabolomic analysis of stress-induced changes

Proteomic and metabolomic analyses complement genomic studies by providing insights into the functional proteins and metabolites that are altered under stress conditions. Proteomic studies have shown that heat stress induces the expression of heat shock proteins (HSPs) and other stress-related proteins, which play a role in maintaining cellular homeostasis (Zhang et al., 2016). Metabolomic analyses have identified significant changes in metabolite profiles under stress conditions. For example, under water stress, there is an increase in reactive oxygen species (ROS) levels and ganoderic acid (GA) content, which are regulated by the interaction between aquaporin (GlAQP) and NADPH oxidase (NOX) (Zhu et al., 2022). Additionally, metabolomic studies under heat stress have shown the accumulation of phosphatidic acid (PA), which is involved in the regulation of secondary metabolism through phospholipid signaling (Liu et al., 2017b). These analyses provide a comprehensive view of the biochemical changes that occur in response to stress.

5.3 Integration of multi-omics for comprehensive insights

The integration of multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, offers a holistic understanding of the stress response in *Ganoderma lucidum*. For instance, a combined transcriptomic and metabolomic analysis under ethylene treatment revealed the coordinated regulation of genes and metabolites involved in the TCA cycle and ganoderic acid biosynthesis (Meng et al., 2022a). Similarly, the integration of transcriptome and metabolome data during the development of *G. lucidum* identified key transcription factors and metabolic pathways involved in GA biosynthesis (Meng et al., 2022b). These integrated approaches enable the identification of regulatory networks and key molecular players that mediate the stress response, providing comprehensive insights into the adaptive mechanisms of *G. lucidum* under environmental stress.

6 Stress-Induced Changes in Key Metabolites

6.1 Impact of temperature stress on triterpenoid biosynthesis

Temperature stress, particularly heat stress (HS), has been shown to significantly influence the biosynthesis of triterpenoids, such as ganoderic acids (GA), in *Ganoderma lucidum*. HS induces the accumulation of reactive oxygen species (ROS), which in turn regulates the expression of heat shock proteins (HSPs) and enhances GA biosynthesis. For instance, HS treatment increases the length between hyphal branches and induces GA accumulation, which is mitigated by ROS scavengers like NAC and VC (Liu et al., 2018b). Additionally, membrane fluidity plays a crucial role in HS-induced GA biosynthesis. Increased membrane fluidity, induced by HS, is associated with elevated GA levels, and this effect can be reversed by membrane rigidifiers such as DMSO (Liu et al., 2017a). Furthermore, phospholipid-mediated signal transduction, particularly involving phosphatidic acid (PA) and phospholipase D (PLD), is essential for HS-induced GA biosynthesis (Liu et al., 2017b). The AMP-activated protein kinase (AMPK)/Sucrose-nonfermenting serine-threonine protein kinase 1 (Snf1) pathway also participates in this process by mediating metabolic rearrangements in response to HS, thereby influencing GA production (Hu et al., 2019).

6.2 Oxidative stress and its effects on polysaccharide production

Oxidative stress, characterized by elevated levels of ROS, significantly impacts the production of polysaccharides in *G. lucidum*. Under oxidative stress conditions, the balance of ROS is crucial for the regulation of secondary metabolite pathways. For example, water stress, which induces oxidative stress, increases intracellular ROS levels and subsequently enhances ganoderic acid content and NADPH oxidase (NOX) activity. The cross-talk between aquaporin (GlAQP) and NOX modulates ROS levels, thereby regulating GA biosynthesis under water stress (Zhu et al., 2022). Additionally, the role of calcium signaling in oxidative stress response is noteworthy. Calcium ions (Ca^{2+}) and their associated signaling pathways, such as the calcineurin pathway, are involved in the regulation of GA biosynthesis under oxidative stress conditions. The addition of Ca^{2+} enhances GA production, suggesting that calcium signaling is integral to the oxidative stress response and secondary metabolite regulation in *G. lucidum* (Xu and Zhong, 2012).

6.3 The role of combined stress factors in metabolite diversity

The interplay of multiple stress factors can lead to a diverse array of secondary metabolites in *G. lucidum*. Combined stress factors, such as temperature and oxidative stress, can synergistically influence metabolite pathways. For instance, HS not only induces ROS accumulation but also increases cytosolic Ca^{2+} concentration, which is crucial for the regulation of hyphal branching, HSP expression, and GA biosynthesis (Zhang et al., 2016). The interaction between ROS and Ca^{2+} signaling pathways highlights the complexity of stress responses in *G. lucidum*. Moreover, the integration of transcriptomics and metabolomics analyses has revealed that ethylene, another stress factor, can modulate key metabolic pathways, including the tricarboxylic acid (TCA) cycle and triterpenoid metabolism, thereby enhancing GA production (Meng et al., 2022a). This indicates that the combined effects of various environmental stressors can lead to a more robust and diverse secondary metabolite profile in *G. lucidum*.

7 Case Studies

7.1 Temperature stress and triterpenoid production

Temperature stress has been shown to significantly impact the biosynthesis of ganoderic acid (GA), a key triterpenoid in *Ganoderma lucidum*. High temperatures increase the levels of nitric oxide (NO) and calcium ions (Ca^{2+}), which interact to regulate GA production. Specifically, heat stress (HS) elevates NO levels by 120%, which in turn modulates GA biosynthesis. The application of a NO scavenger increases GA content by 25%, while a NO donor decreases it by 30%, indicating that NO alleviates HS-induced GA accumulation. Furthermore, the cross-talk between NO and Ca^{2+} signals is crucial, with Ca^{2+} having a more direct and significant effect on GA production under HS conditions (Liu et al., 2018a).

7.2 The role of drought in altering polysaccharide biosynthesis

Water stress, or drought conditions, significantly affects the biosynthesis of polysaccharides and ganoderic acid in *Ganoderma lucidum*. Under water stress, the intracellular reactive oxygen species (ROS) levels, GA content, and NADPH oxidase (NOX) activity increase. The expression of the *G. lucidum* aquaporin (GlAQP) gene, which facilitates water transfer, is induced, enhancing microbial growth. In GlAQP-silenced strains, water stress results in higher ROS levels and GA content compared to wild-type strains. Conversely, in GlAQP-overexpressing strains, ROS levels and GA content are lower. This indicates that the cross-talk between GlAQP and NOX modulates GA biosynthesis via ROS under water stress, with the regulation being positive at the early stage and negative at the late stage of fermentation (Zhu et al., 2022) (Figure 4).

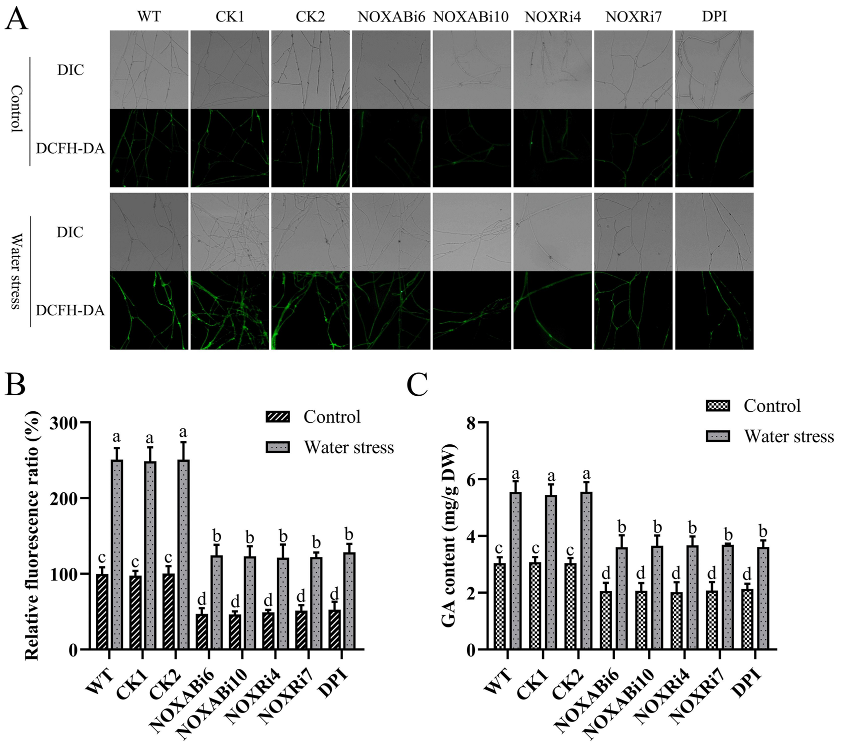

Figure 4 Effect of silencing NOX and adding DPI on ROS level and GA content of *G. lucidum* under water stress (Adopted from Zhu et al., 2022)

Image caption: (A) Change in ROS level detected by DCFH-DA staining in NOX-silenced strains and 10 μM DPI-treated WT under water stress. (B) Change in ROS fluorescence ratio in NOX-silenced strains and 10 μM DPI-treated WT under water stress. (C) The GA content in NOX-silenced strains and 10 μM DPI-treated WT under water stress. The values indicate the mean ± SD of three independent experiments. Different letters indicate significant differences between treatments (Duncan's multiple range test, $P < 0.05$) (Adopted from Zhu et al., 2022)

7.3 Combined salinity and oxidative stress on metabolite diversity

The combined effects of salinity and oxidative stress on *Ganoderma lucidum* lead to a diverse array of secondary metabolites. Salicylic acid (SA) and calcium ions (Ca^{2+}) have been shown to influence the biosynthesis of polysaccharides and triterpenoids. While Ca^{2+} alone does not affect polysaccharide and triterpenoid production, SA increases triterpenoid content by 23.32%. The combined induction of SA and Ca^{2+} results in a 9.02% increase in polysaccharide content and a 13.61% increase in triterpenoid content. This combined stress enhances the expression of key biosynthetic genes, such as ugp, pgm, and gls for polysaccharides, and hmgr, hmgs, mvd, fps, sqs, and ls for triterpenoids, across different developmental stages of *G. lucidum* (Ye et al., 2018).

8 Biotechnological Applications of Stress-Induced Metabolites

8.1 Industrial relevance of stress-enhanced metabolites in pharmaceuticals

Ganoderma lucidum, a well-known medicinal mushroom, produces ganoderic acid (GA), a secondary metabolite with significant pharmacological activities. Environmental stressors such as water stress, heat stress, and nitrogen limitation have been shown to enhance the production of GA, making these stress-induced metabolites highly relevant for pharmaceutical applications. For instance, water stress increases intracellular reactive oxygen species (ROS) levels, which in turn boosts GA content significantly (Zhu et al., 2022). Similarly, heat stress not only induces the accumulation of GA but also enhances the expression of heat shock proteins (HSPs), which are crucial for the organism's stress response (Liu et al., 2018b; Zhang et al., 2018). Nitrogen limitation also promotes GA synthesis by activating specific transcription factors like GCN4, which regulate antioxidant enzyme biosynthesis to manage ROS levels (Lian et al., 2021). These findings highlight the potential of leveraging environmental stress to enhance the production of valuable secondary metabolites for pharmaceutical use.

8.2 Optimization strategies for large-scale cultivation of *Ganoderma lucidum*

To optimize the large-scale cultivation of *Ganoderma lucidum* for enhanced metabolite production, several strategies can be employed. Liquid superficial-static culture (LSSC) has been identified as a superior method for producing higher GA content compared to submerged culture (SC) (Wang et al., 2020). Additionally, the manipulation of environmental conditions such as temperature and nutrient availability can significantly impact metabolite production. For example, the addition of calcium ions to the culture medium has been shown to enhance GA production through the calcineurin signal transduction pathway (Xu and Zhong, 2012). Moreover, understanding the genetic and physiological responses of *G. lucidum* to various stressors can inform the development of more efficient cultivation techniques. For instance, the overexpression of genes involved in ROS management and secondary metabolite biosynthesis can be targeted to improve yield under stress conditions (Hu et al., 2019; Zhu et al., 2022).

8.3 Use of elicitors to stimulate metabolite production under controlled conditions

Elicitors are substances that can stimulate the production of secondary metabolites in fungi under controlled conditions. In *Ganoderma lucidum*, various elicitors such as ethylene, calcium ions, and ROS scavengers have been shown to enhance GA production. Exogenous ethylene, for example, increases both endogenous ethylene and GA levels by up-regulating genes involved in key metabolic pathways (Meng et al., 2022a). Calcium ions also act as effective elicitors by activating the calcineurin signal transduction pathway, which up-regulates the expression of genes involved in GA biosynthesis (Xu and Zhong, 2012). Additionally, the use of ROS scavengers like N-acetyl-l-cysteine (NAC) and ascorbic acid (VC) can modulate the oxidative stress response, thereby influencing GA production (Liu et al., 2018b; Hu et al., 2019). These elicitors provide a valuable tool for optimizing metabolite production in *G. lucidum* under controlled cultivation conditions.

9 Future Prospects

9.1 Advancements in genetic engineering to enhance stress resilience and metabolite production

The application of genetic engineering techniques has shown significant promise in enhancing the stress resilience and secondary metabolite production in *Ganoderma lucidum*. The development of genome-scale metabolic models (GSMM) such as model iZBM1060 has provided a comprehensive framework for understanding and manipulating metabolic pathways. For instance, the addition of phenylalanine was found to significantly increase extracellular polysaccharide (EPS) production, demonstrating the potential of targeted genetic modifications to enhance metabolite yields under stress conditions (Ma et al., 2018). Furthermore, the CRISPR/Cas9 system has emerged as a powerful tool for precise genome editing, enabling the modification of specific genes involved in stress responses and secondary metabolite biosynthesis with high accuracy and efficiency (Devi et al., 2023).

9.2 Application of synthetic biology for optimized biosynthetic pathways

Synthetic biology offers innovative approaches to optimize biosynthetic pathways in *Ganoderma lucidum*. By reconstructing and analyzing genome-scale metabolic models, researchers can identify key enzymes and regulatory nodes that can be targeted for pathway optimization. For example, the GSMM of *G. lucidum* has identified eight key genes for EPS overproduction, providing a roadmap for synthetic biology interventions (Ma et al., 2018). These strategies can be further refined using synthetic biology tools to construct optimized biosynthetic pathways that enhance the production of valuable secondary metabolites while maintaining cellular homeostasis under environmental stress.

9.3 The potential of CRISPR-based editing for regulating stress response genes

CRISPR-based genome editing holds significant potential for regulating stress response genes in *Ganoderma lucidum*. The CRISPR/Cas9 system allows for precise and efficient modification of genes involved in stress responses, enabling the development of strains with enhanced resilience to environmental stressors. This technology has been widely applied in plant metabolic engineering, facilitating the discovery and functional analysis of genes involved in secondary metabolite pathways (Devi et al., 2023). By leveraging CRISPR-based editing, researchers can systematically dissect the genetic basis of stress responses and engineer *G. lucidum* strains with improved stress tolerance and secondary metabolite production capabilities.

10 Conclusion

In this study, we explored the regulation of secondary metabolite pathways in *Ganoderma lucidum* under various environmental stress conditions. Findings reveal that different types of stress, such as water stress, heat stress, and copper stress, significantly influence the biosynthesis of ganoderic acid (GA), a key secondary metabolite with medicinal properties.

Water stress was found to increase intracellular reactive oxygen species (ROS) levels, ganoderic acid content, and NADPH oxidase (NOX) activity. The cross-talk between the *G. lucidum* aquaporin (GlAQP) gene and NOX positively regulated GA biosynthesis via ROS at the early stage of water stress but became negative at the late stage. Heat stress (HS) also induced the production of ROS, which in turn regulated the expression of heat shock proteins (HSPs), hyphal branching, and GA biosynthesis. The AMP-activated protein kinase (AMPK)/Sucrose-nonfermenting serine-threonine protein kinase 1 (Snf1) was found to mediate metabolic rearrangement under HS, negatively regulating GA biosynthesis by removing ROS.

Copper stress was shown to decrease the distance between hyphal branches and increase GA content, with ROS and Ca^{2+} signaling playing crucial roles in this regulation. Increased cytosolic Ca^{2+} levels could reduce ROS by activating antioxidases, thereby modulating hyphal growth and GA biosynthesis. Additionally, the mitogen-activated protein kinase GlSlt2 was found to regulate fungal growth, fruiting body development, cell wall integrity, oxidative stress, and GA biosynthesis.

The insights gained from this study underscore the importance of managing environmental stress to optimize the cultivation of *Ganoderma lucidum* for therapeutic purposes. By understanding the molecular mechanisms underlying stress-induced regulation of secondary metabolite pathways, we can develop strategies to enhance the production of valuable compounds like ganoderic acid. For instance, managing water stress through the modulation of GlAQP and NOX activity can optimize GA production at different stages of fungal growth. Similarly, controlling heat stress by targeting the AMPK/Snf1 pathway and ROS levels can improve GA yield while maintaining fungal health. Copper stress management through the regulation of ROS and Ca^{2+} signaling can also be leveraged to enhance GA biosynthesis.

Acknowledgments

The author would like to express gratitude to the anonymous reviewers for their constructive comments and suggestions on this manuscript.

Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Cao P., Wu C., Dang Z., Shi L., Jiang A., Ren A., and Zhao M., 2017, Effects of exogenous salicylic acid on ganoderic acid biosynthesis and the expression of key genes in the ganoderic acid biosynthesis pathway in the Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (Agaricomycetes), International Journal of Medicinal Mushrooms, 19(1): 65-73. https://doi.org/10.1615/IntJMedMushrooms.v19.i1.70

Devi A., Devi K., Devi P., Devi M., and Das S., 2023, Metabolic engineering of plant secondary metabolites: prospects and its technological challenges, Frontiers in Plant Science, 14: 1171154. https://doi.org/10.3389/fpls.2023.1171154

Hu Y., Xu W., Hu S., Lian L., Zhu J., Ren A., Shi L., and Zhao M., 2019, Glsnf1-mediated metabolic rearrangement participates in coping with heat stress and influencing secondary metabolism in *Ganoderma lucidum*, Free Radical Biology and Medicine, 134: 199-211. https://doi.org/10.1016/j.freeradbiomed.2019.12.041

Lian L., Wang L., Song S., Zhu J., Liu R., Shi L., Ren A., and Zhao M., 2021, GCN4 regulates secondary metabolism through activation of antioxidant gene expression under nitrogen limitation conditions in *Ganoderma lucidum*, Applied and Environmental Microbiology, 87(5): e00156-21. https://doi.org/10.1128/AEM.00156-21

Liu R., Shi L., Zhu T., Yang T., Ren A., Zhu J., and Zhao M., 2018a, Cross talk between nitric oxide and calcium-calmodulin regulates ganoderic acid biosynthesis in *Ganoderma lucidum* under heat stress, Applied and Environmental Microbiology, 84(12): e00043-18. https://doi.org/10.1128/AEM.00043-18

Liu R., Zhang X., Ren A., Shi D., Shi L., Zhu J., Yu H., and Zhao M., 2018b, Heat stress-induced reactive oxygen species participate in the regulation of HSP expression, hyphal branching and ganoderic acid biosynthesis in *Ganoderma lucidum*, Microbiological Research, 209: 43-54. https://doi.org/10.1016/j.micres.2018.02.006

Liu Y., Lu X., Chen D., Lu Y., Ren A., Shi L., Zhu J., Jiang A., Yu H., and Zhao M., 2017b, Phospholipase D and phosphatidic acid mediate heat stress induced secondary metabolism in *Ganoderma lucidum*, Environmental Microbiology, 19(12): 4657-4669. https://doi.org/10.1111/1462-2920.13928

Liu Y., Zhang T., Lu X., Ma B., Ren A., Shi L., Jiang A., Yu H., and Zhao M., 2017a, Membrane fluidity is involved in the regulation of heat stress induced secondary metabolism in *Ganoderma lucidum*, Environmental Microbiology, 19(6): 1653-1668. https://doi.org/10.1111/1462-2920.13693

Lu J., He R., Sun P., Zhang F., Linhardt R., and Zhang A., 2020, Molecular mechanisms of bioactive polysaccharides from *Ganoderma lucidum* (Lingzhi), a review, International Journal of Biological Macromolecules, 150: 765-774. https://doi.org/10.1016/j.ijbiomac.2020.02.035

Ma Z., Ye C., Deng W., Xu M., Wang Q., Liu G., Wang F., Liu L., Xu Z., Shi G., and Ding Z., 2018, Reconstruction and analysis of a genome-scale metabolic model of *Ganoderma lucidum* for improved extracellular polysaccharide production, Frontiers in Microbiology, 9: 3076. https://doi.org/10.3389/fmicb.2018.03076

Meng L., Zhang S., Chen B., Bai X., Li Y., Yang J., Wang W., Li C., Li Y., and Li Z., 2020, The MADS-box transcription factor GlMADS1 regulates secondary metabolism in *Ganoderma lucidum*, Mycologia, 113: 12-19. https://doi.org/10.1080/00275514.2020.1810515

Meng L., Zhou R., Lin J., Wang Q., Wang P., Wang W., Wang L., and Li Z., 2022a, Integrated transcriptomics and nontargeted metabolomics analysis reveal key metabolic pathways in *Ganoderma lucidum* in response to ethylene, Journal of Fungi, 8: 456. https://doi.org/10.3390/jof8050456

Meng L., Zhou R., Lin J., Zang X., Wang Q., Wang P., Wang L., Li Z., and Wang W., 2022b, Transcriptome and metabolome analyses reveal transcription factors regulating ganoderic acid biosynthesis in *Ganoderma lucidum* development, Frontiers in Microbiology, 13: 956421. https://doi.org/10.3389/fmicb.2022.956421

Mu D., Li C., Zhang X., Li X., Shi L., Ren A., and Zhao M., 2014, Functions of the nicotinamide adenine dinucleotide phosphate oxidase family in *Ganoderma lucidum*: an essential role in ganoderic acid biosynthesis regulation, hyphal branching, fruiting body development, and oxidative-stress resistance, Environmental Microbiology, 16(6): 1709-1728. https://doi.org/10.1111/1462-2920.12326

Ren A., Shi L., Zhu J., Yu H., Jiang A., Zheng H., and Zhao M., 2019, Shedding light on the mechanisms underlying the environmental regulation of secondary metabolite ganoderic acid in *Ganoderma lucidum* using physiological and genetic methods, Fungal Genetics and Biology, 128: 43-48. https://doi.org/10.1016/j.fgb.2019.03.009

Sharma C., Bhardwaj N., Sharma A., Tuli H., Batra P., Beniwal V., Gupta G., and Sharma A., 2019, Bioactive metabolites of *Ganoderma lucidum*: factors, mechanism and broad spectrum therapeutic potential, Journal of Herbal Medicine, 19: 100268.
https://doi.org/10.1016/j.hermed.2019.100268

Swallah M., Bondzie-Quaye P., Wu Y., Acheampong A., Sossah F., Elsherbiny S., and Huang Q., 2023, Therapeutic potential and nutritional significance of *Ganoderma lucidum*: a comprehensive review from 2010 to 2022, Food and Function, 14: 1234-1250.
https://doi.org/10.1039/d2fo01683d

Tan X., Sun J., Ning H., Qin Z., Miao Y., Sun T., and Zhang X., 2018, De novo transcriptome sequencing and comprehensive analysis of the heat stress response genes in the basidiomycetes fungus *Ganoderma lucidum*, Gene, 661: 139-151.
https://doi.org/10.1016/j.gene.2018.03.093

Wachtel-Galor S., Tomlinson B., and Benzie I., 2004, *Ganoderma lucidum* ("Lingzhi"), a Chinese medicinal mushroom: biomarker responses in a controlled human supplementation study, The British Journal of Nutrition, 91(2): 263-269.
https://doi.org/10.1079/BJN20041039

Wachtel-Galor S., Yuen J., Buswell J., and Benzie I., 2011, *Ganoderma lucidum* (Lingzhi or Reishi): a medicinal mushroom -- Herbal Medicine: Biomolecular and Clinical Aspects, CRC Press, 2nd edition.
https://doi.org/10.1201/B10787-15

Wang Q., Xu M., Zhao L., Wang F., Li Y., Shi G., and Ding Z., 2020, Transcriptome dynamics and metabolite analysis revealed the candidate genes and regulatory mechanism of ganoderic acid biosynthesis during liquid superficial-static culture of *Ganoderma lucidum*, Microbial Biotechnology, 14: 600-613.
https://doi.org/10.1111/1751-7915.13670

Wang Z., Chen J., Ding J., Han J., and Shi L., 2023, GlMPC activated by GCN4 regulates secondary metabolism under nitrogen limitation conditions in *Ganoderma lucidum*, mBio, 14: e01356-23.
https://doi.org/10.1128/mbio.01356-23

Xu Y., and Zhong J., 2012, Impacts of calcium signal transduction on the fermentation production of antitumor ganoderic acids by medicinal mushroom *Ganoderma lucidum*, Biotechnology Advances, 30(6): 1301-1308.
https://doi.org/10.1016/j.biotechadv.2011.10.001

Ye L., Liu S., Xie F., Zhao L., and Wu X., 2018, Enhanced production of polysaccharides and triterpenoids in *Ganoderma lucidum* fruit bodies on induction with signal transduction during the fruiting stage, PLoS ONE, 13: e0196287.
https://doi.org/10.1371/journal.pone.0196287

Zhang X., Ren A., Li M., Cao P., Chen T., Zhang G., Shi L., Jiang A., and Zhao M., 2016, Heat stress modulates mycelium growth, heat shock protein expression, ganoderic acid biosynthesis, and hyphal branching of *Ganoderma lucidum* via cytosolic Ca2+, Applied and Environmental Microbiology, 82: 4112-4125.
https://doi.org/10.1128/AEM.01036-16

Zhang Y., 2017, *Ganoderma lucidum* (Reishi) suppresses proliferation and migration of breast cancer cells via inhibiting Wnt/β-catenin signaling, Biochemical and Biophysical Research Communications, 488(4): 679-684.
https://doi.org/10.1016/j.bbrc.2017.04.086

Zhu Q., Ren A., Ding J., He J., Zhao M., Jiang A., Zhou X., Wang J., and He Q., 2022, Cross talk between GlAQP and NOX modulates the effects of ROS balance on ganoderic acid biosynthesis of *Ganoderma lucidum* under water stress, Microbiology Spectrum, 10: e01297-22.
https://doi.org/10.1128/spectrum.01297-22

Čižmáriková M., 2017, The efficacy and toxicity of using the Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (Agaricomycetes), and its products in chemotherapy (review), International Journal of Medicinal Mushrooms, 19(10): 861-877.
https://doi.org/10.1615/IntJMedMushrooms.2017024537