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Early Expression of *fps*, *sqs*, and *ls* Genes in *Ganoderma lucidum* and *G. mexicanum* under Static Liquid Culture

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Abstract

The *Ganoderma* genus is recognized for its high content of bioactive compounds, including triterpenoids. The biosynthesis of these metabolites occurs via the mevalonate pathway and is regulated by the expression of key genes such as *fps*, *sqs*, and *ls*. This study analyzed the early expression of these genes in *Ganoderma lucidum* and *G. mexicanum* under static liquid culture. Vineyard pruning extracts prepared with different solvents were used to induce triterpenoid production. Gene expression was evaluated in mycelium cultured at 0, 0.5, 1, and 24 h with elicitor (ht=treatment) or alcohol (hc=control), along with colorimetric quantification of triterpenoids. In *G. lucidum*, *sqs* reached its highest expression at 0.5 ht (20.56-fold increase), while *fps* was higher at 0 h, and *ls* remained repressed. In *G. mexicanum*, *sqs* showed its highest values at 24 ht (11.47-fold increase) and at 1 hc (8.03-fold increase), while *fps* and *ls* were more expressed at 24ht and 1hc. The quantification of triterpenoids partially coincided with expression patterns. These results suggest that vineyard pruning extracts effectively induce metabolite production without genetic modifications, highlighting the utility of optimizing culture conditions.
Keywords: medicinal mushroom, vineyard pruning, fungal cultivation, gene expression.

Expresión temprana de los genes *fps*, *sqs* y *ls* de *Ganoderma lucidum* y *G. mexicanum* en cultivo líquido estático

Resumen

El género *Ganoderma* es reconocido por su alto contenido de compuestos bioactivos, entre ellos los triterpenoides. La biosíntesis de estos metabolitos ocurre mediante la vía del mevalonato, regulada por la expresión de los genes *fps*, *sqs* y *ls*, analizados para este estudio en su etapa temprana en las especies de *G. lucidum* y *G. mexicanum* y cultivo líquido estático. Se utilizaron extractos de madera de vid preparados con distintos solventes para inducir la producción de triterpenoides. La expresión génica se evaluó en micelio cultivado a 0, 0.5, 1 y 24 h con elicitor (ht=tratamiento) y con alcohol (hc=control), junto con la cuantificación colorimétrica de triterpenoides. En *G. lucidum*, *sqs* alcanzó su máxima expresión a los 0.5 ht (20.56 veces más), *fps* fue mayor en 0 h y *ls* permaneció reprimido. En *G. mexicanum*, *sqs* mostró sus valores más altos a las 24 ht (11.47 veces más) y 1 hc (8.03 veces más), *fps* y *ls* en 24ht y 1hc. La cuantificación de triterpenoides coincidió parcialmente con los patrones de expresión. Estos resultados sugieren que los elicitores de madera de vid son efectivos para inducir la producción de metabolitos sin necesidad de modificaciones genéticas y destacan la utilidad de optimizar las condiciones de cultivo.
Palabras clave: hongo medicinal, madera de vid, cultivo de hongos, expresión génica.

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Introduction

Ganoderma P. Karst. (*Polyporaceae*), known as "reishi" and "lingzhi" in Asia, this basidiomycete is renowned for its medicinal value, having been used for centuries in various cultures for its nutraceutical and therapeutic properties (Liu *et al*., 2024; Rosales-López, Arce-Torres, Monge-Artavia & Rojas-Chaves, 2022). *G. lucidum* (Curtis) P. Karst. is the representative species within this genus due to its wide range of medicinal uses throughout millennia in Eastern cultures (Delgado & Ortíz, 2023). Taxonomic studies have identified over 300 *Ganoderma* species, most of which are distributed in tropical regions (Ahmad, Wahab, Ahmad, Ashraf, Abulais & Saad, 2022). Among native desert strains, species such as *G. mexicanum* Pat. (Angulo-Sanchez *et al.*, 2025), initially recorded in Sonora as *G. weberianum* (López-Peña, Gutiérrez, Hernández-Navarro, Valenzuela & Esqueda, 2016), has been less studied. This species has also been reported in other Mexican states (Estado de México, Morelos, and Veracruz), and other countries such as Argentina, Brazil, and Martinique (Cabarroi-Hernández, Villalobos-Arámbula, Torres-Torres, Decock & Guzmán-Dávalos, 2019).

Ganoderma is notable for its high content of bioactive compounds with diverse biological activities. *G. lucidum* produces triterpenoids such as ganoderic acids (GAs), demonstrating antimicrobial, anticancer, antiviral, and immunomodulatory properties (Angulo-Sánchez, López-Peña, Torres-Moreno, Gutiérrez, Gaitán-Hernández & Esqueda, 2022). This anticancer activity is often based on cytotoxic effects, a common feature among many sterols of fungal and plant origin that target cancer cells, harming normal cells (Liu, Ren, Sang, Cheng & Bi, 2025; Woyengo, Ramprasath & Jones, 2009), which underscores the importance of characterizing specific metabolite profiles. The biosynthesis of these triterpenoids in *Ganoderma* occurs via the mevalonate (MVA) pathway (Galappaththi *et al*., 2023). Despite GAs' essential functions, their low yield poses a critical challenge for clinical trials and commercial applications. Currently, these compounds are obtained from the fruiting bodies and mycelia of *G. lucidum*; however, production is limited (Liu & Wang, 2023; Fei, Li, Zhang & Xu, 2019). Notably, the output of GAs through liquid fermentation has shown promising potential (Liu *et al*., 2024).

In *G. lucidum*, elicitation is the primary strategy for increasing triterpenoid production (Gu, Zheng, Lian, Zhong & Liu, 2018). Various elicitors have been evaluated to stimulate the synthesis of these bioactive compounds, optimizing their production through specific culture systems (Pilafidis, Diamantopoulou, Gkatzionis & Sarris, 2022). In Sonora, vineyard pruning is one of the most abundant agricultural residues. It is an ideal substrate for *Ganoderma* cultivation due to its rich cellulose and lignin content, providing an excellent nutrient source for fungal development (Cabrera, López-Peña, Asaff, Esqueda & Valenzuela-Soto, 2018). Additionally, elicitors derived from vineyard pruning extracts contain compounds such as catechin gallate, which have been shown to enhance *Ganoderma* mycelial growth (Angulo-Sánchez *et al*., 2024).

Genes encoding key enzymes involved in GA biosynthetic pathways have been identified (Figure 1). Among them is 3-hydroxy-3-methylglutaryl coenzyme A reductase (*hmgr*), which regulates the production rate of terpene intermediates. Farnesyl diphosphate synthase (*fps*) plays a branching-point role in the pathway for various terpenes, including triterpenes. Squalene synthase (*sqs*) catalyzes the first step in triterpene biosynthesis, and lanosterol synthase (*ls*) plays an essential role in the formation of lanostane-type GA skeletons (Sun, You & Xu, 2021; Fei *et al.*, 2019). This study aimed to elucidate the early expression of *fps*, *sqs*, and *ls* genes involved in triterpenoid biosynthesis, to optimize triterpenoid production.

Materials and Methods

Vineyard pruning extracts

Vineyard pruning (*Vitis vinifera* cv. Ruby Seedless L.) was used as substrate, collected in Pesqueira, Hermosillo, Sonora, Mexico (29°21'20.64''N, 110°51'43.30''W), following the protocol of Harris-Valle, Esqueda, Sánchez, Beltrán-García & Valenzuela-Soto (2007). The elicitor extraction of polar and non-polar compounds, as well as the selection of treatments that promote the highest biomass production of *Ganoderma lucidum* and *G. mexicanum*, were 1 mg/mL and 2 mg/mL, respectively, based on Cruz-Félix *et al*. (2024). A stock solution of vineyard pruning extracts with a concentration of 1 g/mL was prepared for this purpose. For the *G. lucidum* elicitor, an equal-part combination (1:1:1:1) of toluene:chloroform:ethanol:aqueous extracts was used. From this stock, 0.4 mL were added to each culture flask (400 mL) to achieve a final elicitor concentration of 1 mg/mL. For *G. mexicanum*, a 3:1 polar:non-polar ratio (ethanol-aqueous:toluene-chloroform) was used. From this stock, 0.8 mL were added to each flask to reach a final elicitor concentration of 2 mg/mL. As a control, 0.4 mL of 70% ethanol was added to separate cultures.

Static culture of *Ganoderma*

The *G. mexicanum* (BH-21) and *G. lucidum* (FP-34D) strains used in this study are part of the Fungal and Plant Biotechnology Laboratory collection at the Center for Research in Food and Development (CIAD) and from Fungi Perfecti, respectively. Petri dishes with malt extract agar medium were used for inoculum preparation. Static liquid mycelial cultures were established in 1 L flasks containing 400 mL of minimal medium supplemented with 40 g/L glucose and 10 g/L peptone. Incubation was carried out at 25 °C for 21 days in darkness.

Each flask was inoculated with eight 1 cm diameter mycelium disks. Vineyard pruning extracts elicitors were incorporated into the corresponding strain cultures. Four conditions were

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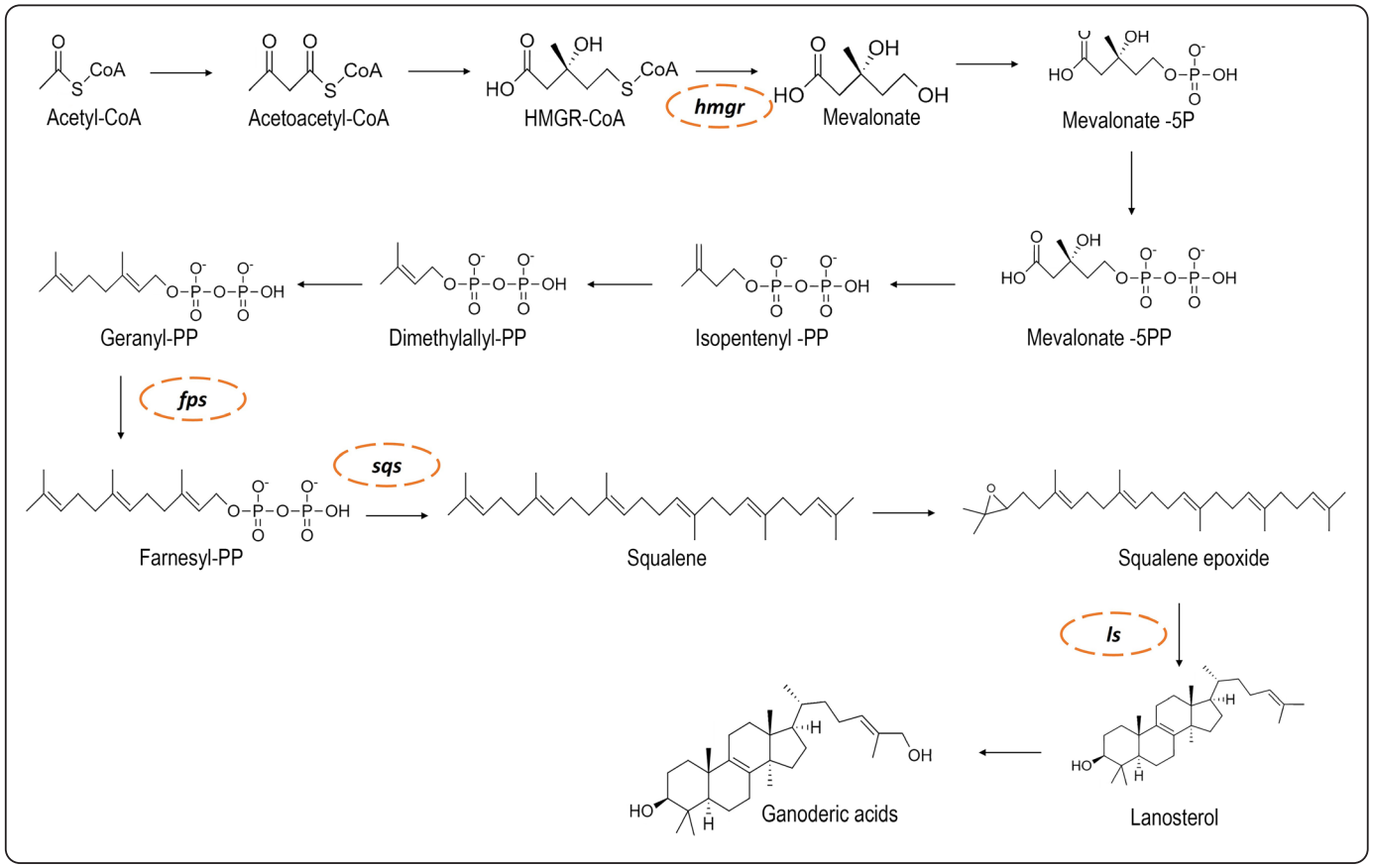

Figure 1. Mevalonate metabolic pathway. Genes encoding enzymes involved in the path are marked in orange. Modified from Angulo-Sanchez *et al*. (2022).

evaluated to analyze gene expression. Samples were taken at different time intervals after extract (t=treatment) or ethanol (c=control) application using separate flasks for each time point: 0.5 h, 1 h, and 24 h. A fifth flask served as a baseline (0 h), from which the sample was collected immediately before the application of the elicitor or ethanol.

Gene expression

Total RNA was extracted using TRIzol (Invitrogen, Massachusetts, USA). Its integrity was verified by 1.2% agarose gel electrophoresis and quantified by spectrophotometry at 260 nm in a NANODROP-1000 (Thermo Fisher Scientific, Massachusetts, USA). Genomic DNA was removed with DNase I (Invitrogen). Complementary DNA (cDNA) was synthesized from 500 ng of RNA, using the Superscript III reverse transcriptase kit (Thermo Fisher Scientific, Massachusetts, USA). A pooled cDNA sample was prepared from three biological replicates for real-time PCR (qPCR) analysis of gene expression. The primers used are shown in Table I. Each reaction included 2 µL of cDNA (100 ng/µL), 0.5 µL of each primer (10-pmol/µL), 5 µL of SYBR Green I (Bio-Rad, California, USA), and 2.4 µL of nuclease-free water. In addition to the target genes, the constitutive 18S rRNA gene was used as a reference gene.

A StepOne Real-Time PCR System (Life Technologies, California, USA) was used according to the manufacturer's instructions (Berumen-Varela *et al*., 2018). Amplification conditions were as follows: an initial denaturation phase at 95 °C for 2 min 30 sec, followed by 40 cycles consisting of denaturation at 95 °C for 30 sec, annealing at 55 °C for 30 sec, and extension at 72 °C for 40 sec; finally, an elongation phase at 72 °C for 5 min. Relative gene expression was analyzed using the $2^{-\Delta\Delta CT}$ method proposed by Livak & Schmittgen (2001). Relative expression for each condition was calculated using the 0h point as the reference, which was assigned the value of 1.

Triterpenoid quantification

Ethanolic extracts were obtained from lyophilized mycelium by sonicating for 30 min and then allowing the mixture (1:10) to stand in the dark for 24 h before recovering the supernatant. Triterpenoid quantification was performed using a colorimetric method described by Bidegain, Postemsky, Pieroni & Cubitto (2019). The resulting-colored product of

Table I. Genetic primer sequences *fps*, *sqs*, *ls*, and 18s.

Genes	Primers	Sequences (5´-3´)	References
fps	*fps*-forward	CCTCATCACCGCTCCAGAA	Zhao, Xu & Zhong (2011)
	fps-reverse	GGCGACGG GAAGGTAGAAG	
sqs	*sps*-forward	TGACGCTTCCTGACGAGA	Xu *et al.* (2010)
	sps-reverse	GTGGCAGTAGAGGTTGTA	
ls	*ls*-forward	CTTCCGCAA GCACTACCCG	
	ls-reverse	AGCAGATGCCCACGAGCC	
18SARNr	*18SARNr*-forward	TATCGAGTTCTGACTGGGTTGT	
	18SARNr-reverse	ATCCGTTGCTGAAAGTTGTAT	

the reaction of triterpenoids with a vanillin-perchloric acid reagent was quantified by measuring its absorbance at 548 nm. Ursolic acid was used as the standard. Quantification was performed against a calibration curve prepared using serial dilutions of the standard, ranging from 95.24 to 333.3 µg/mL, which demonstrated a linear correlation ($R^2 = 0.94$). For the assay, 30 µL of the ethanolic extract (10 mg/mL) was added to each well of a microplate for the reaction. Each sample was analyzed in triplicate.

Statistical Analysis

All experiments, including the mycelial culture and triterpenoid quantification, were performed in triplicate, and the results are presented as the mean ± standard deviation. A completely randomized experimental design with a factorial arrangement (2×2×4) was applied. The NCSS (2022) software was used for the corresponding statistical analyses. A comparison of means was performed using the Tukey-Kramer test with a significance level ($p<0.05$).

Results and Discussion

Expression of *fps*, *sqs*, and *ls* genes

The expression of *fps* in *G. lucidum* (Figure 2A) was the highest at 0 h ($p<0.05$). This indicates that the control (hc) and the treatment (ht) suppressed early gene expression under all conditions, resulting in lower gene expression levels ($p<0.05$). The times relative to the reference gene (18S rRNA) ranged from 0.11±0.01 1ht to 0.45±0.17 24hc (Table II). In *G. mexicanum* (Figure 2D), the conditions with the highest expression ($p<0.05$) were 5.49±1.36 and 3.88±0.26-fold increase for 24ht and 1hc, respectively; while the lowest expression was 0.97±0.13 0.5hc (Table II). Expression of the *sqs* gene in *G. lucidum* (Figure 2B) showed a significantly higher level ($p<0.05$) under the 0.5ht condition, with a 20.56±7.32-fold increase compared to the rest of the samples, which did not exhibit statistically significant differences ($p>0.05$) among themselves, with the lowest increase of 1.11±0.56 at 24ht. For *G. mexicanum* (Figure 2E), the expression varied across conditions. The highest expression ($p<0.05$) was observed in 24ht and 1hc, with 11.47±3.87 and 8.03±3.25-fold increases, respectively. In the latter, expression was equivalent ($p<0.05$) to 1ht (2.27±1.05-fold increase). No differences were observed ($p>0.05$) in the remaining conditions (Table II).

For *ls* in *G. lucidum* (Figure 2C), the expression pattern was similar to *fps*, with gene repression observed under most conditions. The expression changed from 0.04±0 1ht to 1.1±0.25-fold increase 0.5 ht (Table II). In *G. mexicanum* (Figure 2F), following the trend observed in *sqs* and *fps*, the conditions with the highest expression ($p<0.05$) were 24ht, 1hc, and 1ht, with 1.56±0.32, 1.39±0.1, and 1.34±0.16-fold increases, respectively. The conditions with the lowest expression ($p<0.05$) were 0.5hc and 24hc, with 0.05±0.11 and 0.59±0.21, respectively. The relative expression of the genes involved in the triterpenoid biosynthesis pathway showed distinct patterns between the two *Ganoderma* species in response to the elicitors (Table II). In *G. lucidum*, a general repressive effect was observed for *fps* and *ls* genes after the application of both the control and the elicitor, compared to the baseline at 0 h. However, the *sqs* gene showed a notable and significant peak in expression, increasing 20.56-fold at 0.5 ht. Conversely, *G. mexicanum* exhibited a general trend of gene induction. The highest expression levels for all three genes were observed under 24 ht, particularly for *sqs* (11.47-fold increase) and *fps* (5.49-fold increase). Interestingly, the control condition at 1 h also significantly induced all three genes (Table II).

The *sqs* gene has shown high expression in various studies, such as Xu, Zhao & Zhong (2010), where it exhibited the highest expression compared to 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) and *ls*. This finding is consistent with our study's results and highlights the importance of *sqs* in regulating these biosynthetic pathways. This gene encodes squalene synthase, an enzyme critical in the mevalonate

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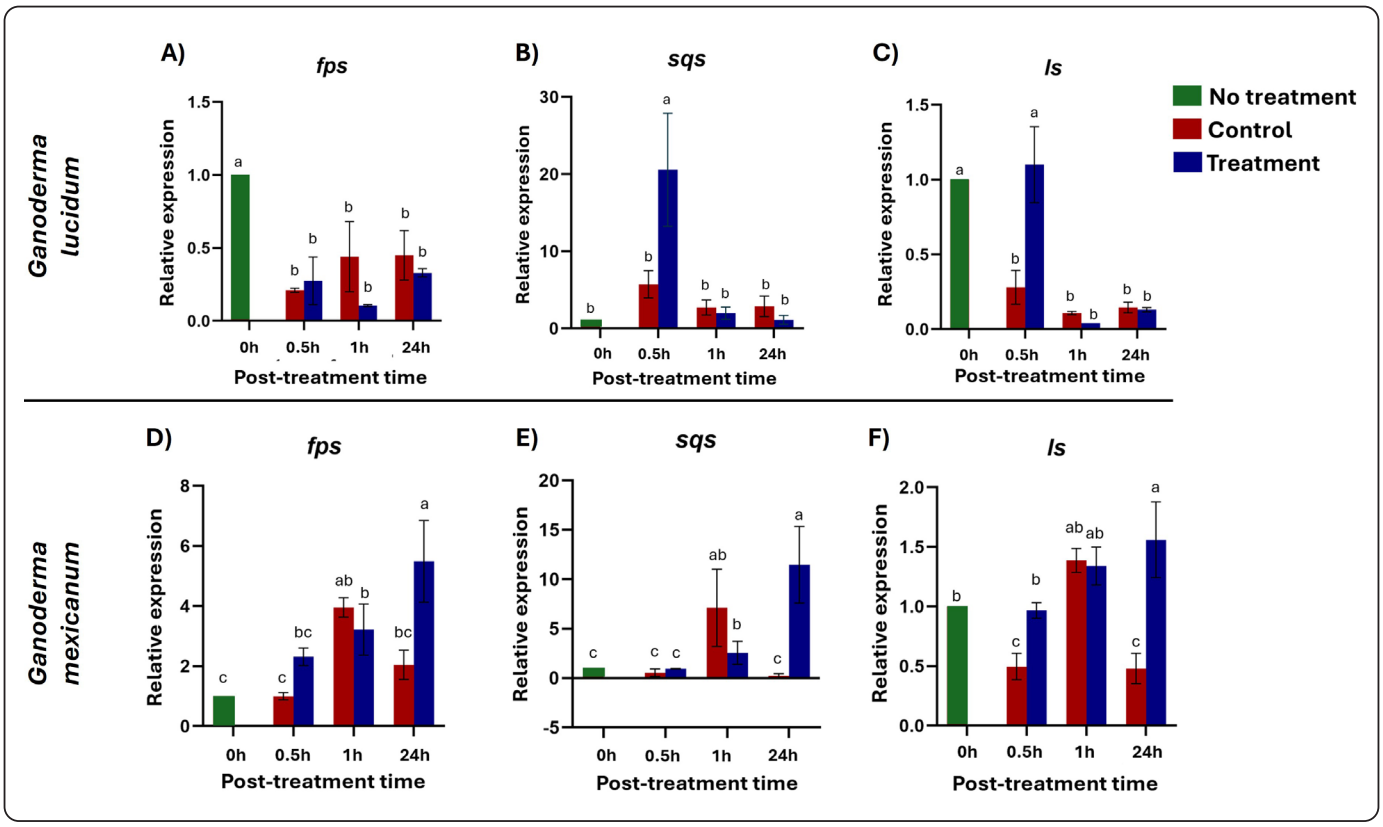

Figure 2. Expression of *fps*, *sqs*, and *ls* genes in *Ganoderma lucidum* and *G. mexicanum*. (A–C) Expression of *fps*, *sqs*, and *ls* in *G. lucidum*, respectively. (D–F) Expression of *fps*, *sqs*, and *ls* in *G. mexicanum*, respectively. Letters indicate statistically significant differences according to the Tukey–Kramer test ($p<0.05$).

Table II. Relative expression of the *fps*, *sqs*, and *ls* genes in *Ganoderma lucidum* and *G. mexicanum*. Expression is shown as relative fold change ± standard deviation, with the 0 h condition (no treatment) serving as the reference and assigned a value of 1. Letters indicate statistically significant differences ($p<0.05$) in the same gene and species.

Species	Gene	Time (h)	Condition	Relative Expression (Fold Change)
Ganoderma lucidum	*fps*	0	Baseline	1.00 ± 0.00^{a}
		0.5	Control	0.21 ± 0.02^{b}
		0.5	Treatment	0.28 ± 0.16^{b}
		1	Control	0.44 ± 0.24^{b}
		1	Treatment	0.10 ± 0.01^{b}
		24	Control	0.45 ± 0.17^{b}
		24	Treatment	0.33 ± 0.03^{b}
	sqs	0	Baseline	1.00 ± 0.00^{b}
		0.5	Control	5.71 ± 1.78^{b}
		0.5	Treatment	20.56 ± 7.33^{a}
		1	Control	2.71 ± 0.98^{b}
		1	Treatment	1.98 ± 0.80^{b}
		24	Control	2.86 ± 1.32^{b}
		24	Treatment	1.11 ± 0.57^{b}

Table II. Relative expression of the *fps*, *sqs*, and *ls* genes in *Ganoderma lucidum* and *G. mexicanum*. Expression is shown as relative fold change ± standard deviation, with the 0 h condition (no treatment) serving as the reference and assigned a value of 1. Letters indicate statistically significant differences (p<0.05) in the same gene and species (*continuation*).

Species	Gene	Time (h)	Condition	Relative Expression (Fold Change)
Ganoderma lucidum	*ls*	0	Baseline	1.00 ± 0.00^{a}
		0.5	Control	0.28 ± 0.11^{b}
		0.5	Treatment	1.10 ± 0.25^{a}
		1	Control	0.11 ± 0.01^{b}
		1	Treatment	0.04 ± 0.00^{b}
		24	Control	0.15 ± 0.04^{b}
		24	Treatment	0.13 ± 0.01^{b}
Ganoderma mexicanum	*fps*	0	Baseline	1.00 ± 0.00^{c}
		0.5	Control	0.97 ± 0.12^{c}
		0.5	Treatment	2.32 ± 0.29^{bc}
		1	Control	3.96 ± 0.30^{ab}
		1	Treatment	3.02 ± 0.73^{b}
		24	Control	2.05 ± 0.49^{bc}
		24	Treatment	5.49 ± 1.36^{a}
	sqs	0	Baseline	1.00 ± 0.00^{c}
		0.5	Control	0.54 ± 0.38^{c}
		0.5	Treatment	0.95 ± 0.02^{c}
		1	Control	8.03 ± 3.25^{ab}
		1	Treatment	2.27 ± 1.05^{b}
		24	Control	0.22 ± 0.22^{c}
		24	Treatment	11.47 ± 3.88^{a}
	ls	0	Baseline	1.00 ± 0.00^{b}
		0.5	Control	0.50 ± 0.11^{c}
		0.5	Treatment	0.96 ± 0.07^{b}
		1	Control	1.39 ± 0.10^{ab}
		1	Treatment	1.34 ± 0.16^{ab}
		24	Control	0.59 ± 0.21^{c}
		24	Treatment	1.56 ± 0.32^{a}

biosynthesis pathway and a potential regulatory point controlling carbon flux toward the biosynthesis of triterpenoids and sterols (Zhou, Ji, Ren, He, Jing & Xu, 2014). This regulatory role may explain why *sqs* shows higher expression under certain conditions. In the study by Xu *et al*. (2022), relative expression levels of *hmgr*, *sqs*, and *ls* in *G. lucidum* peaked at 12 h following induction with methyl jasmonate. The supplementation with sodium acetate (4 mM) significantly increased the GAs content of *G. lucidum* fruiting bodies by 28.63% compared to the control. The acetate ion increased the expression of *hmgs*, *fps*, and *sqs*. Na^{+} supplementation and the consequent exchange of Na^{+}/Ca^{2+} induced GAs biosynthesis (Meng *et al*., 2019). However, culture conditions differed, as these studies used solid medium, whereas our research observed different peak expression times, suggesting that variations in cultivation conditions significantly impact gene regulation.

Total triterpenoid quantification

The production of total triterpenoids was significantly influenced by the interaction between the fungal species and the culture conditions ($p<0.05$). In *G. lucidum* (Figure 3A), the highest concentrations were unexpectedly found in the untreated sample at 0h (55.5±14.5 µg/mL) and in the control at 0.5h (93.94±37.3 µg/mL). This partially aligns with the high initial expression of *fps* and *ls* at 0h, but the peak production in the control condition did not correlate with increased expression of the studied genes.

In *G. mexicanum* (Figure 3B), significantly higher triterpenoid production was observed across several induced conditions: 0.5ht, 1hc, 1ht, and 24hc, with concentrations ranging from approximately 50 to 58 µg/mL. This increased production is consistent with the gene induction patterns observed, where *sqs*, *fps*, and *ls* showed elevated expression in one or more of these conditions. Notably, while the elicitor successfully induced metabolite production in *G. mexicanum*, its maximum yield (~58 µg/mL) was considerably lower than the peak observed in the *G. lucidum* control (~94 µg/mL).

The ability of *G. mexicanum* to synthesize triterpenoids under a broader range of experimental conditions, compared to *G. lucidum*, suggests greater biochemical plasticity and adaptability to environmental conditions. This trait may be crucial for its use as a source of triterpenoids, as its production profile is more adaptable to varying cultivation conditions. In *G. lucidum*, the general repression of *fps* and *ls* suggests that the vineyard pruning extracts, under these conditions, may not be an effective elicitor and could even have an inhibitory effect on the main triterpenoid biosynthesis pathway. The singular, sharp induction of *sqs* at 0.5 ht, which did not translate into a higher triterpenoid yield, indicates a possible bottleneck further down the metabolic pathway or a more complex regulatory mechanism not captured by the expression of these three genes alone. This contrasts with other studies where elicitors such as methyl jasmonate have successfully induced the entire pathway, although under different culture conditions (Ren *et al*., 2013).

The enhanced adaptability of *G. mexicanum* may be attributed to its native origin in a semiarid region of the Sonoran Desert, which likely confers greater genetic variability than the commercial *G. lucidum* strain, optimized for stable, controlled environments. The *sqs* gene, which encodes the first committed enzyme in triterpenoid biosynthesis, was the most responsive in both species, consistent with other studies that identify it as a key regulatory point in this pathway (Fei *et al.*, 2019; Ye, Liu, Xie, Zhao & Wu, 2018; Cao *et al*., 2017). However, our results emphasize that inducing a single gene is not sufficient to guarantee an increase in the final product. A successful elicitation strategy requires coordinated upregulation of the entire biosynthetic path. These findings underscore that optimizing the production of secondary metabolites in fungi is a highly species-specific process, where the strains' genetic background is as crucial as the culture conditions themselves.

Elicitors are compounds that activate an organism's defense response and signal transduction, leading to significant changes in gene expression. Since environmental factors regulate the biosynthesis of secondary metabolites, the mechanisms that control this production in fungi remain incompletely understood. This is due to the complexity of regulatory networks and gene interactions, which complicate the interpretation of responses to external stimuli (Gu *et al.*, 2018; Ren *et al*., 2013). Gene expression in *G. lucidum* and *G. mexicanum* is regulated by environmental conditions, and the production of reactive oxygen

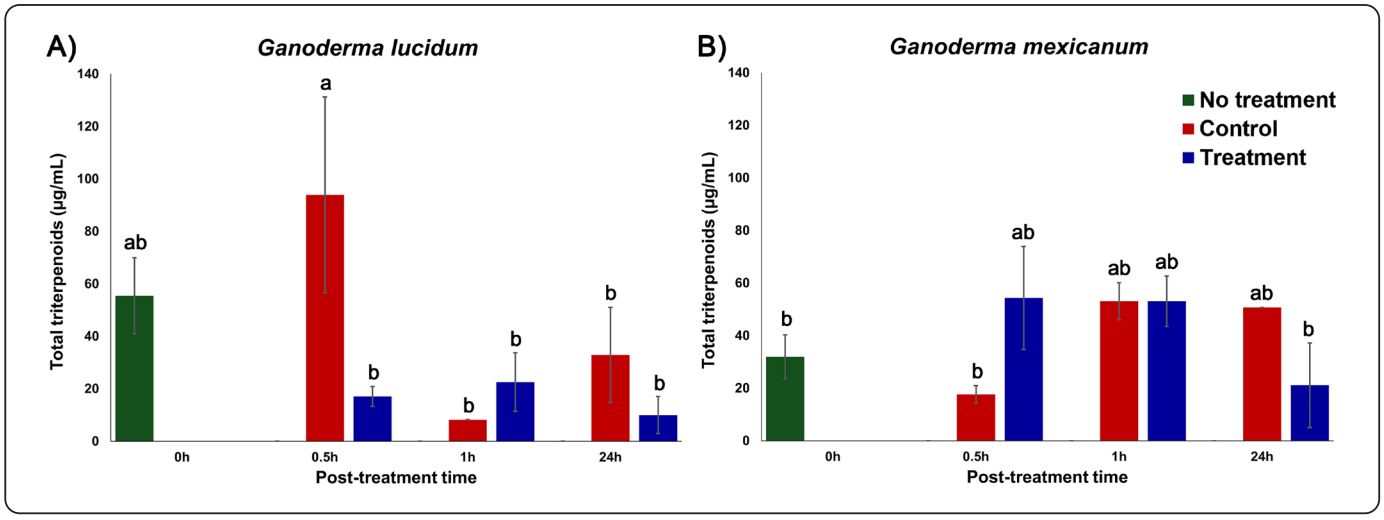

Figure 3. Total triterpenoid quantification. A: Triterpenoids from *Ganoderma lucidum*. B: Triterpenoids from *Ganoderma mexicanum*. Letters indicate statistically significant differences based on the Tukey–Kramer test ($p<0.05$). Two diagonal lines on the x-axis indicate time intervals.

species (ROS) has also been identified as a key factor in GA synthesis (Angulo-Sanchez *et al*., 2022).

In *G. lucidum*, 53 transcription factor families have been characterized, among which the homeobox transcription factor and velvet family protein play key roles in regulating GA biosynthesis (Meng *et al*., 2022). The variability in expression of the *fps*, *sqs*, and *ls* genes between the two species reflects the complexity of their regulation under different treatments and cultivation conditions. In *G. lucidum*, the repression of *fps* and *ls* suggests either stricter transcriptional control or reduced adaptive capacity in the presence of elicitors. *G. mexicanum*, on the other hand, has greater genetic diversity and exhibits more flexible gene expression, which may favor its adaptation to environmental changes and enhance GA production. These results suggest that a complex interaction between environmental factors, transcriptional regulation, and genetic variability influences the secondary metabolite biosynthesis in these species.

Conclusions

Vineyard pruning waste of extracts influences the relatively early expression of the *fps*, *sqs*, and *ls* genes in *Ganoderma mexicanum* and *G. lucidum* under static liquid culture. In *G. mexicanum*, the expression of these genes increases, whereas in *G. lucidum*, *ls* and *fps* are repressed, and *sqs* is only upregulated under specific conditions. The observed differences reflect adaptations of each strain to environmental stimuli and the tested extracts. The quantification of total triterpenoids varied significantly across the studied species, consistent with early and dissimilar gene expression patterns in response to vinewood elicitors. This study will contribute to future strategies to optimize triterpenoid production using mycelium in liquid culture media.

Acknowledgments

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