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Analysis of phytohormone-related gene expression in cotton following *Beauveria bassiana* GHA endophytic association

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Abstract

Cotton (*Gossypium hirsutum* L.) is a vital global crop but suffers heavy losses from insect pests that reduce yield and fiber quality. Conventional pesticide-based control causes environmental harm, underscoring the need for sustainable alternatives. *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), an entomopathogenic fungus, offers promise as both a biocontrol agent and a plant endophyte that can enhance host defense responses. This study investigates how inoculation with live or heat-killed spores of *B. bassiana* GHA affects the expression of defense-related genes in cotton, focusing on key components of the ethylene, salicylic acid, and jasmonic acid signaling pathways. Four genes ERF1 and MPK2 (ethylene pathway), WRKY (salicylic acid pathway), and JAZ1 (jasmonic acid pathway) were analyzed at 2, 8, 24, and, 48 hours post-inoculation. Results showed that live spores markedly upregulated all target genes compared with controls, indicating strong systemic activation of defense signaling. ERF1 and MPK2 expression peaked at 48 hours, showing 46.55-fold and 29.75-fold increases, while WRKY and JAZ1 peaked at 24 hours with 26 and 21.98-fold increases, respectively. Heat-killed *B. bassiana* spores caused a significant increase in ERF1 and MPK2 expression (12.86- and 12.11-fold at 2–8 hours), while JAZ and WRKY genes showed no significant change. These findings demonstrate that *B. bassiana* can induce broad-spectrum systemic resistance in cotton through coordinated activation of multiple hormonal pathways. The results highlight its potential to strengthen plant immunity and reduce dependence on chemical pesticides, offering a promising strategy for sustainable pest management and environmentally resilient cotton production.

Keywords: Biological control, Ethylene, Entomopathogenic fungi, Plant defense system Real-time PCR, Signaling pathway

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ارزیابی بیان ژن های مرتبط با هورمون های گیاهی در پنبه پس از تلقیح با قارچ اندوفیت *Beauveria bassiana*-GHA

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چکیده

پنبه (*Gossypium hirsutum* L.)از مهم‌ترین محصولات کشاورزی است که تحت تأثیر آفات متعدد قرار دارد. استفاده از قارچ‌های بیمارگر حشرات نظیر *Beauveria bassiana* GHA (Balsamo) Vuillemin (Ascomycota: Hypocreales) به‌عنوان اندوفیت، رویکردی نوین در مدیریت زیست‌محور آفات محسوب می‌شود. این پژوهش با هدف بررسی اثر تلقیح گیاه پنبه با اسپورهای زنده و مرده این قارچ بر بیان ژن‌های دخیل در مسیرهای دفاعی اتیلن (ERF1 و MPK2)، اسید سالیسیلیک (WRKY) و اسید جاسمونیک (JAZ1) انجام شد. گیاهان در مرحله چهاربرگی با سه تیمار شامل اسپورهای زنده، اسپورهای مرده و سوسپانسیون (Tween 20) در شرایط گلخانه‌ای تلقیح شدند. نتایج نشان داد که هر دو اسپور مرده و زنده باعث افزایش معنی‌دار در بیان ژن‌های دفاعی شدند. بیشترین بیان ژن‌های مرتبط با مسیر اتیلن (ERF1 وMPK2) ٤٨ ساعت پس از تلقیح با اسپور زنده مشاهده شد (به‌ترتیب ٤٦/٥٥ و ٢٩/٧٥ برابر نسبت به شاهد). بیشترین افزایش بیان ژن‌های WRKY و JAZ1 که به ترتیب در تعامل با مسیرهای دفاعی اسید سالیسیلیک و جاسمونیک هستند، ٢٤ ساعت پس از تلقیح با اسپور زنده رخ داد (به‌ترتیب ٢٦ و ٢١/٩٨ برابر نسبت به شاهد). همچنین، تیمار با اسپورهای مرده افزایش بیان ژن‌های ERF1 و MPK2 را در زمان‌های اولیه (٢ و ٨ ساعت) موجب شد. این نتایج نشان می‌دهد که *B. bassiana* با تحریک مسیرهای مختلف هورمونی، موجب تقویت دفاع سیستمیک گیاه پنبه شده و می‌تواند به‌عنوان بخشی از راهکارهای مدیریت تلفیقی آفات مورد استفاده قرار گیرد.

کلید واژه‌ها: اتیلن، قارچ های بیمارگر حشرات بیان ژن، سیستم دفاعی گیاه، کنترل بیولوژیکی، واکنش زنجیره‌ای پلیمراز در زمان واقعی، مسیر سیگنالینگ

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Introduction

Cotton is a critical natural fiber crop essential to the global textile industry and the livelihoods of millions, especially in developing countries. While economically significant, cotton cultivation confronts challenges from pests, diseases, and environmental stresses, highlighting the need for sustainable farming practices (Chi et al., 2021). Several insect species have been recognized as major pests of cotton, including the aphid *Aphis gossypii* Glover (Hemiptera: Aphididae), the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), the corn earworm *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), the beet armyworm *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), the tobacco budworm *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae), the lygus bug *Lygus hesperus* Knight (Hemiptera: Miridae), the cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), and the cotton boll weevil *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) (Rajendran et al., 2018; Naseri et al., 2023). Exploring effective pest management strategies is of high importance in mitigating the damage caused by these pests and ensuring both economic and production sustainability (Hagenbucher et al., 2013).

The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) has been widely researched for its potential utilization as a biological control agent targeting arthropod pests (Mascarin & Jaronski, 2016; Ebrahimifar et al., 2017; Farrokhzadeh et al., 2025; Zamani et al., 2025). Entomopathogenic fungi are typically administered through spraying techniques (Dannon et al., 2020; Farrokhzadeh et al., 2024). Nevertheless, a major challenge in using these fungi is maintaining the persistence of conidia after application, as abiotic factors such as ultraviolet radiation and drought can greatly reduce their survival, particularly in situations where secondary infection from treated plants plays a key role (Jaronski, 2010; Farrokhzadeh et al., 2024).

Recent research has indicated that the GHA strain of *B. bassiana* is capable of colonizing the host plant and forming a symbiotic association (Donga et al., 2018; Tall & Meyling, 2018; Nishi et al., 2020; Cavazos-Vallejo et al., 2023; Iida et al., 2023). This colonization and establishment of *B. bassiana* within plant tissues is called "endophytic" behavior (Qin et al., 2021; Karooei et al., 2025). The endophyte receives nutrients and protection from environmental stresses through the host plant, which in turn benefits from the symbiotic relationship with the endophyte (Quesada-Moraga et al., 2009; Bamisile et al., 2018). When the plant detects the presence of the endophyte, the plant immune genes linked to salicylic acid, jasmonic acid, and ethylene signaling pathways are upregulated. These upregulation mechanisms lead to signaling transduction involving the production of various chemical compounds, such as antimicrobial peptides, which can have deterrent or toxic effects on insects or other pests as well as plant pathogens (Yan et al., 2019) while also reducing insect performance and/or damage (Christian et al., 2020; Silva et al., 2020).

In cotton, defense against biotic stress is tightly regulated by key phytohormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), which coordinate distinct but interrelated immune responses. SA is primarily implicated in resistance to biotrophic pathogens by activating systemic acquired resistance (SAR), largely through NPR1-mediated signaling and induction of pathogenesis-related (PR) genes. JA and ET, on the other hand, play critical roles in defending cotton against necrotrophic

pathogens and herbivorous insects through regulating defense-related genes such as JAZ1, MPK2, and ERF1. JA signaling is contingent upon the degradation of JAZ repressors, which releases transcription factors to activate defense gene expression (Zhang et al., 2025). ET perception results in a signaling cascade involving EIN2 and EIN3, ultimately enhancing expression of ethylene-responsive factors such as ERF1. All these hormones help cotton plants initiate effective responses to different classes of pathogens and pests (Lorenzo et al., 2003).

These hormonal pathways are not independent but function through complex cross-talk that allows the plant to tailor its defense strategy. SA and JA pathways often act antagonistically; activation of SA signaling along biotrophic infections may suppress JA-mediated defenses to conserve energy (Koornneef & Pieterse, 2008). Conversely, JA and ET act synergistically in cotton's defense against insect herbivory and necrotrophic fungi, jointly regulating genes implicated in wound and stress responses. This regulatory balance is essential for optimizing defense while minimizing growth penalties (Li et al., 2019). The ability of beneficial microbes such as *B. bassiana* to activate multiple signaling pathways suggests a promising strategy for boosting cotton's innate immunity. Understanding how these hormones interact in cotton can inform the development of integrated pest management systems using endophytic biocontrol agents.

Using nontransgenic cotton seed and the *B. bassiana* (GHA), the present study evaluates the expression of cotton plant immune genes associated with ethylene, salicylic acid, and jasmonic acid signaling pathways.

Materials and Methods

Experimental plants

Nontransgenic cotton seeds (*Gossypium hirsutum* L., variety DP 1822 XF) were planted individually in 1.8-liter pots containing potting mix (Beltwide company, Tampa, Florida) and kept inside a greenhouse with conditions set at 25 °C ± 2 °C temperature, 56 ± 4% relative humidity, and a 14-hour light/10-hour dark photoperiod. At the time of the experiment, the plants had reached the four-leaf stage.

Inoculum preparation

For the experiments, dry conidial powders of *B. bassiana* GHA (live spores), serving as the active ingredient in BotaniGard formulations (Certis Bio LLC), were produced via solid substrate fermentation according to the procedures described by Jaronski and Jackson (2012). Conidial viability exceeded 95%, as determined by germination on Potato Dextrose Agar (PDA) (Thermo Fisher Science, USA) following an 18-hour incubation at 25 ± 1 °C (Mehrmoradi et al., 2022). Dead spores of *B. bassiana* GHA were employed to differentiate plant gene expression responses arising from the recognition of fungal surface components from those caused by active fungal infection. To kill the spores, they were incubated at 45 °C for 4 hours, with their non-viability confirmed by testing conidial germination on PDA. The final conidial concentration was ascertained using a Neubauer hemacytometer (0.0025 mm², LW Scientific, New York, NY) (Goettel & Inglis, 1997). When the plants reached the four-leaf stage, the seedlings were treated with one of three treatments: (1) 1×10^8 live *B. bassiana* GHA conidia per ml in 0.01% (v/v) aqueous polyoxyethylene (20) sorbitan monolaurate (Tween 20; Sigma-Aldrich, Rockville, MD); (2) 1×10^8 dead *B. bassiana* GHA conidia per ml in 0.01% (v/v) aqueous Tween 20; or (3) 0.01% (v/v) aqueous Tween 20 suspension without fungal conidia, serving as the control. Inoculation was carried out by spraying 2 ml of the suspension onto each plant using an

adjustable-spray Mini-Wash Bottle (Fisherbrand™ Norristown, PA).

Samples were collected at four time points: 2, 8, 24, and 48 hours post-inoculation. At each interval, a 1.5 cm diameter leaf disk (0.5 g) was excised from the central region of each fully developed leaf on each seedling by a sterilized scalpel and placed into a sterilized microtube containing ceramic beads. The microtube was immediately frozen in liquid nitrogen. Once transferred to the lab, the samples were ground using a laboratory grinder. RNA was extracted following the protocol of the Plant Mini Kit (Qiagen, USA), with the RNA concentration measured using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc, USA).

Real-time PCR

The qRT-PCR reactions were undertaken in a total volume of 10 µL, containing 5 µL of Luna Universal (one-step reaction) master mix (New England Biolabs, USA), 0.5 µL of Luna Warm Start RT enzyme, 0.4 µL of each forward and reverse primer (10 µM) (Table 1), 0.5 µL of RNA template (50 ng/µL), and 3.2 µL of double-distilled water. Amplification was done on a Thermo ABI QuantStudio 5 Real-Time PCR System (96-well) (Applied Biosystems, USA) with the following thermal cycling protocol: reverse transcription at 60 °C for 10 minutes, initial denaturation at 95 °C for 1 minute, followed by 43 cycles of denaturation at 95 °C for 10 seconds, annealing at 60.2 °C for 30 seconds, and extension at 60 °C for 10 seconds. A melt curve analysis from 60 to 95 °C was performed to confirm the specificity of the amplification products. There were five replicates per each treatment/sampling time with the experiment repeated three times.

Table 1. Target genes, primer sequences, and amplicon size used for quantitative real-time PCR analysis of cotton defense-related genes.

Target Gene	Primer name	Sequences	Size	References
mitogen-activated protein kinase 2	MPK2-F	GAACGTCTATTGATGTTTGGTCTGTT	137 bp	(Li et al., 2016)
	MPK2-R	ATCCTCCTCCTTCTGGCTGC		
Ethylene Response Factor (ERF)	ERF1-F	TGGTGGTGGGAGAAGAGGTTG	185 bp	(Li et al., 2016)
	ERF1-R	TCGGGACGAGTATTAGCGGAG		
WRKY transcription factor 40	WRKY-F	ACCATGCACGCCCTTCTCC	139 bp	(Li et al., 2016)
	WRKY-R	CCGTCCCCATACCCCTCTG		
jasmonate-zim-domain protein 1	JAZ1-F	TTATGGTGGACGAGTGATTGTGTT	147 bp	(Li et al., 2016)
	JAZ1-R	TTGATTGGACTTCTGGCTATGCT		
Housekeeping Gene	Ubiquitin-F	AGCTCGGATACGATTGATAACG	321 bp	(Wang et al., 2013)
	Ubiquitin-R	GAAGACGAAGAACAAGGGGAAG		

Statistical analysis

The Ct value was analyzed using the comparative Ct formula ($2^{-\Delta\Delta Ct}$) outlined by Pfaffl (2001) to calculate fold changes in the expression of the target genes. The resulting fold-change data were then analyzed using a repeated measures analysis of variance (ANOVA). Post hoc pairwise comparisons were performed using the Least Significant Difference (LSD) test to identify significant differences among treatment groups. All statistical analyses were undertaken using

IBM SPSS Statistic (version 29, Armonk, NY) (IBM-SPSS, 2024).

Results

Expression of the ERF1 gene varied significantly across treatments and time points following inoculation with live or dead *B. bassiana* spores. At 2 hours post-inoculation, both live and dead spore treatments led to moderate upregulation of ERF1 (5-fold and 12-fold, respectively), with no significant difference between them ($P > 0.05$). At 8 hours after inoculation, expression in the live spore treatment rose to 18-fold, which was not significantly different from the dead spore treatment (10-fold), but both remained meaningfully higher than the control ($P < 0.05$). At 48 hours after inoculation, the expression peaked in the live spore group (55-fold), keeping a higher level compared to the dead spore (8-fold) and control ($P < 0.05$). Overall, the expression of ERF1 gene was significantly influenced by the treatments ($F_{2,732} = 6.544$, $P= 0.002$) and post inoculation interval time ($F_{3,522} = 7.554$, $P= 0.004$). Further, the interaction between post inoculation interval time and treatment ($F_{6, 53} = 33.64$, $P = 0.002$) was significant (Figure 1).

The expression levels of the MPK2 gene indicated significant variation depending on the treatment type and the time elapsed after inoculation with either live or dead *B. bassiana* spores. No significant differences were found among live spores, dead spores, and control groups 2 hours post-inoculation. At 8 hours, MPK2 expression rose in plant treated with both live and dead spore (10–12-fold, respectively), but this growth was not significant between the two treatments. Nevertheless, expression levels in these groups were higher than those of the control plants. At 24 hours, expression in the plant treated with live spore was (28-fold), which was meaningfully higher than both plants treated with the dead spore (11-fold) and control plants ($P < 0.05$). A similar pattern was observed at 48 hours, with the plants treated with live spore group maintaining high expression (29-fold). The expression of the MPK2 gene was significantly impacted by the treatments ($F_{2,632} = 6.544$, $P=0.002$) and post inoculation interval time ($F_{3,422} = 7.554$, $P=0.004$). Further, the interaction between post inoculation interval time and treatment ($F_{6, 63} = 42.43$, $P = 0.003$) was significant (Figure 2).

The differential expression analysis for the WRKY gene revealed that at 2 hours post-inoculation, the changes in expression remained low and comparable across all treatments (live spore, dead spore, and control), with no statistically significant differences found ($P > 0.05$). By 8 hours, the plants treated with live spores exhibited a notable growth in the expression (10-fold), higher than both the dead spore and control groups ($P < 0.001$). The expression peaked at 24 hours post-inoculation in the live spore treatment, reaching 26-fold change, while the dead spore and control treatments continued to show minimal expression. At 48 hours, expression in the live spore group diminished to approximately 15-fold but remained higher than in the dead spore and control treatments ($P < 0.001$). WRKY gene expression was significantly affected by treatments ($F_{2, 472} = 4.544$, $P = 0.005$) as well as the time intervals following inoculation ($F_{3, 532} = 8.254$, $P = 0.003$). The interaction between post inoculation interval time and treatment ($F_{6, 53} = 38.84$, $P = 0.008$) was also significant (Figure 3).

The differential expression analysis of the JAZ gene revealed that at 2 and 8-hours post-inoculation, fold changes in the expression remained low across all treatments (live spore, dead spore, and control), with no significant differences observed ($P > 0.05$). At 24 hours post-inoculation, the plants treated with live spores exhibited a notable rise in the gene expression (22-fold), which was higher than

both the dead spore and control groups (P < 0.001). By 48 hours, the expression in the live spore treatment was still near its peak (21-fold) and remained higher than in the dead spore and control treatments (P < 0.001). The expression of the JAZ gene was significantly influenced by the treatments ($F_{2,572}$ = 14.514, P<0.001) and the post-inoculation interval ($F_{3,622}$ = 11.554, P<0.001). The interaction between post inoculation interval time and treatment ($F_{6, 53}$ = 29.94, P = 0.009) was also significant (Figure 4).

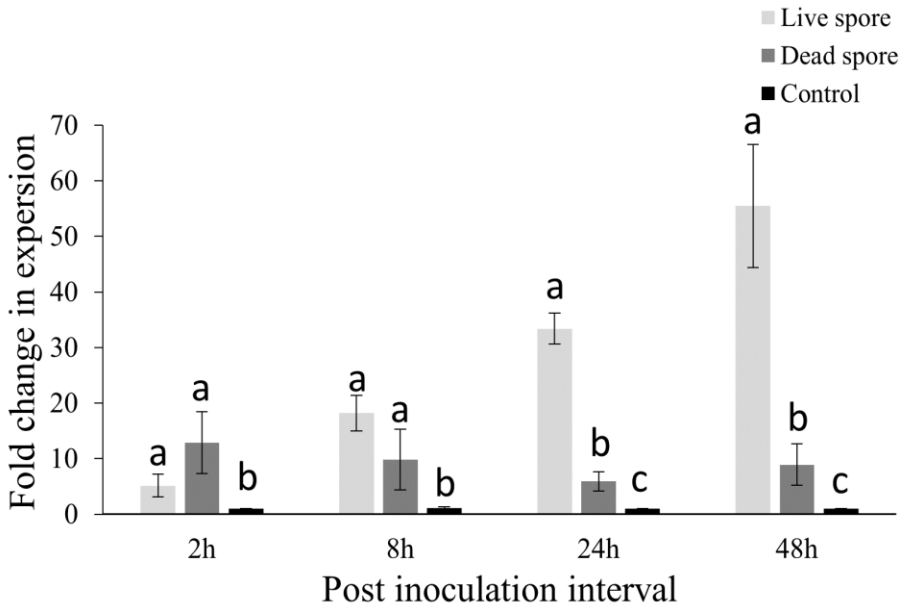


Figure 1. Results for differential expression of the ERF1 gene in the cotton plant between different treatments (P<0.05). The different letters reveal the significant difference between treatments. Error bars represent standard errors (±1SE). (n = 15) per sampling time treatment.

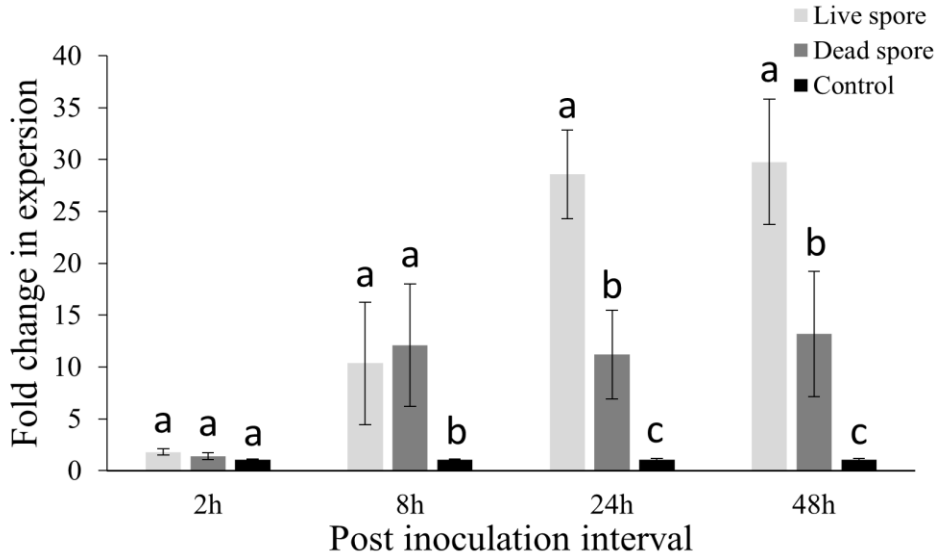


Figure 2. Results of differential expression of the MPK2 gene in the cotton plant between treatments (P<0.05). The different letters signal the significant difference between treatments. Error bars represent standard errors (±1SE). (n = 15) per sampling time treatment.

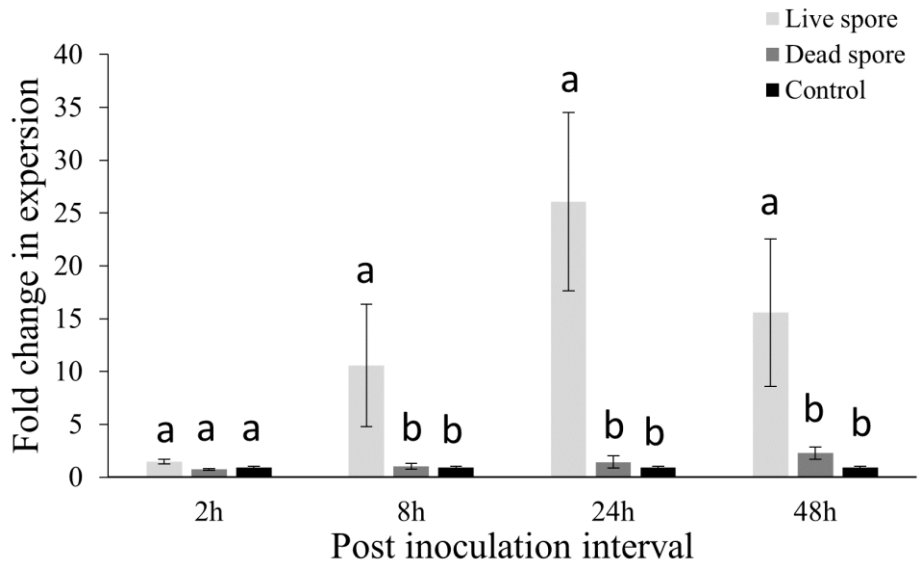


Figure 3. Results of differential expression of the WRKY gene in the cotton plant between treatments (*P*<0.05). The different letters reflect the significant difference between treatments. Error bars represent standard errors (±1SE). (n = 15) per sampling time treatment.

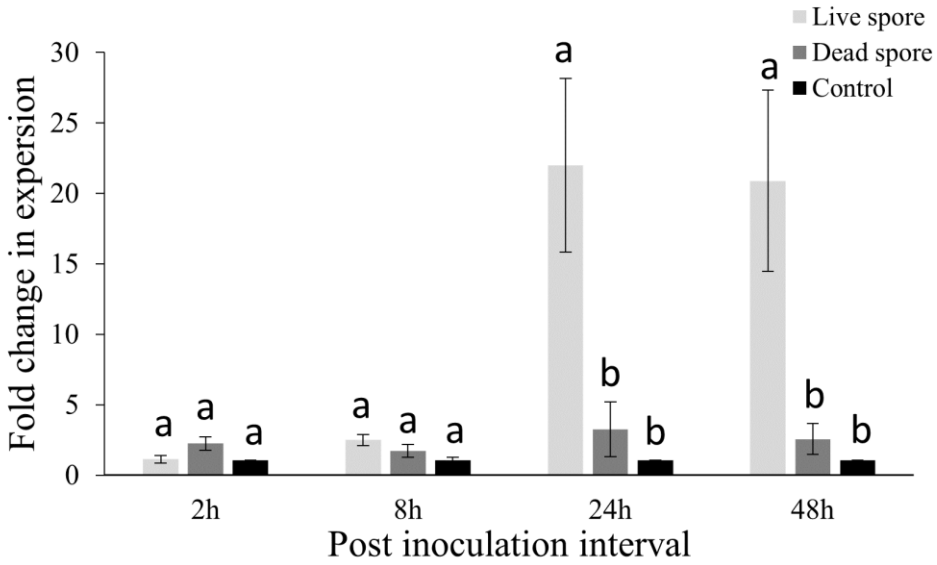


Figure 4. Results of differential expression of JAZ gene in the cotton plant between different treatments. (*P*<0.05). The different letters represent the significant difference between treatments. Error bars represent standard errors (±1SE). (n = 15) per sampling time treatment.

The heat map of results indicated that, between 2 and 48 hours post-inoculation, the expression levels of genes associated with the ethylene, salicylic acid, and jasmonic acid signaling pathways grew in the plants treated with both live and dead spores. Nevertheless, the most significant changes were observed in plants inoculated with live *B. bassiana* spores. Further, the results indicated that the genes involved in the ethylene signaling pathway exhibited greater expression changes over time compared to those related to the salicylic acid and jasmonic acid pathways (Figure 5).

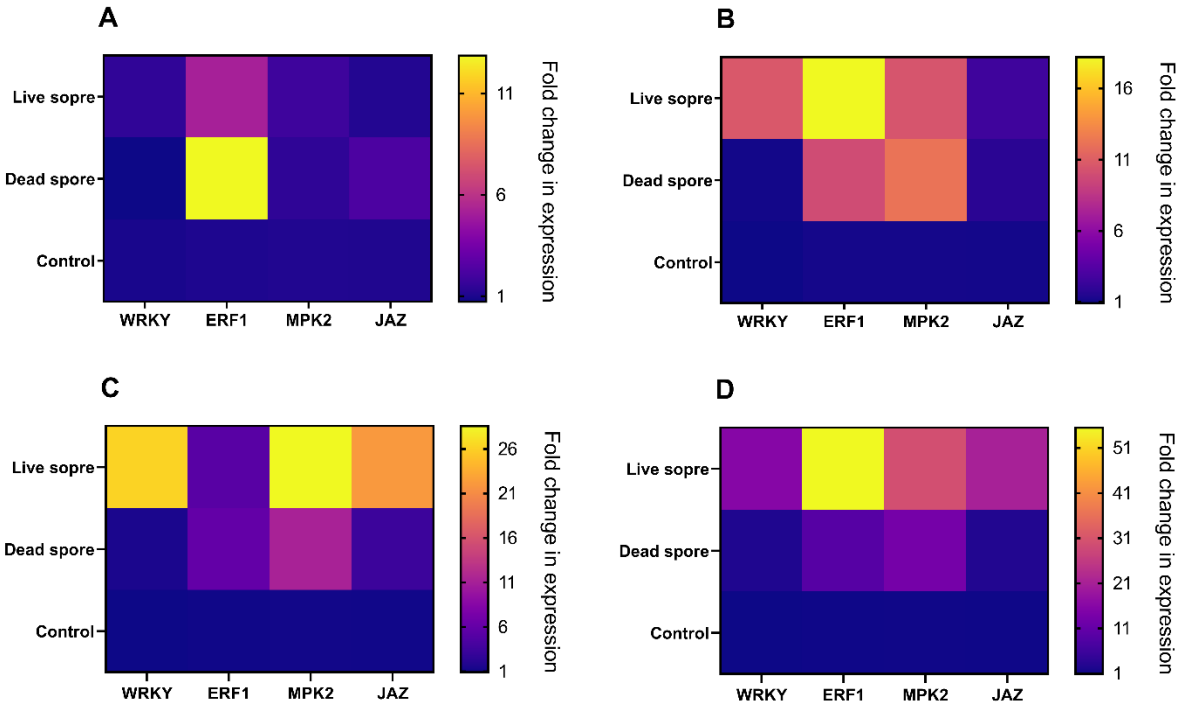


Figure 5. Heat map graph for WRKY, ERF1, MPK2, and JAZ genes at 2 hours (A), 8 hours (B), 24 hours (C), and 48 hours (D) following plant inoculation with live spores, dead spores, and control.

Discussion

This study examined the impact of *B. bassiana* (GHA) on the expression of key immune-associated genes in cotton plants, specifically those involved in the ethylene, salicylic acid, and jasmonic acid signaling pathways. According to the findings, *B. bassiana* could not only act as a biocontrol agent against pests but also induced a substantial immune response in the plant, thus contributing to its enhanced defense mechanisms. These accords with recent studies suggesting that entomopathogenic fungi can modulate plant immunity, offering a novel approach to integrated pest management (IPM) (Christian et al., 2020; Bamisile et al., 2021).

The upregulation of the ERF1 and MPK2 genes, both involved in the ethylene signaling pathway, was particularly striking. These genes indicated substantial increments in expression following the treatment with live spores, with the most pronounced changes occurring at 48 hours post-inoculation (Figures 1, 2). Ethylene is known to play a key role in mediating plant defense responses to biotic stressors, including herbivory and pathogen attack (Qin et al., 2021). The observed upregulation of ERF1 and MPK2 supports the hypothesis that *B. bassiana* triggers a defense response similar to what has previously been reported in other crops exposed to entomopathogenic fungi (Quesada-Moraga et al., 2009). This is in accordance with previous transcriptomic studies in *Arabidopsis thaliana*, where colonization by the endophytic strain *B. bassiana* BG11 led to significant upregulation of ERF1 and other JA/ET pathway markers, such as ORA59 and PDF1.2, suggesting activation of ethylene-mediated immune signaling (Raad et al., 2019). Although the specific involvement of MPK2 has not been previously reported in the context of *B. bassiana* colonization, our findings suggest it may play a

complementary or novel role in ethylene signaling along the plant's response.

The significant induction of the WRKY gene, which is linked to the salicylic acid signaling pathway, suggests that *B. bassiana* also stimulates systemic acquired resistance (SAR) in cotton plants. Salicylic acid is a critical component of SAR, primarily involved in defense against biotrophic pathogens and herbivores (Zhang et al., 2019). Our results reveal that live spores of *B. bassiana* upregulated the WRKY gene (Figure 3). They may also enhance the plant's ability to defend against a wide range of biotic stressors, including both pathogens and pests. This is concordant with previous studies demonstrating that entomopathogenic fungi can interact with plant immune systems, often resulting in enhanced resistance against insect herbivores (Christian et al., 2020; Silva et al., 2020). To date, there have been no studies exploring the effect of *entomopathogenic fungi* on the regulation of WRKY transcription factors in cotton. However, previous research has indicated that infection of cotton plants with *Fusarium oxysporum* induces significant overexpression of WRKY genes, suggesting their potential role in mediating the plant's defense response (Wang et al., 2022).

The significant upregulation of the JAZ1 gene, a key regulator of the jasmonic acid (JA) signaling pathway, further supports the idea that *B. bassiana* activates broad-spectrum immune responses in cotton. Jasmonic acid is crucial in defense against herbivores, and its signaling pathway is often activated by plant pathogen or insect feeding (Yan et al., 2019). The observed rise in JAZ1 expression in live spore treatments suggests that *B. bassiana* could bolster the plant's defense against insect pests such as aphids, bollworms, and armyworms. This finding is in accordance with the notion that entomopathogenic fungi not only act directly against pests but also induce a primed defense state in plants, promoting their resistance to future herbivory (Bamisile et al., 2018).

A notable result of this study was the significant difference in gene expression between the live and dead spore treatments. Although both treatments triggered some level of defense, the response to live spores was far more robust and sustained, particularly for the genes involved in ethylene signaling. This suggests that live *B. bassiana* conidia are more effective at engaging with the plant's immune system, possibly through secreting bioactive compounds or other signaling molecules that trigger defense pathways (Liu et al., 2025). Dead spores, in contrast, may act primarily through their structural components, such as chitin and β-glucans, which can still trigger some immune response but lack the ability to initiate prolonged or dynamic signaling. This finding is corroborated by studies that suggest live fungal spores can establish effective and sustained interaction with plants, which have a more limited role in immune modulation (Fesel & Zuccaro, 2016).

The time-dependent nature of gene expression observed in this study further highlights the dynamic character of plant responses to *B. bassiana* (Figure 5). The persistent activation of defense-related genes over a 48-hour period suggests that the plant's immune system remains primed for a prolonged period, which could boost its overall resistance to pests. This prolonged defense activation may be particularly advantageous in field settings, where pests are often encountered over extended periods, and plants require sustained protection (Hashemi et al., 2020). The observed temporal expression pattern is also in line with other studies that reveal entomopathogenic fungi can induce long-lasting immune responses in plants,

enhancing their resilience to pest attacks (Bamisile et al., 2018).

The expression patterns observed in this study indicate a synergistic interaction between the jasmonic acid (JA) and ethylene (ET) signaling pathways in cotton's defense response to *Beauveria bassiana* inoculation. Notably, ERF1, a key integrator of ET signaling, exhibited strong and sustained upregulation in plants treated with live spores, peaking at a 55-fold growth by 48 hours post-inoculation (Figure 1). Likewise, JAZ, a JA-responsive gene, was significantly upregulated, reaching a 22-fold rise at 24 hours and remaining elevated through 48 hours (Figure 4). The simultaneous upregulation of ERF1 and JAZ supports the presence of JA–ET pathway synergy in mediating the plant's defense response. This finding aligns with previous work by Koornneef and Pieterse (2008), who reported frequent antagonism between the salicylic acid (SA) and JA pathways.

In contrast, the WRKY gene, typically associated with SA signaling, also indicated a substantial rise in expression following live spore treatment, peaking at 26-fold at 24 hours (Figure 3). However, this induction did not coincide with suppression of JA-related gene expression, suggesting the absence of strong antagonism between the SA and JA pathways under these conditions. Instead, the results reveal potential co-activation of both SA and JA/ET pathways in response to *B. bassiana*, reflecting a coordinated, multilayered defense strategy in cotton.

This study highlighted the potential of *B. bassiana* as an effective elicitor of plant immune responses within integrated pest management (IPM) strategies for cotton. Through priming the plant's defense mechanisms, *B. bassiana* may contribute to lowering reliance on chemical pesticides and enhancing crop resilience. Future research should explore the long-term effects of *B. bassiana* on plant health and yield, its interactions with beneficial microbes, and its compatibility with other biocontrol agents. Incorporating known immune elicitors such as BTH or salicylic acid (SA) as positive controls in future studies would provide valuable benchmarks for ascertaining induced immune responses and ameliorating experimental design.

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