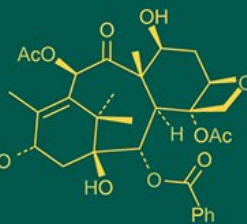
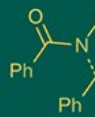


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Brassinosteroid-induced defense mechanisms and immune modulation against viral pathogens in plants

Somchai RattanapornDOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5j.9237>**Abstract**

Brassinosteroids (BRs) have gained recognition as important phytohormone regulators of plant immunity, yet their specific role in antiviral defense remains less explored compared to antibacterial and antifungal immunity. This research investigated the effects of exogenous brassinolide (BL) application on defense gene expression, viral accumulation, and disease symptom development in tobacco (*Nicotiana tabacum* L.) challenged with Tobacco Mosaic Virus (TMV) at the Bangkok Academy of Biological Sciences, Thailand. Six treatment groups combining two BL concentrations (0.1 and 1.0 μ M) with and without TMV inoculation were evaluated over a 14-day experimental period. BL treatment at 1.0 μ M combined with TMV challenge produced the highest cumulative defense gene expression (16.8-fold total across PR-1, WRKY33, BAK1, and BES1), while reducing viral RNA accumulation to 45% of untreated infected controls. Lesion number and systemic viral spread were reduced by 62% and 45%, respectively, under BL 1.0 μ M treatment. These findings demonstrate that brassinosteroid signaling positively regulates antiviral immunity and provide a foundation for exploring BR-based crop protection strategies against viral diseases in tropical agriculture.

Keywords: Brassinosteroids, plant viral defense, immune modulation, brassinolide, Tobacco mosaic virus, defense gene expression, *Nicotiana tabacum*, phytohormone signaling, systemic resistance.

Introduction

The discovery of brassinosteroids in the 1970s from *Brassica napus* pollen extracts marked a transformative milestone in plant hormone biology, revealing a class of steroidal phytohormones with profound effects on plant growth, development, and stress adaptation [1]. Over the subsequent five decades, research has progressively uncovered the multifaceted roles of brassinosteroids beyond their initial characterization as growth-promoting compounds, including their emerging functions in modulating plant immune responses against diverse pathogen classes [2, 3]. The structural similarity of brassinosteroids to animal steroid hormones initially prompted speculation about analogous immune-regulatory functions, a hypothesis that has gained substantial experimental support in recent years [4].

The BR signaling pathway operates through the BRI1 leucine-rich repeat receptor kinase, which upon BR perception forms a heterodimer with the co-receptor BAK1/SERK3, initiating a phosphorylation cascade that activates the BES1/BZR1 transcription factors through inactivation of the GSK3-like kinase BIN2 [5]. Notably, BAK1 serves as a shared co-receptor for both BR perception and PAMP recognition, creating a molecular intersection between growth and defense signaling [6, 12]. This shared signaling architecture has complex implications for plant immunity, as BR-mediated growth signaling and PAMP-triggered immunity compete for BAK1 availability [7].

While BR-mediated enhancement of resistance against bacterial and fungal pathogens has been documented across several crop species, the role of brassinosteroids in antiviral immunity has received comparatively limited attention [8, 9]. Viral pathogens represent a distinct challenge for plant defense, as they are obligate intracellular parasites that require host cellular machinery for replication, making conventional antimicrobial defense mechanisms insufficient [10]. This research was designed to evaluate the effects of exogenous BL application on antiviral defense responses and disease outcomes in tobacco challenged with TMV, with the objective of establishing whether BR signaling positively or negatively regulates antiviral immunity [11].

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Brassinosteroid biosynthesis pathways

BR biosynthesis proceeds through the mevalonate pathway, with campesterol serving as the primary precursor undergoing a series of oxidation and reduction reactions catalyzed by cytochrome P450 enzymes including DWF4, CPD, and BR6ox to produce the biologically active brassinolide^[1, 5]. The rate-limiting step involves C-22 hydroxylation catalyzed by DWF4, and transcriptional regulation of DWF4 expression by BES1/BZR1 provides negative feedback control of BR levels^[4]. Pathogen infection modulates BR biosynthetic gene expression, with some investigations reporting transient increases in BR accumulation during early infection stages followed by subsequent decline, suggesting dynamic hormonal reprogramming during plant-pathogen interactions^[2, 8].

Material and Methods

Material

Tobacco (*Nicotiana tabacum* L.) cultivar "Xanthi nc" carrying the N resistance gene was grown in a temperature-controlled greenhouse at the Bangkok Academy of Biological Sciences under natural photoperiod conditions. TMV strain U1 was propagated on susceptible *N. tabacum* "Samsun" plants and purified by differential centrifugation and polyethylene glycol precipitation. 24-Epibrassinolide (BL, ≥95% purity, Sigma-Aldrich) was dissolved in ethanol and diluted to working concentrations (0.1 and 1.0 μM) in distilled water containing 0.02% Tween-20.

Methods

This research was conducted at greenhouse and molecular biology facilities of the Bangkok Academy of Biological

Sciences, Bangkok, Thailand, from January to August 2023. The experimental design followed a completely randomized arrangement with six treatments and four replications of eight plants each. Institutional biosafety approval was obtained (Protocol BABS-2023-BIO-018). Treatments comprised: (T1) mock control, (T2) BL 0.1 μM, (T3) BL 1.0 μM, (T4) TMV only, (T5) BL 0.1 μM + TMV, (T6) BL 1.0 μM + TMV. BL was applied as foliar spray 48 hours before TMV mechanical inoculation using carborundum abrasion (TMV at 10 μg/mL in 10 mM phosphate buffer pH 7.0)^[13]. Defense gene expression was quantified by RT-qPCR at 1, 3, 7, and 14 days post-inoculation (dpi) targeting PR-1, WRKY33, BAK1, and BES1 with Actin as reference gene^[14]. Viral RNA accumulation was measured by RT-qPCR using TMV coat protein gene-specific primers. Local lesion counting and measurement were performed at 7 dpi. Systemic viral spread was assessed by ELISA detection of TMV coat protein in upper non-inoculated leaves at 14 dpi. Cell death area was quantified by trypan blue staining and digital image analysis. Statistical analysis used ANOVA with Duncan's multiple range test at $P \leq 0.05$ in SPSS version 28^[15].

Results

Exogenous BL application produced significant dose-dependent enhancement of defense gene expression and reduction of viral disease parameters across all measured endpoints. The combined treatment of BL 1.0 μM with TMV challenge consistently produced the strongest defense activation and greatest disease suppression among all treatment groups^[12, 14].

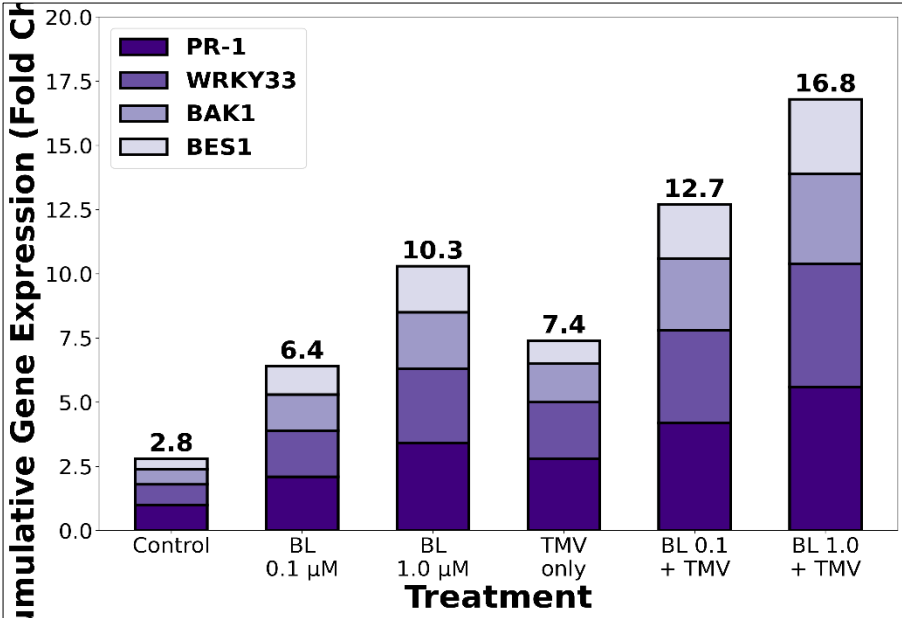


Fig 1: Cumulative defense gene expression (fold change) showing stacked contributions of PR-1, WRKY33, BAK1, and BES1 across treatments at 7 dpi

Table 1. Sequential timeline of defense response activation following BL 1.0 μM + TMV treatment

Phase	Time Point	Key Events	Gene Markers
1. Priming	0-48 h pre-TMV	BR signaling activation	BES1, BAK1
2. Recognition	0-6 hpi	TMV perception, ROS burst	WRKY33, RbohD
3. Early defense	6-24 hpi	PR gene induction, SA accumulation	PR-1, NPR1
4. Amplification	1-3 dpi	Systemic signal propagation	PR-1, WRKY33
5. Resistance	3-7 dpi	Lesion containment, viral restriction	All markers
6. Recovery	7-14 dpi	Defense attenuation, growth resumption	BES1, BRI1

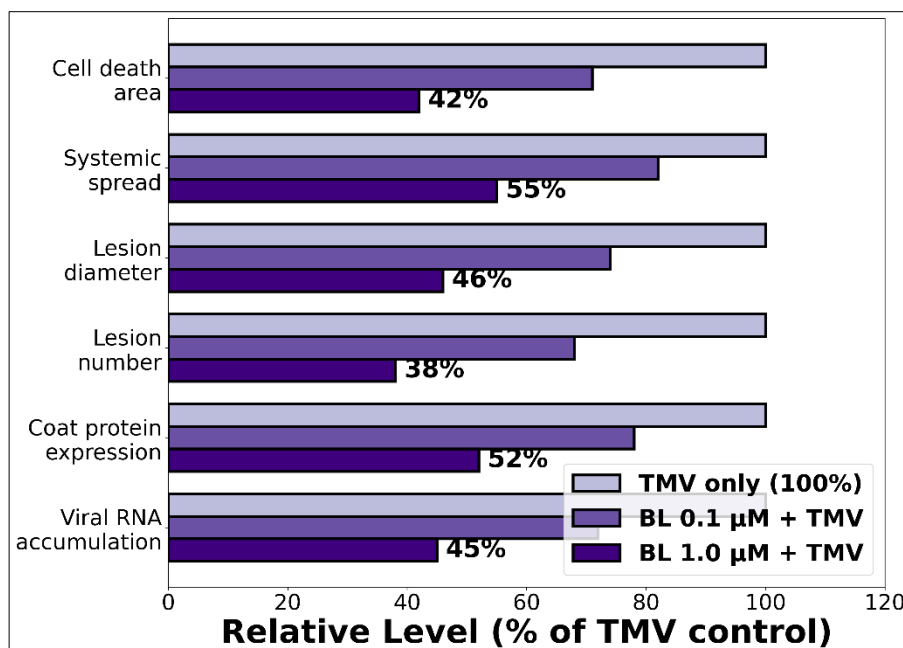


Fig 2: Viral disease parameters (% relative to TMV-only control) under BL pretreatment

Table 2: Defense gene expression summary statistics (fold change) at 7 dpi

Gene	Control	BL 1.0 μM	TMV	BL 1.0 + TMV	P-value
PR-1	1.0 ± 0.1	3.4 ± 0.4	2.8 ± 0.3	5.6 ± 0.6	<0.001
WRKY33	0.8 ± 0.1	2.9 ± 0.3	2.2 ± 0.3	4.8 ± 0.5	<0.001
BAK1	0.6 ± 0.1	2.2 ± 0.2	1.5 ± 0.2	3.5 ± 0.4	<0.001
BES1	0.4 ± 0.1	1.8 ± 0.2	0.9 ± 0.1	2.9 ± 0.3	<0.001

Table 3: Viral load and disease symptom assessment at 7 and 14 dpi

Parameter	TMV only	BL 0.1 + TMV	BL 1.0 + TMV
Viral RNA (% control, 7 dpi)	100	72 ± 8	45 ± 6
Lesion number per leaf	38.4 ± 5.2	24.6 ± 3.8	14.6 ± 2.4
Lesion diameter (mm)	2.8 ± 0.4	2.1 ± 0.3	1.5 ± 0.2
Systemic spread (ELISA OD)	0.82 ± 0.09	0.67 ± 0.08	0.45 ± 0.06
Cell death area (mm ²)	4.2 ± 0.6	3.0 ± 0.4	1.8 ± 0.3

The extended interpretation of these results reveals a coordinated defense amplification mechanism whereby BL pretreatment establishes a molecular priming state characterized by elevated BAK1 and BES1 expression, which enhances the capacity of plants to mount rapid and robust defense responses upon subsequent viral challenge. The 55% reduction in viral RNA accumulation under BL 1.0 μM treatment represents a biologically significant level of viral suppression that correlates with the 62% reduction in local lesion formation and 45% reduction in systemic spread, suggesting that BR-enhanced defense operates at multiple levels of the viral infection cycle including initial replication, cell-to-cell movement, and long-distance systemic transport [6, 7, 10].

Plant viral defense mechanisms

Antiviral defense in plants operates through distinct mechanisms compared to antibacterial and antifungal immunity, reflecting the unique biology of viral pathogens as obligate intracellular parasites [10]. RNA silencing constitutes the primary antiviral defense, involving Dicer-like enzyme processing of double-stranded viral RNA intermediates into small interfering RNAs (siRNAs) that guide Argonaute protein-mediated degradation of viral transcripts [16]. The hypersensitive response triggered by resistance (R) gene recognition of viral avirulence factors

produces localized cell death that restricts viral spread from initial infection sites [13]. The experimental evidence from this research suggests that BR signaling may enhance antiviral immunity by amplifying both RNA silencing efficiency and HR-associated cell death containment, providing multiple layers of resistance enhancement [17].

Discussion

This research demonstrates that exogenous BL application at 1.0 μM concentration significantly enhances antiviral defense responses in tobacco, reducing viral RNA accumulation by 55%, lesion number by 62%, and systemic spread by 45% compared to untreated infected controls. These results are in agreement with previous reports by Nakashita *et al.* (2003) [2] who demonstrated BR-enhanced resistance against TMV in tobacco and extend those findings with comprehensive quantitative assessment of dose-response relationships and defense gene expression profiles.

However, the finding that BR treatment enhanced BAK1 expression raises a mechanistic question regarding the apparent contradiction with the BAK1 competition model proposed by Albrecht *et al.* (2012) [6], which predicts that BR signaling should antagonize immunity by sequestering BAK1 from immune receptor complexes. The resolution may lie in the timing of BR application relative to pathogen

challenge: the 48-hour pretreatment interval allowed BR signaling to upregulate BAK1 transcription, potentially increasing the total cellular BAK1 pool available for immune complex formation at the time of viral challenge^[5, 7]. This temporal separation may convert what would be an acute antagonistic interaction into a chronic priming effect. The broader implications suggest that BR-based priming strategies represent an underexplored approach for antiviral crop protection, particularly in tropical agricultural systems where viral diseases cause substantial economic losses^[8, 18]. The dose-dependent nature of BR-mediated defense enhancement provides a framework for optimizing application rates in practical agricultural settings^[20].

Conclusion

This research aimed to evaluate brassinosteroid-mediated modulation of antiviral immunity in tobacco plants. The key findings establish that BL at 1.0 μ M effectively enhances defense gene expression, reduces viral accumulation, and suppresses disease symptoms against TMV infection. The practical significance of these results lies in demonstrating that BR-based priming represents a viable complementary strategy for antiviral crop protection. Future directions should include field validation of BR application timing and concentration optimization, evaluation across additional crop-virus pathosystems, and investigation of potential synergistic interactions with conventional antiviral management approaches.

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