

Induction of plant virus defense response by brassinosteroids and brassinosteroid signaling in *Arabidopsis thaliana*

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Abstract

Main conclusion Our study demonstrated that CMV resistance was upregulated by brassinosteroids (BRs) treatment, and BR signaling was needed for this BRs-induced CMV tolerance.

Plant steroid hormones, brassinosteroids (BRs), play essential roles in variety of plant developmental processes and adaptation to various biotic and abiotic stresses. BR signal through plasma membrane-localized receptor and other components to modulate several transcription factors that modulate thousands of target genes including certain stress-responsive genes. To study the effects of BRs on plant virus defense and how BRs induce plant virus stress tolerance, we manipulated the BRs levels in *Arabidopsis thaliana* and found that BRs levels were positively correlated with the tolerance to *Cucumber mosaic virus* (CMV). We also showed that BRs treatment alleviated photosystem damage, enhanced antioxidant enzymes activity and induced defense-associated genes expression under CMV stress in *Arabidopsis*. To see whether BR signaling is essential for the plant virus defense response, we made use of BR signaling mutants (a weak allele of the BRs receptor mutant *bri1-5* and constitutive BRs response mutant *bes1-*

D). Compared with wild-type *Arabidopsis* plants, *bri1-5* displayed reversed tolerance to CMV, but the resistance was enhanced in *bes1-D*. Together our results suggest that BRs can induce plant virus defense response through BR signaling.

Keywords *Arabidopsis* · BES1 · Brassinosteroid · BRI1 · CMV · Defense

Abbreviations

BR	Brassinosteroid
BL	Brassinolide
BRZ	Brassinazole
BRI1	BRASSINOSTEROID-INSENSITIVE1
BES1	BRI1 EMS SUPPRESSOR 1
CMV	<i>Cucumber mosaic virus</i>
ROS	Reactive oxygen species
Dpi	Days postinoculation

Introduction

Plants are exposed to a diversity of biotic and abiotic stresses throughout their life cycles and need to constantly regulate their developmental and physiological processes to respond to various internal and external stimuli (Zhang et al. 2009; Ning et al. 2010). Agricultural crops worldwide survey from a vast array of pathogens including bacteria, fungi, and viruses which cause tremendous losses in yield and quality of produce (Culbreath et al. 2003; Rodoni 2009). Multiple methods have been developed to enhance the plant resistance to viruses. One of the fundamental areas for biotechnological advances in plant improvement is boosting plants internal defense mechanism to survive adverse environmental cues.

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Brassinosteroids (BRs) are a group of plant steroid hormones which mainly regulate plant growth and developmental processes such as cell expansion and division, senescence, vascular development. Loss-of-function mutants such as *bri1-5* display dwarf phenotypes with reduced cell elongation (Clouse et al. 1996; Li et al. 1996; Szekeres et al. 1996). By contrast, gain-of-function mutants such as *bes1-D* have long hypocotyls, leaf petioles, curly leaves, and early leaf senescence (Yin et al. 2002). In plants, BR signal through receptor kinase BRI1 (BRASSINOSTEROID-INSENSITIVE1), co-receptor BAK1 (BRI1-ASSOCIATED RECEPTOR KINASE 1), and several other signaling components to control BES1 (BRI1 EMS SUPPRESSOR 1) and BZR1 (BRASSINAZOLE RESISTANT 1) family transcription factors (Zhu et al. 2013). Global gene expression studies demonstrated that BRs can regulate up to 4,000–5,000 genes at different growth stages, with about half induced and half repressed by BRs (Guo et al. 2013). ChIP-Chip analyses indicated that BES1 and BZR1 transcription factors likely account for a majority of the BR-regulated gene expression, as BES1 and BZR1 have thousands of target genes and many of them are regulated by BRs (Sun et al. 2010; Yu et al. 2011).

Although the BR signaling cascades for plant growth and development have been firmly established, the mechanism by which BRs increase tolerance is still unclear. Previous reports showed that exogenous applications of BRs are widely used to improve stress tolerance in plants (Acharya and Assmann 2009) and the application of BRs has become a routine stress management protocol (Divi and Krishna 2009; Peleg and Blumwald 2011; Hayat et al. 2012). Treatment of cucumber (*Cucumis sativus*) with BRs has been reported to enhance tolerance to photo-oxidative and cold stresses and is accompanied by H₂O₂ accumulation and systemic induction of genes associated with stress responses, such as *MAPK1* and *MAPK2* (Xia et al. 2009, 2011). Similarly, application of BRs to radish (*Raphanus sativus*) improves tolerance to Cu and Cr stress owing to the upregulation of a protective antioxidant system and the modulation of endogenous abscisic acid, auxin and polyamine profiles (Choudhary et al. 2010, 2011). Recently, there is increasing evidence that BRs could induce the expression of genes encoding both structural and regulatory proteins such as antioxidants and heat shock proteins (HSPs) (Kagale et al. 2007; Xia et al. 2009; Cui et al. 2011). These results confirmed the roles of BRs in stress responses.

It has been demonstrated that BRs induces resistance of tobacco, rice and cucumber to bacterial, fungal and viral pathogens, establishing a role for BRs in both monocot and dicot plant defense mechanisms. However, signaling cascade mediating BR's action in plant resistance against

viruses remains largely unclear. In this study, we demonstrated that application of BRs offers a unique possibility of enhancing plant virus defense response through activating several key antioxidant enzymes and inducing several resistance genes. Furthermore, we also tested the resistance to *Cucumber mosaic virus* (CMV, genus *Cucumovirus*, family *Bromoviridae*), which is highly virulent on *Arabidopsis*, in *bri1-5* and *bes1-D* and found that compared to wild type, CMV stress tolerance is weaker in *bri1-5* and stronger in *bes1-D*. All of these data illustrated that BR signaling is needed for BR-induced resistant to plant virus. Our results also demonstrated that BR signaling might induce several resistance genes in response to CMV infection.

Materials and methods

Plant material and growth conditions

All *Arabidopsis thaliana* seedlings were grown under 8/16 h light/dark cycles of medium light (100 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for 4 weeks. *Arabidopsis thaliana* seeds of Col-0, WS-2, BR-insensitive mutant *bri1-5* (WS-2 background) and gain-of-function mutant *bes1-D* (Col-0 background), were given by Dr Yanhai Yin (Iowa State University, Ames, IA, USA).

Chemical treatments and pathogen inoculation

Brassinolide (BL, the most active BR) and brassinazole (BRZ, a specific inhibitor of BR biosynthesis) were purchased from Wako Pure Chemical Industries, Ltd (Chuo-Ku, Osaka, Japan) and Santa Cruz Biotechnology, inc (Dallas, TX, USA), respectively. The hormone and inhibitor solutions were prepared in water containing 0.02 % (v/v) Tween 20. For chemical treatment of plants, the 20-day-old seedlings were pretreated by foliar spraying with 1 μM BL or 1 μM BRZ. Distilled water containing 0.02 % (v/v) Tween 20 was used as a control treatment. All experiments were repeated with similar results.

In infection experiments, the chemicals were sprayed 24 h before virus inoculation. Virus isolates of *Cucumber mosaic virus* (CMV) were acquired from Horticulture Institute, Sichuan Academy of Agricultural Sciences, China. The inoculation with virus was carried out as described previously (Xi et al. 2007; Shang et al. 2011). In brief, virus RNA at a concentration of 1 $\mu\text{g}/\text{ml}$ in 10 mM Hepes buffer (pH 7.0) was rubbed onto *Arabidopsis* leaves with cheese cloth. Hepes buffer without virus RNA only was rubbed onto the leaves with cheese cloth as the mock treatment.

Oxidative damage estimation

Electrolyte leakage was measured as previously described (Xu et al. 2012). After measuring the conductivity of the fresh leaves, the leaves were boiled for 15 min to achieve 100 % electrolyte leakage. Lipid peroxidation was estimated by measuring the thiobarbituric acid-reactive substances (TBARS) as previously described (Xu et al. 2012). The lipid peroxides were expressed as TBARS content.

Cell death determination

Tissue staining with trypan blue (1.25 mg/ml, Sigma) to show that the cell death was performed as described previously (He et al. 2007). At least three leaves were used for each staining.

Determination of antioxidant enzymes

For the enzyme assays, 0.3 g of leaf were ground with 3 mL ice-cold 25 mM Hepes buffer (pH 7.8) containing 0.2 mM EDTA, 2 mM ascorbate and 2 % polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 4 °C for 20 min at 12,000g and the resulting supernatants were used for the determination of enzymatic activity (Wang et al. 2011). Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD) activities were assayed as described previously (Wang et al. 2011).

Analysis of chlorophyll fluorescence

Chlorophyll fluorescence was determined with an imaging pulse amplitude modulated fluorometer (IMAG-MINI; Heinz Walz, Effeltrich, Germany). For measurement of F_v/F_m , plants were dark adapted for 30 min. Minimal fluorescence (F_0) was measured during the weak measuring pulses, and maximal fluorescence (F_m) was measured by 0.8 s pulse of light at about 4,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. An actinic light source was then applied to obtain steady-state fluorescence yield (F_s), after which a second saturation pulse was applied for 0.7 s to obtain light-adapted maximum fluorescence (F_m'). F_v/F_m , ΦPSII were calculated as $F_m - F_0/F_m$, $(F_m' - F_s)/F_m'$, respectively (Genty et al. 1989; Bilger and Björkman 1990).

Total RNA extraction and quantitative RT-PCR

Total RNA was extracted from leaves according to a previously described method (Zhang et al. 2010). RNA contents were calculated by measuring the absorbance value taken at 260 nm. The cDNA was amplified using SYBR Premix Ex Taq (TaKaRa). All gene expression levels were measured by quantitative real-time PCR (qRT-PCR). The

C_t (threshold cycle), defined as the PCR cycle at which a statistically significant increase of reporter fluorescence was first detected, was used as a measure for the starting copy numbers of the target gene. Three technical replicates were performed for each experiment. *PP2AA3* (encoding protein phosphatase 2A subunit A3, At1g13320) and *ACTIN1* genes were used as internal controls. All primers are shown in Supplementary Table S1.

Protein extraction and western blotting analysis

Plant tissues collected from systemically infected leaves were obtained. Total proteins were extracted with extraction buffer (50 mM Tris-HCl, 5 % mercaptoethanol, 10 % glycerol, 4 % SDS, 4 M urea, pH 6.8) in an ice bath. The resulting slurries were centrifuged at 8,000g for 10 min at 4 °C, and the supernatants were stored at -80 °C. Protein concentrations were determined by the Bradford method using bovine serum albumin as a standard (Bradford 1976). Western blot analysis was carried out using CMV coat protein-specific antisera.

Statistical analysis

Means of three biological replicates were measured. Student's *t* test was used for comparison between different treatments. A difference was considered to be statistically significant when $P < 0.05$.

Results

BRs increase CMV tolerance in *Arabidopsis thaliana*

To investigate the role of BRs in CMV tolerance, *Arabidopsis thaliana* leaves were pretreated with BL, water and BRZ before CMV infection and then the CMV replication and accumulation levels were detected by qRT-PCR (Fig. 1a) and Western blotting analysis (Fig. 1b) at 3, 6 and 9 days postinoculation (dpi). When the disease symptom appeared, CMV coat protein was easily detected in uninoculated leaves, which indicated that CMV had already spread and replicated largely in systemic leaves. As shown in Fig. 1, the accumulation level of CMV was less and detectable earlier in BL-pretreated plants as compared with water-pretreated plants (Fig. 1; supplementary Fig. S1). However, the viral replication level was significantly higher in BRZ-pretreated plants than water-pretreated plants. These results confirmed the role of BRs in innate immunity.

Electrolyte leakage and TBARS content can indicate the degree of damage in plants caused by stresses. To confirm the effects of virus infection on membrane integrity,

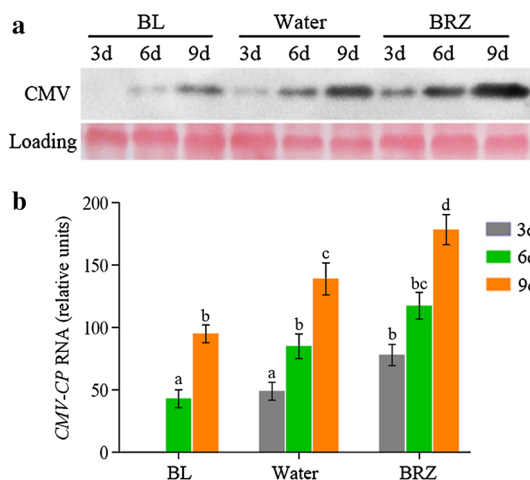


Fig. 1 Virus replication and expression levels of water, 1 μM BL or 1 μM BRZ-pretreated Col-0 infected with CMV. **a** Western blotting analysis of coat protein accumulation of CMV at 3, 6 and 9 dpi. The systemic leaves were used for detection. Rubisco proteins were used as loading controls and were stained by Ponceau S. Experiments were repeated three times with similar results. **b** qRT-PCR analysis of replication levels of CMV at 3, 6 and 9 dpi. Bars represent mean and standard deviation of values obtained from three biological replicates. Significant differences ($P < 0.05$) are denoted by different lowercase letters

TBARS content and electrolyte leakage were determined after virus infections. As shown in Fig. 2, BRZ-pretreated plants had higher oxidative damages (Fig. 2a, b) and more cell-death occurrence than water-treated plants (Fig. 2c). By contrast, oxidative damages and cell-death occurrence

in BL-pretreated plants were lower than those in water-treated plants. These results suggested that virus infection reduced the stability and integrity of the plasma membrane, and BRs were important for plant response to CMV.

BRs alleviate photosystem damage under CMV stress

Most viruses have harmful effects on various biological processes in plants, including the photosystem II (PSII). Chlorophyll fluorescence measurements showed that the PSII photochemical efficiency was decreased by viral infection (Hodgson et al. 1989; Balachandran et al. 1994; Rahoutei et al. 2000; Guo et al. 2005). F_v/F_m and $\phi PSII$ are indicators of PSII photochemistry. To analyze the effects on photosynthetic efficiency, we compared the photochemical efficiency of PSII in the dark-adapted state. As shown in Fig. 3a and b, compared with water-treated plants, CMV-induced reduction of F_v/F_m was less in BL-pretreated plants but greater in BRZ-pretreated plants. The $\phi PSII$ results showed a similar trend (Fig. 3c, d). Taken together, these results indicated that BRs played a protective role in plant photosystem against CMV infection.

Involvement of antioxidant system in BRs-induced CMV defense

Then, we investigated whether antioxidant systems were involved in the BRs-induced CMV defense response. We analyzed antioxidants which protect cells from oxidative

Fig. 2 TBARS content (a), electrolyte leakage (b) and cell death (c) of water, 1 μM BL or 1 μM BRZ-pretreated Col-0 infected with CMV. Leaf samples were harvested at 6 dpi with CMV inoculation. Bars represent mean and standard deviation of values obtained from three biological replicates. Significant differences ($P < 0.05$) are denoted by different lowercase letters

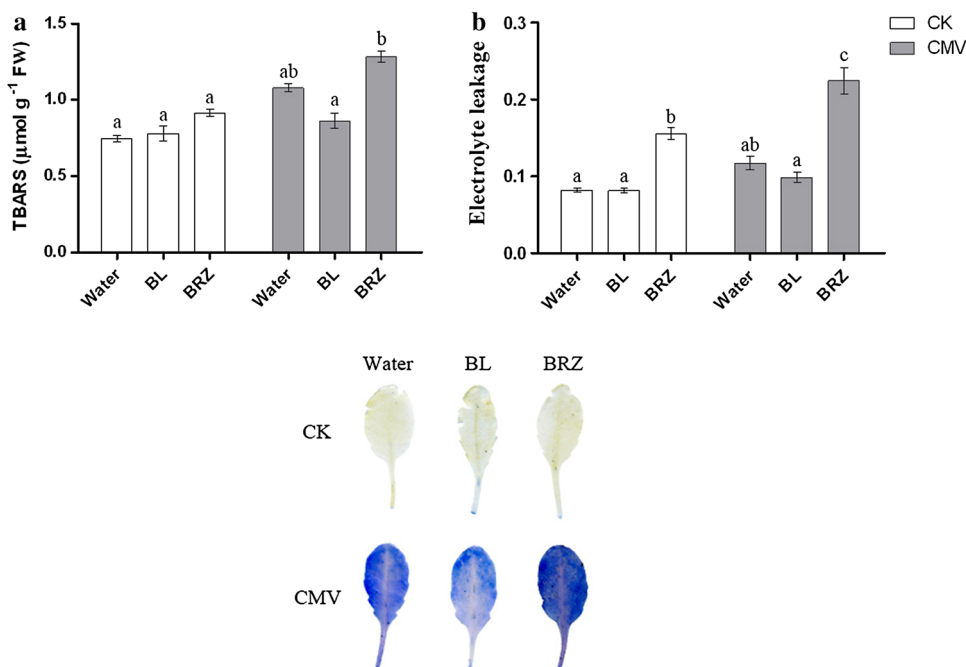


Fig. 3 Chlorophyll fluorescence parameters of water, 1 μ M BL or 1 μ M BRZ-pretreated Col-0 infected with CMV at 6 dpi. Images of F_v/F_m (a), Φ_{PSII} (c). The false color code depicted at the bottom of the image ranged from 0 (black) to 1.0 (purple). Five plants were used for each treatment and the picture of one representative plant is shown. **b** and **d** Average values for the respective chlorophyll fluorescence images. *Bars* represent mean and standard deviation of values obtained from five biological replicates. Significant differences ($P < 0.05$) are denoted by different lowercase letters

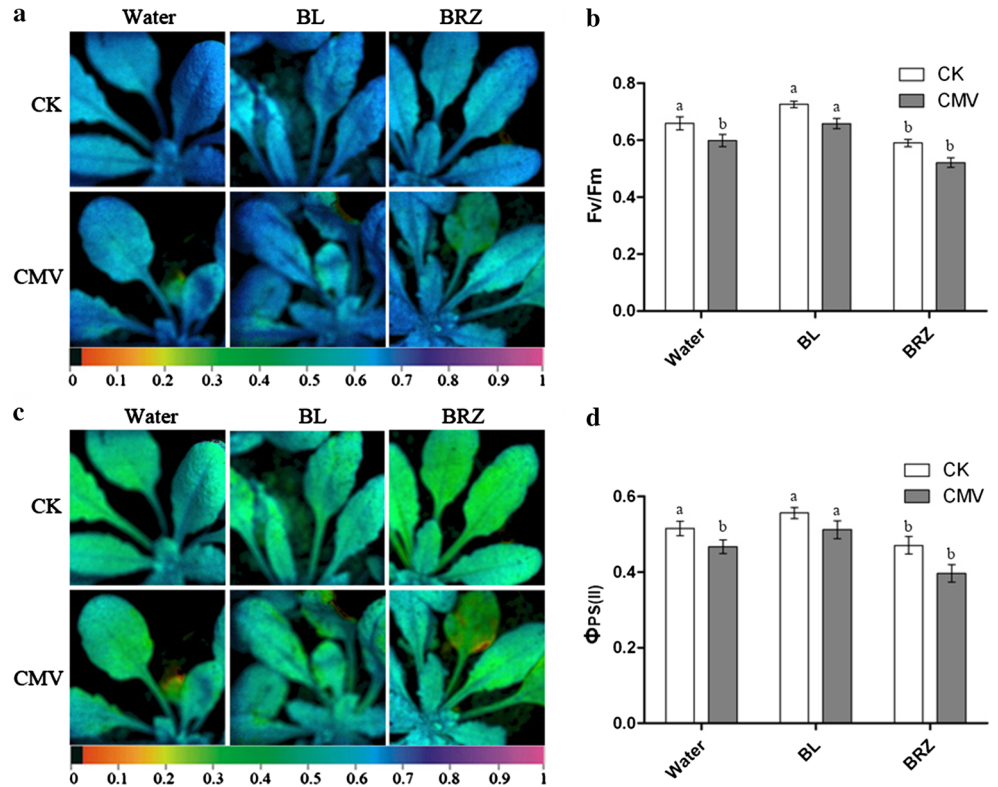
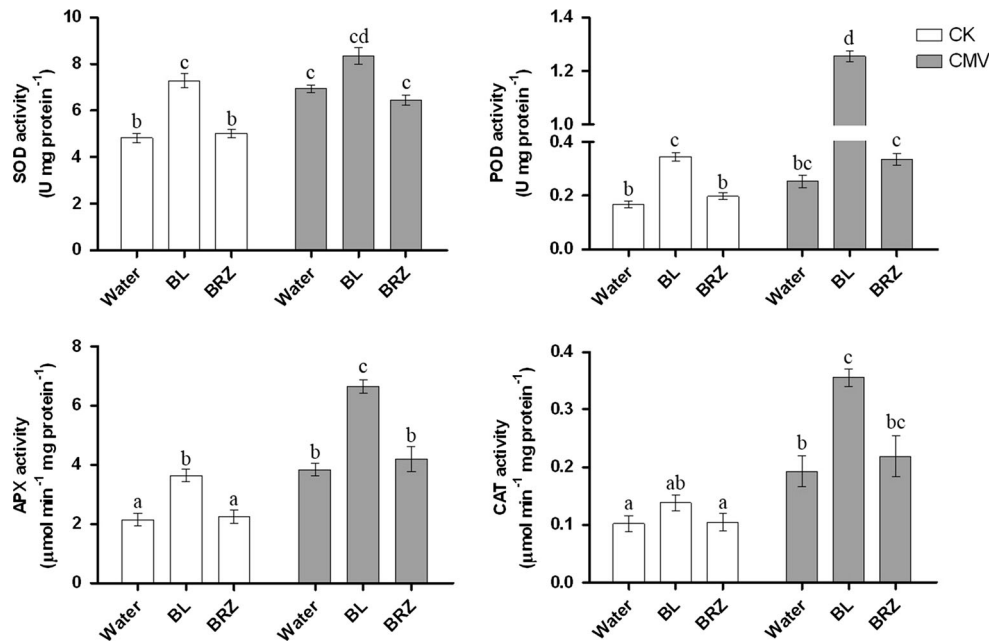


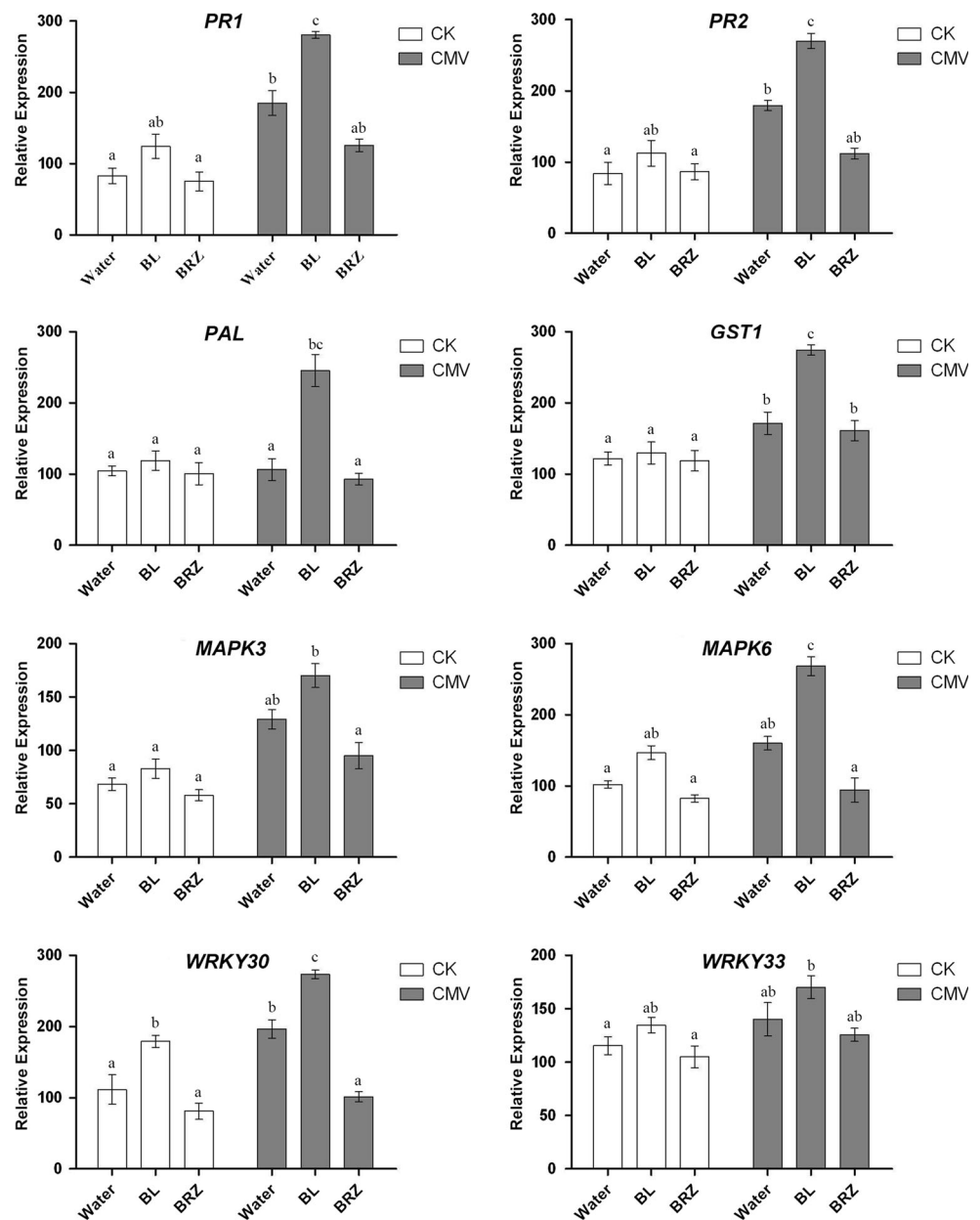
Fig. 4 Antioxidant enzymes activities of water, 1 μ M BL or 1 μ M BRZ-pretreated Col-0 infected with CMV. Leaf samples were harvested at 6 dpi with CMV inoculation, and the activities of antioxidant enzymes were analyzed. *Bars* represent mean and standard deviation of values obtained from three biological replicates. Significant differences ($P < 0.05$) are denoted by different lowercase letters



damage by scavenging reactive oxygen species (ROS). In this work, the enzyme activity of several antioxidative enzymes, such as SOD, POD, CAT and APX, was detected. As shown in Fig. 4, all antioxidant enzymes activity increased in BL-pretreated plants. The activities of these enzymes, however, were little changed in BRZ-pretreated

plants. Viral infection increased the activity of SOD, POD, CAT and APX. Again, the increasing amplitude of all these antioxidant enzymes activity were higher in BL-pretreated plants, compared with water or BRZ-pretreated plants. All these results indicated that BRs-induced CMV defense was related to antioxidant system.

Fig. 5 Defense gene expression levels in Col-0 were detected by qRT-PCR analysis at 6 dpi. Water pretreatment with purified water, BL pretreatment with 1 μ M BL, BRZ pretreatment with 1 μ M BRZ. Bars represent mean and standard deviation of values obtained from three biological replicates. Significant differences ($P < 0.05$) are denoted by different lowercase letters



Defense-associated genes expression in response to BRs Levels

To analyze the underlying molecular mechanisms for BR-induced stress tolerance to CMV, we examined the effects of BRs levels on expression of several genes involved in the defense response. Transcripts of two transcription factors *WRKY30* (Peng et al. 2012), *WRKY33* (Zheng et al. 2006; Lippok et al. 2007; Birkenbihl et al. 2012), pathogen-responsive mitogen-activated protein kinases *MAPK3* and *MAPK6* (Mao et al. 2011), salicylic acid (SA) responsible resistance protein *PR1* and *PR2* (Spoel et al. 2003, 2009; Durrant and Dong 2004), ROS-related resistance protein

glutathione S-transferase (*GST*) (Uquillas et al. 2004), and Phenylalanine ammonia-lyase (*PAL*) (Dixon and Paiva 1995; MacDonald and D’Cunha 2007) in which activity respond to various stresses, were detected. As shown in Fig. 5, transcripts of all these resistance genes were significantly increased in BL-treated plants and almost had little change in BRZ-pretreated plants compared with water-treated plants. In CMV infection plants, compared with water-pretreated plants, expression of all defense genes was upregulated obviously upon treatment with BL but downregulated after BRZ treatment. Among the eight genes, reductions of *PR1*, *PR2*, *MAPK3*, *MAPK6* and *WRKY30* expression by BRZ were more obviously compared with water-pretreated plants.

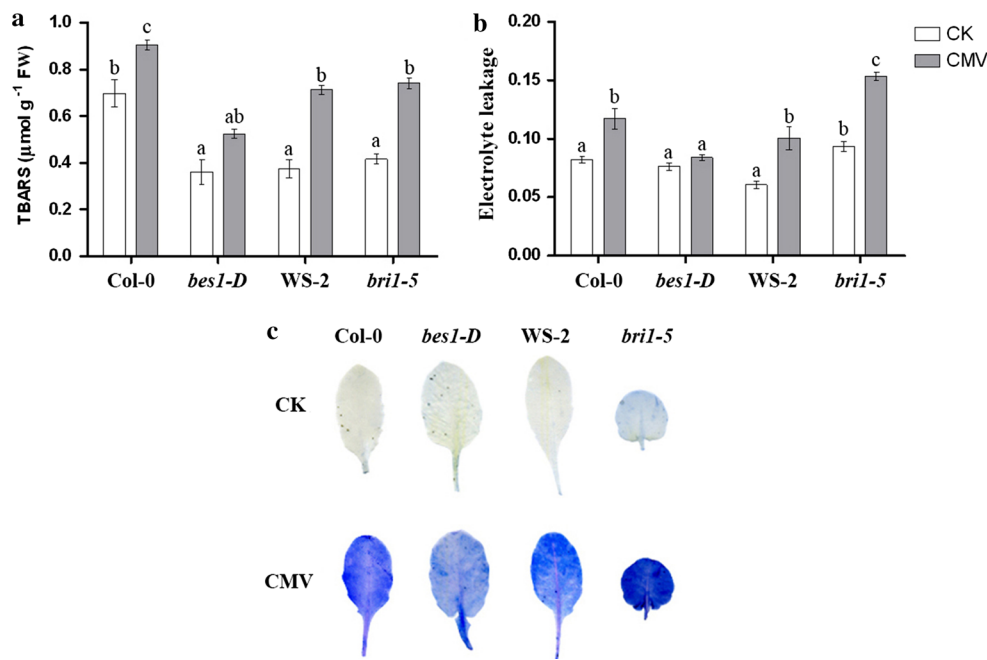
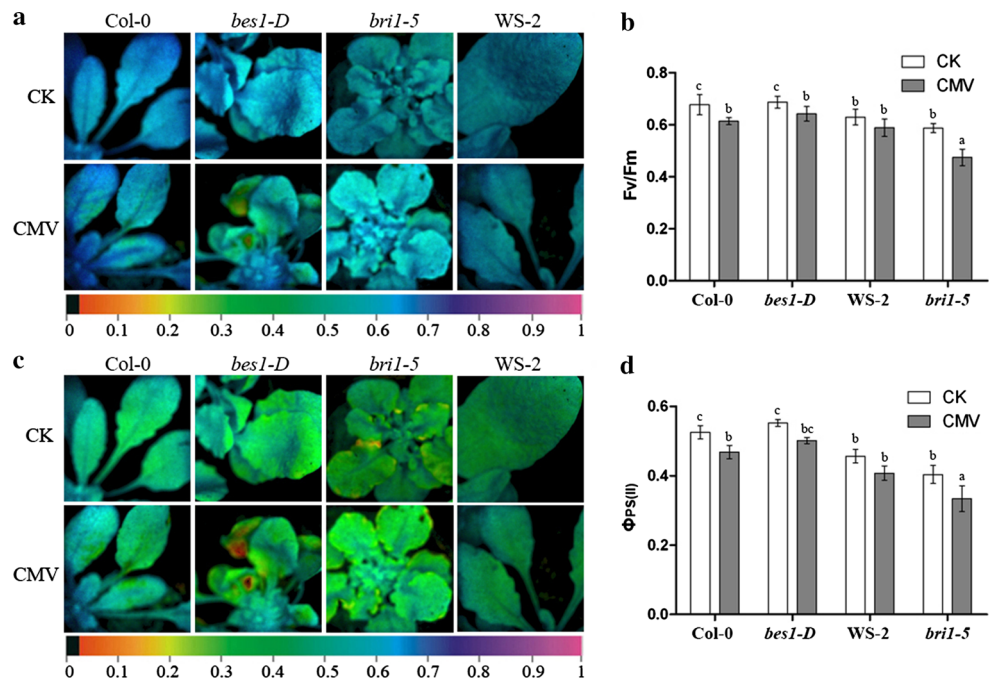


Fig. 6 TBARS content (a), electrolyte leakage (b) and cell death (c) of Col-0, *bes1-D*, WS-2, *bril-5* plants at 6 dpi with CMV inoculation. *bril-5* is in the WS-2 background. Bars represent mean

and standard deviation of values obtained from three biological replicates. Significant differences ($P < 0.05$) are denoted by different lowercase letters

Fig. 7 Chlorophyll fluorescence parameters of Col-0, *bes1-D*, WS-2, *bril-5* plants at 6 dpi with CMV inoculation. Images of F_v/F_m (a), Φ_{PSII} (c). The false color code depicted at the bottom of the image ranged from 0 (black) to 1.0 (purple). Five plants were used for each treatment and the picture of one representative plant is shown. **b, d** Average values for the respective chlorophyll fluorescence images. Bars represent mean and standard deviation of values obtained from five biological replicates. Significant differences ($P < 0.05$) are denoted by different lowercase letters

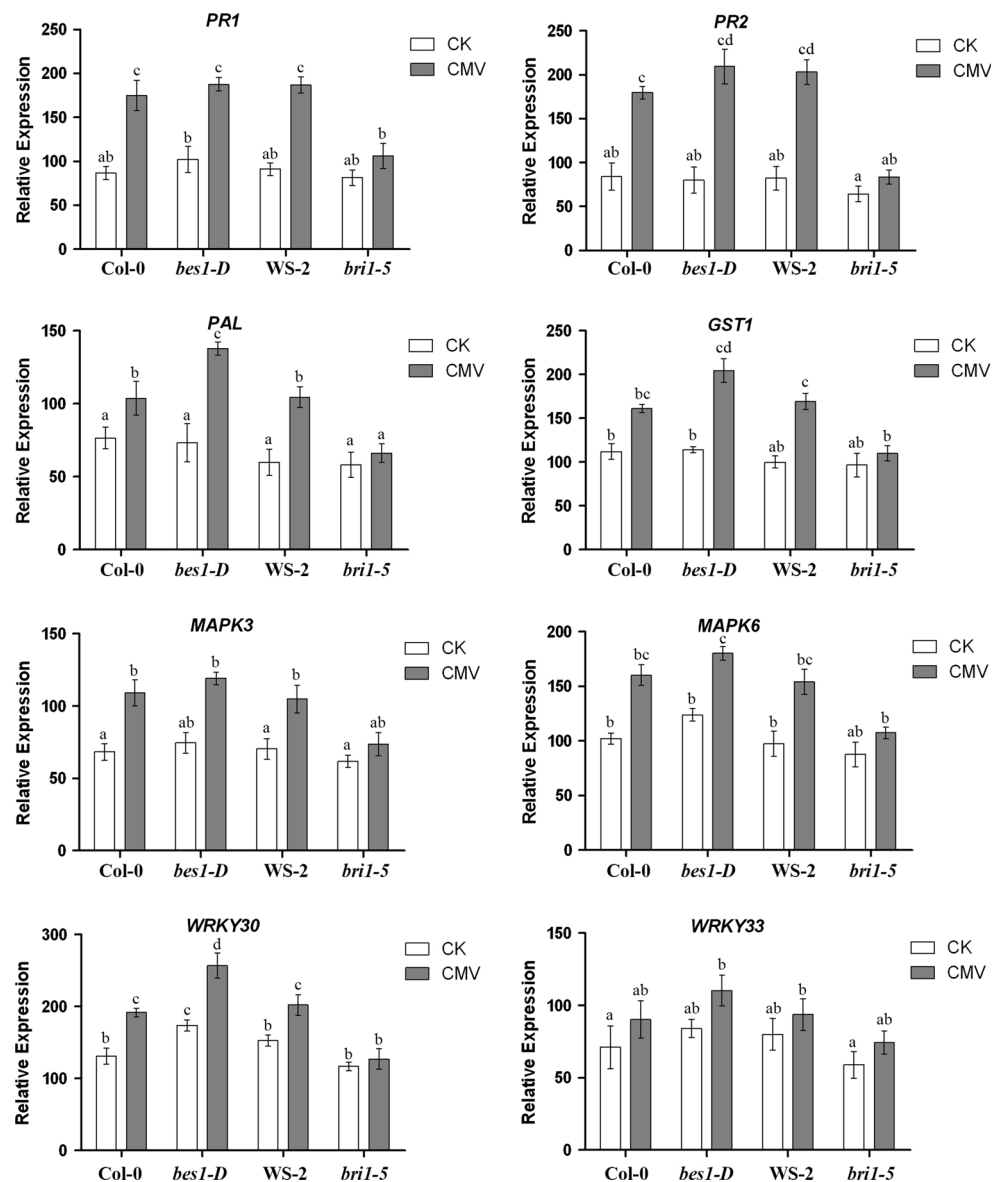


BR signaling pathway plays an essential role in the BRs-enhanced CMV stress tolerance

To determine the possible role of BR signaling pathway in the BRs-enhanced CMV stress tolerance, the BR-insensitive mutant *bril-5* and gain-of-function mutant *bes1-*

D were used to analyze their possible roles in antiviral defenses. Our results suggested that *bril-5* was more susceptible to CMV infection than the wild-type plants WS-2. *bril-5* showed more serious symptoms [higher oxidative damages (Fig. 6a, b), more cell-death occurrence (Fig. 6c), and more compromised in PSII photochemistry (Fig. 7)]

Fig. 8 Resistance gene expression levels of Col-0, *bes1-D*, WS-2, *bri1-5* plants after CMV inoculation. Gene expression levels were detected by qRT-PCR analysis at 6 dpi. Bars represent mean and standard deviation of values obtained from three biological replicates. Significant differences ($P < 0.05$) are denoted by different lowercase letters



than the wild-type plants after virus infection. Furthermore, expression of most resistance genes (Fig. 8) and antioxidant enzymes activity (Fig. 10) cannot be induced after the infections in *bri1-5*, thus resulting in more virus accumulation as compared with WS-2 after inoculation (Fig. 9). However, *bes1-D* adapted to virus infections better, which showed a lighter symptom and had less cell-death occurrence (Fig. 6). Resistance gene transcripts (Fig. 8) and antioxidant enzymes activity (Fig. 10) were significantly higher in *bes1-D* compared with those in Col-0. Moreover, the accumulation levels of CMV were both delayed and decreased in *bes1-D* plants as compared with the wild-type plants (Fig. 9). These data further suggest that BR signaling pathway plays an important role in plant systemic resistance against CMV.

Discussion

Effects of BRs on plant growth and developmental processes such as cell expansion and division, senescence, vascular development, photomorphogenesis and environment stress responses were well studied. But exploration of BRs in plant virus resistance is rarely reported. Plant viral disease is one of the most important factors affecting yield and quality of agricultural products (Rodoni 2009). It is urgent to seek new effective ways to control plant viruses. In this study, we showed that BRs could be used in control of plant viruses.

Our experiments showed that BL treatment increased but BRZ treatment reduced CMV resistance in *Arabidopsis* (Fig. 1). This result confirmed a positive of BRs in virus

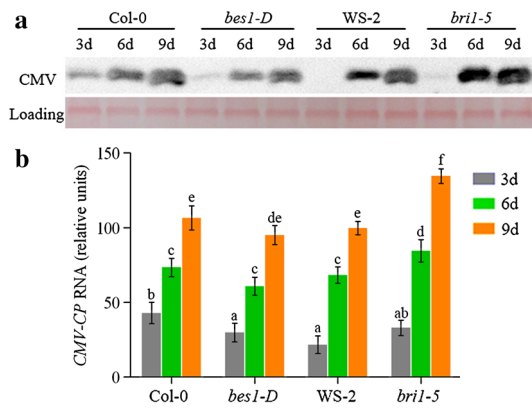


Fig. 9 Virus replication and expression levels of Col-0, *bes1-D*, WS-2, *bri1-5* after CMV inoculation. **a** Western blotting analysis of coat protein accumulation of CMV at 3, 6 and 9 dpi with CMV inoculation. The systemic leaves were used for detection. Rubisco proteins were used as loading controls and were stained by Ponceau S. Experiments were repeated three times with similar results. **b** qRT-PCR analysis of CMV replication levels at 3, 6 and 9 dpi with CMV inoculation. Bars represent mean and standard deviation of values obtained from three biological replicates. Significant differences ($P < 0.05$) are denoted by different lowercase letters

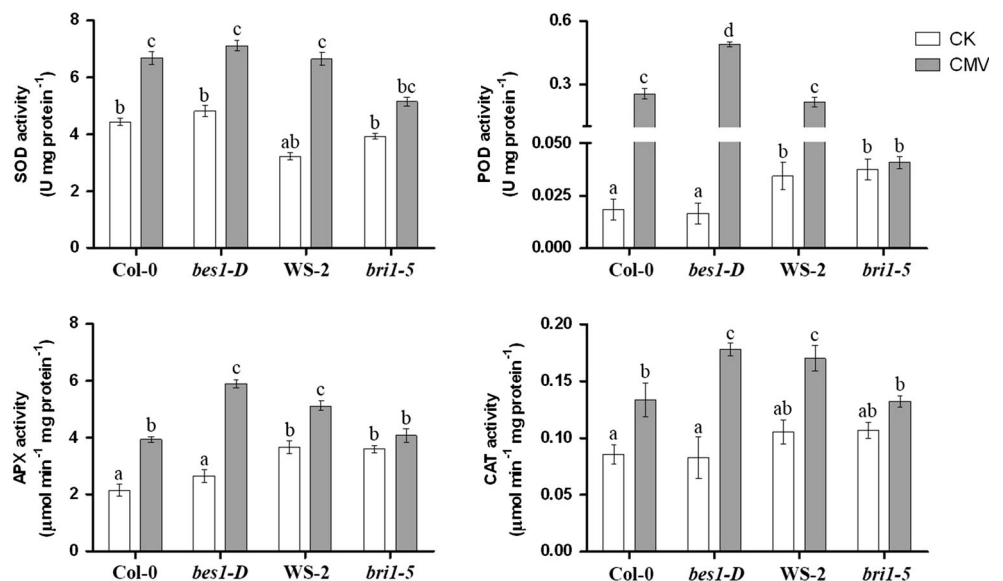
tolerance. Previous studies indicated BRs-enhanced generation of H_2O_2 and induced antioxidative enzymes activity (Xia et al. 2009, 2011). In our study, we found BRs elevated antioxidative enzymes activities (Fig. 4) and defense-associated genes expression (Fig. 5), which were involved in the CMV tolerance. These observations indicated that BRs might participate in variety of physiological functions that activate defense system and enable plants to acquire resistance. Systemic acquired resistance is characterized by micro-oxidative bursts, accumulation of SA, and expression of pathogenesis-related (PR) genes such as

PR1 and *PR2* in systemic tissues (Kwon et al. 2009). The present study showed that the expression of *PR1* and *PR2* was induced after BRs treatment, which was accompanied by the activation of systemic resistance to CMV.

BRs are known to regulate numbers of genes. Among them, there are a cluster of genes involved in stress resistance. Interestingly, we found that a transcription factor *WRKY30* is induced by BL treatment (Fig. 5). Previous study indicated constitutive expression of rice *WRKY30* gene increases the endogenous jasmonic acid accumulation, *PR* gene expression and resistance to fungal pathogens in rice (Peng et al. 2012). Recent CHIP-chip studies have indicated that *WRKY30* was induced by BL and was a direct target of BES1 and BZR1 (Sun et al. 2010; Yu et al. 2011). So we speculated BR signaling regulate defense-associated genes through some transcription factors such as *WRKY30*.

BR signal through receptor kinase BRI1, co-receptor BAK1 and other components to modulate BES1 and BZR1 family transcription factors modulate thousands of target genes including certain stress-responsive genes. Previous studies indicated that BR signaling components play essential roles in innate immunity, while most studies focused on the role of BAK1. Indeed, besides its role in BR signaling, BAK1 is also involved in the regulation of microbe-induced cell death (Kemmerling et al. 2007), and interacts with various pattern recognition receptors (PRRs), including the flagellin receptor FLS2, to drive Pathogen-triggered immunity (PTI) (Albrecht et al. 2012; Belkhadir et al. 2012). In rice, OsSERK1, an ortholog of BAK1, displayed a positive regulator in disease resistance (Hu et al. 2005). We previously reported that BR signaling positive regulators BAK1 and BKK1 are essential for

Fig. 10 Changes in antioxidant enzymes activities in Col-0, *bes1-D*, WS-2, *bri1-5* plants at 6 dpi with CMV inoculation. Bars represent mean and standard deviation of values obtained from three biological replicates. Significant differences ($P < 0.05$) are denoted by different lowercase letters



plants in CMV resistance (Yang et al. 2010). Here, we reported BR signaling receptor BRI1 and transcription factor BES1, which were involved in plant growth regulation, played positive roles in virus resistance. In our study, we found loss-of-function of BRI1 (in *bri1-5*) reduced CMV resistance but active-of-function of BES1 (in *bes1-D*) enhanced CMV tolerance. Furthermore, activities of antioxidative enzymes and transcripts of defense-related genes were downregulated in *bri1-5* but upregulated in *bes1-D* (Figs. 9, 10). So the activated defense system by BR signaling may contribute to virus resistance in plants. In addition, whether other BR signaling-regulated pathways which were downstream of BRI1 are involved in BRs-induced virus resistance need further research.

In summary, our results demonstrated CMV resistance was upregulated by BRs treatment. And BR signaling was needed for this BRs-induced CMV tolerance. Our studies, therefore, offered a hypothesis that BR signaling control virus resistance through regulating antioxidative enzymes and some defense-related genes, such as *WRKY30*. Though the mechanism needs further study, our study contributes to the understanding of signaling cascades mediated by BRs in response to virus.

Author contribution D.-W. Zhang and H.-H. Lin conceived the study and designed the experiments. D.-W. Zhang, X.-G. Deng and F.-Q. Fu performed most of the experiments. D.-W. Zhang, X.-G. Deng and H.-H. Lin wrote the paper.

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