



# Exogenous Brassinosteroid Enhances Plant Defense Against *Colletotrichum gloeosporioides* by Activating Phenylpropanoid Pathway in *Camellia sinensis* L.

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## Abstract

The phytohormones brassinosteroids (BRs) regulate multiple aspects of plant growth, development, and responses to stress. However, the role of BRs in the defense response of tea (*Camellia sinensis* L.), one of the most important beverage crops, remains largely unknown. Previously, we reported that BRs improve tea quality both under normal and unfavorable temperature conditions. Here, we showed that 24-epibrassinolide (EBR, a bioactive BR) enhanced defense against *Colletotrichum gloeosporioides* in tea plants, which was associated with EBR-induced reduction in H<sub>2</sub>O<sub>2</sub> accumulation in tea leaves. *C. gloeosporioides*-caused necrotic lesions and its *actin* gene expression increased over the postinoculation period, but exogenous EBR remarkably suppressed *C. gloeosporioides* spread. Time-course analysis of a key enzyme, phenylalanine ammonia-lyase (PAL), involved in phenylpropanoid biosynthesis, revealed that PAL activity gradually increased from 6 to 24 h postinoculation with *C. gloeosporioides* following an initial decline. Meanwhile, exogenous EBR sharply increased PAL activity of inoculated leaves compared with that of only *C. gloeosporioides* inoculation. Expression analysis of genes involved in phenylpropanoid pathway showed that both exogenous EBR and *C. gloeosporioides* inoculation increased transcript levels of *CsPAL*, *CsC4H*, and *Cs4CL*; however, combined treatment with EBR and *C. gloeosporioides* resulted in a greater increase. Furthermore, *CsPRI* and *CsLOX1* expression analyses revealed that EBR potentially activates systemic induced tolerance, but not the lipoxygenase pathway to enhance tea plant resistance to *C. gloeosporioides*. These findings indicate a positive role of BR in strengthening disease resistance and thus may have potential implications in the control of *C. gloeosporioides*-caused disease in tea plants.

**Keywords** Brassinosteroids · *Camellia sinensis* · *Colletotrichum gloeosporioides* · Disease resistance · Phenylalanine ammonia-lyase · Phenylpropanoid pathway

Lan Zhang and Golam Jalal Ahammed have contributed equally to this work.

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## Introduction

*Camellia sinensis* L. is an evergreen woody perennial plant species, commercially cultivated for manufacturing tea, the most popular healthy drink in the world. It is cultivated across 58 countries in five continents with major production sites in Asia and Africa (Han et al. 2018). People around the

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globe consume tea because of its pleasant taste and numerous health benefits (Kim et al. 2009; Zhang and Tsao 2016). Tea is manufactured from young shoots and leaves which are more prone to abiotic and biotic stressors compared to mature leaves (Han et al. 2018; Li et al. 2018a). Because leaves are the final harvest in tea cultivation, pathogens that cause foliar diseases in tea, not only reduce tea yield but also tea quality. Tea cultivation is affected by hundreds of diseases (Li et al. 2016b). Among various disease causing agents, fungal pathogens from the genera *Colletotrichum* cause the most destructive leaf diseases in tea. The *Colletotrichum* species-caused diseases are commonly called ‘Anthracnose.’ One of the dominant species that causes anthracnose in *Camellia sinensis* is *C. gloeosporioides*. In China, *C. gloeosporioides*-caused disease causes a significant economic loss of tea every year (Guo et al. 2014). Although fungicides can effectively control *C. gloeosporioides*, the presence of pesticide residues in tea has emerged as a matter of public concern due to issues of food safety (Saha et al. 2012). Therefore, exploration of natural biomolecules that can strengthen tea defense against pathogens has received great attention from tea scientists (Li et al. 2016b).

The phenylpropanoid pathway plays a critical role in the defense response of plants to pathogens (Li et al. 2017a; Novo et al. 2017; Yogendra et al. 2015). In general, stimulation of phenylpropanoid metabolism leads to the synthesis and accumulation of a large number of secondary metabolites. Some of these compounds directly function as defense molecules that prevent pathogen expansion and minimize oxidative stress (Wei et al. 2017; Yogendra et al. 2015). Furthermore, accumulation of some other compounds, such as lignin, reinforce defensive structures, such as the cell wall by thickening lignin layers (Dixon et al. 2002; Yogendra et al. 2015). Phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H), and 4-coumarate-CoA ligase (4CL) are three critical enzymes that catalyze phenolic synthesis in the phenylpropanoid pathway (Wei et al. 2017). Derivatives of the phenylpropanoid pathways, such as flavonoids, have strong antimicrobial bioactivity, which restrict fungal pathogens by inhibiting spore germination, germ tube elongation, and mycelia growth (Li et al. 2017a; Novo et al. 2017). Tea plants are rich in secondary metabolites and the activation of phenylpropanoid pathways potentially helps tea plants to ward off pathogen infection (Han et al. 2018; Li et al. 2016a). For instance, anthracnose-resistant *Camellia* varieties show 5- to 10-fold larger levels of polyphenols, catechol, and salicylic acid content than that of susceptible varieties (Xinzhong 2012). In potato, an increased abundance of phenylpropanoid metabolites in stems results in enhanced resistance to *Phytophthora infestans*, the causal agent of late blight disease (Franke et al. 2012). Previous studies have shown that hormones and signal molecules can stimulate the phenylpropanoid pathway, leading to an

enhanced tolerance to abiotic and biotic stresses (Ahmed et al. 2017; Li et al. 2017a). For instance, exogenous nitric oxide enhances disease resistance to *Monilinia fructicola* in peach fruit by activating the phenylpropanoid pathway (Li et al. 2017a). In our previous studies, we also found that application of 24-epibrassinolide increases PAL activity and flavonoid biosynthesis by triggering NO accumulation in tea leaves (Li et al. 2016a, 2017b).

Brassinosteroids are involved in multiple aspects of plant growth, development, and responses to stress (Ahmed et al. 2014; Li et al. 2016a, 2018b). Besides the critical roles of BR in abiotic stress tolerance, its function in disease resistance has been well documented in a range of plants species (Hideo et al. 2003; Li et al. 2018b; Song et al. 2018; Xia et al. 2009, 2011). BR strengthens defense against a broad range of biotic stressors, including fungi, virus, bacteria, and nematodes (Bjornson et al. 2016; Deng et al. 2016; Hideo et al. 2003; Song et al. 2018). In rice, BR application suppresses the fungal pathogens *Magnaporthe grisea* and *Xanthomonas oryzae* that cause blast and bacterial blight diseases, respectively (Hideo et al. 2003). Similarly, BR enhances resistance to the fungus *Oidium* sp., tobacco mosaic virus, and the bacteria *Pseudomonas syringae* pv. Tabaci in tobacco. In barley, suppression of *BRASSINOSTEROID INSENSITIVE 1 (BR1)*, BR receptor enhances susceptibility to *Fusarium culmorum* as reflected by increased necrosis in leaves, indicating that a functional BR1 is essential for disease resistance (Ali et al. 2014). However, overexpression of BR1-associated kinase 1 (BAK1)-interacting receptor-like kinase 3 (BIR3) in tomato and *Arabidopsis thaliana* results in enhanced susceptibility to the necrotrophic fungus *Botrytis cinerea* (Huang et al. 2017). Song et al. (2018) showed that BR deficiency or lack of BR receptor increases susceptibility of tomato plants to root-knot nematode, *Meloidogyne incognita*. However, the role of BR in the defense response of tea plants to *C. gloeosporioides* remains largely unknown. In this study, we showed that exogenous BR could enhance defense against *C. gloeosporioides* in tea plants, which was associated with BR-induced activation of the phenylpropanoid pathway. Our results also suggest that BR potentially stimulates systemic-induced tolerance but not the lipoxygenase pathway to enhance tea plant resistance to *C. gloeosporioides*.

## Materials and Methods

### Plant Materials, Growth Conditions, and Treatments

Two-year-old tea seedlings of Longjing 43 (*Camellia sinensis* (L.) O. Kuntze) grown in the greenhouse of the Tea Research Institute of the Chinese Academy of Agricultural Sciences (TRI, CAAS, N 30°10', E 120°5'), Hangzhou,

China were used in the current study. The growth conditions in the greenhouse were as follows: the mean maximum and minimum temperatures were 28.8 °C and 22.4 °C and relative humidity was about 70–80%. Tea seedlings were treated with 100 nM 24-epibrassinolide (EBR, Sigma-Aldrich, USA) at 24 h and 3 h prior to inoculation with *C. gloeosporioides*. EBR solution was prepared and sprayed following the method as described previously (Li et al. 2016a, 2017b). Control plants were sprayed with distilled water. Each treatment comprised eighteen seedlings. The experiments were replicated three times independently. All plants were watered with Hoagland's solution.

### Culture of Fungi *C. gloeosporioides* and Methods of Inoculation

A pure culture of *C. gloeosporioides* isolates was obtained following the protocol that we described previously (Li et al. 2016b). To study the infection behavior of cultured *C. gloeosporioides*, a *C. gloeosporioides* spore suspension at a density of  $1 \times 10^5$  spores per ml was sprayed onto the leaves. Tea seedlings were sealed hermetically by plastic wrap to make sure the humidity is 100%.

### Determination of H<sub>2</sub>O<sub>2</sub> Accumulation

Histochemical staining of H<sub>2</sub>O<sub>2</sub> was performed as previously described (Xia et al. 2009). Tea leaves were vacuum infiltrated with 1 mg ml<sup>-1</sup> 3,3'-diaminobenzidine (DAB) in 50 mM TRIS-acetate buffer (pH 3.8) and incubated at 25 °C in for 6 h. DAB-stained leaves were then decolorized in boiling ethanol (95%) for 15 min and photographed with a digital camera. H<sub>2</sub>O<sub>2</sub> concentration in tea leaves was measured spectrophotometrically (Willekens et al. 1997).

### Assay of PAL Enzyme Activity

A tea leaf sample (0.3 g) was homogenized in 3 ml 50 mM potassium phosphate buffer (pH 8.8, containing 2 mM ethylenediaminetetraac (EDTA), 2% polyvinyl polypyrrolidone (PVPP), and 0.1% mercaptoethanol). The resulting homogenates were centrifuged at 15,000 rpm for 20 min at 4 °C and crude enzyme extracts were obtained as the supernatants. The PAL activity was assayed with L-phenylalanine as substrate based on the yield of cinnamic acid and spectrophotometrically determined by monitoring the change in absorbance at 290 nm as described by Zheng et al. (2005).

### Total RNA Extraction and Gene Expression Analysis

For gene expression analysis, leaf samples were collected at 12 h postinoculation and immediately frozen into liquid nitrogen and kept at -80 °C until isolation of RNA. Total

RNA from tea leaves was extracted using an RNA extraction kit (TIANGEN BIOTECH, Beijing, China), according to the manufacturer's recommendations. Total RNA was reverse transcribed using a ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan), following the manufacturer's instructions. Gene-specific quantitative real-time PCR (qRT-PCR) primers were designed based on their cDNA sequences. qRT-PCR was performed using the ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Each reaction (20 µl) consisted of 10 µl SYBR Green PCR Master Mix (Takara, Chiga, Japan), 2 µl diluted cDNA, 0.4 µl ROX reference Dye II (Takara, Chiga, Japan), and 0.2 µmol forward and reverse primers. The cycling conditions were as follows: 95 °C for 30 s, and 40 cycles of 95 °C for 5 s and 60 °C for 34 s. *CsPTB* was used as an internal control. Relative gene expression was calculated as previously described (Livak and Schmittgen 2001).

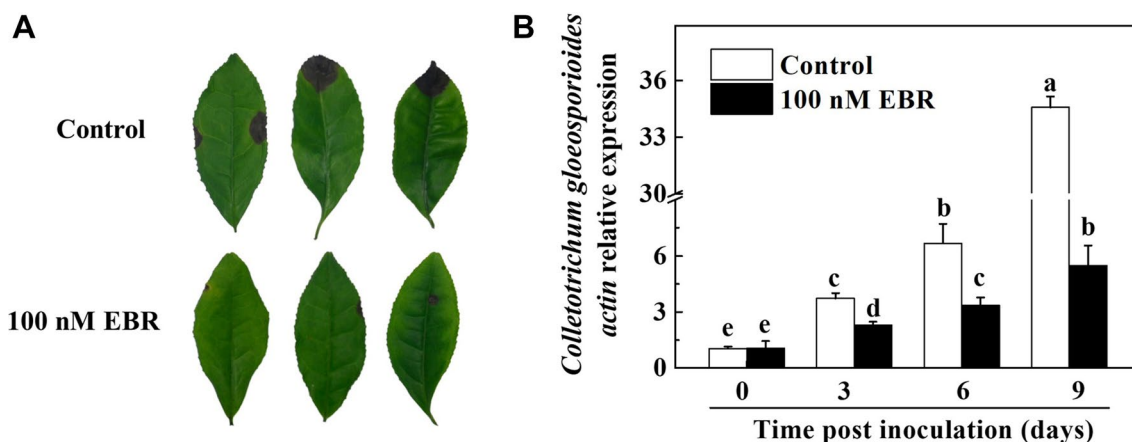
### Statistical Analysis

The data were statistically analyzed using SAS 8.1 software package (SAS Institute Inc., Cary, NC, USA). Differences between treatments means were separated by the Tukey' test at a significance level of  $P < 0.05$ .

## Results

### Exogenous Brassinosteroid Enhances Defense Against *C. gloeosporioides*

To explore the effects of BR on tea plant defense against *C. gloeosporioides*, we pretreated tea plants with exogenous 24-epibrassinolide (EBR, 100 nM) and then inoculated with *C. gloeosporioides*. Necrotic lesions caused by *C. gloeosporioides* expanded gradually over the postinoculation period (data not shown). It is evident from the leaf phenotypes (Fig. 1a) that the lesion area of controls was clearly larger than that of EBR-pretreated leaves, indicating that exogenous EBR remarkably suppressed *C. gloeosporioides* spread as reflected by very small lesions. We also assayed relative expression of *C. gloeosporioides actin* in tea leaves following exogenous EBR and/or *C. gloeosporioides* inoculation (Fig. 1b). The expression of *C. gloeosporioides actin* showed 3.6-, 6.4-, and 33.2-fold increases at 3, 6, and 9 days postinoculation (dpi), respectively, compared with that at 0 dpi. However, exogenous EBR significantly reduced *C. gloeosporioides actin* expression resulting in only at 1.6-, 2.0-, 6.3-fold changes at 3, 6, and 9 dpi, respectively, compared with that of only *C. gloeosporioides*-inoculated tea leaves. These results clearly indicate that exogenous EBR enhances tea plants defense against *C. gloeosporioides*.



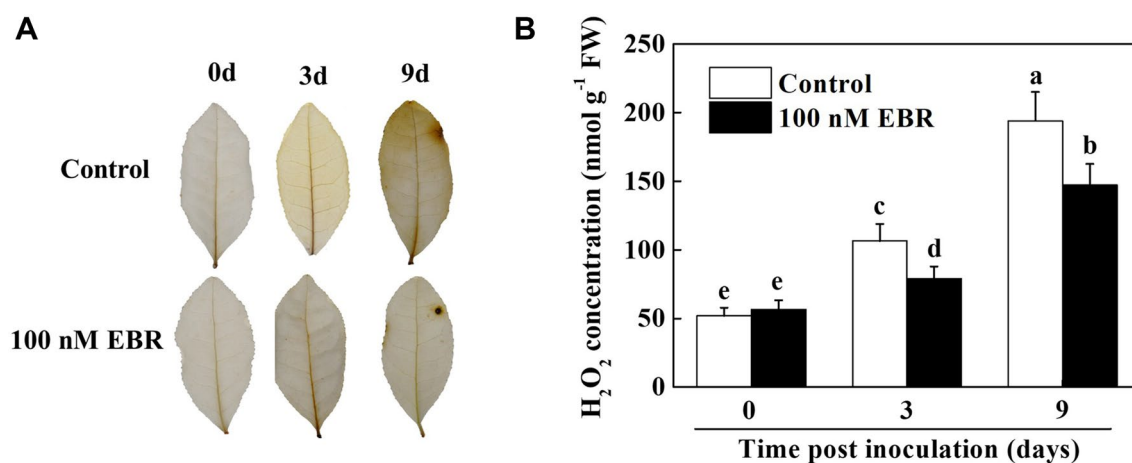
**Fig. 1** Exogenous brassinosteroid suppressed *C. gloeosporioides* spread on tea leaves. **a** Phenotypes of leaves at 9 days postinoculation (dpi) with *C. gloeosporioides*. **b** Relative expression of *C. gloeosporioides actin* at different postinoculation time periods. Two-year-old tea

seedlings were treated with 100 nM EBR (24-epibrassinolide) at 24 h and 3 h prior to inoculation with *C. gloeosporioides*. Gene expression data comprised six replicates. Means followed by the same letter are not significantly different according to Tukey's test ( $P < 0.05$ )

### Brassinosteroids Attenuate *C. gloeosporioides*-Induced $H_2O_2$ Accumulation in Tea Leaves

Reactive oxygen species (ROS) accumulation and subsequent oxidative stress are common phenomena that occur when a plant is challenged with biotic and abiotic stressors. Therefore, we analyzed  $H_2O_2$  accumulation in tea leaves by using both histochemical and biochemical methods. In situ visualization of  $H_2O_2$  using DAB staining

showed that  $H_2O_2$  accumulation increased over the post-inoculation period and became highest at 9 dpi (Fig. 2a). Consistent with DAB staining results,  $H_2O_2$  concentrations in tea leaves increased by 104.52% and 194.01% at 3 and 9 dpi, respectively (Fig. 2b). However, exogenous EBR remarkably decreased  $H_2O_2$  accumulation in *C. gloeosporioides*-inoculated tea leaves as reflected by relatively light brown colored leaves and lower concentrations of  $H_2O_2$ . These findings suggest that exogenous EBR potentially alleviated *C. gloeosporioides*-induced oxidative stress by strengthening antioxidant potential.



**Fig. 2** Exogenous brassinosteroids minimized *C. gloeosporioides*-induced  $H_2O_2$  accumulation in tea leaves. **a** Visualization of in situ  $H_2O_2$  accumulation by 3,3'-diaminobenzidine (DAB) staining. The deep brown spots indicate the polymerization products produced by the reaction of DAB with  $H_2O_2$ . **b**  $H_2O_2$  concentration. Two-year-old

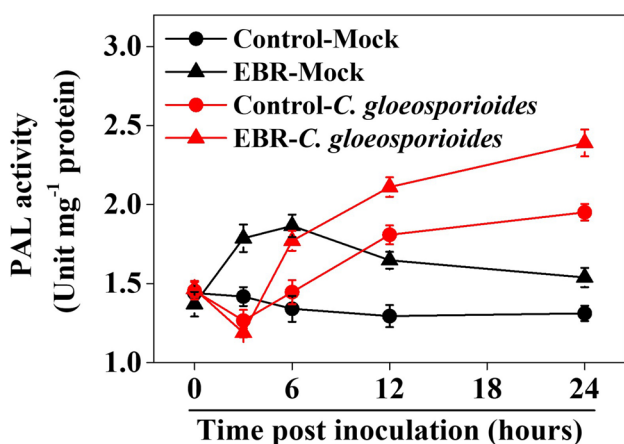
tea seedlings were treated with 100 nM EBR at 24 h and 3 h prior to inoculation with *C. gloeosporioides*. Biochemical data are expressed as mean  $\pm$  SD ( $n = 6$ ). Means denoted by the same letter did not significantly differ at  $P < 0.05$  according to Tukey's test



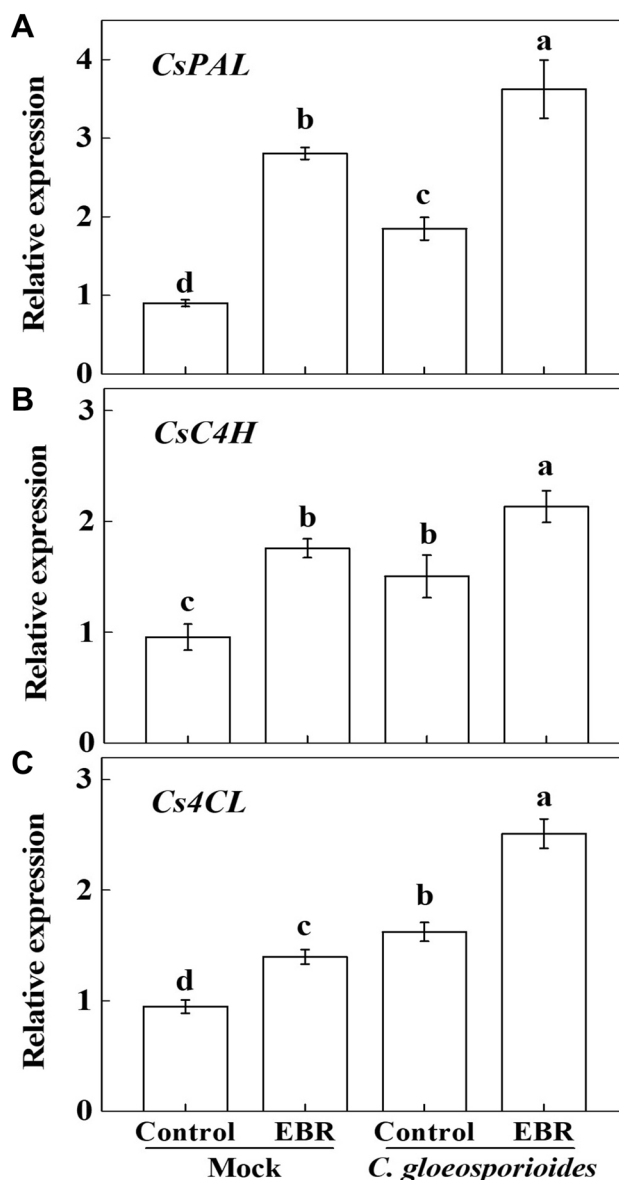
## Brassinosteroids Stimulate Phenylpropanoid Pathway to Enhance Resistance to *C. gloeosporioides*

Activation of the phenylpropanoid pathway contributes to plant defense against pathogenic fungi. Therefore, we analyzed the activity of PAL, the first key enzyme in the phenylpropanoid pathway. Time-course analysis of PAL activity revealed that inoculation with *C. gloeosporioides* caused an initial decline in the early few hours; however, PAL activity, then, gradually increased with postinoculation time period (Fig. 3). At 24 h postinoculation, PAL activity in *C. gloeosporioides*-inoculated tea leaves increased by 48.74% compared with that of control. On the other hand, in the absence of *C. gloeosporioides*, exogenous EBR increased PAL activity during the initial 6 h, which then slowly declined over time. At 24 h postinoculation, PAL activity in only EBR-treated tea leaves was 17.30% higher than that of control. However, combined treatment of exogenous EBR and *C. gloeosporioides* increased PAL activity by 22.47% compared with only *C. gloeosporioides* inoculation.

To further explore the mechanism of EBR-induced enhanced resistance to *C. gloeosporioides*, we assayed the transcript abundance of *CsPAL*, *CsC4H*, and *Cs4CL*, which encode the first three key enzymes of the phenylpropanoid pathway, respectively. As shown in Fig. 4, EBR, *C. gloeosporioides*, and EBR + *C. gloeosporioides* treatments caused 3.12-, 2.05-, and 4.03-fold increases in *CsPAL*, 1.84-, 1.57-, and 2.23-fold increases in *CsC4H* and 1.47-, 1.71-, and 2.65-fold increases in *Cs4CL* expression, respectively, as compared to the control. Interestingly, transcript levels of *CsPAL*, *CsC4H*, and *Cs4CL* in EBR and *C. gloeosporioides*-treated tea leaves were 1.96-, 1.42-, and 1.55-fold larger than that of only *C.*



**Fig. 3** Time-course analysis of phenylalanine ammonia-lyase (PAL) activity as influenced by exogenous brassinosteroid pretreatment and *C. gloeosporioides* inoculation. Two-year-old tea seedlings were treated with 100 nM EBR at 24 h and 3 h prior to inoculation with *C. gloeosporioides*. Data are expressed as mean  $\pm$  SD ( $n=6$ )



**Fig. 4** Exogenous brassinosteroids activated phenylpropanoid pathway in response to *C. gloeosporioides* infection in tea leaves. Relative expression of **a** PHENYLALANINE AMMONIA-LYASE (*CsPAL*), **b** CINNAMATE-4-HYDROXYLASE (*CsC4H*), and **c** 4-COUMARATE-COA LIGASE (*Cs4CL*). Two-year-old tea seedlings were treated with 100 nM EBR at 24 h and 3 h prior to inoculation with *C. gloeosporioides*. Transcript levels of genes were analyzed by qRT-PCR using gene-specific primer pairs (Supplementary Table S1). The samples were harvested for gene expression analysis at 12 h postinoculation with *C. gloeosporioides*. Data are the means  $\pm$  SD of three biological replicates. Means denoted by the same letter did not significantly differ at  $P < 0.05$  according to Tukey's test

*gloeosporioides*-inoculated tea leaves, respectively. This implies that exogenous EBR activated transcription of key enzymes of the phenylpropanoid pathway, which potentially increased resistance to *C. gloeosporioides* in tea plants.

## Brassinosteroids Induce Defense Gene Expression But Not LOX Pathway to Enhance Defense Against *C. gloeosporioides*

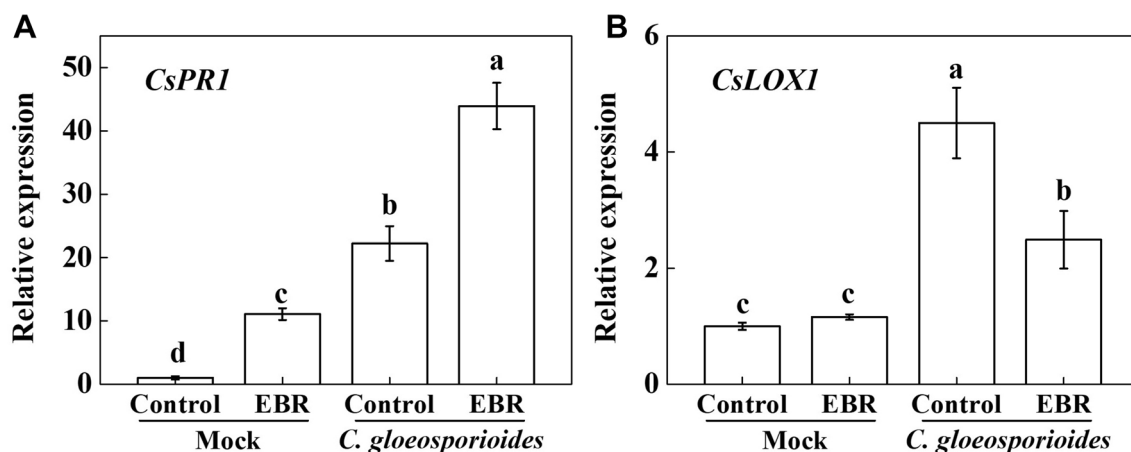
Three classical hormones, such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), play critical roles in plant defense against pathogens (Yang et al. 2014). Most often, the SA and JA pathways function antagonistically to mediate a defense response. However, BR-induced resistance to *Meloidogyne incognita* is not dependent on SA, JA, and ET accumulation (Song et al. 2018). In our previous study, we found that exogenous caffeine enhances defense against *C. gloeosporioides* by increasing LOX activity and JA concentration in tea leaves (Li et al. 2016b). Furthermore, a close association between expression of defense genes, such as *PATHOGENESIS-RELATED GENE 1 (PR1)* and an effective defense response has been reported in various plant-pathogen systems (Ahammed et al. 2018; Xia et al. 2011). Therefore, we analyzed transcript abundance of *CsPR1* and *CsLOX1* following treatment with EBR and/or *C. gloeosporioides*. As shown in Fig. 5a, exogenous EBR and *C. gloeosporioides* inoculation increased the transcript levels of *CsPR1* accounting for 10.93- and 21.93-fold changes, respectively, compared with the non-inoculated control. Meanwhile, EBR pretreatment on *C. gloeosporioides*-inoculated plants caused a 1.98-fold increase in *CsPR1* expression as compared with that of only *C. gloeosporioides*-inoculated tea leaves. Moreover, we found 1.16- and 4.50-fold increases in *CsLOX1* expression after exogenous EBR treatment and *C. gloeosporioides* inoculation, respectively, compared with the non-inoculated control (Fig. 5b). Unlike the transcript abundance of *CsPR1*, *CsLOX1* expression was not altered

by EBR-only treatment. Moreover, exogenous EBR rather attenuated *C. gloeosporioides*-induced upregulation in *CsLOX1* expression, indicating that exogenous EBR potentially inhibits the LOX-mediated defense pathway to enhance resistance to *C. gloeosporioides* in tea plants.

## Discussion

Global tea production is being influenced by climate change (Han et al. 2018). The key drivers of climate change not only exert direct effects on tea physiology, but also affect tea production indirectly by altering insect and disease abundance. Previously, we showed that elevated CO<sub>2</sub> increases susceptibility of tea plants to *C. gloeosporioides* by decreasing caffeine biosynthesis (Li et al. 2016b). In the current study, we found that exogenous BR improves tea plant resistance to *C. gloeosporioides* which is largely attributed to the BR-induced activation of the phenylpropanoid pathway (Figs. 1, 2, 3, 4). Moreover, exogenous BR upregulated expression of the defense gene, *CsPR1*, but downregulated expression of *CsLOX1* to enhance the defense response of tea plants to *C. gloeosporioides*.

Plants have developed intricate strategies to restrict pathogen development to a limited area (Yogendra et al. 2015). These include activation of signaling molecules, transcriptional reprogramming, and biosynthesis of defense metabolites that limit pathogen infection. Most often a high concentration of secondary metabolites belonging to phenylpropanoid pathway largely contributes to the enhanced resistance in resistant genotypes (Li et al. 2017a; Novo et al. 2017; Wei et al. 2017; Yogendra et al.



**Fig. 5** Exogenous brassinosteroids augmented the expression of defense gene in *C. gloeosporioides*-inoculated tea leaves. Relative expression of **a** *PATHOGENESIS-RELATED GENE 1 (CsPR1)* and **b** *LIPOXYGENASE 1 (CsLOX1)*. Two-year-old tea seedlings were treated with 100 nM EBR at 24 h and 3 h prior to inoculation with *C. gloeosporioides*. Transcript levels of genes were analyzed by qRT-

PCR using gene-specific primer pairs (Supplementary Table S1). The samples were harvested for gene expression analysis at 12 h postinoculation with *C. gloeosporioides*. Data are the means  $\pm$  SD of three biological replicates. Means denoted by the same letter did not significantly differ at  $P < 0.05$  according to Tukey's test

2015). In the current study, inoculation with *C. gloeosporioides* caused necrotic lesions in tea leaves; however, exogenous EBR reduced the lesion expansion by limiting the pathogen spread beyond the site of infection (Fig. 1). Some evidence suggests that BRs alter the efficiency of innate immunity responses to microbe-associated molecular patterns (MAMPs) which are often perceived in association with the co-receptor, Brassinosteroid Insensitive1-Associated Kinase 1 (BAK1), leading to a rapid and transient production of apoplastic ROS as well as activation of mitogen-activated protein kinase (MAPK) cascades (Bjornson et al. 2016). The MAPK signaling pathway plays a critical role in defense against biotic stressors (Song et al. 2018; Yogendra et al. 2015). In potato, MAPKs stimulate the WRKY1 transcription factor which directly triggers 4-coumarate:CoA ligase (4CL) synthesis to enhance defense against *Phytophthora infestans* by secondary cell wall thickening (Yogendra et al. 2015). On the other hand, in tomato, suppression of *MAPK1*, *MPAK2*, and *MAPK3* compromises BR-induced resistance to root-knot nematode (Song et al. 2018), suggesting that BR might activate MAPK and thus WRKY to stimulate the phenylpropanoid pathway to enhance defense against *C. gloeosporioides* in tea plants. However, at this point, such an assumption is just speculation and thus requires further molecular intervention to dissect the in-depth mechanism of BR-induced phenylpropanoid biosynthesis and subsequent disease resistance.

ROS play a dual role in plant stress responses (Xia et al. 2009). ROS overload causes oxidative burst and damage to proteins, lipids, and nucleic acids. On the other hand, a rapid and transient ROS accumulation in the apoplast mediates BR-induced disease resistance (Song et al. 2018; Xia et al. 2011). In the current study, *C. gloeosporioides*-caused necrotic lesions were associated with in situ accumulation of  $H_2O_2$  (Fig. 2). However, exogenous EBR minimized  $H_2O_2$  accumulation, possibly by activating the phenylpropanoid pathway that produces compounds with high antioxidative property. In cucumber, exogenous EBR enhances PAL activity and flavonoid content, leading to the attenuation of organic pollutant-induced ROS accumulation and oxidative stress (Ahammed et al. 2017). As the key enzymes of phenylpropanoid metabolism pathway, enhanced activity of PAL, C4H, and 4CL potentially increases the biosynthesis of polyphenols including flavonoids, in plants (Wei et al. 2017). Furthermore, nitric oxide plays a critical role in BR-induced flavonoid biosynthesis in tea plants (Li et al. 2017b). While exogenous NO enhances defense against fungal pathogen *Monilinia fructicola* by activating the phenylpropanoid pathway in peach fruits (Li et al. 2017a), scavenging of NO in the upper untreated leaves blocks BR-induced systemic virus resistance in tobacco (Deng et al. 2016). Therefore, it is plausible that BR-induced NO production and subsequent

secondary metabolite synthesis might largely contribute to the BR-enhanced resistance to *C. gloeosporioides* in tea plants.

Plant hormones play a vital role in plant immune responses (Yang et al. 2014). These defense responses are not activated by a single hormone, rather through a complex intertwined network of pathways involving multiple hormones and signaling intermediates. The SA, JA, and ET pathways are primary mediators in the defense response, which function synergistically or antagonistically to fine-tune resistance of a plant species to a particular pathogen (Yang et al. 2014). Previous studies have revealed that the BR-induced systemic defense response is independent of SA, JA, and ET accumulation (Hideo et al. 2003; Xia et al. 2009, 2011), but possess some characteristics that are similar to SA-dependent systemic acquired resistance (SAR). For instance, exogenous EBR augments expression of stress- or defense-related genes such as *PATHOGENESIS-RELATED 1 (PR1)*, leading to enhanced tolerance to wilt disease caused by *Fusarium oxysporum* in cucumber (Xia et al. 2011). EBR treatment results in a rapid and transient upregulation of *PR-1* and *PAL* transcripts as early as at 3 h, which reaches the maximum level at 12 h without any significant effect on SA accumulation in cucumber (Xia et al. 2009). Likewise, we found that EBR treatment induced an increase in *CsPR1* and *CsPAL* mRNA levels in the absence of *C. gloeosporioides* inoculation (Fig. 5a). However, combined treatment of EBR and *C. gloeosporioides* caused the maximum upregulation in *CsPR1* expression, which was significantly higher than that of only EBR or *C. gloeosporioides* inoculation. In contrast to *CsPR1*, *CsLOX1* expression was suppressed by exogenous EBR (Fig. 5b). Many oxylipins are derived through the 9-lipoxygenase (9-LOX) oxylipin pathway, which play important roles in plant defense. Previous studies have revealed close interactions between the 9-LOX and BR pathways in modulating plant defense in *Arabidopsis* (Marcos et al. 2015). Furthermore, a specific branch of the LOX pathway, the 13-LOX pathway, synthesizes JA in plants. Therefore, it cannot be excluded that exogenous EBR-induced suppressed *LOX1* expression might result in decreased JA biosynthesis. Notably, BR suppresses JA biosynthesis in rice and thus attenuates plant defense against root-knot nematodes (Nahar et al. 2013). This observation contrasts with the report of Song et al. (2018), who demonstrated a positive role of BR in enhancing resistance to root-knot nematode in tomato. Therefore, BR-induced enhanced defense against *C. gloeosporioides* might be mediated by a complex interconnecting signal transduction pathway that demands further elucidation in tea plants.

In conclusion, we clarified a positive role of BR in strengthening plant resistance to *C. gloeosporioides* in tea plants. Exogenous EBR pretreatment substantially reduced *C. gloeosporioides*-induced lesion expansion in inoculated

tea leaves and was accompanied by decreased transcript levels of *C. gloeosporioides actin* and a reduced accumulation of H<sub>2</sub>O<sub>2</sub>. Exogenous EBR activated the phenylpropanoid pathway as reflected by increased PAL activity and transcript levels of *CsPAL*, *CsC4H*, and *Cs4CL*. Moreover, expression of *CsPRI* and *CsLOXI* was upregulated and downregulated, respectively, by exogenous EBR in fungi-inoculated leaves, indicating that BR potentially activates the systemic defense response to enhance tea plant resistance to *C. gloeosporioides*. Taken together, our results suggest a positive role of BR in the immune response of tea plants which can be implicated in *C. gloeosporioides*-caused disease control in an eco-friendly manner.

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare no conflict of interest.

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