Resistance-related physiological response of rice leaves to the compound stress of enhanced UV-B radiation and *Magnaporthe oryzae*

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**ABSTRACT**

An enhanced UV-B radiation (5.0 kJ m\(^{-2}\)) was supplied before, during, and after *Magnaporthe oryzae* infection. The effects of single and compound stress of the UV-B radiation and *M. oryzae* on the resistance physiology and gene expression of rice leaves were examined. Results revealed that UV-B radiation given before *M. oryzae* infection (UV-B → M.) significantly increased the pathogenesis-related proteins (PRs) activities of phenylalanine ammonialyase (PAL), lipoxigenase (LOX), chitinase (CHT), and β-1,3-glucanase, the resistance-related substances (flavonoids and total phenols) content, and resistance-related genes (OsPAL and OsCHT) expression, thereby improving the disease resistance of rice leaves. Simultaneous exposure to UV-B radiation and *M. oryzae* (UV-B/M.) significantly increased the OsLOX2 expression and the PRs activities. Exposure to UV-B radiation after *M. oryzae* infection (M. → UV-B) decreased the flavonoid content, did not improve the PRs activity, and increased OsLOX2 expression. Compound treatments of UV-B → M., UV-B/M., and M. → UV-B reduced the disease index by 62.3%, 40.2%, and 26.6%, respectively, indicating UV-B radiation inhibited the occurrence of *M. oryzae* disease, but its inhibitory effect weakened when it was provided after *M. oryzae* infection. Hence, rice responded to the compound stress of UV-B radiation and *M. oryzae* through a resistance-related physiological mechanism associated with the sequence of stress occurrence.

**Introduction**

Crops usually experience abiotic and biotic stress during growth. For example, crops are exposed to a decreased ozone concentration in the stratosphere and an increased ultraviolet-B (UV-B) radiation (280–315 nm) flux reaching the surface (Williamson et al. 2014). Crops are also affected by pathogens as one of the major biotic stress (Sharma et al. 2012; Samalova et al. 2014).

The resistance of crop leaves presents an active response when crops cope with environmental stress, such as enhanced UV-B radiation and pathogens. This physiological response involves various enzymes. For instance, phenylalanine ammonialyase (PAL) is a key rate-limiting enzyme in phenylpropanoid compound metabolism, and it regulates the biosynthesis of flavonoids, which is a substance implicated in the resistance of crop leaves (Rawal et al. 2013; Chandrasekaran et al. 2017). Enhanced UV-B radiation and pathogens induce an increase in PAL activity in leaves, promote flavonoid biosynthesis, protect crops from UV-B damage, help them resist pathogen invasion, and improve crop resistance (Li et al. 2018). Therefore, PAL participates in resistance-related mechanisms against UV-B-induced stress and in the inhibition of pathogen infections in crops leaves (Liu et al. 2012; Zhang et al. 2014).

Lipoxigenase (LOX) is a multifunctional enzyme that can metabolize endogenous and exogenous chemicals. LOX can actively respond to UV-B and pathogens by catalyzing the generation of reactive oxygen species (ROS) (He et al. 2014). When stress cannot be timely and effectively eliminated, LOX is activated, and the reaction products of LOX can further generate ROS, elicit a destructive effect on the cell membrane, and cause a disease-resistant necrotic reaction in crop leaves (Pushpalatha et al. 2011; Singh et al. 2012).

Chitinase (CHT) and β-1,3-glucanase are important pathogenesis-related proteins (PRs) that participate in the resistance-related mechanism of crops under abiotic and biotic stress, such as enhanced UV-B radiation. Enhanced UV-B radiation induces an increase in the activities of PRs, such as chitinase and β-1,3-glucanase (Oka et al. 2011). Chitin, which is an important component in the cell wall of pathogenic fungi, stimulates defense reaction and activates CHT, β-1,3-glucanase, and other PRs (Chehab et al. 2007; Wan et al. 2008) to inhibit pathogen growth in crop leaves and to improve disease resistance.

Rice is one of the most important grain crops worldwide, and its production is threatened by *Magnaporthe oryzae*, which is the most harmful pathogen affecting this crop (Law et al. 2017). The colonization and growth of *M. oryzae* are remarkably influenced by the enhanced UV-B radiation. Under the enhanced UV-B radiation, spores and appresoria are significantly inhibited, accompanied by a reduced number of lesions, declined pathogenicity, and weakened state of *M. oryzae* on rice leaves (Li et al. 2018). Rice blast occurrence is closely related to the resistance mechanism of rice (Vergne et al. 2010). Under *M. oryzae* stress, the levels of resistance-inducing substances increase, thereby improving enzymatic activities in the phenylpropanoid pathway in rice leaves.
(Gao et al. 2010). Under some circumstances, rice can rapidly generate a response to the M. oryzae infection and form a necrotic reaction at infection sites, thereby preventing further infection (Dodd et al. 2010). When exposed to UV-B radiation, rice leaves effectively filter UV-B radiation by synthesizing defense-related substances (Schreiner et al. 2014) and by increasing PR activities (Oka et al. 2011) to activate a stress-resistant defense system, trigger and activate multi-signal transduction, and stimulate resistance-related genetic expression, thereby alleviating M. oryzae occurrence (Duan et al. 2014). Therefore, the physiological mechanism associated with the disease resistance of rice leaves presents an active response to enhanced UV-B radiation and M. oryzae stress. However, few reports have described the resistance of rice leaves in response to the compound stress of enhanced UV-B radiation and M. oryzae. Enhanced UV-B radiation (5.0 kJ m$^{-2}$) exposure was conducted at different phases of M. oryzae infection (before, during, and after) in rice leaves. The response of rice to the single and compound stress of enhanced UV-B radiation and M. oryzae was analyzed by determining the resistance-related physiological mechanism and gene expression in rice leaves. The following assumptions were made: (1) The resistance of rice involved an active response to the compound stresses of UV-B radiation and M. oryzae. (2) When the rice was exposed to the enhanced UV-B radiation before M. oryzae infection occurred, the resistance mechanism was activated, and disease resistance was improved. (3) When the rice was irradiated with UV-B after M. oryzae infection occurred, the resistance of rice leaves to stress could not be further improved.

**Materials and methods**

**Materials**

A native rice variety (Baijiaolaojing) obtained from seeds grown by farmers was used in this experiment. M. oryzae strain (PEX22A-PCB1004) was provided by the Key Laboratory for Plant Pathology of Yunnan Province, with confirmed disease-causing genes and stable pathogenicity. Furthermore, 40 W UV-B lamps (wavelength 280–320 nm) were purchased from Shanghai GuCun Instrument Plant, and a UV-B radiation determinator (Beijing 720) was procured from Photo-electric Instrument Plant of Beijing Normal University.

Soil was collected from the experimental farm of Yunnan Agricultural University. Soil background values were as follows: total nitrogen, phosphorus, and potassium contents of 1.65, 9.98, and 26.32 g kg$^{-1}$, respectively; alkali-hydrolyzable nitrogen, available phosphorus, and potassium contents of 123.52, 97.50, and 316.60 mg kg$^{-1}$, respectively; organic matter content of 29.0 g kg$^{-1}$; and pH of 6.79.

**Cultivation in pots in a glasshouse**

Rice seeds were soaked in carbendazim solution diluted by 1000 times for 20 min and placed in a tray covered with two layers of gauze soaked in sterile water. The seeds were cultivated in the dark for 5 days in an incubator at 28°C until they germinated. Afterward, 9 seedlings were sown into each of the 20 pots filled with 20 kg soils in a greenhouse. The greenhouse had a daily temperature varied 20–35°C, a nightly temperature of 15–24°C, and a humidity of 80–90%. After the seedlings started growing 3 leaves, 18 pots with a consistent growth were selected for the enhanced UV-B radiation and M. oryzae inoculation treatments.

**Experimental design**

The 40 W UV-B lamps (wavelength 280–320 nm) were used to supply UV-B radiation above the rice seedlings. The UV-B radiance here as biologically effective UV-B irradiance (UVBE) weighted according to the generalized plant action spectrum of Caldwell (1971) and normalized at 300 nm. The UV-B lamps were filtered by 0.13 mm thick cellulose diacetate membrane which was pre-burned for stability and replaced each week with a new pre-burned film. The height of the lamp tubes was measured using a determinator and adjusted to ensure that 5.0 kJ m$^{-2}$ UV-B radiation reached the rice seedlings. The 5.0 kJ m$^{-2}$ UV-B radiation represent 20% summer ozone depletion at Kunming City (25°N, 1950 m), Yunnan Province, China during a clear day at the summer solstice (10 kJ m$^{-2}$ day$^{-1}$ UVBE) according to a mathematical model of Madronich et al. (1995). The UV-B lamps were not installed in the control group. UV-B radiation was administered for 7 h at 10:00–17:00 every day until the physiological and disease indices were identified. Each treatment was performed four times.

Six treatments were prepared in the experiment: (1) natural light for control group (CK), (2) single treatment of enhanced UV-B radiation (UV-B), (3) single treatment of M. oryzae inoculation (M.), (4) compound treatment of enhanced UV-B radiation supplied 3 days before M. oryzae inoculation/pre-inoculation (UV-B → M.), (5) compound treatment of enhanced UV-B radiation and during M. oryzae infection (UV-B/M.), and (6) compound treatment of enhanced UV-B radiation supplied 3 days after M. oryzae inoculation/post-inoculation (M. → UV-B). All of the indices were determined 7 days after the experiment.

**Inoculation of M. oryzae**

M. oryzae strain was cultured for 7 days at 26°C on a yeast culture medium. A mycelial lawn with a diameter of 2 mm was cut using a borer, transferred to a potato glucose agar (PDA) medium, and cultured for 10 days with 10 h/day light conditions at 26°C. M. oryzae sporulation was observed under a microscope. Spore suspensions were utilized as inoculants with a 3 × 10$^5$ mL$^{-1}$ concentration as determined by a cell counting chamber. Inoculation was performed by spraying M. oryzae spores onto the leaves of rice seedling.

**Determination of resistance-related enzymes in rice leaves**

The fresh rice leaves (0.3 g) were homogenized to extract the soluble proteins using a pre-chilled mortar and pestle with the addition of quartz sand in ice-cold saline water. The homogenate was centrifuged at 2000 × g for 10 min at 4°C. A supernatant (50 μL) was used to determine the total soluble protein content. The binding of the dye (Coomassie Brilliant Blue G-250) to protein caused an increase in absorbance at 595 nm. Bovine serum albumin (BSA) was used as the standard (0.563 g L$^{-1}$), and double-distilled water was used in blanks (Bradford, 1976).
**Determination of PAL activity (Engelsma 1974)**

In this procedure, 0.3 g of rice leaves was sampled, added to 3 mL of 0.1 mol L\(^{-1}\) boric acid buffer (pH = 8.8, containing 5 mmol L\(^{-1}\) β-mercaptoethanol and 0.1 g L\(^{-1}\) PVP), ground in an ice bath, and centrifuged at 10,000 × g at 4°C by using a high-speed freezing centrifuge for 10 min. The supernatant (20 μL) was taken and added with 780 μL of 0.1 mol L\(^{-1}\) boric acid buffer and 200 μL of 0.02 mol L\(^{-1}\) phenylalanine. The reaction solution was kept at 30°C in a thermostatic water bath for 0.5 h. The change in optical density at 290 nm (OD\(_{290}\)) was determined with a UV-vis spectrophotometer. The PAL activity expressed as 1 U mg\(^{-1}\) protein referred to the change in absorbance for 0.1 unit at 290 nm per minute per milligram of protein in rice leaves.

**Determination of LOX activity (Todd et al. 1990)**

In this procedure, 0.30 g of rice leaves was added to 4.5 mL of 50 mmol L\(^{-1}\) phosphate buffered solution (PBS) (containing 1.33 mmol L\(^{-1}\) ethylenediaminetetraacetic acid (EDTA) and 1% polyvinylpyrrolidone [PVPP], pH = 7.8), ground in an ice bath, and centrifuged at 16,000 × g at 4°C by using the high-speed freezing centrifuge for 20 min. For reaction tubes, 280 μL of the reaction solution (40 mL of 0.1M PBS, 200 μL of Tween 20, and 40 μL of linoleic acid, pH 7.0) and 20 μL of the enzyme extracting solution were added. Absorbances at 15 and 75 s at 234 nm were recorded. The LOX activity was expressed as 1 U mg\(^{-1}\) protein. One unit of enzymatic activity per milligram of protein catalyzed the change in the absorbance of 0.001 unit per minute at 25°C.

**Determination of CHT activity (Reissig et al. 1955)**

In this procedure, 0.10 g of rice leaf was weighed and ground into powder after liquid nitrogen was added. Afterward, 1 mL of acetic acid extraction solution was added (0.05 mol L\(^{-1}\), pH 5.0). The resulting mixture was centrifuged at 12,000 rpm at 4°C for 20 min, and 0.4 mL of the supernatant was added to 0.2 mL of acetic acid buffer solution (0.05 mol L\(^{-1}\), pH 5.0) and 0.4 mL of 1% colloid chitin solution. The mixture was incubated in a water bath at 37°C for 1 h and centrifuged at 5000 rpm for 10 min. To determine N-acetylglucosamine content, we obtained 0.4 mL of the supernatant and added it to 0.2 mL of saturated borax solution. The mixture was subsequently heated in a boiling water bath for 7 min. After the mixture was cooled, 0.2 mL of glacial acetic acid and 0.2 mL of 1% paraformaldehyde (PDAB) solution were added. The mixture was incubated in a water bath at 37°C for 15 min, and absorbance was obtained at 585 nm. The CHT activity was expressed as 1 U mg\(^{-1}\) protein. One unit of enzymatic activity referred to 1 mg of N-acetylglucosamine generated per gram of tissue per hour through chitin decomposition at 37°C.

**Determination of β-1,3-glucanase activity (Ippolito et al. 2000)**

In this procedure, 100 μL of crude enzyme solution was taken and added with an equal volume of 0.05 mol L\(^{-1}\) sodium acetate solution (containing 5 mg mL\(^{-1}\) laminarin, pH 5.0). The mixture was incubated in a water bath and maintained at 37°C for 1 h. Afterward, 600 μL of 3.5-dinitrosalicylic acid (DNS) was added, and the resulting mixture was heated in a water bath for 5 min at 100°C. The mixture was cooled and diluted with distilled water. The reducing sugar content was determined by using a colorimeter at 540 nm. The enzyme solution heated and boiled for 5 min was used as the control. One unit of enzymatic activity was expressed as a gram of tissue generating 1 mg of reducing sugar per hour.

**RT-qPCR analysis**

RNA was extracted with a QIAGEN RNeasy Plant mini kit (74903). The extracted RNA was eluted with 30 μL of RNase-free water, and its concentration was measured using a UV spectrophotometer (NanoDrop).

Diethyl pyrocarbonate (DEPC) water, 0.5 μL of RNA enzyme inhibitor (50 U μL\(^{-1}\)), 2 μL of random primer (50 pmol μL\(^{-1}\)), and 0.2 μg of RNA were successively added to a 1.5 mL centrifuge tube. The volume of DEPC water and RNA was 8 μL. Afterward, 10.5 μL of the reactant was placed in water at 65°C for 5 min, exposed to room temperature for 10 min, and centrifuged at 8000 × g for 5 s. Then, 0.5 μL of RNA enzyme inhibitor (50 U μL\(^{-1}\)), 4 μL of 5x buffer (Invitrogen), 2 μL of dNTP mix (10 mM each), 2 μL of DTT, and 1 μL of AMV (200 U μL\(^{-1}\)) were successively added, maintained at 40°C in a water bath for 1 h, and exposed to 90°C for 5–10 min. Afterward, the resulting mixture was cooled in an ice bath for 5 min and centrifuged at 8000 × g for 5 s.

The sequence and primers are shown in Table 1. In this procedure, 6.6 μL of double-distilled water, 8 mL of 2× PCR mix (QIAGEN), 0.2 μL of primer (up 50 pmol μL\(^{-1}\)), 0.2 μL of primer (down 50 pmol μL\(^{-1}\)), and 1 μL of the template were added to a reaction tube. The mixture was cycled for 2 min at 95°C, followed by 40 cycles of 94°C for 10 s, 60°C for 10 s, and 72°C for 40 s. RT-qPCR samples were tested in triplicates on an ABI ViiA 7 PCR instrument. The Ct data acquisition in the reaction was set using a corrected threshold value. Real-time fluorescence quantification PCR was performed, and the Ct of the control was set as the reference gene in the control group, and a 2\(^{-ΔΔCt}\) method was utilized for relative quantification.

**Determination of stress-resistant substances in rice leaves**

Fresh rice leaves (0.1 g) was sampled by a punch with a diameter of 7 mm and placed in a test tube. The samples were extracted for 30 min with 10 mL of acidified methanol (methanol:water:hydrochloric acid, 79:20:1, v/v) according to the procedure of Chhiphano et al. (2003). The hydrochloric acid used was 36% HCl. Extract absorbance at 300 nm was measured using an ultraviolet–visible spectrophotometer (UV-5800), because the major extracted compounds with 300 nm absorbance were flavonoids, and expressed on the basis of leaf surface area (A\(_{300}\) g\(^{-1}\) FW).

**Table 1. Sequence and primer design.**

| Primer | Direction | Primer sequence (5′–3′) |
|--------|-----------|------------------------|
| OsLOX2 | Forward   | GCGATGGACATTTGAGAGGAG |
| OsPAL  | Reverse   | TGAATGAGTCAGCAATACGGG |
| OsCHT  | Reverse   | GTCGTCACTGAGACCCCAAC  |
| Actin  | Reverse   | ATCGAATCCAAACGAGCC   |
|        | Forward   | ACCGATAGGGCGCTTGGAGG |
|        | Reverse   | GTCGCTGACATCGCGCTCQTG |
|        |           | GTCCTCATCAAATTTCCGGCT |
According to the method of Kakani et al. (2004), five 38.5 mm² leaf discs were placed in a test tube and extracted for 24 h with 10 mL of acidified methanol. The test tubes were kept in dark at room temperature for 24 h to completely extract phenolic compounds. The absorbance was measured at 300 nm with an ultraviolet–visible spectrophotometer (UV-5800). The phenolic content was calculated using the equation, \[ C = 16.05 \times A \], where \( A \) is an absorbance at 300 nm and \( C \) is phenolic concentration (mg·mL\(^{-1}\)). The pigment concentrations were expressed on a leaf area basis (μg·cm\(^{-2}\)).

**Investigation of disease index**

After \( M. \) oryzae was inoculated for 1 week, the disease index (DI) of rice blast was observed. In accordance with the international rice disease level classification standard (IRRI 2002), DI was divided into 10 levels based on the size of the scab area. Each level represented the corresponding value.

\[
\text{DI} = \frac{\left\{ \sum (\text{disease level leaf number} \times \text{representative value}) \right\}}{\text{total leaf number} \times \text{heavy disease representative value}} \times 100\%.
\]

**Data analysis**

Data were processed using Excel 2010 and drawn using Origin 9.0. Results were expressed as means ± standard deviations (SDs). Duncan’s new multiple range test at a 0.05 probability level was conducted to detect differences between treatments by using SPSS20 (SPSS Inc, Chicago, IL, USA).

**Results**

**Activities of resistance-related enzymes in rice leaves**

The enhanced UV-B radiation (UV-B) significantly increased LOX, PAL, CHT, and \( \beta \)-1,3-glucanase activities in rice leaves in comparison with those in CK. \( M. \) oryzae inoculation (M.) significantly increased \( \beta \)-1,3-glucanase activity, whereas PAL activity was significantly inhibited. In the compound treatments of UV-B radiation and \( M. \) oryzae inoculation, the activities of LOX, PAL, CHT, and \( \beta \)-1,3-glucanase of UV-B \( \rightarrow \) M. and UV-B/M. were significantly higher than those of the rice seedlings exposed to single stressor only. The activities of LOX, PAL, and CHT in the seedlings exposed to the compound treatment of UV-B radiation supplied after \( M. \) oryzae infection were significantly lower than those under the UV-B-only treatment. The LOX and PAL activities were significantly higher than those under the M. only treatment. The CHT and \( \beta \)-1,3-glucanase activities were not different from those under the M. treatment but were significantly higher than those of CK. The activities of LOX, PAL, CHT, and \( \beta \)-1,3-glucanase were the highest under the UV-B \( \rightarrow \) M. and UV-B/M. treatments, followed by the UV-B and M. \( \rightarrow \) UV-B treatments (Figure 1). Thus, the compound stress of UV-B radiation and \( M. \) oryzae resulted in increased enzymatic activities in the rice leaves.

**Resistance-related gene expression in rice leaves**

The expression levels of resistance-related genes, namely, OsLOX2, OsPAL, and OsCHT, were quantitatively measured...
through qRT-PCR under the enhanced UV-B radiation and *M. oryzae* stresses (Figure 2). The single UVB, simultaneous, and post-inoculation groups had significantly elevated the OsLOX2 expression, but not for the single M. and pre-inoculation group. All the five stress treatments significantly increased the OsPAL and OsCHT expression. The OsLOX2 expression was the highest under the UV-B treatment, followed by the simultaneous and post-inoculation treatments. The OsPAL expression was the greatest in the pre-inoculation treatment, followed by the single UV-B and simultaneous treatments. The OsCHT expression was the greatest in single UV-B and pre-inoculation treatments, followed by the single M. and simultaneous treatments. Thus, UV-B radiation and *M. oryzae* infection caused an increased expression of genes encoding resistance-related enzymes in rice leaves.

### Content of stress-resistant substances in rice leaves

The flavonoid contents were the highest in pre-inoculation treatment, followed by the single UV-B and single M. treatment. All the five stress treatments significantly increased the total phenol content in rice leaves, and the increased values were higher in the single UV-B, pre-inoculation, and simultaneous treatments than in the other treatments (Figure 3). Thus, UV-B radiation and *M. oryzae* infection enhanced the contents of the stress-resistant substance in rice leaves.

### Disease index of *M. oryzae* in rice leaves

The enhanced UV-B radiation significantly inhibited *M. oryzae* growth in rice leaves. The inhibitory effects of UV-B→M., UV-B/M., and M.→UV-B treatments on *M. oryzae* disease were 62.3%, 40.2%, and 26.6%, respectively. The compound treatment of the enhanced UV-B radiation supplied before the *M. oryzae* infection (UV-B→M.) elicited the highest inhibitory effect on *M. oryzae* growth on rice leaves (Figure 4).

### Discussion

In the present study, rice leaves actively responded to the compound stress of enhanced UV-B radiation and *M. oryzae*, and this resistance mechanism was related to the sequence of stress exposure. When the UV-B radiation intensity is within the tolerable limits of rice leaves, UV-B activates the related enzymatic activity and gene expression of the stress resistance pathway, thereby inducing immunity to infection (Shehzadi et al. 2018). The UV-B radiation stimulates the plant’s defensive mechanism similar to antioxidants and promotes the scavenging of free radicals in cells (Karthishwaran et al. 2018).

The compound treatment of the enhanced UV-B radiation supplied before the *M. oryzae* inoculation (UV-B→M.) could activate the resistance-inducing mechanism and improve the stress tolerance of rice leaves. Therefore, the UV-B→M. treatment had the lowest incidence of *M. oryzae* disease. The PAL and CHT activities and the flavonoid content under the UV-B→M. treatment were significantly higher than those under other treatments. In addition, positive correlations were found between the OsPAL expression and the PAL activity (*r* = 0.914, *P* < .01) and between the OsCHT expression and the CHT activity (*r* = 0.648, *P* < .05).
control process in phytogenetics to form a necrotic reaction at causes apoptosis. Moreover, this high expression is an active glucanase (Oka et al. 2011), and inhibits pathogen growth an increase in the activities of PRs, such as CHT and (Agati et al. 2013). Enhanced UV-B radiation also triggers tive oxygen and enhance the stress resistance of plants from damage caused by UV-B radiation and oxidative reac-
tion (SD), n = 4. Different letters indicate significant differences (P < .05) according to Duncan’s new multiple range test.

Figure 4. Disease index of rice leaves under single and compound stress of UV-B radiation and M. oryzae infection. All values represent the mean ± standard deviation (SD), n = 4. Different letters indicate significant differences (P < .05) according to Duncan’s new multiple range test.

< .01). Thus, OsPAL and OsCHT genes played an important role in enzymatic activities that helped generate disease resistance.

OsPAL is a key gene in the phenylpropanoid pathway that produces physical and chemical barriers to resist pathogen invasion and induce defensive genetic signal transduction in the entire plant (Dixon et al. 2010). Therefore, OsPAL performs a regulatory role in disease resistance-related enzyme activities and resistance-inducing substance contents (Das et al. 2017; Govender et al. 2017). The expression of OsPAL controls the corresponding production of flavonoids (Rawal et al. 2013; Chandrasekaran et al. 2017), and this process is activated by UV-B radiation. Flavonoids can protect crops from damage caused by UV-B radiation and oxidative reactive oxygen and enhance the stress resistance of plants (Agati et al. 2013). Enhanced UV-B radiation also triggers an increase in the activities of PRs, such as CHT and β-1,3-glucanase (Oka et al. 2011), and inhibits pathogen growth on leaf tissues, thereby improving the disease resistance of crops (Cheng et al. 2014). UV-B radiation stimulates many different signal pathways in crops and causes crops to form a light defense system (Jenkins 2009).

UV-B/M. increases PR and LOX activities and OsLOX2 gene expression. A significantly negative correlation between M. oryzae DI and OsLOX2 expression (r = −0.607, P < .01) was observed, indicating that rice leaves could generate an oxidative burst reaction. OsLOX2 plays a key role in plant leaf senescence and death (Huang et al. 2014). A high OsLOX2 expression corresponds to a serious foliage disease. This process belongs to plant’s defensive mechanism, which causes apoptosis. Moreover, this high expression is an active control process in phytogenetics to form a necrotic reaction at infection sites (Dodds and Rathjen 2010). For instance, if the epidermal cells of a tomato fruit undergo plasmolysis due to UV-B radiation, dead cells form a physical barrier to reduce nutrient release through the wound, block pathogen entry, and restrict the extent of damage (Charles et al. 2009). The activity of PR under UV-B/M. was significantly higher than that under M. treatment, suggesting the occurrence of oxidative burst reaction in rice exposed to stress. Appropriate UV-B radiation levels can activate defensive mechanisms and relieve oxidative damage (Hideg et al. 2013).

Pathogens are sensitive to UV-B radiation that can alter their morphological characteristics and weaken their pathogenicity. Therefore, the inoculation process of pathogenic spores may be hindered by UV-B radiation (Cheng et al. 2014). When pathogens are exposed to UV-B radiation, the molecular structure of their genetic materials changes (Rastogi et al. 2010). Enhanced UV-B radiation decreases the number of spores and appressoria of M. oryzae and the number of specks/lesions on rice leaves (Li et al. 2018).

In M.→UV-B, radiation did not increase the PR activity in the infected leaves; however, OsLOX2 gene expression was significantly higher than that of M. oryzae-only inoculation group. After the crop leaves were infected, the physiological system suffered from irreversible damaged, and the crops consequently lost their disease functions. In the experiment, the leaves suffered from UV-B radiation stress, so plant cells generated ROS, which causes tissue damage (He et al. 2014). Hence, the disease resistance of crops is not improved by UV-B radiation, that is, the disease in crop foliage is even aggravated (Marroquinguzman et al. 2017). In the present study, the degree of rice leaf blight was significantly lower in the M.→UV-B treatment group than in the M. treatment group, although the OsLOX2 gene expression in the former was lower than that in the latter. This result indicated that the UV-B radiation supplied after M. oryzae inoculation did not aggravate leaf blight possibly because UV-B radiation slows down disease progression, inhibits the expansion of lesions, and weakens the growth of pathogens (Matsuura and Ishikura 2014). No significant difference was found between the PR contents in the M.→UV-B and M. treatment groups, indicating that UV-B radiation did not increase stress tolerance of M. oryzae-infected leaves. However, the mechanisms involved in the present study should be further investigated.

UV-B radiation supplied before M. oryzae inoculation helped activate the resistance mechanism of rice and inhibited M. oryzae growth in the leaves. As M. oryzae infection progressed, the inhibitory effect of UV-B radiation decreased. Disease resistance mechanism in crops mainly involves oxidative burst reaction that mainly improves PAL and LOX activities (Vera et al. 2011) and produces defensive phenolic compounds in crops (Wasternack and Hause 2013). This mechanism consumes energy and promotes a reduced growth. In our study, the UV-B→M. treatment might accelerate the oxidative burst reaction in rice leaves. The enhanced UV-B radiation within the tolerable range could help control disease. Damage on crops caused by compound stress was less than the damage caused independently by either UV-B radiation or M. oryzae.

It is an important way for the crop disease prevention and control by developing an inherent resistance mechanism in crops to enhance the controllability and preventability, and therefore transited the disease control from an external system of the interaction between the pathogen and host crop to an interactive internal system (Huang et al. 2010). To prevent the spread of a recognized infection, crops rapidly generate defensive mechanisms (Yang et al. 2017), such as oxidative burst reaction (Azarabadi et al. 2017) and PR and phytoalexin synthesis (Loon 2007; Anushree et al. 2016). However, limited studies have been performed in these aspects because the crop resistance mechanism and the interaction between UV-B radiation and pathogens are complicated. In the future, the response of different pathogens, the crop sensitivity to UV-B radiation, and the interaction between stress factors should be explored using compound
UV-B radiation and pathogen stressors. Further studies should be conducted to enrich the knowledge on crop growth under complicated and various environmental conditions.

Conclusions
The resistance-related mechanism of rice leaves positively responded to the single and compound stress of the enhanced UV-B radiation and *M. oryzae*. Their response was related to the sequence of stress exposure. Exposure to UV-B radiation prior to *M. oryzae* infection significantly activated the stress-related response and resulted in minimal blighting in rice leaves. The enhanced UV-B radiation and the simultaneous *M. oryzae* infection also activated the resistance mechanism and alleviated *M. oryzae* disease. The enhanced UV-B radiation administered after *M. oryzae* infection could not improve the activity of resistance-related enzymes. The enhanced UV-B radiation inhibited *M. oryzae* disease, but its inhibitory effect weakened when *M. oryzae* progressively infected the rice leaves.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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