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Research article

The role of phytochromes in *Nicotiana tabacum* against *Chilli veinal mottle virus*

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ABSTRACT

It has been reported that phytochrome A (phyA) and phytochrome B (phyB) are potent regulators of plant defense. However, the mechanisms that phytochromes use to interfere with plant resistance to viral infection remain largely unclear. In this study, *Chilli veinal mottle virus* (ChiVMV) was used to investigate the role of phytochromes in response to biotic stress. Our results showed that the phytochromes mutant *phyAphyB28* plants displayed more serious necrosis and dwarf phenotypes compared to that of wild type plants (WT) after ChiVMV infection. qRT-PCR and Western blot analyses indicated that the expression and accumulation of ChiVMV were higher in *phyAphyB28* mutants than that in WT plants. The leakage (EL) and the content of thiobarbituric acid-reactive substance (TBARS) suggested that *phyAphyB28* mutants suffered more severe membrane damage than that of WT plants. In addition, extensive ROS accumulated in *phyAphyB28* mutants after ChiVMV infection, whereas ROS production in WT plants were much less than mutant plants. The activities of antioxidant enzymes were down-regulated in *phyAphyB28* mutants when compared with that in WT plants under ChiVMV infection. Besides, the contents of endogenous SA, JA and the expression of both hormones signaling related genes were lower in *phyAphyB28* mutants compared to that in WT plants. Application of exogenous SA and JA could alleviate disease symptoms. Taken together, these results demonstrated that phyA and phyB positively regulated plant defense responses to ChiVMV infection and this process was dependent on the SA and JA defense pathways.

1. Introduction

In the volatile natural environment, plants are exposed to diverse biotic and abiotic stresses, including pathogens, herbivores and competitors. Plants have successfully evolved sophisticated mechanisms to adapt to various situations. A number of complexes signaling networks that sense and respond to pathogens attacks have been defined. Upon invasion by microbial pathogens, plants modify endogenous plant molecules and produce signals to activate numerous defense responses to combat the attacking intruders (Pandey et al., 2016; Rojas et al., 2014).

The interaction of plant-pathogen usually results in the synthesis of pathogenesis-related (PR) proteins, generation of reactive oxygen species (ROS), the induction of hypersensitive responses (HRs) that limit pathogen spread, programmed cell death (PCD), the deposition of callose, cytoskeletal reorganization, cell wall fortification, and synthesis of phytoalexins and structural cell wall changes (Rojas et al., 2014; Kim et al., 2005; Ahuja et al., 2012; Hardham et al., 2007). Plant defense and resistance activated by pathogens are dynamic and can be affected by changing environmental conditions (Colhoun, 1973).

Light is an essential environmental stimulus. In the course of evolution, the quantity and quality of light from the environment is perceived and responded to by a family of plant photoreceptors, including red/far-red light-absorbing phytochromes (phy), UV-A, blue light photoreceptors cryptochromes (cry), phototropins (phot), and several others (Karpinski et al., 2003). These photoreceptors have a profound influence on many aspects of plant growth and development, as well as on plant stress responses against both biotic and abiotic factors (Griebel and Zeier, 2008).

Phytochromes are the best characterized photoreceptors. Molecular phylogenetic analyses indicate that the phytochromes are encoded by a small gene family in numerous species. There are five phytochrome genes (PHYA to PHYE) in *Arabidopsis thaliana* (Carvalho et al., 2011). Unique amongst light receptors, phytochromes exist as two forms, Pr

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and Pfr. In the dark, phytochromes are synthesized in cytoplasm as inactive red-light absorbing forms (Pr). After absorption of red light, Pr is photoactivated to active forms (Pfr) and translocated to nucleus (Nagatani, 2004). Subsequently, this change results in phosphorylation of PHYTOCHROME-INTERACTING FACTORS (PIFs) by the direct interactions between Pfr and PIFs (Soy et al., 2012).

The role of phytochromes in plant-pathogen interactions had been demonstrated in some previous studies. For instance, phyA and phyB acted antagonistically in cold tolerance by perceiving far-red light and red light (R), respectively (Wang et al., 2016). PhyB inactivation in tomato attenuated direct anti-herbivore defense (Cortés et al., 2016). *Oryza sativa* (rice) plants lacking phyA, phyB and phyC were more susceptible to the *Magnaporthe grisea.* (Xie et al., 2011). The results of the abovementioned studies suggest that phytochromes can interact with plant hormones, such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), to adjust plant immune responses (Wang et al., 2016; Xie et al., 2011). Our previous works had also demonstrated that phyA and phyB are essential for the defense strategies of *N. tabacum* against CMV (Chen et al., 2018a; Li et al., 2015).

Chilli veinal mottle virus (ChiVMV) is a type member of the genus *Potyvirus*, family *Potyviridae*. Plants infected by ChiVMV exhibit symptom of leaf mottling, vein-clearing, leaf chlorosis and necrosis. Disease caused by ChiVMV infection contributed to significant yield losses of some *Solanaceae* crops in Asian countries (Lee et al., 2017). Our previous works had demonstrated that *N. tabacum* plants infected with ChiVMV displayed punctate necrosis leaves, leaf distortion, systemic necrosis on leaves and stems and caused the death of the whole plant in the end (Zhu et al., 2014a; Yang et al., 2018). To date, the roles of phyA and phyB in plant resistance against necrotic pathogen remain largely unclear. In the present study, we investigated the role of phyA and phyB in modulating ChiVMV resistance in *N. tabacum* plants, and the possible mechanism of phyA and phyB mediated resistance to necrotic virus are discussed.

2. Materials and methods

2.1. Plant growth and virus inoculation

Phytochrome mutant seeds were obtained from our previous study (Chen et al., 2018a). Plants of *N. tabacum* NC89 (WT) and phytochrome mutants were grown in a greenhouse under 25 °C with cycles of 16-h-light/8-h-dark (100 μmol m^{-2}·s^{-1}). ChiVMV was suspended in 0.02 M sodium phosphate buffer (PBS) at 4 °C. Five-week-old tobacco seedlings were used for chemical treatment and virus inoculation. The fifth leaves of tested plants were mechanically inoculated with ChiVMV inocula, and PBS buffer was used as a control.

2.2. Hormone treatments

Hormone treatments were performed according to the methods described previously with some modifications (Zhu et al., 2014b). SA and JA solutions were prepared in water containing 0.02% Tween-20 (vol/vol) respectively. Seedlings were sprayed with respective hormone 24 h before ChiVMV inoculation. Tween-20 solution was used as a control treatment. SA and JA were purchased from Sigma-Aldrich (http://www.sigmaaldrich.com). The hormone concentrations used were as follows: SA (300 μM) and JA (100 μM) respectively. All experiments were repeated with similar results.

2.3. Protein extraction and Western blot analysis

Plant tissues collected from the second systemically infected leaves were obtained at 5 days post inoculation (dpi). Total protein was extracted using extraction buffer (50 mM Tris-Cl, pH 6.8, 5% mercaptoethanol, 10% glycerol, 4% SDS, and 4 M urea) in an ice bath. Protein concentrations were determined with the Bradford method using bovine serum albumin as the standard (Bradford, 1976). Western blotting analysis was performed according to the methods described previously (Xi et al., 2007) and by using coat protein-specific antisera of ChiVMV.

2.4. Total RNA extraction and qRT-PCR

Total RNA was extracted from *N. tabacum* leaves using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations. The expression levels of related genes were measured by quantitative real-time PCR (qRT-PCR), for which SYBR Green PCR Master Mix was used as previously described (Chen et al., 2018b). Three technical replicates were performed for each experiment. The EF1α gene was used as an internal control. All primers used in this study are shown in Supplementary Table 1.

2.5. Staining of trypan blue, DAB and NBT

Nitroblue tetrazolium (NBT), 3, 3′-diaminobenzidine (DAB) and trypan blue were employed as described previously (Zhu et al., 2014a). For detection of H_2O_2 and O^{2-}, tobacco leaves were excised at the base with a razor blade and submerged in NBT (0.5 mg .mL^{-1}) solutions for 2 h or DAB (2 mg. mL^{-1}) solutions for 8 h. Leaves were then boiled in 95% ethanol for 15 min. For detection of cell death, viral infected leaves were submerged in trypan blue for 8 min (1.25 mg. mL^{-1}, Sigma), and then boiled in 95% ethanol for 30 min.

2.6. Detection of electrolyte leakage, thiobarbituric acid-reactive substance, the content of H_2O_2 and the ratio of GSH/GSSG

For the electrolyte leakage, the leaves were boiled for 15 min to achieve 100% electrolyte leakage after measuring the conductivity of the fresh leaves. The method was implemented as described previously (Zhu et al., 2014a). The H_2O_2 content of leaves and the level of thiobarbituric acid-reactive substance (TBARS) were measured with the method reported previously (Xi et al., 2007; Xu et al., 2012). The ratio of GSH/GSSG was measured with a kit (Nanjing Jianchen, China).

2.7. Activity determination of antioxidant enzymes

To obtain the extracts used to determine the enzyme activities, 0.3 g fresh leaf tissue was ground in 3 mL of ice-cold 25 mM Hepes buffer (pH 7.8) containing 0.2 mM EDTA, 2 mM ascorbic acid and 2% (w/v) polyvinylpyrrolidone. The homogenized material was centrifuged at 12000 × *g* for 20 min at 4 °C, and the supernatant was used to determine the enzymatic activity. Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) activities were assayed as previously described (Wang et al., 2011).

2.8. Measurement of JA, SA and ET levels

SA and JA were quantified by high-performance liquid chromatography-mass spectrometry (HPLC-MS) according to the previously reported procedures (Pan et al., 2010). 2-Hydroxybenzoic acid-[^{2}H6] (d_6-SA) was obtained from Sigma-Aldrich, and dihydrojasmonic acid (H_2JA) were obtained from OlChemim as internal standards. Ethylene (ET) extraction from tobacco and analysis were performed as previously described (Zhu et al., 2014a).

2.9. Statistical analysis

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

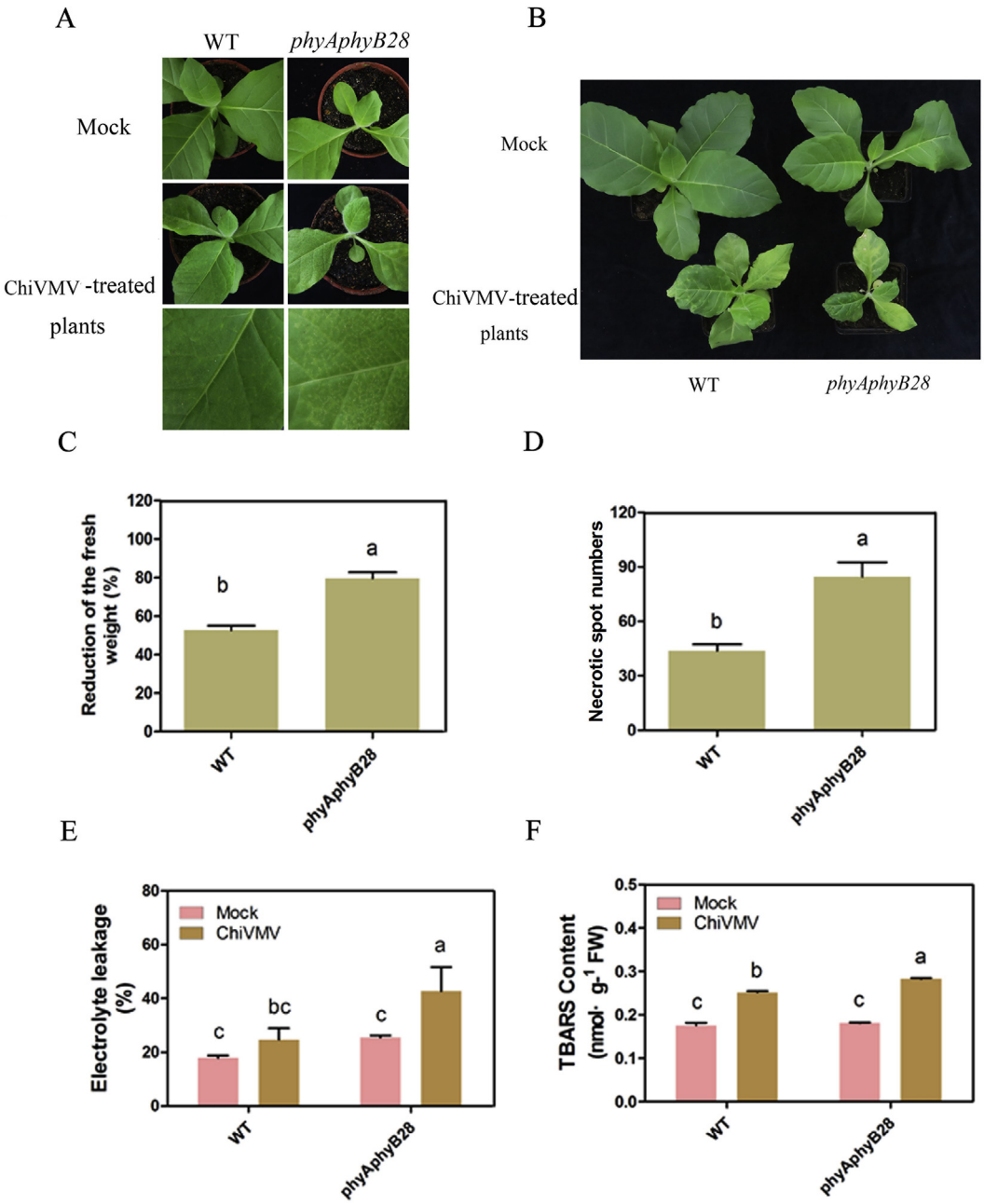

Fig. 1. *phyAphyB28* plants were more susceptible to ChiVMV infection. A. Phenotypes of WT and *phyAphyB28* plants at 5 dpi. B. Phenotypes of WT and *phyAphyB28* plants at 15 dpi. C. Reduction of aboveground fresh weight of WT and *phyAphyB28* plants at 15 dpi. D. Necrotic spot numbers on infected leaves of WT and *phyAphyB28* plants at 15 dpi. E. Electrolyte leakage in systemic leaves of *phyAphyB28* plants and WT plants at 5 dpi. F. TBARS contents in systemic leaves of *phyAphyB28* plants and WT plants at 5 dpi. Bars represent the mean and standard deviation of values obtained from three biological replicates per genotype. Significant differences ($P < 0.05$) are denoted by different *lowercase letters*.

3. Results

3.1. The phyAphyB28 mutant plants were more susceptible to ChiVMV infection

To investigate the role of phytochromes in ChiVMV tolerance, we compared the susceptibility of the phytochrome-defective mutant (*phyAphyB28*) and wild-type (WT) plants to ChiVMV infection. The results showed that the *phAphyB28* mutant plants exhibited necrotic spots in inoculated leaves at 5 days post of ChiVMV inoculation, (Fig. 1A). At 15 dpi, the systemic leaves of *phyAphyB28* mutants showed more serious necrosis and dwarf phenotypes than that in WT plants (Fig. 1B–D). Compared to the untreated plants, plant height was reduced 53.15% in WT plants, whereas that was reduced 79.53% in *phyAphyB28* mutant plants (Fig. 1C).

The electrolyte leakage (EL) and content of thiobarbituric acid-reactive substances (TBARS) can reflect the damage in plants caused by biotic stresses. To examine the effect of ChiVMV on membrane damage in viral infected plants, the EL and TBARS content were measured at 5 dpi. As shown in Fig. 1E, the level of EL was significantly increased in *phyAphyB28* plants than that in WT plants. The change in the content of TBARS displayed a similar trend (Fig. 1F). These results suggested that ChiVMV infection reduced the stability and integrity of the plasma membrane, and *phyAphyB28* plants were subjected to more serious plasma membrane damage under ChiVMV infection. Taken together, the results demonstrated that *phyAphyB28* mutants were more

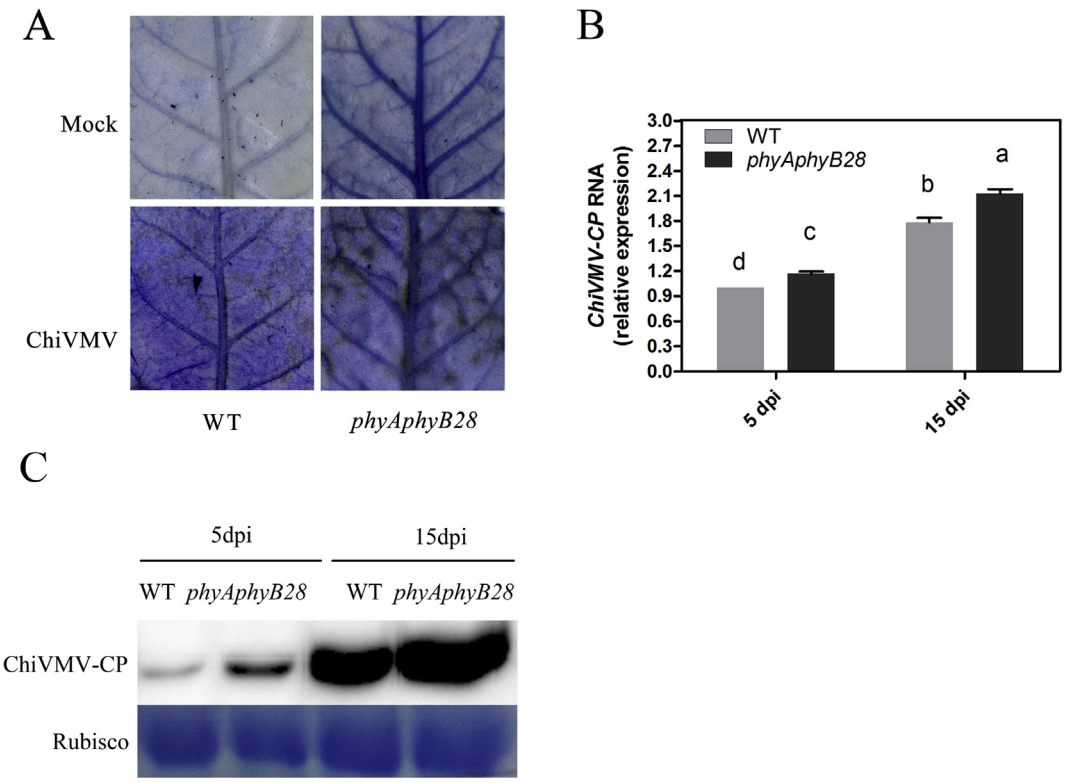

Fig. 2. Detection of cell death and virus expression in ChiVMV infected plants. A. Trypan blue-staining of systemic leaves from *phyAphyB28* plants and WT plants at 5 dpi. B. Quantitative real-time PCR analysis of virus replication levels at 5 dpi and 15 dpi, respectively. C. Western blotting analyses of ChiVMV-CP accumulation in systemic leaves of *phyAphyB28* plants and WT plants at 5 dpi and 15 dpi. Bars represent the mean and standard deviation of values obtained from three biological replicates per genotype. Significant differences ($P < 0.05$) are denoted by different *lowercase letters*.

susceptible to ChiVMV infection.

3.2. The phyAphyB28 mutant plants showed more cell death and virus accumulation

To investigate the effect of viral infection on internal change in plants, we then monitored the level of cell death in *phyAphyB28* and WT plants. The results displayed that leaves of *phyAphyB28* mutants showed more necrotic spots when leaves tissue were stained with trypan blue (which indicates dead cells) under ChiVMV infection, whereas the number of necrotic spots and the level of staining were much less in WT plants (Fig. 2A). The results indicated more cell-death occurrence in the mutant plants than that in the WT plants after virus infection. In addition, the accumulation of viruses was detected in systemic leaves of mutant and WT plants by quantitative real-time PCR (qRT-PCR) and Western blotting analysis at 5 dpi and 15 dpi respectively. The results of qRT-PCR demonstrated that the expression of ChiVMV-coat protein (ChiVMV-CP) gene was much higher in *phyAphyB28* mutants than that in WT plants (Fig. 2B). Western blotting analysis also revealed more accumulation of viral coat proteins in *phyAphyB28* plants than that in WT plants (Fig. 2C).

3.3. The phyAphyB28 mutant plants accumulated higher levels of ROS under ChiVMV infection

ROS, which include superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2) and singlet oxygen, play a crucial role in plant defense mechanisms (Kotchoni and Gachomo, 2006). The levels of O^{2-} and H_2O_2 in the leaves were measured at 5 dpi, via NBT and DAB staining, respectively. The results exhibited that NBT and DAB stained stronger in *phyAphyB28* mutant plants than those of WT plants (Fig. 3A and B). The respiratory burst oxidase homologue D gene (*RbohD*) is a key defense-related gene involved in the generation of ROS (Zhu et al., 2014a). It was noted that the transcription level of *NtRbohD* in *phyAphyB28* plants was higher than that in WT plants (Fig. 3C). To further quantify the ROS level in infected leaves, we detected the contents of H_2O_2. As shown in Fig. 3D, ChiVMV inoculation led to higher H_2O_2 accumulation in *phyAphyB28* leaves than that in WT leaves. These data suggested that more ROS were accumulated in phytochrome defective mutants under ChiVMV infection. Because reduced GSH could participate in redox regulation of the expression of related genes like *NPR1*, *GSTs*, *PR1* in infected cells and regeneration of oxidized thiol groups of proteins (Gullner and Kômíves, 2006), the ratios of GSH/GSSG were measured in WT and *phyAphyB28* plants after ChiVMV infection. The results showed that the ratios of GSH/GSSG in WT plants were almost 1.7-fold more than those in *phyAphyB28* mutant plants (Fig. 3E), indicating that *phyAphyB28* plants had a weaker ability to scavenge free radicals compared to WT plants.

3.4. ChiVMV infection caused inefficient activities of antioxidant enzymes in phyAphyB28 mutant plants

Several reports showed that excessive accumulation of ROS ultimately leads to necrotic lesions and cell death (Doke, 1997). To prevent or minimize oxidative damage, plants evolved antioxidant mechanisms, such as peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT), to regulate the metabolic balance of ROS production (Kotchoni and Gachomo, 2006). Therefore, the activities of antioxidant enzymes were measured. The activity of SOD increased after virus inoculation and was significantly up-regulated in WT plants compared to that in *phyAphyB28* plants (Fig. 4A). The activities of POD and CAT displayed similar tendencies (Fig. 4B and C). However, the activities of APX showed no obvious differences between *phyAphyB28* and WT plants under ChiVMV infection (Fig. 4D). These

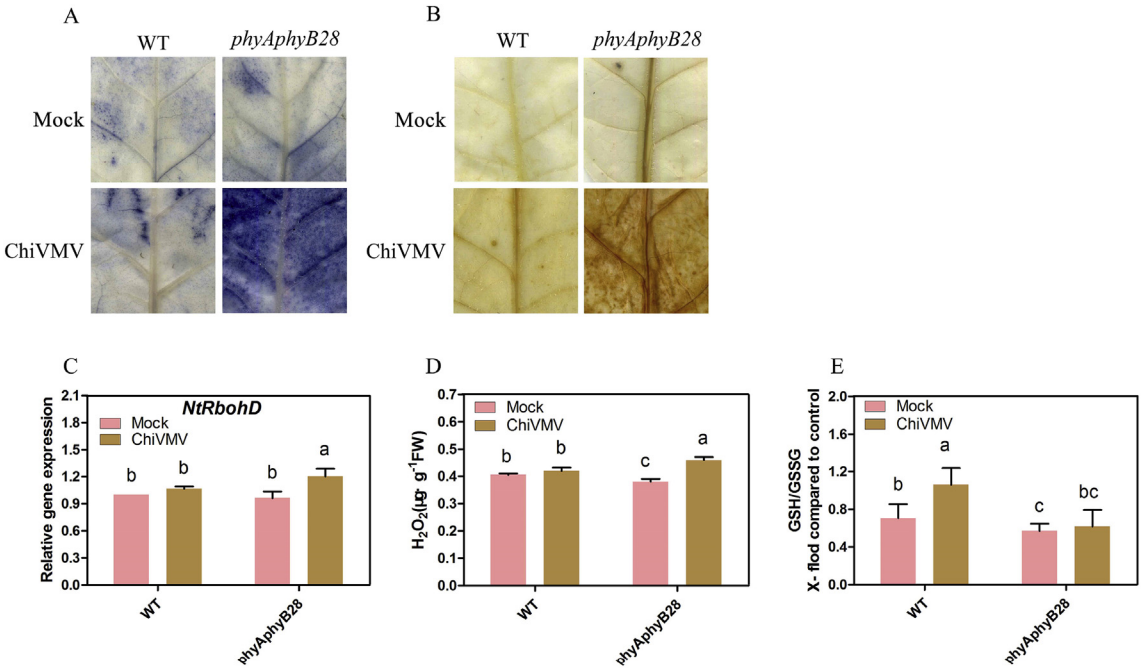

Fig. 3. Reactive oxygen species (ROS) and reductive substances in ChiVMV-inoculated *N. tabacum* plants. (A) NBT staining, (B) DAB staining, (C) Quantitative real-time PCR analysis of the transcript levels of *NtRbohD*. (D) the content of H_2O_2 and (E) the ratios of GSH/GSSG in systemic leaves from WT and *phyAphyB28* plants at 5 dpi. Bars represent the mean and standard deviation of values obtained from three biological replicates per genotype. Significant differences ($P < 0.05$) are denoted by different *lowercase letters*.

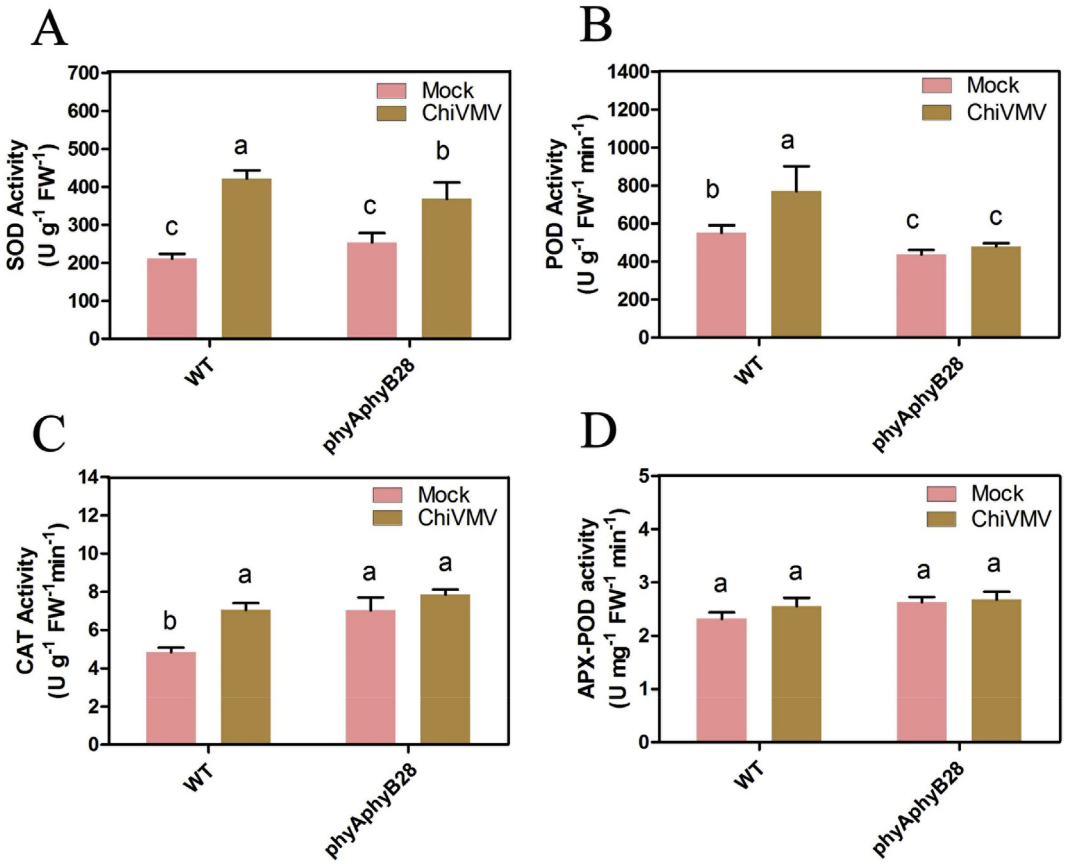

Fig. 4. Analysis of enzyme activities of systemic leaves in WT and *phyAphyB28* plants at 5 dpi. A. Activity of superoxide dismutase (SOD). B. Activity of peroxidase (POD). C. Activity of catalase (CAT). D. Activity of ascorbate peroxidase (APX). Bars represent the mean and standard deviation of values obtained from three biological replicates per genotype. Significant differences ($P < 0.05$) are denoted by different *lowercase letters*.

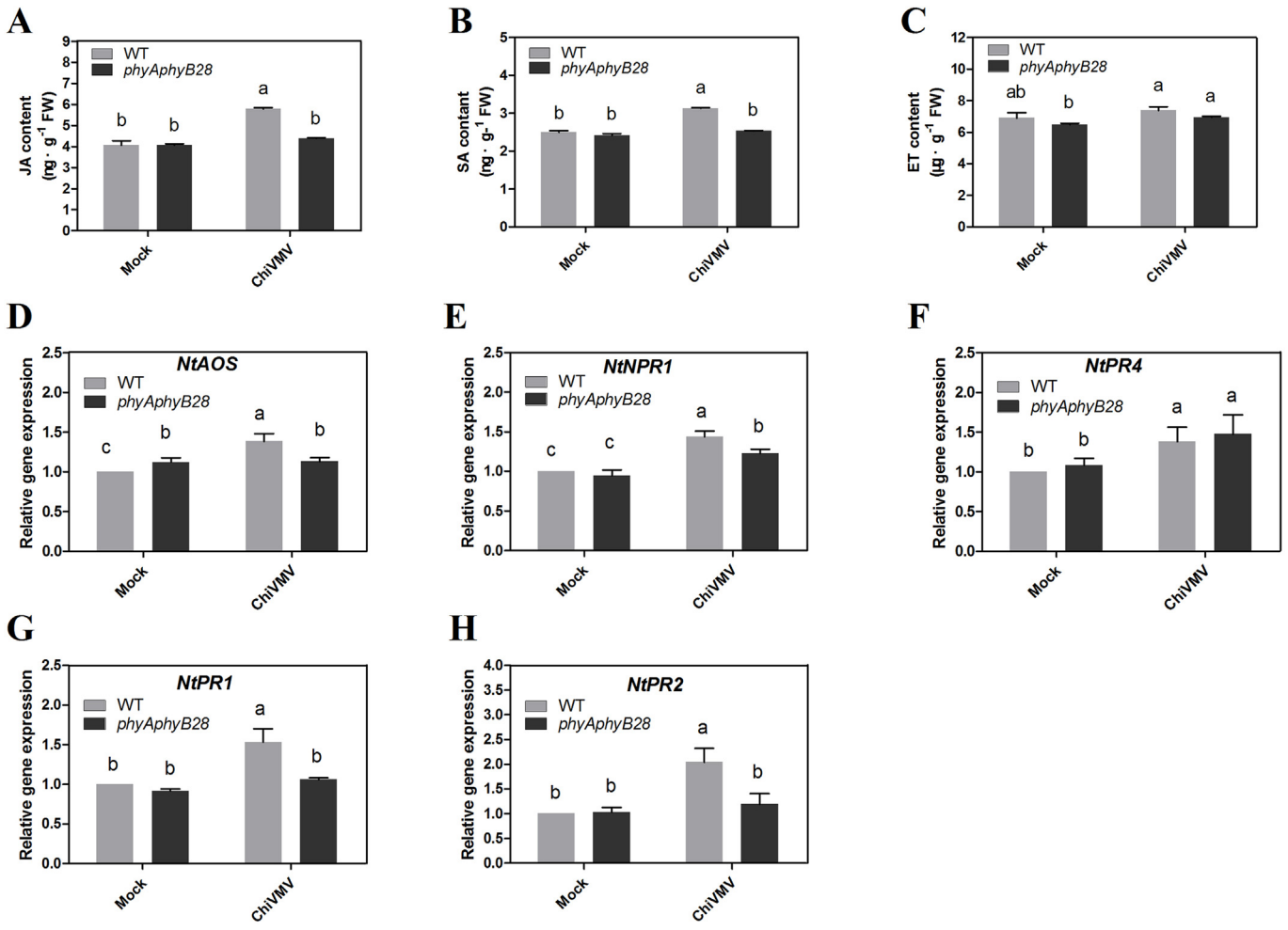

Fig. 5. Accumulation of endogenous JA, SA, and ET and transcription levels of related genes in systemic leaves of WT and *phyAphyB28* plants at 5 dpi. (A, B, C). Content of endogenous JA, SA, and ET in systemic leaves of WT and *phyAphyB28* plants at 5 dpi. (D, E, F, G, H). Quantitative real-time PCR analysis of the expression of *NtAOS*, *NtNPR1*, *NtPR4, NtPR1 and NtPR2* in systemic leaves of WT and *phyAphyB28* plants at 5 dpi. Bars represent the mean and standard deviation of values obtained from three biological replicates per genotype. Significant differences ($P < 0.05$) are denoted by different *lowercase letters*.

results suggested that the deficiency of phytochromes had depressed the antioxidant abilities of tobacco plants under ChiVMV infection.

3.5. Phytochrome regulated plant defense depended on SA and JA signaling

The defense signaling molecules SA, JA and ET are well documented as being key factors in response towards pathogens (Kunkel and Brooks, 2002). To explore whether SA-, JA-, and ET-mediated defense pathways are required for phyA and phyB against ChiVMV, the contents of endogenous SA, JA and ET in plants were measured at 5 dpi. Our results showed that the contents of endogenous SA and JA were higher in all ChiVMV-inoculated plants than those in mock-inoculated plants (Fig. 5A and B), suggesting that SA- and JA-mediated signaling pathways played important roles in ChiVMV resistance. Additionally, the accumulation of SA and JA in WT plants exhibited much higher increases than that in *phyAphyB28* plants (Fig. 5A and B). In the meantime, endogenous levels of ET showed no obvious differences between WT and *phyAphyB28* plants (Fig. 5C). We further investigated the expression of SA-, JA-, and ET-related genes in tobacco plants after ChiVMV infection. The results indicated that the expression levels of *NtAOS* (JA signaling related gene), *NtNPR1, NtPR1, NtPR2* (SA signaling related genes) and *NtPR4* (ET signaling related gene) were up-regulated in all ChiVMV-inoculated plants compared with that in mock-inoculated groups (Fig. 5D–H). In ChiVMV-inoculated plants, the expression levels of *NtAOS*, *NtNPR1, NtPR1* and *NtPR2* displayed much higher increases in WT plants than those of *phyAphyB28* plants (Fig. 5D, E, G, H). However, the transcript levels of *NtPR4* displayed a similar degree of increase in WT and *phyAphyB28* plants following ChiVMV infection (Fig. 5F).

3.6. Pre-treatment of JA and SA decreased the susceptibility of phyAphyB28 plants to ChiVMV infection

The abovementioned results indicated that effects of phytochromes on *N. tabacum* defense induced by ChiVMV were dependent on the SA and JA signaling pathways. To test this hypothesis further, the phytochrome mutants and WT were analyzed after foliar application of exogenous SA and JA in ChiVMV infection. As expected, application of exogenous SA and JA significantly decreased necrosis in the *phyAphyB28* and WT plants (Fig. 6A). Then, the expression levels of ChiVMV coat protein were detected by Western blots at 5 dpi. Results showed that lower levels of virus accumulation were found in ChiVMV-*phyAphyB28* and ChiVMV-WT plants after SA and JA pretreatment (Fig. 6B).

4. Discussion

Phytochromes perceive light signals from the environment and play important role for plants in response to biotic stresses (Griebel and Zeier, 2008; Xie et al., 2011). In this study, we compared the

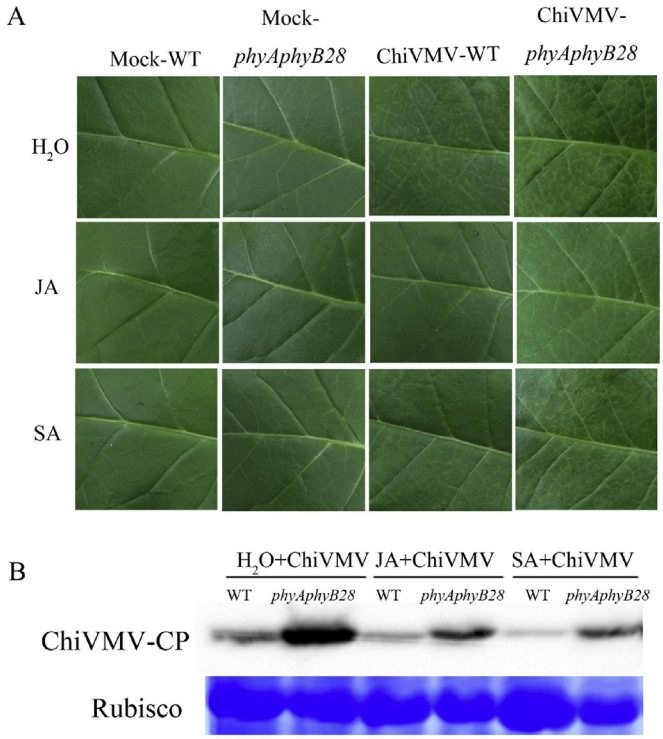

Fig. 6. Phenotype (A) and virus accumulation (B) of WT and *phyAphyB28* plants pretreated with JA and SA at 5 dpi. "H_2O", "JA" and "SA" indicates that plants were pretreated with H_2O, JA and SA, respectively; "Mock-WT" and "Mock-*phyAphyB28*" represent mock-infected plants, respectively; "H_2O + ChiVMV", "JA + ChiVMV", "SA + ChiVMV" indicates that ChiVMV-infected plants were pretreated with H_2O, JA and SA, respectively.

susceptibility of WT plants and phytochrome deficient plants to necrotic virus. We found that *phyAphyB28* plants displayed higher susceptibility to ChiVMV infection (Figs. 1–2), which suggested that phyA and phyB played positive role in tobacco plants response to ChiVMV infection.

ROS burst constitutes an early response to pathogen attack by strengthening cell walls through cross-linking glycoproteins and by activating defense signaling components (Li et al., 2017). However, elevated levels of ROS may be affect the growth of plants (You et al., 2016). The observations of relevant parameters in our study imply that the *phyAphyB28* plants accumulated higher levels of ROS under ChiVMV infection (Fig. 3). In addition, the activities of ROS scavenging enzymes (CAT, SOD, POD) were lower in mutant plants than that in WT plants after inoculation with ChiVMV (Fig. 4). These results indicated that phyA and phyBplay protective roles in plant defense against ChiVMV infection. Furthermore, these data also indicated that phyAand phyB may affect the metabolic balance of antioxidant enzymes and the generation of ROS, thus affect the plant resistance to virus infection.

As natural phytohormones, salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) contribute to plant defense against pathogen infection (Abe et al., 2012). The signaling pathways between the three phytohormones act through a complex network of synergistic and antagonistic interactions (Koornneef and Pieterse, 2008). In many cases, SA signaling enhances plant resistance against biotrophic and hemibiotrophic pathogens, while JA/ET-mediated responses act predominantly against necrotrophic pathogens and herbivorous insects (Robertseilaniantz et al., 2011). It has been demonstrated that JA and SA are also involved in a number of light-regulated plant responses (Kazan and Manners, 2011; Chen et al., 2018a). In the present study, we observed that endogenous SA and JA accumulation were decreased in *phyAphyB28* plants than that in WT plants under ChiVMV inoculation. Simultaneously, the expression of SA- and JA-related genes were down-regulated in *phyAphyB28* plants than that in WT plants after ChiVMV inoculation (Fig. 5). In addition, foliar application of tobacco plants with SA and JA significantly decreased viral accumulation in both mutants and WT plants (Fig. 6). These data suggested that the increased viral susceptibility in phytochromes mutant plants was related to SA and JA signaling mediated resistance.

Our previous study demonstrated that phyA and phyB regulated tobacco against CMV via SA signaling pathway, but not JA signaling pathway (Chen et al., 2018a). JA has been shown to participate in the defense responses against necrotrophic pathogens (Pandey et al., 2016). Our works also showed that JA and SA played different roles for tobacco responses against ChiVMV at different stages post viral infection (Zhu et al., 2014a; Yang et al., 2018). The present results are somewhat consistent with a previous study that showed phytochromes regulate SA and JA signaling pathways in rice for age-related resistance to *Magnaporthe grisea* (Xie et al., 2011). Our findings suggested that phytochromes could regulate plant immunity via different plant hormone pathways in different plant-virus interaction systems.

In summary, our results provided evidence that *phyAphyB28* tobacco plants are more vulnerable to ChiVMV infection. Our collective evidence supports the conclusion that phyA and phyB are positive regulators of plant immunity. This study further reveals that phyA and phyB could regulate plant responses to ChiVMV infection via SA and JA signal pathways. Future research works are needed to deeply elucidate the molecular link between phytochromes and virus resistance.

Declaration of interest

The authors declare that they have no conflict of interest.

Author contributions

Chunyan Fei, Dehui Xi and Honghui Lin designed experiments; Chunyan Fei, Lijuan Chen, Ting Yang and Wenshan Zou performed experiments; Chunyan Fei wrote the manuscript; Dehui Xi revised the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.04.002.

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