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PopW activates PAMP-triggered immunity in controlling tomato bacterial spot disease

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ABSTRACT

PopW, a protein elicitor, reduces tomato bacterial spot disease caused by *Xanthomonas euvesicatoria* (X.e.) with a significantly decreased titer of X.e., achieving more than 50% biocontrol efficacy. Along with phenotype changes, apparent reactive oxygen species (ROS) burst and callose deposition were triggered by PopW at the cellular level; The mRNA abundance of PAMP-triggered immunity (PTI)-associated marker genes (*PTI5*, *LRR22*, *GRAS2*) was increased by PopW at the molecular level. These results demonstrated that PopW, as a PAMP, triggers early immunity of tomato against X.e. to reduce tomato bacterial spot disease.

1. Introduction

Tomato bacterial spot disease is a widespread foliar disease in tomato and pepper caused by *Xanthomonas euvesicatoria* (X.e.). X.e. infection affects the foliage, fruit, blossoms, and stems of tomato plants, causing more than 60% yield loss during high temperature and humidity [1].

Chemical control and culture management are the most selective measures in the field [2]. Along with green consumerism turning into the mainstream of our daily life, biocontrol has been adopted for controlling crop diseases because of its lack of pollution and harm. Previous studies showed that mutants of X.e. strain 75-3 (*hrpG*, *hrpX*, *hrpF* and *hrpE1*) were useful for controlling bacterial spot disease under greenhouse and field conditions, achieving 21–58% biocontrol efficacy [3]. Plant growth-promoting rhizobacteria (PGPR), systemic acquired resistance inducers and host-specific unformulated bacteriophages were also evaluated with 20–60% biocontrol efficacy in the greenhouses [4,5].

PopW is a harpin protein composed of pectase and harpin domains from *Ralstonia solanacearum* ZJ3721 [6]. PopW has shown a broad-spectrum biocontrol capacity and plant growth-promoting ability in both greenhouse and field conditions, displaying high potential for industrialization [7–9]. Moreover, PopW significantly decreased titers of X.e. in tomato leaves, achieving more than 50% biocontrol efficacy. This study analyzes the mechanism of PopW-triggered tomato resistance against X.e.

PAMPs (Pathogen-Associated Molecular Patterns) trigger plant early immunity, which is known as PTI (PAMP-Triggered Immunity). PTI is an innate immunity based on recognition of PAMPs via PRRs (Pathogen-Recognition Receptors) located on plant cells [10]. A series of events in PTI, such as reactive oxygen species (ROS) burst, callose deposition and expression of PTI-associated marker genes, take place in a few minutes to several days [11–13]. On the other hand, plant pathogenic bacteria have to overcome PTI before they can successfully infect plant cells.

In this study, we attempted to associate PTI triggered by PopW, as a PAMP, with its actual role as a biocontrol agent (BCA) to better understand the mechanism of biocontrol of tomato bacterial spot disease by PopW.

2. Materials and methods

2.1. Plant conditions and PopW and X.e. preparation

Tomato seeds (*Solanum lycopersicum* cultivar Hezuo 903 developed by the Shanghai Tomato Research Institute in Shanghai,

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China) were sown in artificial soil composed of black clay and vermiculite in a ratio of 4:1. Two-week-old tomato seedlings with 3–4 true leaves were transferred into a pot (upper diameter = 14.7 cm, lower diameter = 12.8 cm, height = 11 cm) containing artificial soil and incubated in a greenhouse maintained at 25–28 °C with a relative humidity of 70% and a 16/8 h day/night photoperiod. Two weeks after being transferred, the plants were used to conduct the experiment.

The extraction and purification of PopW was performed as described previously [6]. X.e. was grown in liquid NYGA medium (yeast extract 3 g, peptone 10 g and glycerin 20 g for 1 L, pH 7.0–7.2), containing 100 mg of rifampicin per liter at 28 °C for 72 h. Cultured X.e. cells were pelleted by centrifugation (4 °C, 6000 rpm for 10 min) and then resuspended in sterile water with 0.01% Silwet L-77 (vol/vol), and adjusted to 3×10^8 CFU/mL by serial dilutions for spray inoculation.

Additionally, flg22 peptide at a final concentration of 1 μM (synthesized by Zhongya Co., Beijing, China) was used for the following experiment based on previous reports [14].

2.2. Biocontrol assay of tomato bacterial spot disease

Tomato seedlings with 3–4 true leaves were transferred into a pot. After 2 weeks, 25, 50, 125 and 250 mg/L PopW was sprayed onto the leaves of seedlings with 0.01% Silwet L-77 (vol/vol), and X.e. with 0.01% Silwet L-77 (vol/vol) was spray-inoculated onto the tomato leaves after 5 days. Tomato plants were grown under 100% humidity conditions for one week to promote X.e. infection and then transferred to normal growth conditions (greenhouse conditions at 25–28 °C with a relative humidity of 70% and a 16/8 h day/night photoperiod). The disease index was calculated as described by Horsfall and Barratt [15], from bacterial spot occurrence to no visually increased bacterial spots for water controls.

2.3. Monitoring of X.e. proliferation in tomato leaves

Tomato leaves were treated with PopW and X.e. as described in Section 2.2. To monitor X.e. growth in plants, tomato leaves were sampled at 0, 1, 3 and 4 days post-inoculation with X.e., and 0-day samples were collected at 2 h post-X.e. inoculation. Each sample (1 g) was soaked in 70% ethanol for 30–40 s for surface sterilization, washed 2–3 times in sterile water, air dried, and then homogenized in 9 mL sterile 0.85% NaCl with 0.5 g silica sand using a sterile pestle and mortar. Subsequently, appropriate dilutions were plated onto NYGA agar supplemented with 100 mg of rifampicin per liter at 28 °C. After incubation for 72 h, X.e. colonies grown with rifampicin resistance were counted on plates, and X.e. density in the leaves was determined (CFU per gram of leaf fresh weight (fw)). Three replicates were performed for each treatment, and each experiment was repeated two times.

2.4. Detection of hydrogen peroxide

ROS burst was determined as described by Niu et al. [16]. Tomato leaves were sampled at 10 and 24 h. Each sample (four leaves) was soaked in 1 mg/mL diaminobenzidine (DAB, pH 3.8) for 8 h at 25 °C for staining. Stained leaf tissues were boiled in 95% ethanol for 20 min for destaining. Then, leaf tissues were suspended with sterile water and observed under a fluorescence microscope. H_2O_2 as a main form of ROS was also quantitatively determined by ELISA with an H_2O_2 detection kit (produced by Jiancheng Biotech, Nanjing) according to the manufacturer's protocol.

2.5. Determination of callose deposition

Callose deposition was determined as described by Reuber et al. [17]. Tomato leaves were sampled at 10 and 24 h. To destain the samples, each sample was soaked in destaining solution (phenol:glycerol:lactate:water:ethanol = 1:1:1:1:8), infiltrated by applying a vacuum for 5–10 min, and boiled at 100 °C until all of the samples were completely destained. To stain callose, the destained samples were vacuum-treated in 0.01% aniline blue solution (150 mM K_2HPO_4, PH = 9.5) for 5–10 min, placed in the dark for 8 h at 25 °C, rinsed in sterile water, and then photographed in UV light by a fluorescence microscope.

2.6. Q-PCR analysis of mRNA abundance

Tomato leaves were treated with PopW or water and then inoculated with X.e. after 5 days. Each sample (0.1 g) was collected at 6, 12, 36 and 48 h and ground with liquid N_2 into powder. Total RNA was extracted by using 1 ml TRIZOL reagent (Invitrogen Co.) and was treated with RNase-free DNAse (Takara Co.) to remove genomic DNA. Reverse transcription of RNA was performed with 1 μg of total RNA using M-MLV reverse transcriptase according to the manufacturer's protocol (Invitrogen Co.). The primers used were listed by Kim et al. [18]. A constitutively expressed gene (*18S rRNA*) was used as a housekeeping gene in quantitative real-time PCR analysis. Three replicates were performed for each treatment.

2.7. Statistical analysis

Data were statistically analyzed for significance by a least significant difference (LSD) test ($P < 0.05$) using the statistical software Data Processing System (DPS version 7.05).

3. Results

3.1. PopW shows potential biocontrol capacity toward X.e

The first bacterial spot was initially observed in tomato leaves treated with PopW or water 9 days after X.e. inoculation. However, the disease index in PopW treatments was obviously lower than water controls from 9 through 29 days post-inoculation (dpi), and the lowest disease index was obtained with 50 mg/L PopW (Fig. 1A). PopW (50 mg/L) achieved the best biocontrol efficacy (54.57%) at 30 dpi, and the other PopW treatments (250, 125 and 25 mg/L) obtained more than 40% biocontrol efficacy (Table 1).

To determine the effect of PopW on plant resistance toward X.e. infection, we monitored the growth of X.e. in tomato leaves of seedlings treated with 50 mg/L PopW or water at 0, 1, 3 and 4 dpi. The density of X.e. was less at 0, 1 and 3 dpi in tomato leaves of seedlings treated with PopW and was significantly reduced at 4 dpi by PopW relative to leaves pretreated with water (Fig. 1B).

3.2. PopW elevates the level of ROS burst in tomato leaves

ROS, as an important immunity system in plants under stress, might activate a series of resistance reactions in plant cells as a second messenger [19,20]. Considering the ROS burst as a non-negligible segment of PTI in plant immunity [13,21], experiments were designed to investigate the timing for PopW to induce the ROS burst in treated tomato plants. By DAB-histochemical staining of ROS formation in vivo, it was shown that PopW apparently triggers the ROS burst at 10 and 24 (hours post-treatment) hpt in treated tomato leaves compared with water controls (Fig. 2A). The ROS burst was also enhanced in tomato leaves treated with PopW

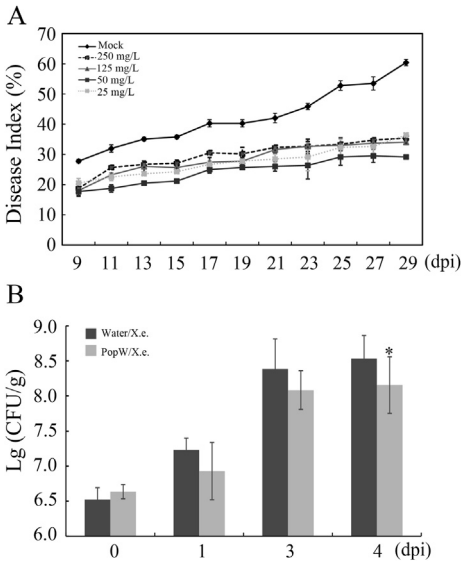

Fig. 1. The effect of PopW to control tomato bacterial spot disease. Tomato seedling leaves were sprayed with (A, B) 50 mg/L PopW or water and spray-inoculated with 3×10^8 CFU/mL X.e. after 5 days. (A) The disease index in tomato leaves pretreated with water or PopW at different concentrations (25, 50, 125 and 250 mg/L) after challenge inoculation with X.e. (B) The growth of X.e. in tomato leaves of seedlings treated with water or PopW. The density of X.e. in tomato leaves was detected at 0, 1, 3 and 4 dpi, and the 0 dpi sample was collected at 2 hpi. Each column represents an average of three replicates, and the bars indicate SD. The asterisk indicates a significant difference (least significant difference test, $P < 0.05$) in the density of X.e. in tomato leaves pretreated with PopW relative to leaves pretreated with water.

Table 1
Effects of PopW at various concentrations on resistance induction in tomato against X.e. (30 dpi).

Treatment	Disease index (%)	Biocontrol efficacy (%)
250 mg/L PopW	35 ± 1.04b	41.38
125 mg/L PopW	34 ± 0.60b	43.68
50 mg/L PopW	27 ± 2.06c	54.57
25 mg/L PopW	35 ± 1.53b	42.41
Mock	60 ± 1.04a	/

Note: Data represent means ± SD; Different letters indicate statistically significant differences between treatments (least significant difference test; $P < 0.05$).

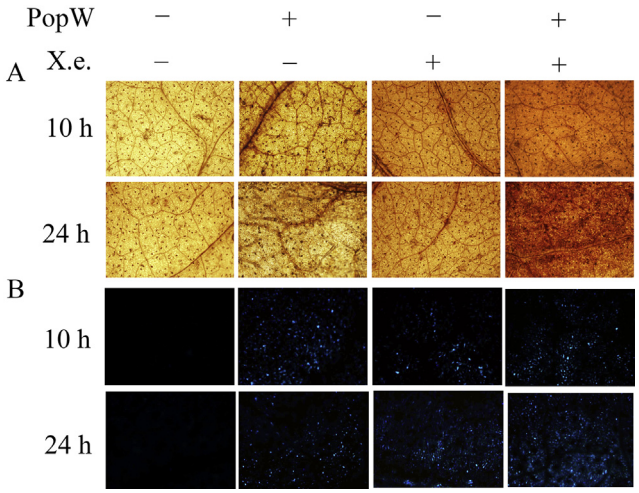

Fig. 2. PopW activates ROS burst and callose deposition in tomato leaves against X.e. Tomato seedlings were sprayed with 50 mg/L PopW or water and spray-inoculated with 3×10^8 CFU/mL X.e. after 5 days. (A) The ROS burst in tomato leaves at 10 and 24 h. (B) Callose deposition in tomato leaves at 10 and 24 h.

followed by X.e. at 10 and 24 (hours post-inoculation) hpi relative to leaves treated with X.e. and water (Fig. 2A). Quantitative detection of H_2O_2 showed that PopW (50 mg/L) obviously increased the level of H_2O_2 in tomato leaves from 6 h through 48 h without X.e. pressure (Fig. 3A) and from 10 h through 72 h under the pressure of X.e. (Fig. 3B).

A previous study showed that flg22-induced H_2O_2 accumulation is an early signaling event that occurs within a few minutes [22]. Flg22 is a reported PAMP factor. To determine if PopW activates an early PTI-associated signaling event, we detected the level of H_2O_2 at 5 min after treatment with PopW or flg22. PopW significantly increased H_2O_2 accumulation at 5 min in tomato leaves, which was similar to leaves treated with flg22 (Fig. 3C). These results imply that PopW might act as a PTI inducer in the early stage of controlling tomato bacterial spot disease.

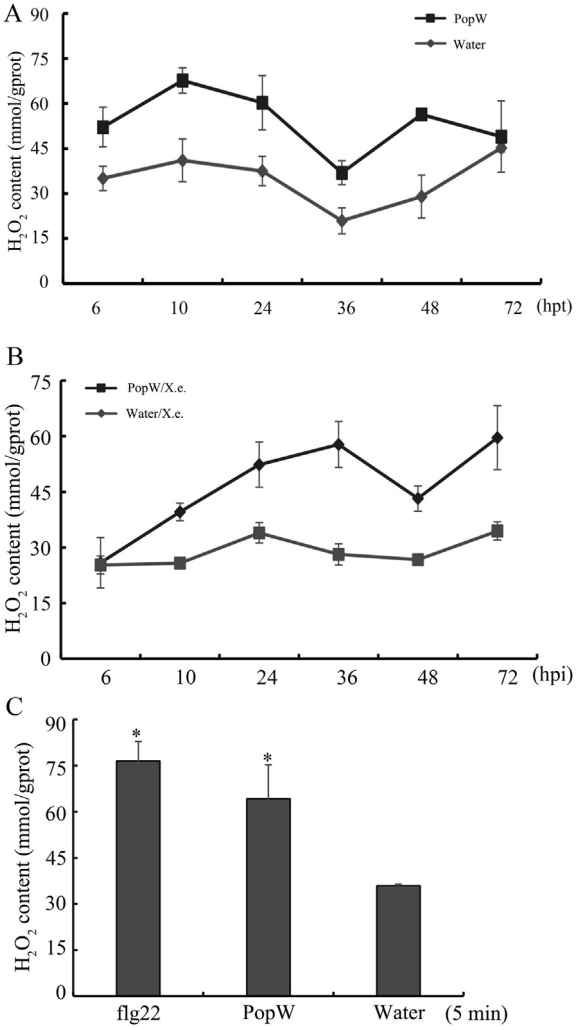

Fig. 3. Dynamic accumulation of H_2O_2 in tomato leaves in response to PopW. Tomato seedling leaves were sprayed with (A, B and C) 50 mg/L PopW, water or 1 μM flg22 and spray-inoculated with (B) 3×10^8 CFU/mL X.e. after 5 days. The gprot/mL stands for grams of the protein per milliliter of liquid. (A) H_2O_2 accumulation in a 72-h period was observed in tomato leaves treated with PopW or water. (B) H_2O_2 accumulation in a 72-h period was observed in tomato leaves inoculated with X.e. after PopW or water treatment. (C) H_2O_2 accumulation in tomato leaves treated with PopW, flg22 or water at 5 min. These data represent one experiment. The experiment was performed twice. Data represent means ± SD, $n = 3$. The asterisk indicates a significant difference (least significant difference test, $P < 0.05$) relative to water controls.

3.3. PopW activates callose deposition in tomato leaves

Different from the ROS burst, cell wall defenses, exemplified by callose deposition, are typically triggered by PAMPs [12]. To further analyze the function of PopW as a PAMP factor, we determined the callose deposition in tomato leaves treated with PopW. As shown in Fig. 2B, massive callosities accumulated in PopW-treated tomato leaves at 10 and 24 hpt compared to tomato leaves treated with water. Additionally, callose deposition was obviously more intensive in tomato leaves treated with PopW followed by X.e. than in tomato leaves treated with water followed by X.e. at 10 and 24 hpi. These results demonstrated that callose deposition is involved in the plant resistance triggered by PopW against X.e. (Fig. 2B).

3.4. PopW increases mRNA abundance of PTI marker genes

Three marker genes of PTI were analyzed by Q-PCR to determine whether they were induced in tomato leaves against X.e. in response to PopW [18]. The three marker genes included the following: (1) *PTI5*, which encodes a transcription factor in the ethylene response factor family; (2) *LRR22*, which encodes a tomato homolog of the *Arabidopsis* LRR22 receptor-like kinase; and (3) *GRAS2*, which encodes a transcription factor linked to abiotic and biotic stress [18]. The mRNA level of *PTI5*, *LRR22* and *GRAS2* was significantly increased by PopW in tomato leaves inoculated with X.e. at 6 and 12 hpi relative to leaves treated with water followed by X.e. (Fig. 4). The increased mRNA abundance of the above three marker genes indicated that PTI is triggered by PopW in tomato leaves against X.e. infection.

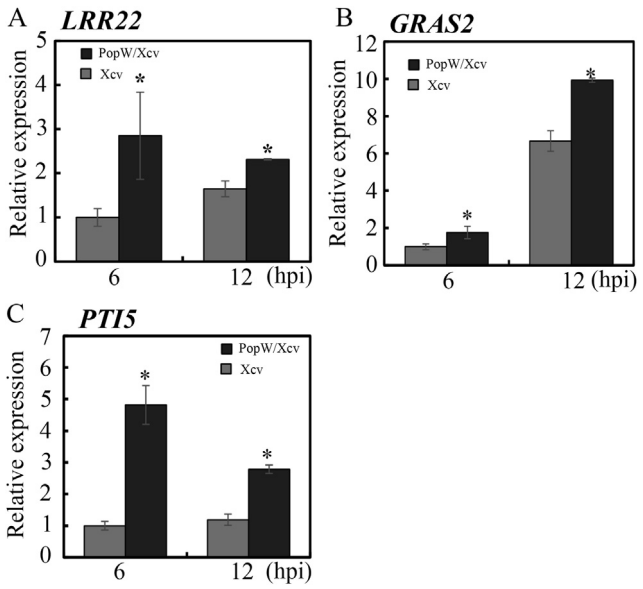

Fig. 4. PopW increases the mRNA abundance of tomato PTI-related genes at early stages after X.e. inoculation. Tomato leaves were treated with PopW (50 mg/L) or water and spray-inoculated with 3×10^8 CFU/mL X.e. after 5 days. Relative mRNA levels of (A) *LRR22*, (B) *GRAS2*, (C) *PTI5* at 6 and 12 h in tomato plants inoculated with X.e. after PopW treatment or water treatment. The mRNA levels of all of the genes were determined by quantitative real-time PCR. Relative mRNA levels were normalized using the transcripts of *SI18S rRNA*. Each column represents an average of three replicates, and the bars indicate SD. The asterisk indicates a significant difference (least significant difference test, $P < 0.05$) in mRNA abundance in tomato leaves treated with PopW followed by X.e. relative to leaves treated with water followed by X.e.

4. Discussion

As a newly identified harpin protein, PopW manifests potential broad-spectrum biocontrol capacity in controlling many plant diseases, e.g., tobacco mosaic virus disease caused by tobacco mosaic virus (TMV) [7], cucumber downy mildew disease caused by *Pseudoperonospora cubensis* [8], tomato leaf mold disease caused by *Fulvia fulva* and rice false smut disease caused by *Ustilaginoidea virens* [9]. The salicylic acid (SA) signaling pathway is responsible for the tomato resistance induced by PopW against TMV [7]. Meanwhile, PopW transgenic tobacco lines acquired resistance against TMV and *R. solanacearum*, and SA and jasmonic acid/ethylene signaling pathways are involved in transgenic tobacco resistance against *R. solanacearum* [23,24]. In this study, our data showed that PopW decreases the titers of X.e. in tomato leaves and achieves more than 50% biocontrol efficacy (Fig. 1 and Table 1). However, the association of PopW with changes in tomato leaves in response to X.e. infection is unknown.

Previous studies showed that the ROS burst rapidly occurs in response to PAMPs. The ROS burst peaks at 5–10 min in plants triggered by flg22 [12,14,21], at 10–20 min by elongation factor Tu (EF-Tu) [25], and at 35–40 min by *Pseudomonas syringae* [26]. In our study, the accumulation of H_2O_2, as a main form of ROS, peaks at 5 min in tomato leaves triggered by PopW (Fig. 3C).

The quantitative analysis of the dynamics of H_2O_2 accumulation in tomato leaves triggered by PopW was examined. The data indicated that PopW prolongs H_2O_2 accumulation for more than 48 h compared to water controls (Fig. 3A), while flg22-induced H_2O_2 accumulation lasted up to more than 24 h [27]. When tomato leaves of seedlings were challenge inoculated with X.e., H_2O_2 accumulation was triggered again by PopW and lasted more than 72 h (Fig. 3B). That H_2O_2 accumulation is reactivated by PopW in the pressure of X.e. (Fig. 3B) seems to priming—an enhanced capacity to mobilize infection-induced cellular defense [28].

PAMP-induced callose deposition in the leaves of *Arabidopsis* has emerged as an important marker response to study the signaling pathways controlling PTI. Callose deposition is normally determined in PTI, including induction by flg22 and chitosan [27] and suppression by effectors from pathogen [29,30]. In this study, PopW intensively activates callose deposition under the pressure of X.e. (Fig. 2B). The result suggested that PopW might prime PTI in tomato leaves against X.e.

The PTI relevant genes *PTI5*, *GRAS2* and *LRR22* were selected as PTI marker genes [18]. Kim et al. showed that PTI is suppressed by XopN (X.e. T3S Effector), and at 6 hpi, the mRNA abundance for all three genes was significantly higher in leaves inoculated with an *XopN* mutant compared to X.e. wild-type strain controls. In our study, the mRNA abundance of *PTI5*, *GRAS2* and *LRR22* was increased by PopW at 6 and 12 h (Fig. 4). These data indicated that PopW induces the plant PTI process at the genetic level in tomato.

Stomatal immunity is now a well-documented subdiscipline of PTI [31,32], and X.e. is reported to infect plant tissue through stomata on the leaf surface. We intend to investigate whether PopW modulates the closure of stomata to prevent the access of pathogenic bacteria into plant tissues.

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References

[1] J. Byrne, A. Dianese, P. Ji, H. Campbell, D. Cuppels, F. Louws, S. Miller, J. Jones, M. Wilson, Biological control of bacterial spot of tomato under field conditions at several locations in North America, Biol. Control 32 (2005) 408–418.

[2] L. Long, H.M. Liu, Z.M. Xia, X.L. Yu, Y. Xiong, L. Li, Occurrence regularity of scab disease on hot pepper and its control techniques, Guizhou Agric. Sci. (2009) 75–76.

[3] W. Moss, J. Byrne, H. Campbell, P. Ji, U. Bonas, J. Jones, M. Wilson, Biological control of bacterial spot of tomato using *hrp* mutants of *Xanthomonas campestris* pv. *vesicatoria*, Biol. Control 41 (2007) 199–206.

[4] H.H. El-Hendawy, M.E. Osman, N.M. Sorour, Biological control of bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* by *Rahnella aquatilis*, Microbiol. Res. 160 (2005) 343–352.

[5] A. Obradovic, J. Jones, M. Momol, S. Olson, L. Jackson, B. Balogh, K. Guven, F. Iriarte, Integration of biological control agents and systemic acquired resistance inducers against bacterial spot on tomato, Plant Dis. 89 (2005) 712–716.

[6] J.G. Li, H.X. Liu, J. Cao, L.F. Chen, C. Gu, C. Allen, J.H. Guo, PopW of *Ralstonia solanacearum*, a new two-domain harpin targeting the plant cell wall, Mol. Plant Pathol. 11 (2010) 371–381.

[7] J.G. Li, J. Cao, F.F. Sun, D.D. Niu, F. Yan, H.X. Liu, J.H. Guo, Control of tobacco mosaic virus by PopW as a result of induced resistance in tobacco under greenhouse and field conditions, Phytopathology 101 (2011) 1202–1208.

[8] L. Zheng, Y.M. Luo, Q.Y. Xue, S.M. Li, H.X. Liu, J.H. Guo, Control and growth promotion of PopW to cucumber downy mildew under greenhouse and field conditions, Acta Phytopathol. Sin. 43 (2013) 179–186.

[9] C. Wang, J. Cao, T.T. Wang, L. Zheng, C. Wang, H.X. Liu, Induction of Protein PopW from *Ralstonia solanacearum* in plants disease resistance, Chin. J. Biol. Control 30 (1) (2014) 86–93.

[10] J.D. Jones, J.L. Dangl, The plant immune system, Nature 444 (2006) 323–329.

[11] M.A.E. Alvarez, R.I. Pennell, P.J. Meijer, A. Ishikawa, R.A. Dixon, C. Lamb, Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity, Cell 92 (1998) 773–784.

[12] L. Gómez-Gómez, G. Felix, T. Boller, A single locus determines sensitivity to bacterial flagellin in Arabidopsis thaliana, Plant J. 18 (1999) 277–284.

[13] K. Apel, H. Hirt, Reactive oxygen species: metabolism, oxidative stress, and signal transduction, Annu. Rev. Plant Biol. 55 (2004) 373–399.

[14] M. Desclos-Theveniau, D. Arnaud, T.Y. Huang, G.J.C. Lin, W.Y. Chen, Y.C. Lin, L. Zimmerli, The Arabidopsis lectin receptor kinase LecRK-V. 5 represses stomatal immunity induced by *Pseudomonas syringae* pv. *tomato* DC3000, PLoS Pathog. 8 (2012) e1002513.

[15] J.G. Horsfall, R. Barratt, An improved grading system for measuring plant diseases, Phytopathology 35 (1945), 655–655.

[16] D.D. Niu, H.X. Liu, C.H. Jiang, Y.P. Wang, Q.Y. Wang, H.L. Jin, J.H. Guo, The plant growth-promoting rhizobacterium *Bacillus cereus* AR156 induces systemic resistance in Arabidopsis thaliana by simultaneously activating salicylate-and jasmonate/ethylene-dependent signaling pathways, Mol. Plant Microbe Interact. 24 (2011) 533–542.

[17] T.L. Reuber, J.M. Plotnikova, J. Dewdney, E.E. Rogers, W. Wood, F.M. Ausubel, Correlation of defense gene induction defects with powdery mildew susceptibility in Arabidopsis enhanced disease susceptibility mutants, Plant J. 16 (1998) 473–485.

[18] J.G. Kim, X. Li, J.A. Roden, K.W. Taylor, C.D. Aakre, B. Su, S. Lalonde, A. Kirik, Y. Chen, G. Baranage, *Xanthomonas* T3S effector XopN suppresses PAMP-triggered immunity and interacts with a tomato atypical receptor-like kinase and TFT1, Plant Cell. 21 (2009) 1305–1323.

[19] A. Levine, R. Tenhaken, R. Dixon, C. Lamb, H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response, Cell 79 (1994) 583–593.

[20] C. Lamb, R.A. Dixon, The oxidative burst in plant disease resistance, Annu. Rev. Plant Biol. 48 (1997) 251–275.

[21] J. Zhang, F. Shao, Y. Li, H. Cui, L. Chen, H. Li, Y. Zou, C. Long, L. Lan, J. Chai, A *Pseudomonas syringae* effector inactivates MAPKs to suppress PAMP-induced immunity in plants, Cell. Host Microbe 1 (2007) 175–185.

[22] G. Felix, J.D. Duran, S. Volko, T. Boller, Plants have a sensitive perception system for the most conserved domain of bacterial flagellin, Plant J. 18 (1999) 265–276.

[23] C. Wang, H.X. Liu, J. Cao, J.H. Guo, Construction of transgenic tobacco expressing PopW and analysis of its biological phenotype, Chin. J. Biotechnol. 30 (2014) 569–580.

[24] C. Wang, C. Wang, H.W. Li, T. Wei, H.X. Liu, Overexpression of a harpin-encoding gene PopW in tobacco enhances resistance against *Ralstonia solanacearum* through SA, JA/ET Signal Pathw. (2015) (unpublished).

[25] A.P. Macho, B. Schwessinger, V. Ntoukakis, A. Brutus, C. Segonzac, S. Roy, Y. Kadota, M.H. Oh, J. Sklenar, P. Derbyshire, A bacterial tyrosine phosphatase inhibits plant pattern recognition receptor activation, Science 343 (2014) 1509–1512.

[26] J.M. Smith, A. Heese, Rapid bioassay to measure early reactive oxygen species production in Arabidopsis leave tissue in response to living *Pseudomonas syringae*, Plant Methods 10 (2014) 6.

[27] E. Luna, V. Pastor, J. Robert, V. Flors, B. Mauch-Mani, J. Ton, Callose deposition: a multifaceted plant defense response, Mol. Plant Microbe Interact. 24 (2011) 183–193.

[28] U. Conrath, C.M. Pieterse, B. Mauch-Mani, Priming in plant–pathogen interactions, Trends Plant Sci. 7 (2002) 210–216.

[29] M.G. Kim, L. da Cunha, A.J. McFall, Y. Belkhadir, S. DebRoy, J.L. Dangl, D. Mackey, Two *Pseudomonas syringae* type III effectors inhibit RIN4-regulated basal defense in Arabidopsis, Cell 121 (2005) 749–759.

[30] B. Hurley, D. Lee, A. Mott, M. Wilton, J. Liu, Y.C. Liu, S. Angers, G. Coaker, D.S. Guttman, D. Desveaux, The *Pseudomonas syringae* type III effector HopF2 suppresses Arabidopsis stomatal immunity, PLoS One 9 (2014) e114921.

[31] M. Melotto, W. Underwood, J. Koczan, K. Nomura, S.Y. He, Plant stomata function in innate immunity against bacterial invasion, Cell 126 (2006) 969–980.

[32] A.S. Kumar, V. Lakshmanan, J.L. Caplan, D. Powell, K.J. Czymmek, D.F. Levia, H.P. Bais, Rhizobacteria *Bacillus subtilis* restricts foliar pathogen entry through stomata, Plant J. 72 (2012) 694–706.