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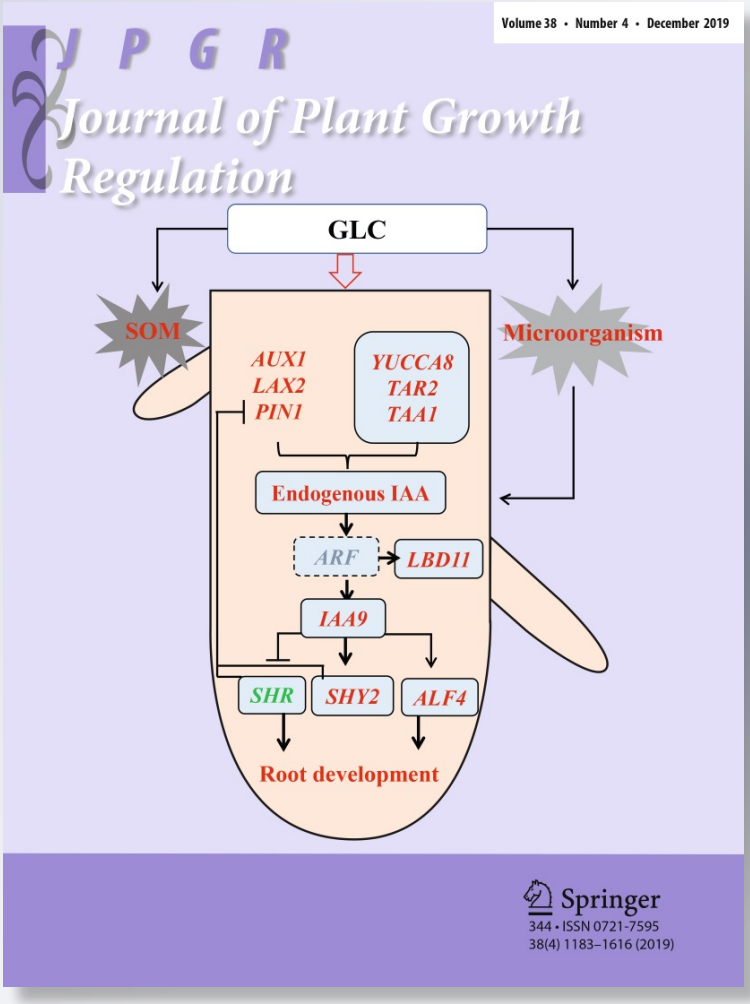

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Comparative Analysis of Tolerant and Susceptible Citrus Reveals the Role of Methyl Salicylate Signaling in the Response to Huanglongbing

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Abstract

Huanglongbing (HLB), associated with *Candidatus* Liberibacter asiaticus (Las), is the most devastating disease of citrus worldwide. Tolerance to HLB has been observed in some citrus varieties, but its molecular mechanisms are not well understood. Methyl salicylate (MeSA), involved in salicylic acid (SA) signaling, is a critical mobile signal for plant systematic acquired resistance (SAR). This study compared the response of tolerant sour pomelo (*Citrus grandis* Osbeck) and susceptible Jincheng orange (*Citrus sinensis* Osbeck) to Las infection. During 18 months of resistance evaluation, sour pomelo displayed significantly delayed and milder symptoms, and tolerated higher levels of Las growth, compared with Jincheng orange. High levels of MeSA were detected in sour pomelo and MeSA responded positively to Las infection. Little MeSA was found in Jincheng orange regardless of Las infection. Correspondingly, the SA content in sour pomelo was significantly higher than that in Jincheng orange. During Las infection, SA levels decreased significantly in sour pomelo but increased in Jincheng orange. These data indicated that MeSA was correlated with tolerance to HLB in citrus. Gene expression analysis showed that Cs*SAMT1* and Cs*SABP2-1*, involved in the interconversion of MeSA and SA, were related to MeSA accumulation in sour pomelo, and sour pomelo possesses a strong SAR response. Our study indicates that MeSA-mediated SAR plays an important role in citrus tolerance to HLB. This study provides new insights into HLB tolerance in citrus and useful guidance for improving citrus resistance to HLB by manipulation of MeSA signaling in the future.

Keywords Citrus · HLB · Tolerance · MeSA · SA · SAR

Introduction

Huanglongbing (HLB) is the most destructive disease of citrus. It has caused substantial economic losses in many affected areas and has recently gained worldwide notoriety (Bove 2006; da Graca et al. 2016; Hodges and Spreen 2012). The associated causal agent of HLB is a non-cultured, phloem-limited bacterium of the genus *Candidatus* Liberibacter (Duan et al. 2009). Three different bacterial species are associated with HLB in citrus (Wang et al. 2017): *Candidatus* Liberibacter asiaticus (Las), found in most HLB-affected countries, *Candidatus* Liberibacter africanus, restricted to South Africa, and *Candidatus* Liberibacter americanus, limited to the Americas. Las is the most prevalent species in citrus and is transmitted naturally by the Asian citrus psyllid, *Diaphorina citri*, or experimentally by grafting (Shokrollah et al. 2009). Typical symptoms of HLB in infected citrus include asymmetric blotchy mottling of older leaves and

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a range of chlorotic patterns, followed by leaf drop, twig dieback, reduced fruit production, and tree decline at later stages (Bove 2006). Anatomical aberrations in infected plants include the over-accumulation of starch in vascular parenchyma, massive deposition of callose in phloem elements, phloem plugging, necrosis and collapse, and finally the disruption of chloroplast structure (Aritua et al. 2013; Bove 2006; Fu et al. 2015; Kim et al. 2009). There are no efficient curative treatments for HLB and current management strategies mainly include the removal of infected trees, elimination of citrus psyllids and planting of HLB-free trees (Bove 2006; Wang et al. 2017; Zou et al. 2017).

At present, the molecular basis of the citrus response against HLB attack is poorly understood, although the gene regulatory networks involved in the citrus response to HLB have been predicted by many omics methods (da Graca et al. 2016; Martinelli et al. 2013; Wang et al. 2017; Zheng and Zhao 2013). The relationship between citrus and the causal agent of HLB is shaped by multiple elements such as host resistance, tolerance or susceptibility, citrus psyllid spread and environmental stress, in which the host defense response plays a vital role (da Graca et al. 2016; Fan et al. 2011; Martinelli et al. 2013, 2012; Xu et al. 2015; Zheng and Zhao 2013; Zhong et al. 2015). Different citrus varieties show different tolerance levels, but no HLB-resistant cultivars have been found. For example, *C. reticulata, C. sinensis*, and *C. reshni* cv. Cleopatra show susceptibility to HLB, while *C. aurantium, C. aurantifolia, C. limonia* Osbeck, *C. grandis* cv. Limau Bali, and *Poncirus trifoliata* (L.) Raf. display HLB tolerance (Folimonova et al. 2009). Thus, one of the important questions in citrus HLB research is what makes some citrus cultivars show tolerance to HLB. Answering this question is crucial for understanding the mechanisms of interaction between HLB and citrus and is the basis for developing effective disease management strategies.

Several comparative studies of tolerant and susceptible citrus varieties have shown that salicylic acid (SA)-mediated defense, such as systemic acquired resistance (SAR) and basal resistance, plays an important role in the response to HLB (Albrecht and Bowman 2012; Aritua et al. 2013; Martinelli et al. 2013; Wang et al. 2016). Additionally, it was suggested that Las represses citrus SA-mediated basal defense or SAR to promote the establishment of Las colonies in the phloem (Aritua et al. 2013; Li et al. 2017). Obviously, there is strong cross talk between SA signaling and Las. However, the exact mechanisms involved in this cross talk remain elusive.

SA signaling is critical for plant defenses against pathogens and is involved in multi-layered defense responses, from PTI (pathogen-associated molecular pattern-triggered immunity)-triggered basal defense, ETI (effector-triggered immunity)-mediated defense, and local acquired resistance (LAR) to SAR (Kumar 2014). Methyl salicylate (MeSA), a SA derivative, is a critical phloem-mobile signal for SA-mediated SAR during pathogen infection (Lu et al. 2016; Shine et al. 2018). Park et al. (2007) demonstrated a working model for MeSA signaling in tobacco in response to TMV virus infection: MeSA is synthesized from SA by SA methyltransferase (SAMT1) in the primary infected tissue, and the accumulated MeSA is translocated through the phloem to the systemic tissue; once at the systemic tissue, MeSA is converted back by salicylic acid-binding protein 2 (SABP2) to active SA, and finally the active SA triggers plant SAR to suppress further infection and pathogen spread. It is important to note that MeSA has no biological activity and has to be reconverted to SA to trigger SAR. The working model of MeSA signaling has also been proposed to apply to *Arabidopsis* and potato (Manosalva et al. 2010). However, there have been no reports about this working model in citrus to date. Recently, Dutt et al. (2016) showed that overexpression of the *Arabidopsis NPR1* gene in the sweet orange cultivars 'Hamlin' and 'Valencia' enhanced resistance against HLB, indicating that altering SA signaling can confer resistance to HLB in citrus (Wang et al. 2016). Therefore, elucidating the mechanisms by which MeSA participates in SA signaling in citrus in response to HLB would potentially provide targets for manipulating SA signaling to battle HLB in citrus breeding.

In this study, we compared the MeSA signaling responses to Las infection between tolerant sour pomelo (*Citrus grandis* Osbeck) and susceptible Jincheng orange (*Citrus sinensis* Osbeck). Sour pomelo displayed high tolerance to HLB compared with Jincheng orange. Our results indicate that, compared with SA signals, MeSA plays an important role in activating SAR against HLB in citrus. Possible mechanisms of MeSA signaling in response to HLB are also discussed.

Materials and Methods

Plant and Las Bacteria Materials and Growth Conditions

Sour pomelo (*Citrus grandis* Osbeck) and Jincheng orange (*Citrus sinensis* Osbeck) scions were collected from Yunnan province, China, and the National Citrus Germplasm Repository, Chongqing, China, respectively, and were grafted onto *Citrus junos* Sieb. ex Tanaka rootstock in a greenhouse.

Citrus scions containing Las were harvested from naturally infected sweet orange (*C. sinensis*) trees in Guilin, Guangxi Province, China. The scions were reproduced and maintained in Jincheng orange by grafting in a greenhouse with restricted access. Using the primers Las16S-f/Las16S-r (Table S1), the presence of Las in plants was confirmed by PCR.

Citrus DNA and RNA Isolation

Citrus genomic DNA was prepared using the Plant DNeasy Prep Kit (Aidlab, Beijing, China). Citrus RNA extraction was performed using the EASYspin Plant RNA Extraction kit (Aidlab). cDNA was synthesized from 1 µg total RNA with an iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA).

HB Infection Analysis

Two-year-old sour pomelo and Jincheng orange plants growing in the greenhouse were selected to evaluate tolerance to HLB. Using the primers Las16S-f/Las16S-r (Table 1), Las-free plants were identified by PCR. Twenty well-grown Las-free plants per variety were infected by graft infection as described by Zou et al. (2017). Each plant was infected with three buds containing Las. The Las-infected and non-infected plants were maintained in a greenhouse. Every 3 months after infection, Las growth in the plants was detected by PCR using the primers Las16S-f/Las16S-r (Table S1), and the development of HLB symptoms was recorded with photographs. Eighteen months after infection, the number of symptomatic leaves per plant was counted and the percentage of symptomatic leaves per plant was calculated. The experiment was replicated two times.

The Las pathogen growth in plants was quantified using the quantitative PCR (qPCR) method (Zou et al. 2017). Three leaves per plant were selected randomly to be tested. Their midrib tissues were pooled, and DNAs were isolated from the pooled tissues. Using 2 × iQ™ SYBR Green Supermix (Bio-Rad), the Las 16S and citrus 18S genes were amplified with the qLas16S-f/qLas16S-r and qCt18S-f/qCt18S-r primers, respectively (Table S1). PCRs were carried out as follows: pretreatment at 95 °C for 1 min, followed by 40 amplification cycles of 95 °C for 10 s and 60 °C for 1 min. The experiments were repeated three times. Using non-infected plants as a control, the bacterial population per µg citrus DNA was calculated using the formula reported by Zou et al. (2017).

Callose and Starch Grain Observation

Midribs of similar age, position, and developmental stage from Jincheng orange and sour pomelo leaves were prepared for microscopic observations. The midribs were cut into 1-cm sections and immediately transferred to FAA solution (100 ml of 37% formaldehyde, 100 ml glacial acetic acid and 900 ml of 70% ethanol). After 72 h incubation in FAA solution, the samples were maintained in 70% ethanol. Callose and starch grains were detected by counterstaining with methylene blue-azure A and basic fuchsin staining and aniline blue, respectively (Kim et al. 2009). Briefly, the midribs were cut into 20-µm-thick transverse sections on a KD-1508A microtome (KEDI Instrumental Equipment Co. Ltd, Zhejiang, China). For starch grain detection, the slides were stained with 1% basic fuchsin for 15 min and then with 0.25% methylene blue-azure A for 30 s. For callose detection, the slides were stained with 0.01% aniline blue (0.1 M phosphate buffer, pH 9.0) for 10 min. The samples were observed using a BX51 fluorescence microscope system equipped with a DP70 digital camera (Olympus, Tokyo, Japan). For starch grain observation, specimens were examined under white light, and for callose observation, specimens were examined under UV illumination. Callose deposition was quantified by counting the number of fluorescent spots in the phloem of each sample (Boava et al. 2017).

Starch Quantification

Starch isolation and content determination were performed according to the protocol of the Starch Assay Kit (G-clone Biotechnology Co., Ltd; Beijing, China). Briefly, 0.1 g fresh midribs were ground in liquid nitrogen and extracted with 80% ethanol (v/v) at 80 °C for 30 min. Starch grains were precipitated by centrifugation at 3000*g* for 5 min, suspended in 0.5 ml of water, and boiled for 15 min. After adding 0.35 ml of 60% perchloric acid, the solution was shaken for 3 min, diluted by adding 0.85 ml of water, and centrifuged at 3000*g* for 10 min. The starch content in the supernatant was quantified by the sulfuric acid-anthrone colorimetric method (DuBois et al. 1956). Absorbance was measured at 620 nm in a Spectra-Max M2 microplate reader (Molecular Devices Corporation, Menlo

Table 1 PCR analysis for the presence of Las in plants with *Ca*. L. asiaticus infection

Test[a]	Total no. of plants tested	No. of plants with the presence of Las									
		3 MAI		6 MAI		9 MAI		12 MAI		18 MAI	
		JC	SP	JC	SP	JC	SP	JC	SP	JC	SP
Test 1	20	20	0	20	0	-	4	20	7	-	10
Test 2	20	18	0	20	0	-	5	20	6	-	11

[a]The tolerance evaluation test was replicated two times as follows: graft infection, PCR confirmation, and qPCR quantification. Every 3 months after graft infection, the presence of Las in the midribs of plants was detected by PCR using the primers Las16S-f/Las16S-r (Table S1). MAI, month after infection

Park, CA, USA) using glucose as a standard. The starch content in fresh midribs was calculated using the formula: Starch content (mg/g fresh weight) = 2.89×(Absorbance + 0.0295). All analyses were repeated three times.

Hormone Content Determination

Hormones were extracted from the leaves of citrus plants and quantified as described previously (Seskar et al. 1998). SA and MeSA were extracted from 1 g infected mesophyll (in this manuscript, "mesophyll" indicates leaf tissues without the midrib, but with minor veins) and midrib tissues of Jincheng orange and sour pomelo after 18 months of Las infection. Tissue samples (1 g fresh weight) were frozen in liquid nitrogen, ground to a fine powder, and sequentially extracted with 15 ml isopropanol/hydrochloric acid buffer (1 M) for 30 min and then with 20 ml dichloromethane for 30 min at 4 °C. The extraction was centrifuged at 13,000*g* for 5 min. The lower organic phase was dried with nitrogen gas. The extracted hormones were redissolved with 200 µl methanol containing 0.1% methanoic acid and then filtered with a 0.22-µm membrane. The SA and MeSA contents were determined using HPLC-MS/MS at Zoonbio Biotechnology Co. (Nanjing, China). The test was repeated three times.

Gene Expression Analysis

Gene expression was analyzed using the quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The tested genes and the primers used in this study are listed in Table S1. cDNAs were amplified in 10-µL reaction mixtures using 2×iQ™ SYBR Green Supermix (Bio-Rad). PCRs were carried out as follows: pretreatment at 95 °C for 5 min, followed by 40 amplification cycles of 95 °C for 20 s and 60 °C for 1 min. The experiments were repeated three times. Using the citrus *actin* gene (GenBank No. GU911361.1) as an internal control, relative expression values were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Statistical Analysis

Data analysis was performed using the SPSS v22.0 statistical package (IBM Corp., Armonk, NY, USA). Data are presented as means ± standard deviations. Significant differences were identified by Tukey's test ($P < 0.05$).

Results

HLB Tolerance Differences Between Sour Pomelo and Jincheng Orange

To compare differences in Las growth between sour pomelo and Jincheng orange, the presence of the Las pathogen in leaf tissues was first determined by PCR (Fig. S1) and then confirmed by qPCR using non-infected plants as controls (Fig. 1). Based on these data, 20 and 18 Jincheng orange plants showed the presence of Las in two independent tests 3 months after graft infection (Fig. 1a; Table 1). Las was not detected in any of the tested sour pomelo plants 3 and 6 months after graft infection (Fig. 1b). At 9, 12, and 18 months, the number of sour pomelo plants with Las increased gradually (Table 1; Fig. 1c). In total, Las was detected in all the Jincheng orange plants and in 10 and 11 sour pomelo plants in the two independent tests during the 18 months of infection (Table 1). The results showed that the Las pathogen takes longer to successfully colonize sour pomelo, compared with Jincheng orange.

After 6 months of infection, most Jincheng orange plants began to display symptoms in their new leaves and shoots and showed severe symptoms (such as blotchy mottling and midrib yellowing) in the following 6 months, while no visible symptoms were found in any tested sour pomelo plants. After 18 months, mild symmetric blotchy mottling symptoms were detected in some new leaves of sour pomelo plants with the Las pathogen (Fig. 2a). Similar development of symptoms was observed in the two replicates.

To evaluate the tolerance differences between Jincheng orange and sour pomelo, the symptomatic leaves per plant were counted in the two varieties. As shown in Fig. 2b, the frequency (43%) of symptomatic leaves in Jincheng orange was significantly higher than that (25%) in sour pomelo, showing that sour pomelo has stronger tolerance to HLB.

To compare Las population characteristics between sour pomelo and Jincheng orange, the pathogen growth in asymptomatic and symptomatic leaves was quantified using qPCR 18 months after infection (Fig. 2c). Statistical analysis of 10 plants per variety showed that there were no differences in pathogen population among the asymptomatic and symptomatic leaves of Jincheng orange and asymptomatic leaves of sour pomelo. Interestingly, the Las population in symptomatic leaves of sour pomelo was significantly higher than that in symptomatic leaves of Jincheng orange. In this study, the Las concentration that caused sour pomelo to display visible symptoms was more than 10 times that for Jincheng orange.

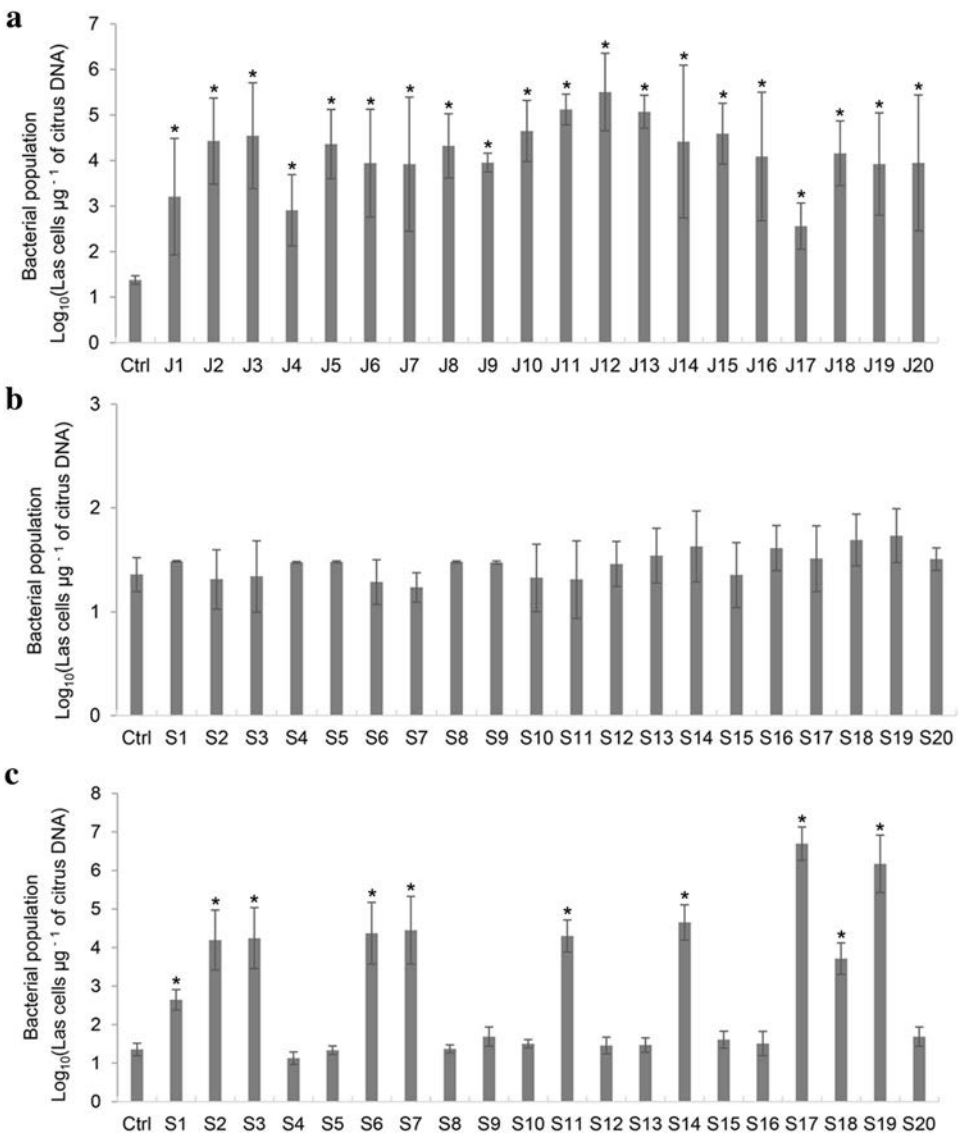

Fig. 1 Quantification of Las growth (Las cells μg^{-1} of citrus DNA) in Jincheng orange and sour pomelo after *Ca*. L. asiaticus infection. **a** and **b** Las growth in Jincheng orange and sour pomelo 3 months after *Ca*. L. asiaticus infection, respectively. **c** Las growth in sour pomelo at 18 months after *Ca*. L. asiaticus infection. The presence of bacteria was determined relative to non-infected control plants ($P < 0.05$, Tukey's test), which means if $P < 0.05$, the plant tested was classified into the group with Las cells. Standard errors were calculated from three leaves per plant. Error bars indicate standard error of means. * represents a significant difference compared with the non-infected control plant ($P < 0.05$, Tukey's test). Ctrl: non-infected control plant

Anatomical Response to HLB of Sour Pomelo and Jincheng Orange

Light microscopy analysis showed anatomical differences between midribs from sour pomelo and Jincheng orange leaves after 18 months of HLB infection. Compared with control plants, the cell wall thickness and the number of cell layers in the phloem of infected Jincheng orange plants were increased (Fig. 3a). No obvious changes were detected in the phloem of sour pomelo during HLB infection (Fig. 3a). Obvious accumulation of starch was observed in phloem parenchyma cells from infected Jincheng orange leaves but was not observed in infected sour pomelo leaves (Fig. 3a). Starch content changes during Las infection were quantified by the phenol–sulfuric acid method. The starch contents in control leaves showed no obvious difference between Jincheng orange and sour pomelo (Fig. 3b). After Las infection, the starch levels increased significantly in both Jincheng orange and sour pomelo leaves, but the starch level in Jincheng orange leaves was significantly higher and was about two times that in sour pomelo leaves (Fig. 3b).

Callose deposition was determined by aniline blue staining. Obvious callose deposition was observed in sieve elements from infected midribs but not from the control (Fig. 4a). Quantification analysis showed that callose contents in Las-infected midribs of Jincheng orange and sour pomelo were five and two times greater than in the control, respectively (Fig. 4b). Infected Jincheng orange displayed significantly greater callose deposition in midribs, which was more than two times that in infected sour pomelo (Fig. 4b).

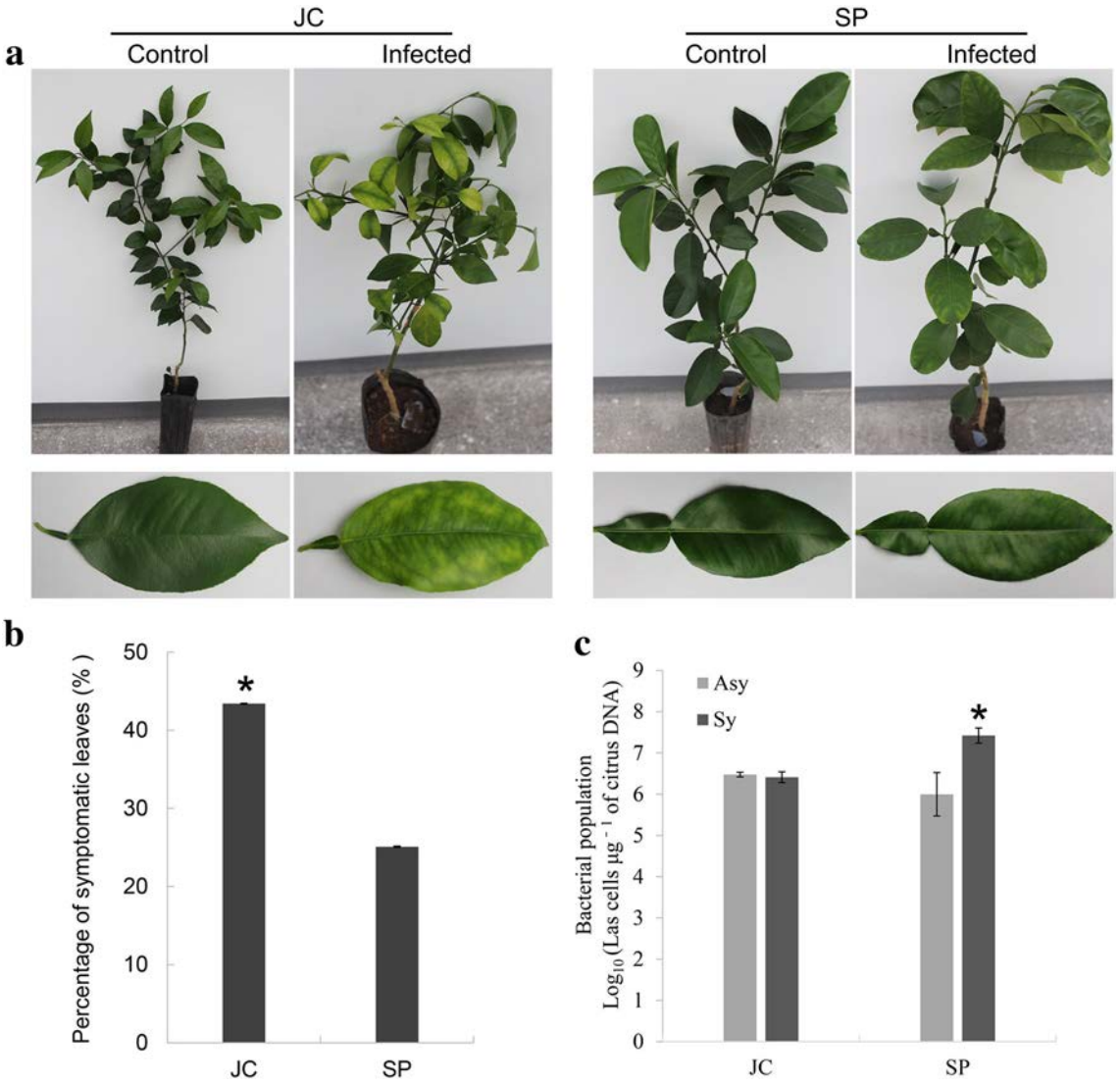

Fig. 2 Comparative analysis of HLB tolerance between Jincheng orange and sour pomelo in a greenhouse. **a** Representative HLB symptoms in the two varieties 18 months after *Ca*. L. asiaticus infection. **b** Comparative analysis of disease frequency between Jincheng orange and sour pomelo 18 months after *Ca*. L. asiaticus infection. Eighteen months after infection, the percentage of symptomatic leaves per variety was determined. Each bar is the mean of 10 plants per variety. **c** Quantification analysis of Las populations (Las cells μg^{-1} of citrus DNA) between Jincheng orange and sour pomelo 18 months after *Ca*. L. asiaticus infection. Standard errors were calculated from 10 plants per variety in three independent tests. Error bars indicate standard error of means. *represents a significant difference between Jincheng orange and sour pomelo based on Tukey's test ($P < 0.05$). JC, Jincheng orange; SP, sour pomelo. Asy, asymptomatic leaf; Sy, symptomatic leaf

MeSA and SA levels in sour pomelo and Jincheng orange

To investigate the response of MeSA to Las infection, the MeSA and SA hormone contents in sour pomelo and Jincheng orange were measured by HPLC analysis (Table 2). MeSA contents were very low (0.01–0.5 ng g^{-1} FW) in both control and infected leaves of Jincheng orange. Conversely, high levels of MeSA were detected in both the midrib (12.33 ng g^{-1} FW) and mesophyll (8.19 ng g^{-1} FW) in sour pomelo before Las infection. After Las infection, the hormone levels in midrib and mesophyll tissues of this variety significantly increased to 16.02 and 14.79 ng g^{-1} FW, respectively. The SA levels in the midrib and mesophyll of sour pomelo were 9.02 and 3.72 ng g^{-1} FW, respectively, which were significantly higher than those (1.86 and 1.35 ng g^{-1} FW, respectively) in Jincheng orange. After Las infection, the SA levels in sour pomelo leaves decreased more than twofold, whereas the SA levels in Jincheng orange leaf increased compared with control plants. In particular, the SA level in the midrib of infected Jincheng orange leaves markedly increased more than 10 times (from 1.86 to 19.69 ng

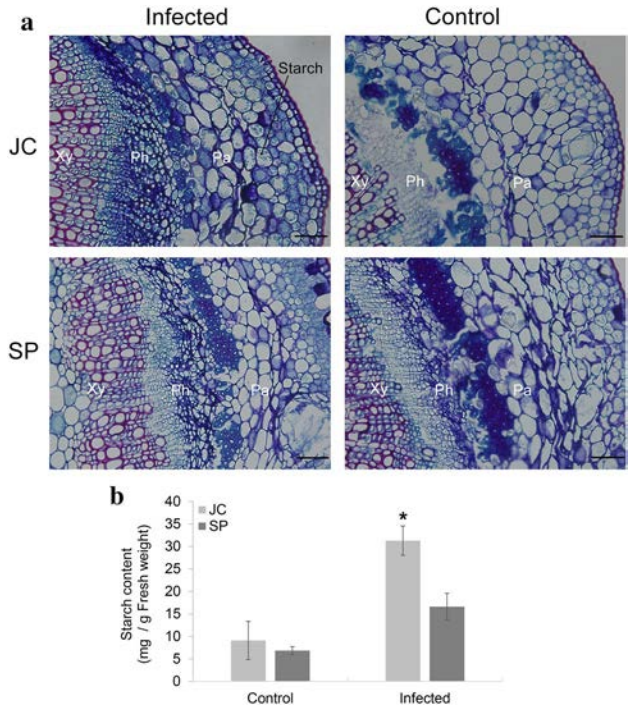

Fig. 3 Starch accumulation in leaf tissue. **a** Anatomical analysis of midrib phloem tissues of Las-infected and control citrus leaves 18 months after *Ca*. L. asiaticus infection. Midribs were collected from leaves with representative symptoms as shown in Fig. 2a. The slides were stained with methylene blue-azure A and basic fuchsin. Starch particles (arrows) were observed predominantly in the mesophyll parenchyma cells of Las-infected Jincheng orange leaves. **b** Starch content in leaf tissue of Jincheng orange and sour pomelo 18 months after *Ca*. L. asiaticus infection. Starch contents are expressed relative to fresh weight. Vertical bars indicate standard deviations of the means of three tests. * represents a significantly difference between Jincheng orange and sour pomelo based on Tukey's test ($P < 0.05$). JC, Jincheng orange; SP, sour pomelo. Pa, parenchyma; Ph, phloem; Xy, xylem. Bar = 20 μm

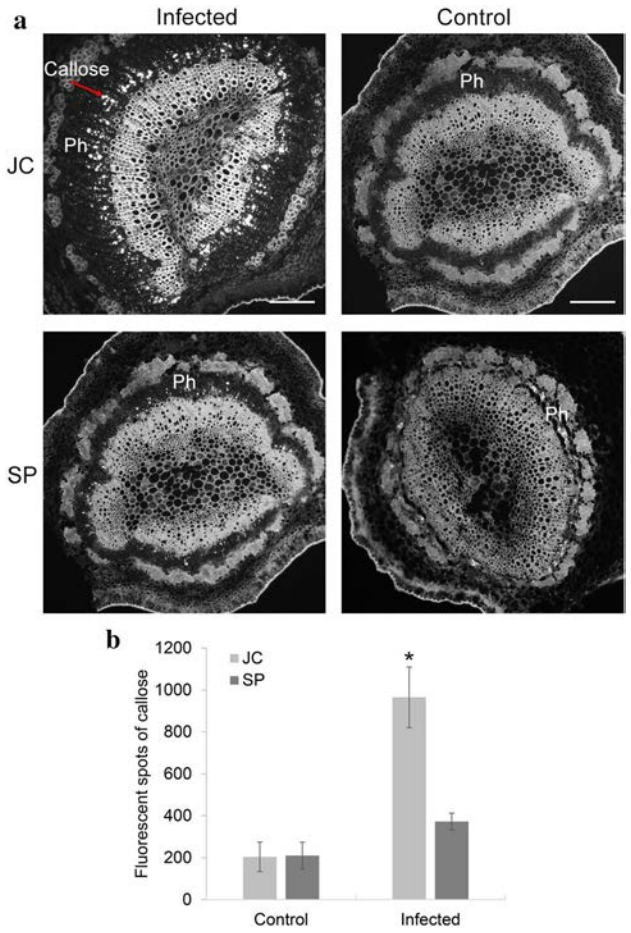

Fig. 4 Callose deposition in leaf midribs. **a** Anatomical analysis of callose in midrib phloem tissues 18 months after *Ca*. L. asiaticus infection. Midribs were collected from leaves with representative symptoms as shown in Fig. 2a. The slides were stained with 0.05% aniline blue solution and observed under a fluorescent microscope with a UV filter. Light areas represent callose deposits in the phloem. **b** Callose quantification in leaf midribs 18 months after *Ca*. L. asiaticus infection. Callose was quantified by the number of fluorescent spots of callose in the phloem from each sample. Twenty slides were counted per sample. Vertical bars indicate standard deviations of the means of three tests. * represents a significant difference between Jincheng orange and sour pomelo based on Tukey's test ($P < 0.05$). JC, Jincheng orange; SP, sour pomelo. Ph, phloem

g^{-1} FW) and was about 5.3-fold that in infected sour pomelo midribs. These data clearly showed that the tolerant sour pomelo accumulated high levels of MeSA, and that MeSA responded positively to Las infection, indicating MeSA was correlated with tolerance to HLB in sour pomelo.

Expression Characteristics of *CsSAMT1* and *CsSABP2-1* in Sour Pomelo and Jincheng Orange

The SA methyltransferase SAMT1 and salicylic acid-binding protein 2 (SABP2) are key enzymes in the interconversion of SA and MeSA in SA-mediated SAR (Park et al. 2007). Thus, using the protein sequences of *Arabidopsis* AtSAMT1 (Lin et al. 2013) and tobacco NtSABP2 (Park et al. 2007) as queries, we performed Basic Local Alignment Search Tool (BLAST) searches against the reference genome of *Citrus sinensis* to identify homologous citrus genes. The searches showed that the protein sequences of *CsSAMT1* (Cs1g24440) and *CsSABP2-1* (Cs1g23200) were highly homologous to those of AtSAMT1 and NtSABP2, respectively (Figs. S1 and S2). Multiple sequence alignment showed that most amino acid residues in the active sites of CsSAMT1 and CsSABP2-1 were conserved compared with other known SAMTs and SABP2s, respectively (Figs. S1 and S2).

Then, we compared the expression characteristics of *CsSAMT1* and *CsSABP2-1* between sour pomelo and Jincheng orange (Fig. 5). Before graft infection, the expression levels of *CsSAMT1* in the midrib and mesophyll of sour pomelo were 57- and 195-fold that in non-infected Jincheng orange, respectively. After Las infection, *CsSAMT1* displayed significantly

Table 2 Hormone contents in citrus leaves

Treatment	Variety	SA (ng/g FW)		MeSA (ng/g FW)	
		Midrib	Mesophyll	Midrib	Mesophyll
Control	Jincheng orange	1.86 ± 0.74	1.35 ± 0.56	0.04 ± 0.02	0.01 ± 0.00
	Sour pomelo	9.02 ± 2.01*	3.72 ± 0.13*	12.33 ± 4.18*	8.19 ± 3.73*
Las	Jincheng orange	19.69 ± 1.94	1.64 ± 0.08	0.51 ± 0.51	0.01 ± 0.01
	Sour pomelo	3.74 ± 1.42*	1.80 ± 0.08*	16.02 ± 2.26*	14.79 ± 1.70*

*Indicates the difference between Jincheng orange and sour pomelo was significant (Tukey's test, $P < 0.05$). FW, Fresh Weight

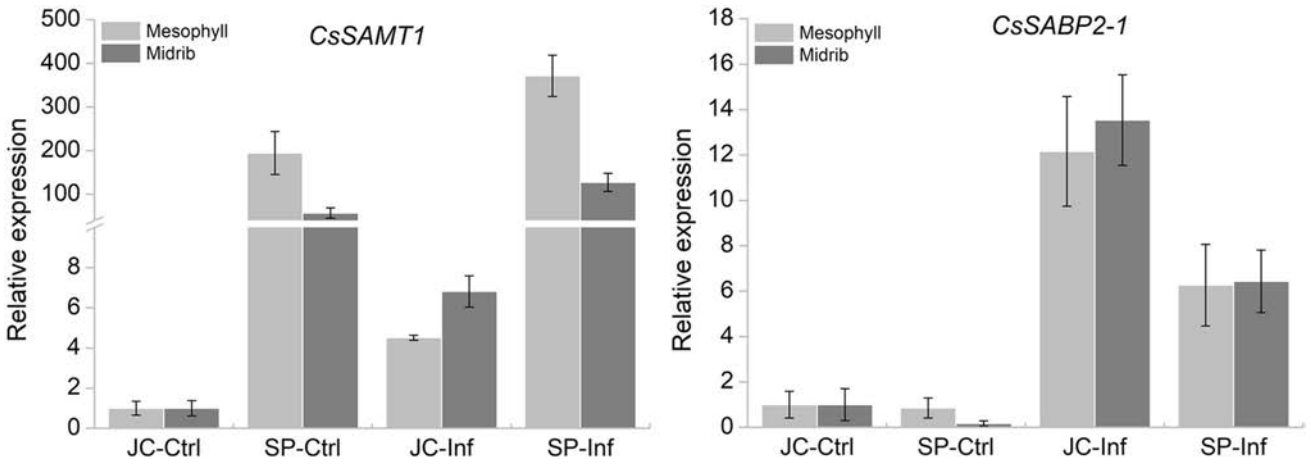

Fig. 5 Quantitative RT-PCR comparison of *CsSAMT1* and *CsSABP2-1* gene expression between sour pomelo and Jincheng orange 18 months after *Ca*. L. asiaticus infection. Gene expression was normalized to the expression of the actin gene, and gene expression in non-infected Jincheng orange (JC-Ctrl) was set to "1". Expression levels are presented as mean fold differences relative to JC-Ctrl. JC-Ctrl, non-infected Jincheng orange; JC-inf, *Ca*. L. asiaticus-infected Jincheng orange; SP-Ctrl, non-infected sour pomelo; SP-Ctrl, *Ca*. L. asiaticus-infected sour pomelo

induced expression in both sour pomelo and Jincheng orange. Compared with non-infected Jincheng orange, the expression levels of *CsSAMT1* were increased to 127- and 371-fold in the midrib and mesophyll of infected sour pomelo, respectively, and were still markedly higher than those (sevenfold in the midrib and fivefold in mesophyll) in infected Jincheng orange. These data showed that *CsSAMT1* had high base expression levels but also displayed strong Las-inducible expression in sour pomelo.

For *CsSABP2-1*, there was no difference in gene expression between sour pomelo and Jincheng orange before Las infection (Fig. 5). After Las infection, the expression of *CsSABP2-1* increased by 12- to 14-fold in Jincheng orange, which was significantly higher than that (about sixfold) in sour pomelo.

Expression Characteristics of SAR-Associated Genes in Sour Pomelo and Jincheng Orange

To compare the difference in SAR levels between sour pomelo and Jincheng orange, we investigated the expression of the pathogenesis-related *PR1, PR2* and *PR5* genes, which are involved in plant SAR and among which *PR2* is a SAR marker gene (Dutt et al. 2016; Li et al. 2017) (Fig. 6). Before Las infection, the expression levels of *CsPR1* and *CsPR2* in sour pomelo were more than three times that in Jincheng orange, while the expression of *CsPR5* in the mesophyll of sour pomelo was about five times that in Jincheng orange. No difference in *CsPR5* expression was detected in the midrib between the two varieties before infection. These data indicated that the basic resistance mediated by *CsPR1, CsPR2* and *CsPR5* in sour pomelo was obviously stronger than that in Jincheng orange. During Las infection, *CsPR1, CsPR2* and *CsPR5* showed various expression patterns in the two varieties. Cs*PR1* had 13- and 33-fold induced expression in the midribs of sour pomelo and Jincheng orange, respectively, but its expression was obviously decreased in the mesophyll of Jincheng orange. *CsPR2* had 18- and 33-fold induced expression in the midrib and mesophyll of sour pomelo, respectively. In Jincheng orange, this gene had only sevenfold induced expression in the mesophyll tissue, while its expression was downregulated by Las infection

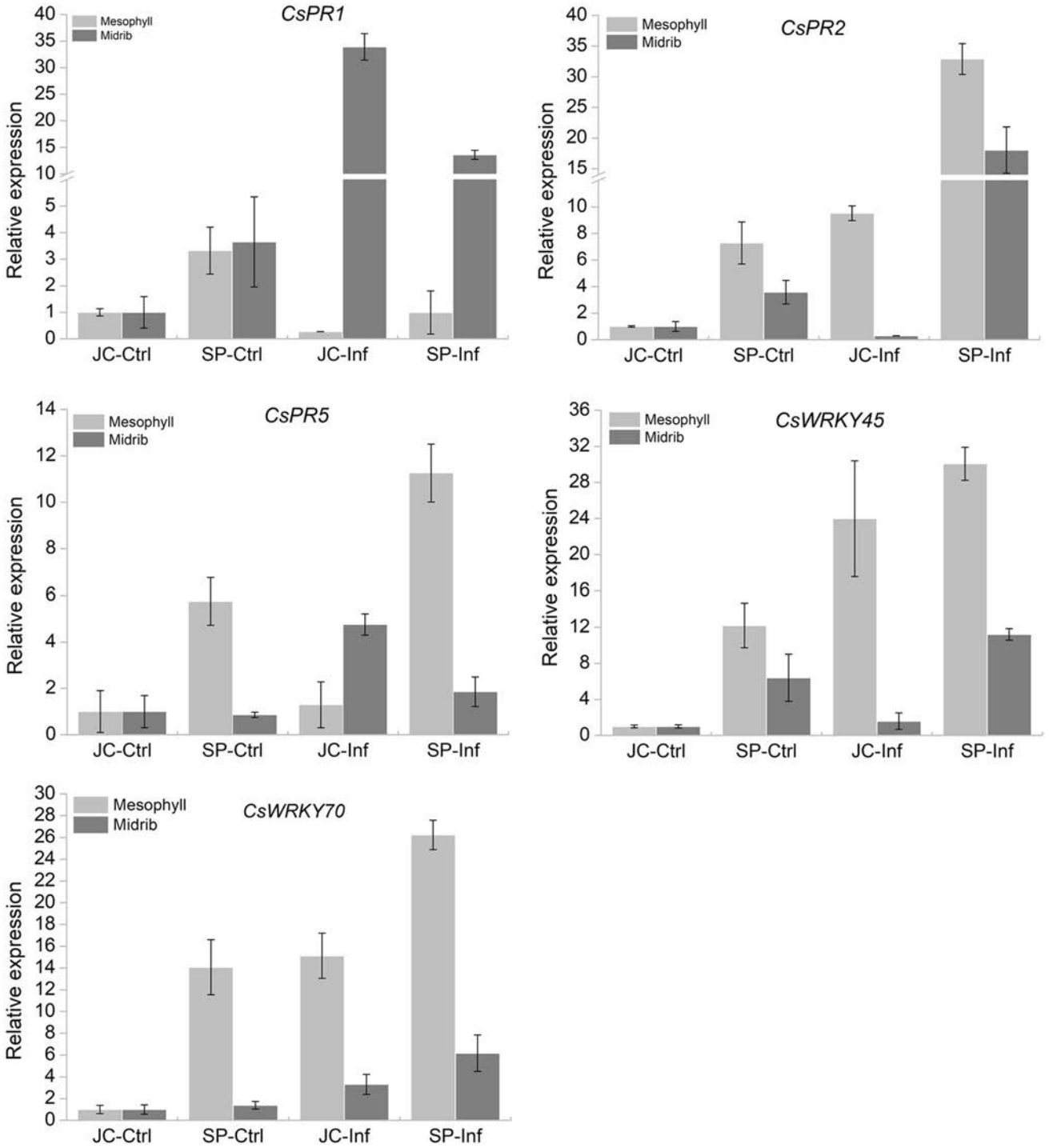

Fig. 6 Quantitative RT-PCR comparison of SAR-associated gene expression between sour pomelo and Jincheng orange 18 months after *Ca*. L. asiaticus infection. Gene expression was normalized to the expression of the actin gene, and gene expression in non-infected Jincheng orange (JC-Ctrl) was set to "1". Expression levels are presented as mean fold differences relative to JC-Ctrl. JC-Ctrl, non-infected Jincheng orange; JC-inf, *Ca*. L. asiaticus-infected Jincheng orange; SP-Ctrl, non-infected sour pomelo; SP-Ctrl, *Ca*. L. asiaticus-infected sour pomelo

in the midrib. Induced expression of *CsPR5* was detected in the midrib of Jincheng orange and in the mesophyll of sour pomelo, but no difference in expression was detected in the mesophyll of Jincheng orange or in the midrib of sour pomelo. According to these data, *CsPR2* showed high levels of basic and induced expression in both the midrib

and mesophyll of sour pomelo compared with Cs*PR1* and *CsPR5*, indicating *CsPR2* plays an important role in the tolerance of sour pomelo to HLB disease.

CsWRKY45 and *CsWRKY70*, which are associated with the regulation of SA signaling during HLB infection (Martinelli et al. 2013), were also investigated in this study (Fig. 6). Compared with Jincheng orange, *CsWRKY45* displayed higher expression levels (6–12-fold) before graft infection and was significantly upregulated by Las infection in both the midrib and mesophyll of sour pomelo. *CsWRKY70* had a high expression level in the mesophyll of sour pomelo before infection and was significantly induced by Las infection in both sour pomelo and Jincheng orange.

Discussion

Huanglongbing disease affects all citrus species and relatives. However, there is substantial variation among different citrus genotypes in their responses to infection. Based on symptomatic variation and the presence of Las, citrus genotypes are generally grouped into three categories: sensitive, showing severe chlorosis in leaves and branches, greatly reduced growth and eventual death; tolerant, with some scattered distinct or very minimal symptoms and containing detectable Las but with no plant death; and resistant, without any symptoms or detectable Las (Folimonova et al. 2009; Shokrollah et al. 2009; Wang et al. 2016). No resistant genotypes have been identified to date. In this study, the tolerance differences between sour pomelo and Jincheng orange were investigated by analyzing symptoms, pathogen presence, bacterial titer, anatomical dimensions, starch accumulation and callose deposition during 18 months of resistance evaluation. Compared with Jincheng orange, sour pomelo showed the presence of Las 3 months later and symptoms 12 months later and had mild symptoms. The data showed that sour pomelo, a *C. grandis* variety originating from Yunnan province, China, is a tolerant variety. Shokrollah et al. (2009) showed that another *C. grandis* variety, Limau Bali, displayed no symptoms and no Las presence 6 months after HLB infection, and thus, the cultivar was classified into the resistant group. In our test, the *C. grandis* variety sour pomelo also showed no symptoms and no Las presence 6 months after HLB infection. However, after more than 6 months, symptoms and the presence of Las were detected in about 50% of plants. It may be that the two *C. grandis* varieties really have different tolerance levels, or it may be that the different test conditions caused different tolerance levels.

A minimal Las concentration is required for HLB symptoms in citrus. In some sweet orange varieties, symptomatic leaves have more than 6.0×10^6 bacterial cells per μg citrus genome (Trivedi et al. 2009). However, the distribution of Las is very uneven in different tissues from the same tree and shows great variation between individual trees, although high levels of Las are usually found in midrib tissues (Li et al. 2009; Tatineni et al. 2008). In this study, to minimize the effects of uneven pathogen distribution when determining the Las concentration, the bacterial growth in asymptomatic and symptomatic leaves was investigated on a large scale in 10 plants per variety. Statistical analysis showed that Las required more than a 10 × greater titer in symptomatic leaves of the tolerant sour pomelo to achieve a similar degree of symptoms to the susceptible Jincheng orange, suggesting that sour pomelo tolerates higher levels of Las growth. Folimonova et al. (2009) detected similar levels of the Las pathogen in most of the citrus genotypes they examined, despite different disease severities. Based on our results, we speculate that tolerant cultivars could well serve as reservoirs of HLB.

Many studies have indicated that HLB infection suppresses citrus immunity by interfering with SA signaling, which plays an important role in the tolerance of citrus varieties to HLB (Albrecht and Bowman 2012; Li et al. 2017; Wang et al. 2016). MeSA is a critical phloem-mobile signal for SA-mediated SAR during pathogen infection and is required for SAR signaling in plant defense (Lu et al. 2016; Manosalva et al. 2010; Shine et al. 2018). These reports encouraged us to investigate the differences in MeSA signaling in the SAR response to Las infection between sour pomelo and Jincheng orange. To better understand the response characteristics of MeSA to Las infection, the mesophyll and midrib were considered systemic and primary infected tissues, respectively (Kumar 2014), because Las mainly colonizes the phloem of midribs (Bove 2006). We found that the tolerant sour pomelo had markedly higher levels of MeSA in both the primary infected and systemic tissues before Las infection and that its levels were significantly upregulated by Las infection. However, the susceptible Jincheng orange had little MeSA in either the primary infected or systemic tissue regardless of infection. Simultaneously, we showed that in both tolerant sour pomelo and susceptible Jincheng orange, the SA content was lower than the MeSA content, although the hormone levels were increased significantly by Las infection. These findings clearly indicate that MeSA signals play a more important role in the tolerance of sour pomelo to HLB disease, compared with SA.

We further explored the molecular regulation of MeSA accumulation in sour pomelo using qRT-PCR. Our data showed that *CsSAMT1* had a high expression level in sour pomelo. Compared with *CsSAMT1, CsABP2-1* had very low expression in sour pomelo. In plants, SAMT is responsible for the formation of MeSA from SA, while SABP2 mediates the production of SA from MeSA (Park et al. 2007). Thus, we speculated that high expression of *CsSAMT1* and low expression of *CsABP2-1* are responsible for the high

levels of MeSA accumulation in sour pomelo. Theoretically, MeSA accumulation enhances the SAR response in plants (Lin et al. 2013; Manosalva et al. 2010; Park et al. 2007). In the present study, Cs*PR1*, Cs*PR2*, Cs*PR5*, and Cs*WRKY45*, Cs*WRKY70*, which are involved in plant SAR, displayed high expression in tolerant sour pomelo compared with susceptible Jincheng orange, which indicates that the SAR-mediated basal resistance of sour pomelo is stronger than that of Jincheng orange. It has been shown that activation of SABP2 is required for SAR induction in systemic tissues during pathogen infection (Park et al. 2007) and that interconversion of SA and MeSA is crucial for the induction of MeSA-mediated SAR resistance (Lin et al. 2013; Manosalva et al. 2010; Park et al. 2007; Shine et al. 2018). After infection, both *CsSAMT1* and *CsSABP2-1* showed significantly increased expression in the two varieties, indicating the interconversion of SA and MeSA was promoted by Las infection. The above results suggest that MeSA positively regulates citrus tolerance to HLB disease through a similar molecular model (Fig. 7) to that reported by Park et al. (2007): accumulation of MeSA is induced by the CsSAMT1 enzyme in phloem parenchyma cells and companion cells, and the accumulated MeSA moves through the phloem to systemic tissues, in which it is converted back to active SA by CsSABP2-1, and finally the active SA triggers plant SAR against HLB disease. MeSA has no bioactivity and must be converted to bioactive SA to promote a SAR response. The levels of SA in tolerant sour pomelo were markedly higher than in susceptible Jincheng orange before infection, indicating constitutive accumulation of MeSA should favor the early accumulation of SA, which leads to a high SAR level for a fast and strong response by citrus to pathogen infection (Dutt et al. 2016; Friedrich et al. 2001). However, during Las infection, SA levels declined dramatically in the tolerant sour pomelo. This decrease may be due to increased SA consumption for a strong SAR response. In susceptible Jincheng orange, the SA level increased significantly by more than tenfold in the primary infected tissues after Las infection. However, this high accumulation of SA was not enough to efficiently activate host SAR as shown by in the tolerant sour pomelo. Martinelli et al. (2013) thought that adequate activation of SAR in infected young leaves is required to enhance the defense response to HLB disease. Young leaves are generally considered the sites where most new Las infections occur. Thus, as in sour pomelo, the early activation of sufficient SAR could be a potential strategy for efficient control of HLB spread. Additionally, the achievement of SA-mediated SAR signaling needs a threshold level of active SA, and when SA levels rise above this threshold, SAR and other defense responses are triggered to defend against pathogen attack (Manosalva et al. 2010). Our study showed that the threshold levels of SA are about 2 and 4 ng g^{-1} FW in systemic and infected tissues of

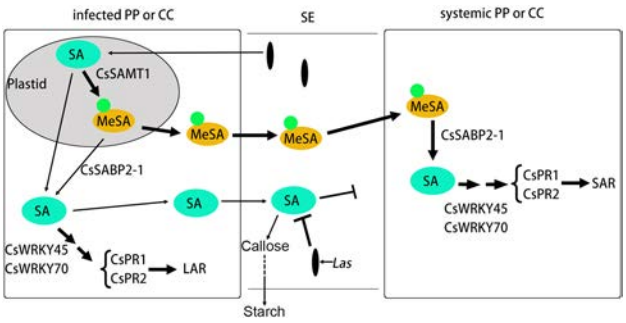

Fig. 7 A supposed model for MeSA signaling in response to HLB infection in the citrus phloem. Las in SE activates SA accumulation in the plastids of infected PP or CC where MeSA is synthesized from SA by CsSAMT1. The accumulated MeSA diffuses into the cytoplasm where it is converted back to SA by CsSABP2-1 and the increased SA activates LAR in infected sites. MeSA and SA move through the SE to activate SAR in distal systemic tissues. In the SE, SA signaling is repressed by Las (for example, SA degradation by Las-encoded SA hydroxylase) and cannot efficiently translocate the SAR signal over long distances. However, MeSA can avoid Las hijacking and translocate the SAR signal to the systemic PP or CC. Once in the systemic tissue, MeSA is converted back to active SA by CsSABP2-1, and finally the active SA triggers plant SAR to suppress further infection and pathogen spread. Overaccumulation of SA in the SE may enhance Las-induced callose deposition and eventually starch deposition in PP. PP, Phloem parenchyma cells; CC, companion cells; SE, sieve elements

tolerant sour pomelo, respectively, which are far lower than those in tobacco (Seskar et al. 1998) and rice (Silverman et al. 1995), demonstrating that a low level of SA is enough to activate SAR to enhance pathogen tolerance in citrus as noted by Zhang et al. (2010).

Our data showed that starch accumulation and callose deposition in tolerant sour pomelo were lower than in susceptible Jincheng orange. Several reports have shown that callose over-deposition and starch over-accumulation occur specifically in the midribs of leaves in HLB-infected citrus (Kim et al. 2009). Callose is involved in the gating of sieve plates, which are basic gates of the phloem (Ellinger and Voigt 2014). Callose deposition between the plasma membrane and cell wall at sites of pathogen attack is believed to provide a physical barrier to pathogen spread (Nishimura et al. 2003). However, in HLB-infected sweet orange leaves, over-accumulation of callose plugs the sieve pores and blocks sugar transport, resulting in excessive starch accumulation in the vascular parenchyma, which disrupts chloroplasts and ultimately causes greening symptoms in the leaves (da Graca et al. 2016). Moreover, Nishimura et al. (2003) showed that *Arabidopsis* mutants deficient in stress-induced callose accumulation constitutively express SA-dependent SAR and have enhanced pathogen resistance. Conversely, SA enhances callose deposition during pathogen infection (Fernández-Crespo et al. 2017; Yi et al. 2014). Thus, we speculate that the low level of callose deposition in tolerant

sour pomelo may be partly due to the low concentration of bioactive SA, suggesting that accumulation of non-bioactive MeSA does not induce over-deposition of callose.

The data presented here show that MeSA signaling plays an important role in the response to HLB, and we speculate that MeSA signaling positively regulates citrus tolerance to HLB disease through the supposed working model (Fig. 7), but this needs to be further dissected in the future. In the supposed model, the *CsSAMT1* and *CsSABP2-1* genes have vital roles in activating SAR against HLB. SAR can confer systemic, long-lasting and broad-spectrum resistance to plants (Shine et al. 2018). Thus, it is possible that durable resistance of citrus against HLB could be engineered by manipulating *CsSAMT1* and *CsSABP2-1* expression to regulate the interconversion of MeSA and SA, and increase the effectiveness of the SAR response, which may have significant practical applications for citrus disease resistance.

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Author contribution XZ designed the experiments and wrote the manuscript. XB performed resistance evaluations. QW performed citrus hormone. ZX performed starch content analysis. LW performed microscopic observations and RT-qPCR analysis. AP and YH performed PCR analysis. LX and ZX performed qPCR analysis. SC analyzed the data and revised the manuscript. All authors read and approved the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Albrecht U, Bowman KD (2012) Transcriptional response of susceptible and tolerant citrus to infection with *Candidatus* Liberibacter asiaticus. Plant Sci 185–186:118–130. https://doi.org/10.1016/j.plantsci.2011.09.008

Aritua V, Achor D, Gmitter FG, Albrigo G, Wang N (2013) Transcriptional and microscopic analyses of citrus stem and root responses to *Candidatus* Liberibacter asiaticus infection. PloS ONE 8:e73742 https://doi.org/10.1371/journal.pone.0073742

Boava LP, Cristofaniyaly M, Machado MA (2017) Physiologic, anatomic, and gene expression changes in Citrus sunki, Poncirus trifoliata and their hybrids after After '*Candidatus* Liberibacter asiaticus'. Infect Phytopathol 107:590–599. https://doi.org/10.1094/PHYTO-02-16-0077-R

Bove JM (2006) Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J Plant Pathol 88:7–37. https://doi.org/10.4454/jpp.v88i1.828

da Graca JV, Douhan GW, Halbert SE, Keremane ML, Lee RF, Vidalakis G, Zhao H (2016) Huanglongbing: an overview of a complex pathosystem ravaging the world's citrus. J Integr Plant biol 58:373–387. https://doi.org/10.1111/jipb.12437

Duan Y et al (2009) Complete genome sequence of citrus huanglongbing bacterium, '*Candidatus* Liberibacter asiaticus' obtained through metagenomics Mol Plant Microbe Interact 22:1011–1020. https://doi.org/10.1094/MPMI-22-8-1011

DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related. Subst Analy Chem 28:350–356. https://doi.org/10.1021/ac60111a017

Dutt M, Barthe G, Irey M, Grosser J (2016) Transgenic citrus expressing an arabidopsis NPR1 gene exhibit enhanced resistance against huanglongbing (HLB; citrus greening). PLoS ONE 11:e0147657. https://doi.org/10.1371/journal.pone.0147657

Ellinger D, Voigt CA (2014) Callose biosynthesis in Arabidopsis with a focus on pathogen response: what we have learned within the last decade. Ann Bot 114:1349–1358. https://doi.org/10.1093/aob/mcu120

Fan J, Chen CX, Yu QB, Brlansky RH, Li ZG, Gmitter FG (2011) Comparative iTRAQ proteome and transcriptome analyses of sweet orange infected by "*Candidatus* Liberibacter asiaticus". Physiol Plantarum 143:235–245. https://doi.org/10.1111/j.1399-3054.2011.01502.x

Fernández-Crespo E, Navarro JA, Serra-Soriano M, Finiti I, García-Agustín P, Pallás V, González-Bosch C (2017) Hexanoic acid treatment prevents systemic MNSV movement in *Cucumis melo* plants by priming callose deposition correlating SA and OPDA accumulation. Front Plant Sci 8:1793. https://doi.org/10.3389/fpls.2017.01793

Folimonova SY, Robertson CJ, Garnsey SM, Gowda S, Dawson WO (2009) Examination of the responses of different genotypes of citrus to huanglongbing (citrus greening) under different conditions Phytopathology 99:1346–1354. https://doi.org/10.1094/PHYTO-99-12-1346

Friedrich L, Lawton K, Dietrich R, Willits M, Cade R, Ryals J (2001) *NIM1* overexpression in arabidopsis potentiates plant disease resistance and results in enhanced effectiveness of fungicides. Mol Plant Microbe 14:1114–1124 https://doi.org/10.1094/MPMI.2001.14.9.1114

Fu SM, Hartung J, Zhou CY, Su HN, Tan J, Li ZA (2015) Ultrastructural changes and putative phage particles observed in sweet orange leaves infected with '*Candidatus* Liberibacter asiaticus'. Plant Dis 99:320–324. https://doi.org/10.1094/PDIS-01-14-0106-RE

Hodges AW, Spreen TH (2012) Economic impacts of citrus greening (HLB) in Florida, 2006/07-2010/11 Food & Resource Economics

Kim JS, Sagaram US, Burns JK, Li JL, Wang N (2009) Response of sweet orange (*Citrus sinensis*) to '*Candidatus* Liberibacter asiaticus' infection: microscopy and microarray analyses Phytopathology 99:50–57. https://doi.org/10.1094/PHYTO-99-1-0050

Kumar D (2014) Salicylic acid signaling in disease resistance. Plant Sci 228:127–134. https://doi.org/10.1016/j.plantsci.2014.04.014

Li W, Levy L, Hartung JS (2009) Quantitative distribution of '*Candidatus* Liberibacter asiaticus' in citrus plants with citrus huanglongbing Phytopathology 99:139–144. https://doi.org/10.1094/PHYTO-99-2-0139

Li JY, Pang ZQ, Trivedi P, Zhou XF, Ying XB, Jia HG, Wang NA (2017) '*Candidatus* Liberibacter asiaticus' encodes a functional salicylic acid (SA) hydroxylase that degrades SA to suppress plant defenses Mol Plant Microbe 30:620–630 https://doi.org/10.1094/Mpmi-12-16-0257-R

Lin J et al (2013) Overexpression of a soybean salicylic acid methyltransferase gene confers resistance to soybean cyst nematode. Plant Biotechnol J 11:1135–1145. https://doi.org/10.1111/pbi.12108

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time Quantitative PCR and the $2^{-\Delta\Delta CT}$. Method Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Lu H, Greenberg JT, Holuigue L (eds) (2016) Salicylic Acid Signaling Networks. Lausanne: Frontiers Media. Lausanne: Frontiers Media. https://doi.org/10.3389/978-2-88919-827-6

Manosalva PM, Park SW, Forouhar F, Tong L, Fry WE, Klessig DF (2010) Methyl esterase 1 (StMES1) is required for systemic acquired resistance in potato Mol Plant Microbe Interact 23:1151–1163. https://doi.org/10.1094/MPMI-23-9-1151

Martinelli F et al (2012) Transcriptome profiling of citrus fruit response to Huanglongbing disease PloS one 7:e38039 https://doi.org/10.1371/journal.pone.0038039

Martinelli F et al (2013) Gene regulatory networks elucidating huanglongbing disease mechanisms PLoS ONE 8:e74256 https://doi.org/10.1371/journal.pone.0074256

Nishimura MT, Stein M, Hou BH, Vogel JP, Edwards H, Somerville SC (2003) Loss of a callose synthase results in salicylic acid-dependent disease resistance Science 301:969–972. https://doi.org/10.1126/science.1086716

Park SW, Kaimoyo E, Kumar D, Mosher S, Klessig DF (2007) Methyl salicylate is a critical mobile signal for plant systemic acquired resistance Science 318:113–116. https://doi.org/10.1126/science.1147113

Seskar M, Shulaev V, Raskin I (1998) Endogenous methyl salicylate in pathogen-inoculated tobacco plants Plant Physiol 116:387–392 doi. https://doi.org/10.1104/Pp.116.1.387

Shine MB, Xiao XQ, Kachroo P, Kachroo A (2018) Signaling mechanisms underlying systemic acquired resistance to microbial pathogens Plant Science https://doi.org/10.1016/j.plantsci.2018.01.001

Shokrollah H, Abdullah TL, Sijam K, Siti Nor AA, Nur Ashikin PA (2009) Differential reaction of citrus species in Malaysia to Huanglongbing (HLB) disease using grafting method. Am J Agric Biol Sci 4:32–38. https://doi.org/10.3844/AJAB.2009.32.38

Silverman P, Seskar M, Kanter D, Schweizer P, Metraux JP, Raskin I (1995) Salicylic acid in rice (biosynthesis, conjugation, and possible role). Plant Physiol 108:633–639

Tatineni S, Sagaram US, Gowda S, Robertson CJ, Dawson WO, Iwanami T, Wang N (2008) In planta distribution of '*Candidatus Liberibacter asiaticus*' as revealed by polymerase chain reaction (PCR) and real-time. PCR Phytopathol 98:592–599. https://doi.org/10.1094/PHYTO-98-5-0592

Trivedi P et al (2009) Quantification of viable *Candidatus* Liberibacter asiaticus in hosts using quantitative PCR with the aid of ethidium monoazide (EMA). Eur J Plant Pathol 124:553–563. https://doi.org/10.1007/s10658-009-9439-x

Wang Y, Zhou L, Yu X, Stover E, Luo F, Duan Y (2016) Transcriptome profiling of Huanglongbing (HLB) tolerant and susceptible citrus plants reveals the role of basal resistance in HLB tolerance Front Plant Sci 7:933. https://doi.org/10.3389/fpls.2016.00933

Wang N et al (2017) The *Candidatus* Liberibacter-Host Interface: Insights into Pathogenesis Mechanisms and Disease Control Ann Rev Phytopathol 55:451–482. https://doi.org/10.1146/annurev-phyto-080516-035513

Xu MR, Li Y, Zheng Z, Dai ZH, Tao Y, Deng XL (2015) Transcriptional Analyses of Mandarins Seriously Infected by '*Candidatus* Liberibacter asiaticus' PLoS ONE 10:e0133652. https://doi.org/10.1371/journal.pone.0133652

Yi SY, Shirasu K, Moon JS, Lee S-G, Kwon S-Y (2014) The activated SA and JA signaling pathways have an influence on flg22-triggered oxidative burst and callose deposition. PLoS ONE 9:e88951. https://doi.org/10.1371/journal.pone.0088951

Zhang XD, Francis MI, Dawson WO, Graham JH, Orbović V, Triplett EcW, Mou ZL (2010) Over-expression of the Arabidopsis NPR1 gene in citrus increases resistance to citrus canker European. J Plant Pathol 128:91–100. https://doi.org/10.1007/s10658-010-9633-x

Zheng ZL, Zhao Y (2013) Transcriptome comparison and gene coexpression network analysis provide a systems view of citrus response to '*Candidatus* Liberibacter asiaticus' infection BMC Genomics 14:27 https://doi.org/10.1186/1471-2164-14-27

Zhong Y et al (2015) Comparative Transcriptome and iTRAQ Proteome Analyses of Citrus Root Responses to *Candidatus* Liberibacter asiaticus infection. PLoS ONE 10:e0126973. https://doi.org/10.1371/journal.pone.0126973

Zou X et al (2017) Transgenic citrus expressing synthesized cecropin B genes in the phloem exhibits decreased susceptibility to Huanglongbing. Plant Mol Biol 93:341–353. https://doi.org/10.1007/s11103-016-0565-5

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检 索 报 告

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日　　期：2021 年 09 月 22 日

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二 O 二 O 年制

3	通讯作者	CRISPR/Cas9-mediated editing of CsWRKY22 reduces susceptibility to Xanthomonas citri subsp. citri in Wanjincheng orange (Citrus sinensis (L.) Osbeck)	000493657100008	IF_{2019}=1.462	中科院类别基础版 / 分区 小类 BIOTECHNOLOGY & APPLIED MICROBIOLOGY 生物工程与应用微生物 / 4区 小类 PLANT SCIENCES 植物科学 / 4区 大类 生物 / 4区	2019	英文	国外
4	1	Comparative Analysis of Tolerant and Susceptible Citrus Reveals the Role of Methyl Salicylate Signaling in the Response to Huanglongbing	000496316900029	IF_{2019}=2.672	中科院类别基础版 / 分区 小类 PLANT SCIENCES 植物科学 / 3区 大类 生物 / 4区	2019	英文	国外
5	1	Overexpressing GH3.1 and GH3.1L reduces susceptibility to Xanthomonas citri subsp. citri by repressing auxin signaling in citrus (Citrus sinensis Osbeck)	000534070200003	IF_{2019}=2.74	中科院类别基础版 / 分区 小类 MULTIDISCIPLINARY SCIENCES 综合性期刊 / 3区 大类 综合性期刊 / 3区	2019	英文	国外