Systemic Acquired Resistance Induced by Agrobacterium tumefaciens in Peach and Differential Expression of PRI Genes

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Abstract. Crown gall disease caused by Agrobacterium tumefaciens affects a wide range of horticultural plants, and has no effective treatment. During the evaluation of crown gall resistance of peach germplasm resources, we observed enhanced resistance to subsequent invasion that was activated by virulence of A. tumefaciens in two peach cultivars. To further verify the phenotype observed in field experiments, systemic acquired resistance (SAR)-related salicylic acid (SA) and PRI genes were investigated. The levels of SA were elevated in two cultivars, and these high levels were maintained for 35 days post-inoculation. Compared with mock-inoculated controls, eight of the 22 candidate PpPRI genes in A. tumefaciens-inoculated samples were significantly upregulated and three were downregulated in response to inoculation with A. tumefaciens. These data suggested that SA-induced SAR was activated in two peach cultivars by virulent A. tumefaciens infection. In addition, the eight induced PpPRI genes can be used as molecular markers in defense studies in peach.

Plants have evolved an array of strategies to defend themselves against pathogens. The constitutive structural and chemical barriers are the first line of defense. When the pathogens go past these barriers, two-branched inducible defenses are recruited to halt further ingress (Pieterse et al., 2009). Pattern-triggered immunity (PTI) is a non-host resistance initiated by the recognition of pathogen-associated molecular patterns (Jones and Dangl, 2006; Mishina and Zeier, 2007). For a successful colonization, pathogens deliver effectors into the plant cells to suppress the PTI. In turn, plants develop resistance proteins that recognize these attacker-specific effectors, activating effector-triggered immunity (ETI) (Jones and Dangl, 2006). Effector-triggered immunity is an amplified PTI response, resulting in gene-for-gene resistance and usually a hypersensitive cell death response at the infection site (Jones and Dangl, 2006).

Besides resistance responses at the site of attempted ingress, plants can initiate SAR in tissues distant from the initial infection, which confers stable and broad-spectrum systemic resistance against subsequent attackers (Cameron et al., 1994; Durrant and Dong, 2004). The phytohormone SA is known to participate in regulating SAR response (Loake and Grant, 2007). Once the pathogen is detected, the plant amplifies the production of SA and induces the expression of pathogenesis-related (PR) protein genes, which leads to the establishment of SAR (van Loon and van Strien, 1999). Routinely, the expression of PRI genes and increased levels of SA are used as markers to manifest the SAR induction (Anand et al., 2008; Govrin and Levine, 2002; Wu et al., 2013). Induced systemic resistance (ISR), elicited by plant growth-promoting rhizobacteria, is another form of induced resistance in plants, and phenotypically it is similar to the SAR. Unlike SAR, the ISR is not associated with the expression of PR genes or accumulation of SA (Vallad and Goodman, 2004; van Loon et al., 1998).

Agrobacterium tumefaciens, a soil-borne bacterium, causes formation of crown galls in many plant species. During infection, a specific segment of the tumor-inducing plasmid, the transfer DNA (T-DNA), is transferred from the bacterium and integrated into the plant genome (Lee et al., 2009; Pitzschke and Hirt, 2010). Genes encoded by T-DNA are expressed, resulting in the over production of auxins and cytokinins and leading to an abnormal cell proliferation and tumor formation at the infection site.

Although, the pathogen infection process has been elucidated, little is known about the defense mechanism in host plants. Resistance genes that were screened from the Rhizobium vitis-inoculated cDNA library in grapevine suggest that SA signaling is involved in defense responses (Choi et al., 2010). Previous studies have also demonstrated that SAR regards Agrobacterium infection in Nicotiana benthamiana (Anand et al., 2008). In the course of Arabidopsis–Agrobacterium interaction, the transcript of PRI gene could not be detected, indicating that SAR was not induced in the host organism (Lee et al., 2009).

Crown gall disease is a serious problem in horticultural crops worldwide, including peach, and using plant resistance remains an alternative approach to combat this disease (Escobar and Dandekar, 2003; Zoina and Raio, 1999). During the evaluation of crown gall resistance in peach wild germplasm resources and cultivars, we observed two peach cultivars that presented enhanced resistance when infested with A. tumefaciens. Further investigation indicated that the SA-induced SAR was activated instead of ISR. To our knowledge, this is the first example of SAR activated by A. tumefaciens in horticultural crops. In addition, two PRI genes have been isolated from a peach tree infected by Xanthomonas campestris pv. pruni (Sherif et al., 2012), but no data have shown the expression profiles of PRI gene family at the SAR. In this study, we assayed transcript expression of PRI genes in peach SAR induced by A. tumefaciens infection.

Materials and Methods

Plant material. Plant material used for evaluation of the resistance to crown gall was classified into two classes based on the propagation methods. The first class included 40 accessions or cultivars, most of which were obtained from the wild germplasm resources collection and some cultivars were placed in this class because of the same propagation method (Table 2). They were propagated from seeds and every accession or cultivar was presented with 10–70 seedlings. The second class was propagated by grafting and contained 189 peach germplasms including Chinese landraces, breed cultivars, and related species (data not shown). Four replications of each germplasm were grafted on the rootstock of Prunus persica. Seedlings and grafted saplings were prepared previously in a fumigated soil and were considered to be intact and transplanted to experimental field in the spring of the following year.

To characterize the enhanced resistance observed in field experiments, two cultivars ‘Honggangsuanuotu’ and ‘Xibe13-1’ were used for their inducible resistance, and further investigation proved that they were the most resistant and the most susceptible to crown gall disease, respectively. Fifty
replications of each cultivar were prepared by grafting on the rootstock of *P. persica*. Dormant 1-year-old saplings were planted in pots, 40 cm diameter and 50 cm high, filled with soil mixture (1 part peatsoil:1 part sand). Shoot tips were cut 15 cm long and then planted in pots, 40 cm diameter and 50 cm high, filled with soil mixture (1 part peatsoil: 1 part sand) and water. The density of the suspension was adjusted to 109 colony-forming units/mL. Bacterial inoculations. *Agrobacterium tumefaciens* at different time periods. Bacterial cells were cultured in yeast extract and beef extract (YEB) medium (1 g L−1 yeast extract, 5 g L−1 beef extract, 5 g L−1 tryptone, 5 g L−1 sucrose, and 0.5 g L−1 MgSO4·7H2O) on a rotary shaker at 160 rpm at 30 °C for 16 h. Bacterial cells were harvested by centrifugation and suspended in sterilized distilled water. The density of the suspension was adjusted to 109 colony-forming units/mL.

The inoculation was performed as described by Bliss et al. (1999). Twigs were wounded at three sites by cutting into the cambial area with a scalpel and removing a piece of tissue about 1 cm long from the stem surface. One drop of prepared bacterial suspension was placed on each wound site, which was wrapped with parafilm to prevent drying. Mock inoculation of the control was performed in a similar manner, but sterilized distilled water was used instead of the bacterial suspension. Two months later, we recorded the maximum diameter of each tumor. The resistance of the strain used for inoculation; x = all the seedlings were inoculated twice; w = 7–10 seedlings of each accession or cultivar were inoculated randomly and the selected saplings were infected only once.

### Table 1. Primers used in qPCR of *Pp-PR1s* and reference genes.

| Name                  | Locus name | Forward primer sequence (5′−3′) | Reverse primer sequence (5′−3′) | Amplicon size (bp) |
|-----------------------|------------|---------------------------------|---------------------------------|-------------------|
| *PpPR1* 809a          | ppa012617m | ACCAGGGCCACCCCACTACTGT          | ACCACGACGGAGTGTG            | 57                |
| *PpPR1* 806a          | ppa022704m | GGGCCCAAGATIGGCTGTA            | TGCACTCGGGACCTTGT            | 68                |
| *PpPR1* 601           | ppa011521m | GTTCGCGAGGACCACCTTGT          | GGCCTCTCCCTTATTGTG           | 237               |
| *PpPR1* 801           | ppa026522m | GTGATGTATGATGGACCTTGT         | AGGTGCTTCTGGTTGTG           | 248               |
| *PpPR1* 802           | ppa027034m | TGGGATGATGATGCCGACCTTGT        | GTGCCAACTAGTGTTGAGG           | 105               |
| *PpPR1* 803           | ppa017707m | TTGTCGCGAGGACCACCTTGT         | ATGCCAACACTCAAAGCCTTGT        | 113               |
| *PpPR1* 804           | ppa018679m | TTGTCGCGAGGACCACCTTGT         | CATGTCGCGCCTTGTG             | 194               |
| *PpPR1* 805           | ppa023001m | ATGACGACCCGCACTTTGT           | GGGCTTGCGCCCAACTAG           | 233               |
| *PpPR1* 807           | ppa021743m | CAGGACCTTACCCACTTGT          | CTGTGATGCTTCTGAGG            | 226               |
| *PpPR1* 808           | ppa014967m | AAAGGAGGCTGGCCCAACAC            | CGAGGCTGCTGATCTTGT            | 134               |
| *PpPR1* 810           | ppa012599m | GAAGCTGCACTTGCAAGG           | ACATAAGAACCTCATCAAGC           | 178               |
| *PpPR1* 811           | ppa0127196m | GTGACGACGGCATGCTGTTGT        | GCTGAGAAGACTCAGACCTTGT        | 283               |
| *PpPR1* 812           | ppa013370m | ATAGGCTGACGCTGTGTTGT          | CAGCCACACTGATGGTTG             | 279               |
| *PpPR1* 813           | ppa015888m | GCGTTCGGGCTAGAACTTGT         | TGGCCTCGGGCTTGTG             | 243               |
| *PpPR1* 814           | ppa018857m | AGAGCCGACGACGCTGTTGT        | CGGCTTGACGATCTTGAGG           | 212               |
| *PpPR1* 815           | ppa024466m | GCAGATGACGCTGCTGCG          | CTGGGCTGCTGATCTTGT            | 261               |
| *PpPR1* 816           | ppa019711m | TTGCGCTATGGACGCTCTTA            | TGGGCTGCTGATTGTGAGG           | 217               |
| *PpPR1* 817           | ppa015211m | GCACCAACAGGCTCGTA            | CATAGCGACGTCAACAACTTGT        | 109               |
| *PpPR1* 818           | ppa017637m | AAGATTTGCGGACGTGCTTGT        | TGACGCATTGTTGACTTGT           | 146               |
| *PpPR1* 819           | ppa018993m | GTGAGAACTTGGCCTCTGCGTTG       | TGGACCGATTTGTTGACG             | 103               |
| *PpPR1* 820           | ppa026973m | GCCCATATGGCAGGCTAGAAT         | TCTAGCGTACGAGGCTTGT             | 103               |
| *PpPR1* 821           | ppa025062m | TTGCGTATGGTTCTTGTCA          | CATATTCGTTGCGCATACTTG          | 194               |
| TEF2                  | —           | GTTGGGACGACTGAAAATGCTG        | TGGAGGAGGGAGAAGGTAAGGAGG            | 129               |
| **RPII**              | —           | TGAAGATACACCCATGATGAGTGAAGA  | CTTTGACGACGACGATGAGTCC           | 128               |

* The two pairs of primers were obtained from Sherif et al. (2012).

### Table 2. Frequency of tumor occurrence and tumor size in seven peach accessions/cultivars of seedlings inoculated with virulence strains of *Agrobacterium tumefaciens* at different time periods.

| Accession/cultivar | Genetic origin | Code | D (mm) | 5-23 | 6-22 | 5-18 | 6-07 | 6-27 |
|--------------------|----------------|------|--------|------|------|------|------|------|
| Honggengansutao    | *P. kansensis*  | D (mm) | 6.15 ± 4.46 | 3.10 ± 2.56 | 2.45 ± 2.00 | 3.62 ± 3.28 | 2.18 ± 2.27 |
| Xibei 13-1         | *P. persica × P. davidiana* | D (mm) | 13.22 ± 4.96 | 2.22 ± 2.02 | 3.42 ± 3.56 | 3.80 ± 3.66 | 2.67 ± 2.91 |
| Zhongtaokangzhen 1 | *P. persica*    | D (mm) | 95.1 ± 18.4 | 16.7 ± 15.6 | 16.2 ± 16.6 | 17.1 ± 17.8 | 17.8 |
| Hongxinmaotao 2    | *P. mira*       | D (mm) | 11.88 ± 2.89 | 9.57 ± 4.05 | 9.84 ± 3.75 | 10.84 ± 4.94 | 8.85 ± 3.79 |
| Wanzhouyetao 1     | *P. persica*    | D (mm) | 75.2 ± 7.20 | 72.0 ± 75.5 | 73.8 ± 73.2 | 11.34 ± 5.31 | 92.7 |
| Rutgers Redleaf 1   | *P. persica*    | D (mm) | 14.34 ± 5.31 | 14.76 ± 6.43 | 13.28 ± 5.31 | 13.79 ± 6.92 | 11.34 ± 5.31 |
| Okinawa 1           | *P. persica*    | D (mm) | 73.1 ± 3.32 | 5.28 ± 3.01 | 6.47 ± 2.82 | 7.22 ± 4.83 | 6.86 ± 3.63 |

D = average diameter of tumor in an accession/cultivar; F = frequency of tumor occurrence in an accession/cultivar; the accessions or cultivars were selected because of: 1 = potential use as a rootstock; 2 = growing trend and size were highly consistent; 3 = known rootstocks and being susceptible to *A. tumefaciens*; used as a reference standard to confirm the oncogenicity of the strain used for inoculation; x = all the seedlings were inoculated twice; w = 7–10 seedlings of each accession or cultivar were inoculated randomly and the selected saplings were infected only once.
at the inoculation site was harvested at 3, 6, 9, and 35 d after inoculation and immediately frozen in liquid N$_2$ and stored at –80 °C.

**RNA extraction and gene expression.**

Plant material pooled from five plants was ground with a mortar and pestle under liquid nitrogen into a fine powder. The total RNA was extracted from 200 mg of inoculated or mock tissues using RNeasy Kit (TaKaRa, Tokyo, Japan). RNA samples were treated with RNase-free DNase I (TaKaRa) before the synthesis of cDNA to remove any traces of genomic DNA. The first-strand cDNA was synthesized from 5 μg of total RNA using RevertAid™ Premium First Strand cDNA Synthesis Kit (Fermentas UAB, Burlington, ON, Canada) according to the manufacturer’s instructions.

To verify the genes of SAR pathway that were affected by infestation with *A. tumefaciens*, we measured the expression of the PRI genes and contrasted it against RPII and TEF2 (Tong et al., 2009) as reference genes (Table 1). The polymerase chain reaction (PCR) reaction was performed in 20 μL of reaction mixture containing 1 μL cDNA, 10 μL LightCycler® 480 SYBR Green Master (Roche, Penzberg, Germany), and 0.5 μL (10 μmol·L$^{-1}$) of each primer. The specificity of the individual PCR amplification was confirmed by the melting curve analyses following the final cycle of the PCR. The fold-change in gene expression was calculated using the comparative cycle threshold ($C_{\text{t}}$) method ($2^{-\Delta C_{\text{t}}}$) (Schmittgen and Livak, 2008).

**Genomics database search and in silico analysis of sequences.**

Gene and protein sequences were retrieved by homology search in the NCBI and the Genome Database for Rosaceae (http://www.rosaceae.org/species/prunus_persica/genome_v1.0). The peach EST database contained 79,824 entries as of 1 Jan. 2013 (http://www.ncbi.nlm.nih.gov/genbank/dbest/dbest_summary/) and was not abundant enough. Therefore, the PpPRI gene was confirmed by EST evidence in genus *Prunus* (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignments of PpPRI genes with orthologs from diverse plant species were conducted using ClustalX (Jeanmougin et al., 1998). The phylogenetic tree was constructed with MEGA6 software using the Neighbor-Joining method with...
1000 bootstrap replications (Tamura et al., 2013).

**Quantification of endogenous SA.** Endogenous SA was quantified as described by Forcat et al. (2008). Ground samples (150 mg) were weighted and transferred to 2-mL microfuge tubes and each sample was dissolved with 400 μL of 10% methanol containing 1% acetic acid. Samples were shaken on a rotary shaker at 25 °C for 10 min and placed on ice for 30 min. The mixtures were centrifuged at 13,000 g for 10 min at 4 °C. The supernatant was carefully removed and the pellet was resuspended with 400 μL of 10% methanol containing 1% acetic acid. The resuspended mixtures were incubated on ice for 30 min and centrifuged again. The supernatants resulting from the two extractions were mixed and preserved for later analysis.

Samples were analyzed by liquid chromatography–tandem mass spectrometry (MS) using Agilent 6460B (Agilent Technologies, Santa Clara, CA). Chromatographic separation was carried out on a ZORBAX SB-C18 column (2.1 mm × 100 mm × 1.8 μm) (Agilent Technologies). The mobile phase consisted of water–formic acid (99:1) as solvent A and acetonitrile as solvent B. The gradient profile began at 90% solvent A in equal intervals. The flow rate was 0.3 mL·min⁻¹ and the column temperature was set at 30 °C. The injection volume was 5 μL. For MS analyses, nitrogen was used as a drying and nebulizing gas, and nebulizer pressure was set at 275,790 Pa. Gas flow was set at 10 L·min⁻¹ and temperature was 350 °C. Data were collected and analyzed by MassHunter Workstation B02.06 data acquisition and processing software (Agilent Technologies).

**Results**

**Verification of the induced resistance by virulent A. tumefaciens invasion in certain peach germplasms.** In 2013, a total of 1100 seedlings were screened. All of the inoculation sites that were treated with sterilized distilled water did not result in tumor formation (data not shown). To increase the frequency of inoculation replication, the seedlings were inoculated for the second time at 30 d after the first inoculation. Except the two accessions ‘Honggengansutao’ and ‘Xibei 13-1’, the gall size and/or occurrence frequency in 40 accessions or cultivars did not differ between the two inoculation periods (Table 2).

In 2014, 189 germplasms were screened and 35 germplasms were selected randomly and inoculated for the second and third time at 20 and 40 d after the first inoculation. The gall sizes between the first and the second, the first and the third, and the second and the third inoculations in 35 germplasms were positively correlated ($R^2 = 0.83, 0.79, \text{and } 0.91$, respectively; data not shown). Meanwhile, seven were selected from 40 accessions or cultivars that were inoculated the previous year, and they were inoculated three times successively. The gall sizes of two accessions did not differ between the inoculation periods, and they were smaller than the galls formed after the first inoculation in 2013. The same results were confirmed for the frequency of gall occurrence (Table 2). These data indicated that these two cultivars displayed reinforced resistance to subsequent invasion.

**Quantitative analysis of SA.** Changes in the levels of SA in the host during the infection with A. tumefaciens are presented in Figure 1. SA was elevated in mock-inoculated and A. tumefaciens-inoculated plants, peaking at 3 d postinoculation (dpi). Over time (at 9 and 35 dpi), the levels of SA slightly decreased and were maintained at a stable level in bacteria infected ‘Honggengansutao’. By contrast, the
level of SA remained similar at 3, 6, 9, and 35 dpi in bacteria-infected ‘Xibei 13-1’. The level of SA was much higher in ‘Honggengansutao’ than in ‘Xibei 13-1’, and in mock-treated ‘Honggengansutao’ the levels were higher than in bacteria infected ‘Xibei 13-1’.

Identification of the active peach PR1 genes from ‘Lovell’. An extensive search of the NCBI and the Genome Database for Rosaceae for all possible PR1 genes in peach yielded 30 sequences, excluding nine sequences with no EST evidence. These predicted PR1 protein sequences were aligned and the duplicates with identical sequences were consolidated. Subsequently, a total of 22 non-redundant PR1 proteins were identified from the genome of ‘Lovell’. Proposed names of genes were designated according to the location and order on the scaffolds to differentiate each gene. For example, ppa026522m, the first one to appear on scaffold 8, was followed by ppa027034m, and these were denoted as PpPR1 801 and PpPR1 802, respectively (duplicates were not counted).

The phylogenetic analysis of PpPR1 proteins and PR1 proteins from other species resulted in two clades. One clade (group I) included nine PpPR1 and 11 orthologs, and the other clade (group II) consisted of 13 paralogs (group II) and received high bootstrap support of 100% (Fig. 2).

Differential expression of PpPR1 genes in response to A. tumefaciens infection. In this study, the expression of PpPR1 genes was monitored to indicate the SAR status. We identified 22 candidate PpPR1 genes from the peach genome through mining of genomic databases. The transcript profiles of individual PpPR1 genes were analyzed by quantitative reverse transcription-PCR after inoculation with A. tumefaciens (Fig. 3). The transcription of PpPR1 802, 803, 805, 806, 810, 813, 820, and 821 was induced by bacteria, reaching the maximum levels at 3 or 6 dpi and seven of them maintained these high levels at 35 dpi except PpPR1 820. However, the transcription of PpPR1 601, 809, and 811 was depressed and maintained at low levels. Transcription of the remaining 10 PpPR1 genes did not change and they were constitutively expressed, indicating that, with the exception of PpPR1 804 induced by wounds, these genes had other functions.

Discussion

In this study, we show that SAR is activated as a consequence of both compatible and incompatible (less compatible) A. tumefaciens–peach interactions. The induced resistance protected the plants against the subsequent colonization and development by the same virulent bacterial pathogen, resulting in a lower frequency of tumor occurrence and a smaller gall size (Table 2). Meanwhile, the groove at the site of inoculation became deeper and wider in bacteria-infected branches than in mock-treated branches (Fig. 4). This phenotypical difference may have originated from the PTI and/or ETI responses. The SAR can be triggered by both PTI and/or ETI
response to pathogens varied among plant species. We hypothesized that the failure of the SAR induction was due to the SAR pathway rather than bacteria recognition. On the other hand, the SAR induction and the level of protection provided by SAR varies in different plant species or cultivars infected with different pathogens (Bonnet et al., 1996; Cameron et al., 1994; Mishina and Zeier, 2007). When infected with A. tumefaciens, the SAR was activated in tobacco, whereas the opposite was reported in Arabidopsis (Anand et al., 2008; Lee et al., 2009). Thus, we speculated that the SAR was induced only in few genotypes/cultivars or the low SAR efficiency was neglected.

The results showed that the levels of SA increased at 3 dpi in infested ‘Honggengansutao’ and ‘Xibei 13-1’. The increase in the levels of SA was manifested as SAR induced instead of ISR induced. Salicylic acid is not only a signal molecule involved in regulation of plant defense, but it also directly affects bacteria (Prithiviraj et al., 2005). The expression of virB1 gene, corresponding to the T-DNA transfer and integration, was inhibited for more than 50% by SA at concentration of 2 μM (≈276 ng·g⁻¹FW) (Yuan et al., 2007). The levels of SA in intact ‘Honggengansutao’ and ‘Xibei 13-1’ cultivars were 220 and 82 ng·g⁻¹FW, respectively, coinciding with their resistant and susceptible phenotypes. Although the content of SA was less than 2 μM, it perhaps contributed to the restriction of the A. tumefaciens infection.

In the present study, eight PpPR1 genes were upregulated in response to inoculation with A. tumefaciens. One of 22 PR1 genes identified in Arabidopsis was induced by pathogens (van Loon et al., 2006). Similarly, none of the three identified PR1 genes identified in apple were induced by infection with fire blight bacteria (Bonasera et al., 2006). By contrast, the 12 selected PR1 genes in rice were all upregulated in response to the infection by blast fungus (Mitsuhara et al., 2008). Thus, induction of PR1 genes in response to pathogens varied among plant species. Moreover, additional three PpPR1 genes were downregulated in resistance and susceptibility, exhibiting negative regulation to the bacterial infection. All of the 11 differentially expressed PpPR1 genes were resolved in different clades on the phylogenetic tree (Fig. 2). The expression of PpPR1 802 was upregulated in response to the inoculation with A. tumefaciens, and it differed from its nearest ortholog in apple (MdPR1a) (Bonasera et al., 2006). In contrast, its nearest paralog PpPR1 811 was down regulated, whereas PpPR1820 and its nearest paralog PpPR1 821 were both induced. These results indicated that, despite their sequence similarities, PR1 genes underwent evolutionary divergence as a consequence of functional selection and that several of the genes are associated with the host defense.

Many factors, such as wound and low temperature, have been described to induce the expression of PR1 genes as well as SAR (Mitsuhara et al., 2008; van Loon et al., 2006). The wound, which is essential for SAR expression, was excluded. Studies have shown that SAR was established 24–48 h after pathogen infection (Cameron et al., 1994; Ross, 1961). Once the SAR is activated, plants express a set of PR and other defense genes, and the PR proteins including PR1 and products of other defense genes such as camalexin (Mishina and Zeier, 2007) and coronatine (Spel and Dong, 2008) provide a multifaceted protection against successive invasions. In this study, the elevated levels of SA and upregulated expression of eight PpPR1 genes indicated that the SAR has been activated, but obvious morphological changes were apparent in few and small-size tumors in resistance and in large tumors in susceptible at the inoculation sites (Fig. 4) at 35 dpi. The opposing results originated from the unique pathology of A. tumefaciens invasion. After A. tumefaciens integrated successfully their T-DNA into the host genome, the transformed plant cells proliferated and were irrelevant to pathogens.

We confirmed that SAR was induced by the virulence of A. tumefaciens infection in two peach cultivars, but the underlying mechanism of SAR induction needs to be elucidated to facilitate the development of efficient methods for the control of crown gall disease. In addition, although researchers have made many efforts to select crown gall resistant Prunus resources, there are still a limited number of resistant genotypes/cultivars (Bliss et al., 1999; Pierronnet and Salessees, 1996; Zoina and Raio, 1999). Therefore, the inducible crown gall resistance should be considered in evaluation experiments.

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