A Conserved Basal Transcription Factor Is Required for the Function of Diverse TAL Effectors in Multiple Plant Hosts

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Many Xanthomonas bacteria use transcription activator-like effector (TALE) proteins to activate plant disease susceptibility (S) genes, and this activation contributes to disease. We recently reported that rice basal transcription factor II A gamma subunit, OsTFIIAγ5, is hijacked by TALE-carrying Xanthomonas oryzae infecting the plants. However, whether TFIIAγs are also involved in TALE-carrying Xanthomonas-caused diseases in other plants is unknown. Here, molecular and genetic approaches were used to investigate the role of TFIIAγs in other plants. We found that TFIIAγs are also used by TALE-carrying Xanthomonas to cause disease in other plants. The TALEs of Xanthomonas citri pv. citri (Xcc) causing canker in citrus and Xanthomonas campestris pv. vesicatoria (Xcv) causing bacterial spot in pepper and tomato interacted with corresponding host TFIIAγs as in rice. Transcriptionally suppressing TFIIAγ led to resistance to Xcc in citrus and Xcv in pepper and tomato. The 39th residue of OsTFIIAγ5 and citrus CsTFIIAγ is vital for TALE-dependent induction of plant S genes. As mutated OsTFIIAγ5V39E, CsTFIIAγV39E, pepper CaTFIIAγV39E, and tomato SlTFIIAγV39E also did not interact with TALEs to prevent disease. These results suggest that TALE-carrying bacteria share a common mechanism for infecting plants. Using TFIIAγV39E-type mutation could be a general strategy for improving resistance to TALE-carrying pathogens in crops.

Keywords: bacterial disease, basal transcription factor, transcription activator-like effector, crop, Xanthomonas

INTRODUCTION

Many Xanthomonas bacteria use transcription activator-like effector (TALE) proteins to induce transcription of host disease susceptibility (S) genes, which can cause devastating diseases in plants (Zhang et al., 2015). The TALEs are the largest family of type III effectors in Xanthomonas, a particularly widespread phytobacterial genus consisting of almost 30 species that cause disease in more than 400 plant species, including 11 monocotyledonous and 57 dicotyledonous families (Ryan et al., 2011). Each TALE typically has a modular architecture containing an N-terminal secretion signal that guides the protein’s translocation into host cells through a type III secretion system; a central repeat domain that binds to the effector binding element (EBE) of the host gene promoter via its repeat variable di-residues (RVDs); a newly identified transcription factor binding...
(TFB) motif that interacts with the host basal transcription factor IIA gamma subunit (TFIIAYγ); three nuclear localization signals that guide the protein's translation into the host nucleus; and a highly conserved C-terminal acidic activation domain that permits the TALE protein to activate transcription (Doyle et al., 2013; Zhang et al., 2015; Yuan et al., 2016).

**Xanthomonas oryzae** pv. *oryzae* (Xoo) and **Xanthomonas oryzae** pv. *oryzicola* (Xoc) cause bacterial blight and bacterial streak diseases in rice (*Oryza sativa*), respectively. A recent study revealed that the binding of Xoo and Xoc TALEs to rice EBEs via their RVDs alone cannot efficiently induce rice S gene expression to cause disease. The TFB motif of TALEs is vital for Xoo and Xoc to successfully invade plant cells (Yuan et al., 2016). Xoo and Xoc TALEs directly interact with rice TFIIAY (OsTFIIAY)5 via their TFB motif to activate the transcription of rice S genes and enable colonization. The attenuation or absence of this interaction could eliminate or weaken the activation of rice S gene expression (Yuan et al., 2016). For example, rice recessive disease resistance gene *xa5*, which encodes a mutated OsTFIIAY5 in which valine (V) is changed to glutamic acid (E) at the 39th amino acid residue (OsTFIIAY5V39E), confers broad-spectrum resistance to both Xoo and Xoc because most TALEs interact either weakly or not at all with OsTFIIAY5V39E. Partial suppression of OsTFIIAY5 expression also results in resistance to Xoo and Xoc (Yuan et al., 2016). Further, the rice carrying OsTFIIAY5V39E is indistinguishable from carrying OsTFIIAY5 in plant development and yield.

At least eight other **Xanthomonas** species [**Xanthomonas campestris** pv. *musacearum*, **Xanthomonas axonopodis** pv. *manihotis*, **Xanthomonas citri** subsp. *citri* (Xcc), **Xanthomonas campestris** pv. *malavacearum*, **Xanthomonas campestris** pv. *campestris*, **Xanthomonas campestris** pv. *vesicatoria* (Xcv), **Xanthomonas axonopodis** pv. *vasculorum*, and **Xanthomonas translucens**) also use TALEs to infect plants, including banana, cassava, citrus, cotton, brassicaceae, pepper, tomato, sugarcane, or cereal species, with severe adverse effects on crop yield and quality (Schornack et al., 2013; Boch et al., 2014). In addition to the **Xanthomonas** species, the plant bacterium **Ralstonia solanacearum**, the endosymbiont **Burkholderia rhizoxinica**, and unclassified marine microorganisms also carry TALEs that may also have a role in host–microbe interactions (de Lange et al., 2013, 2015; Juillerat et al., 2014).

TFIIA is a conserved transcription factor of eukaryotes, and it is involved in RNA polymerase II-dependent transcription. It contains two subunits, the large subunit TFIIAαβ and the small subunit TFIIAγ, encoded by separate genes (Orphanides et al., 1996). TFIIAγs from different species have high amino acid sequence similarity (Yuan et al., 2016). TFIIA interacts with other transcriptional regulatory proteins to form a transcription pre-initiation complex, which binds to the TATA-box of promoters and initiates gene expression (Hoiby et al., 2007). However, it is unknown whether other TALE-carrying bacteria also hijack their corresponding host TFIIAγ to cause plant disease.

To investigate whether host TFIIAγs are required for TALE-carrying bacteria to infect other crops, we analyzed the interactions between TFIIAγs of citrus, pepper and tomato and TALEs from Xcc, which causes canker disease in citrus, and Xcv, which causes bacterial leaf spot disease in pepper and tomato. We also analyzed the importance of citrus, pepper, and tomato TFIIAγs in diseases caused by Xcc and Xcv. The results suggest that Xcc and Xcv TALEs also interact with plant TFIIAγs via their TFB motifs. The 39th amino acid residue of TFIIAγs is essential for the interaction between TFIIAγs and TALE TFB motifs. Suppressing the expression of citrus TFIIAγ gene significantly improves resistance to Xcc, which is associated with compromised induction of host S gene. Suppressing the expression of pepper and tomato TFIIAγ genes also improves resistance to Xcv in these plants. Therefore, our results suggest that TALE-carrying bacteria use a common mechanism to hijack host basal transcription factor for successful infection.

**MATERIALS AND METHODS**

**Plant and Bacterial Materials**

Indica rice (*O. sativa ssp. indica*) IR24 and IRBB5 are near-isogenic lines. IR24 carries OsTFIIAY5 gene and is susceptible to Xoo and Xoc. IRBB5, with the genetic background of IR24, carries recessive disease resistance gene *xa5* encoding a mutated OsTFIIAY5 (OsTFIIAY5V39E) and is resistant to Xoo and Xoc. Plants were grown during a normal rice-growing season under natural field conditions. The wild *Oryza* species used in this study were from our collection (Yuan et al., 2014). Sweet orange (*Citrus sinensis* L. Osbeck cv. Newhall) plants were grown in a greenhouse at 25 to 30°C. Pepper (*Capsicum annuum* L. cv. Hua 50) and tomato (*Solanum lycopersicum* L. cv. Ailsa Craig) plants were germinated and grown in a growth chamber under a photosynthetic photon flux density of approximately 120 μmol photons m⁻² s⁻¹ with a 16-h light/8-h dark photoperiod cycle at 25°C and a relative humidity of approximately 60%.

The Philippine Xoo strains PXO99 and PXO341 have commonly been used in studies of rice resistance to bacterial blight disease (Li et al., 2012). The Xoc strain RH3 is also frequently used in research (Yuan et al., 2016). Xcc strain X02-007 (Fu et al., 2012) and Xcv strain 23-1 (Sun et al., 1999) were also previously used in research. All the Xanthomonas strains were grown at 28°C on nutrient agar medium. When genetic manipulation of bacteria was undertaken, antibiotics were used at the following final concentrations as required: ampicillin at 100 μg ml⁻¹, rifampicin at 75 μg ml⁻¹, and kanamycin at 25 μg ml⁻¹.

**Transformation**

For stable transformation of rice, Agrobacterium-mediated transformation was performed using calli derived from mature embryos of indica rice line IRBB5 according to previously reported methods (Ge et al., 2006). For construction of P<sub>Ubi</sub>OsTFIIAY5 derivative vectors, the 2055-bp promoter, 321-bp full-length cDNA and 1229-bp terminator of OsTFIIAY5 were amplified with primers listed in Supplementary Table 1 and inserted into vector pCAMBIA1301 in order. For construction of P<sub>Ubi</sub>OsTFIIAY5 derivative vectors, 321-bp full-length cDNA of OsTFIIAY5 and its derivatives were amplified with primers listed...
in Supplementary Table 1 and inserted into vector pU1301 (Yuan et al., 2010).

For transiently suppressing the target gene in sweet orange, Xcc-facilitated agroinfiltration was performed. In brief, a 162-bp cDNA fragment of CsTFIIAy was amplified with primers listed in Supplementary Table 1 and inserted into vector pDS1301, and the construct was then transformed into Agrobacterium tumefaciens strain GV3101 (Yuan et al., 2010). Sweet orange young leaves were inoculated with Xcc [5 × 10^8 colony-forming unit (cfu)/ml] by the penetration method using a needleless syringe. Eight hours later, the same inoculated leaf areas were subjected to agroinfiltration with Agrobacterium carrying the recombinant construct (Hu et al., 2014; Jia and Wang, 2014). The Xcc-facilitated agroinfiltration plants were grown in a greenhouse at 25 to 30°C with a 16-h light/8-h dark photoperiod cycle.

For transiently suppressing target gene in pepper and tomato, virus-induced gene silencing (VIGS) was performed. In brief, the DNA segment of the target gene or green fluorescence protein (GFP) gene was inserted into the tobacco rattle virus (TRV) vector pTRV2 as described previously (Liu et al., 2002a). The recombinant vectors were introduced into GV3101. Agrobacterium culture was infiltrated into the cotyledons of germinating pepper plants or the first two true leaves of the four-leaf stage tomato plants using a 1-ml needleless syringe (Liu et al., 2002b; Chung et al., 2004). The Agrobacterium-infiltrated plants were first kept in a growth chamber at 16°C for 1 day and then grown in a chamber at 25°C with a 16-h light/8-h dark photoperiod cycle.

### Protein–Protein Interaction

To study the interaction between TALE and host proteins in yeast two-hybrid assays (Yuan et al., 2010), the TFB motif of TALE genes and plant TFIIAy genes were amplified using PCR primers listed in Supplementary Table 1. The amplified bacterial DNA segments were ligated into pGBK7 vector, and the amplified plant DNA segments were ligated into pGADT7-Rec vector. The pGBK7 and pGADT7 plasmids were then transformed into yeast strain AH109 (Yuan et al., 2010). The yeast clones were scribed on the synthetic defined premixes (SD) medium lacking leucine (L) and tryptophan (W) (−LW) and selective SD medium lacking L, W, histidine (H), and adenine (A) (−LWHA). The interactions of examined proteins were assessed by growth of yeast cells on selective medium and by examination of β-galactosidase activity as described previously (Yuan et al., 2010).

To study the interaction between TALE and plant proteins in planta, co-immunoprecipitation (CoIP) assays were performed (Yuan et al., 2016). The DNA segments of TFB motifs of TALEs were ligated into the pU1031-9myc vector, and the DNA segments of plant genes were ligated into the pU1301-3FLAG vector (Yuan et al., 2010). The recombinant vectors were introduced into GV3101. Agrobacterium-mediated transformation was performed by infiltrating into Nicotiana benthamiana leaves using a needleless syringe. CoIP assays were conducted using anti-FLAG antibody (Sigma-Aldrich, St. Louis, MO, United States) and anti-myc antibody (Tiangen, Beijing, China) as described previously (Yuan et al., 2016). Each CoIP assay was repeated at least twice. The original western blotting images are provided in Supplementary Figure 13.

### Site-Directed Mutation

Mutation of plant genes was performed using the GeneTailor Site-Directed Mutagenesis System (Invitrogen Life Technologies, Carlsbad, CA, United States) as described previously (Yuan et al., 2011). The mutagenic primers are listed in Supplementary Table 1.

### Pathogen Inoculation

To evaluate rice bacterial blight disease, rice plants were inoculated with Xoo by the leaf-clipping method at the booting (panicle development) stage (Chen et al., 2002). Disease was scored by measuring the lesion length at 14 days after inoculation.

To evaluate rice bacterial streak disease, rice plants were inoculated with Xoc strains by the needle stab method at the tillering stage (Yuan et al., 2016). The disease was scored by measuring the lesion length at 14 days after inoculation.

To evaluate citrus bacterial canker disease, sweet orange leaves were inoculated with Xcc at a concentration of 5 × 10^8 cfu/ml in 10 mM MgCl_2 using a needleless syringe. The bacterial growth rate in sweet orange leaves was measured by counting the cfu as described previously (Hu et al., 2014).

To evaluate bacterial spot disease of pepper and tomato, 4-week-old VIGS-treated plants were sprayed with Xcv at a concentration of 5 × 10^5 cfu/ml in 10 mM MgCl_2. The bacterial growth rate in pepper or tomato leaves was measured by counting the cfu as described previously (Bonas et al., 1991).

### Gene Expression Analysis

For gene expression analysis, 2-cm rice leaf fragments near the bacterial infection sites or citrus, pepper and tomato leaf tissues next to the infiltration sites were collected for RNA isolation. Quantitative reverse transcription-PCR (qRT-PCR) was conducted using gene-specific primers (Supplementary Table 2) as described previously (Yuan et al., 2010). The expression level of rice, pepper or tomato actin gene or citrus EF1a gene was used to standardize the RNA sample of rice, pepper, tomato or citrus, respectively. The expression level relative to that of controls was assessed. Each qRT-PCR assay was repeated at least twice with similar result, with each repetition having three replicates.

### Statistical Analysis

Differences between samples were analyzed for statistical significance by using the SPSS software and the Student’s t-test (two tailed).

### RESULTS

#### Xcc Uses Host TFIIAy to Cause Disease in Sweet Orange

Xcc causes citrus canker. Xcc strain X02-007 carries TALE genes, which contained TFB motifs of TALEs that showed...
high sequence similarity with the motifs of Xoo and Xcc TALEs (Supplementary Figure 1; Yuan et al., 2016). The TFB motifs of Xcc TALEs interacted with sweet orange TFIIAγ (CsTFIIAγ) both in yeast and in planta (Figures 1A,B). To investigate whether CsTFIIAγ is required for TALE-dependent induction of the host S gene during Xcc invasion, we suppressed CsTFIIAγ gene expression in the leaves of sweet orange by RNA interference (RNAi). The plants with suppressed expression of CsTFIIAγ appeared markedly less diseased, with less cell division and proliferation or hyperplasia after Xcc inoculation (Figures 1C–E) and significantly lower (P < 0.01) Xcc growth rate compared to control plants (Figure 1F). Citrus CsLOB1 is a S gene to Xcc (Hu et al., 2014; Li et al., 2014). Consistent with reduced disease symptoms, the CsTFIIAγ-RNAi leaves showed significantly suppressed Xcc-induced expression of CsLOB1 compared with control (Figure 1G).

Further, different citrus species, such as hongkong kumquat, grapefruit and trifoliate orange, have a TFIIAγ protein sequence identical to CsTFIIAγ in sweet orange, although the gene sequences differ for a few nucleotides (Supplementary Figure 2A). All the citrus species were similarly susceptible to Xcc (Supplementary Figure 2B). These results suggest that, like OsTFIIAγ5, CsTFIIAγ is also hijacked by TALE-carrying bacteria, with the host becoming infected via TALE-induced expression of the host S gene.

The 39th Amino Acid Residue of Plant TFIIAγs Is Essential for the Virulence of TALE-Carrying Bacteria

Rice plants carrying recessive xa5, which encodes the mutated OsTFIIAγV39E, have broad-spectrum resistance to TALE-carrying bacteria and no effect on rice yield and development (Yuan et al., 2016). To analyze whether OsTFIIAγV39E is the only type of mutation that confers disease resistance in rice and whether mutations similar to OsTFIIAγV39E can confer disease resistance in other plant species, we first examined the sequences of OsTFIIAγ5 coding region in different Oryza species.

The Oryza genus consists of two cultivated rice species, Asian cultivated rice (O. sativa, AA genome) and African cultivated rice (O. glaberrima, AA genome), and wild (undomesticated) rice species (AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, KKLL genomes) (Ge et al., 1999; Ammiraju et al., 2010). We sequenced the OsTFIIAγ5 locus in 15 different Oryza genomes, including nine accessions of AA genome (O. sativa indica, O. sativa japonica, O. rufipogon, O. nivara, O. glaberrima, O. longistaminata, O. meridionalis, O. glaucapatula, and O. barthii), one accession of BB genome (O. punctata), two accessions of CC genome (O. officinalis and O. rhizomatis), one accession of EE genome (O. australiensis), one accession of FF genome (O. brachyantha), and one accession of GG genome (O. meyeriana). Sequence alignment analysis showed
that different Oryza species contained the same sequences of the OsTFIIAγ5 coding region and encoded the identical OsTFIIAγ5 protein, which is consistent with its molecular function as a basal transcription factor.

We searched the putative OsTFIIAγ5 variant from database OryzaGenome1, which contains genotype information of 463 accessions of wild rice O. rufipogon, which is evolutionally closely related to cultivated rice (Ohyanagi et al., 2016). Thirty-four single nucleotide polymorphisms (SNPs) were distributed in the OsTFIIAγ5 gene region, all within the introns and none in the exons. Thus the 463 O. rufipogon accessions encode the identical OsTFIIAγ5 protein. Moreover, we also searched the putative OsTFIIAγ5 variants from Rice SNP-Seek Database2, which contain genotype information of 3024 O. sativa varieties from the International Rice GenBank Collection Information System (IRGCIS3). There were 93 SNPs distributed in the 6260-bp region of OsTFIIAγ5 gene, and 105 rice varieties contained SNPs in the coding region causing amino acid mutation. Of these 105 rice varieties, 104 have a mutated OsTFIIAγ5 gene encoding OsTFIIAγ5V39E, identical to the protein encoded by recessive disease resistance gene xa5. One variety has a different type of mutated OsTFIIAγ5 gene, which encodes a protein with valine changed to aspartic acid (D) at the 39th residue (OsTFIIAγ5V39D) (Supplementary Table 3). Eighty-five of the 104 rice varieties carrying the mutated OsTFIIAγ5V39E gene and one carrying the mutated OsTFIIAγ5V39D gene belong to the Aus group, which is mainly from South Asia, and the other 19 carrying the mutated OsTFIIAγ5V39E gene belong to Indica II, Indica IB, Indx and Admix groups, mainly from Southeast Asia (Huang et al., 2011). The distribution of OsTFIIAγ5V39E resistance allele in these groups likely reflects the high disease pressure in these regions.

1http://viewer.shigen.info/oryzagenome/mapview/Top.do
2http://snp-see.irci.org/
3http://irgcis.irci.org:81/grc/irgcishome.html
where Xoo is a devastating disease. The results suggest that the OsTFIIAγ5 gene mutation probably appeared in cultivated rice.

Amino acid sequence alignment of TFIIAγ5s from different species showed that they share a high sequence similarity, with the 39th amino acid residue having three types, V, leucine (L), and E (Yuan et al., 2016). The V type occurs in plants (O. sativa, Arabidopsis thaliana, Citrus sinensis, Capsicum annuum, and Solanum lycopersicum). The L type occurs in animals (Rattus norvegicus, Homo sapiens, Danio rerio, and Drosophila melanogaster). The E type was only detected in rice OsTFIIAγ5 locus (OsTFIIAγ5V39E), which is recessively inherited. In addition, we identified a D type in OsTFIIAγ5 locus (OsTFIIAγ5V39D) from rice germplasm (Supplementary Table 3). To determine whether the 39th amino acid of OsTFIIAγ5 is vital for TALE-dependent induction of rice genes, we first produced four OsTFIIAγ5 derivatives at the 39th residue, substituting V with alanine (A), D, L or glutamine (Q); V, A and L are hydrophobic amino acids, and D and Q are acidic hydrophilic amino acids. The TFB motifs of TALE PhhXo1 from Xoo and TALE Tal3c from Xoc interacted as strongly with mutants OsTFIIAγ5V39A, OsTFIIAγ5V39D, OsTFIIAγ5V39L, and OsTFIIAγ5V39Q as with wild-type OsTFIIAγ5 in yeast cells (Supplementary Figure 3). The PhhXo1 TFB interacted weakly and Tal3c TFB did not interact with OsTFIIAγ5V39E (E being an acidic hydrophilic amino acid) as reported previously (Yuan et al., 2016).

We then generated OsTFIIAγ5 transgenic plants with the four different amino acid substitutions (A, D, L, and Q) at the 39th residue driven by the native promoter of OsTFIIAγ5 (P Osborne5γ5) in the xas5 (OsTFIIAγ5V39E) resistance allele background (IRBB5). The P Osborne5γ5:OsTFIIAγ5V39A, P Osborne5γ5:OsTFIIAγ5V39L, P Osborne5γ5:OsTFIIAγ5V39D, and P Osborne5γ5:OsTFIIAγ5V39Q plants showed susceptibility to Xoo strains PXO99 and PXO341, also susceptibility to Xoc strain RH3 compared to the wild-type plant (Figure 2A). The susceptibility of these transgenic plants to Xoo was associated with more efficiently induced expression of known rice S genes (Xa13/Os8N3/SWEET11, OsTFIIAγ1, and OsTFX1) to Xoo (Figure 2B; Chu et al., 2006; Sugio et al., 2007). The susceptibility of these transgenic plants to Xoc was associated with more efficiently induced expression of the known rice S gene (OsSULTR3;6) to Xoc (Figure 2B; Cernadas et al., 2014). We also generated OsTFIIAγ5 overexpressing plants with the same mutations listed above driven by maize ubiquitin promoter (P Ubi ). These transgenic plants also showed increased susceptibility to Xoo and Xoc, which was associated with increased induced expression of rice susceptibility genes, compared to wild-type plants (Supplementary Figures 4, 5). These results suggest that the 39th amino acid residue of OsTFIIAγ5 is essential for TALE-dependent induction of rice S genes, and it seems that only the V to E mutation at this residue (OsTFIIAγ5V39E ) affects the interaction between OsTFIIAγ5 and TFB of TALEs.
Furthermore, we overexpressed CsTFIIAγ in rice IRBB5 carrying the recessive xa5 gene. The transgenic plants displayed susceptibility to Xoo strains PXO99 and PXO341 (Figure 4A). The susceptibility was associated with overexpression of CsTFIIAγ, which was further confirmed in two independent T1 families (Supplementary Figure 9). The induced expression of known S genes Xa13/Os8N3/SWEET11, OsTFIIAγ1, and OsTFX1, each of which is targeted by a different TALE, was significantly higher (P < 0.01) in transgenic plants than in wild-type plants (Figure 4B). Similarly, overexpressing CsTFIIAγ in rice increased susceptibility to Xoc, which was associated with significantly promoted Xoc-induced expression of rice S gene OsSULTR3;6 compared to wild-type plants (Supplementary Figure 10). However, the transgenic plants with overexpression of CsTFIIAγV39E showed similar lesion length compared to wild-type plants upon inoculation with Xoo strain PXO99 (Supplementary Figure 11). These results suggest that CsTFIIAγ can also facilitate the infection of Xoo and Xoc in rice by helping TALE-induced activation of rice S genes, and the 39th amino acid residue of CsTFIIAγ is also essential for TALE-dependent induction of host S genes.

**Xcv Also Uses Host TFIIAγ to Cause Disease in Pepper and Tomato**

We also examined the relationship between pepper (Capsicum annuum L. cv. Hua 50) CaTFIIAγ or tomato (Solanum lycopersicum L. cv. Ailsa Craig) SITFIIAγ and Xcv, which causes bacterial spot disease in the two crops. CaTFIIAγ and SITFIIAγ have identical sequences, and Xcv strain 23-1 carries TALE genes (Yuan et al., 2016). The TFB motifs of Xcv 23-1 TALEs, which featured high sequence similarity with the TFB motifs of Xoo, Xoc, and Xcc TALEs (Supplementary Figure 1), interacted with CaTFIIAγ/SITFIIAγ both in yeast and in planta (Figures 5A,B), but they did not interact with Ca/SITFIIAγV39E (Supplementary Figures 8B, 12). Transiently suppressing CaTFIIAγ or SITFIIAγ by VIGS enhanced the resistance of pepper and tomato to Xcv. The pepper plants with suppressed expression of CaTFIIAγ (TRV2:CaTFIIAγ) and tomato plants with suppressed expression of SITFIIAγ (TRV2:SITFIIAγ) showed markedly reduced disease symptoms and significantly lower (P < 0.01) Xcv growth rates compared to corresponding control plants expressing TRV2:GFP (Figures 5C,D). These results suggest that TALE-containing Xcv causes diseases in pepper and tomato and may also need the help of host TFIIAγs.

**DISCUSSION**

In addition to TALE-carrying Xoo and Xoc needing the help of host TFIIAγ (OsTFIIAγS) to cause disease in rice (Yuan et al., 2016), the TFB motifs of CsTFIIAγ (Supplementary Figures 7A, 8A), although these TFBs interacted strongly with both OsTFIIAγ5 and CsTFIIAγ5 in yeast cells and in planta (Figures 1, 3 and Supplementary Figure 7B). The only exception was the TFB motif of Xoo Tal7a. It interacted with CsTFIIAγV39E (Supplementary Figure 7A), which is consistent with this TFB also interacting with OsTFIIAγ5V39E (Yuan et al., 2016).

The 39th amino acid residue of citrus CsTFIIAγ is also V, and CsTFIIAγ and OsTFIIAγ5 share 93/89% sequence similarity/identity (Supplementary Figure 6; Yuan et al., 2016). Furthermore, the TFB motifs of Xcc TALEs have high sequence homology with TFBs of Xoo and Xoc TALEs (Supplementary Figure 1). To investigate whether the OsTFIIAγ5V39E type mutation in CsTFIIAγ (CsTFIIAγV39E) could prevent TALE-carrying bacteria from infecting plants, we first examined the interactions between the TALE TFB motifs of different Xanthomonas bacteria and CsTFIIAγV39E. The TFB motifs of Xoo, Xoc, and Xcc TALEs could not interact with CsTFIIAγV39E (Supplementary Figures 7A, 8A), although these TFBs interacted strongly with both OsTFIIAγ5 and CsTFIIAγ5 in yeast cells and in planta (Figures 1, 3 and Supplementary Figure 7B). The only exception was the TFB motif of Xoo Tal7a. It interacted with CsTFIIAγV39E (Supplementary Figure 7A), which is consistent with this TFB also interacting with OsTFIIAγ5V39E (Yuan et al., 2016).

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et al., 2016), the present results show that TALE-carrying Xcc also requires host TFIIAγ (CsTFIIAγ) for successful colonization in sweet orange. Transcriptional suppression of CsTFIIAγ can reduce disease symptoms. Xoo and Xoc employ OsTFIIAγ5 to facilitate TALE-induced expression of host S genes for infection (Yuan et al., 2016). Efficient binding of the TFB motifs of Xoo and Xoc TALEs to OsTFIIAγ5 is important for the induction of S genes. The present results suggest that Xcc likely uses the same mechanism as Xoo and Xoc to infect its host. This inference is supported by the following evidence. First, the TFBs of Xcc TALEs also interacted with CsTFIIAγ. Second, transcriptional suppression of CsTFIIAγ resulted in reduced disease of sweet orange and was associated with compromised induction of S gene CsLOB1. Last, because of the high amino acid homology among TALE TFBs of different Xanthomonas species, such as Xoo, Xoc, Xcc, and Xcv (Supplementary Figure 1), and among different TFIIAγs of different eukaryote species, such as OsTFIIAγ5, CsTFIIAγ, GaTFIIAγ, and SITFIIAγ (Yuan et al., 2016), CsTFIIAγ could be used by Xoo and Xoc to cause disease in rice, which was associated with induced expression of rice S genes. Although high amino acid sequence similarity was present among TALE TFBs of different Xanthomonas species, there was still some dissimilarity in the motif (Supplementary Figure 1; Yuan et al., 2016). Protein residues diversity may influence
the interaction strength with plant TFIIAγ, which might be supported by the relatively weak band in some of the CoIP assays.

The present results also show that Xcv TALEs can bind to pepper CaTFIIAγ and tomato SITFIIAγ through their TFBSs. Suppression of CaTFIIAγ and SITFIIAγ reduces Xcv-caused disease symptoms. Although the present study does not identify which host gene is targeted by the TALEs of Xcv strain 23-1, the results suggest that CaTFIIAγ and SITFIIAγ may also be hijacked by Xcv for infection. This inference is also supported by the localization characteristic of the TALE binding sites (EBEs) of host gene promoters in different plants. We analyzed 45 EBEs of known S genes and executor resistance genes that can be directly targeted by Xoo, Xoc, Xcv, Xcc, Xam, or Xg TALEs in rice, pepper, citrus, cassava, or tomato (Supplementary Table 4). These EBEs either overlapped their corresponding TATA-box or were located close to it, and some TATA-boxes are the core part of EBEs, which is consistent with computational model-based predictions that target sites of TALEs are preferentially located at most 300 bp upstream and 200 bp downstream of the transcription start site of target genes (Grau et al., 2013). TFIIAγ is a component of a transcription pre-initiation complex that binds to the TATA-box of promoters to initiate gene expression in eukaryotes (Høiby et al., 2007). The overlap or adjacent physical position of TALE-binding sites in relation to the positions of TATA-box brings the possibility that Xcv TALEs also use CaTFIIAγ and SITFIIAγ for infection in pepper and tomato. Furthermore, the localization relationship between EBE and TATA-box also suggests that other TALE-carrying bacteria, in addition to Xoo, Xoc, Xcc, and Xcv, may also require the help of host TFIIAγ for infection.

Our previous and present results indicate that the difference in interaction strength between different TFBS motifs from diverse Xanthomonas TALEs and corresponding host plant TFIIAγ with V39E mutation could be correlated with specific differences in the amino acids of the TFBS motifs (Supplementary Figures 1, 3, 7, 12). The present results undoubtedly suggest that the 39th amino acid residue of OsTFIIAγ5 is important for TALE-dependent induction of host genes. The OsTFIIAγ5V39E encoded by recessive disease resistance gene xa5 is the only type of mutation identified so far that can prevent or attenuate the infection of Xoo and Xoc in rice (Supplementary Table 3; Yuan et al., 2016). The V39E substitution of CsTFIIAγ (CsTFIIAγV39E) also prevented its interaction with TFBSs of Xcc TALEs and abolished or compromised CsTFIIAγ-facilitated infection of Xoo and Xoc in rice, suggesting that the CsTFIIAγV39E mutation may make citrus resistance to Xcc. In addition, the TFBSs of Xcv TALEs cannot interact with CsTFIIAγV39E and SITFIIAγV39E, suggesting that the TFIIAγV39E-type mutation may also be used to prevent Xcv-caused diseases in pepper and tomato. Moreover, TFBSs with mutation in the 134 amino acids motif might be found with more Xcc or Xcv strains been sequenced, which can break CsTFIIAγV39E, CaTFIIAγV39E, and SITFIIAγV39E mediated resistance to Xcc or Xcv. An example is that the TFBSs of pthXo1, tal7a, and tal8a of Xoo pv. PXO99 differed by 1 to 20 residues from the other 12 TFBSs could interact with OsTFIIAγV39E, and the rice variety IRBB5 carrying OsTFIIAγV39E is susceptible to PXO99 (Yuan et al., 2016).

In summary, the previous report (Yuan et al., 2016) and the present results suggest that TALE-carrying bacteria probably use a common mechanism that is employing host TFIIAγs to cause disease in rice, citrus, pepper, and tomato. The genus Xanthomonas infects a wide variety of hosts. Because of the high amino acid sequence homology among TFIIAγs from different plant species and among the TFBS motifs of TALEs from different Xanthomonas species, other TALE-carrying Xanthomonas species, which have not been examined, likely also hijack host TFIIAγs for successful infection. Since rice plant OsTFIIAγ5V39E confers broad-spectrum resistance to Xoo and Xoc without affecting rice development and yield (Yuan et al., 2016) and the present results suggest that CsTFIIAγV39E may also compromise bacterial infection, use of TFIIAγV39E-type mutation—either by exploring germplasm for natural mutants or using gene-editing technologies—may provide a general strategy for improving resistance to Xcv and other TALE-carrying pathogens in crops in addition to rice and citrus.

ADDITIONAL INFORMATION

Gene sequences of CsTFIIAγ from different Citrus species have been deposited in GenBank with the following accession codes KU377725 (in Citrus sinensis Hongkong Kumquat), KU377726 (in Citrus sinensis Sweet orange), KU377727 (in Citrus sinensis Grapefruit), and KU377728 (in Citrus sinensis Trifoliate orange).

AUTHOR CONTRIBUTIONS

MY, RH, and SH performed most of the experiments and analyzed the data; MZ and PL helped to generate transgenic rice plants, analyze protein–protein interactions, and amplify TALE; JX and XL provided biochemical and molecular analysis support and management; MY and SW designed the research, supervised the project, interpreted data, and drafted and revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2017.01919/full#supplementary-material
REFERENCES

Ammiraju, J. S., Fan, C., Yu, Y., Song, X., Cranston, K. A., Pontaroli, A. C., et al. (2010). Spatio-temporal patterns of genome evolution in allotetraploid species of the genus Oryza. Plant J. 63, 430–442. doi: 10.1111/j.1365-313X.2010.04251.x

Boch, J., Bonas, U., and Labaye, T. (2014). TAL effectors-pathogen strategies and plant resistance engineering. New Physiol. 204, 823–832. doi: 10.1111/nph.13015

Bonas, U., Schulte, R., Fenselau, S., Minsavage, G., Staskawicz, B., and Stall, R. (1991). Isolation of a gene-cluster from Xanthomonas campestris pv. vesicatoria that determines pathogenicity and the hypersensitive response on pepper and tomato. Mol. Plant Microbe Interact. 4, 81–88. doi: 10.1094/MPMI-4-081

Cernadas, R. A., Doyle, E. L., Nino-Lui, D. O., Wilkins, K. E., Bancroft, T., Wang, L., et al. (2014). Code-assisted discovery of TAL effector targets in bacterial leaf streak of rice reveals contrast with bacterial blight and a novel susceptibility gene. PLOS Pathog. 10, 10003972. doi: 10.1371/journal.ppat.10003972

Chen, H., Wang, S., and Zhang, Q. (2002). New gene for bacterial blight resistance in rice located on chromosome 12 identified from minghui 63, an elite restorer line. Phytopathology 92, 750–754. doi: 10.1094/PHYTO.2002.92.7.750

Chu, Z., Yuan, M., Yao, J., Ge, X., Yuan, B., Xu, C., et al. (2006). Promoter mutations for rapid functional analysis of transgenes in citrus leaves. Mol. Cells 21, 390–398. doi: 10.1016/j.molcel.2005.11.002

de Lange, O., Wolf, C., Thiel, P., Krüger, J., Kleusch, C., Kohlbacher, O., et al. (2015). DNA-binding proteins from marine bacteria expand the known sequence diversity of TALE-like repeats. Nucleic Acids Res. 43, 10065–10080. doi: 10.1093/nar/gkv1053

Doyle, E. L., Stoddard, B. L., Voytas, D. F., and Bogdanove, A. J. (2013). TAL effectors: highly adaptable phytobacterial virulence factors and readily sequence diversity of TALE-like repeats. Trends Cell Biol. 23, 390–398. doi: 10.1016/j.tcb.2013.04.003

Fu, X., Gong, X., Zhang, Y., Wang, Y., and Liu, J. (2012). Different transcriptional response to Xanthomonas citri subsp. citri between kumquat and sweet orange with contrasting canker tolerance. PLOS ONE 7:e41790. doi: 10.1371/journal.pone.0041790

Ge, S., Sang, T., Lu, B. R., and Hong, D. Y. (1999). Phylogeny of rice genomes with emphasis on origins of allotetraploid species. Proc. Natl. Acad. Sci. U.S.A. 96, 14400–14405. doi: 10.1073/pnas.96.25.14400

Ge, X., Chu, Z., Lin, Y., and Wang, S. (2006). A tissue culture system for different Xanthomonas oryzae pv. oryzae of citrus is targeted by a major virulence effector PthA of Xanthomonas citri subsp. citri. Mol. Plant-Microbe Interact. 20, 1250–1255. doi: 10.1099/mpmi.0.101927-0

Jia, H., and Wang, N. (2014). Xcc-facilitated agroinfiltration of citrus leaves: a tool for rapid functional analysis of transgenes in citrus leaves. Plant Cell Rep. 33, 1993–2001. doi: 10.1007/s00299-014-1673-9

Juillerat, A., Bertonati, C., Dubois, G., Guyot, V., Thomas, S., Valton, J., et al. (2014). BurrH: a new modular DNA binding protein for genome engineering. Sci. Rep. 4:3831. doi: 10.1038/srep03831

Li, H., Li, X., Xiao, J., Wang, R. A., and Wang, S. (2012). Ortholog alleles at Xa3/Xa26 locus confer conserved race-specific resistance against Xanthomonas oryzae in rice. Mol. Plant 5, 281–290. doi: 10.1093/mp/sts079

Li, Z., Zou, L., Ye, G., Xiong, L., Li, Z., Zakria, M., et al. (2014). A potential disease susceptibility gene CsLOB of citrus is targeted by a major virulence effector PthA of Xanthomonas citri subsp. citri. Mol. Plant 7, 912–915. doi: 10.1093/mp/sts176

Liu, Y., Schill, M., and Dinesh-Kumar, S. (2002a). Virus-induced gene silencing in tomato. Plant J. 31, 777–786. doi: 10.1046/j.1365-313X.2002.01394.x

Liu, Y., Schill, M., Marathe, R., and Dinesh-Kumar, S. (2002b). Tobacco Rar1, EDS1 and NPR1/NIWI like genes are required for N-mediated resistance to tobacco mosaic virus. Plant J. 30, 415–429. doi: 10.1046/j.1365-313X.2002.01297.x

Ohyanagi, H., Ebata, T., Huang, X., Gong, H., Fujita, M., Mochizuki, T., et al. (2016). OsRgaGenome: genome diversity database of wild Oryza species. Plant Cell Physiol. 57, e1. doi: 10.1093/pcp/pcv171

Orphanides, G., Largrange, T., and Reinberg, D. (1996). The general transcription factor TFIIA. Biochim. Biophys. Acta 1297, 1–41. doi: 10.1016/0005-2736(96)02510-6

Su, G., Yang, B., Zhu, T., and White, F. (2007). Two type III effector genes of Xanthomonas oryzae pv. oryzae control the induction of the host genes OsTFIIA1 and OsTFXI during bacterial blight of rice. Proc. Natl. Acad. Sci. U.S.A. 104, 10720–10725. doi: 10.1073/pnas.0701742104

Sun, F., Dz, Z., Zhao, T., and Cheng, B. (1999). Pathogen and race identification of bacterial spot of pepper and tomato. Acta Physiophytochem. Sin. 29, 265–269. doi: 10.13926/j.cnki.aps.1999.03.014

Yuan, M., Chu, Z., Li, X., Xu, C., and Wang, S. (2010). The bacterial pathogen Xanthomonas oryzae overcomes rice defenses by regulating host copper redistribution. Plant Cell 22, 3164–3176. doi: 10.1105/tpc.110.078022

Yuan, M., Ke, Y., Huang, R., Ma, L., Yang, Z., Chu, Z., et al. (2016). A host basal transcription factor is a key component for infection of rice by TALE-carrying bacteria. eLife 5:e19605. doi: 10.7554/eLife.19605

Yuan, M., Zhao, J., Huang, R., Li, Xiao, J., and Wang, S. (2014). Rice MiN3/saliva/SWEET gene family: evolution, expression profiling, and sugar transport. J. Integr. Plant Biol. 56, 539–570. doi: 10.1111/jipb.12173

Yuan, T., Li, X., Xiao, J., and Wang, S. (2011). Characterization of Xanthomonas oryzae-responsive cis-acting element in the promoter of rice race-specific susceptibility gene Xa13. Mol. Plant 4, 300–309. doi: 10.1093/mp/ssq076

Zhang, J., Yin, Z., and White, F. (2015). TAL effectors and the executor R genes. Front. Plant Sci. 6:641. doi: 10.3389/fpls.2015.00641

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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