
Figures and figure supplements

Xanthomonas citri subsp. *citri* type III effector PthA4 directs the dynamical expression of a putative citrus carbohydrate-binding protein gene for canker formation

Xinyu Chen et al.

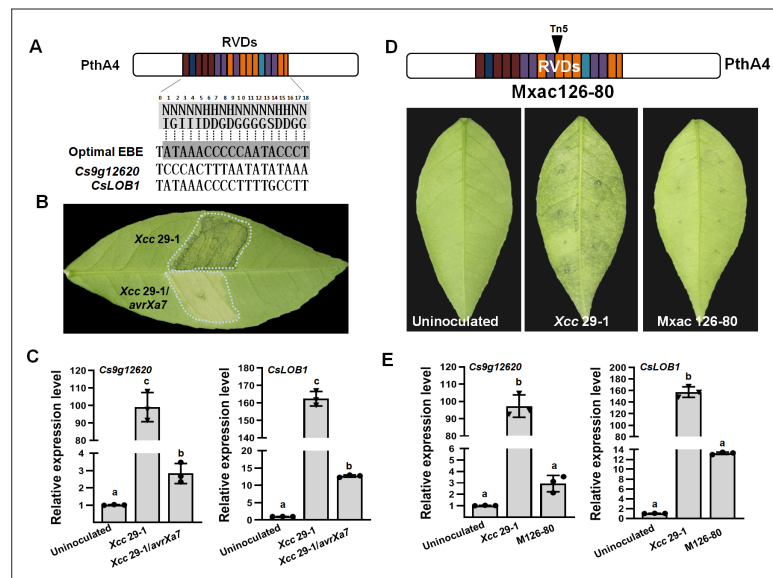


Figure 1. The expression of *Cs9g12620* depends on PthA4 during *Xcc* infection. **(A)** Predicted PthA4-binding elements of the *Citrus sinensis* genes upregulated in response to *Xcc* infection. The optimal effector-binding element (EBE) that corresponded to the PthA4 repeat variable di-residues (RVDs) were predicted based on the transcriptional activator-like (TAL) effector–DNA-binding code. Putative PthA4 EBEs in promoters of *Cs9g12620* and *CsLOB1* are shown below the optimal EBE of PthA4. **(B)** The disease symptoms of *Xcc* 29-1/*avrXa7* on *C. sinensis* leaves. The cell suspension (10^8 CFU/ml) was infiltrated into plant leaves, and the phenotype was recorded at 5 dpi. All the experiments were repeated three times with similar results. **(C)** A qRT-PCR analysis of the expression of *Cs9g12620* and *CsLOB1* in plants inoculated with *Xcc* that expressed *avrXa7*. The level of expression of each gene in the uninoculated samples was set as 1, and the levels in other samples were calculated relative to those baseline values. Values are the mean results from three biological replicates and are the means \pm SD (ANOVA, $p < 0.01$). The experiment was repeated three times. **(D)** The phenotype elicited by the *pthA4* mutant *Mxac126-80*. The analyses were performed as described in **B**. **(E)** qRT-PCR analysis of the transcript levels of *Cs9g12620* and *CsLOB1* plants inoculated with *Xcc* 29-1 and *Mxac126-80*. The analyses were the same as those conducted in **C**. ANOVA, analysis of variance; dpi, days post-inoculation; qRT-PCR, real-time quantitative reverse transcription PCR; SD, standard deviation; *Xcc*, *Xanthomonas citri* subsp. *citri*.

```
AAGTCAACATTAGCACGAACCTTGTACTTATTGGATTTCATAATGAG
AATGAATGATTAGTACTAACATGTCCGTACAAGTATGATTCATATG
TAACAACAGAGTCTTGATTAAGTTTTGTACTTAGAAATATTGTAGA
ACTAAGACTATAGGAGCAAATCACGGCAACCAATGAAGCTTCAT
TTGGGGGCTGCTCCAACACTTGAATCAAGACTTTTGAGTGGAAA
AACAAGAATTAATTGAAATTATGGGAAATGGGGAGACCAAGTGG
CACATCTCACGGCTAATTTGGTGGCCCTATAACGTGGTTCCTTA
TCTTCTTTCTTCCTTTCTTTTTGTCTCCCACTTTAATATATAAAAAA
                                     PthA4 EBE
ATCATAACACGTACATGCTCCCGTTGAGCGTTTTTCATTCTATAAAT
ACTGTATTCATCCTAGTTGCTTCATCAATATAGAAAAGATGCCTT
GGTTTCTCTCTCTT
```



Figure 1—figure supplement 1. The location of the PthA4 effector-binding element (EBE) in the promoter of *Cs9g12620* in *Citrus sinensis*. The promoter sequences were retrieved from the citrus genome database. The location of binding site of PthA4 EBE is indicated by an underline. Translation initiation site is indicated by an arrow.

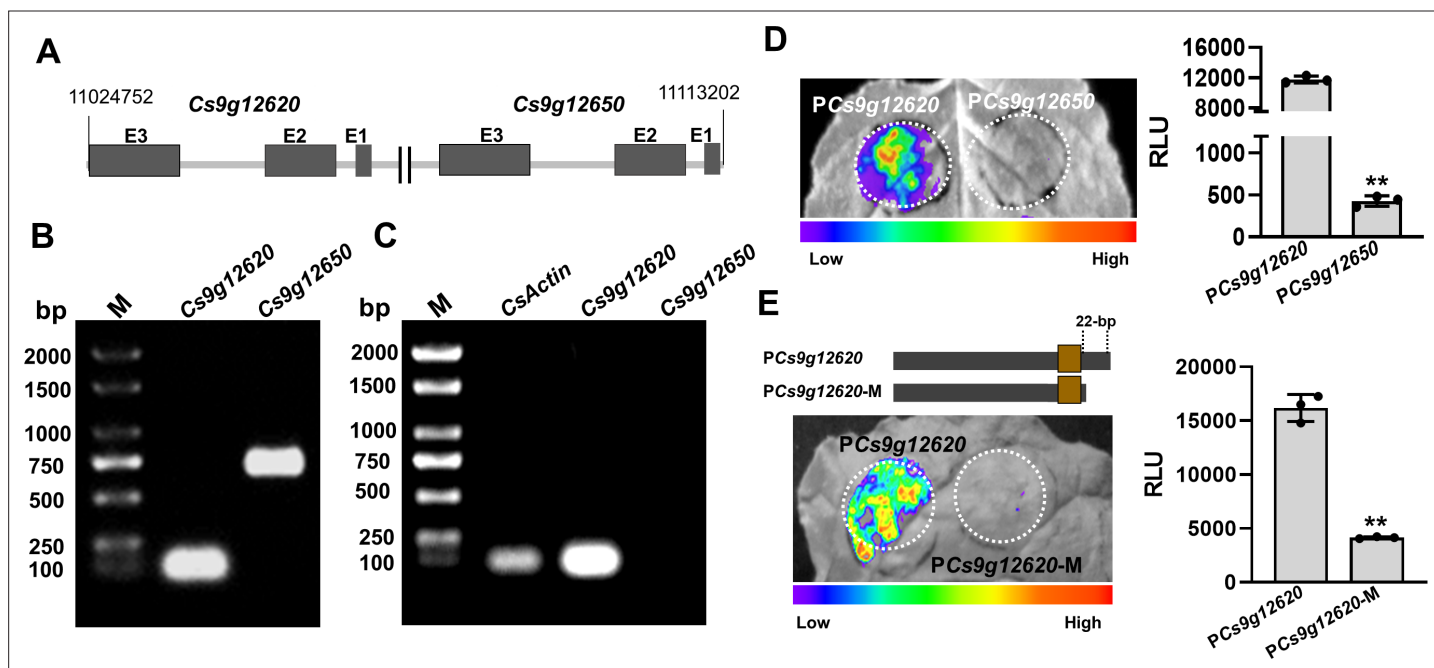


Figure 2. *Cs9g12620* and *Cs9g12650* show different profiles of expression owing to the genetic variation in promoter. **(A)** The location of *Cs9g12620* and *Cs9g12650* in the *Citrus sinensis* chromosome. The location was revealed by a BLAST search of the Citrus Genome Database (<https://www.citrusgenomedb.org/>). **(B)** Verification of the existence of *Cs9g12620* and *Cs9g12650* in the *C. sinensis* genome by PCR amplification. The specific primers for either gene were used for qRT-PCR analysis, which are shown in **Supplementary file 1b**. M, DL2000 DNA marker. **(C)** Semi-quantitative RT-PCR detection of the transcription of *Cs9g12620* and *Cs9g12650*. *CsActin* was used as the internal control gene. A total of 2 μ g of total RNA extracted from *C. sinensis* was used to synthesize the first single-stranded cDNA. The specific primers for each gene were the same as those of the qRT-PCR analysis and are shown in **Supplementary file 1b**. M, DL2000 DNA marker. **(D)** Luciferase assays of *Cs9g12620* and *Cs9g12650* promoter activity. The *Cs9g12620* and *Cs9g12650* promoter luciferase fusions were transiently expressed in the *Nicotiana benthamiana* leaves. Luciferase activity was measured with a CCD imaging system at 2 days post-agroinfiltration. The image on the right shows the quantification of luciferase signal using a microplate luminescence reader. Values are the means \pm SD ($n = 3$ biological replicates; Student's t -test, ** $p < 0.01$). **(E)** Luciferase assays of *Cs9g12620* and *Cs9g12620-M* with the deletion of 22 bp at the 3'-terminus. The *Cs9g12620* and *Cs9g12620-M* promoter luciferase fusions were transiently expressed in the *N. benthamiana* leaves. Luciferase activity was measured with a CCD imaging system at 2 days post-agroinfiltration. The luciferase signal was quantified as described in **D**. CCD, charge-coupling device; qRT-PCR, real-time quantitative reverse transcription PCR; RT-PCR, reverse transcription PCR; SD, standard deviation.

Cs9g12620	AAGTCAACAT	TAGCACGAAC	TTGTACTTAT	TGGATTCATA	ATGAGAATGA
Cs9g12650	GATTTATTAT	GAGAACGA--	TTAAATATAT	TAT-CTGTTA	ATGTGAAAGA
Cs9g12620	ATGATTAGTA	CTAACATGTC	CGTACAAGTA	TGATTCA--T	ATGTAACA--
Cs9g12650	TTGTAAAGTA	TTGTTCTGCT	ATTATCGGCA	GCATGTGCCT	CTGTAATATG
Cs9g12620	--ACAGAGTC	TTGATTAAGT	TTTGTACTTA	GAAATATTGT	AGAACTAAGA
Cs9g12650	AAATAGAATT	TGTACTTTAT	AT-GTACTTC	AAAATGTTAG	CTGATAGAAT
Cs9g12620	CTA---TAGG	AGCAAATCAC	GGCAACC---	-----AAT	GAAGCTTCAT
Cs9g12650	TCAAAGTAGG	AGCAAATCAC	GGCAACCGGC	AACCAGTAAT	GAAACTTCAT
LB1					
Cs9g12620	TTGGGGCTGC	TCCAACACTT	GAATCAAGAC	TTTTGAGTGG	AAAAACAAGA
Cs9g12650	GTGGGG---C	TCCAACGCAT	TAATGAAGAA	ATTTGTGTGA	AAATACAAGA
Cs9g12620	ATTAATTGAA	ATTATGGGAA	ATGGGGAGAC	CAAGTGGCAC	ATCTCAGCGC
Cs9g12650	CTT----GAG	ATT-TGGGAA	ATGCTTGGAC	AAAGGGACAC	TTCTCGCGGC
LB2					
Cs9g12620	TAATTTGGTG	GCCCTATAAC	GTGGTT--CC	TTATCTTCTT	T--CTTCCTT
Cs9g12650	TAATTCGGTG	GCCCTATAAC	GTGGTTTTCT	TTTTCTTCTT	TTTCTTCCTT
Cs9g12620	TCTTTTTGTC	TCCCACTTTA	ATATATAAAA	AAATCATACA	CGTACATGCT
Cs9g12650	TGCTTTTTGTC	TTCAAATTCA	ATATA-AAAA	TCATTATTTG	CGTAC-TGAT
PthA4 EBE					
Cs9g12620	CCCGTTGAGC	GTTTTCATTC	TATAAATACT	GTATTCATCC	TAGTTGCTTC
Cs9g12650	CTTGTTGAAC	TAATTCATTC	TATAAATATC	CTATTCAGTC	TACTTGCTTC
Cs9g12620	ATCAATATAG	AAAAGATGCC	TTGGTTTCTC	TCTCTT	
Cs9g12650	TTCATCATAG	AAAA-----	-----	-----	

Figure 2—figure supplement 1. Alignment of the promoter regions of Cs9g12620 and Cs9g12650 in *Citrus sinensis*. The promoter sequences were retrieved from the citrus genome database. The putative binding sites of CsLOB1 (LB1 and LB2) are shadowed with blue. The location of binding site of PthA4 (effector-binding element, EBE) is indicated by an underline. A green shadow shows the location of predicted promoter core structure. The lack of 22 bp at the 3' terminus is shown by a red box.

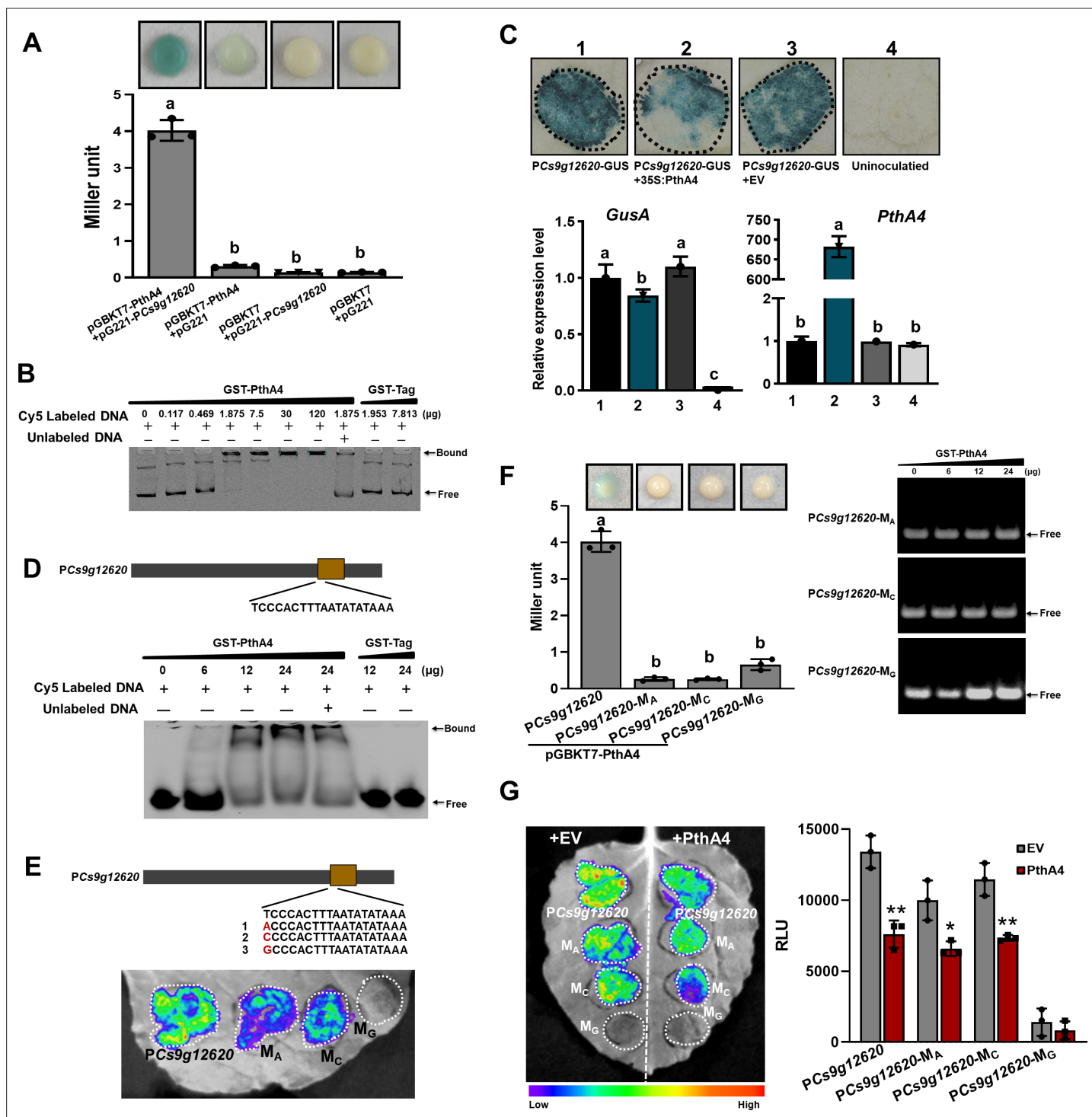


Figure 3. PthA4 binds to and suppresses the *Cs9g12620* promoter. **(A)** A yeast one-hybrid assay shows that PthA4 interacts with the *Cs9g12620* promoter. pGBKT7-pthA4 and pG221-PCs9g12620 were co-transformed into the yeast strain EGY48 and screened on synthetic dropout SD/-Ura and SD/-Ura/-Trp media. The transformed cells were dissociated by repeated freeze-thaw treatment and then used for β -galactosidase assays on filters soaked with Z buffer that contained 20 μ g/ml X-gal. The pGBKT7 and pG221 vectors were used as negative controls by co-transformation with the corresponding constructs. The columns below show the quantitative assay of β -galactosidase by the Miller method. Values are the means \pm SD ($n = 3$ biological replicates). Columns labeled with different letters indicate significant difference among means (ANOVA, $p < 0.01$). **(B)** An electrophoretic mobility shift assay (EMSA) shows that PthA4 binds to the *Cs9g12620* promoter. Purified GST-PthA4 was incubated with 25 ng of promoter DNA in gel shift binding buffer at 28°C for 30 min and then analyzed in 6% non-denaturing PAGE. A GST tag was used as a negative control. **(C)** The overexpression of PthA4 suppressed the *Cs9g12620* promoter. PthA4 was transiently co-expressed with a *Cs9g12620* promoter GUS fusion in *Nicotiana benthamiana*.

Figure 3 continued on next page

Figure 3 continued

The promoter-driven GUS activity was assayed at 2 days post-agroinfiltration. The upper images show histochemical staining of the *Cs9g12620* promoter-driven GUS activity. The bar chart below shows the qRT-PCR analysis of the transcript levels of *gusA* and *pthA4*. The level of expression of each gene in the samples that expressed the promoter alone was set as 1, and the levels of the other samples were calculated relative to that. Values are the mean results from three biological replicates and are the means \pm SD. Columns labeled with different letters indicate significant difference among means (ANOVA, $p < 0.01$). **(D)** EMSA shows that PthA4 binds to the 19 bp predicted binding site (effector-binding element, EBE) in the *Cs9g12620* promoter. The purified GST-PthA4 was incubated with 25 ng of synthetic 19 bp EBE DNA fragment in gel shift binding buffer at 28°C for 30 min and then analyzed in 6% non-denaturing PAGE. **(E)** Luciferase assays of the activity of *Cs9g12620* promoter with site-directed mutation in PthA4 EBE. The first nucleotide acid 'T' in EBE was mutated into A, C, or G, which generated PCs9g12620-M_A, PCs9g12620-M_C, and PCs9g12620-M_G, respectively. The mutants were fused with luciferase and transiently overexpressed in *N. benthamiana* leaves. The luciferase activity was measured with a CCD imaging system at 2 days post-agroinfiltration. **(F)** PthA4 did not bind to PCs9g12620-M_A, PCs9g12620-M_C, and PCs9g12620-M_G. The left image represents a yeast one-hybrid assay that did not show an interaction of PthA4 between PCs9g12620-M_A, PCs9g12620-M_C, and PCs9g12620-M_G. The experiments were conducted in the same manner as those in **A**. Columns labeled with different letters indicate significant difference among means (ANOVA, $p < 0.01$). The right image represents an EMSA that shows that PthA4 does not bind to the promoter mutants. Purified GST-PthA4 was incubated with 25 ng of promoter DNA in gel shift binding buffer at 28°C for 30 min and then analyzed in 6% non-denaturing PAGE. **(G)** Luciferase assays showing the suppression of PthA4 on PCs9g12620-M_A, PCs9g12620-M_C, and PCs9g12620-M_G. The promoter luciferase fusions were co-expressed with PthA4 in *N. benthamiana*. Co-expression with the empty binary vector pHB was used as the control. The analysis was the same as that described in **E**. The image on the right shows the quantification of luciferase signal using a microplate luminescence reader. Values are the means \pm SD ($n = 3$ biological replicates). ANOVA, analysis of variance; CCD, charge-coupling device; GST, glutathione-S transferase; PAGE, polyacrylamide gel electrophoresis; qRT-PCR, real-time quantitative reverse transcription PCR; SD, standard deviation. * $p < 0.05$. ** $p < 0.01$ (Student's *t*-test).

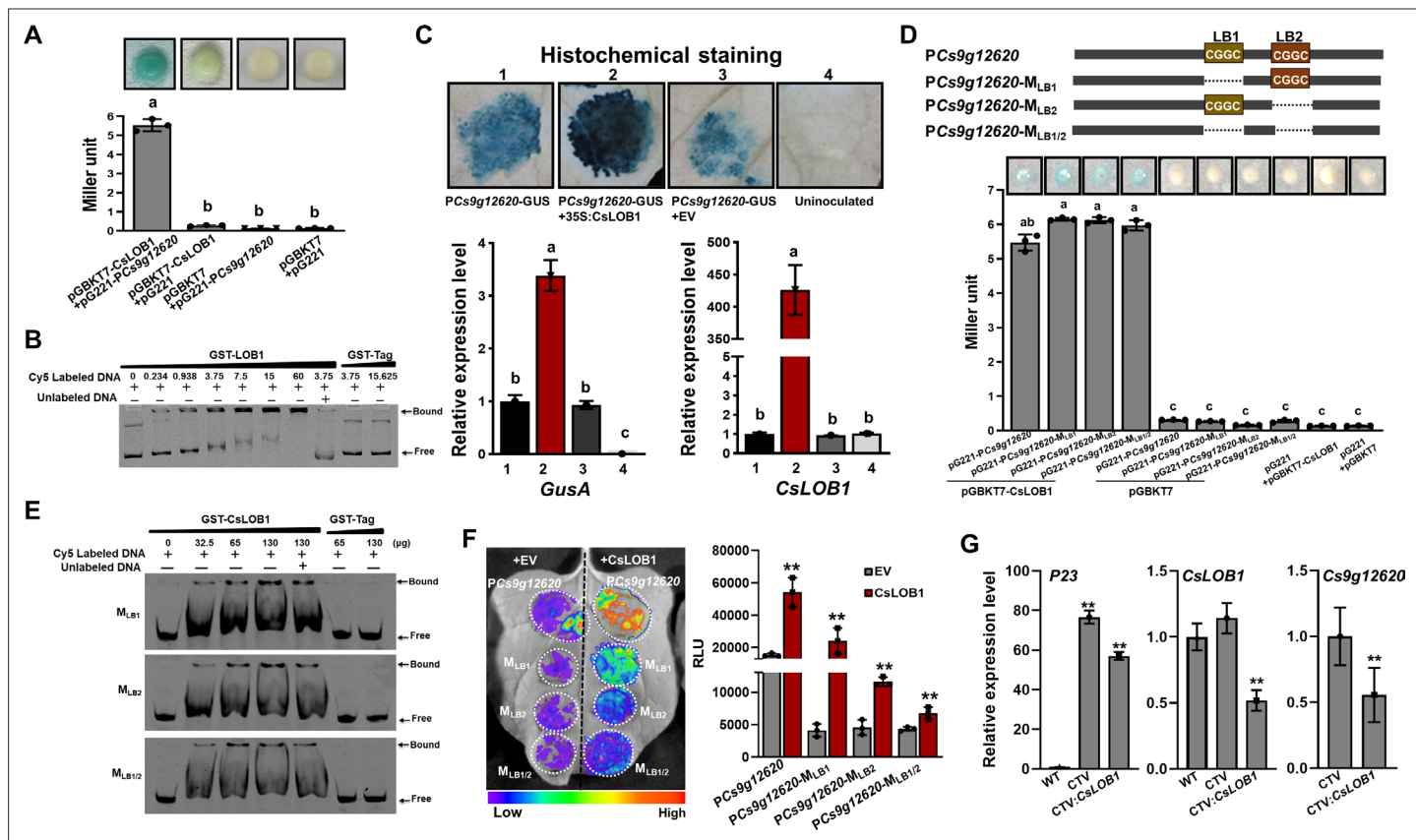


Figure 4. The *Cs9g12620* promoter is directly activated by *CsLOB1*. **(A)** A yeast one-hybrid (Y1H) assay shows that *CsLOB1* interacts with the *Cs9g12620* promoter. pGBKT7-*CsLOB1* and pG221-*PCs9g12620* were co-transformed into yeast strain EGY48 and screened on synthetic dropout SD/-Ura and SD/-Ura/-Trp media. The transformed cells were dissociated by repeated freeze-thaw treatment and then used for β -galactosidase assays on filters soaked with Z buffer that contained 20 μ g/ml of X-gal. pGBKT7 and pG221 vectors were used as the negative control by co-transformation with corresponding constructs. The columns below show the quantitative assay of β -galactosidase by the Miller method. Values are the means \pm SD ($n = 3$ biological replicates). Columns labeled with different letters indicate significant difference among means (ANOVA, $p < 0.01$). **(B)** An electrophoretic mobility shift assay (EMSA) shows that *CsLOB1* binds to the *Cs9g12620* promoter. Purified GST-*CsLOB1* was incubated with 25 ng of promoter DNA in gel shift binding buffer at 28°C for 30 min and was then analyzed by 6% non-denaturing PAGE. **(C)** *Cs9g12620* promoter activity is induced by *CsLOB1*. *CsLOB1* was transiently co-expressed with the *Cs9g12620* promoter *GUS* fusion in *Nicotiana benthamiana*. Promoter-driven *GUS* activity was assayed at 2 days post-agroinfiltration. The upper images show the histochemical staining of *Cs9g12620* promoter-driven *GUS* activity. The bar chart below shows the qRT-PCR analysis of transcript levels of *gusA* and *pthA4*. The level of expression of each gene in the samples that expressed the promoter alone was set as 1, and the levels of the other samples were calculated relative to that. Values are the mean results from three biological replicates and are the means \pm SD. Columns labeled with different letters indicate significant difference among means (ANOVA, $p < 0.01$). **(D)** A Y1H assay shows that *CsLOB1* interacts with the *Cs9g12620* promoter *PCs9g12620* and *CsLOB1*-binding site deletion mutants *PCs9g12620-M_{LB1}*, *PCs9g12620-M_{LB2}*, and *PCs9g12620-M_{LB1/2}*. The upper diagram shows the putative *CsLOB1*-binding sites and the corresponding deletion mutants. The bar chart below represents a Y1H assay that shows the interaction of *CsLOB1* with the promoter mutants. The analyses were same as those conducted in **A**. Columns labeled with different letters indicate significant difference among means (ANOVA, $p < 0.01$). **(E)** An EMSA showed that *CsLOB1* binds to *Cs9g12620-M_{LB1}*, *PCs9g12620-M_{LB2}*, and *PCs9g12620-M_{LB1/2}*. Purified GST-*CsLOB1* was incubated with 25 ng of promoter DNA in gel shift binding buffer at 28°C for 30 min. The analyses were same as those in **B**. **(F)** Luciferase assays show the role of *CsLOB1* on *PCs9g12620-M_{LB1}*, *PCs9g12620-M_{LB2}*, and *PCs9g12620-M_{LB1/2}*. Promoter luciferase fusions were co-expressed with *CsLOB1* in *N. benthamiana*. Co-expression with the empty binary vector pHB was used as the control. Luciferase activity was measured with a CCD imaging system at 2 days post-agroinfiltration. The image on the right shows the quantification of luciferase signal using a microplate luminescence reader. Values are the means \pm SD ($n = 3$ biological replicates, $**p < 0.01$ [Student's *t*-test]). **(G)** qRT-PCR analysis of the transcript level of *Cs9g12620* in *CsLOB1*-silenced plants. *CsLOB1* was silenced in *Citrus sinensis* using the citrus tristeza virus (CTV)-based gene silencing vector CTV33. The effective infection by CTV was evaluated by the expression of *P23* gene harbored in CTV33. The silencing efficiency of *CsLOB1* was verified by comparison with the levels of expression in the WT and empty CTV33-infected control plants. The reduced expression of *Cs9g12620* is shown by comparison with the level of expression in the empty CTV33-infected plants. The qRT-PCR was performed as described in **C**. ANOVA, analysis of variance; CCD, charge-coupled device; *GUS*, β -glucuronidase; PAGE, polyacrylamide gel electrophoresis; qRT-PCR, real-time quantitative reverse transcription PCR; SD, standard deviation; WT, wild type.

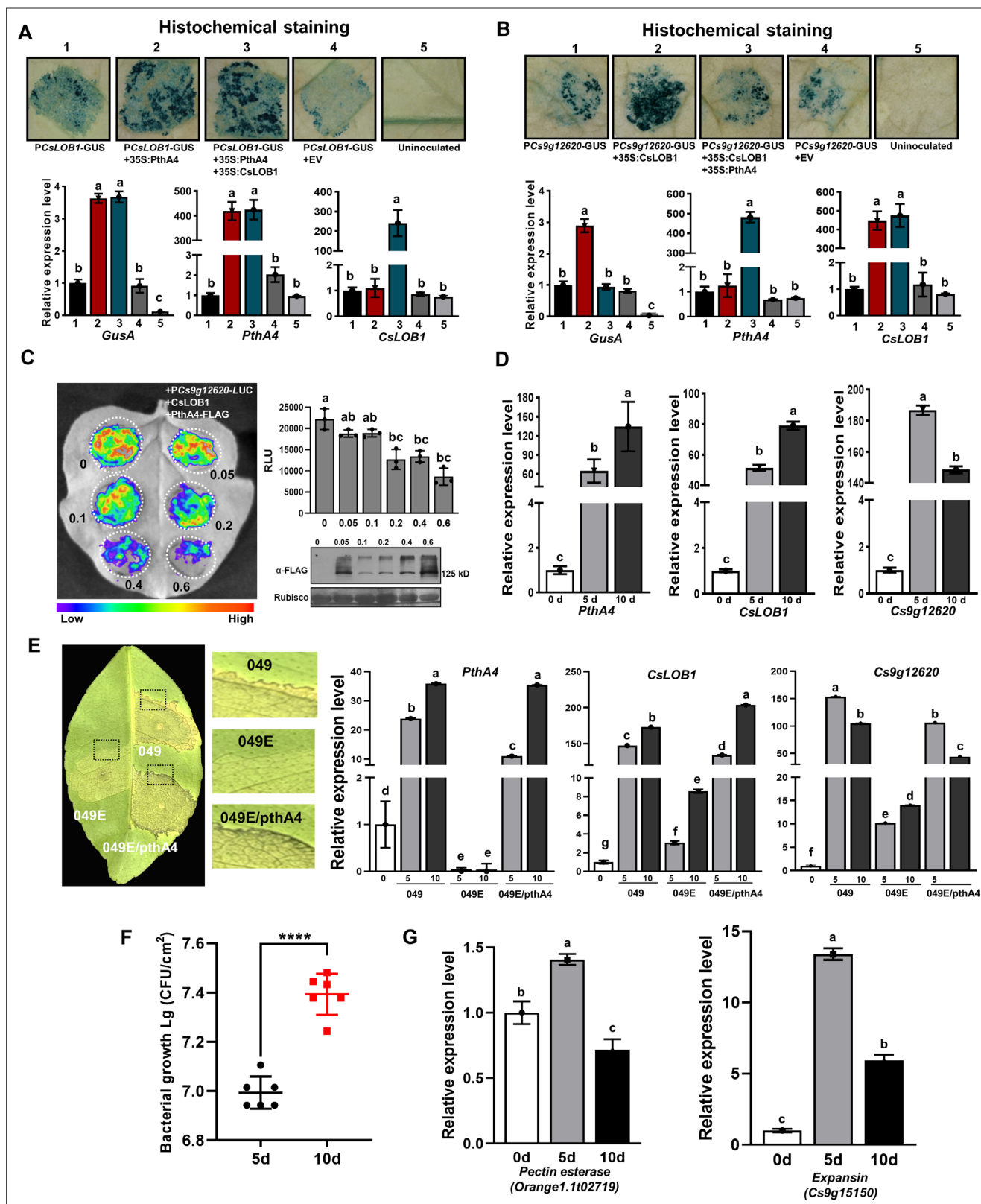


Figure 5. CsLOB1-induced *Cs9g12620* promoter activity is suppressed by PthA4. (A) PthA4 induced the activity of *CsLOB1* promoter. PthA4 was transiently co-expressed with the *CsLOB1* promoter GUS fusion in *Nicotiana benthamiana*. GUS activity was assayed at 2 days post-agroinfiltration. The upper images show the histochemical staining of *CsLOB1* promoter (PCsLOB1)-driven GUS activity. The bar chart below shows the qRT-PCR analysis of the transcript levels of *gusA*, *CsLOB1*, and *pthA4*. The transcript level of each gene in the samples that expressed PCsLOB1-GUS was set as

Figure 5 continued on next page

Figure 5 continued

1, and the levels of the other samples were calculated relative to that. Values are the mean results from three biological replicates and are the means \pm SD. Columns labeled with different letters indicate significant difference among means (ANOVA, $p < 0.01$). **(B)** PthA4 suppressed the CsLOB1-induced activity of the *Cs9g12620* gene promoter. The suppression was evaluated by the co-expression of PthA4 with CsLOB1 and *PCs9g12620*-GUS. Histochemical staining and a qRT-PCR analysis were performed as described in **A**. Columns labeled with different letters indicate significant difference among means (ANOVA, $p < 0.01$). **(C)** PthA4 suppressed the CsLOB1-induced *PCs9g12620* activity in a dose-dependent manner. CsLOB1 and *PCs9g12620*-LUC were co-expressed with PthA4-FALG in *N. benthamiana*. The agrobacteria that expressed PthA4-FLAG were arranged to a cell concentration series of OD₆₀₀ values of 0.05, 0.1, 0.2, 0.4, and 0.6. The luciferase signal was quantified with a microplate luminescence reader. Values are the means \pm SD ($n = 3$ biological replicates). Columns labeled with different letters indicate significant difference among means (ANOVA, $p < 0.01$). The image on the bottom right shows the expression of PthA4-FLAG by immunoblotting with anti-FLAG. **(D)** A qRT-PCR analysis of the levels of expression of *pthA4*, *CsLOB1*, and *Cs9g12620* in *Citrus sinensis* leaves inoculated with Xcc 29-1. The cell suspension (10^8 CFU/ml) was infiltrated into the plant leaves. qRT-PCR was performed at 0, 5, and 10 days post-inoculation (dpi). The level of expression of each gene at 0 dpi was set as 1, and the level in other samples was calculated relative to those baseline values. Values are the mean results from three biological replicates and are the means \pm SD. Columns labeled with different letters indicate significant difference among means (ANOVA, $p < 0.01$). **(E)** The level of expression of *Cs9g12620* was dynamically related with that of PthA4 during Xcc infection. The disease symptoms caused by WT Xcc 049, transcriptional activator-like (TAL)-free mutant 049E, and Xcc 049E/*pthA4* on *C. sinensis* leaves. The phenotype was recorded at 10 dpi. A qRT-PCR analysis was conducted to evaluate the levels of expression of *pthA4*, *CsLOB1*, and *Cs9g12620*. The experiment was performed as described in **D**. Columns labeled with different letters indicate significant difference among means (ANOVA, $p < 0.01$). **(F)** Growth of wildtype Xcc 29-1 in *C. sinensis* plants. The Xcc 29-1 cells of 10^8 CFU/ml were infiltrated into citrus leaves. At 5 and 10 days post inoculation, bacteria were recovered from leaves and counted on Nutrient Agar (NA) plates. Error bars represent the standard deviation from three independent experiments (Student's *t*-test, **** $p < 0.0001$). **(G)** qRT-PCR analysis of *pectin esterase* (*orange1.1t02719*) and *expansin* (*cs9g15150*) genes expression levels in *C. sinensis* leaves inoculated with Xcc 29-1. The experiment was performed as described in **D**. Columns labeled with different letters indicate significant difference among means (ANOVA, $p < 0.01$). ANOVA, analysis of variance; GUS, β -glucuronidase; qRT-PCR, real-time quantitative reverse transcription PCR; SD, standard deviation.

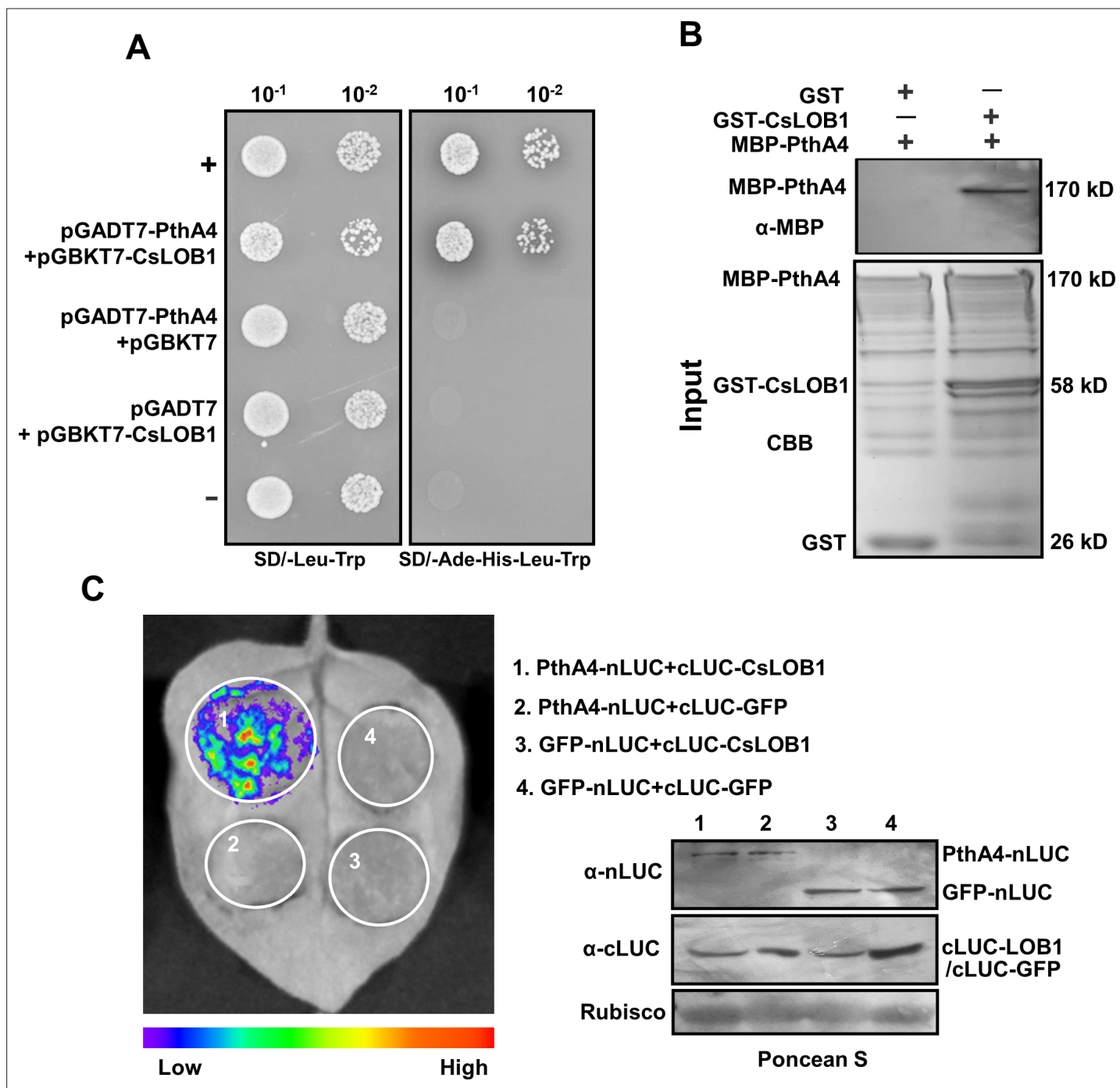


Figure 6. PthA4 interacts with CsLOB1. **(A)** A yeast two-hybrid assay shows that PthA4 interacts with CsLOB1. pGADT7-PthA4 and pGBKT7-CsLOB1 were co-transformed in yeast cells and screened on synthetic dextrose media that lacked leucine and tryptophan (SD/-Leu-Trp). A single yeast colony was then selected for serial dilution and grown on SD/-Leu-Trp and SD/-Ade-His-Leu-Trp to examine the interaction. The yeast co-transformed with pGADT7-T and pGBKT7-53 served as a positive control. The yeast co-transformed with pGADT7-T and pGBKT7-lam served as a negative control. **(B)** GST pull-down assay of the interaction between PthA4 and CsLOB1. The recombinant GST-CsLOB1 and MBP-PthA4 proteins were used for GST pull-down assays. The GST protein served as a negative control. The pull-down of MBP-PthA4 was verified by anti-MBP immunoblotting. The experiment was repeated three times with similar results. **(C)** A split luciferase assay for the interaction of PthA4 and CsLOB1 in vivo. *Nicotiana benthamiana* leaves were co-infiltrated with agrobacteria that harbored 35S:PthA4-nLUC and 35S:cLUC-CsLOB1. The image on the right shows the expression of respective proteins. Images of chemiluminescence were obtained by treatment with 0.5 μ M luciferin 48 hr post-infiltration. Similar results were obtained in three biological repeats. GUS, β -glucuronidase.

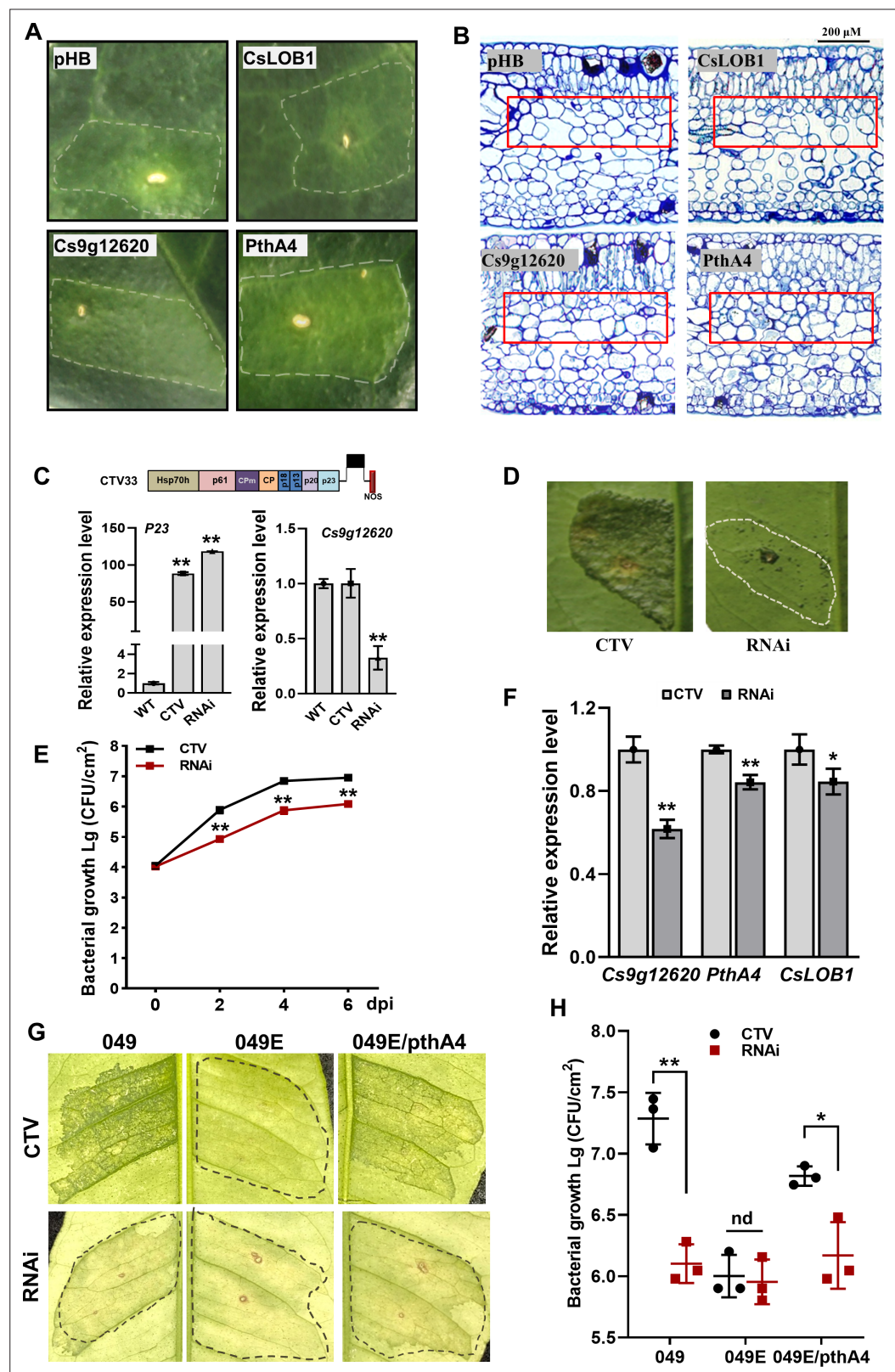


Figure 7. Cs9g12620 is involved in the formation of canker symptoms. (A) The transient overexpression of Cs9g12620 results in hypertrophy in the *Citrus sinensis* leaves. The expression of *pthA4* was used as the positive control. The cultured *Agrobacterium* cells were adjusted to an OD₆₀₀ of 0.01 and infiltrated into young *C. sinensis* leaves. The phenotypes were recorded at 15 days post-agroinfiltration. The area of infiltration for each gene is

Figure 7 continued on next page

Figure 7 continued

indicated by a dotted line. **(B)** Transmission electron micrograph of *C. sinensis* leaf tissues. Transmission electron micrographs of the cross sections of citrus leaf tissue. The leaves were sampled at 15 days post-agroinfiltration. The changes in tissue structure induced by *Cs9g12620* and *pthA4* are indicated by a box. The bars represent 200 μ M. **(C)** A real-time quantitative reverse transcription PCR (qRT-PCR) analysis of the silencing of *Cs9g12620* in *C. sinensis*. *Cs9g12620* was silenced using the citrus tristeza virus (CTV)-based gene silencing vector CTV33. The effective infection by CTV was evaluated by measuring the level of expression of the *P23* gene harbored in CTV33. The silencing efficiency of *Cs9g12620* was verified by comparison with the levels of expression in the WT and empty CTV33-infected control plants. Values are the mean results from three biological replicates (Student's *t*-test, ***p* < 0.01). The experiment was repeated three times. **(D)** The canker symptoms on *Cs9g12620*-silenced citrus plants were caused by the WT *Xcc* 29-1. The *Xcc* 29-1 cells of 10^7 CFU/ml were infiltrated into citrus leaves to analyze the development of canker symptoms. The canker symptoms were recorded at 6 days post-inoculation (dpi). The areas of infiltration are indicated by dotted lines. The inoculation of the plants infected by the empty vector CTV33 was used as the negative control. **(E)** Growth of *Xcc* 29-1 in *Cs9g12620*-silenced citrus plants. A volume of 10^7 CFU/ml of *Xcc* 29-1 cells were infiltrated into citrus leaves. At 2, 4, and 6 dpi, bacteria were recovered from leaves and counted on Nutrient Agar (NA) plates. Error bars represent the standard deviation from three independent experiments (Student's *t*-test, **p* < 0.05, ***p* < 0.01). **(F)** A qRT-PCR analysis of *Cs9g12620*, *CsLOB1*, and *pthA4* in *Cs9g12620*-silenced citrus plants inoculated with *Xcc* 29-1. The experiment was performed as described in **C**. **(G)** The canker symptoms on *Cs9g12620*-silenced citrus plants were caused by the WT *Xcc* 049, transcriptional activator-like (TAL)-free mutant 049E, and 049E/*PthA4*. The experiment was performed as described in **D**. **(H)** Growth of WT *Xcc* 049, 049E, and 049E/*PthA4* in *Cs9g12620*-silenced citrus plants. At 6 dpi, bacteria were recovered from leaves and counted on nutrient-rich (NA) plates. The experiment was performed as described in **E**. dpi, days post-inoculation; WT, wild type.

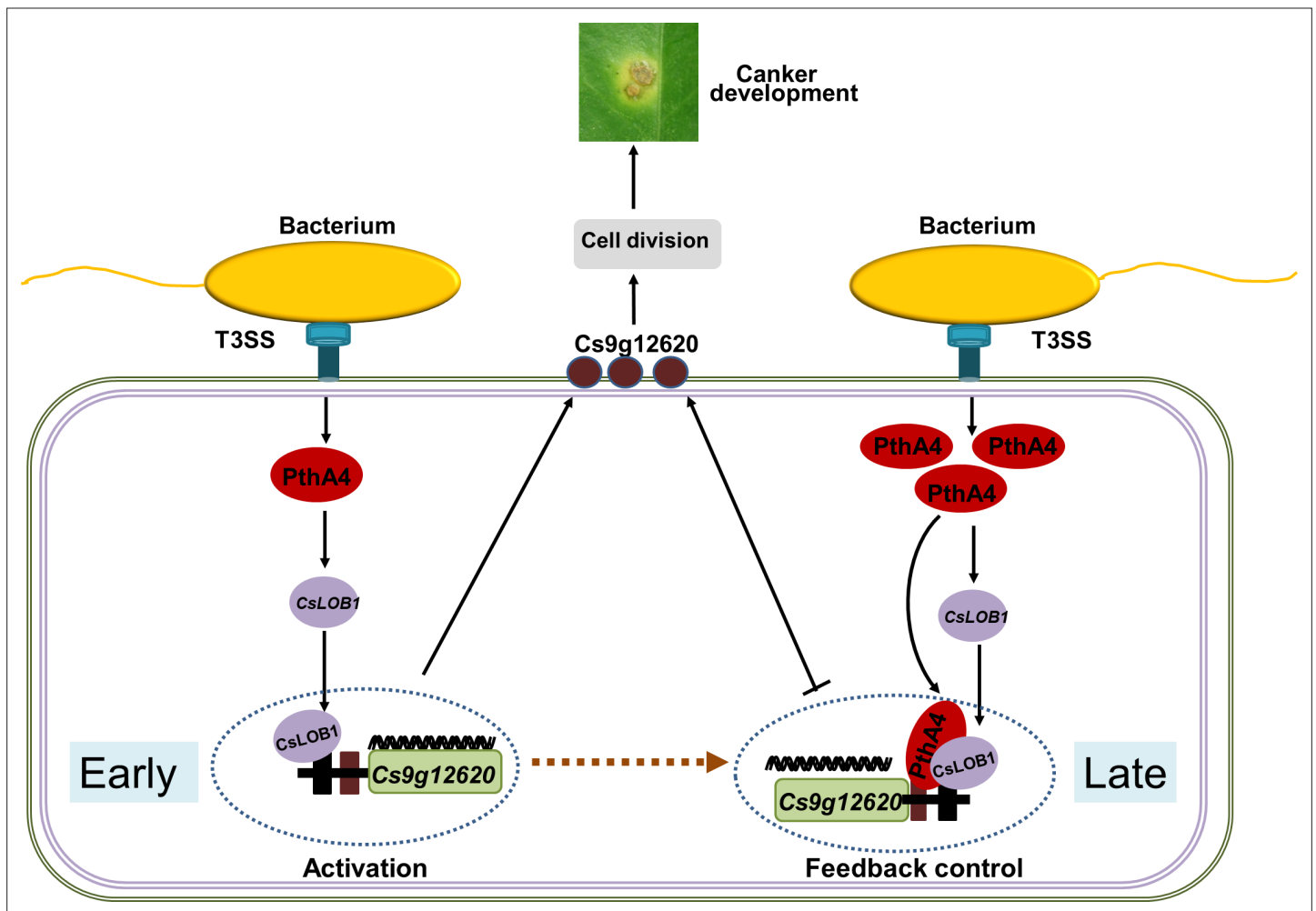


Figure 8. Proposed model of the expression of *Cs9g12620* directed by PthA4-mediated induction of CsLOB1. The regulation of putative carbohydrate-binding protein gene *Cs9g12620* was divided into two steps. At the early infection stage, the presence of PthA4 is responsible for the induction of CsLOB1, which is responsible for the induction of *Cs9g12620*. At the late infection stage, the over-redundancy of PthA4 exerts a feedback suppression effect on the activation of *Cs9g12620* by interacting with CsLOB1.