Activation of the pathogen-inducible *Gst1* promoter of potato after elicitation by *Venturia inaequalis* and *Erwinia amylovora* in transgenic apple (*Malus* × *domestica*)

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Abstract

Rather than using a constitutive promoter to drive transgenes for resistance against fungal and bacterial diseases in genetic engineering of apple (*Malus* × *domestica*) cultivars, a promoter induced only after infection was preferred. The ability of the *Pgst1* promoter from potato (*Solanum tuberosum* L.) to drive expression of the *gusA* reporter gene was determined in two genotypes of apple: the fruit cultivar Royal Gala and the M.26 rootstock. β-glucuronidase activity in the transgenic lines grown in a growth chamber was determined quantitatively using fluorometric assays and compared to the activity in Cauliflower Mosaic Virus (*CaMV*) 35S promoter-driven transgenic lines. In both apple genotypes, the *Pgst1* promoter exhibited a low level of expression after bacterial and fungal inoculation compared to the level obtained with the *PCaMV35S* promoter (15% and 8% respectively). The *Pgst1* promoter was systematically activated in apple at the site of infection with a fungal pathogen. It was also activated after treatment with salicylic acid, but not after wounding. Taken together, these data show that, although the *Pgst1* promoter is less active than the *PCaMV35S* promoter in apple, its pathogen responsiveness could be useful in driving the expression of transgenes to promote bacterial and fungal disease resistance.

Introduction

Constitutive promoters and non-specific promoters such as *PCaMV*35S (Cauliflower Mosaic Virus) and *nos* (nopaline synthase) have been frequently used as experimental tools to assess the effects of transgene expression in many plant species. With such promoters, a gene is expressed in the majority of tissues during most phases of plant growth and development. This limited temporal and spatial regulation may be suitable for proof of concept experiments, but presents a number of potential drawbacks for use in genet-ically improved crops. For example, the presence of transgenes driven by constitutive promoters may result in homology dependent gene silencing, particularly when the promoter is highly active (Vaucheret et al., 1998). Thus gene expression under the control of inducible promoters is preferred in any strategy to produce transgenic plants with transgene-mediated improvements in resistance to pathogens. Such a pathogen-inducible promoter should be strictly regulated, primarily responsive to multiple pathogens and not responsive to abiotic stress factors (Stuiver & Custers, 2001). A relatively small number of these promoters have been studied extensively.

Most apple (*Malus* × *domestica*) cultivars are susceptible to two important major diseases, fire

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blight, caused by the necrogenic bacterium *Erwinia amylovora* (Burr) and apple scab caused by the ascomycete fungus *Venturia inaequalis* (Cooke). Several strategies have been used to enhance resistance against fire blight, including the use of lytic peptides such as attacin (Reynoird et al., 1999), and lactoferrin (Malnoy et al., 2003a), and against apple scab including the use of endochitinase (Bolar et al., 2000, 2001). However, all the transgenes tested so far were under the control of the *PCaMV*35S promoter. This may lead to undesirable accumulation of transgenic proteins in the consumed fruits. An evaluation of gene promoters specifically induced by bacterial and fungal pathogens is therefore needed in apple. To address these concerns we have undertaken a study of the efficacy of the inducible promoter *Pgst1* in transgenic apple plants.

The potato defense gene *Pgst1* (formerly called *Pprp1–1*) encodes a glutathione S transferase (Hahn & Strittmatter, 1994). The promoter of this gene has been previously shown to mediate rapid and local transcriptional activation in response to different types of plant microbe interactions (fungus and virus). Transcription from this promoter is not induced by environmental abiotic stimuli such as wounding or heat shock (Martini et al., 1993; Strittmatter et al., 1996).

In this study, we report the analysis of expression profiles of the potato *Pgst1* gene promoter in apple genotypes in response to biotic and abiotic stresses.

Materials and methods

Bacterial strains

The wild-type strain of *E. amylovora* (Ea 273) and its transposon mutants (Table 1) were grown overnight at 28°C on Kado medium containing kanamycin or rifampicin (20 mg ml^{-1}) for the transposon mutants. Inocula were prepared by suspension in distilled water at a concentration of 7.5×10^7 cfu ml^{-1}. *Pseudomonas syringae* pv. *tabaci* (B86–7) strain was grown at 28°C on Kado, inoculum was prepared as described for *E. amylovora*.

Fungus strain

A mixed inoculum of *Venturia inaequalis* isolates 1805-2, 1777-8, 1771-2, 1778-6 and 1810-1, representing the five races, 1–5, was used (Table 1). Inocula were prepared by suspension of the conidia in distilled water at a concentration of 2.7×10^7 conidia ml^{-1}.

Plasmid constructs

Two binary expression vectors, pBI121 and pBI101.1:Pprp1-1 were used. pBI121 (BD Bioscience Clontech, Palo Alto, CA) contained an *npt*II-based expression cassette as the selectable marker and a *gusA*-intron expression cassette adjacent to the right border of the T-DNA. pBI101.1:Pprp1–1

Table 1. Bacterial strains and fungal isolates used in this study

Strains and isolates	Relevant characteristics[a]	Reference or source
Erwinia amylovora		
Ea273 (CU 0273)	Wild-type isolated from apple	S.V. Beer
E.a 273 G73 (CU 4277)	hrc V:: Tn5-gusA1; HR$^-$ P$^-$	Wei et al., 1992
E.a 273 dspEΔ1521	Deletion of 5' dspE; Hr$^+$; P$^-$	Bogdanove et al., 1998
E.a 273 Δ N–W (CU 5114)	Δ (hrpN-hrpW)::aphII; Hr^r; V^r (almost HR$^-$)	S.V. Beer
Pseudomonas syringae		
pv *tabaci*	Wild type, isolated from *Nicotiana tabacum*	
Venturia inaequalis		
Isolate 1805-2	Race 1	
Isolate 1777-8	Race 2	
Isolate 1771-2	Race 3	
Isolate 1778-6	Race 4	
Isolate 1810-1	Race 5	

[a]Hrc, hypersensitive response conserved (HRP secretion mutant); HR, hypersensitive response; P, pathogenecity.

contained an *nptII* based expression cassette as the selectable marker and the *gusA*-intron under the control of the P*gst1* promoter. These binary vectors were introduced by electroporation into the supervirulent *Agrobacterium tumefaciens* strain EHA105 (Hood et al., 1993).

Plant material and transformation

The apple cultivar Royal Gala (RG) and the rootstock M.26 were chosen for this study because they can be genetically transformed at high efficiency (Borejsza-Wysocka et al., 1999; Norelli et al., 1999). Leaf segments were excised from *in vitro* grown shoots of these two cultivars three weeks after subculturing. Transformation experiments were carried out as previously reported (Borejsza-Wysocka et al., 1999; Norelli et al., 1999) using *Agrobacterium tumefaciens* strain EHA105 containing pBI121 or pBI101.1:Pprp1-1 binary vectors. DNA was isolated from the youngest leaf of putative transgenic plants and non-transformed control plants as described by Cheung et al. (1993) and the PCR procedure was as described by Bolar et al. (1999). In order to check for the presence of promoter-GUS fusions, specific primers were designed to amplify a fragment overlapping the promoter region and the *gusA* sequence (Table 2). Non-transgenic RG, M.26 and transgenic clones were propagated *in vitro* (Norelli et al., 1998). Acclimation of the plants was performed as described by Bolar et al. (1998).

Determination of ploidy level

Ploidy level in the transgenic and untransformed clones was estimated by flow cytometry. Nuclei were isolated from *in vitro* leaves by manual chopping with a razor blade directly into the buffer described by the manufacturer (Partec, Münster, Germany). After addition of 4,6 diamino 2 phenyl indole dihydrochloride (DAPI; 2% v/v) and filtration through a 20 μm nylon mesh, the mixture was analyzed with a cytometer (Partec II; Partec).

Inoculation procedures and plant treatments

Erwinia amylovora
The youngest expanded leaves from self-rooted plants in a growth chamber were inoculated by cutting the leaves transversely with scissors dipped in a suspension (7.5×10^7 cfu ml^{-1}) of the virulent *E. amylovora* strain Ea273 or water (control).

Venturia inaequalis
The youngest expanded leaf was tagged and the plants were inoculated with a suspension of *V. inaequalis* (2.7×10^6 conidia ml^{-1}), using an atomizer connected to compressed air (Yepes & Aldwinckle, 1993). The plants were incubated in a mist chamber (16 h photoperiod of 40 micromol m^{-2} s^{-1}, 18 ± 1°C and 100% relative humidity) for 48H and later moved to a growth chamber.

Abiotic elicitor
Salicylic acid (Sigma, St Louis, MO) was applied by spraying with an atomizer.

Fluorometric GUS assay

GUS activity in apple was quantified using the procedure described by Gittins et al. (2000). Leaf tissue (20 mg) was ground in 500 μl of assay buffer (40 mM sodium phosphate pH7, 4 mM EDTA, 0.08% SDS (w/v), 0.08% Triton ×100 (v/v), 10 mM β-mercaptoethanol) containing methanol (1/4 v/v). After centrifugation for 15 min at 13000 *g*, 150 μl of supernatant was mixed with 350 μl of assay buffer and 200 μl of 4 methylumbelliferyl β-D-glucuronide (4 MUG ; 4 mM) substrate was added. An aliquot of 200 μl was

Table 2. Primer pairs used in this study

Primer	Sequence	Size of amplified fragment (bp)
Gst1–GUS F	CTAGCCACCAGATTTGACCA	957
Gst1–GUS R	TCCCGCTAGTGCCTTGTCCAG	
CaMV35S GUS F	GACGTAAGGGATGACGCACAAT	1450
CaMV35S GUS R	CAGCAGCAGTTTCATCAATCA	

immediately added to 800 μl of stop buffer (0.2 M Na$_2$CO$_3$) to be used as a time zero control. The remaining mixture was incubated at 37°C for 60 min. The amount of 4-MU product was determined using the DyNa Quant 200 fluorometer (λ excitation 365 nm/λ emission 455 nm; Hoefer LKB, Lorton, VA). β-glucuronidase activity was normalized to the protein content of extracts determined by the method of Bradford using bovine serum albumin as a standard (Bradford, 1976). For each treatment, 6 to 10 replicates from 2 or 3 separate experiments were assayed.

Results

Generation of transgenic lines and analysis of gusA *expression following fungal and bacterial infection*

Transformation experiments with binary vectors pBI121 and PBI101.1:Pprp1-1 harboring the *PCaMV35S* and *Pgst1* promoters, respectively, were performed on leaves detached from *in vitro* shoots of M.26 and RG. The transformation rate per leaf varied from 15% to 80% according to the genotype. Integration of the promoter-*gusA*-fusion was confirmed by PCR analysis of lines growing on kanamycin selective medium. Flow cytometry showed that all transgenic lines were diploid. Several vigorous 2n lines harboring the *Pgst1* (5 RG and 6 M.26 lines) and *PCaMV35S* (5 RG and 5 M.26 lines) promoters selected after PCR analyses were micropropagated *in vitro* and acclimated in a growth chamber. For comparison, untransformed lines of RG and the rootstock M.26 were propagated in an identical manner.

Expression of the *PCaMV35S-gusA* and *Pgst1–gusA* fusions was measured in extracts from the transgenic lines without treatment or 10 days after spraying with water or with *V. inaequalis* conidia (Figure 1). Among the *PCaMV35S–gusA* lines GUS activity varied from 65 nM 4 MU min^{-1} mg protein^{-1} in transgenic RG line TRG-171 plants to 135 nM 4 MU min^{-1} mg protein^{-1} in transgenic M.26 line T26.642 plants 10 days after water or *V. inaequalis* inoculation. GUS activity values were similar in the untreated leaves (data not shown). In both RG and M.26, expression of the *PCaMV35S* promoter was very similar. A lower range of variation was observed among the *Pgst1-gusA* lines 10 days after inocula-

tion with *V. inaequalis*. GUS activity after water treatment was 1.5 to 7.9 fold less than after *V. inaequalis* spraying in M.26 lines and 2.8 to 4.6 fold less for RG lines. GUS activity in untreated leaves of untransformed M.26 and RG *Pgst1-gusA* lines was negligible (data not shown).

The pattern of expression of the same transgenic lines 48 h after inoculating leaves with the virulent Ea273 strain of *E. amylovora* was similar to that after *V. inaequalis* inoculation. There were similar differences in activation of *gusA* between the control and the *E. amylovora* treatment, as between the control and the *V. inaequalis* treatment.

Based on these analyses, two transformants with each promoter for each apple genotype that had high GUS activity when sprayed with virulent races of *V. inaequalis* or inoculated with a virulent strain of *E. amylovora* were selected for further studies.

Effect of wounding on PCaMV35S *and* Pgst1 *promoter activity in transgenic lines of apple*

In potato, the *Pgst1* promoter is not responsive to abiotic stimuli such as wounding (Strittmatter et al., 1996). Some experiments were performed to characterize the effect of the wounding alone or coupled with a fungal inoculation procedure, on the activity of these two promoters in apple. Leaves from two transgenic lines per promoter fusion were sprayed with *V. inaequalis* or water. Similar leaves were wounded by scissor cuts and sprayed with conidia or water. All these treatments induced the *PCaMV35S* promoter weakly (data not shown). The *Pgst1* promoter was weakly activated following wounding, and strongly activated after inoculation with and without wounding (data not shown).

These data indicated that the *Pgst1* promoter is not wound-inducible in apple and that scissor cutting is a reliable technique of inoculation for further assessment of *E. amylovora* effects on these promoters.

Time course of activation of Pgst1 *promoters following pathogen inoculation and the effect of mutants of* Erwinia amylovora *and of other bacterial pathogens*

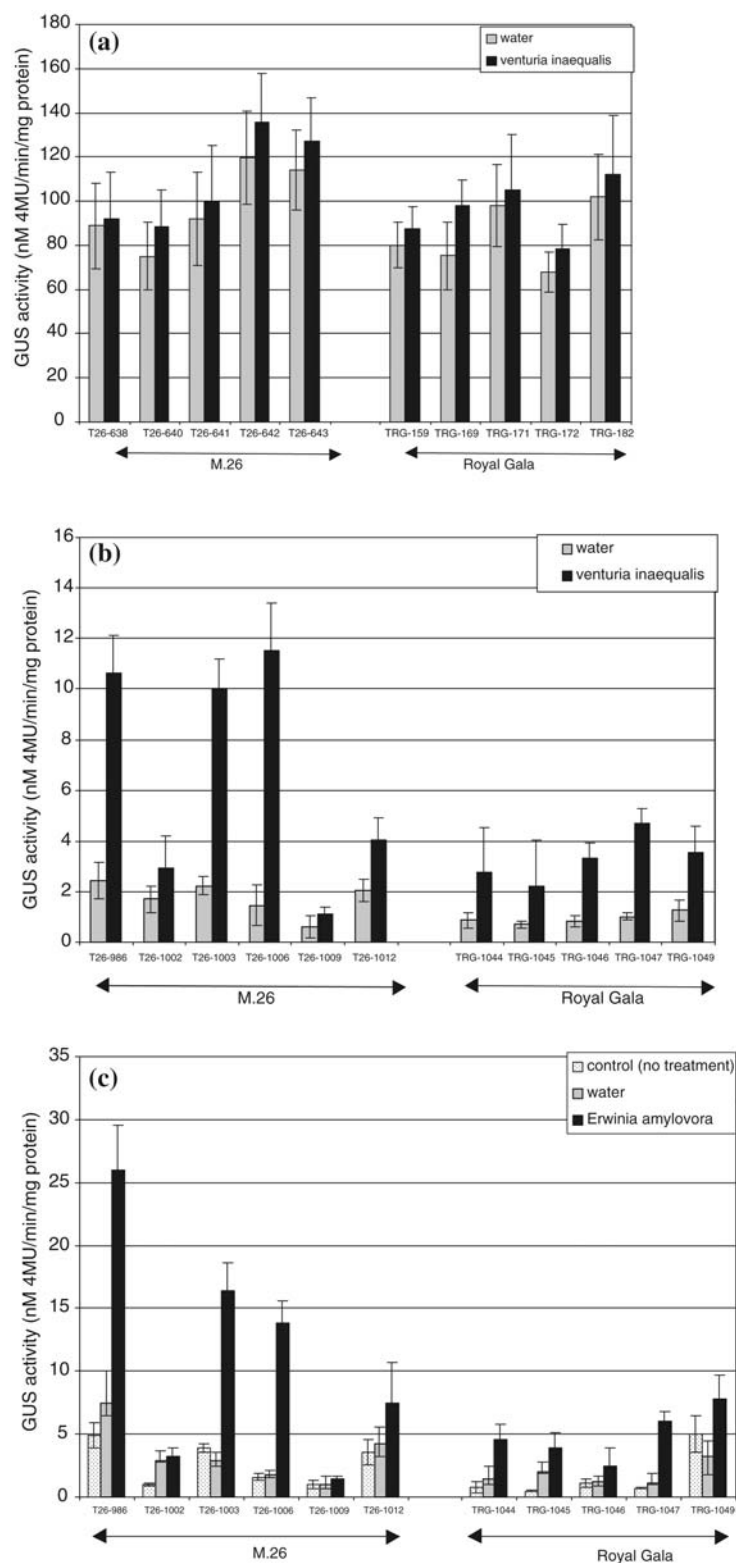GUS activity was monitored in leaves from 6 selected transgenic lines (T26-986, T26-1003,

Figure 1. GUS activity in independent transgenic lines of apple containing the P*CaMV35SP-GusA* (a) or P*gst1*-gusA fusions (b and c). GUS activity was measured 10 days after inoculation with *Venturia inaequalis* at 2.5×10^5 conidia ml^{-1}, 48 h after inoculation with *Erwinia amylovora* at 7.5×10^7 cfu ml^{-1} or with water. Bars represent confidence interval at $\alpha = 0.05$ among 10 replicates obtained from 3 separate experiments.

T26-643, TRG-1046, TRG-1047 and TRG-171) at 0, 3, 7, 10 and 12 days after inoculation with *V. inaequalis* or 0, 3, 6, 12, 24, 48, 72 and 96 h after inoculation with *E. amylovora*, or after water treatment (Figure 2). The *PCaMV35S-gusA* lines showed weak activation after treatment with *V. inaequalis*, *E. amylovora* and water in the first hours following the treatment (data not shown). This activity decreased 72 h after inoculation of the leaves with *E. amylovora*, at the time when necrosis first became visible in the inoculated leaves.

In P*gst1-gusA* lines, maximum GUS activity was observed in both apple genotypes 10 days after inoculation with *V. inaequalis* and 48 h after inoculation with *E. amylovora*. This activity reached 11% and 6% of the GUS activity of *PCaMV35S–gusA* levels of transgenic lines M.26 and RG, respectively, after inoculation with *V. inaequalis*. After inoculation with *E. amylovora*, GUS activity increased and reached 20% and 10% of the GUS activity of *PCaMV35S-gusA* transgenic lines of M.26 and RG, respectively. At this time point, GUS activity increased modestly in the P*gst1–gusA* transgenic M.26 lines after water treatment. However, this activation was 4 and 5 fold less than obtained after inoculation with *E. amylovora* and *V. inaequalis*, respectively. In P*gst1–gusA* transgenic RG lines, GUS activity remained low after water treatment.

In response to another bacterial pathogen (*Pseudomonas syringae* pv *tabaci*), the P*gst1* promoter was activated during an incompatible interaction in M.26 (data not shown) and RG transgenic lines (data not shown).

Avirulent mutant strains of *E. amylovora* with mutations affecting the *hrp* and *dsp* regions were investigated in order to understand the role of pathogenicity factors of *E. amylovora* in induction of the disease. The *Pgst1-gusA* transgenic M.26 line T26-986 showed different GUS activity in response to these mutants (data not shown). Similar results were obtained with the *Pgst1-gusA* transgenic RG line TRG-1047 (data not shown). These results show the important role of the *hrp* and *dsp* genes in activation of the P*gst1* promoter. It is activated weakly after inoculation with water and with the hrpN-W and dspE/A mutants of *Erwinia amylovora* (22% and 42% respectively, of the GUS activity obtained after treatment with *E. amylovora* wild type). This suggests that the pathogenicity factors harpin N and W, and DspE encoded by these genes are essential for induction of this promoter. When inoculated with these bacterial mutants, *PCaMV35S-gusA* transgenic lines showed no significant difference in GUS activation (data not shown).

Spatial and temporal expression of Pgst1 *promoter in response to fungal inoculation*

In tobacco the *Pgst1*promoter shows local and rapid activation in response to infection by *Phytophthora infestans*, which causes an incompatible reaction (Martini et al., 1993). Experiments were done to confirm that this promoter shows the same pattern of spatial induction in apple. GUS activity was measured in the inoculated leaf and also in upper and lower untreated leaves of the selected *Pgst1-gusA* transgenic lines. The *Pgst1* promoter showed maximum activity in the untreated lower leaves 10 days after inoculation with *V. inaequalis* (Figure 3). This activity was almost two fold more than that obtained in the inoculated leaves in the *Pgst1-gusA* transgenic M.26 line T26-986. No GUS activity was detected in the upper leaves of the *Pgst1-gusA* transgenic M.26 line T26-986. No difference in GUS activity was detected in the treated and neighboring untreated leaves of the *Pgst1-gusA* transgenic RG line TRG-1047 (Figure 3). The *PCaMV35S-gusA* transgenic lines showed stable expression of GUS activity in treated and untreated leaves throughout the duration of the experiment (data not shown).

Effect of salicylic acid concentration on the activation of the Pgst1 *promoter*

The presence of a phytohormone responsive cis element in the *Pgst1* promoter was sought by spraying salicylic acid on the surface of transgenic leaves. Salicylic acid weakly increased GUS activity in the *Pgst1-gusA* transgenic M.26 line T26-986 during the first 12 h post inoculation. During these first 12 h no difference of induction could be observed after a treatment with two concentrations of SA (5 and 50 mM). A strong increase in GUS activity appeared in the transgenic M.26 line 24 hours after treatment. No difference in GUS activity was observed in the *PCaMV35S-gusA* line after these treatments (data not shown).
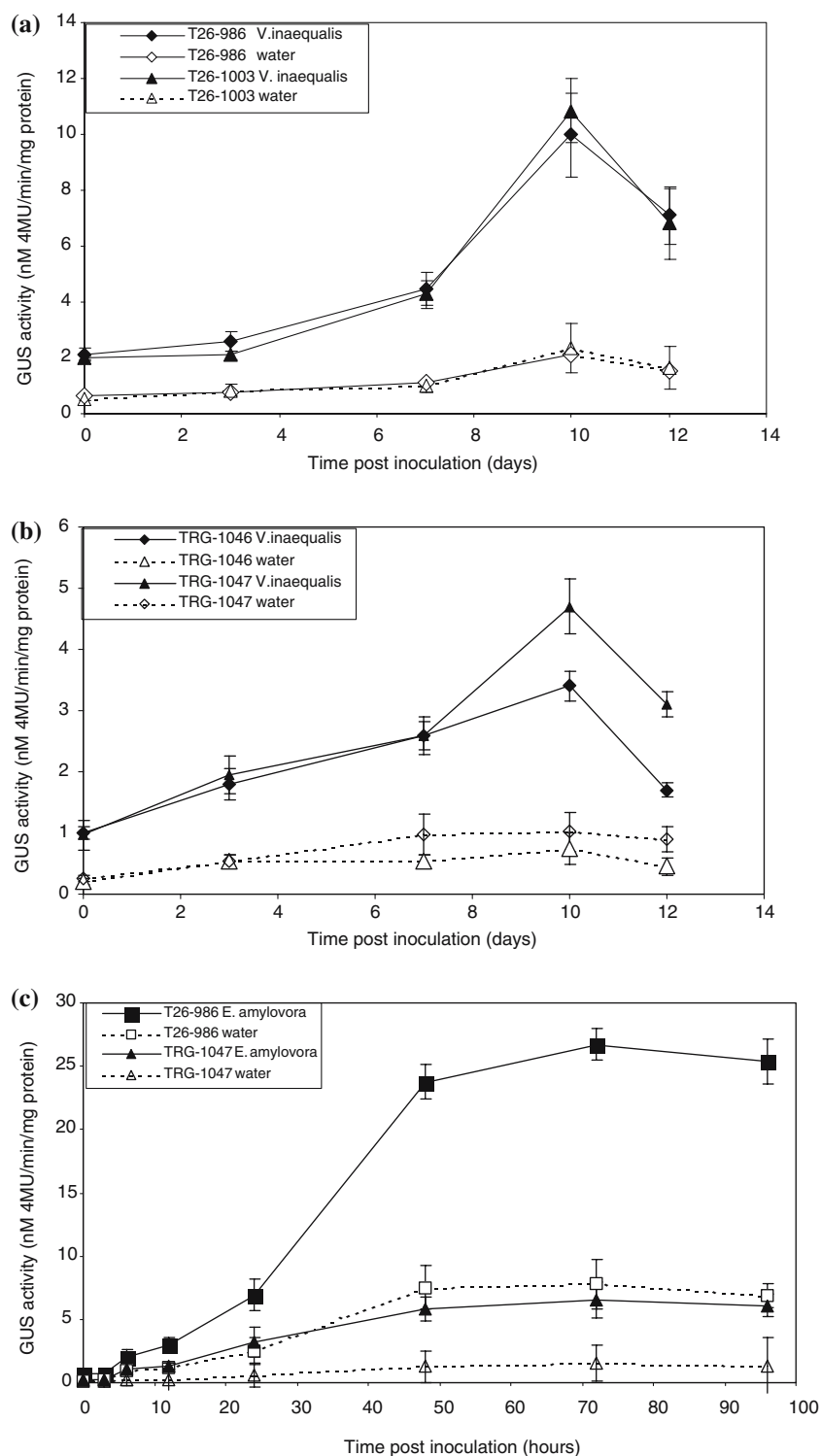
Figure 2. Time courses of activation of promoter *Pgst1* fusions in leaves of representative transgenic apple lines, M.26 (T26.986 and T26.1003) (a and c) or Royal Gala (TRG.1046 and TRG.1047) (b and c) inoculated with *Venturia inaequalis* (a and b) or with *Erwinia amylovora* (c). Each datum point represents the mean of 6 replicates obtained from 3 separate experiments. Bars represent confidence interval at $\alpha = 0.05$.
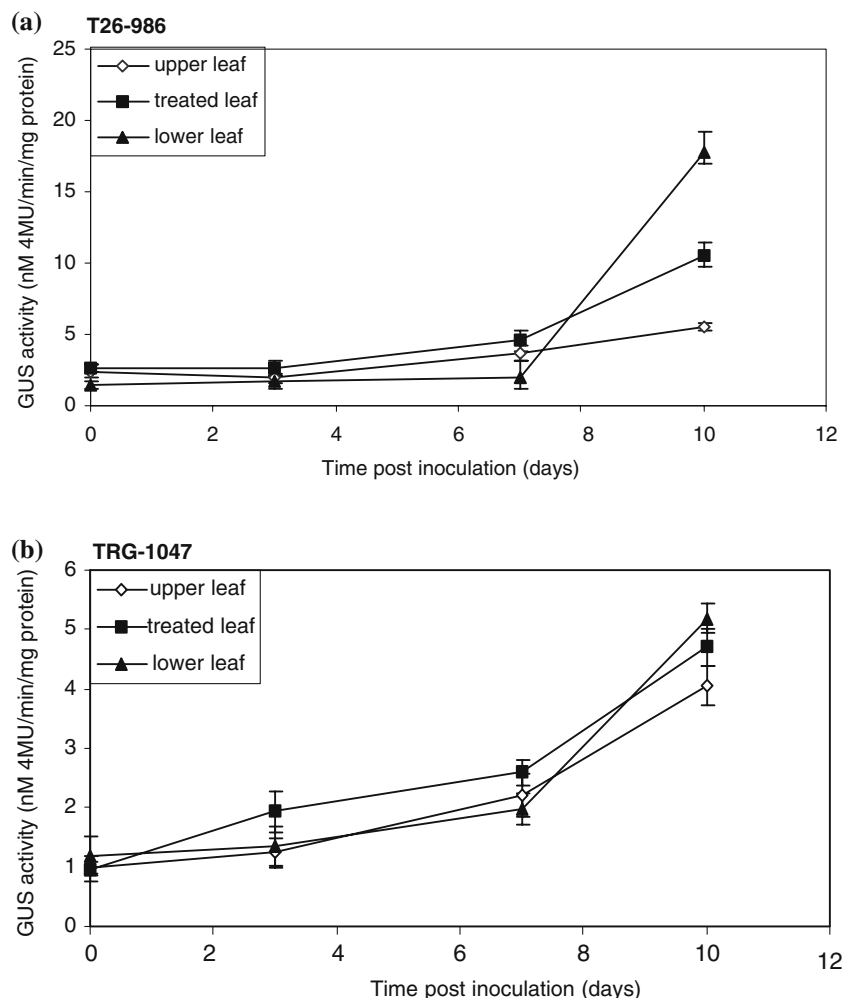
Figure 3. Time courses of *Pgst1-gusA* activation in the upper and lower leaves of representative transgenic apple lines M.26 (a, T26.986) or Royal Gala (b, TRG.1047) inoculated with *V. inaequalis*. Each datum point represents the mean of 6 replicates obtained from 3 separate experiments. Bars represent confidence interval at $\alpha = 0.05$.

Discussion

Genetic engineering is a potentially powerful tool for creating disease resistant plants, thereby offering an alternative method of disease control, or in some cases control of a disease inadequately controlled at present. Researchers have identified numerous plant and pathogen genes that can be used to increase crop resistance toward invading pathogens. These strategies involve both expression of gene products toxic to certain pathogens, and also enhancement of the plant's own natural defense mechanism. Such introduced genes usually are placed under the control of strong promoters, such as the *PCaMV35S* promoter which yields

constitutive expression of the gene product in all plant tissues at all stages of growth. Use of a promoter that allowed restricted expression of the desired gene product exclusively at the sites of pathogen invasion would be a preferred effect in many cases. Ideally, expression of the gene product would be limited to cells surrounding infection sites and would not occur in other parts of the plant.

In order to improve fire blight resistance of apple cultivars without undesirable effects on potentially beneficial plant-associated bacteria or risk to human health, we have undertaken a search for this type of promoter. Knowledge of genes involved in plant-pathogen interactions is still very

limited in the case of fire blight, and no homologous promoters have been characterized, with the exception of the promoter *PYPR10* in apple which is inducible by fungal and abiotic stress (Pühringer et al., 2000). We therefore selected the *Pgst1* promoter from potato (also referred as *Pprp1-1*) (Martini et al., 1993) and designed experiments to evaluate its performance in apple using the *gusA* reporter gene, prior to using it to control the expression of a functional transgene.

In this study, we produced a population of transgenic lines of two genotypes of apple in which the expression of the *gusA* gene was driven by the constitutive *PCaMV35S* promoter and the inducible *Pgst1* promoter. Activation was measured by GUS assay in 5 or 6 transgenic lines for each genotype and construct. A high level of variability was observed in the M.26 and Royal Gala transgenic lines with the *Pgst1* promoter. The range of variation observed for the *PCaMV35S* promoter was similar to the variation of *gusA* expression under the same promoter in transgenic apple (Pühringer et al., 2000) but the level of expression of the *gusA* gene was more than six times higher in our experiments. This difference can be explained by the different vectors that we and Pühringer et al., 2000 used. Several hypotheses could explain the high variability of expression of the *gusA* gene between the different lines, including the influence of the number, position and integrity of the transgene insertion. Therefore, in such a study, the individual transgenic lines selected for more detailed promoter analysis should be chosen carefully. In this work we have selected two transgenic lines with strong GUS expression, avoiding the extreme levels of GUS activity.

The pattern of expression of the *Pgst1-gusA* fusion in apple revealed that the *Pgst1* promoter is functional in apple and retains most of its typical features of expression in potato. In fact, it conserved a similar pattern of induction compared to that found by Martini et al. (1993) and Strittmatter et al. (1996) in potato. As in potato, this promoter was not activated by wounding, but it was activated by different pathogens (bacterial and fungal) in apple. Furthermore, we demonstrated that the kinetics of activation of the *Pgst1* promoter differed following the pathogen study. It reached a maximum 48 h and 10 days after inoculation with a bacterium (*E. amylovora*) and with a

fungus (*V. inaequalis*), respectively, in the two genotypes of apple studied. At that time, GUS activity differed between the two genotypes of apple and also between the treatments with the pathogens. The genotype M.26 had GUS activity that was 2.5 fold higher than that of the genotype Royal Gala regardless of the pathogen inoculated. Similar results were obtained following inoculation with *E. amylovora* and *V. inaequalis*. GUS activity was 1.7 fold higher after inoculation with *E. amylovora* than the activity observed after inoculation with *V. inaequalis*, and this difference was consistent between the two genotypes of apple.

Our study in apple also provided evidence of the systemic induction of the *Pgst1* promoter following infection with a mixture of five virulent races of the fungus *V. inaequalis*. *GusA* expression in neighboring upper and lower non-treated leaves closely followed the expression in the treated leaf. A systemic signal that directly activates the *Pgst1* promoter appears to be involved in the two genotypes of apple. This result correlates with the induction of this promoter by salicylic acid and with the low level of systemic induction previously reported in potato after inoculation with *Phytophthora infestans* (Martini et al., 1993).

Our study provided detailed insight into the role of various pathogenicity regions of the *E. amylovora* genome. Taking advantage of a panel of well characterized mutants of *E. amylovora* provided by S.V. Beer (Cornell University, Ithaca, NY), we were able to study the differential response of this promoter to several gene products. The response of this promoter to treatment with an *E. amylovora hrp* secretion mutant was almost identical to the response to water treatment. This indicates that a functional secretory *hrp* system is necessary for the activation of this promoter in apple. Treatment with mutant strains unable to synthesize either harpin N and W or DspE/A protein clearly resulted in lower activation of the *Pgst1* promoter than the treatment with wild type *E. amylovora*. Thus, these pathogenicity factors, which are secreted via the *hrp* system, play a complementary role in the activation of the *Pgst1* promoter. These two pathogenicity factors were also reported to play an important role in the activation of two other inducible promoters in pear (Malnoy et al., 2003b). Recently, Venisse et al. (2003) have demonstrated the important role of these factors in the induction of fire blight infection in apple.

Taken together, our data indicate that the potato promoter *Pgst1*, is functional in apple and exhibits an induction pattern similar to that observed in potato. It is interesting to note that it is not the first time that a promoter isolated from another botanical family was functional in the *Rosaceae*. Indeed, the *str246C* and *sgd24* promoters isolated from *Solanaceae*, are functional in pear (*Rosaceae*), which is botanically quite distant from *Solanaceae* (Malnoy et al., 2003b). But cross functionality is not true of all pathogen inducible promoters isolated from the *Solanaceae*. In fact, the *hsr203J* promoter isolated from tobacco was weakly functional in the *Rosaceae*, apple (Reynoird et al., 2000) and pear (Malnoy et al., 2003b).

The *Pgst1* promoter could be used to drive the expression of transgenes for bacterial and fungal disease resistance, as was done with the harpin gene in potato (Li & Fan, 1999), albeit with some potential drawbacks. Its systemic activation in apple could be beneficial, as it may help the plant to mount an active defense in advance of bacterial and fungal spread in the tissues. However, this fact may cause unwanted accumulation of the transgene product in fruits. The average level of *gusA* expression by the *Pgst1* promoter in response to bacterial and fungal induction is much lower (15% and 8% respectively) than the constitutive expression driven by the *PCaMV35S* promoter in apple. Thus, transgenes with a high efficacy in combating *E. amylovora* or *V. inaequalis* at low levels will be required. Another strategy will be to define synthetic plant promoters that directly control local gene expression in response to pathogens. Pathogen inducible plant promoters contain multiple *cis*-acting elements which are responsible for the induction patterns of the promoter, but only some of these may contribute to pathogen inducibility. Therefore, a synthetic promoter containing specific cis-acting elements can prevent a local and elevated gene expression after a pathogen attack. Using a range of *cis*-acting elements (boxes W1, W2, GCG, JERE, S, GST1, and D), Rushton et al. (2002) were able to develop different synthetic plant promoters that produced varied patterns of induction after treatment with compatible or incompatible pathogens and abiotic stress. Some of these promoters showed an expression higher than the expression obtained with the original vector.

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