Research Note

# Infections with Various Types of Organisms Stimulate Transcription from a Short Promoter Fragment of the Potato *gst1* Gene

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**By histochemical GUS staining, we demonstrate that transcription from a short promoter fragment of the potato *gst1* gene is locally induced after infection of a host plant with various types of pathogenic or symbiotic organisms. This regulatory unit is not active in noninfected tissues, except root apices and senescing leaves. Measuring the expression of a fusion between the promoter fragment and the *gus* gene in transgenic plants, therefore, allows comparison of the induction of defense reactions in different types of plant-microbe interactions, in one and the same plant.**

*Additional keyword:* pathogen defense–related gene.

The analysis of cellular processes leading to the induction of defense responses depends on markers specific for the infection of a host plant with potentially pathogenic microorganisms. Several molecular responses are rapidly and locally induced in plants upon infection by pathogens. These include the activation of defense genes (Hahlbrock and Scheel 1989; Linthorst 1991), the deposition of callose (Cuypers and Hahlbrock 1987) or modifications in photosynthetic electron transport (Koch et al. 1994). However, most of these reactions are not restricted to plant/microbe interactions, and only a few comparative studies have been performed addressing the question whether and how such responses are induced in interactions between a same host plant and different infecting agents, including both pathogenic and symbiotic organisms (Gianinazzi-Pearson et al. 1994). Molecular markers, such as the transcriptional activation of promoter GUS-gene fusions, indicative for the stimulation of defense reactions in a broad spectrum of interactions, would facilitate the comparison of signal transduction processes activated in response to different types of organisms.

The potato defense gene *gst1* (formerly called *prp1-1*) encodes a glutathione *S*-transferase (Hahn and Strittmatter 1994). A 273-bp promoter fragment of this gene (positions −402 to −130) has previously been shown to mediate rapid and local transcriptional activation in response to infection with the late blight fungus *Phytophthora infestans* (Mont.) de Bary (Martini et al. 1993). Transcription from this promoter fragment is not induced by abiotic stimuli, such as wounding, light/dark switches, or heat shock. To further characterize the transcriptional activation pattern mediated by the 273-bp fragment, we have now tested its activity in noninfected tissues and its responsiveness to infection with various types of pathogenic and symbiotic organisms.

### Activity in noninfected tissues.

Two different promoter fragments of the potato *gst1* gene were fused to the *Escherichia coli uidA* (*gus*) gene. A longer one, covering positions −1521 to +31, was inserted into the vector pPTVgus (Martini et al. 1993) giving the construct PG150; a shorter one, from positions −402 to −130, was inserted upstream of the CaMV 35S TATA-box region (positions −46 to +8) of the vector pETVgus (Martini et al. 1993) giving the construct EG27. After transformation of potato cv. Désirée, various independent transgenic lines were regenerated harboring one of the two chimeric constructs. In previous investigations, these lines had been used to test the transcriptional inducibility of the chimeric constructs in response to infection with *P. infestans* (Martini et al. 1993; Hahn and Strittmatter 1994). In the present work, the level of expression in noninfected tissues was assayed in two of the transgenic lines, PG150-7 and EG27-04, exhibiting a high

induction rate after *P. infestans* infection. As a control, potato plants harboring only the *gus* gene (construct PG0 in Martini et al. 1993) or the *gus* gene in combination with the CaMV 35S TATA-box region (–46 to +8) (construct EG0 in Martini et al. 1993) were analyzed. Various types of organs were detached from greenhouse grown plants, vacuum infiltrated with a solution consisting of 100 mM sodium phosphate (pH 7) and 0.5 mg of X-Gluc (5-bromo-4-chloro-3-indolyl β-D-glucuronide) per ml, and incubated for 16 h at 37°C. Afterwards, leaves were cleared in ethanol. From leaves, stems, shoot apices, and various flower parts, semithin sections were prepared after Historesin embedding, which was performed following the instructions of the manufacturer (Jung, Heidelberg, Germany); roots were analyzed as whole pieces. In transgenic lines harboring the *gst1/gus* fusions, GUS enzyme activity was only detectable in roots and senescing leaves (Table 1). About 44% of root apices from 4-week-old cuttings of EG27-04 plants showed GUS enzyme activity. Light GUS staining was also sometimes detectable in the central cylinder of roots, in addition to the intense GUS staining in root apices. The same GUS staining pattern was found in roots from axenically grown plants (Fig. 1), excluding the possibility that contact with microorganisms caused the expression of the chimeric *gst1/gus* construct. In some rare cases, GUS enzyme activity was also observed at sites of lateral root formation. No GUS staining was found in any tissue of transgenic lines harboring the EG0 or PG0 constructs. As identical expression patterns were found with the 273-bp and 1.5-kb promoter fragments, we conclude that this pattern is an intrinsic feature of the intact *gst1* promoter and not the result of deleting *cis*-acting elements during the generation of the 273-bp fragment.

### Responsiveness to viral infection.

Potato virus Y (PVY), a single-stranded RNA virus representing the type member of the group of potyviruses, is transmitted either by mechanical inoculation or by aphids, and its host range is limited mainly to *Solanaceae*. On potato, the common O strain, PVY$^O$, causes severe necrosis, whereas the veinal necrotic strain, PVY$^N$, induces comparatively mild symptoms characterized by a vague mottling on leaves (De Bokx and Huttinga 1981). These two strains (kindly provided by H.-L. Weidemann, Biologische Bundesanstalt, Braunschweig) were applied to infect a line of potato cv. Bintje carrying the EG27 construct. In these experiments, potato cv. Bintje was used because cv. Désirée is known to exhibit a high level of field resistance to PVY infection. Plants from one transgenic line in which transcription of the EG27 construct had previously been shown to be responsive to fungal attack were mechanically infected with a 1:5 diluted inoculum prepared from PVY-infected *Nicotiana tabacum* L., as described by Fang and Grumet (1993). Leaves of five plants were harvested 1 day and 3 days after inoculation and analyzed for GUS enzyme activity, according to Ashfield et al. (1994). The responsiveness of *gus* expression to PVY$^N$ and PVY$^O$ is illustrated in Figure 2 A to D. No GUS enzyme activity was detectable 1 day after inoculation with either of the virus strains (Fig. 2A, C), but after 3 days, infection with these strains resulted in the formation of blue spots indicating GUS expression (Fig. 2B, D). The pattern of GUS activity paralleled the spread of PVY in the leaves, detected by hybridization of leaf squashes with PVY-specific RNA-probes

(data not shown). No GUS activity was found in mock-inoculated leaves.

Multiplication of potato leaf roll virus (PLRV), a luteovirus, is limited to the phloem (Waterhouse et al. 1988). Infection causes leaf rolling and chlorosis. No qualitative differences in symptom expression occur depending on the type of isolate infecting a plant (Hooker 1983). The responsiveness of the 273-bp *gst1* promoter fragment to PLRV infection was studied in the transgenic line EG27-04 of potato cv. Désirée. Plants were graft-inoculated with PLRV, and tubers from these plants were then regrown to obtain leaves from secondarily infected plants (Barker et al. 1992). Lower leaves of plants showing clear symptoms of virus infection were histochemically assayed for GUS enzyme activity (Ashfield et al. 1994). Blue staining was restricted to the vascular system of the leaves (Fig. 2F), indicating that the activity of the *gst1* promoter fragment was strictly limited to the tissue in which the virus multiplied. GUS activity in the vicinity of necrotic areas of leaves from mock-inoculated EG27-04 plants (Fig. 2 E) is very likely due to senescence. No GUS-staining was observed after viral infection of potato plants harboring the construct EG0.

### Responsiveness to nematode infection.

Potatoes are attacked by cyst nematodes of the genus *Globodera* and root-knot nematodes of the genus *Meloidogyne*. After penetration of roots, cyst nematodes migrate intracellularly and cause extensive necrotic lesions, whereas root-knot nematodes migrate intercellularly, not leading to similar tissue destruction (Sijmons et al. 1994). Both types then induce the formation of sophisticated, hypertrophic feeding cells on which they fully depend until the completion of their life cycle. After infection with root-knot nematodes, the host cells surrounding the feeding cells start to divide, giving rise to a typical root-knot or gall.

Internodes of the in vitro propagated potato line EG27-04 (cv. Désirée) or a control line harboring construct EG0 were put onto B5 medium. One week later, newly formed roots were inoculated with about 20 larvae per root, either from surface-sterilized *Globodera pallida* (Stone) Behrens (pathotype 3) (Heungens et al., in press) or from monoxenically grown *Meloidogyne incognita* (Kofoid & White) Chitwood (Niebel et al. 1993). Root samples were taken at various time points after inoculation and histochemically assayed for GUS

**Table 1.** Activity of *gst1* promoter fragments in noninfected tissues of transgenic potato plants (cv. Désirée)

| | Histochemical detection of GUS activity | |
| Plant tissue | PG150-7 | EG27-04 |
|---|---|---|
| Cotyledon | – | NT[a] |
| Green leaf | – | – |
| Senescing leaf | + | + |
| Shoot apex | – | – |
| Petiole | – | – |
| Stem | – | – |
| Root | + | + |
| Petal | – | – |
| Anther | – | – |
| Pollen | – | – |
| Ovule | – | – |

[a] Not tested.

enzyme activity, as described above for noninfected tissues. For each time point, a total of about 50 roots were inoculated and analyzed, in comparison with noninfected control roots. After placing the samples on slides, they were observed and photographed under the microscope.

Noninfected roots of EG27-04 plants revealed the same GUS staining pattern as described in Figure 1. Three days after inoculation with *G. pallida,* GUS enzyme activity started to increase in roots from these plants; the GUS staining reached its maximum approximately 4 days after inoculation (Fig. 3A), and stayed at this level until about 1 week after inoculation. The indigo blue accumulated in tissues surrounding the necrotic lesions that occurred at the penetration and migration sites of the nematode. Semithin sections of infected roots indicated that the GUS staining was localized in the cortex of roots, whereas the epidermal cell layer and the stele exhibited no GUS enzyme activity (Fig. 3B). Inoculation of roots from EG27-04 plants with *M. incognita* resulted in a different GUS staining pattern. Only in a few cases, small regions of GUS enzyme activity were observed in the center of galls, 4 days after inoculation (Fig. 3C). The roots of potato plants harboring the EG0 construct did not show any sign of GUS staining, irrespective of the type of nematode used for infection.

Only a few pathogen defense–related plant genes, such as genes encoding extensin (Niebel et al. 1993) or catalase (Niebel et al. 1995) from tobacco, have previously been

shown to be activated by nematode infection. Transcription of the chimeric *gst1/gus* gene construct is strongly activated in the vicinity of necrotic lesions induced during early stages of *Globodera* infection. Possibly, this response is mediated by



Fig. 2. Promoter activity in virus-infected leaves. Leaves from a transgenic line of potato cv. Bintje transformed with the EG27 construct were mechanically inoculated with potato virus Y (PVY) strain N (**A** and **B**) or PVY strain O (**C** and **D**). Leaflets were harvested 1 day (**A** and **C**) or 3 days (**B** and **D**) after inoculation, infiltrated with the GUS substrate X-Gluc and then cleared in ethanol. Tubers from mock-inoculated (**E**) or potato leaf roll virus (PLRV)–inoculated (**F**) transgenic potato line EG27-04 were regrown to obtain secondarily infected plants; leaves from these plants were harvested when leaf rolling and chlorosis occurred on infected individuals. GUS activity was checked by incubation in X-Gluc followed by destaining in ethanol.
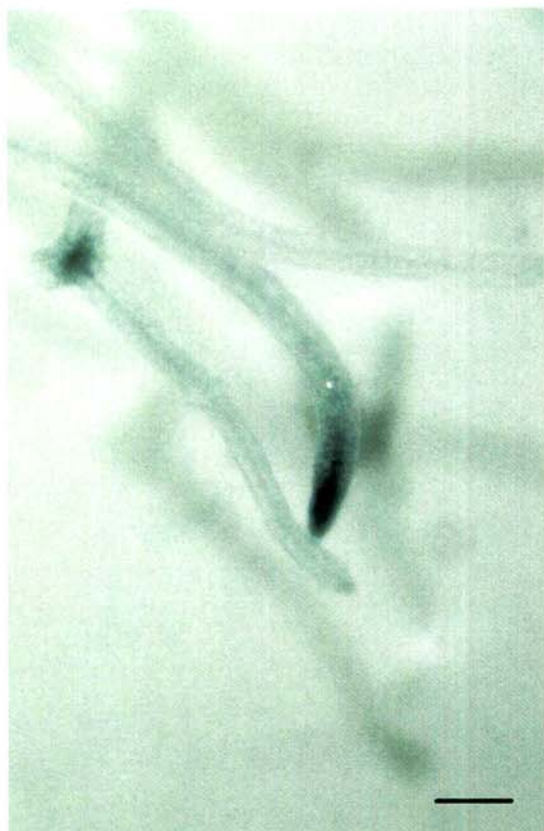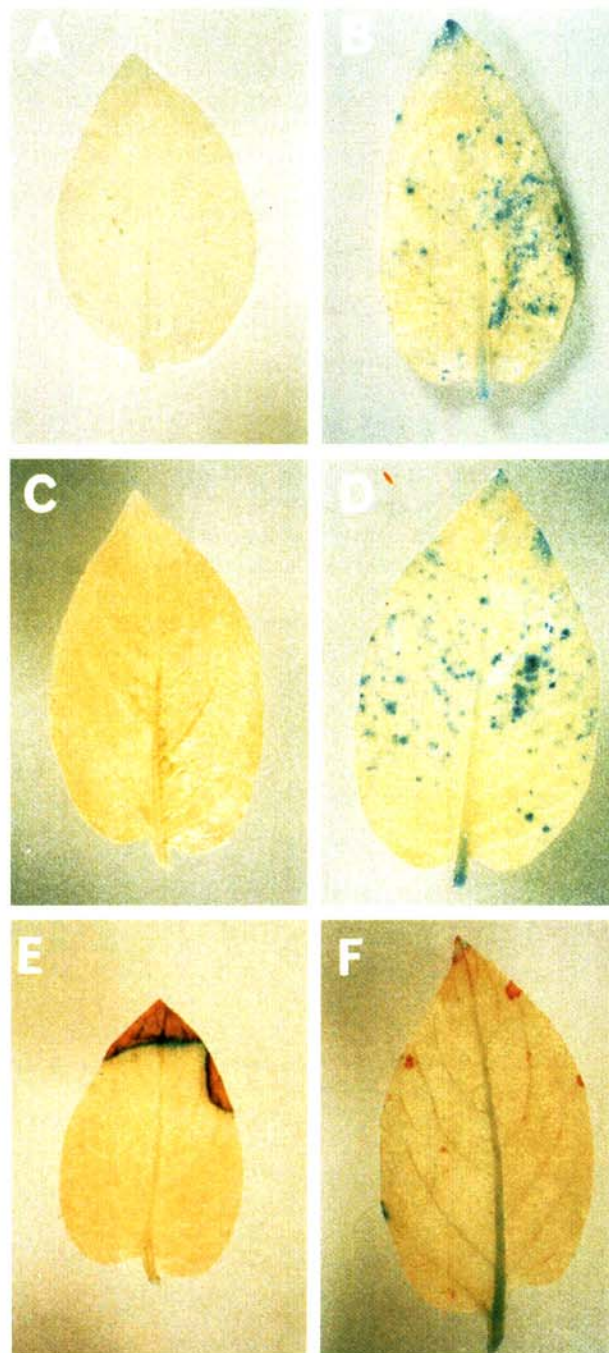


Fig. 1. *gst1* promoter activity in noninfected roots. Roots from axenically grown transgenic potato line EG27-04 were incubated overnight with the GUS substrate X-Gluc. Activity of the GUS enzyme is indicated by the deposition of blue indigo. Bar = 220 μm.

ethylene, which has previously been demonstrated to accumulate in cyst nematode–infected oat roots (Volkmar 1991). Interestingly, almost no response of the promoter fragment is detectable after *Meloidogyne* infection. The nondestructive migration mechanism of *Meloidogyne* larvae might allow this pathogen to partly prevent the activation of defense reactions in the host plant.

## Responsiveness to mycorrhization.

Plant genes regulating symbiotic interactions between glomalean fungi and roots have yet to be identified, although evidence for their existence has been provided through plant mutants defective in their ability to form the arbuscular mycorrhiza association (Duc et al. 1989; Gianinazzi-Pearson et al. 1991; Gollotte et al. 1993). The possibility that defense gene activation may play some role in controlling symbiotic fungal development within roots has been investigated, but no clear-cut picture has emerged because of the variability in responses depending on the plant species involved or the gene studied (Gianinazzi-Pearson et al. 1992; Lambais and Mehdy 1993; Harrison and Dixon 1994; Franken and Gnädinger

1994). However, the overall conclusion that can be drawn is that in each case some of the defense genes show no change in expression, while others are only weakly or transiently elicited. Localization of PR-1, an antifungal protein in tobacco (Alexander et al. 1993), or mRNA encoding enzymes involved in the flavonoid/isoflavonoid pathways in alfalfa, suggests that this may be due to a very localized plant response that is limited to the parenchymal cortical cells harboring the intracellular haustoria, arbuscules (Gianinazzi-Pearson et al. 1992; Harrison and Dixon 1994).

The transgenic potato line EG27-04 was used to study the activation of the chimeric *gstl/gus* construct in potato roots during arbuscular mycorrhiza development by the glomalean fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe (BEG 12). Two independent experiments were performed, one using potato sprouts as starting material and the other rooted cuttings of potato shoots. Both were planted into plastic pots containing 300 g of an α-irradiated (10 Gy) clay loam soil (pH 7.8, 20 ppm Olsen P) mixed with 100 g of a sieved (5 mm) soil-based inoculum (containing spores, hyphae, and
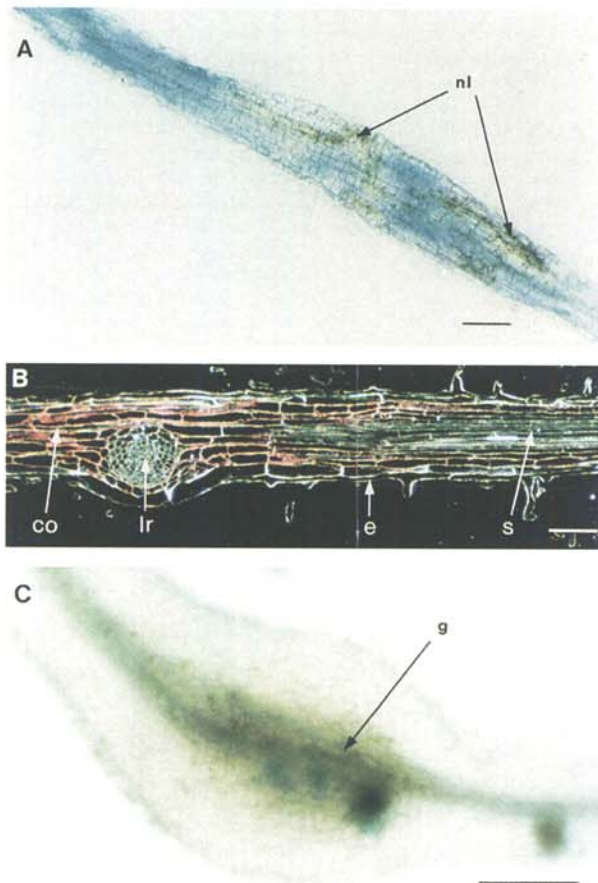
**Fig. 3.** Promoter activity in nematode-infected roots. Root samples were harvested 4 days after inoculation of EG27-04 plantlets with larvae of *Globodera pallida* (Pa3) (**A** and **B**) or *Meloidogyne incognita* (**C**). GUS enzyme activity was detected by incubation in the substrate X-Gluc. (**A** and **C**, Micrographs of whole root pieces. **B**, The dark field micrograph of a longitudinal root section; GUS staining is indicated by red color. co, cortex; e, epidermis; g, gall; lr, lateral root; nl, necrotic lesion; s, stele. Bars = 100 μm.
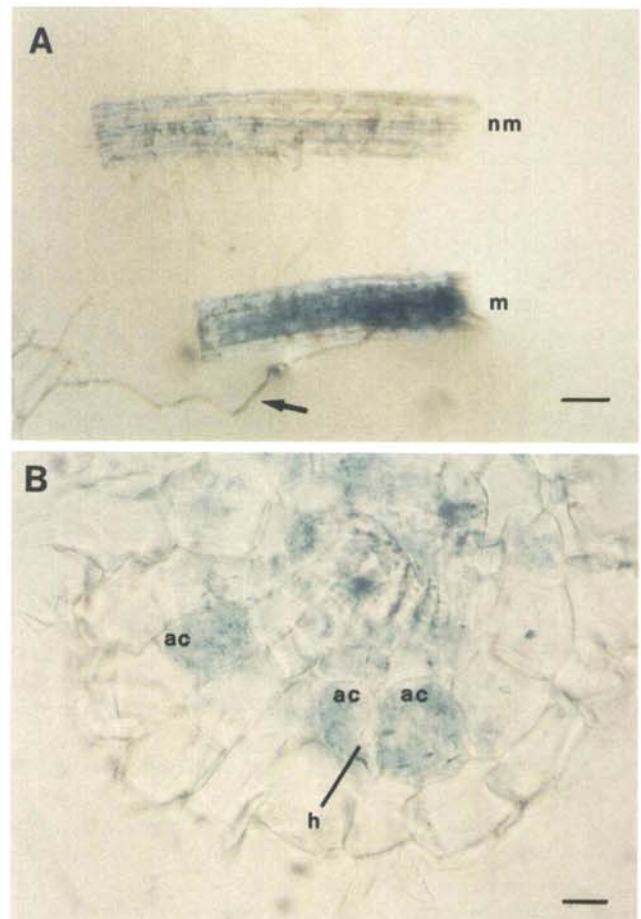
**Fig. 4.** Promoter activity in response to mycorrhization of roots. **A,** Histochemical GUS staining of roots from transgenic potato line EG27-04. Light GUS staining is detectable in the central cylinder of nonmycorrhizal root portions (nm) and intense GUS staining in the *G. mosseae*-infected portions (m). External fungal hyphae do not stain (arrow). Bar = 150 μm. **B,** Section of a GUS-stained *G. mosseae*–infected root showing GUS activity in parenchyma cortical cells containing arbuscules (ac). h, fungal hypha. Bar = 17 μm.

infected root pieces) of *G. mosseae*. As a control, transgenic sprouts or cuttings were planted into 400 g of irradiated soil only. Identical experiments were carried out with plants harboring the EG0 construct. Pots were randomized in a constant environment chamber (300 $\mu$E s$^{-1}$ m$^{-2}$, 16-h photoperiod, 18 to 20°C, 70% relative humidity), watered daily with reverse-osmosed water and once weekly with a modified (P/10) Long Ashton solution. Plants were harvested 6 weeks after root development began for sprouts and after 4 weeks growth for cuttings. Root samples were taken for estimating mycorrhizal infection (Trouvelot et al. 1986) and histochemical staining for GUS enzyme activity. GUS staining was performed with 1-cm-long detached root pieces according to Couteaudier et al. (1993). Observations for GUS staining were made by light microscopy on whole root pieces and fresh root sections.

Mycorrhizal infection reached 20 to 25% of the root system, irrespective of the type of transformant. No GUS activity occurred in the cortex of uninoculated roots from EG27-04 plants, but it was clearly associated with root regions colonized by *G. mosseae* in inoculated plants (Fig. 4A). About 60% of infected root pieces showed strong staining, 30% weaker, and 10% none. No GUS activity was found in external living fungal hyphae infecting roots (arrow in Fig. 4A), and no signs of GUS staining were detectable in roots from plants transformed with the EG0 construct. Cross sections of stained mycorrhizal roots revealed that the GUS activity was only associated with parenchyma cortical cells containing hyphae of *G. mosseae* and was completely absent from uncolonized cells (Fig. 4B).

The localization of GUS activity in root tissue colonized by the symbiotic fungus *G. mosseae* indicates transcriptional activation of the chimeric *gst1/gus* construct, with activity of the *gst1* promoter fragment limited to infected cells. This contrasts with the findings after infection with pathogenic fungi or nematodes. In these cases, transcriptional activity of the chimeric *gst1/gus* construct was also located in the vicinity of infected host cells, indicating a difference in gene activation between symbiotic and pathogenic interactions. The present observations on *gst1* promoter activation during arbuscular mycorrhiza formation provide further evidence that symbiotic fungi can elicit a very localized defense response in infected host cells.

**General conclusions.**

The 273-bp *gst1* promoter fragment is sufficient for transcriptional activation in response to infection of potato with different types of organisms. This feature, in combination with the lack of activity in most noninfected tissues, suggests that measuring the expression of a fusion between the 273-bp promoter fragment and the GUS gene allows comparison of signaling processes in different types of plant-microbe interactions, using one and the same host plant. Furthermore, the 273-bp regulatory region might be applicable in a variety of genetic engineering approaches either aiming at the generation of conditional mutations or at the protection of plants against pathogen attack.

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