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Infection and Stress Activation of Bean Chalcone Synthase Promoters in Transgenic Tobacco

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Chalcone synthase (CHS) catalyzes a key regulatory step in the synthesis of pterocarpan phytoalexins that is characteristic of many legumes. The 5′-flanking sequences from two bean *CHS* genes, *CHS8* and *CHS15*, were each fused upstream of the coding region of the β-glucuronidase (*GUS*) reporter gene and transformed into tobacco. Shortwave UV light or 1 mM $HgCl_2$ induced the expression of both gene fusions in transgenic tobacco leaves. The response to UV irradiation occurred within 6 hr and was more rapid than the response to Hg. Increased GUS activity was restricted to tissue in the immediate vicinity of localized treatments with these inducers. Application of 100 mM oxalate, a treatment that induces disease resistance in cucumbers, or infiltration of an incompatible isolate of *Pseudomonas syringae* pv. *syringae* induced the expression of the *CHS8-GUS*, but not the *CHS15-GUS* gene fusion. Both oxalate and *P. s.* pv. *syringae* induced the *CHS8* promoter in tissues 30–40 mm from localized sites of application, indicating the involvement of a secondary signal. These data imply the operation of several distinct mechanisms for stress activation of defense genes. These mechanisms are conserved between tobacco and bean such that the bean *CHS* promoters are induced even though CHS plays no role in phytoalexin biosynthesis in tobacco.

Additional keywords: abiotic stress, *Nicotiana tabacum*, promoter analysis.

Chalcone synthase (CHS, EC 2.3.1.74) catalyzes the first reaction in a branch pathway of phenylpropanoid metabolism leading to the synthesis of flavonoid compounds (Dixon *et al.* 1983; Hahlbrock and Scheel 1989). These compounds play important roles in plants as UV protectants, flower pigments, phytoalexins, and inducers of the expression of nodulation genes in *Rhizobium* (Peters *et al.* 1986; Redmond *et al.* 1986; Hahlbrock and Scheel 1989). cDNA sequences complementary to CHS mRNA have been cloned from bean (*Phaseolus vulgaris* L.) cells (Ryder *et al.* 1984) and used to isolate genomic clones, allowing examination of the organization and expression of *CHS* genes in bean. There is a family of seven *CHS* genes per haploid bean genome, and infection with *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib., treatment with fungal elicitor or light, or wounding causes differential accumulation of transcripts encoded by members of this family (Ryder *et al.* 1987). Analysis of *in vitro* transcription by isolated bean nuclei has shown that transcriptional activation of *CHS* genes initiates the accumulation of CHS mRNA in elicited cells and in wounded or infected hypocotyls (Lawton and Lamb 1987). Transient expression of a bean *CHS* promoter-reporter gene construct in electroporated soybean and alfalfa protoplasts has indicated that the sequences necessary for induction by the elicitor reside within 326 base pairs (bp) upstream of the transcriptional initiation site of the gene *CHS15* (Dron *et al.* 1988; Choudhary *et al.* 1990).

The object of this study was to define the inducibility of two *CHS* promoters in transgenic tobacco and to establish a system to investigate physical and biological agents that activate *CHS* promoter-driven transcription. Gene fusions between the 5′-flanking regulatory sequences of two *CHS* genes (*CHS8* and *CHS15*) and the *GUS* gene region encoding for β-glucuronidase (GUS, EC 3.2.1.31) were constructed and used to transform tobacco. We show that the 5′ sequences of these *CHS* genes are sufficient to specify induction of GUS activity in response to environmental stresses such as wounding and chemical treatments. Furthermore, one of these *CHS* promoters contains sequences that control expression associated with a bacterially induced hypersensitive response.

MATERIALS AND METHODS

Construction of vectors and transformation of tobacco. *CHS8* and *CHS15* are members of a family of seven *CHS* genes in the bean genome (Ryder *et al.* 1987). The structures and nucleotide sequences of these genes will be described elsewhere (S. D. Clouse and C. J. Lamb, unpublished). The *CHS8* and *CHS15* genes both contain a *Dde*I site 1 bp upstream of the translation initiation codon. The *CHS8* promoter, contained in a 1.4-kilobase *Eco*RI-*Dde*I fragment, and the *CHS15* promoter, contained in a 490-bp *Hin*dIII-*Dde*I fragment (Dron *et al.* 1988), were ligated into the *Sma*I site of the promoterless *GUS* expression vector pBI101.1 (Jefferson 1987) after filling in the *Dde*I site. The *CHS8-GUS* and *CHS15-GUS* contructs were verified by sequencing (Sanger *et al.* 1977) over the ligation site using a *GUS*-specific primer (Clonetech, Palo Alto,

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CA). The *CHS-GUS* vector constructs were used to transform tobacco (cv. Xanthi) leaf disks by standard procedures (Horsch *et al.* 1985), and plants were regenerated under kanamycin selection. Of 15 independent *CHS15-GUS* transformants, 14 expressed GUS activity in root tips as a test of correct expression. Three of these were studied in detail and showed appropriate developmental regulation: high GUS activity in the roots, active in petals, and inducible by wounding and $HgCl_2$ (Schmid *et al.* 1990). Two of the three, CHS15-GUS-6 and CHS15-GUS-11, were used in this study. Of 10 independent *CHS8-GUS* transformants, nine showed appropriate regulation as defined above and two, CHS8-GUS-3 and CHS8-GUS-5, were used in this study. Resultant transgenic plants were grown in a greenhouse, prior to analysis for induction of GUS activity after various inducing treatments.

Inducing treatments. Disks (1.5-cm diameter) were cut with a cork borer from fully expanded leaves and placed on moistened filter paper in petri dishes. Leaf disks to be irradiated with UV light were placed abaxial side up and exposed to shortwave UV light in a Chromato-Vue cabinet, model CC-60 (UV Products, Inc., San Gabriel, CA). Irradiation for 2 min (540 $\mu W/cm^2$) was used because this exposure provided the maximum induction of GUS activity. Leaf disks to be treated with 1 mM $HgCl_2$ were placed adaxial side up, and a 10-μl droplet of the treatment solution was applied to the center of the disk. Disks from both treatments were incubated at room temperature in darkness until assayed for GUS activity. To determine if UV light activated the *CHS-GUS* fusion genes at a distance from the treatment site, a portable UV lamp (model UV GL-25, UV Products) covered with cardboard containing a 1.0-cm diameter opening was placed directly on the abaxial side of a tobacco leaf. A small area of leaf tissue was exposed to shortwave UV light for 30 sec while the leaf was still attached to the plant.

Oxalate solutions were prepared by titrating 1 M oxalic acid with KOH to pH 6.1. Various concentrations of oxalate were applied as 25-μl droplets to the adaxial leaf surface of intact tobacco plants. Disks (1.5 cm) centering on the droplet were cut from the leaf at the indicated times and assayed immediately for GUS activity. *Pseudomonas syringae* pv. *syringae* van Hall D20, an isolate pathogenic on wheat, was grown overnight in nutrient broth enriched with glucose (8 g Bacto-nutrient broth [Difco Laboratories, Detroit, MI] and 8 g D-glucose per liter). Approximately 20-μl aliquots of the resulting bacterial suspension ($A_{600} = 0.2$) were infiltrated into leaf panels with a syringe. At the times indicated, 1.5-cm leaf disks centering on the lesions were cut out and immediately assayed for GUS activity. In leaf dissection experiments, the leaves were treated as described above and remained on the plant for 48 hr at which time the leaves were cut into sections and assayed.

Fluorogenic assay for GUS activity. GUS activity in cell extracts was determined with the fluorogenic assay as described by Jefferson (1987). Disks (1.5 cm) or squares (1.27 cm^2) were cut from leaves, and each leaf piece was homogenized in 700 μl of fluorogenic assay extraction buffer (Jefferson 1987). After a brief centrifugation, 20 μl of the supernatant was added to 100 μl of GUS assay buffer containing the fluorogenic substrate 4-methylumbelliferyl β-D-glucuronide (Sigma, St. Louis, MO) and incubated at 37° C for 1 hr. The reaction was stopped by adding

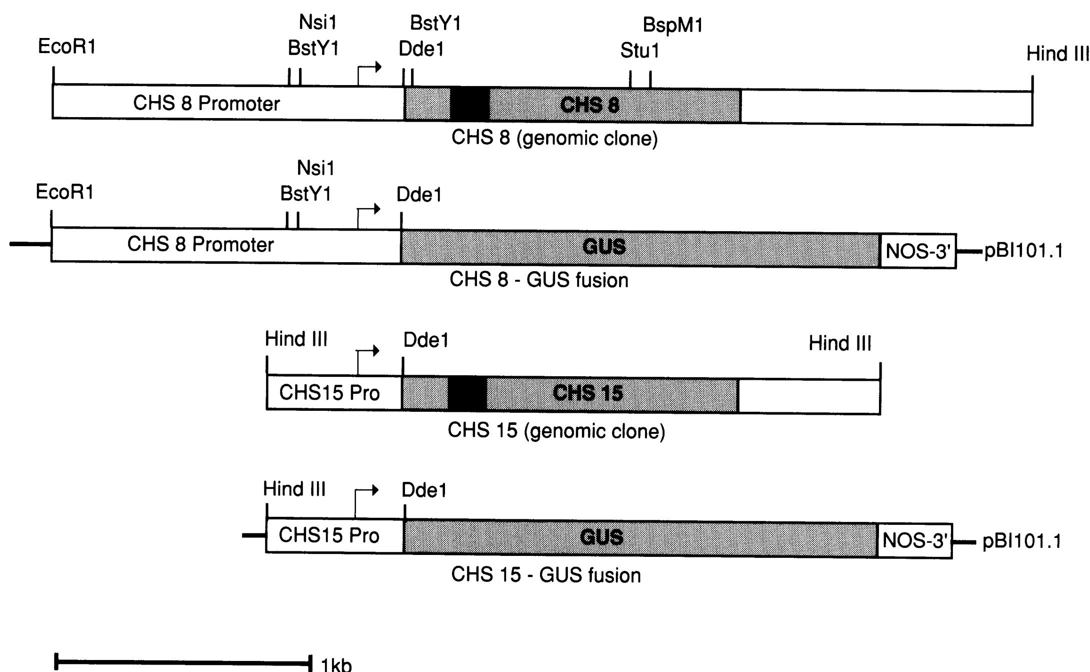

Fig. 1. Restriction maps of the chalcone synthase (*CHS*) genomic clones *CHS8* and *CHS15* and their corresponding promoter fusion with the β-glucuronidase (*GUS*) reporter gene. The *CHS8* promoter, contained in a 1.4-kilobase *Eco*RI-*Dde*I fragment, and the *CHS15* promoter, contained in a 490-base pair *Hin*dIII-*Dde*I fragment, were ligated into the *Sma*I site of the promoterless *GUS* expression vector pBI101.1 after filling in the *Dde*I site.

900 μl of 0.2 M Na_2CO_3. Concentrations of 4-methylumbelliferone were determined with a spectrofluorometer, excitation at 365 nm, emission at 455 nm.

Histochemical staining for GUS activity. Freshly cut leaf pieces were vacuum-infiltrated with 1 mM 5-bromo-4-chloro-3-indolyl glucuronide (X-gluc, Clonetech) in 50 mM Na_2HPO_4, pH 7. The tissue was then incubated at 37° C for 4 to 24 hr as appropriate. For incubations longer than 8 hr, 10 μg/ml of rifampicin was added to the X-gluc solution. Following incubation, the tissues were both cleared and stored in 70% ethanol.

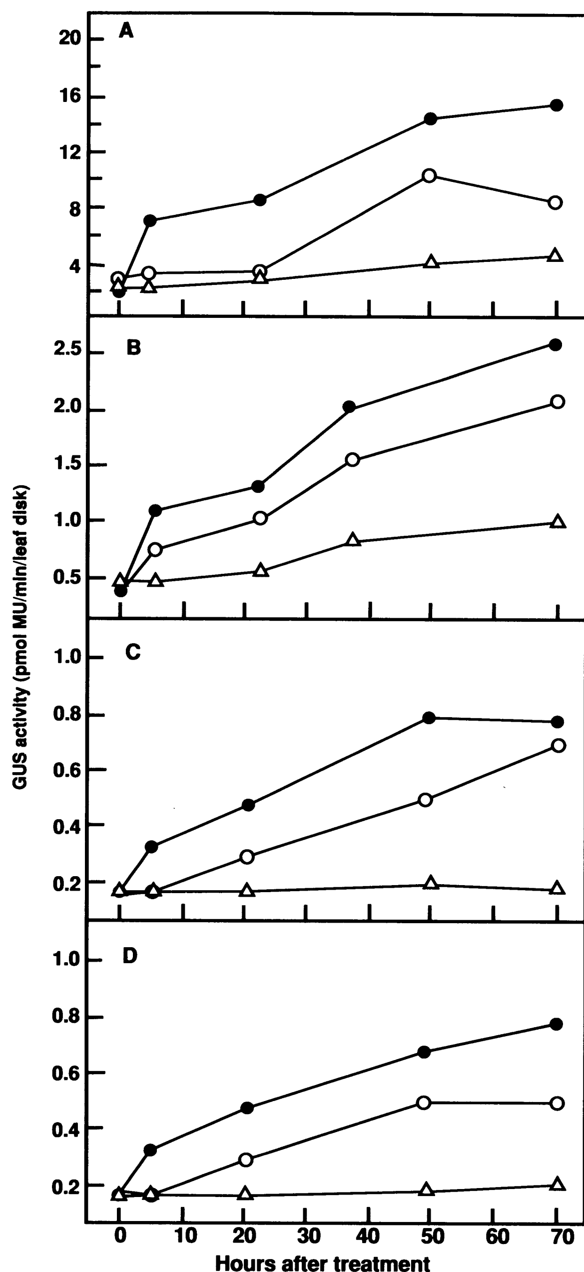

Fig. 2. Expression of chimeric genes in transgenic tobacco tissue after treatment with UV light or Hg. Leaf disks were irradiated with shortwave UV light in a Chromato-Vue cabinet for 2 min (●), treated with 10 μl of 1 mM $HgCl_2$ applied to the center of the disks (○), or treated with 10 μl of water (△). The leaf disks were incubated in petri dishes at room temperature in darkness. β-Glucuronidase (GUS) activity was determined at various times after treatment by the fluorogenic assay. The tobacco transformants examined were (**A**) CHS8-GUS-5, (**B**) CHS8-GUS-3, (**C**) CHS15-GUS-6, and (**D**) CHS15-GUS-11.

RESULTS

Transcriptional fusions. The stress responsiveness of two bean *CHS* promoters was examined by analysis of GUS activity in transgenic plants containing gene fusions in which the *GUS* reporter gene was placed under the control of the 1.4-kilobase *CHS8* or the 490-bp *CHS15* promoter (Fig. 1). A high proportion of kanamycin-resistant *CHS8-GUS* transformants and kanamycin-resistant *CHS15-GUS* transformants showed strong GUS activity in root tips where these *CHS* promoters are known to be highly active (Schmid *et al.* 1990; J. Schmid and C. J. Lamb, unpublished). Of these, the independent transformants CHS8-3 and CHS8-5, containing the *CHS8-GUS* gene fusion, and CHS15-6 and CHS15-11, containing the *CHS15-GUS* gene fusion, were selected for study. The structure of the transcriptional fusions is shown in Figure 1.

Promoter activation by UV light or Hg. Environmental and biological stresses known to elicit plant responses induced the expression of the *CHS-GUS* fusion genes in transgenic tobacco. All plants tested with the *CHS8* or the *CHS15* promoter showed increased GUS activity after treatment with UV light or Hg (Fig. 2). Expression kinetics of the *CHS8-GUS* fusion gene was similar for transformants 3 and 5; likewise, the *CHS15-GUS* fusion gene was expressed similarly in transformants 6 and 11. UV light induced activation of both fusion genes within 6 hr after treatment, resulting in severalfold increases in *GUS*

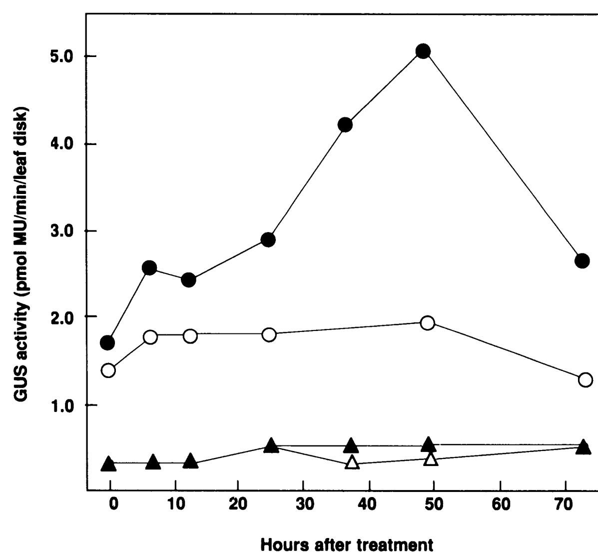

Fig. 3. Induction of β-glucuronidase (*GUS*) reporter gene activity in leaves of transformant CHS8-GUS-5 and pBI101.1-2-transformed tobacco after infiltration with an incompatible isolate of *Pseudomonas syringae* pv. *syringae*. The bacterial suspension was infiltrated into a localized interveinal area of a leaf with a syringe. Disks centering on the lesion caused by the treatment were cut out of the leaves immediately before they were assayed for GUS activity. Symbols are as follows: (●) CHS8-GUS-5 inoculated with *P. s.* pv. *syringae*; (○) CHS8-GUS-5 mock inoculated; (▲) pBI101.1-2-transformed tobacco inoculated with *P. s.* pv. *syringae*; and (△) pBI101.1-2-transformed tobacco mock inoculated. Similar results were obtained for CHS8-GUS-5 in three separate experiments with time courses out to 48 hr. No induction of GUS activity was ever seen in pBI101.1-2-transformed tobacco plants.

expression. The response to Hg was slower and resulted in less marked increases in GUS activity. Although the kinetics of expression were similar for both *CHS8-GUS* transformants, transformant 5 always had higher levels of GUS activity in response to the same treatment (Hg or UV).

Promoter activation by infection. We were particularly interested in examining responses to infection because CHS is involved in phytoalexin production in bean, but not in tobacco. Infiltration of transgenic tobacco leaves with an isolate of *P. s.* pv. *syringae* that caused a hypersensitive response resulted in an increase in GUS activity within 6 hr (Fig. 3). The gene fusion containing the *CHS8* regulatory sequence was induced with a maximum of GUS activity at about 48 hr after infiltration. A small increase in *GUS* expression was seen in the same transformant infiltrated with only the bacterial nutrient medium, indicating a slight wound effect. Infiltration of *P. s.* pv. *syringae* into leaves of a tobacco plant transformed with the "promoterless" *GUS* construct pBI101.1 did not lead to any induction of GUS activity. No significant GUS activity, or fluorescence that would be misinterpreted as GUS activity in the fluorogenic assay, was seen. Thus, neither *Pseudomonas* itself, the nutrient medium, nor any wound-induced endogenous compounds can be responsible for the observed GUS activity, since these factors would also be present in the plants containing pBI101.1. A hypersensitive response was evident in CHS8-GUS-5 tobacco leaves infiltrated with *P. s.* pv. *syringae* within 9 hr of treatment, closely following the initial increase in GUS activity. This indicates that the timing of activation of the *CHS8* promoter correlates with the development of the hypersensitive response. Although all transgenic plants tested with the *CHS8* or the *CHS15* promoter showed induction of GUS activity after UV or Hg treatment, neither of the transformants containing the gene fusion under the control of the *CHS15* promoter showed induction in response to infiltration with *P. s.* pv. *syringae*.

Spatial patterns of fusion gene expression. Oxalate can induce disease resistance in cucumbers, possibly by production of a systemic signal. Therefore, oxalate was tested here to see if it could also activate expression of the *CHS-GUS* chimeric genes in transgenic tobacco. Oxalate was an effective inducer of GUS activity in plants with the *CHS8* construct (Fig. 4). A concentration of 100 mM oxalate gave the maximum response. Like *P. s.* pv. *syringae*, oxalate was unable to induce GUS activity in plants carrying the *CHS15* transcriptional fusion. In the *CHS8* transformants, the response to oxalate was slower but more prolonged than to *P. s.* pv. *syringae* inoculation, leading to higher levels of GUS activity after 3 days (Fig. 5).

Because oxalate and *P. s.* pv. *syringae* are able to cause systemic responses in cucumber plants, these treatments were examined for their possible systemic actions on the *CHS8* and *CHS15* promoters in transgenic tobacco. Assays of sections of leaf tissue around sites treated with *P. s.* pv. *syringae* or oxalate showed the induction of reporter gene activity in the surrounding tissues (Fig. 6a and 6b). For both stimuli, levels of GUS activity were highest around the treatment sites and gradually declined to background levels 3 to 6 cm from the sites. Maximum induction of GUS activity varied in leaves from twofold to fivefold. No induction of activity at a distance was seen in *CHS8-GUS* transformants infiltrated with sterile nutrient medium or irradiated with UV light, or in plants carrying the *CHS15*

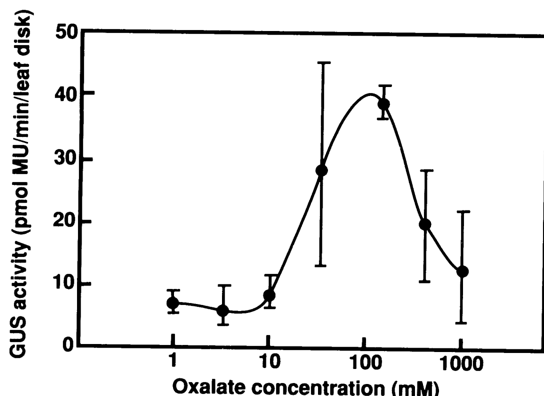

Fig. 4. Induction of β-glucuronidase (*GUS*) reporter gene activity in CHS8-GUS-5 transgenic tobacco plants in response to oxalate. Oxalic acid was titrated to pH 6.1 with KOH, and 25-μl drops of various concentrations were applied to the leaf. Leaf disks centering on lesions caused by the oxalate were cut out of the leaves 48 hr after treatment and assayed for GUS activity. Error bars denote standard deviation ($n = 4$).

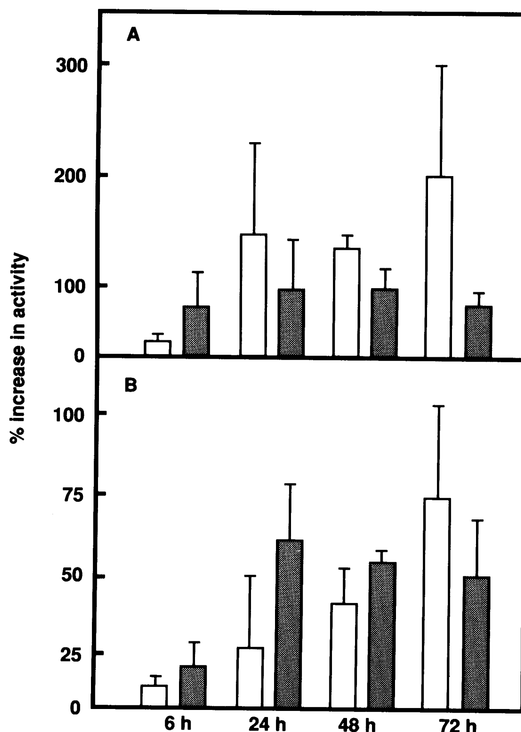

Fig. 5. Comparison of β-glucuronidase (*GUS*) reporter gene activity induced by *Pseudomonas syringae* pv. *syringae* or oxalate. Leaves of transgenic tobacco plants CHS8-GUS-3 (**A**) and CHS8-GUS-5 (**B**) were infiltrated locally with either 100 mM oxalate (open bar) or *P. s.* pv. *syringae* (shaded bar). Disks centering on the lesion resulting from the treatments were cut out of the leaves at the indicated times for determination of GUS activity. Values are the means and standard errors for four experiments. Control values for transformants CHS8-GUS-3, CHS8-GUS-5, CHS15-GUS-6, and CHS15-GUS-11 at 6 hr are 0.68, 7.8, 0.68, and 0.85 pmol of 4-methylumbelliferone per hour per disk, respectively. Neither of the transformants containing the *CHS15-GUS* construct showed induction of GUS activity by infiltration with *P. s.* pv. *syringae* or oxalate.

constructs (Fig. 6c, 6d, and 6e). However, plants containing the *CHS15* promoter did show a wound-induced response in the immediate area of infiltration with nutrient medium. Histochemical analysis of GUS activity *in situ* confirmed the pattern of gene activation deduced from the tissue dissection experiments (Fig. 7a and b). Thus, wounding alone resulted in a narrow zone of indigo dye, indicating GUS activity at the point of injury. In contrast, induction of a hypersensitive response with *P. s.* pv. *syringae* led to the staining of a large area of the leaf corresponding

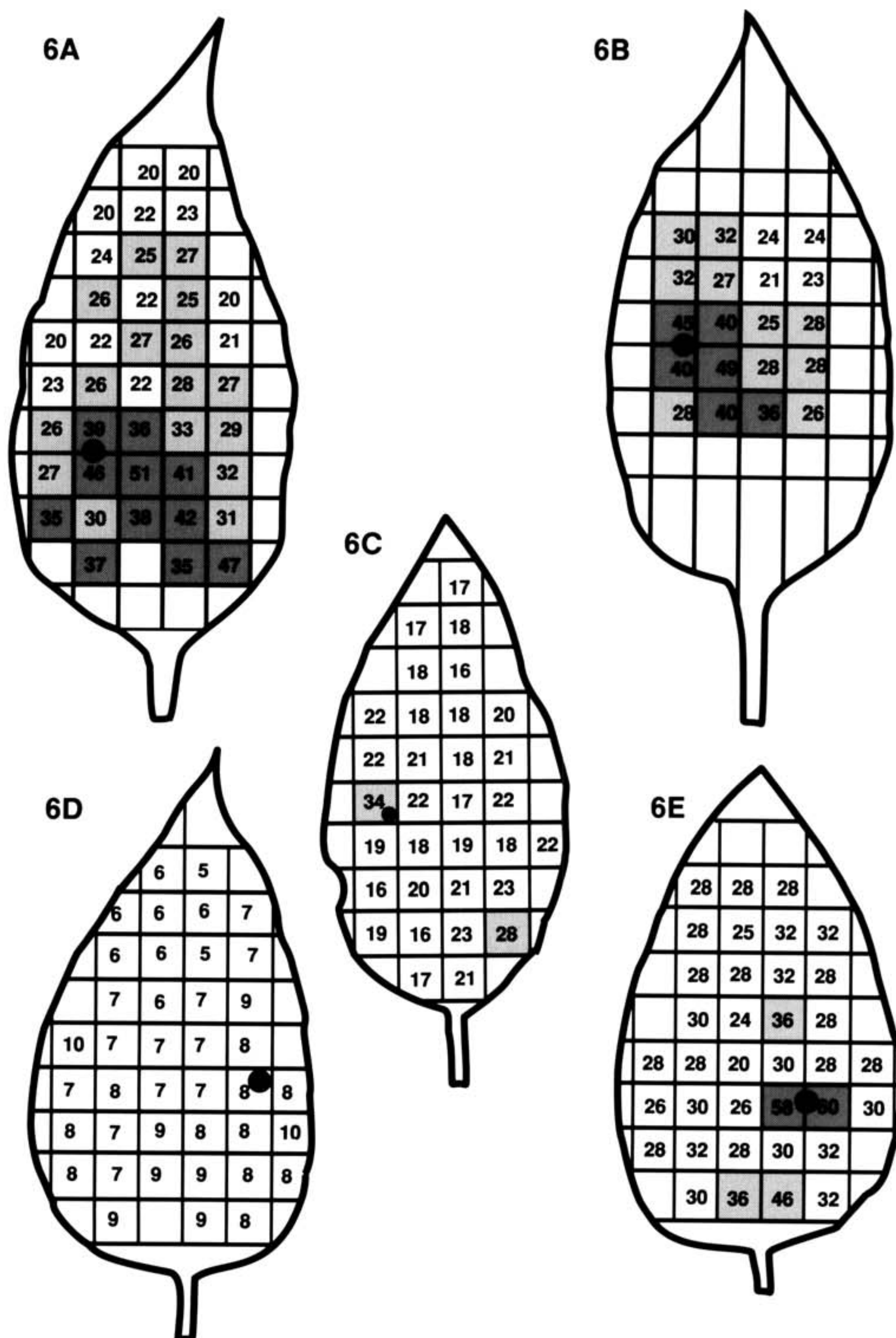

Fig. 6. Spatial patterns of β-glucuronidase (*GUS*) reporter gene induction in leaves of transgenic tobacco. Black circles delineate the small areas of leaf tissue that received the treatment. Leaves were removed from the plant 48 hr after treatment and cut into 1.27 cm^2 sections for determination of GUS activity. Values in each section are levels of GUS activity expressed as fluorescence in the assay (100 = 1.0 μM 4-methylumbelliferone). The shading highlights low, intermediate, and high expression within an individual leaf. **A**, transformant CHS8-GUS-5 infiltrated with *Pseudomonas syringae* pv. *syringae*; **B**, CHS8-GUS-5 treated with 100 mM oxalate; **C**, CHS8-GUS-5 infiltrated with sterile *P. s.* pv. *syringae* nutrient broth; **D**, CHS15-GUS-6 infiltrated with *P. s.* pv. *syringae*; and **E**, CHS8-GUS-5 irradiated in a small area with UV light.

to enhanced levels of GUS activity.

Induction of GUS activity from the *CHS8* promoter was seen only in the treated leaf; there was no enhancement of GUS activity in the untreated leaves of the same plant (Fig. 8). The decline in GUS activity as a function of the age of the leaf pair analyzed (Fig. 8) is a developmental phenomenon.

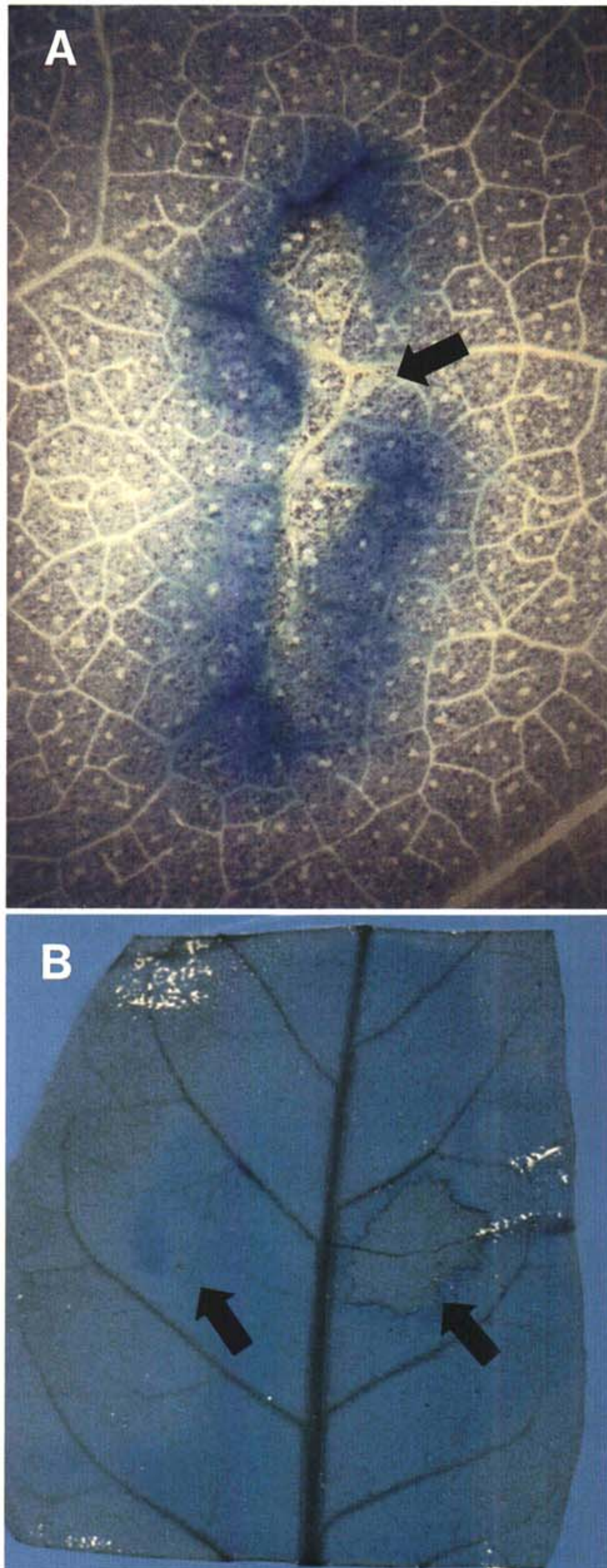

Fig. 7. Histochemical staining of leaf tissue from CHS8-GUS-5 transgenic tobacco plants for determining β-glucuronidase (GUS) activity after wounding or infiltration with *Pseudomonas syringae* pv. *syringae*. To visualize GUS activity, tissues were incubated overnight in 1 mM 5-bromo-4-chloro-3-indolyl glucuronide and then partially cleared in 70% ethanol. **A**, Tissue 7 days after wounding with a pipette tip. **B**, Tissue expressing a hypersensitive response induced by *P. s.* pv. *syringae* on the right side of the leaf and a response induced by wounding on the left side 48 hr after treatment.

DISCUSSION

Analysis of the expression of gene fusions in transgenic plants or electroporated protoplasts is a powerful approach for the study of promoter function and gene regulation mechanisms. The expression of *CHS* promoter-reporter gene fusions in electroporated protoplasts has been intensively studied, and functional dissection of *CHS* promoters using these assay systems has defined *cis*-acting elements involved in the induction of the bean *CHS15* promoter by fungal elicitor or glutathione (Dron *et al.* 1988; Choudhary *et al.* 1990; A. D. Choudhary and R. A. Dixon, unpublished results) and in the induction of antirrhinum and parsley *CHS* promoters by UV irradiation (Lipphardt *et al.* 1988; Schulze-Lefert *et al.* 1989). Although transient assay in protoplasts is a simple and convenient experimental system for analysis of the properties of stress-inducible promoters, this approach does not allow examination of the spatial pattern and tissue specificity of defense gene induction, neither can it address key features peculiar to the molecular response of intact plants to microbial attack, namely defense gene regulation in relation to race-cultivar specific interactions and induced systemic immunity. Details of the developmental expression of the promoter in *CHS8-GUS* transformed tobacco have been presented elsewhere (Schmid *et al.* 1990).

Previous studies have shown ethylene induction of a gene fusion comprising a bean chitinase promoter-GUS reporter gene fusion in transgenic tobacco (Broglie *et al.* 1989) and wound induction of gene fusions under the control of promoters from potato proteinase inhibitor and *wun1* genes (Thornburg *et al.* 1987; Longemann *et al.* 1989). We have shown here that the 5′-flanking sequences of the bean phytoalexin biosynthetic genes *CHS8* and *CHS15* are sufficient for UV and Hg induction in transgenic tobacco leaves. In addition, the *CHS8* promoter contains sequences for induction in tobacco leaves by inoculation with *P. s.* pv. *syringae* or treatment with oxalate. This is the first report of transgenic plants containing a gene fusion responsive to microbial attack. Our data show that attempted infection by *P. s.* pv. *syringae*, a bacterial pathogen of wheat that elicits a hypersensitive response on the nonhost tobacco, activates the *CHS8* promoter.

Tobacco phytoalexins are furanocoumarin or sesquiterpenoid compounds, rather than flavonoid derivatives as in legumes, and endogenous *CHS* genes are not stress induced in tobacco. Yet, the bean *CHS* promoters are

activated by stress signals, showing appropriate control in transgenic tobacco, even though CHS has no function in stress metabolism of tobacco. Therefore, the bean *CHS8* and *CHS15* promoters appear to respond to conserved signal systems for stress induction of defense genes that were probably established prior to the evolution of chemically distinct phytoalexins in the Leguminosae and Solanaceae.

In addition, the *CHS8* promoter apparently contains additional *cis*-acting regulatory sequences required for induction by microbial attack that are not present in the shorter *CHS15* promoter. The additional elements involved in *CHS8* activation by microbial attack appear to be distinct from the Hg and UV responsive elements present in both the *CHS8* promoter and the shorter *CHS15* promoter fragment, implying the existence of more than one signal transduction pathway for stress induction of phytoalexin biosynthetic genes. Moreover, while induction of the *CHS8* promoter by Hg (Doerner *et al.* 1990) or UV is localized to the immediate vicinity of the site of application of these stimuli, both oxalate and *P. s.* pv. *syringae* cause induction of the *CHS8* promoter at a distance. Since the bacteria are restricted to the hypersensitive lesion, the *CHS8* promoter contains *cis*-acting elements responsive to both local induction and induction at a distance.

Our data suggest that UV light cannot be used as a model for infection, since induction of the bean *CHS8* promoter by bacterial infection requires different or additional signals, and possibly therefore separate *cis*-elements, from those involved in UV and fungal elicitor induction. An elicitor-responsive element and related silencer and enhancer sequences are located within 326 bp upstream of the transcription initiation site of the *CHS15* gene (Dron *et al.* 1988; M. A. Lawton, R. A. Dixon, and C. J. Lamb, unpublished; A. D. Choudhary, M. J. Harrison, and R. A. Dixon, unpublished), but this region of the promoter does not confer induction by bacterial infection in transgenic tobacco.

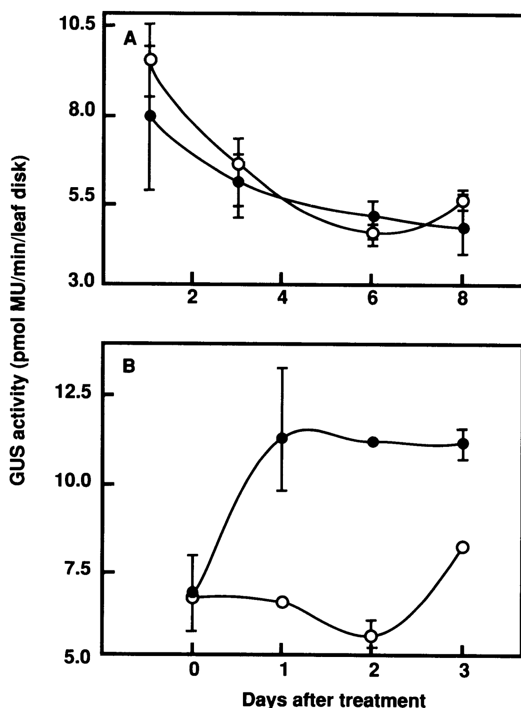

Fig. 8. Effect of *Pseudomonas syringae* pv. *syringae* infiltration on the β-glucuronidase (GUS) activity of adjacent untreated leaves. CHS8-GUS-5 transgenic tobacco plants received leaf infiltrations of *P. s.* pv. *syringae* (●) or were mock inoculated (○). Disks centering on the lesion caused by treatment, or similarly located disks on untreated plants, were cut out of the leaves and immediately assayed for GUS activity. **A**, Activity in leaves immediately above infiltrated and control leaves. **B**, Activity in the infiltrated leaves and in the corresponding leaves of control plants. Error bars denote standard deviation ($n = 4$ for **A**; $n = 2$ for **B**).

In cucumber, oxalate has been shown to mimic the effects of infection leading to systemic induction of resistance to *C. lagenarium* (Passerini) Ellis & Halsted (Doubrava *et al.* 1988). The mode of oxalate action may be through the sequestering of Ca^{2+}, which could disrupt membrane or pectic structures causing the production of a systemic signal. Phosphates, another class of Ca^{2+} chelator, were also recently reported to induce resistance in cucumbers (Gottstein and Kuć 1989). The induction of GUS activity in *CHS8-GUS* transformed plants exposed to oxalate or *P. s.* pv. *syringae* decreased as a function of the distance from the treatment site, the induction decreasing to zero over distances of several centimeters. However, no induction was seen in leaves above the treated leaf. No systemic induction of *CHS8-GUS* was detectable under the conditions used in these experiments. Like tobacco, bean is, however, reported to have the ability to respond with systemic resistance after infection (Cloud and Deverall 1987; Tuzun and Kuć 1985), although this may not involve the activation of *CHS* genes.

In contrast to oxalate treatment or infection, and in common with wounding, UV light treatment of *CHS8* transgenic tobacco leaves induced expression of the gene fusion only in the tissues actually exposed to the treatment. The *GUS* reporter gene was similarly induced by UV light when fused with the promoter sequence from a gene encoding for a bean hydroxyproline-rich glycoprotein (K. Wycoff and B. A. Stermer, unpublished results). This gene is not known to be induced by UV light, but is induced by wounding (Corbin *et al.* 1987). Thus, although our data do not challenge the concept that UV light is a specific inducer of *CHS*, it is nevertheless possible that, in the transgenic plants studied in the present work, UV treatment may also result in wound effects.

It will be of great interest to determine whether the *CHS8-GUS* gene fusion shows the clear temporal differences in expression in incompatible and compatible race-cultivar specific interactions between tobacco and tobacco pathogens, which were previously established for transcription of endogenous *CHS* genes in the bean-*C. lindemuthianum* system. Our efforts to examine the effects of fungal inoculation on the expression of the *CHS8-GUS* and *CHS15-GUS* gene fusions have been hindered by moderate to high levels of GUS activity in the filamentous fungi examined so far, including *Alternaria, Cladosporium*, and *Colletotrichum* species. Hence, other reporter genes may be necessary for infection studies with these fungi and to address the regulation of these and other defense gene promoters in race-cultivar specific interactions.

Our study has defined induction systems in transgenic tobacco that may now be applied to functional dissection,

by deletion analysis, of the *CHS* promoter to define further the factors which regulate defense gene expression in response to attempted infection and related stresses. Our study has also indicated the existence of different signaling systems (local and at a distance) for activation of *CHS* promoters under different stress situations. Such inducible promoters could be used to screen for compounds that activate plant defenses or could be used to drive expression of foreign genes encoding proteins antagonistic to microbial activities, and hence may be useful in the design of novel crop protection strategies.

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