CELLULAR & MOLECULAR BIOLOGY LETTERS
Volume 9, (2004) pp 843 – 853
http://www.cmbl.org.pl

Received 8 June 2004
Accepted 17 September 2004

TRANSCRIPTIONAL REGULATION OF THE *gluB* PROMOTER DURING PLANT RESPONSE TO INFECTION

AGNIESZKA MAC, MAGDALENA KRZYMOWSKA, ANNA BARABASZ and JACEK HENNIG*
Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5A, 02-106 Warsaw, Poland

Abstract: Several studies suggest that plant hydrolytic enzymes, such as 1,3-β-glucanases, may be components of a general defense system against pathogen invasion in several different plant species. We isolated and characterized a genomic sequence coding for a new acidic 1,3-β-glucanase (*gluB*) from *Solanum tuberosum*. The 5' flanking region of the *gluB* gene was also characterized. A chimeric gene composed of 2998 bp of the promoter sequence from the *gluB* gene was fused to the β-glucuronidase (GUS) coding region and used to transform potato and tobacco plants. Transcriptional activation of the *gluB* promoter was investigated in response to inoculation with *Phytophthora infestans* (*Pi*) or tobacco mosaic virus (TMV). In pathogen inoculated transgenic plants, GUS activity was strongly induced locally around necrotic lesions.

Key Words: Plant-Pathogen Interactions, 1,3-β-glucanase, Transcriptional Regulation

INRODUCTION

Plants possess a complex set of defence mechanisms that are responsible either for preventing unfavourable interactions with other living organisms in their natural environment or for reducing the negative effects of such interactions. One common form of resistance to disease is the synthesis of a large number of novel proteins with putative roles in defense. The pathogenesis-related (PR) proteins are a subset of this type of protein. The PR proteins are classified into 17 unrelated families (see: http://www.bio.uu.nl/~fytopath/PR-families).

*Corresponding author; tel: +48 22 8237186, e-mail: jacekh@ibb.waw.pl
Abbreviations used: GUS - β-glucuronidase; HR – hypersensitive response; IF-induction factor; *Pi* – *Phytophtora infestans*; PVY – potato virus Y; PR – pathogenesis related; RACE – rapid amplification of cDNA ends; SAR – systemic required resistance; TMV – tobacco mosaic virus.

Proteins belonging to the PR-2 family are hydrolytic enzymes with 1,3-β-glucanase activity [glucan endo-1,3-β-glucosidases, E.C. 3.2.1.39] [1]. The 1,3-β-glucanases are abundant, highly up-regulated enzymes that are widely distributed in plants. Although the main stream of 1,3-β-glucanase research focuses on their possible role in the defense response of plants to microbial infection, there is strong evidence that these enzymes are also involved in diverse physiological and developmental processes in uninfected plants. Some of the tobacco genes encoding 1,3-β-glucanases were shown to be up-regulated at the transcriptional level [2-4], and their expression was associated with both the hypersensitive response (HR) and systemic required resistance (SAR) in tobacco plants infected with Tobacco Mosaic Virus (TMV) [5] and in the response to developmental signals [3, 6, 7]. In this paper, we describe the isolation of a 1,3-β-glucanase (*gluB*) promoter and its analysis *via* fusion with the GUS reporter gene. The expression of this chimeric gene was studied in transgenic tobacco and potato plants in order to check whether the isolated promoter is sufficient to drive pathogen-dependent gene induction.

MATERIALS AND METHODS

Plant material and growth conditions

Plants of *Solanum tuberosum* L. cv. Désirée or *Nicotiana tabacum* cv. Xanthi nc were grown *in vitro* or in the soil in growth chambers using a 16 h period of light (22°C) and an 8 h period of darkness (18°C). The light intensity was 5000 to 6000 lux, and the humidity was maintained at 70-80%. For the majority of our experiments, 6-8-week old plants were used.

Isolation of the *gluB* genes and their regulatory regions

The cDNA library from PVY infected potato was created by Talarczyk [8] using a Stratagene kit. The library was prepared in the lambda Uni-Zap XR vector (Stratagene), with its construction performed strictly according to the manufacterer's protocol. A commercially available potato genomic library (Clontech) in the λEMBL-3 SP6/T7 vector was screened according to the manufacturer's instructions for clones hybridizing to sequence encoding potato 1,3-β-glucanase (*gluB*). The library was diluted prior to plating to ensure the formation of distinct plaques. These plaques were transferred onto Hybond N (Amersham Biotech) membrane and hybridized at 65ºC overnight with radioactively labelled DNA. After hybridization, the membranes were washed: 1×10min. in 2×SSC, 0.1% SDS at room temperature; 1×30min. in 2×SSC, 0.1% SDS at 50ºC; and 1×30min. in 0.2×SSC, 0.1% SDS at 65ºC. Plaques positive for 1,3-β-glucanase were cut out from of the plate, diluted, and re-plated to obtain clear-cut plaques. The screening procedure was repeated until distinct clones were isolated. Phage DNA of the 8-1-3 clone was isolated and analyzed *via* restriction enzyme digestion or hybridization with specific probes, and finally, the sequence of nucleotides was determined.

Construction of the gluB/GUS fusion genes

The 8-1-3 clone was modified by creating a *Nco*I site at the ATG translation initiation codon using the Kunkel method [9]. The sequence upstream to the *gluB* translation initiation site was then fused to GUS/3'NOS sequences to form chimeric constructs. The *Nco*I-*Sst*I fragment from the pRAJ 275 vector [10] containing GUS coding sequence was ligated with the 3' nopaline synthase (NOS) sequence from pBI101.2. Then the GUS/3'NOS construct was inserted into the *Nco*I-*Kpn*I sites of the pSK^+ vector with the 5'-untranslated regulatory region of the *gluB* gene (2998bp). The expression cassette containing the *gluB promoter*/GUS gene fusion was cloned as *Eco*RI-*Kpn*I fragments into the binary vector pGA482 [11] and mobilized into *Agrobacterium tumefaciens* strain LBA4044.

Plant transformation

Leaf discs from *N. tabacum* cv. Xanthi nc or leaflets of *S. tuberosum* cv. Désirée plants, cultivated *in vitro*, were transformed, and plants were regenerated using the standard methods [12]. Kanamycin-resistant plants were regenerated on MS medium [13]. After shoot regeneration, the presence of the transgene was checked by PCR with specific primers. Control plants were constructed *via* empty binary vector transformation. Primary potato transformants were propagated vegetatively on MS medium with 50 μg/ml kanamycin at least five times. Selected Km^r lines were transferred into soil and grown for a few weeks before the experiments. Primary tobacco transformants were allowed to self-fertilize and F1 seeds were collected and germinated on MS agar medium with 200 μg/ml kanamycin. Transgenic seedlings were transferred to soil. The presence of the expression cassette was confirmed as follows: the PCR reaction was performed using two pairs of primers. First, two primers were complementary to the 5' regulatory region of the *gluB*: forward primer 5'-AAGCAGAAGCCACTAAGATT-3', and reverse primer 5'-GCATGTATG GCTTTTGTATG-3', with amplified fragment length 1052 bp. The following PCR cycle parameters were used: 94°C, 5 min.; [94°C, 40 sec.; 50°C, 40 sec.; 72°C, 1 min. 20 sec.] x 30; 72°C, 7 min. The other pair was: forward primer 5'-CGTAGGGTAACTTGACTTGG-3', complementary to the 3' end of regulatory region, and reverse primer 5'-AGGCACAGCACATCAAAGAG-3', complementary to the GUS coding sequence. Cycling parameters were: 94°C, 5 min.; [94°C, 40 sec.; 52°C, 40 sec.; 72°C, 1 min. 40 sec.] x 30; 72°C, 7 min. With this pair of primers, a 1489-bp long fragment was amplified.

Induction of the *gluB*/GUS gene

Six- to eight-week old potato or tobacco plants were used for GUS assays following inoculation with *Pi* or TMV, respectively. For *Pi* inoculation, one or two droplets (10μl each) of zoospore suspension (10^5/ml) were spotted on the abaxial side of leaflets of potato plants grown on MS agar medium in Magenta boxes. To maintain a high level of humidity, 2ml of sterile water were added to

the containers. Subsequently, the plants were incubated at 15ºC over five days under limited light conditions. For inoculation with TMV, the leaves of plants were dusted with carborundum and rubbed with a 1 μg/ml suspension of TMV strain U1 in 50 mM phosphate buffer, pH 7.5. Leaf discs were punched out from the inoculated leaves at three or seven days after treatment. The leaf material was either immediately subjected to histochemical staining or frozen and stored at -80ºC for fluorometric assay.

Detection of GUS activity

β-Glucuronidase activity was assayed using either a fluorometric method or histochemical staining. For the fluorometric GUS assay, four 1 cm diameter leaf discs were homogenized in 0.5 ml lysis buffer. The homogenate was centrifuged for 10 min in an Eppendorf centrifuge at 4ºC, and the GUS activity of the supernatant was measured, essentially as described by Jefferson ***et al***. [14]. The GUS activity was normalized to the protein concentration for each crude extract and calculated as pmol of 4-methylumbelliferone (4-MU) produced per minute per milligram of soluble protein. Protein content was measured by the Bradford method [15] using BSA as a standard. For histochemical staining, hand-cut tissue sections were vacuum infiltrated with a staining solution containing 1 mg/mL X-gluc (5-bromo-4-chloro-3-indolyl β-glucuronide), 100 mM phosphate buffer, pH 7.0, 5 mM $K_3[Fe(CN)_6]$, 5 mM $K_4[Fe(CN)_6]$ and 10 mM EDTA, and incubated for 4 to 20 hours at 37ºC. After staining, the tissue sections were fixed by incubation in a solution of 5% formaldehyde, 5% acetic acid and 20% ethanol, for 10 min at room temperature, and then cleared by incubation sequentially in 50% ethanol for 10 min at room temperature and 100% ethanol for 3 hours at 60ºC. Stained sections were viewed and photographed with the OLYMPUS B071 Stereomicroscope.

RESULTS AND DISCUSSION

Isolation of the genomic copy of the *gluB* gene with its regulatory region

Talarczyk performed and described an isolation of a cDNA of the *gluB* genes [8]. To isolate the *gluB* promoter sequence, a 1.9-kb long probe homologous to *gluB20-2* was prepared. This probe contained the full coding sequence plus the intron. It was used to screen a commercially available genomic library made of leaves collected from 2-month old potato plants (cv. Désirée) in the λEMBL-3 vector. According to the manufacturer's specification, the library contained 2.0×10^6 independent clones. Approximately 1.2×10^5 clones were screened; initially, 19 gave a positive signal (see *Materials and Methods* for a detailed description of the screening protocol). When the screening procedure was completed, 14 clones remained. One clone (8-1-3) was chosen for further detailed analysis.

Structural analysis of the *gluB* gene isolated from the potato genomic library

A 5500-bp long fragment of the potato genome present in clone 8-1-3 (Acc. No. AJ586738) contained an open reading frame of 1014 bp, consisting of two exons: short exon 1 (64 bp) and long exon 2 (950 bp), separated by an intron of 523 bp. Both exons of the 1,3-β-glucanase gene isolated from the genomic library exhibit 98.62% identity to the *gluB20-2* (Acc. No. AJ586575) and 94.40% to the *gluB* (Acc. No. AJ009932) genes isolated from a cDNA library from potato infected with PVY [8]. The putative protein encoded by the isolated gene is 338-amino acid peptide. This protein shows 99.68% identity to the GLUB*20-2*, isolated from the potato plant. The percents of identity to the proteins coded by other genes isolated from potato were as follows: 90.86% identity to the protein coded by the *gluA2* gene (AY170827), 89.91% to the *gluA1* (Acc. No. AY170826), 89.64% to the *gluB* (Acc. No. AJ009932), 49.84% to the *gluB1* (Acc. No. U01900), 49.67% to the *gluB3* (Acc. No. U01902), 49.26% to the 1,3-β-glucan glucanohydrolase gene (Acc. No. AF067863), and 49.12% to the *gluB2* gene (Acc. No. U01901). The identity values for both the *gluB* open reading frames and the putative proteins were obtained using the FASTA3 program, available at www.ebi.ac.uk/fasta33. For proteins, the PAM250 matrix was used. It may be assumed that the beginning of the cDNA sequence obtained previously using the 5'-RACE technique [8] is the beginning of the transcribed region within the *gluB* gene. The untranslated leader is 50 bp long. There is a putative TATA-box sequence (TATATAA) located at pos. -34 from the start of transcription. The 3'-terminal untranslated region is 200 bp long, as may be deduced by a sequence comparison of several clones obtained from the cDNA library (data not shown). An analysis performed with the PLOTSTRUCTURE program indicated that the N-terminus of the peptide was strongly hydrophobic (data not shown). An analysis of the amino acid sequence with the MOTIFS and PROFILESCAN programs against the PROSITE database and tested with a database search against SWISS-PROT showed the presence of glycosyl hydrolase family 17 signature, characteristic for 1,3-β-glucanase from various plant species.

A search through the GenBank/EMBL database revealed several regions that are within the putative regulatory sequence upstream of the gene and that share a significant level of homology with various entries present in the database (Tab. 1). Three are particularly interesting: (1) a 140-bp region of homology to the minus strand of the tomato polygalacturonase 7 gene (80% identity); (2) a 43-bp region of homology to the minus strand of the *A. thaliana* 12-oxo-phytodienate reductase gene (93% identity); and (3) two blocks of 34- and 32-bp respectively exhibiting 94% and 90% identity to two similar blocks from the tobacco PR-*2d* gene promoter.

This is especially interesting because the PR-*2d* gene encodes an acidic isoform of 1,3-β-glucanase, which is specifically induced in response to infection [3].

Tab. 1. Sequence blocks of high homology to various sequences deposited in databases. These sequences are present in the regulatory region upstream of the *gluB* gene (clone 8-1-3), isolated from the potato genomic library. Only hits with lengths over 30 bp have been included. The search was performed with the BLASTN program using a gapped BLAST algorithm with the default word size.

Region	Length	Homologous sequence	Acc. No.	Identity
81-220	140	*Lycopersicon esculentum* polygalacturonase7	AF072732	80%
342-384	43	*A.thaliana* 12-oxo-phytodienoate reductase	AF218257	93%
1293-1326/ 1383-1414	34/32	*N. tabacum PR-2 d*	X69794	94% / 90%
1001-1068	68	*Solanum demissum* chromosome V	AC150162	85%
172-220/	49/41	*A. thaliana* chromosome I	AC055769	85%/90%
342-382		*A. thaliana* chromosome I	AC017118	85%/90%
		A. thaliana chromosome V	AB022213	85%/90%
342-382	41	*A. thaliana* chromosome II	AC006413	88%
81-127	47	*A. thaliana* chromosome III	AL162507	87%
108-145	38	*A. thaliana* chromosome V	AB019224	89%
1001-1037	37	*L. pimpinellifolium Cf-9* resistance gene cluster	AJ002236	89%
369-403	35	*A. thaliana* chromosome IV	AF074021	91%
		A. thaliana chromosome IV	AL161501	91%

Pathogen-induced expression of a chimeric *gluB*/GUS gene in transgenic tobacco plants

To analyze *gluB* gene regulation, a chimeric *gluB*/GUS reporter gene was constructed and introduced into tobacco plants. The chimeric *gluB*/GUS gene was constructed by a fusion of the upstream promoter region of the *gluB* gene (-2949 to +51) to the ATG initiation codon of the GUS coding sequences and the 3' nopaline synthase (NOS) regulatory sequences. In 9 of the 10 independent kanamycin-resistant (Kan^r) transformants, the chimeric gene was activated during the hypersensitive response to TMV infection. GUS activity was detected histochemically, by staining leaves with X-gluc, after inoculation with TMV.

The chimeric gene driven by the *gluB* promoter was expressed at detectable levels in the tissue surrounding the local lesions as indicated by the distinct blue staining around each lesion at three days post inoculation (dpi) (Fig. 1A). At seven dpi, when the necrotic lesions were fully developed, GUS activity increased and was detected at greater distances from the lesions and along veins, which passed near or through the lesions (Fig. 1B). It is known that β-glucan is deposited in the phloem during physical stress and during pathogen attack, perhaps to prevent the spread of phloem mobile pathogens [16]. It is possible that the vascular location of the *gluB* promoter activity is associated with this

phenomenon. No GUS activity could be detected before inoculation or after mock inoculation. In contrast, GUS activity was detected throughout the whole leaf in the control intact plants carrying a CaMV 35S/GUS reporter gene (data not shown), consistent with the earlier observations that the expression from the CaMV 35S promoter is constitutive and relatively non-tissue specific. To more precisely define the activation level of the analyzed promoter, a quantitative GUS assay was performed. Fig. 2 shows the average GUS activities measured in the leaf tissue of plants carrying the ***gluB***/GUS cassette. In tissue collected immediately after virus treatment, no significant GUS activity was observed (data not shown). On average, a 2.5- to 12-fold increase in GUS activity was observed in inoculated plants over H_2O-treated plants seven dpi with TMV.

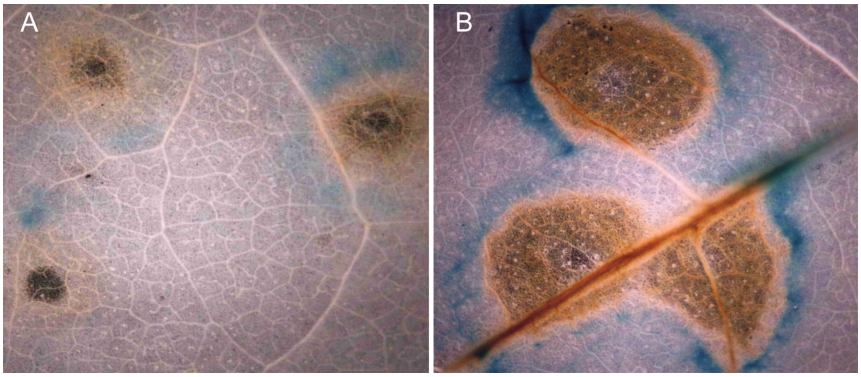

Fig. 1. The histochemical localization of GUS activity during the hypersensitive response to TMV infection. Leaf discs were punched out of leaves of F2 transformants carrying the ***gluB***/GUS construct 3 days (A) and 7 days (B) after inoculation. Chlorophyll was removed with ethanol washes to facilitate detection of the blue stain.

However, in two lines (line 20 and 24), the induction factor reached even level thirty. In contrast, very low induction was observed in one out of ten analyzed lines (line 7). No GUS activity was detected in the leaves of control plants transformed with the vector without the expression cassette (line pGA). To eliminate the effect of the gene copy number on GUS activity, only a single copy of the F2 progeny of the primary transformants was used for these studies.

We found considerable variations in the basal level of GUS activity, as well as in its level after induction by TMV infection. However, in the majority of the tested lines, IF lies between 3 and 10. In other published studies where transgenic tobacco plants expressing glucanase promoter/GUS fusions were used, IF after TMV infection was: for basic glucanases (***glb50***), 3.2 [17] or 3.7 (***glB***) [7]; and for acidic glucanase promoter/GUS fusions, 14 (***gl9***) [17] or 300 (PR-***2d***) [3]. We ascribe the observed variations, like the high level activities in line 12 or the dramatically pronounced activation in lines 20 and 24, to previously observed phenomenon referred to as the position effect.

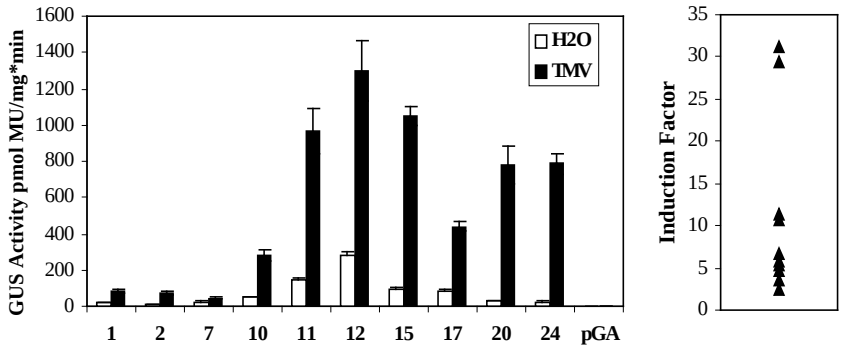

Fig. 2. Local induction of GUS activity in *gluB*/GUS transgenic tobacco plants after TMV inoculation. GUS activity was measured in the F2 progeny of ten independent. *gluB*/GUS transformants at seven days post mock- or TMV-inoculation. The data represents the average GUS activity calculated for three plants of each transgenic line. Specific activity was expressed as pmol 4-MU/min/mg protein. The induction factor (IF, right panel) was calculated as the ratio of the GUS activity in the pathogen-inoculated plants to the activity in the mock-inoculated plants. The IF for the vector transformed plants is not shown. Error bars represent the standard deviation.

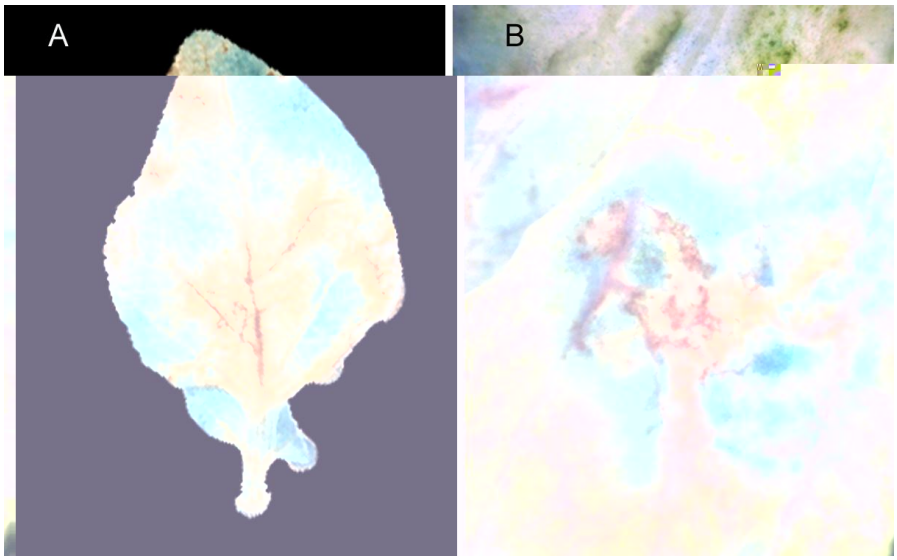

Fig. 3. Localization of GUS activity during the response to *P. infestans* infection. The leaf (A) was cut from a transgenic potato plant carrying the *gluB*/GUS construct and stained for GUS 5 days after *Pi*-treatment. (B) Indigo dye deposition was observed in the area located close to the disease symptoms.

Induced expression of a *gluB*/GUS gene fusion in transgenic potato plants
To analyze *gluB* gene regulation in a homologous system, a chimeric *gluB*/GUS gene was introduced into potato plants. Detached leaflets were used for histochemical GUS assays following inoculation with *Pi*. Five days post inoculation, a strong induction of GUS activity was observed in whole leaflets (Fig. 3A), but the strongest signal was located in the tissue directly surrounding sites necrotized by *Pi* (Fig. 3B).

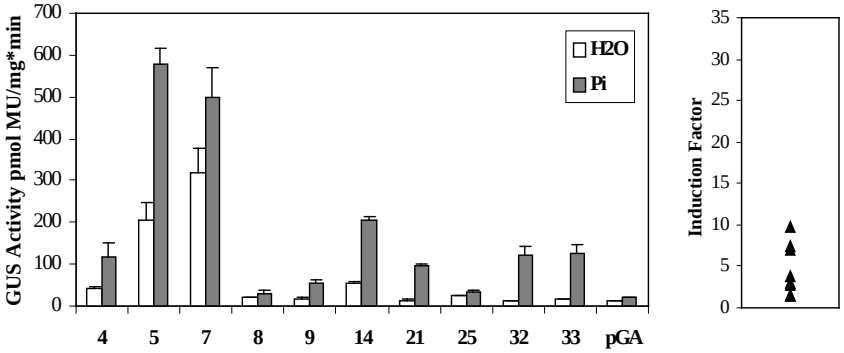

Fig. 4. The induction of GUS activity in *gluB*/GUS transgenic potato plants. GUS activity was assayed in *Pi*- or mock (H_2O)-infected plants. The plants were inoculated *in vitro* with a suspension of *Pi* zoospores in water, and kept for 5 days under conditions of high humidity. The results were the mean calculated from 3 vegetatively propagated plants. Error bars represent the standard deviation.

gluB/GUS heterozygous potato lines exhibited high inducibility after *Pi*-treatment. In all the tested lines, 5 dpi, there was a 2- to 11-fold increase in GUS activity (Fig. 4). Similarly to transgenic tobacco plants, variations in the GUS basal levels between the various lines were observed. Inducibility of the promoter correlates well with the increase in 1,3-β-glucanase activities observed after *Pi* infection ([18] and Barabasz – data not published).
Interestingly, strong and spatially precise activation of the *gluB* promoter was clearly visible in both compatible (*Pi* with potato) and incompatible interaction (TMV with tobacco), supporting the idea that the timing of host defense reactions could play a crucial role for the outcome of pathogen invasion.

Acknowledgements. This work was funded by grant No. PBZ/KBN/ 029/P06/2000 from the State Committee for Scientific Research (KBN).

REFERENCES

1. Leubner-Metzger, G. and Meins, F. Jr. Function and regulation of plant β-1,3-glucananses (PR-2). in: **Pathogenesis Related Proteins** (Datta, S.K. and Muthukrishnan, S. Eds), CRS Press, Washington, DC, 1999, 49-66.

2. Ward, E.R., Payne, G.B., Moyer, M.B., Williams, S.C., Dincher, S.S., Sharkey, K.C., Beck, J.J., Taylor, H.T., Ahl-Goy, P., Meins, F. Jr. and Ryals, J.A. Differential regulation of β-1,3-glucanase messenger RNAs in response to pathogen infection. **Plant Physiol.** 96 (1991) 390-397.
3. Hennig, J., Dewey, R.E., Cutt, J.R. and Klessig, D.F. Pathogen, salicylic acid and developmental dependent expression of a 1,3-β-glucanase/GUS fusion in transgenic tobacco plants. **Plant J.** 4 (1993) 481-493.
4. Shah, J. and Klessig, D.F. Identification of a salicylic acid-responsive element in the promoter of the tobacco pathogenesis-related 1,3-β-glucanase gene, PR-2d. **Plant J.** 10 (1996) 1089-1101.
5. Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl-Goy, P., Metraux, J.-P. and Ryals, J.A. Coordinate gene activity in response to agents that induce systemic acquired resistance. **Plant Cell** 3 (1991) 1085-1094.
6. Leubner-Metzger, G., Frundt, C., Vogeli-Lange, R. and Meins, F. Jr. Class I [beta]-1,3-glucanases in the endosperm of tobacco during germination. **Plant Physiol.** 109 (1995) 751-759.
7. Vogeli-Lange, R., Frundt, C., Hart, C.M., Nagy, F. and Meins, F. Jr. Developmental, hormonal, and pathogenesis-related regulation of the tobacco class I beta-1,3-glucanase B promoter. **Plant Mol. Biol.** 25 (1994) 299-311.
8. Talarczyk, A. Identyfication of new elements involved in plant response to viral infection. **Ph.D. Thesis**, IBB-PAS (2001)
9. Sambrook, J., Fritsch, E.F. and Maniatis, T. **Molecular Cloning. A Laboratory Manual** Cold Spring Harbor, Cold Spring Harbor Laboratory Press (1989).
10. Jefferson, R.A., Kavanagh, T.A. and Bevan, M.W. GUS fusions: β-Glucuronidase as a sensitive and versatile gene fusion marker in higher plants. **EMBO J.** 6 (1987) 3901-3907.
11. An, G. Development of plant promoter expression vectors and their use for analysis of differential activity of nopaline synthase promoter in transformed tobacco cells. **Plant Physiol.** 81 (1986) 86-91.
12. Horsch, R.B., Fry, J.E. Hoffmann, N.L., Eichholtz, D., Rogers, S.G. and Fraley, R.T. A simple and general method for transferring genes into plants. **Science** 227 (1985) 1229-1231.
13. Murashige, T. and Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiol. Plant.** 15 (1962) 473-479.
14. Jefferson, R.A., Burgess, S.M. and Hirsh, D. β-Glucuronidase from *Escherichia coli* as a gene fusion marker. **Proc. Natl. Acad. Sci. USA** 83 (1986) 8447-8451.
15. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Anal. Biochem.** 72 (1976) 248-254.

16. Goodman, N., Kiraly, Z. and Wood, K.R. **The Biochemistry and Physiology of Plant Disease**, University of Missouri Press, Columbia, 1986, 352-365.
17. Van de Rhee, M.D., Lemmers, R. and Bol, J.F. Analysis of regulatory elements involved in stress-induced and organ-specific expression of tobacco acidic and basic beta-1,3-glucanase genes **Plant Mol. Biol.** 21 (1993) 451-461,
18. Borkowska, M., Krzymowska, M., Talarczyk, A., Awan, M.F.M., Yakovleva, L., Kleczkowski, K. and Wielgat. B. Transgenic potato plants expressing soybean beta-1,3-endoglucanase gene exhibit an increased resistance to *Phytophthora infestans*. **Z. Naturforsch.** 53 (1998) 1012-1016.