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A Pathogen-Induced Wheat Gene Encodes a Protein Homologous to Glutathione-S-Transferases

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Winter wheat (*Triticum aestivum*) shows local, induced resistance against the plant-pathogenic fungus *Erysiphe graminis* f. sp. *tritici* following exposure to the nonpathogen *E. g.* f. sp. *hordei*. The onset of this resistance has been shown to be correlated with the activation of putative defense genes, and cDNA clones representing transcripts of induced genes have been obtained (P. Schweizer, W. Hunziker, and E. Mösinger, Plant Molecular Biology 12:643-654, 1989). We have cloned and sequenced a gene corresponding to one of these cDNAs, *WIR5*. Sequence analysis indicated that this gene contains three exons and encodes a protein of 229 amino acids. S1 mapping showed that transcripts homologous to this gene are at least 20 times more abundant in leaves infected 14 hr earlier with *E. g.* f. sp. *hordei* than in control leaves. Sequence comparison showed that the *WIR5* gene product is highly homologous to glutathione-S-transferases (GSTs; EC 25.1.18) of maize. This, together with the fact that the intron positions of both the wheat gene and the maize GSTI gene are conserved, suggests that the cloned pathogen-induced gene, named *GstA1*, encodes a wheat glutathione-S-transferase.

Additional keywords: disease resistance.

Wheat (*Triticum aestivum* L.) shows the phenomenon of induced resistance to *Erysiphe graminis* DC. f. sp. *tritici* Ém. Marchal (wheat powdery mildew). The success of an infection with this pathogen was found to be remarkably reduced locally by a previous infection with *E. g.* f. sp. *hordei* Ém. Marchal, which is not pathogenic to wheat (Schweizer *et al.* 1989). The onset of this induced resistance is associated with the activation of host genes. To study this phenomenon at a molecular level and to determine the function and regulation of putative defense genes, we cloned genes corresponding to pathogen-induced cDNA clones obtained previously by Schweizer *et al.* (1989). Here we report the sequence of a gene corresponding to one of the induced cDNA clones, *WIR5*. From the sequence information we conclude that this gene probably encodes a glutathione-S-transferase (GST; EC 2.5.1.18). These enzymes, which occur ubiquitously in the animal and plant kingdoms, catalyze the conjugation of the tripeptide glutathione to electrophilic centers of lipophilic compounds, thereby detoxifying them (for an overview, see Sies and Ketterer 1988). In plants, GSTs have been implicated in the detoxification of herbicides (for a review, see Timmerman 1989). A possible role for GSTs in pathogen defense has so far not been described.

MATERIALS AND METHODS

Plant material and infection. Growth and cultivation conditions of winter wheat (cultivars Fidel and Cheyenne) and *E. g.* f. sp. *hordei* were as described by Schweizer *et al.* (1989). Five- to 7-day-old wheat seedlings were inoculated with conidiospores by brushing barley plants (cultivar Gerbel), infected 7 days earlier, over the test plants.

Library screening, Southern hybridization, and sequence analysis. A wheat (*T. aestivum* cv. Cheyenne) genomic library constructed in λ EMBL3 was purchased from Clonetech Laboratories, Inc., Palo Alto, CA. Approximately 10^6 recombinant plaques were screened with ^{32}P-labeled cDNA *WIR5* (Schweizer *et al.* 1989) according to standard procedures. Subcloning of DNA fragments into pBluescript vectors KS+ and SK+ (Stratagene, La Jolla, CA) and Southern blot hybridizations with nick-translated ^{32}P-labeled probes were performed according to standard procedures (Maniatis *et al.* 1982). DNA sequences were determined at least twice on both strands of overlapping deletion fragments created by exonuclease III digestion. Sequencing reactions were done with single-stranded pBluescript KS+ and SK+ DNA using the Sequenase kit (U.S. Biochemical Corporation, Cleveland, OH). Sequences were assembled and analyzed using Staden Plus (Amersham International PLC, Amersham, U.K.) and PC/Gene (Genofit S.A., Geneva) software.

DNA and RNA isolation. DNA was isolated from 5- to 7-day-old wheat plants (cultivar Fidel). Leaf tissue (15 g) was homogenized in liquid nitrogen with mortar and pestle and added to 150 ml of lysis buffer (7 M urea, 350 mM NaCl, 50 mM Tris-HCl, pH 8, 20 mM EDTA, 1% sarcosyl) and carefully extracted with 150 ml of phenol/chloroform/isoamyl alcohol 25:24:1. The organic extraction was repeated twice, and the nucleic acids were spooled out after the addition of 15 ml of 3 M NaAc, pH 5, and 1 volume of ethanol. The precipitate was dissolved in 10 ml of TE (10 mM Tris-HCl, pH 8, 1 mM EDTA) and dialyzed against 3 L of TE overnight. RNA was digested with 250 μg/ml of RNase A for 30 min at 37° C. After another organic extraction, the DNA was redialyzed against TE

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overnight and used for Southern blotting.

Total RNA from leaves of 5- to 7-day-old wheat plants, which were infected or not infected with spores of *E. g.* f. sp. *hordei* 14 hr earlier, was isolated by the hot phenol method. One to 2 g of tissue were powdered in liquid nitrogen and added immediately to a 1:1 mixture of phenol, and 2× NETS (200 mM NaCl, 2 mM EDTA, 20 mM Tris-HCl, pH 7.5, 1% SDS) was preheated to 80° C. The mixture was thoroughly vortexed, cooled on ice, and centrifuged. After two more phenol extractions at room temperature, the nucleic acids were precipitated with ethanol and redissolved in water. RNA was then precipitated by adding 1 volume of 5 M LiCl and incubating on ice for 4 hr. The RNA was pelleted and dissolved in water.

S1 probe and S1 mapping. A 371-base pair (bp) *Sac*I fragment (between *Sac*I sites at position 298 and 664; Fig. 1) was subcloned into a *Sac*I-digested pBluescript KS+ vector. This construct was multiplied as a single-stranded phage that was used as a template for the synthesis of a uniformly ^{32}P-labeled single-stranded probe by extension from the universal sequencing primer. The product was cut with *Eco*RI, which cleaves in the cloning box after the insert, denatured, and run on a sequencing gel, from which the newly synthesized radioactive strand was eluted and used as a probe. S1 mappings were performed with 10 μg of LiCl-precipitated RNA exactly as described previously (Dudler and Travers 1984), except that 220 units of S1 nuclease (Boehringer, Mannheim, Germany) were used per reaction.

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GGATCCAGTTCTGATATTGAGACCTCGACGATGGGAGGAAGGGCGCGATCGATGTGGAGT
      10        20        30        40        50        60
AATTTGAATTTCAAATCTATCTATCTGGGGTATATTGGTCCTTCACCGATGTTTGGGGGG
      70        80        90       100       110       120
CTGTCGGAAATTGGTTCCGCGATCTACAAAAGTGAATGGAGGGAGTAGTTGTTTCTCCAA
     130       140       150       160       170       180
TCCGTACCAACGCACGTGTTTCTAACTAGTACTTACTTCCTTCGCACCACAATATGGAAT
     190       200       210       220       230       240
AGAGGGAGTATCGATAAACTAACAAAGATGATTACTTACCCGGTTTAAATGATTCAAGAG
     250       260       270       280       290       300
CTCATTTAATTTGGCACTCATCATTTCATATATCTTTTTGGTAGAAATGAAATAAAGCA
     310       320       330       340       350       360
GATCTAGACACTAGCTAAAAAGTCGATGTAGCCTTGTTATTTCCTTGGGCCACGCGGGCC
     370       380       390       400       410       420

GGGTGTGGTGCTCCCTGCTCTGTGTATAAATGGAGATCAACATCCAAGGCCTCCTCCCAC
     430       440       450       460       470       480

ACACACACGCTACAGAGCAGAGCAGAGTCTTGCTCCAGTATCTGCCCTCTCCTGCCTGCC
     490       500       510       520       530       540
                                    M   S   P   V   K   V   F   G
TGTAGAGCATCCATCACGTGAAGTTCACGGACAAACATGTCTCCGGTGAAGGTGTTCGGG
     550       560       570       580       590       600
 H   P   M   L   T   N   V   A   R   V   L   L   F   L   E   E   V   G   A   E
CACCCGATGTTGACAAACGTCGCACGGGTGCTGCTCTTCCTGGAGGAGGTCGGCGCCGAG
     610       620       630       640       650       660
 Y   E   L   V   P   M   D   F   V   A   G   E   H   K   R   P   Q   H   V   Q
TACGAGCTCGTGCCCATGGACTTCGTCGCCGGCGAGCACAAGAGGCCCCAACACGTCCAG
     670       680       690       700       710       720
 L   N
CTAAACgtaagcagacgacaattcgtcgatcggtcaccgtatcaggttcaggtgatgatt
     730       740       750       760       770       780
                                    P   F   A   K   M   P   G   F
tctgacagagatgctacgtgtttttcttccttgcagCCGTTTGCGAAGATGCCTGGGTTC
     790       800       810       820       830       840
 Q   D   G   D   L   V   L   F
CAAGATGGCGATCTCGTCCTGTTCGgtgagccgtctaacacatcagtgcacacgacacga
     850       860       870       880       890       900
atcattcctgtctaggagtatatatcatgttaaaaatccattggttggtttctctaatct
     910       920       930       940       950       960
                                    E   S   R   A   I   A   K   Y   I   L   R   K
aacaaatcgatgagtgagtgagtgcagAGTCGCGCGCCATCGCCAAGTACATCCTCCGCA
     970       980       990      1000      1010      1020
   Y   G   G   T   A   G   L   D   L   L   G   E   N   S   G   I   E   E   L   A
AGTACGGGGGGACAGCCGGCCTGGACCTCCTCGGAGAAAACAGCGGAATCGAAGAATTAG
    1030      1040      1050      1060      1070      1080
   M   V   D   V   W   T   E   V   E   A   Q   Q   Y   Y   P   A   I   S   P   V
CAATGGTGGACGTGTGGACGGAGGTGGAGGCCCAGCAGTACTACCCGGCCATCTCGCCCG
    1090      1100      1110      1120      1130      1140
   V   F   E   C   I   I   I   P   F   I   I   P   G   G   G   A   A   P   N   Q
TGGTGTTCGAGTGCATCATCATCCCCTTCATCATCCCTGGCGGTGGCGCGGCGCCGAACC
    1150      1160      1170      1180      1190      1200
   T   V   V   D   E   S   L   E   R   L   R   G   V   L   G   I   Y   E   A   R
AGACCGTCGTGGACGAGAGCCTGGAGCGGCTGAGGGGTGTGCTGGGGATCTACGAGGCCC
    1210      1220      1230      1240      1250      1260
   L   E   K   S   R   Y   L   A   G   D   S   I   T   F   A   D   L   N   H   I
GGCTGGAGAAGAGCAGGTACTTGGCCGGGGACTCCATCACGTTCGCCGATCTGAACCACA
    1270      1280      1290      1300      1310      1320
   P   F   T   F   Y   F   M   T   T   P   Y   A   K   V   F   D   D   Y   P   K
TCCCGTTCACCTTCTACTTCATGACCACCCCGTACGCCAAGGTGTTTGATGACTACCCCA
    1330      1340      1350      1360      1370      1380
   V   K   A   W   W   E   M   L   M   A   R   P   A   V   Q   R   V   C   K   H
AGGTGAAGGCCTGGTGGGAGATGCTCATGGCGAGGCCGGCGGTGCAGAGGGTCTGCAAGC
    1390      1400      1410      1420      1430      1440
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(continued next column)

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   M   P   T   E   F   K   L   G   A   Q   Y   *
ATATGCCTACCGAGTTTAAGCTAGGTGCGCAGTACTAGGTGTTTATGCACTCCGCGCGGG
      1450      1460      1470      1480      1490      1500
TGTGTGCCGTGCCGTGCCGTGCCGTGCCGGTGATGATTCCGTTCAAGAAAAGAGACCTCC
      1510      1520      1530      1540      1550      1560
CTCCCGTGGGTGGGTGATCATCTGCCCGTCTACTTCCAGTAGTATACGCTTTCAGTTTCA
      1570      1580      1590      1600      1610      1620
GCAGCACGAGGAGTCCGCTTGCGCTTCTGTTCGTGCGCTGGATTCCTTGCTTTAATTTAT
      1630      1640      1650      1660      1670      1680

GCACTAGTACTAGTTAGTAAATAAACAACGGACTGGTCAATGCTGCTAATCCGTACTGTA
      1690      1700      1710      1720      1730      1740
CGTCCTATATAGTAGTACCCCACTAGAGATCAGTATATATATACATCTTGGATACGTGTG
      1750      1760      1770      1780      1790      1800
CATTTGCATGCAAATATATATGGACAGGGATAACTGGCACATGTGTGCCCGTTAGGAAAA
      1810      1820      1830      1840      1850      1860
TTACGATCGCTCTCCTTCGGTCGGTGGCCCGCTCGCTCGATCGAGCGTTCCTTCGGTCGG
      1870      1880      1890      1900      1910      1920
TGTCCCGCTCGCCCTTCGGTCCGTGGCCCGCTCGCTCGATCGAGCGTTCCTTCGGTCGGT
      1930      1940      1950      1960      1970      1980
GTCCCGCTCGCTCGATCGAGCGTTGAGGCCAGTTGGCTCGATCGCTCCGCTTTGATCGAG
      1990      2000      2010      2020      2030      2040
TTGTGAATGCTTTCACCGGTTTTTTTTTCTTCTGATTTTTATTTTCAGAACGGTCTACTA
      2050      2060      2070      2080      2090      2100
AATAGTCACTACACCCAATAACCAACTCGGCCATCCTACCTTGTTATCCACAATACGTGA
      2110      2120      2130      2140      2150      2160
ACAACGGAAGTTGAATTC
      2170
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Fig. 1. Nucleotide sequence of the *GstA1* gene and amino acid sequence of the encoded protein. The amino acid sequence is written in the single-letter code above the nucleotide sequence. Intron sequences are written in lowercase letters. The translation stop codon is marked with an asterisk. The presumed TATA box and the polyadenylation signal are overlined. The broken arrow denotes the transcription initiation region.

cDNA cloning by polymerase chain reaction (PCR) amplification. As amplification primers a 23mer oligonucleotide with the sequence 5′-TTGGATCCAGTATCTGCCCTCTC (oligo 1; bases 6 to 23 correspond to the gene sequence between positions 514 and 531 in Fig. 1, whereas the 5′ end creates a *Bam*HI site for later cloning) and a 21mer oligonucleotide with the sequence 5′-TTGAGCTCGAACACCACGGGC (oligo 2; bases 6 to 21 are complementary to the gene sequence between positions 1136 and 1151; the 5′ end creates a *Sac*I site) were synthesized. The reactions were done essentially as described by Kawasaki and Wang (1989). Briefly, first strand cDNA synthesis was primed with 50 pmol of oligo 2 using 2 μg of LiCl-precipitated RNA extracted from infected wheat leaves (cultivar Cheyenne) as a template in a 20-μl reaction mixture containing 2 μl of 10× PCR buffer (500 mM KCl, 200 mM Tris-HCl, pH 8.4, 25 mM $MgCl_2$, 1 mg/ml bovine serum albumin), 1 unit per microliter of RNasin, 1 mM of each dNTP, and 200 units of M-MLV reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD). The reaction was first incubated for 10 min at room temperature and then for 1 hr at 37° C. After heat inactivation (3 min at 90° C), a 10-μl aliquot of the reaction product

was directly amplified by the PCR in a volume of 100 μl containing 9 μl of 10× PCR buffer, 1.25 mM of each dNTP, 1 μm each of oligo 1 and 2, and 2 units of Taq polymerase (Perkin-Elmer, Norwalk, CT) through 25 cycles (1 min at 94° C, 30 sec at 50° C, and 1 min at 72° C). The product was extracted with phenol, precipitated, and cloned into a pBluescript KS+ vector.

RESULTS

The isolation of the cDNA clone *WIR5* by differential screening of cDNA libraries has been described previously (Schweizer *et al.* 1989). This clone represents a wheat (cultivar Fidel) transcript that is induced upon infection of wheat with *E. graminis*. The clone was reported to contain an insert of about 700 bp and, therefore, does not represent the complete mRNA of 1.4 kilobases (kb) as determined by northern blots (Schweizer *et al.* 1989). We have used the *WIR5* cDNA clone as a probe to screen a λ EMBL3 wheat genomic library (cultivar Cheyenne). Three positive λ clones were obtained, one of which, λ *WIR56*, was analyzed further. Digestion of this clone with restriction enzymes yielded a 2.2-kb *Bam*HI-*Eco*RI fragment hybridizing with the *WIR5* cDNA clone. This genomic fragment, as well as the *WIR5* cDNA, was completely sequenced on both strands. The analysis of these sequences and the experiments described below led us to the conclusion that the fragment contains a gene with three exons which encode a protein of 229 amino acids with a calculated relative molecular mass of 25,828. The sequence of this gene and the encoded protein are shown in Figure 1. This conclusion is based on the following data:

1) The *WIR5* cDNA sequence, which consists of 634 nucleotides, not counting the poly(A) tail, contains an open reading frame corresponding to part of exon 3. The 5′ end of the truncated cDNA corresponds to nucleotide position 1071 of the gene sequence (Fig. 1). The cDNA contains a poly(A) tail 35 nucleotides 3′ of the polyadenylation signal (corresponding to position 1700, overlined in Fig. 1). The coding part is 98% identical to the gene sequence, the eight base substitutions resulting in two conservative and one nonconservative amino acid changes (Fig. 2). Except for several small (1 to 10 bp) insertions and deletions, the nontranslated trailer sequence is also very similar.

2. To confirm the exon-intron structure shown in Figure 1, we cloned the cDNA parts bridging the exon-intron boundaries. cDNA was synthesized from mRNA of wheat plants (cultivar Cheyenne) infected with powdery mildew, and the relevant species was amplified in a PCR using synthetic oligonucleotides corresponding to sequences in the first and third exons as primers, respectively (see Materials and Methods). The product was cloned and one clone was sequenced. In this way a cDNA sequence was obtained starting in exon 1 and extending to exon 3. The exon-intron boundaries as shown in Figure 1 were confirmed. This cDNA sequence and the exon sequences of the gene are 99% identical over the coding regions, the three base substitutions resulting in one conservative amino acid change (Fig. 2).

3. The methionine codon at position 577 (Fig. 1) is likely to be the translation initiation codon, because six codons upstream of it there is an in-frame TGA stop codon (position 559). Between this initiation codon and an upstream potential transcription initiation site defined by the TATA box at position 445 (Fig. 1, overlined), there is no other initiation codon. That transcription does indeed start in the expected region (Fig. 1, broken arrow) was confirmed by S1-mapping the 5′ end of the transcripts. For this purpose, a 371-bp *Sac*I fragment of the cloned gene (between *Sac*I sites at positions 298 and 664, Fig. 1) was subcloned, and a continuously labeled single-stranded probe was prepared representing the antisense strand of the gene. This probe was hybridized to equal amounts of total RNA extracted from plants (cultivar Cheyenne), which were or were not infected with *E. graminis*. As can be seen in Figure 3, the major protected fragment has a length of about 190 bases, indicating that transcription is initiated around position 479, that is about 30 nucleotides downstream of the putative TATA box. It is evident from Figure 3 that mRNA homologous to the cloned gene is indeed pathogen-induced. RNA extracted from infected plants gives a signal at least 20 times stronger than RNA from control plants, as determined by densitometry of appropriately exposed autoradiograms. The weaker bands with sizes between 110 and 136 nucleotides map to the nontranslated leader sequence. We interpret these signals to result most likely from transcripts of homologous genes with slightly diverged leader sequences. The presence of several related *WIR5* genes in wheat is evident from the Southern blot experiment shown in Figure 4. About six to 10 bands hybridize to the *WIR5* cDNA

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                    20                  40                  60
WC  --------------------------------v-------------------------
WG  MSPVKVFGHPMLTNVARVLLFLEEVGAEYELVPMDFVAGEHKRPQHVQLNPFAKMPGFQD
M1  -a-m-ly-AV-SW-lt-CATA---A-sd--i--iN-AtA---S-E-lVR---GQv-AL--
M3  -a-l-ly-M-lSP--V--ATV-N-K-Ldf-i--v-LTt-A--Q-DFlA----GQi-ALV-

                    80                  100                 120
WC  ----------------------------------======--------------------
WG  GDLVLFESRAIAKYILRKYGGTAGLDLLGENSGIEELAMVDVWTEVEAQQYYPAISPVVF
M1  ---Y-------C--AA--NK    Pe--R-GN l--A------I----n--TA-lN-il-
M3  --E--------Nr--AS--AS E-T---PATaSAAK-E    --L---sHHfH-NA--l--

                    140                 160                 180
WC  ------------------------------------------------s---------
WG  ECIIIPFIIPGGGAAPNQTVVDESLERLRGVLGIYEARLEKSRYLAGDSITFADLNHIPF
M1  QVl-S-   ML--tt DEK----N--k-kK--Ev-----T-Ck-----FlsL-----vSV
M3  QLlvR-Ll     -G--DAa--eKHA-Q-AK--Dv---H-ArNk-----EF-L--A--ALL

                    200                 220
WC  - ------------e-----------------------------R---
WG  T FYFMTTPYAKVFDDYPKVKAWWEMLMARPAVQRVCKHMPTEFKLGAQY
M1  - LCLFa----S-L-A--H-----SG--E--s--k-AAL-KPSA
M3  PALTSARP-RPGCVAAR-H------AiA----F-kTVAAI-LPPPPSSSA
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Fig. 2. Optimized sequence alignment of the proteins encoded by the wheat *GstA1* gene (WG) or the cDNAs (WC) with GSTI (M1) and GSTIII (M3) of maize (Grove *et al.* 1988). The sequence of the *GstA1*-encoded protein is given in the single-letter amino acid code. Positions in the other sequences with identical amino acids are indicated with hyphens; conserved substitutions (A,S,T; D,E; N,Q; R,K; I,L,M,V; F,Y,W) are denoted by lowercase letters. Empty spaces indicate gaps introduced to optimize the alignment. Double hyphens indicate the overlap of the wheat cDNA-derived sequences as translated from cDNA amplified by the polymerase chain reaction (amino acids 1 to 100) and *WIR5* (amino acids 95 to 229) cDNA, respectively.

in *Hin*dIII and *Eco*RI digests of wheat (cultivar Fidel) genomic DNA.

The sequence of the putative protein encoded by the λ *WIR56* gene was compared to the Swiss-Prot protein sequence data base. Significant similarity to the GSTs (GST, EC 2.5.1.18) of maize was detected. Figure 2 shows the optimized alignment of the wheat protein with the two published maize GST sequences (GSTI and GSTIII). The wheat protein shows 51% identical and 13.5% conserved amino acids compared to GSTI, and 40% identical and 14.5% conserved amino acids compared to GSTIII of maize, respectively (Moore *et al.* 1986; Shah *et al.* 1986; Grove *et al.* 1988). The sequence similarity between the two maize GSTs is about the same: 50% identical and 14% conserved amino acids. We conclude from this analysis that the λ *WIR56* clone contains a gene which is likely to encode a GST. We name this gene *GstA1*.

DISCUSSION

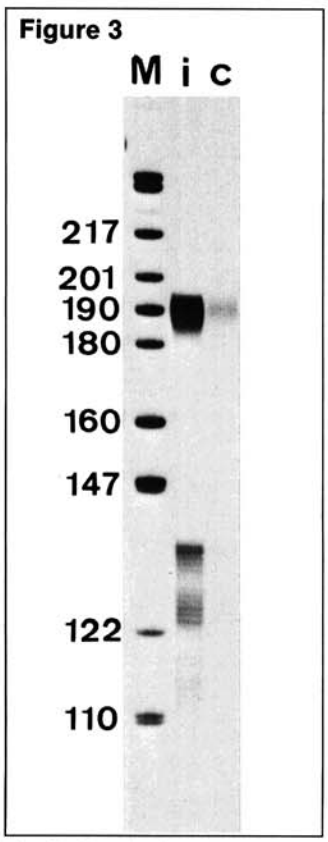
In this study we present evidence that pathogen attack of wheat results in a strong increase of mRNA coding for a protein homologous to GST. We have not shown the enzyme activity of this protein. However, the following considerations strongly suggest that the protein is indeed a GST. First, the wheat protein and the maize GSTs are similar over their entire sequence. With 40–50% sequence identity, the wheat protein sequence is as similar to the maize GSTs as these are to each other. Second, both the wheat *GstA1* and the maize GSTI (Shah *et al.* 1986) genes are interrupted by two introns, the positions of which are exactly conserved in the two species. This suggests that these genes are homologous.

Our S1-mapping experiments show that transcripts homologous to the *GstA1* gene are about 20 times more abundant in wheat leaves infected with powdery mildew than in uninfected ones. This increase results, at least in part, from transcriptional induction (Schweizer *et al.* 1989). Our S1-mapping data are compatible with the assumption that the cloned *GstA1* gene is activated. However, this can only be proven by a promoter analysis in appropriate assay systems.

We have measured GST activity in leaves 24 and 48 hr after infection with *E. g.* f. sp. *hordei* in a spectrophotometric assay using 1-chloro-2,4-dinitrobenzene as a substrate. Compared to control plants, GST activity increased by a factor of two after 48 hr (unpublished results). Although this confirms that GSTs are induced by pathogen attack, the factor of induction measured in this assay is much lower than the one implied by the S1-mapping experiments. This might be due to the relatively high background GST activity in noninduced leaves, or due to the possibility that 1-chloro-2,4-dinitrobenzene may be a poor substrate for the induced isozyme(s), as has been reported for the GST isozymes reactive with metolachlor in sorghum (Gronwald *et al.* 1987) and with atrazine in maize (Timmerman and Tu 1987), respectively. Clarification of this point will have to await production of specific antibodies directed against fusion proteins and the availability of the *GstA1*-encoded protein expressed in *Escherichia coli*.

The sequences of the cDNAs are very similar, but not identical, to the sequence of the genomic clone. The sequence heterogeneities between the *WIR5* cDNA and the *GstA1* gene may be attributable to the fact that they were obtained from different cultivars (Fidel and Cheyenne, respectively). However, the cDNA part cloned by PCR amplification originated from the same cultivar as the genomic clone. Thus, the sequence heterogeneities indicate the presence of several genes similar to *GstA1* in the wheat genome. Indeed, Southern analysis showed that between six to 10 bands are hybridizing to the *WIR5* cDNA insert in both *Hin*dIII- and *Eco*RI-digested wheat DNA (cultivar Fidel). Since *T. aestivum* is an allohexaploid species, the number of bands seems compatible with the presence of one gene per monohaploid genome, that is six alleles in the allohexaploid genome, assuming heterozygosity (Fidel is a hybrid line). If this interpretation is correct, the situation in wheat parallels the one in the diploid maize, where both GSTI and GSTIII genes were reported to be present in one copy per haploid genome (Moore *et al.* 1986; Shah *et al.* 1986; Grove *et al.* 1988).

A widely assumed hypothesis is that pathogen-induced host genes code for products which are involved in host defenses against pathogens. Our results strongly suggest that one such pathogen-activated gene encodes a GST. Assuming this to be true, the question arises as to how



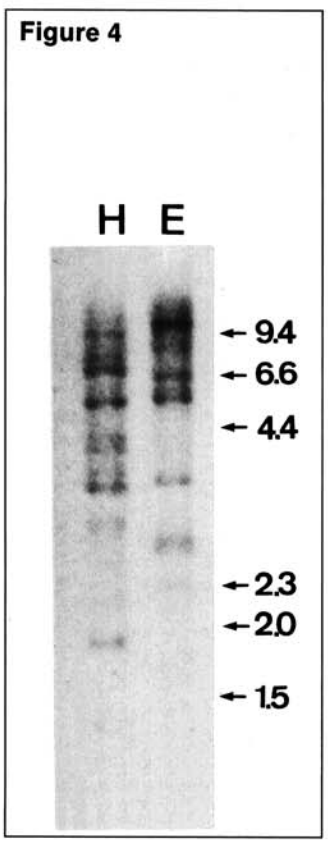


Fig. 3. S1 mapping of the transcripts. Ten micrograms of total RNA from infected (lane i) or control (lane c) plants was hybridized with a single-stranded probe. Numbers on the left of the figure indicate lengths of marker bands (lane M) in bases.

Fig. 4. Southern blot hybridization of 10 μg of wheat DNA cut with *Hin*dIII (lane H) or with *Eco*RI (lane E). The probe was the *WIR5* cDNA insert.

these enzymes might be involved in the defense against pathogens. GSTs are widely found in animals and plants. They constitute a family of multifunctional dimeric enzymes catalyzing the conjugation of the tripeptide glutathione (γ-L-glutamyl-L-cysteinylglycine) to electrophilic centers of lipophilic compounds, thereby detoxifying them (for a recent overview, see Sies and Ketterer 1988). In plants, GSTs have been shown to play a role in the detoxification of herbicides. Best characterized in maize (for a recent review, see Timmerman 1989), certain isozymes were found to detoxify atrazine and other herbicides. Moreover, treatment with safeners increased herbicide tolerance, and this correlated with an increase in GST activity. This increase was shown to be at least partly due to induction of gene transcription (Wiegand *et al.* 1986). Similar results were reported in sorghum (*Sorghum bicolor* (L.) Moench), where various herbicide antidotes induced the *de novo* synthesis of GST isozymes reactive with the chloroacetanilide herbicide metolachlor (Gronwald *et al.* 1987; Dean *et al.* 1990). It is not known whether GST genes are induced by pathogens in these systems.

A possible connection between glutathione metabolism and pathogen defense of plants was recently revealed by the intriguing observation that glutathione (i.e. a substrate of GST) induces transcription of defense genes in cultured cells (Wingate *et al.* 1988; Dron *et al.* 1988). Furthermore, in animal systems, the highly toxic products of membrane lipid peroxidation have been found to be substrates of GST isozymes, which may thus contribute to protection from oxidative tissue damage (for a review, see Pickett and Lu 1989). Since formation of active oxygen species and membrane lipid peroxidation are known to occur in plants in response to tissue damage, elicitor treatment, and pathogen attack (Thompson *et al.* 1987; Rogers *et al.* 1988; Chai and Doke 1987; Kato and Misawa 1976; Keppler and Novacky 1987; Croft *et al.* 1990), it is conceivable that GST plays a similar protective role in plants. Thus, GST genes could be members of a class of general stress response genes, which are activated by many different stimuli. In addition to infection, we have tested one other stimulus: wounding of wheat leaves (by Carborundum treatment) does not result in increased abundance of *GstA1* homologous mRNA, as determined by S1 mapping (data not shown), thus indicating a more specific role. However, whether or how GSTs contribute to the defense of wheat against *E. graminis* remains a matter of speculation at this time. Although genetic manipulation of wheat is as yet not possible, we hope that the cloned wheat *GstA1* gene will help to attack experimentally some of the questions concerning GST and pathogen defense in other systems. It will also be interesting to investigate the relation between GST induction and herbicide resistance in wheat.

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